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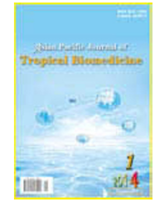

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Characterization of larval habitats for anopheline mosquitoes in a malarious area under elimination program in the southeast of Iran

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PEER REVIEW

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Comments

This is a valuable research work in which authors have investigated the correlation between some environmental characteristics of anopheline larval habitats including intensity of light, water current, vegetation situation, the presence of algae, substrate type, water conductivity, total alkalinity, turbidity, total dissolved solid, pH and ions such as chloride, sulphate, calcium, and magnesium and anopheline larvae abundance, which can be considered for effective planning and implementing malaria elimination program.

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ABSTRACT

Objective: To determine the effects of environmental characteristics of larval habitats on distribution and abundance of anopheline mosquitoes in Bashagard county, a malarious area in southeast of Iran.

Methods: Larvae were collected monthly using the standard dipping method and identified using a morphological-based key. Environmental characteristics of the larval habitats were recorded. Water samples were taken from habitats during larval collection for physico-chemical characterization. Statistical analyses were performed.

Results: In total 5 150 anopheline larvae from 36 larval habitats were collected and identified. They comprised of six species: *Anopheles culicifacies* (29.36%), *Anopheles moghulensis* (25.20%), *Anopheles dthali* (18.02%), *Anopheles superpictus* (17.24%), *Anopheles turkhudi* (5.17%) and *Anopheles stephensi* (5.01%).

The most common larval habitats were natural and clear water bodies such as riverbeds with sandy substrates and still water. Furthermore, the anopheline larvae were abundant in permanent and full sunlight habitats without vegetation and algae. Larval density was positively correlated with water temperature. Chemical characteristics including conductivity, total alkalinity, sulphate and chloride had significant effects on distribution and abundance of anopheline species.

Conclusions: The result of this study indicates a correlation between some environmental characteristics and anopheline larvae abundance which can be considered for effective planning and implementing malaria elimination program in Iran.

KEYWORDS

Anopheles, Larval habitats, Malaria, Bashagard, Iran

1. Introduction

Malaria is one of the most widespread diseases in the world, especially in the tropical and subtropical regions[1]. It is one of the most important vector borne diseases and public health problems in Iran. The disease is endemic in the southeast of country with two seasonal peaks mainly in spring and autumn. According to the report of Iranian

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Ministry of Health and Medical Education, the annual malaria cases have been reported from 66075 to 3000 during 1995–2010, indicating the sharp decline of disease which has led World Health Organization to categorize Iran in the elimination phase[1,2]. The national malaria strategic plan has recently set goals to reduce local malaria transmission by taking strategies targeting vector control through indoor residual spraying, distribution of long lasting insecticidal nets and application of larvicides. In this regard, Iran is aiming to eliminate *Plasmodium falciparum* by 2015 and to become malaria-free by 2025[2,3].

In many parts of the world, larval control through source reduction and routine application of larvicides is considered as a key intervention in eradicating malaria[4]. Larval control measures are intended to reduce malaria transmission by preventing propagation of mosquito vectors and subsequently reducing human vector pathogen contacts[5,6]. Control of larval mosquito populations is often advantageous because the larvae are usually concentrated, relatively immobile, and often readily accessible. Moreover, mosquito larvae unlike adults cannot change their habitat to avoid control activities[6].

Several environmental characteristics affect larval density which may influence the development and survival rate of the malaria vector larvae. These characteristics include climate, physical and chemical conditions of the aquatic habitats, vegetation type, and biological characteristics[7,8]. Finding of local change in environmental characteristics of anopheline larval habitats can help in conducting suitable vector control programs[9,10].

Bashagard is a malaria endemic focus in the southeast of Iran where local transmission occurs every year. Mosquito larval control measures, consisting of regular application of *Bacillus thuringiensis* and environmental management are important activities of malaria control programme in this county. Five anopheline species including: *Anopheles stephensi* (*An. stephensi*), *Anopheles dthali* (*An. dthali*), *Anopheles culicifacies* (*An. culicifacies*), *Anopheles fluviatilis* (*An. fluviatilis*) and *Anopheles superpictus* (*An. superpictus*) are widespread and constitute the malaria vectors in this area[11]. Understanding the larval ecology of these vectors is of particular importance for monitoring and control of malaria in this county. Knowledge of larval vector ecology is a key factor in risk assessment and establishment of effective control measures, because the most effective method for controlling vector populations is to control the larvae in their aquatic habitats before they emerge as adults[12].

The aim of this study was to determine the environmental characteristics of anopheline larval habitats and their potential influence on the distribution and abundance of malaria vectors in Bashagard county, southeast of Iran. The results of this study will provide information that would help in planning and implementing an effective program for larval control by the National Malaria Control Program in the country.

