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Research Article

A LONGITUDINAL STUDY ON ANOPHELES MOSQUITO LARVAL ABUNDANCE IN DISTINCT GEOGRAPHICAL AND ENVIRONMENTAL SETTINGS AND EMERGENCE IN RANCHI AND RAMGARH OF JHARKHAND

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ABSTRACT
In selected 16 villages of high endemic type larvae study was conducted during Jan'12 to Mar'2014 presented here. To verify the possible impact on malaria transmission larvae emergence was conducted. In the cold as well as dry seasons larvae of Anopheles species were identified in all possible water bodies and irrigation systems of the tribal remote areas. Larvae and Pupae dipping surveys (LDPD) are an important tool in mosquito control surveillance programs. The dipping surveys do show where mosquitoes are breeding. Period of emergence varies with sampled larvae and pupae stage. Physical factors, Enzyme activity, hormonal activity and gene regulation in larvae, pupae may also be possible factors for late emergence.

Keywords: *Anopheles Sps; Larval Density, Larval Habitats*

INTRODUCTION
Local environmental characteristics, such as altitude, climate and land use, can significantly impact on phenology and population dynamics of mosquito larvae, and indirectly affect the dynamics of mosquito-borne diseases (Imbahale *et al.*, 2011). The breeding habitat is crucial for mosquito population dynamics where many important life cycle processes such as oviposition, larval development, and emergence take place (Overgaard *et al.*, 2001).

Anopheles mosquitoes originating from specific breeding habitats that transmit human malaria and transmission normally occurs within a certain radius (within flight range of adult vectors) from breeding habitats (Carter *et al.*, 2000).

Some important measures in mosquito control to be followed are: discourage egg-laying, prevent development of eggs into larvae and adults, kill the adult mosquitoes, do not allow adult mosquitoes into places of human dwelling, prevent mosquitoes from biting human beings and deny them blood meals (Mosquito Control, 2008). Though the World Health Organization adopted a formal policy on the control and eradication of the malaria since 1955 ("Malaria Eradication"2010).

Aquatic habitats are an important component of the process that results in malaria transmission. These habitats are crucial in determining the types of malaria vectors present in an area, their abundance and also the population dynamics of emerging adult mosquitoes (Gillies and de Meillon, 1968; Ndenga *et al.*, 2006).

Immature stages of malaria vectors prefer different habitat types (Munga *et al.*, 2006; Mutuku *et al.*, 2006). These habitats differ in their physical, chemical and biological characteristics (Edillo *et al.*, 2006). Therefore, understanding habitat bio-physicochemical characteristics, anopheline larval dynamics and productivity of adult malaria vectors can be useful in improving Larval Source Management (LSM) operations.

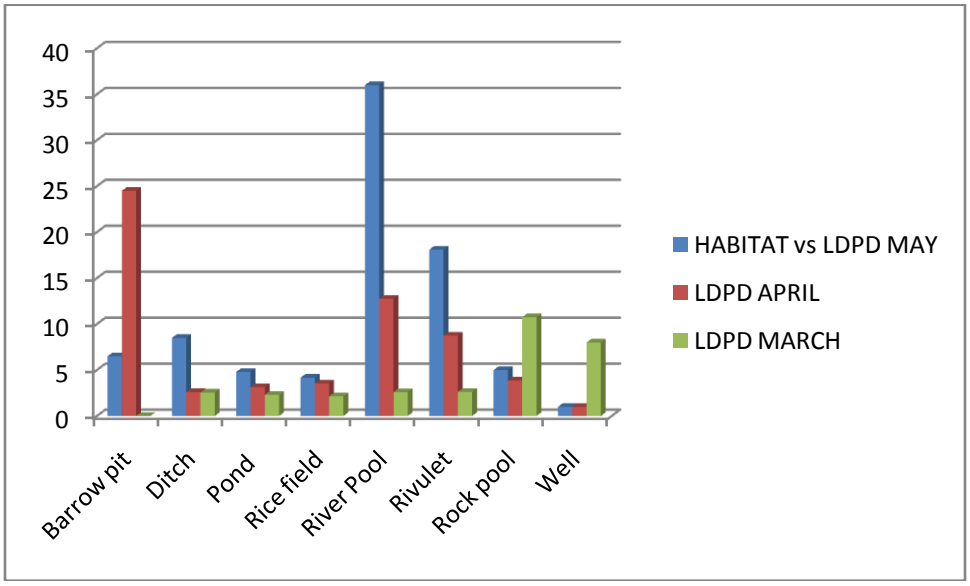
However, these vectors have been found breeding in a great variety of aquatic habitats (Muturi *et al.*, 2007; Sattler *et al.*, 2005). Several factors including oviposition behavior of female mosquitoes Chen *et al.*, (2006), physical, chemical and biological characteristics of habitats (Fillinger *et al.*, 2009) land cover and change in land use (Munga *et al.*, 2006) local climatic characteristics (Lindsay *et al.*, 1998) and topography (Himeidan *et al.*, 2009).

