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Research Article

ENVIRONMENTAL CHARACTERISTICS OF ANOPHELINE MOSQUITO LARVAL HABITATS IN ENDEMIC AREAS AND STUDIES ON LARVAE EMERGENCE FOR ANOPHELINE DIVERSITY

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ABSTRACT
The study was conducted in 8 villages located in the basin and Valley of district Kolasib and Aizwal of Mizoram. In selected villages, four are located at high altitude and four at lower altitude, all villages were of high endemic type, study was conducted during Jun'11 to Mar'2014 to verify the malaria transmission in cold, rainy as well as summer dry seasons. Larvae of Anopheles were identified visually, sampled and larval emergence was perform in field lab and Microscopically adult were identified.

Keywords: *Anopheles, LDPD, Habitats Diversity, Emergence*

INTRODUCTION
Human malaria is transmitted by Anophelines originating at specific breeding habitats and transmission normally occurs within a certain radius (within flight range of adult vectors) from breeding sites (Carter *et al.*, 2000).
The breeding habitat is crucial for mosquito population dynamics, since it is the location where many important life cycle processes and emergence take place (Overgaard *et al.*, 2001).
Anopheline mosquito breeding generally occurs in different types of water and a wide range of habitats which may be natural or man-made, temporary or permanent, shaded or sunny (Machault *et al.*, 2009). Certain environmental parameters are particularly influential in determining larval habitat suitability for the different anopheline vectors, including size and permanence of the water body, water salinity and turbidity, amount of sunlight, and presence of emergent or floating vegetation (Service, 1989).
Knowledge of the ecological characteristics of the breeding habitats and the environmental factors affecting mosquito abundance can help in designing optimal vector control strategies (Overgaard *et al.*, 2001; Surendran & Ramasamy, 2005).
Each anopheline species has its preferred breeding site for oviposition, depending on factors such as weather conditions, physical geography and human activity (Liu *et al.*, 2012). The physico-chemical parameters of the water probably determine the selection of larval habitats.
Various physico-chemical properties of the larval habitat such as pH, optimum temperature, concentration of ammonia, nitrate and sulphate have been found to affect larval development and survival (Mutero *et al.*, 2004).
The breeding habitat is crucial for mosquito population dynamics, since it is the location where many important life cycle processes such as oviposition, larval development, and emergence take place (Overgaard, 2002).
A strong association exists between the density and distribution of the mosquito larval stages and that of the adult vectors. Control of larval mosquito populations is often advantageous because the larvae are usually confined, relatively immobile and occurs in minimal habitat area compared with adults that can rapidly disperse over large areas (Floore, 2006).
Larval control measures are intended to reduce malaria transmission indirectly by reducing the vector population density near human habitations. As the larvae are exclusively aquatic, their distribution is determined by the locations of suitable water bodies.
Therefore, knowledge of the ecological characteristics of the larval habitats and the environmental factors affecting mosquito abundance can help in designing optimal vector control strategies (Overgaard, 2002; Gimnig *et al.*, 2001).

This work was carried out in order to collect basic information to control malaria by mosquito abatement with GECH project conducted at district Kolasib and Aizwal of NE State Mizoram.

MATERIALS AND METHODS

The Studied Area

The study was conducted at eight villages in two district of state Mizoram. Kolasib area covers about 1382.51 km_2 with total Population (as per census 2011; 83054) with latitude of 722m (2370ft) from sea level and Aizawl is located north of the Tropic of Cancer in the northern part of Mizoram and is situated on a ridge 1132 metres (3715 ft) above sea level, with Population (2011 Census; 404,054). Temp in summer (20-30^{0}C) and winter (11-21^{0}C) of state Mizoram. It rains heavily from May to September with annual average rainfall is 254 cm and Lunglei has 350 cm.

Larval Sampling and Processing

Mosquito larvae were sampled fortnightly from Oct 2011 up to early Jan 2014 which covered the dry season (December–February) and short rainy season (March–May).