2. Materials and methods

2.1. Study area

The study was done in Bashagard county in the Hormozgan province, southeast of Iran. The county is located between latitudes 26°04′–26°58′ N and longitudes 57°23′–59°02′ E with an approximately 31000 population in 2011. The average of annual rainfall is about 265 mm while annual mean relative humidity is 40%. It has a warm climate with mean annual temperature of 26.2 °C ranging from 9.4 °C to 44.2 °C. It is an underdeveloped area with majority of the population living in straw houses on hills and foot-hills, close to rivers. Socio-economic condition of villagers is poor and they solely depend on livestock herding. In the study area, natural earth dams block the water flow and create suitable places for mosquitoes breeding. Malaria is a major public health problem in Bashagard which occurs year-round with peaks after the two annual rainy seasons (April–June and October–December)[3].

In this study, 11 villages were selected based on similarity in ecology and human population densities as fixed places for anopheline larval collection. The study villages had exhibited documented consistent endemic malaria transmission[3].

2.2. Larval sampling and identification

Anopheline larvae were collected form selected villages monthly for a period of 12 months from September 2009 to August 2010. In each village, all larval habitats present in and within a 500-m radius of the village were sampled for anopheline larvae using a standard 350 mL capacity mosquito dipper or a white plastic pan with the same capacity according to WHO procedures[13]. 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 breeding places where dippers were not effective, larval collection was performed using plastic pipettes. Samplings were always done by the same individual in the morning (08:00–12:00 h) or afternoon (14:00–17:00 h) for about 30 min at each larval habitat. All third and fourth instars of anopheline larvae were passed though a 100 mesh sieve and 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 characters[14,15].

2.3. Characterization of larval habitats

Environmental characteristics of each larval habitat were measured and recorded during the larval collection. Environmental data which were determined in this study included habitat hydrological variables and water physico-chemical characteristics. The habitat hydrological variables including intensity of light, being natural or artificial, water current, vegetation covering, the presence of algae, substrate type, and permanence of the habitat were recorded. Light intensity was visually categorized as full sunlight, partial sunlight and shade. The substrate type was categorized as mud, sand and gravel. Habitats were categorized as artificial or natural.

For analysis of physico-chemical characteristics, water samples were collected from different habitats in 1 000 mL polyethylene bottles and transferred to laboratory with cold boxes. Cold boxes were used for transportation of water samples to the laboratory for analysis. The samples were analyzed for conductivity, total alkalinity, turbidity, total dissolved solid (TDS), pH and ions such as chloride, sulphate, calcium, and magnesium. Water temperature, pH, and turbidity were determined using thermometric, potentiometeric, and nephelometric methods, respectively. Alkalinity and total hardness were determined via titration while wonductivity and TDS were measured using spectrophotometric technique. Spectrophotometer Hach DR2800® was used for measurement of sulphate and chloride while calcium and magnesium were measured using Flame Atomic Absorption Spectrometry. Water quality chemical indicators were measured according to the standard methods for the examination of water and wastewater, as described by Eaton *et al*[16].

2.4. Statistical analysis

The data were analyzed using SPSS Ver. 16. Means comparison and one-way analysis of variance (ANOVA) tests were used for analyzing the variation in larval densities among habitat types and environmental factors of habitat characteristics. Larval densities were calculated as number of larvae per 10 dips. Pearson correlation analysis was used to assess the relationship between physico-chemical characteristics of the larval habitats and larval densities. For each of physico-chemical characteristics, simple correlation between larvae abundance and individual characteristics were first checked and only significant associations further analyzed by step-up multiple logistic regressions to determine the best predictor variables associated with relative abundance of the anopheline larval species.

3. Results

3.1. Species composition of anopheline larvae

The results of monthly larval collection showed that the varied densities of the anopheline larvae vary markedly with the minimum in January and maximum in April (Figure 1).

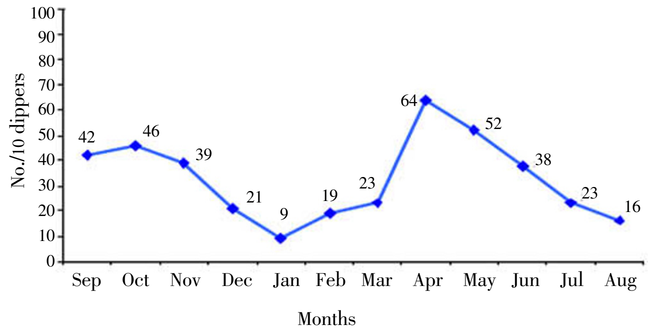

Figure 1. Monthly activity of anopheline larvae in Bashagard county, southeast of Iran during 2009-2010.