MATERIALS AND METHODS

The Study Area

The study was conducted in eight high altitute and eight at lower altitude villages, in the basin and Valley in of Ranchi and Ramgarh of Jharkhand, where malaria is endemic and transmitted by the Anopheles mosquito species. The Ranchi area lies approximately between latitudes 23.35˚N and longitudes 85.23˚E at about 2140 ft above the sea level. It covers about 5231(sq km) and RAMGARH area lies approximately between latitudes 23˚ 38' N and longitudes 85˚ 34' E at about 2930 ft above the sea level. The total population of the study Ranchi area was approximately >29 Lakhs individuals in Sensus 2011 and Ramgarh area was approx. >9.4 Laks individuals in Sensus 2011.

Habitat	LDPD May	LDPD April	LDPD Mar	LDPD Feb	LDPD Jan
Barrow pit	6.5	24.5	0	0	-
Ditch	8.5	2.61	2.55	3.06	-
Pond	4.8	3.15	2.3	2.58	1.71
Rice field	4.18	3.54	2.15	3.23	0
River Pool	36	12.75	2.6	2.3	-
Rivulet	18.1	8.75	2.62	2.43	2.64
Rock pool	5	3.86	10.75	4	1.25
Well	1	1	8	0	-



Ranchi a fairly warm climate with winter min 5.3˚C max 20.9˚C and summer min 20.6˚C max 41.2˚C and Ramgarh with winter min 5.3˚C max 20.9˚C and summer min 27.˚C max 42.˚C mean annual variance in temperature respectively. The Ranchi annual rainfall and the mean relative humidity are about 1530mm Annual and 56% and Ramgarh its 1344mm annual and 52% respectively. Ambient temperature, rainfall and relative humidity were recorded on a 21 × Micro-data logger in each study site.

It was also a low populated area inhabited by local tribals and they migrated to different cities for job and other economic activities. The area is semi-arid and relatively flat, with the natural vegetation mainly of scattered varities of *trees* and thorn bushes. The major irrigation of agriculture is natural rain, drainages and tube well that are used for irrigation-based agriculture and other economic activities include Lake, Basin and River.

These studied localities were selected purposely, based on past and recent reports of local malaria situation and its transmission. The all anopheline positive habitats present within a 500 m radius of each

irrigated village and 700 m along the major drainages (basin or river) which are located adjacent to the villages were sampled to study larval habitats found closer to houses.

Larval Sampling and Processing

Mosquito larvae were sampled fortnightly interval from Jan 2012 up to early Mar 2014 which covered the dry season (Mar-June) and rainy season (July–Oct) and winter season (Nov-Feb). During sampling the physical characteristic of the larval habitat, including the vegetation cover were noted. Anopheles larvae were more preferably found in grassy compared to other habitats rainfall intensity led to an increase or decrease in mosquito larval abundance depending on the location. This is a single month June 2012 research survey studies and pattern scenario varies with seasons.

During each survey, a habitat was first inspected for the presence of mosquito larvae visually, then by dipping using a standard dipper (11.5 cm diam and 350 ml capacity), pipettes, and white plastic pans (Sharma, 1990; Service, 1993). When mosquito larvae were present, 1–5 dips were taken depending on the size of each larval habitat at intervals along the edge. Samplings were always done by the same individual in the morning (0700–1200 hrs) at each larval habitat. All I-IV instar larvae and pupae of anopheline were collected and identified by visually criteria.