Use of the Dipper: Various kinds of dippers were used, including small frying pans, soup ladles and photographic dishes. It was important to use the right type and size for each breeding sites. A white enameled dipper was preferred, because this allows easy recognition of larvae.

The dipper was gently moved into the water at an angle of about 45°, until one side was just below the surface.

Using a Small Spoon or Pipette: A small spoon or wide-mouth pipette was used for collecting larvae and pupae from small collections of water, e.g. the gum collecting bowls attached to rubber trees, bamboo stumps, in a tin can or a hoof-print.

Larval Sampling: Samplings were conducted in the morning (0900– 1200 hrs) for about 30 min or less at each larval habitat using a standard dipper (11.5 cm diam and 350 ml capacity), pipettes and white plastic pans (Sharma, 1990; Service, 1993). When larvae were present, 5-10 dips were taken depending on the size of habitat at intervals along the edge. All I, II, III and IV instar anopheline larvae and pupa were collected in screwed cap bottle or vial.

Transporting Live Larvae and Pupae to the Laboratory: The bottles or vials which contained larvae or pupae, should filled with water to provide enough oxygen for larvae and pupae. Each bottle or vial was tightly closed with a stopper.

Where the laboratory was more than two hours away, the stoppers were removed every two hours to provide the specimens with fresh air.

Immergence of Larvae into Adult: Larva and pupae were fed to grow till emergence using fish powder. They all kept in early morning sunlight for an hour then sifted to shade at room temperature. Every morning water and food keep on changing till larvae and pupae samples get moulted to mosquitoes in white enameled bowl. They were prevented to escape by using cotton net with rubber band.

Microscopic Identification: Emerged adult were suction using tube captured and kept in test tube was then kept in direct sunlight for death.

Simultaneously, microscopic examination to identify the new hatch mosquitoes using standard key book of identification.

Keys for the identification of emerged anopheline mosquitoes of India have been published by several authors, of which the most important are those by Christophers (1933) and Puri (1954, adults; 1960, larvae).

Larval Habitat Characterization and Recording of Environmental Variables

Simultaneously, environmental characteristics of each larval habitat were also taken into consideration. The environmental variables noted were water temperature, water pH, water depth, elevation, intensity of light, turbidity, vegetation type, water current, substrate type, distance to the nearest house, natural or human made habitat, the presence of algae and permanence of the habitat.

Water temperature was measured using scaled Thermometer whereas, water pH was measured using pH indicator paper.

Ethical Considerations

This study was approved by NIMR-ICMR Ethical Review Committee. Verbal consent to access compounds and farms was obtained from local leaders and residents during village administrative in each of the study areas.

RESULTS AND DISCUSSION

Results

Analyses the association between environmental habitat and mosquito larvae abundance. The relative abundance of anopheline species emerges in the different larval habitats was significantly varied. Total 1942 early instars (I and II), late instars (III and IV) and pupa of Anopheles from 15 larval habitats and identified using morphological characters. Emergence was perform in Field Unit Lab microscopically for species characterization.

Species Composition and Seasonal Abundance of Emerged Anopheline Larvae

Four months' study on larvae Emergence on 557 out of 1942 larvae in Environmental Epidemiology Field Laboratory emerged adult named after microscopic examination. They comprised of seven species: *An barbirostris* (32.2%), *An kochi* (24.4%), *An macuautus* (18.8%), *An. nivipes* (1.6%), *A. philippinensis* (2.2%), *An subpictus* (6.6%) and *An vagus* (13.8%) (Table 1; Figure 1).

The most abundant species was *A barbirostris*, *A Kochi*, *A macuautus* and *A vagus* whereas *An. nivipes (*1.6%), *A. philippinensis* (2.2%) and *A subpictus* were generally scarce (Table 1; Figure 1) which were collected from the study areas. Larvae of two malaria vectors, *A kochi* and *A vagus or A macuautus* are co-existed and collected in a wide range of habitats with different physico-chemical parameters (table 1, 2).