During this study, in total 5 150 anopheline larvae were collected and identified. They comprised of six species: *An. culicifacies* (29.36%), *Anopheles moghulensis* (*An. moghulensis*) (25.20%), *An. dthali* (18.02%), *An. superpictus* (17.24%), *An. turkhudi* (5.17%) and *An. stephensi* (5.01%).

An. culicifacies was the most common and predominant species, being distributed in a wide range of habitats and widespread across the studied area whereas *An. stephensi* was the least abundant and found in seven villages (Table 1).

The mean larval density differed significantly between villages ($P<0.05$). The least and the most anopheline larval mean density were (12.8±2.29) and (37.40±3.66) larvae/10 dips in Islamabad and Daranar villages, respectively.

An. culicifacies Giles *s.l.* was collected throughout the year except of March. This species was collected in 77.2% of larval habitats including natural breeding sites in riverbeds with sandy substrates and still water. Permanent and clear water bodies in full sunlight without vegetation and algae were preferred by *An. culicifacies* (Table 2). This species was found with *An. dthali*, *An. superpictus*, *An. stephensi An. moghulensis* and *An. turkhudi* in larval habitats (Table 1).

An. dthali Patton was collected throughout the year and found in 69.3% of habitats. The most common larval breeding sites for this species were usually natural riverbeds with clear water in full sunlight, without vegetation and algae. This species also preferred permanent habitats characterised by still water and sandy substrates. *An. dthali* larvae were associated with *An. culicifacies*, *An. superpictus*, *An. stephensi*, *An. moghulensis* and *An. turkhudi* (Table 1).

An. superpictus Grassi was distributed in the study area and was collected from 32.5% of larval breeding habitats including permanent habitats in riverbeds mostly without vegetation and algae. This species also preferred still and clear water with full sunlight and sandy substrates (Table 2). This species was not found in February and September. *An. superpictus* was found to be accompanied by *An. culicifacies*, *An. dthali*, *An. stephensi*, *An. moghulensis* and *An. turkhudi* (Table 1).

Table 1
Mean density of anopheline larvae in Bashagard county, southeast of Iran during 2009–2010 (larvae/10 dips).

Villages	Mean density of anopheline larvae[a]						
	An. culicifacies	*An. dthali*	*An. superpictus*	*An. stephensi*	*An. moghulensis*	*An. turkhudi*	All species
Tisur	4.84±0.70	11.21±1.35	3.65±0.84	2.32±0.45	13.81±1.63	3.12±0.52	35.21±3.14
Nasary	8.12±1.04	3.35±0.67	7.23±0.86	5.47±0.21	6.85±0.42	5.63±0.48	34.66±1.45
Sarzeh	13.63±1.64	3.10±0.56	2.04±0.38	0.00±0.00	1.24±0.15	0.00±0.00	18.35±2.96
Daranar	21.70±2.32	8.16±1.23	2.27±0.55	3.14±0.67	1.38±0.27	5.70±0.86	37.40±3.66
Poshtgar	10.53±1.24	5.12±0.83	8.15±1.05	0.00±0.00	7.13±0.82	2.02±0.54	34.32±2.73
Ashkan	1.28±0.34	6.72±0.84	9.53±1.64	1.64±0.33	10.86±1.25	0.00±0.00	32.12±2.32
Islamabad	3.68±0.75	2.42±0.55	4.82±0.65	0.00±0.00	3.92±0.47	0.00±0.00	12.80±2.29
Gurichi	25.64±2.18	5.82±0.43	0.00±0.00	1.27±0.43	5.12±0.64	0.00±0.00	35.17±2.40
Piskav	4.21±0.61	5.02±0.45	2.23±0.76	3.65±0.71	3.65±0.71	2.05±0.37	18.46±3.32
Sardasht	1.94±0.42	5.45±0.27	1.32±0.22	2.14±0.16	18.45±2.43	3.14±0.67	28.12±2.93
Kahno	3.75±0.86	4.15±0.64	3.12±0.65	0.00±0.00	7.21±0.61	0.00±0.00	17.60±1.42

[a]: Mean density of anopheline larvae is expressed as mean±SE.

An. stephensi Liston was found abundantly in natural habitats, especially in riverbeds with sandy substrates. Permanent and clear water in full sunlight without vegetation and algae was the most common habitat for this species (Table 2). *An. stephensi* larvae were collected from 28.7% of habitats and associated with *An. culicifacies*, *An. dthali*, *An. superpictus*, *An. moghulensis*, and *An. turkhudi* (Table 1). This species was collected throughout the year during the study period.

An. moghulensis Christophers, a non-vector species, was collected from 62.3% of habitats and accompanied with *An. culicifacies*, *An. dthali*, *An. superpictus*, *An. stephensi*, and *An. turkhudi* (Table 1). As shown in Table 2, this species oviposits more in riverbeds with permanent and still water than in other water. *An. moghulensis* was collected monthly during the study period.