Emergence study in Laboratory

Samples of Larvae and pupae were kept in bowl having fresh water and supplemented with fish powder. They were prevented to scape by using cotton net with rubber band. All kept at in early morning sunlight for an hour then kept at room temp away from direct sun light. Every morning water and food keep on changing till larvae and pupae get moulted to mosquitoes. Hatched adult using suction tube captured and kept in test tube were then kept in direct sunlight for death. The larvae hatched to adults in laboratory identified morphologically by light microscopy using the key of Gillies and De Meillon (1968).

Larval Habitat Characterization and Recording of Environmental Variables

Simultaneously, with larval sampling, the environmental characteristics of water temperature, water pH, turbidity, water current, the presence of algae and natural or human made habitat variables were recorded. Water temperature was measured using LCD portable Digital Multistem Thermometer (ST-9269 A/B/C-Model, USA), whereas, water pH was measured using pH indicator (Viac. Imbonati 2420159 Milano, Italy). Turbidity was estimated by taking water samples in glass test tubes and holding them against a white background to categorize them as either clear or turbid (Minakawa *et al.*, 1999).

Intensity of light was visually categorized as light and shade. The type and presence of aquatic vegetation was observed and recorded and the presence or absence of mats of algae (green algae) was visually determined. These were then categorized into 3 classes (e.g.1 = 0–100 m, 2 = 100–300 m, and 3 for distances >300 m) (Minakawa *et al.*, 1999). Larval density was expressed as number of larvae density per dip. Since number of larvae sampled for some anopheline species was low and different numbers of dips (1–5) were taken based on the size of the habitats.

Ethical Considerations

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

RESULTS AND DISCUSSION

Observation and Results

Table 1: Habitat characteristic, larval density (LDPD), PH and Temp

SN	Habitat	Larvae + Pupa	LDPD May	Temp.	PH	LDPD April	LDPD Mar	LDPD Feb	LDPD Jan
1	Barrow pit	52+0=52	6.5	26°C-27°C	7.2-8.0	24.5	0	0.0	-
2	Ditch	80+05 = 85	8.5	26°C-29°C	8.0	2.61	2.55	3.06	-
3	Pond	129 + 06= 135	4.8	23°C -	7.1-7.5	3.15	2.3	2.58	1.71

				30°C						
4	Rice field	147+11=158	4.18	23°C	-	7.3-8.0	3.54	2.15	3.23	0
				30°C						
5	River Pool	729+17=746	36.0	25°C	-	7.2-8.0	12.75	2.6	2.30	-
				30°C						
6	Rivulet	236+01=237	18.1	25°C	-	7.6-8.0	8.75	2.62	2.43	2.64
				29°C						
7	Rock pool	45+03=48	5.0	25°C		7.7-8.0	3.86	10.75	4.0	1.25
8	Well	02+0=02	1.0	24°C		7.1	1.0	8.0	0.0	-
	Larvae count	1463					1236	440	410	326

Table 2: Characteristics of anopheline larval habitats sampled for Emergence into lab

Characteristics	Variables	B.Pit	Ditch	Pond	Rice field	River Pool	Rivulet	Rock Pool	Well
Intensity of light	Light			+	+	+	+	+	
	Shade	+	+						+
Turbidity	Clear	+		+	+	+	+	+	+
	Turbid		+						
Vegetation	Emergent								
	Floating	+			+	+	+	+	
	Emergent+floating	+	+	+					
	None								+
Permanence	Permanent			+		+	+		+
	Semi-permanent							+	
	Temporary	+	+		+				
Water current	Still	+	+	+	+			+	+
	Slow flowing					+	+		
Distance to the nearest house	0–100 m	+	+	+					+
	100–300 m	+			+	+	+	+	
	>300 m								
Origin of habitat	Natural	+	+	+		+	+	+	
	Human made		+		+				+
Presence of algae	Present	+	+	+					
	Absent				+	+	+	+	+

Table 3: Distribution of Anopheles species in different types of larval habitats and Details of Larvae Immergence