Monthly Anopheline Larval Variations

Marked monthly variations were observed in densities of the anopheline larval populations with their minimum mean density in January (LDPD 0.97) and maximum mean density in March (LDPD 28.4) and gradually no rose up during the short rainy season (Table 2).

In selected localities densities of larvae increased towards the end of the short rainy season (May) and beginning of the main rainy season (June) and during the initial larval survey of the dry season in all the localities (December–February) which gradually decreased during the short rainy season (Table 2).

Studied Locality and Emerged Anopheline

Max anopheline emergence was found in Lengpui (39.7%) followed by Bairabi (23%) and Sairang (20.5%) rest 3 places cover 17% emergence (Table 3).

Habitat Diversity and Larval Abundance

The spatial distribution of the anopheline larvae in different aquatic habitats in the study localities. as *A barbirostris* 58(32.2%), *A Kochi* 44(24.4%), *A macuautus* 34(18.8%) and *A vagus* 25(13.8%) larvae were the predominant species occurring in a wide range of habitats. and *A barbirostris* larvae were collected most abundantly from pond (total emerge 58.5%), whereas *An Kochi (*24.4%) larvae and *Anopheles vagus* ((13.8%)) larvae were mostly coexisting in barrow pit and seepage (28.8% and 13.3% respectively) followed by Rice field and River bed pool (5.5% and 3.8% respectively). Larvae of *A. philipinensis* and *A subpictus*, also occurred in rice field and pond co-existing with the former two species, but were scarce and generally absent from other habitat types (Table 4).

Monthly Rain Fall in Studied Area

Three locality having min and max rain fall (Table 6) in April min 2.5mm max 77.5 min and min 6 mm max 95mm in July reported from Bairabi (Ksb).

In Kawnpui (Ksb) in May min 0.30 mm max min 41mm while in June min 2.2mm max 93 mm. Sihhmui (Azl) min 0.2 mm max 56.0 mm in May and July min 0.2mm max 86.5mmwere reported (Table 6).

There is a definite role and correlation exists among rain fall, temperature, humidity, larvae abundance and also environmental settling.

Table 1: Emerged Larvae into Female Anopheles sps. in Various Habitats in Studied Areas (June 2011– Sept 2011)

Larval Habitat	*An. Barbi Rostris*	*An. Kochi*	*An. Maculatus*	*An. Nivipus*	*An. Phili Pinensis*	*An. Sub Pictus*	*An. Vagus*	Habitat Type Emerges
Borrowpit	01	28	-	-	-	10	13	52 (28.8%)
Pond	54		-	03	03	01	04	65 (36.1%)
Rice Field	-	06	01	-	01	01	01	10 (5.5%)
River Bed Pool	-	05	-	-	-	-	02	07 (3.8%)
Seepage	-	05	14	-	-	-	05	24 (13.3%)
Stream Bed Pool	-	-	09	-	-	-	-	09 (5%)
Ditch	02	-	-	-	-	-	-	02 (1.1%)
Rivulet	01	-	-	-	-	-	-	01 (.5%)
Rock Pool	-	-	01	-	-	-	-	01 (.5%)
Tyre Print	-	-	09	-	-	-	-	09 (5%)
Adult Type Emerges	58(32.2%)	44(24.4%)	34(18.8%)	03(1.6%)	04(2.2%)	12(6.6%)	25(13.8%)	180 (100%)

Table 2: Monthly Larval Emergence Density (LED) into Female Anopheles Adults

Month	Female Emerged	*A. barbirostris*	*A. kochi*	*A. macuautus*	*A. nivipes*	*A. philipinensis*	*A subpictus*	*A. vagus*
JUNE'11	38	11 (28.9%)	09(23.6%)	04 (10.5%)	0	0	0	14 (36.8%)
JULY'11	79	34 (43%)	15(18.9%)	09 (11.3%)	03(3.7%)	0	10(12.6%)	08 (1%)
AUG'11	29	10 (34.4%)	5 (17.2%)	12 (41.3%)	0	01 (.03%)	01 (.03%)	0
SEPT'11	27	09 (31%)	11 (40.7%)	3 (11.1%)	0	3 (11.1%)	1 (.03%)	1 (.03%)

