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Environmental characteristics of anopheline mosquito larval habitats in a malaria endemic area in Iran

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ABSTRACT

Objective: To determine the effects of environmental parameters of larval habitats on distribution and abundance of anopheline mosquitoes in Rudan county of Iran. **Methods:** This cross-sectional study was conducted during the mosquito breeding season from February 2010 to October 2011. The anopheline larvae were collected using the standard dipping method. The specimens were identified using a morphological-based key. Simultaneously with larval collection, environmental parameters of the larval habitats including water current and turbidity, sunlight situation, and substrate type of habitats were recorded. Water samples were taken from breeding sites during larval collection. Before collection of samples, the water temperature was measured. The water samples were analysed for turbidity, conductivity, total alkalinity, total dissolved solid, pH and ions including chloride, sulphate, calcium, and magnesium. Statistical correlation analysis and ANOVA test were used to analyze the association between environmental parameters and larval mosquito abundance. **Results:** In total 2 973 larvae of the genus *Anopheles* were collected from 25 larval habitats and identified using morphological characters. They comprised of six species: *An. dhali* (53.21%), *An. stephensi* (24.22%), *An. culicifacies* (14.06%), *An. superpictus* (4.07%), *An. turkhudi* (3.30%), and *An. apoci* (1.14%). The most abundant species was *An. dhali* which were collected from all of the study areas. Larvae of two malaria vectors, *An. dhali* and *An. stephensi*, co-existed and collected in a wide range of habitats with different physico-chemical parameters. The most common larval habitats were man-made sites such as sand mining pools with clean and still water. The anopheline mosquitoes also preferred permanent habitats in sunlight with sandy substrates. The results indicated that there was a significant relationship between mean physico-chemical parameters such as water temperature, conductivity, total alkalinity, sulphate, chloride, and mosquito distribution and abundance. **Conclusions:** The results of this study showed a correlation between certain environmental parameters and mosquito larvae abundance, and these parameters should be considered in planning and implementing larval control programs.

1. Introduction

Malaria is one of the most important vector borne diseases that is currently endemic in the southeast of Iran. According

to the report of Ministry of Health and Medical Education of the country, burden of the disease has been successfully reduced and a nationwide campaign was launched by the Iranian Government in 2010, to eliminate malaria in most endemic regions by 2025[1]. The National Malaria Control Programmes in Iran currently rely on strategies targeting vector control, which focused on indoor residual spraying, application of larvicides and the use of long-lasting insecticidal nets[1,2].

Anopheline mosquito breeding generally occurs in different types of water and a wide range of habitats which

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may be natural or man-made, temporary or permanent, shaded or sunny^[3]. 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^[4]. Each anopheline species has its preferred breeding site for oviposition, depending on factors such as weather conditions, physical geography and human activity^[5]. 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^[6]. 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^[7].

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 concentrated, relatively immobile, and occupy minimal habitat area compared with adults that can rapidly disperse over large areas^[8]. 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^[7,9].

In Iran, five anopheline species including *Anopheles stephensi*, *An. dthali*, *An. culicifacies*, *An. fluviatilis* and *An. superpictus* are widespread and constitute the malaria vectors in south and south-east of the country^[10–13]. Rudan is a malaria endemic focus in the southeast of Iran and local transmission occurs in this county. Larval control through environmental management and the use of chemical and biological larvicides is implemented as part of an integrated approach to malaria control in this area. However, for larval control is an integral part of a vector management program, it is crucial to understand the environmental characteristics and factors affecting larval activity of the principal malaria vectors^[14].

This study was conducted to determine the environmental characteristics of anopheline larval habitats and their potential influence on the distribution and abundance of malaria vectors in Rudan county, southeast of Iran.

2. Materials and methods

2.1. Study area

The study was conducted at seven villages (Abnama, Dehematen, Shorani, Hisbandegan, Ghale-Dezh, Chiromabad, and Jannatabad), which are located along the Rudan river with

an area of approximately 60 km² in the Rudan county, southeast of Iran.

The study villages were chosen based on similarity in ecology and human population densities. In the study area, natural and man-made obstacles such as sand mining pools and temporary earth dams block the water flow and create places in which mosquitoes breed. The selected study villages had exhibited documented consistent and recurring endemic malaria transmission in the Rudan county. The county is located between 27°05'–27°59' N latitudes and 56°50'–57°29' E longitudes with approximately 112 423 individuals in 2010. Relative humidity and temperature are ranged between 26%–74% and 15–44 °C, respectively, while average of annual rainfall is about 162 mm. The main economic activities in the area are farming and livestock herding. Malaria is a major public health problem in this county and occurs year-round with peaks after the two annual rainy seasons (April–June and October–December).