SN	Habitat	Emm.	Died L/P	%	♂	Scientific name ♀	No ♀	%
1	Barrow Pit	**85**	215	28.3	41	*An. subpictus/ An. vagus*	*10/34*	22.7/ 77.2
2	Ditch	**20**	50	40	9	*An. annularis/An.fluvitalis/An. subpictus*	*01/02/08*	9.0/18.1/72.7
3	Pond	103	84	55	53	*An. annularis/An. culicifacies/An. fluvitalis/ An. splendidus/An. thebeld/An. vagus*	*15/12/14/ 69/01/02*	13.2/10.6/12.3/ 61.0/0.08/1.76
4	Rice field	107	98	51	50	*An. annularis/An. culicifacies/An. fluvitalis/ An. jeyporienni/An. splendidus An. stephensi An. theobeld*	03/ 06/12 /04/05/01 /02	9.0/18.1/36.3/ 11.1/ 13.8/3.0/ 6.0
5	River	**130**	49	72.6	51	*An. annularis/An.*	*01/74/03*	1.2/94.8/3.8

	Pool					*culicifacies/An. theobeld*		
6	Rivulet	**56**	151	27	22	*An. annularis/An. culicifacies/An. fluvitalis/ An. splendidus/An. theobeld*	*01/22/03/ 03/05*	2.9/64.7/8.8/ 8.8/14.7
7	Rock Pool	**08**	08	50	04	*An. fluvitalis/An. splendidus/An. subpictus/ An. vagus*	*01/01/01/01*	25/25/25/25
8	Well	**02**	0	100	0	*An. theobeld/An. vagus*	*01/01*	50/50

Species Composition and Seasonal Abundance of Anopheline Larvae

In total 319 anopheline larvae and pupae were collected from 8 larval habitats. They were allow to emerge in Laboratory condition of food, water, temperature and humidity. After final adult hatching all are simultaneously kill and identified microscopically. They comprised of nine species: An. annularis (6.58%), An. culicifacies (35.73%), An. fluvitalis (10.03%), An. jeyporienni (1.25%), An. splendidus (24.45%), An. stephensi(0.31%), An. subpictus (5.95%), An. theobeld (3.76%) and An. vagus (11.9%) *and An. sergenti (0.001%)* were generally scarce (Table 3). Larvae were observed during every month of the study period. Marked monthly variations were observed in densities LPD of the anopheline larval populations with their minimum LDPD in Nov-Dec and maximum LDPD density in MAY-JUN.

Habitat Diversity and Larval Abundance

Table 3 shows the spatial distribution of the anopheline larvae in different aquatic habitats in the study localities. *An. culicifacies* and *An. fluvitalis* larvae were the predominant species occurring in a wide range of habitats. Larvae of *An. sergenti*, also occurred in sippage and co-existing with other species, but were scarce and generally absent from other habitat types.

It confirm latter as *Anopheles culicifacis* s and *An. fluvitalis* are the two malaria vectors in this epidemic prone area, the former being the major vector.

Expressed as number of larvae per number of sampling dips, the relative abundance of anopheline species in the different larval habitats was significantly variable (Graph 1). Thus, based on comparison of mean densities of larvae, it was revealed that *An. culicifacis in river pool, rivulet* and *An. fluvitalis* were the most abundant larvae in rice field, pond and rock pool respectively. *Anopheles subpictusAnn. vagus* also colonized many habitat types. Abundance of tadpoles in habitats of high anopheline presence is due to the fact that they predate less on anopheline larvae (Kramer, 1964).

Environmental Factors Associated with Larval Occurrence and Abundance

Accordingly, the relative abundance of *Anopheles vagus, Ann. Subpictus and Ann. annularis* larvae was negatively associated with aquatic vegetation and water current while that of *An. culicifacis* was positively associated with the presence of mats of algae and water temperature. *An. fluvitalis* larval abundance was also positively associated with the presence of algae in the water bodies and distance to the nearest house (table 3).

Conclusion

The anopheline larvae were abundant in permanent and full sunlight habitats with or without vegetation and algae (Graph 2). The pH of sampled water was almost alkaline 7.4 in all habitat reported harbour by mostly Larvae and few Pupae with temp range of 23-31°C. Larval density was positively correlated with physical Characteristics like Intensity of light, water temperature, Water pH, Water current and Origin of habitat etc and Chemical characteristics including conductivity, total alkalinity etc may had significant effects on distribution and abundance of anopheline species.

Death of larvae and pupae may be due predators and low oxygen content in water. Period of emergence varies with sampled larvae and pupae stage. Late emergence is due to Physical factors like handling, predators, oxygen and light intensity, temp, humidity may also play important role. Low Enzyme activity, hormonal activity, low light intensity and gene regulation in larvae, pupae may also be possible factors for delayed emergence that is affected by factors mentioned.

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