An. turkhudi Liston, another non-vector species, was collected from 21.2% of larval habitats only in July and September. It was mainly associated with *An. culicifacies*, *An. dthali*, *An. superpictus*, *An. stephensi*, and *An. moghulensis* (Table 1). Environmental characteristics of larval habitats for *An. turkhudi* are presented in Table 2.

3.2. Environmental characteristics of larval habitats

During this study 44 different types of habitats in the eleven villages were investigated, of which 81.8% (36) were found to be positive for anopheline larvae. Distribution of *Anopheles* larvae collected from various habitats is shown in Table 2.

Permanent and natural habitats with still and clear water in full sunlight were the most productive breeding sites

Table 2
Characteristics of larval habitats and mean densities of anopheline larvae in Bashagard county, southeast of Iran during 2009–2010.

Characteristic	Variables		Percentage of anopheline larvae (%)						Mean density[a]	*P*
			An. culicifacies	*An. dthali*	*An. superpictus*	*An. stephensi*	*An. moghulensis*	*An. turkhudi*		
Permanence	Permanent		86.7	92.3	64.5	76.2	62.8	94.4	32.16±2.08	<0.001
	Temporary		13.4	7.7	35.5	23.8	37.2	5.6	10.43±2.03	
Water current	Slow flowing		23.5	34.3	8.8	16.4	6.3	43.7	9.02±1.85	<0.001
	Still		76.5	65.7	91.2	83.6	93.7	56.3	31.87±2.70	
Intensity of light	Full sunlight		82.4	78.5	85.3	89.2	71.1	77.6	34.15±2.45	0
	Partial sunlight		12.3	15.0	9.4	7.6	22.5	14.0	7.31±1.96	
	Shaded		5.3	4.5	5.3	3.2	6.4	8.4	3.23±2.60	
Turbidity	Clear		84.5	81.7	78.4	88.5	85.8	94.6	32.48±1.40	0.001
	Turbid		15.5	18.3	21.6	11.5	14.2	5.4	5.85±1.20	
Vegetation situation	With vegetation		34.7	35.8	32.2	29.4	31.8	34.6	19.16±1.45	0.002
	Without vegetation		65.3	64.2	67.8	70.6	68.2	65.4	34.18±1.70	
Presence of algae	Present		38.7	42.3	31.6	33.3	37.3	42.4	21.60±2.12	0.002
	Absent		61.3	57.7	68.4	66.7	62.7	57.6	33.39±2.05	
Substrate type	Mud		34.4	26.5	20.7	28.2	30.1	18.8	14.29±1.98	0.042
	Sand		56.2	65.6	67.3	61.3	58.7	59.2	34.22±2.30	
	Gravel		9.4	7.9	12.0	10.5	11.2	22.0	8.53±2.62	
Habitat type	Natural	River edge	25.5	30.2	29.8	18.8	48.6	42.1	20.14±2.20	0.001
		Riverbed	56.5	52.2	61.7	62.3	39.6	48.4	33.85±2.32	
	Artificial	River edge	5.2	7.6	2.3	4.4	6.1	5.9	5.42±1.92	
		Water leakage	12.8	10.0	6.2	14.5	5.7	3.6	7.71±2.95	

[a]: Mean density of anopheline larvae is expressed as mean±SE.

Table 3
Physico-chemical characteristic of larval habitats in Bashagard county, southeast of Iran during 2009-2010.

Parameters	Villages										
	Tisur	Nasary	Sarzeh	Daranar	Poshtgar	Ashkan	Islamabad	Gurichi	Piskav	Sardasht	Kahno
Temperature (°C)	25.3±0.6	25.2±0.5	25.4±0.3	27.2±0.8	24.4±0.4	23.7±0.2	23.1±0.6	25.2±0.5	24.4±0.4	24.2±0.5	23.2±0.4
pH	8.2±0.1	8.3±0.2	7.2±0.1	8.6±0.1	8.3±0.3	8.4±0.1	7.1±0.2	8.1±0.3	7.5±0.1	8.2±0.2	7.3±0.3
Conductivity (μS/cm)	2138.6±11.6	1982.1±12.5	958.6±10.8	2421.2±9.3	1446.3±19.2	1324.2±13.2	941.3±11.7	2076.6±8.3	1088.1±12.5	1323.2±10.4	974.2±8.7
Total dissolved solids (mg/L)	922.3±8.5	1731.1±7.9	941.4±11.2	981.5±19.1	1509.6±13.7	487.0±8.3	1228.7±12.7	1363.3±7.3	683.4±3.5	572.2±6.2	774.2±11.8
Turbidity (NTU)	8.2±0.5	8.7±0.4	28.1±0.7	7.8±0.2	8.5±0.4	9.3±0.6	10.1±0.3	11.7±0.5	25.4±0.7	10.3±0.4	24.7±0.4
Sulphate (mg/L)	322.3±1.8	316.7±1.4	188.4±1.6	485.8±0.2	302.2±0.6	282.2±1.2	166.5±1.2	320.5±0.6	192.2±1.6	215.6±1.4	174.5±1.5
Chloride (mg/L)	118.2±4.6	109.6±5.4	83.4±3.5	231.2±1.5	106.6±4.6	98.4±2.1	63.5±2.3	112.0±6.8	89.1±4.4	95.5±5.6	73.2±3.5
Total alkalinity (mg/L)	461.7±1.2	412.6±1.1	271.3±1.1	523.2±0.7	462.1±1.5	512.2±2.1	142.6±2.2	412.3±3.2	234.7±1.8	328.2±0.6	148.8±2.1
Total hardness (mg/L)	128.6±1.5	126.4±2.8	131.5±2.1	362.3±1.6	358.4±1.2	146.5±1.5	355.7±2.8	318.2±1.5	248.3±1.1	241.4±2.2	185.5±0.7
Calcium (mg/L)	21.1±0.4	20.8±0.2	23.1±0.3	18.2±0.4	64.5±0.2	31.2±0.4	61.1±0.2	29.7±0.5	56.6±0.4	57.4±0.1	47.7±0.2
Magnesium (mg/L)	16.3±0.5	14.4±0.2	14.5±0.2	23.1±0.3	36.2±0.4	33.6±0.1	36.2±0.5	48.7±0.4	23.5±0.8	19.2±0.5	10.5±0.2