Table 3: Emerged Adult from Anopheline Larvae Collected from Eight Localities of Kolasib and Aizawl (June 2011– Sept 2011)

Emerged Adult	Bairabi	Bualpui	Lengpui	Pangbalkawn	Sairang	Sairang Dinthar	Total	%
A. barbirostris	5		28				33	42.3
A. kochi	-				14	1	15	19.2
A. macuautus	-	5		4			9	11.5
A. nivipes	-		3				3	3.8
A. subpictus	10						10	12.8
A. vagus	3				2	3	8	10.2
Total	18(23%)	5(6.4%)	31(39.7)	4(5.1)	16(20.5)	4(5.1)	78	100%

Table 4: Spatial Distribution of the Anopheline Larvae Density (LDPD) Monthly in Different Aquatic Habitats in the Study Localities

Period↓ Habitat→	Boat	Ditch	Drain	Pond	Rice Field	River Bed Pool	Rivulet	Rock Bed Pool	Minor Seepage	UGT	Stream Bed Pool	Borrow pit
DEC'10	-	-	-	1.98	-	2.5	-	-	-	0.6	-	-
JAN'11	-	0.87	1.14	0.91	-	-	-	-	1.8	-	-	-
FEB'11	-	-	1.07	1.14	-	3.05	-	-	-	-	-	-
MAR'11	-	-	-	-	-	-	28.4	-	-	-	-	-
APR'11	5.66	3.62		0.71		9.56	1.5		1.0	0.75		
MAY'11	1.87	-	1.66	-	7.52	-	30.2	-	-	-	-	1.0
JUN'11	4.0	-	-	-	1.11	-	2.5	-	-	-	-	2.0
JULY'11	4.61	-	-	-	3.33	-	1.35	-	-	1.55	-	2.0
AUG'11	-	3.0	-	-	0.6	2.0	-	-	0.5	6.0	-	TyrePrint
SEPT'11	0.66	-	2.8	-	2.42	3.0	-	-	-	-	-	4.0
NOV'11	-	-	-	12.6	4.0	0.33	5.4	1.08	-	-	-	2.0

Table 5: Monthly Average Larval Density

Period	Larvae (I-IV) + Pupae no	LDPD; Average Number of Larvae-Pupae/Dip
DEC'10	144+0	1.97
JAN'11	74+1	0.97
FEB'11	148+1	1.47
MAR'11	142+0	28.4
APR'11	341+7	4.52
MAY'11	337+11	4.09
JUN'11	114+3	1.72
JULY'11	218+5	2.89
AUG'11	100+1	1.87
SEPT'11	114+2	2.23
NOV'11	145+1	4.91