2.2. Larval collection

Larval specimen collection was carried out in the selected villages at different times during the mosquito breeding season from February 2010 to October 2011. In each village, all larval habitats present in and within a 500-m radius of the village were sampled for anopheline larvae using the standard dipper (11.5 cm diameter and 350 mL capacity) according to WHO procedures^[15]. When mosquito larvae were present, 10–30 dips were taken depending on the size of each larval habitat at intervals along the edge. In small habitats, dippers were not effective and larvae were collected individually using plastic pipettes. Samplings were always done by the same individual in the morning (08:00–12:00) or afternoon (14:00–17:00) for about 30 min at each larval habitat. All third and fourth instar anopheline larvae were preserved in lacto-phenol. In the laboratory, each larva was individually mounted in Berlese's medium on a microscope slide and identified to species by morphological criteria^[16,17].

2.3. Environmental characteristics of larval habitats

Simultaneously with larval sampling, the environmental characteristics of each larval habitat were measured and recorded. Environmental characteristics recorded in the current study included habitat hydrological variables and water physico-chemical characteristics. The habitat hydrological characteristics recorded were intensity of light, water current, substrate type, being natural or man made and permanence of the habitat. Intensity of light was visually categorized as full sunlight, partial sunlight and shade. The type of substrate was observed and recorded as mud, sand and gravel. Water current was determined by visual inspection and categorized as slow flowing or still. Categorizing of habitats as natural or human made was performed visually.

For physico-chemical analysis, water samples were collected from different habitats in 1 000 mL polyethylene

bottles, tightly closed and labeled with date of collection and habitat number. These bottles were transported in cold box to the laboratory for analysis. The samples were analyzed for turbidity, conductivity, total alkalinity, total dissolved solid (TDS), pH and ions such as chloride, sulphate, calcium, and magnesium. Water temperature was determined using thermometric, pH using potentiometric and turbidity using nephelometric method. Conductivity and TDS were determined using spectrophotometric technique while alkalinity and total hardness were measured via titration. Sulphate and chloride were measured using spectrophotometer Hach DR/2800 while calcium and magnesium were measured using flame atomic absorption spectrometry. Chemical indicators of water quality were measured according to the procedures in the standard methods for the examination of water and wastewater, as described by Eaton *et al*[18].

2.4. Data analysis

The data were subjected into SPSS V. 16 and then were analyzed. Variation in larval densities among habitat types and environmental factors of habitat characteristics were analyzed using mean comparison and one-way analysis of variance (ANOVA) test. Since the numbers of larvae sampled for some mosquito species was low, larval densities were expressed as number of larvae per 10 dips. Pearson correlation analysis was used to assess the relationship between larval densities and physico-chemical characteristics of the larval habitats. For each of physico-chemical characteristic, simple correlation between larvae abundance and individual parameters were first checked and only significant associations were further examined by

step-up multiple logistic regressions to determine the best predictor variables associated with relative abundance of the larval species of anopheline.

3. Results

3.1. Environmental characteristics of larval habitats

During this study, all of the different types of habitats were found to be positive for anopheline larvae. Most of the larvae were collected predominantly from river edges with permanent, still and clear water. Artificial habitats like sand mining pools with sandy substrate and full sunlight were the most common habitats for anopheline larvae (Table 1). Physico-chemical characteristics of the larval habitats are shown in Table 2.

The mean water pH was almost the same in all of the samples. However, total alkalinity was variable and ranged from 52.06 to 493.66 mg/L. The sulphate and chloride ions in water samples showed considerable variation. There was an average sulphate and chloride content of 55.86–254.04 and 271.46–295.80 mg/L, respectively. The overall range of mean conductivity varied from 826.13 to 2 767.06 μ s/cm, while the mean total dissolved solids ranged from 515.04 to 1 722.26 mg/L.

The results of this study showed that physico-chemical characteristics which had effects on larval density were sulphate ($r=0.23$, $P<0.04$), chloride ($r=0.19$, $P<0.02$), total alkalinity ($r=0.16$, $P<0.01$), conductivity ($r=0.29$, $P<0.03$) and water temperature ($r=0.17$, $P<0.01$). The other measured chemical parameters such as calcium, magnesium, TDS and total hardness were not significantly associated with larval density in different habitats.