Data are expressed as mean±SE.

for the anopheline species (Figure 2). Moreover, lack of vegetation and algae was significantly associated with larval density in the studied habitats (P=0.002).

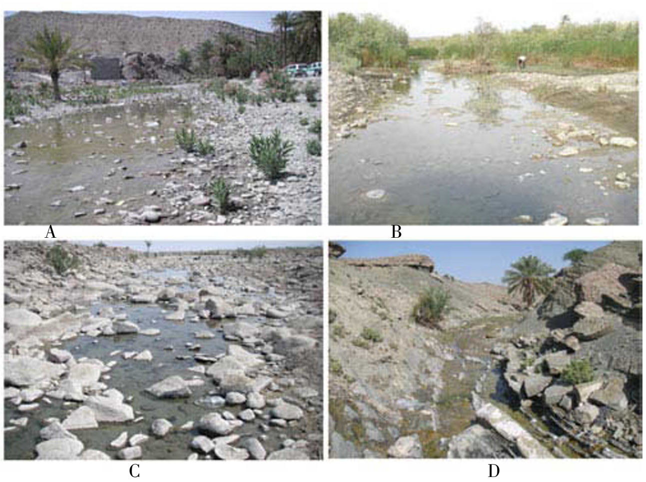

Figure 2. Typical potential anopheline larval habitats in Bashagard county, southeast of Iran during 2009-2010.
A and B: river edge, C: riverbed, D: Water leakage.

The mean water temperature was variable among different larval habitats ranging from 23.1-27.2 °C and it was positively correlated with larval density (r=0.15, P<0.01) (Table 3).

Results concerning the chemical characteristics of the larval habitats showed that larvae of all anopheline species had been collected from water bodies with mean pH ranging from 7.1 to 8.6, mean total alkalinity ranging from 142.6 to 523.2 mg/L, and mean conductivity ranging from 941.3 to 2421.6 μS/cm. In addition, sulphate and chloride ions in water samples showed considerable variation (Table 3). There was an average sulphate and chloride content of 166.5-485.8 mg/L and 63.5-231.2 mg/L, respectively. Analysis of the correlation between chemical characteristics of larval habitats and mean density of anopheline larvae revealed that pH (r=0.2, P<0. 02), total alkalinity (r=0.14, P<0.02), conductivity(r=0.32, P<0.03), chloride (r=0.12, P<0.01) and sulphate (r=0.18, P<0.02) had significant effects on larval density. These characteristics were found to be the key factors which are associated with occurrence and abundance of anopheline larvae species.

In this study, the rest measured chemical characteristics such as calcium, magnesium, TDS and total hardness differed between larval habitats but these parameters were not significantly associated with larval density.

4. Discussion

During this study, four out of the eight known malaria vector species in Iran, including *An. stephensi*, *An. culicifacies*, *An. dthali* and *An. superpictus* were collected.

An. stephensi is considered to be the primary vector and other species, *An. dthali*, *An. culicifacies* and *An. superpictus*, play the main role as secondary vectors in the south and south-east of country[17-19]. The most abundant anopheline larvae were *An. culicifacies*, *An. moghulensis*, *An. dthali*, *An. superpictus*, *An. turkhudi* and *An. stephensi*, respectively. These species were reported previously from the area[11,20]. Anopheline species were abundantly collected from riverbeds in natural habitats. These habitats are permanent with still and clear water in sunlight, making conditions suitable for the development of anopheline mosquitoes. The study area has a dry and hot climate with low level of seasonal precipitation. Therefore, the rivers in most of the times have low levels of still and clear water which creates favourite conditions for mosquitoes breeding. Our results are similar to Hanafi-Bojd *et al.*, demonstrating that riverbeds in natural habitats with still and clear water are important larval habitats for anopheline mosquitoes[20].

