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Larval habitats and biodiversity of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of southern Iran

A.A. Hanafi-Bojd[1], H. Vatandoost[1], M.A. Oshaghi[1], Z. Charrahy[2], A.A. Haghdoost[3], M.M. Sedaghat[1], F. Abedi[4], M. Soltani[5] & A. Raeisi[1,6]

[1]*Department of Medical Entomology & Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran;* [2]*Department of Education, Jehad-e-Daneshgahi of Tehran University, Tehran;* [3]*Research Center for Modeling in Health, Kerman University of Medical Sciences, Kerman;* [4]*Mashhad University of Medical Sciences, Mashhad;* [5]*Advanced Information & Communication Technology Research Center, Sharif University of Technology, Tehran;* [6]*Malaria Unit, CDC, Ministry of Health and Medical Education, Tehran, Iran*

ABSTRACT

Background & objectives: Malaria is the most important mosquito-borne disease in Iran. It is endemic in south to southeastern part of the country. Knowledge about bio-ecology of vectors will support authorities for appropriate management of the disease. Bashagard district is one of the main endemic areas for malaria in south of Iran. This study was conducted to determine anopheline fauna, diversity and affinity in the area, characterization of larval habitats, and mapping their potential distribution across the district.

Methods: The potential aquatic habitats for *Anopheles* larvae were extracted from Indian Remote Sensing Satellite (IRS) image and digital elevation model of the area using GIS. Surface water bodies were sampled monthly during 2009–10 for anopheline larvae, while characteristics of their physical environment were recorded and water samples were analyzed.

Results: A total of 4511 *Anopheles* larvae were found during the year with the lowest and highest frequencies in February and April, respectively. Dominant species was *Anopheles culicifacies*. The Shanon diversity index ranged from 0.570–0.829 at fixed collection sites, while the affinity index was significant among some vector species.

Conclusion: Riversides and riverbeds were the main breeding places which provided sandy, rocky, and clay beds for different species. The potential breeding places as well as distribution of collected species were mapped. Knowledge about ecology of malaria vectors provides information to health sector for effective control programs.

Key words *Anopheles;* ecology; Iran; larval habitats

INTRODUCTION

The distribution pattern of adult mosquitoes is related to habitat preferences of the immature stages. These habitats may be natural or man-made, temporary or permanent. Moreover, each species has specific needs and habitats[1]. Larval control through larviciding and environmental management are the main intervention methods for malaria vector control around the world. Identifying the mosquito larval habitats has a critical role in each control program. Actually it is difficult to find all potentially breeding sites of mosquitoes over a large geographic area (e.g. at district level) based on field survey. This method requires time and money and reduces the efficiency of control program due to missing of some breeding places. Remote sensing is an appropriate method for rapid and accurate determination of breeding places of mosquitoes and environmental factors associated with their distribution.

Malaria is one of the most important vector borne diseases in Iran. The endemic foci of the disease are located mainly in south and southeast parts of the country, especially in three provinces of Sistan and Baluchistan, Hormozgan and Kerman. The last report of Malaria Unit, CDC of Iran shows about 3000 cases in 2011, mostly caused by *Plasmodium vivax.* Based on the last report of WHO, Iran is classified in the elimination phase of malaria control[2]. There are seven proven vectors for the disease in the country, namely *Anopheles stephensi* Liston, *An. dthali* Patton, *An. culicifacies* Giles, *An. fluviatilis* James, *An. superpictus* Grassi, *An. maculipennis* and *An. sacharovi*[3–6]. *Anopheles pulcherrimus* is also reported as a suspected malaria vector in the southeast of Iran[7]. Six out of these vectors are reported from the southern part of the country. Characteristics of Culicidae mosquito larval habitats had been studied for some species in Iran[8–23].

Bashagard is an important endemic area for malaria in south of Iran and local transmission occurs in this district. There is a report of eight *Anopheles* species in this area[24]. Larval control through larviciding with *Bacillus thuringiensis* and surface water management are the in-

tervention methods for malaria vector control in the area. Higher knowledge on the ecology of vectors will result in better management of the control strategies, especially in the elimination phase. Therefore, this study was conducted to determine some ecological parameters of the anopheline species, physicochemical characteristics of their breeding places, and mapping the potential mosquito larval habitats in Bashagard district, southern Iran.

MATERIAL & METHODS

Study area

Bashagard district is located in latitude and longitude of 26° 21' N, 57° 54' E with a population of >30,000 in 2009. It is an under developed area in Hormozgan province, southern Iran. Relative humidity and temperature ranged between 30–65% and 7–45°C, respectively, while average of annual rainfall is 265 mm. Population of this hilly district is scattered and mainly inhabited close to main and seasonal rivers. The soil texture is mainly sandy and is not able to preserve the precipitation as surface water. In 2009, five villages with different climatic conditions were selected as fixed places for anopheline larval collection (Sardasht, Molkan, Chowkhoon, Bolbolabad and Dargazan) although random sampling was also conducted during the one-year study period from 7 other villages.

Larval collection

Within a radius of 1 km around the selected villages all potential breeding sites of anopheline mosquitoes were sampled using a standard mosquito dipper (350 ml) monthly for a period of 12 months from November 2009. Collected larvae were transferred to the laboratory of Malaria Research Center of Bashagard district and conserved in lacto-phenol. The III and IV instars of anopheline larvae were mounted in Berlese's medium and identified morphologically using taxonomic keys of Azari-Hamidian and Harbach[25], and Shahgudian[26].