Table 1

Environmental characteristic of larval habitats and mean density of anopheline larvae in Rudan county, southeast of Iran (February 2010–October 2011).

Characteristics	Variables	Mean density ^a	p	Percentage of anopheline larvae (%)					
				<i>An. dthali</i>	<i>An. stephensi</i>	<i>An. culicifacies</i>	<i>An. superpictus</i>	<i>An. turkhudi</i>	<i>An. apoci</i>
Permanence	Permanent	31.12±2.07	0.000	85.6	73.4	79.0	51.7	96.2	91.5
	Temporary	19.78±1.93		14.3	26.6	21.0	48.3	3.8	8.5
Water current	Slow flowing	20.05±2.67	0.001	38.2	14.3	21.6	5.1	41.8	8.7
	Still	30.22±1.92		61.8	85.7	78.4	94.9	58.2	91.3
Intensity of light	Full sunlight	31.13±1.92	0.041	82.2	90.3	83.6	87.4	80.8	93.2
	Partial sunlight	18.21±1.96		12.5	6.5	10.1	7.5	13.6	6.8
	Shaded	12.85±2.70		5.3	3.2	6.3	5.1	5.6	0
Turbidity	Turbid	19.28±1.20	0.002	18.4	17.9	21.1	24.3	6.2	34.5
	Clear	30.48±1.93		81.6	82.1	78.9	75.7	93.8	65.5
Substrate type	Mud	21.39±2.05	0.504	24.5	31.8	37.6	14.6	21.1	25.4
	Sand	33.12±2.40		67.3	59.6	52.2	64.2	55.5	47.3
	Gravel	18.85±2.13		8.2	8.6	10.2	21.2	23.4	27.3
Origin of habitat (River edge)	Natural	20.52±2.32	0.045	28.6	32.3	41.0	8.6	14.7	2.0
	Human made	30.10±1.95		71.4	67.7	59.0	91.4	85.3	98.0

^aMean density of anopheline larvae is expressed as mean±SE.

Table 2

Physico-chemical parameters of larval breeding places in Rudan county, southeast of Iran.

Parameters	Villages						
	Jannatabad	Chiromabad	Ghale-Dezh	Hisbandegan	Shorani	Dehematen	Abnama
Temperature (°C)	25.40±0.79	24.70±0.47	23.10±0.48	25.96±1.06	24.32±0.78	24.30±0.61	27.18±0.80
pH	8.29±0.07	8.18±0.05	8.40±0.10	8.19±0.05	8.49±0.13	8.16±0.04	8.19±0.06
Conductivity (μ s/cm)	1 588.66±13.81	1 921.40±19.54	2 767.06±15.5	2 440.86±11.68	826.13±9.84	1 983.20±16.10	1 603.66±8.73
TDS (mg/L)	1 051.60±19.73	1 307.26±17.27	1 770.00±8.48	1 722.26±7.33	515.40±4.15	1 404.00±2.37	1 081.06±11.85
Turbidity (NTU)	0.54±0.04	0.42±0.02	0.52±0.03	0.56±0.04	0.50±0.02	0.51±0.01	0.56±0.00
Sulphate (mg/L)	182.74±1.60	159.24±0.69	254.04±1.27	183.58±1.48	55.86±0.28	159.71±0.83	187.12±0.99
Chloride (mg/L)	273.86±1.25	272.80±6.06	295.80±2.41	271.46±1.57	264.26±6.29	277.60±0.33	274.06±1.48
Total alkalinity (mg/L)	207.60±2.10	196.46±0.68	493.66±1.24	174.26±1.07	52.06±0.62	200.26±0.84	211.00±1.25
Total hardness (mg/L)	194.60±1.11	202.40±2.80	293.86±1.21	274.73±2.09	276.33±0.68	199.46±0.69	196.93±0.75
Calcium (mg/L)	21.13±0.44	23.13±0.30	44.73±0.65	44.80±0.39	41.60±0.45	22.40±0.32	21.33±0.30
Magnesium (mg/L)	29.95±0.46	21.36±0.50	41.39±0.32	36.38±0.42	33.96±0.45	23.86±0.41	30.12±0.61

Data are expressed as mean±SE. TDS: Total dissolved solids.