Our findings showed that *An. culicifacies* and *An. dthali* co-exist and breed in different habitats. These observations are consistent with previous investigations which showed that these larval species share the same habitats with varied environmental characteristics[20]. The frequent occurrence of *An. culicifacies* and *An. dthali* in habitats with different physico-chemical characteristics may mean that their life is adaptable to a wide range of environmental conditions.

In the current study, the mean water temperatures in larval positive habitats ranged from 23-28 °C. The results showed that two most commonly collected species including *An. culicifacies* and *An. dthali* had the widest range of observed water temperatures, indicating that species which use open habitats can show a wider range of tolerance against habitats water temperature. Moreover, there was a positive correlation between habitats temperature and the larval density. It is because higher temperatures can be detrimental to the presence of many other aquatic arthropods, including predators which subsequently increases the survivorship of anopheline larvae[21]. Similarly, studies conducted by Russell and Rao in India show that mosquito larvae of *An. culicifacies* normally found in sunny habitats. They also stated that egg-laying female *An. culicifacies* may be quite sensitive to light conditions, which may lead to the differential larval distribution[22].

Water movement in habitats was also important in species distribution. The results showed that, anopheline larvae were abundantly collected from still waters. All of these habitat types were previously reported from elsewhere in the country[8,23].

Similar observation in Eritrea and Ethiopia confirms that stagnate and clear water is preferred by *An. gambiae s.l.* as larval habit[22,24,25]. The main reason for the high abundance of anopheline larvae in still waters may be that still waters provide 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 is detrimental to *Anopheles* larval survival as a result of the physical harm to the larvae and reduction in their oxygen tension[26].

In agreement with previous studies which have shown that chemical characteristics such as sulphate, chloride, alkalinity and conductivity may affect survival and breeding activity of the anopheline mosquitoes[27-29]. The present study clearly indicated a significant relationship between chemical characteristics such as pH, total alkalinity, conductivity, chloride, sulphate and mosquito distribution and abundance. The results showed that anopheline larvae were abundantly collected from water bodies with mean pH ranging from 7.1 to 8.6. It has previously been demonstrated that mosquito larvae favoured a neutral pH or slightly alkaline environment[30]. The results of another study also showed that pH levels of anopheline larval habitats are close to the neutrality[20]. Moreover, the results of a more recent investigation by Soleimani-Ahmadi *et al.*, emphasizes that the favourable pH for the majority of anopheline mosquitoes ranges between 8-8.5[8]. The alkaline tendency of anopheline larvae have been previously reported from Ghana, Ethiopia, Sri Lanka, China and India[4,25,27,29,31]. It may be concluded that larvae of anopheline species mostly prefer habitats with neutral to slightly alkaline environments.

In the present study, although chemical parameters such as calcium, magnesium, TDS, and total hardness were different in habitats, they had no significant effect on anopheline larval density. The difference of such chemical parameters in larval habitats may be due to the soil particles chemical characteristics and edaphic factors in the area. It seems further studies are required to investigate the influence of these parameters on development and abundance of anopheline larvae.

In the field studies which conducted under natural conditions it was suggested that biotic and physico-chemical factors could have played an important role in the development of mosquito larvae in their habitats[32,33]. In addition to measured chemical characteristics of larval habitats in this study, it is thought that other factors such as phosphate, nitrate, dissolved oxygen, biochemical oxygen demand, better to measure due to their influence immature mosquito population. Moreover, we did not control some confounders such as nutrients of the water, predators, pathogens, and plant odours which could have affected anopheline mosquito larvae abundance and distribution. 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.

In conclusion, the current study demonstrates the diversity and abundance of larval habitats and their significance for anopheline larval production and development in Bashagard couny. It was found that much of the larval production goes on in the natural and permanent habitats such as riverbeds and chemical characteristic such as pH, total alkalinity, conductivity, chloride and sulphate were found to be the key factors determining the occurrence and abundance of anopheline larvae in the habitats. These habitats are responsible for continuous production of the adult vectors throughout the year.

Since most of the anopheline species encountered are potential vectors of malaria, results of the present study provide a basis for appropriate management of larval habitats which may help in suppression of vector density, and consequently, malaria transmission in the study area. Furthermore, these finding could be useful in planning and implementing larval control programs and elimination of malaria in Iran.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Malaria is the most important mosquito-borne disease in Iran. It is endemic in south to southeastern part of the country. Bashagard is an endemic area for malaria in south of Iran and local transmission occurs in this district. Larval control through larviciding with *Bacillus thuringiensis* and surface water management is one of the intervention methods for malaria vector control in this area. Knowledge about bio-ecology of vectors will support authorities for appropriate management of the disease.