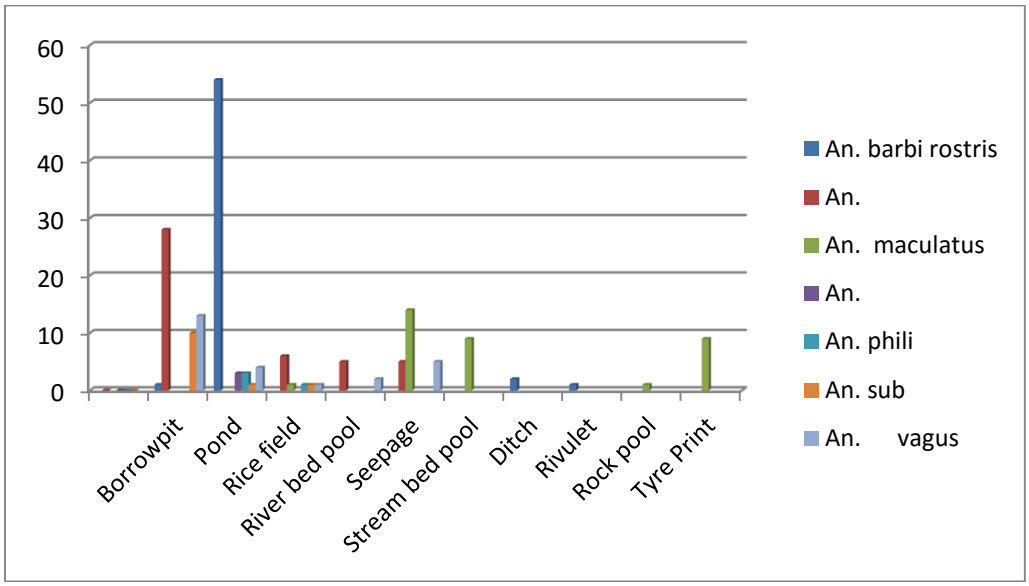

Figure 1: Emerged Larvae into Female Anopheles sps. in Various Habitats in Studied Areas (June 2011– Sept 2011)

Table 6: Monthly Rainfall Reading Details

Month/Site	Bairabi (Ksb)		Kawnpui (Ksb)		Sihhmui (Azl)	
	Min	Max	Min	Max	Min	Max
DEC'10	-	56.7mm	4mm	81mm	1.8mm	64.2mm
Mar'11	3.4mm	38.3mm	15mm	34mm	0.4mm	35.2mm
April'11	2.5mm	77.5mm	3.0mm	62.2mm	4 mm	11.2 mm
May'11	8.5 mm	78mm	0.30 mm	41mm	0.2 mm	56.0 mm
June'11	6.0mm	95mm	2.2mm	93 mm	0.2mm	83.4mm
July'11	2.5mm	33mm	2.0mm	45.7mm	0.2mm	86.5mm
Aug'11	5.0mm	64.7mm	1.5mm	71.3mm	0.3mm	45.4mm

Discussion

Although control of the immature mosquito population is an important component of the NVDCP Program, Government of India, larval habitat productivity and the impact of this program on the population dynamics of vector species have well understood. Knowledge of larval habitats and their distribution would be an important step for planning and implementing larval control strategies effectively.

This study has documented the occurrence of five species of *Anopheles* larvae emergence (*An barbirostris, An kochi, An macuautus, An. nivipes, A. philippinensis, An subpictus and An vagus*) the former three were the predominant species in the area. Previous studies have shown that the *An. minimus and An. dirus* was the primary vector and other anopheline species, play the main role as secondary vectors in the south and north east states of India.

Environmental Factors Associated with Larval Occurrence/Abundance

Higher mean densities of *An. barbirostris* larvae were obtained from aquatic habitats that had clear and standing water, free of vegetation and temporary habitats near to human dwellings (<100 m). *An. kochi* larvae were also significantly collected from permanent and natural habitats that had clear and standing

water with floating algae. *An. vagus* larvae were collected from aquatic habitats which are clear, permanent, still, having emergent and floating vegetation with algae near human dwellings.

Anopheline larvae were significantly correlated with elevation, aquatic vegetation, habitat permanence, water current and distance to the house negatively associated with aquatic vegetation and water current while some Anopheline larvae were positively associated with the presence of algae and water temperature.

Hatching of Larvae

Early in the spring, when the water is cold, and the mosquito's metabolism is slow, it can take as long as a month, or even more from the time the eggs hatch until there are flying mosquitoes. Later in the summer, some species emerges from egg to adult in as little as 4 or 5 days. The hatching and emerges of fully adult mosquitoes depends food availability and also on temperatures of water they endure.

Precaution of Sampling

While dipping, don't agitate water the larvae may swim downwards. Moving along the breeding site, the surface of the water was skimmed with the dipper. The dipper was carefully lifted out of so that the larvae and pupae were not spilled out with the water and transferred to a bottle or vial. When the area was thickly surrounded by vegetation, the dipper was pressed through the plants and lowered into the water allowing the water to flow in dipper.

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