3.2. Species composition and abundance of Anopheline larvae

During this study, 2 973 third- and fourth-instar anopheline larvae were collected, including *An. dthali* (53.21%), *An. stephensi* (24.22%), *An. culicifacies* (14.06%), *An. superpictus* (4.07%), *An. turkhudi* (3.30%), and *An. apoci* (1.14%). *An. dthali* was the most predominant species, being represented in a wide range of habitats and widespread across the study area. *An. apoci* was generally the least abundant of the six species and found in only one of the study villages (Table 3).

In this study, the mean larval density differed significantly between villages ($P<0.05$). The Galeh-Ddezh village recorded the highest anopheline mosquito mean density with 49.26±5.14 larvae/10 dips. Variation in species diversity of anopheline mosquitoes among study villages was evident. The highest number of anopheline species was found in Shorani ($n=5$), while at most three species were found in each of the other villages. Details of the larval composition and mean density of mosquitoes are shown in Table 3.

An. dthali was collected in 82.3% of larval habitats. These breeding habitats are characterized by having clear and still water with sandy substrates. Man-made larval habitats with full sunlight were the most common habitats for this species (Table 1). This species was accompanied by *An. stephensi*, *An. culicifacies*, *An. superpictus*, *An. turkhudi*, and *An. apoci*.

An. stephensi was distributed in the study area and found

in 73.4% of larval habitats. Larval breeding sites of this species were usually man-made bodies of clear water in full or partial sunlight. Permanent habitats with still water and sandy substrates were preferred by this species. *An. stephensi* larvae associated with *An. dthali*, *An. culicifacies*, *An. superpictus*, *An. turkhudi*, and *An. apoci*.

An. culicifacies s.l were collected from 62.3% of larval breeding habitats including permanent habitats in river edges mostly with still and clear water with full sunlight and sandy substrates (Table 1). This species was found to be accompanied by *An. stephensi*, *An. dthali*, *An. superpictus*, and *An. turkhudi*.

An. superpictus was found abundantly in artificial habitats, especially mining pools in river edges. Permanent and still body of water in full sunlight with clear water and sandy substrates were the most common habitats for this species (Table 1). *An. superpictus* larvae were collected from 36.3% of habitats and associated with *An. dthali*, *An. stephensi*, *An. culicifacies*, *An. turkhudi*, and *An. apoci*.

An. turkhudi, a non-vector species, was collected from 25.6% of larval habitats. It was mainly accompanied by *An. stephensi*, *An. dthali*, *An. culicifacies*, and *An. superpictus*. Environmental characteristics of breeding sites for *An. turkhudi* are presented in Table 1.

An. apoci, another non-vector species, was found in 12.5% of breeding sites and its larvae associated with *An. stephensi*, *An. dthali*, and *An. superpictus*. Larval habitats

Table 3Mean densities of *Anopheles* larvae in Rudan county, southeast of Iran (February 2010–October 2011) (larvae/10 dips).

Villages	<i>An. dthali</i>	<i>An. stephensi</i>	<i>An. culicifacies</i>	<i>An. superpictus</i>	<i>An. turkhudi</i>	<i>An. apoci</i>	All species
Jannatabad	7.10±0.89	3.86±0.83	2.33±0.50	0	0	0	35.13±3.66
Chiromabad	18.46±2.29	5.06±0.67	0	2.06±0.39	0	2.26±0.56	35.86±3.96
Ghale-Dezh	10.73±1.34	1.30±0.32	4.93±0.08	0	0	0	49.26±5.14
Hisbandegan	4.20±0.69	11.46±1.56	0	0	4.02±0.82	0	19.86±2.33
Shorani	25.53±2.67	15.93±2.35	3.13±0.68	2.06±0.38	2.33±0.50	0	17.00±2.10
Dehematen	20.93±2.61	8.26±1.09	2.53±0.56	4.13±0.59	0	0	27.66±2.97
Abnama	18.46±2.02	1.93±0.35	14.73±1.66	0	0	0	13.40±1.75

Mean density of anopheline larvae is expressed as mean±SE.

of *An. apoci* are characterized in Table 1 for environmental characteristics.

4. Discussion

Iran is considered to be in pre-elimination phase by World Health Organization^[19] and in this regard, larval control is now apart of operational strategies adopted by the National Malaria Control Program, Government of Iran^[1]. Although control of the immature mosquito population is an important component of the malaria control program in Iran, larval habitat productivity and the impact of this program on the population dynamics of vector species have not been well understood. Knowledge of larval habitats and their distribution would be an important step for planning and implementing larval control strategies effectively.