Research frontiers

This study is performed to determine the effects of environmental parameters of larval habitats on distribution and abundance of anopheline mosquitoes in Bashagard county, a malarious area in southeast of Iran. Knowledge about ecology of malaria vectors provides information to health sector for effective control programs.

Related reports

The results of this study are in agreement with Shililu *et al.* (2003) study in Eritrea, which showed that still waters were the main larval habitats for anopheline mosquitoes. In addition, this finding confirm the results of Liu *et al.*(2012) study which showed chemical characteristics such as sulphate, chloride, alkalinity and conductivity may affect survival and breeding activity of the anopheline mosquitoes.

Innovations and breakthroughs

This is the first time that special chemical characteristics of anopheline mosquito larval habitats and their relationship with distribution and abundance of anopheline species in the studied area is being considered.

Applications

Since most of the anopheline species encountered are potential vectors of malaria, results of the present study provide a basis for appropriate management of larval habitats which may help in suppression of vector density, and consequently, malaria transmission in the study area.

Peer review

This is a valuable research work in which authors have investigated the correlation between some environmental characteristics of anopheline larval habitats including intensity of light, water current, vegetation situation, the presence of algae, substrate type, water conductivity, total alkalinity, turbidity, total dissolved solid, pH and ions such as chloride, sulphate, calcium, and magnesium and anopheline larvae abundance, which can be considered for effective planning and implementing malaria elimination program.

References

[1] World Health Organization. World Malaria Report 2011. Geneva: WHO; 2011. [Online] Available from: http://www.who.int/malaria/world_malaria_report_2011/en/index.html [Accessed on 2 August, 2013].

[2] Hemami MR, Sari AA, Raeisi A, Vatandoost H, Majdzadeh R. Malaria elimination in iran, importance and challenges. *Int J Prev Med* 2013; **4**(1): 88-94.

[3] Soleimani-Ahmadi M, Vatandoost H, Shaeghi M, Raeisi A, Abedi F, Eshraghian MR, *et al.* Field evaluation of permethrin long-lasting insecticide treated nets (Olyset®) for malaria control in an endemic area, southeast of Iran. *Acta Trop* 2012; **123**(3): 146-153.

[4] Kudom AA, Mensah BA, Agyemang TK. Characterization of mosquito larval habitats and assessment of insecticide-resistance status of *Anopheles gambiae* senso lato in urban areas in Southwestern Ghana. *J Vector Ecol* 2012; **37**(1): 77-82.

[5] Keiser J, Singer BH, Utzinger J. Reducing the burden of malaria in different eco-epidemiological settings with environmental management: a systematic review. *Lancet Infect Dis* 2005; **5**(11): 695-708.

[6] Floore TG. Mosquito larval control practices: past and present. *J Am Mosq Control Assoc* 2006; **22**(3): 527-533.

[7] Gouagna LC, Rakotondranary M, Boyer S, Lempérière G,

Dehecq JS, Fontenille D. Abiotic and biotic factors associated with the presence of *Anopheles arabiensis* immatures and their abundance in naturally occurring and manmade aquatic habitats. *Parasit Vectors* 2012; **5**(1): 96.

[8] Soleimani-Ahmadi M, Vatandoost H, Hanafi-Bojd AA, Zare M, Safari R, Mojahedi A, et al. Environmental characteristics of anopheline mosquito larval habitats in a malaria endemic area in Iran. *Asian Pacific J Trop Med* 2013; **6**(7): 510-515.

[9] Shiff C. Integrated approach to malaria control. *Clin Microbiol Rev* 2002; **15**(2): 278-293.

[10] Imbahale SS, Mweresa CK, Takken W, Mukabana WR. Development of environmental tools for anopheline larval control. *Parasit Vectors* 2011; **4**: 130.

[11] Soleimani-Ahmadi M, Vatandoost H, Shaeghi M, Raeisi A, Abedi F, Eshraghian MR, et al. Vector ecology and susceptibility in a malaria-endemic focus in Southern Islamic Republic of Iran. *East Mediterr Health J* 2012; **18**(10): 1034-1041.

[12] Diallo D, Diagne CT, Hanley KA, Sall AA, Buenemann M, Ba Y, et al. Larval ecology of mosquitoes in sylvatic arbovirus foci in Southeastern Senegal. *Parasit Vectors* 2012; **5**: 286.

[13] World Health Organization. Manual on practical entomology in malaria. Part II. Methods and techniques. Geneva: WHO; 1975. [Online] Available from: http://whqlibdoc.who.int/offset/WHO_OFFSET_13_(part2).pdf [Accessed on 2 August, 2013]

[14] Azari-Hamidian S, Harbach RE. Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa* 2009; **2078**: 1-33.

[15] Shahgudian ER. A key to Anophelines of Iran. *Acta Med Iran* 1960; **3**: 38-48.

[16] Eaton AD, Clesceri LS, Rice EW, Greenberg AE. *Standard methods for the examination of water and wastewater*. 21th ed. Washington D.C.: American Public Health Association; 2005.

[17] Nateghpour M, Akbarzadeh K, Farivar L, Amiri A. Detection of asymptomatic malaria infection among the Afghani immigrant population in Iranshahr district of southeastern Iran. *Bull Soc Pathol Exot* 2011; **104**(4): 321-323.

[18] Vatandoost H, Akbarzadeh K, Hanafi-Bojd AA, Mashayekhi M, Saffari M, Elfatih M, et al. Malaria stratification in a malarious area, a field exercise. *Asian Pacific J Trop Med* 2010; **3**(10): 807-811.

[19] Hanafi-Bojd AA, Azari-Hamidian S, Vatandoost H, Charrahy Z. Spatio-temporal distribution of malaria vectors (Diptera: Culicidae) across different climatic zones of Iran. *Asian Pacific J Trop Med* 2011; **4**(6): 498-504.

[20] Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, Charrahy Z, Haghdoost AA, Sedaghat MM, et al. Larval habitats and biodiversity of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of Southern Iran. *J Vector Borne Dis* 2012; **49**(2): 91-100.

[21] Tuno N, Okeka W, Minakawa N, Takagi M, Yan G. Survivorship of *Anopheles gambiae* sensu stricto (Diptera: Culicidae) larvae in western Kenya highland forest. *J Med Entomol* 2005; **42**(3): 270-277.

[22] Russell PF, Rao TR. On relation of mechanical obstruction and shade oviposition of *Anopheles culicifacies*. *J Exp Zool* 1942; **91**(2): 303-329.

[23] Azari-Hamidian S. Larval habitat characteristics of the genus *Anopheles* (Diptera: Culicidae) and a checklist of msquitoes in Guilan province, Northern Iran. *Iran J Arthropod-Borne Dis* 2011; **5**(1): 37-53.

[24] Shililu J, Ghebremeskel T, Seulu F, Mengistu S, Fekadu H, Zerom M, et al. Larval habitat diversity and ecology of anopheline larvae in Eritrea. *J Med Entomol* 2003; **40**(6): 921-929.

[25] Kenea O, Balkew M, Gebre-Michael T. Environmental factors associated with larval habitats of anopheline mosquitoes (Diptera: Culicidae) in irrigation and major drainage areas in the middle course of the Rift Valley, Central Ethiopia. *J Vector Borne Dis* 2011; **48**(2): 85-92.

[26] Okogun GR. Life table analysis of *Anopheles* malaria vectors: generational mortality as tool in mosquito vector abundance and control studies. *J Vector Borne Dis* 2005; **42**(2): 45-53.

[27] Piyaratne MK, Amerasinghe FP, Amerasinghe PH, Konradsen F. Physico-chemical characteristics of *Anopheles culicifacies* and *Anopheles varuna* breeding water in a dry zone stream in Sri Lanka. *J Vector Borne Dis* 2005; **42**(2): 61-67.

[28] Edillo FE, Tripét F, Touré YT, Lanzaro GC, Dolo G, Taylor CE. Water quality and immatures of the M and S forms of *Anopheles gambiae s.s.* and *An. arabiensis* in a Malian village. *Malar J* 2006; **5**: 35.

[29] Liu XB, Liu QY, Guo YH, Jiang JY, Ren DS, Zhou GC, et al. Random repeated cross sectional study on breeding site characterization of *Anopheles sinensis* larvae in distinct villages of Yongcheng City, People's Republic of China. *Parasit Vectors* 2012; **5**: 58.

[30] Abdullah MA, Merdan AI. Distribution and ecology of the mosquito fauna in the southwestern Saudi Arabia. *J Egypt Soc Parasitol* 1995; **25**(3): 815-837.

[31] Saxena OP, Kumar ML, Saxena A, Sharma MC, Saxena RC. Study on the physico-chemical characteristics of breeding grounds in relation to the population density of *Anopheles stephensi*. *J Commun Dis* 1992; **24**(2): 109-115.

[32] Imbahale SS, Paaijmans KP, Mukabana WR, van Lammeren R, Githeko AK, Takken W. A longitudinal study on *Anopheles* mosquito larval abundance in distinct geographical and environmental settings in western Kenya. *Malar J* 2011; **10**: 81.

[33] Koenraadt CJ, Takken W. Cannibalism and predation among larvae of the *Anopheles gambiae* complex. *Med Vet Entomol* 2003; **17**(1): 61-66.