During this study, six species of *Anopheles* were identified in the area. The most abundant anopheline larvae were *An. dthali*, *An. stephensi*, *An. culicifacies* and *An. superpictus*, respectively. Previous studies have shown that *An. stephensi* is primary vector and other anopheline species, such as *An. dthali*, *An. culicifacies* and *An. superpictus*, play the main role as secondary vectors in the south and south-east of Iran^[10]. All the species identified here had previously been reported from Hormozgan province^[2].

Results of this study have revealed that river edges and sand mining pools were important mosquito larval habitats. These habitats are permanent with still and clear water in sunlight, making conditions favourable for the development of anopheline mosquitoes. All of these habitat types were previously reported from elsewhere in the country except sand mining pools^[20,21]. Artificial habitats such as sand mining pools were more important for anopheline mosquito breeding. These habitats block water flow and create pools which offer ideal situations for the proliferation of anopheline mosquitoes. These results are similar to the study of Kenea *et al.* in Ethiopia, who reported that sand mining pools were suitable for proliferation of anopheline mosquitoes^[22].

In this study, *An. dthali* and *An. stephensi* co-existed and bred in a wide range of habitats with different physico-chemical characteristics. Co-existence of these two malaria vectors in the same habitats has previously been reported in the southeast of Iran^[21]. The frequent occurrence of *An. dthali* and *An. stephensi* in different habitats may mean that their life is adaptable to a wide range of environmental conditions.

It was observed that larvae of *An. superpictus* and *An. culicifacies* were the most abundant in permanent and still water in full sunlight, along the edge of Rudan river. Previous studies indicated that *An. superpictus* mainly breeds in ground pools in river edges, mostly with still and clear water in sunlight^[20]. In a similar study conducted by Surendran *et al.* in Sri Lanka, *An. culicifacies* larvae were observed to breed abundantly in rock pools and sand pools along river margins^[23]. Russell and Rao indicated that mosquito larvae of *An. culicifacies* were normally found in

sunny situations. They also stated that egg-laying female *An. culicifacies* may be quite sensitive to light conditions, which may lead to the differential larval distribution^[24].

The results showed that all the species were abundantly collected from still waters. Similar observation was made in sand pools in Eritrea, where high numbers of *An. gambiae* s.l were sampled from stagnated water^[25]. Results of another study conducted in Ethiopia showed that *An. gambiae* s.l mostly prefers still waters^[22]. Still and clear water with suitable pH, temperature and nutrient composition has been found to encourage breeding in *Anopheles* species^[26]. The explanation for the abundance of anopheline larvae in still waters may be that still water provides favourite situations in which larvae can stay close to the surface with their spiracle open to the air for breathing. Moreover, high water current and flooding are detrimental to *Anopheles* larval survival due to reduction in their oxygen tension and physical harm to the larvae^[27].

In this study, larvae of non-vector species, *An. turkhudi* and *An. apoci* were found to have low abundance and were restricted to only a few aquatic habitats. Both species preferred sunny and permanent water bodies, suggesting that conditions favourable for their development is met by these types of habitats.

Certain physico-chemical characteristics such as turbidity and pH were similar in potential or actual larval habitats. This may be due to the edaphic factors in the area. The present study clearly indicates a significant relationship between physico-chemical parameters such as sulphate, chloride, total alkalinity, conductivity, water temperature and mosquito distribution and abundance. Previous studies have shown that physico-chemical parameters such as sulphate, chloride, temperature, alkalinity, and pH may provide conducive environment for survival and breeding activity of the anopheline species^[28–31]. Finally, in addition to environmental parameters which were considered in this study, factors such as differential survivorship of larvae, adaptive differences of adult females and their oviposition behaviour may affect the abundance and distribution of anopheline larvae.

A limitation of this study is without measuring chemical parameters such as dissolved oxygen, nitrate, phosphate, which could play a role in the results obtained. Another limitation was that we did not controlled nutrients of the water, predators and pathogens which could have affected abundance and distribution of anopheline mosquito larvae.

In conclusion, the current study demonstrates a low level of abundance in larval habitats and it was found that mosquito larval abundance depends mainly on the stability of these habitats. Much of the larval production goes on in the man-made habitats such as sand mining pools. These habitats are responsible for continuous production of the adult vectors throughout the year. Such artificial habitats can easily be managed through participation of community. Since most of the anopheline species encountered are potential vectors of malaria, results of present study provide baseline information that would guide decisions on larval control by the National Malaria Control Program in the country.

Conflict of interest statement

The authors declare no conflict of interest.

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