Physical characteristics of larval habitats

The coordinates of each habitat were recorded using the global positioning system (GPS) during the study period. Physical characteristics for each habitat including water depth, turbidity, and presence of floating and/or emergent vegetation, light/shadow and temperature were also recorded.

Chemical analysis of the water

Water samples were collected from the fixed breeding places during October 2010 and transferred to the laboratory under cold condition. Salinity, pH, conductivity, total hardness and dry residue of samples were analyzed in the water quality laboratory, Hormozgan Province Health Center.

Data analysis

A frame of IRS image of the study area was obtained from the National Cartographic Center (NCC) and analyzed using ER-Mapper 7.1 to extract the surface water pixels and preparing the relevant layer for the area. A digital elevation model (DEM) of the study area was also obtained based on a contour map of 1:50,000 with a contour interval of 30 m. Topographic parameters associated with mosquito breeding places such as elevation and slope of land-surface were extracted from DEM. Based on our findings in the field, the main rivers of the area were close to the villages and found to be the primary breeding places, while the temporary rivers considered as the secondary source for mosquitoes breeding after seasonal rainfall. These potential habitats were extracted from the IRS image and saved as a layer in ArcGIS 9.3. We considered the slope lower than 8% with the higher potential for preserving water bodies. Also breeding places will establish close to human or animal dwellings, because females need blood feeding. With the maximum flight range of 2 km for seeking breeding places and egg-laying, another layer was prepared and overlaid the classified slope and potential habitats layers, then the potential breeding places were classified and mapped in 5 categories. SPSS 18 was employed to compare the habitats of different species.

Biodiversity of species

The Shannon diversity index (H) was used to characterize species diversity at five fixed study sites. Shannon's index accounts for both abundance and evenness of the species present[27].

$$H' = -\sum_{i=1}^{s} (p_i \ln p_i)$$

Affinity index between species

This index was calculated using Fager & McGowan test[28] to find the affinity between pairs of anopheline species occurring in the same habitats. The formula is as follows:

$$[J/(N_A N_B)^{1/2}] - 1/2(N_B)^{1/2}$$

Where, J is the number of joint occurrences; N_A is the total number of occurrences of species A; N_B is the total number of occurrences of species B; and species are assigned to the letters so that $N_A \leq N_B$. The expressions equal to or higher than 0.5 were considered to show affinity.

RESULTS

During the study period a total of 4511 *Anopheles* larvae were identified. These comprised of 8 species: *An. culicifacies* (33.9%), *An. dthali* (17%), *An. stephensi* (13.3%), *An. superpictus* (8.6%), *An. fluviatilis* (0.1%), *An. moghulensis* Christopher (17.2%), *An. turkhudi* Liston (7.2%) and *An. apoci* Marsh (2.7%). There are two peaks of activity in April and September–October (Fig. 1).

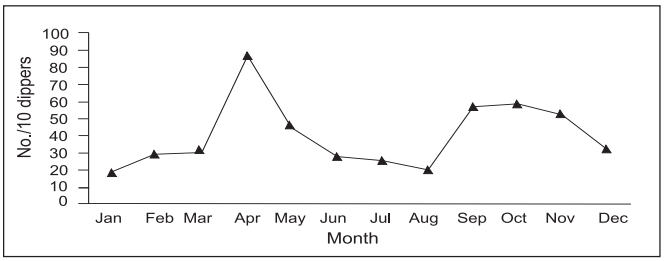

Fig. 1: Monthly prevalence of anopheline larvae in Bashagard district, southern Iran during 2009–10

Larval habitat characteristics

During this survey we found *Anopheles* larvae from four natural habitats, *e.g*. (a) river edges, (b) riverbeds, (c) stream edges, and (d) marshes; and as well as two artificial habitats: (e) palm irrigation channels and water leakages (Fig. 2a–e). Among these habitats, water pools in riverbeds were the main breeding places for anopheline mosquitoes (Table 1). Temperature of breeding sites during the larval collection ranged from 17–32°C, while the altitude of these sites ranged between 420 and 1021 m above the sea level. Most of the larvae were collected from water bodies that have < 20 cm depth. These species were mainly found in habitats with temperature of 20–30°C, without vegetation, having sand and mud beds, clear water and full sunlight (Table 1). Results of chemical analysis of the larval habitats are shown in Table 2.

Anopheles culicifacies Giles *s.l.* was collected from 73.9% of all visited breeding places during the study period. The other anopheline species recorded were *An. dthali* (58.9%), *An. stephensi* (41.1%), *An. superpictus* (23.3%), *An. fluviatilis* (1.4%), *An. apoci* (19.2%), *An. moghulensis* (48.8%) and *An. turkhudi* (28.8%) species (Table 3). Chemical analysis of water showed that it is tolerance to a range of conditions. The results are shown in Table 2.

The analysis of occurrence and co-occurrence of different collected species is presented in Table 3. *Anopheles dthali* was collected in 67.1% of breeding places searched in this survey. Characteristics of breeding places for this species are described in Tables 1 and 2. *Anoph-*

Table 1. Habitat characteristics of anopheline larvae (%) in Bashagard district, southern Iran during 2009–10

Characteristics of breeding sites	Classes	*An. culicifacies*	*An. dthali*	*An. stephensi*	*An. superpictus*	*An. fluviatilis*	*An. turkhudi*	*An. moghulensis*	*An. apoci*
Habitat temperature (°C)	17–20	7.4	8.2	8.6	14.3	0	12	12.2	14.3
	20.1–25	40.7	38.8	40	33.3	0	40	43.9	57.1
	25.1–30	44.4	46.9	48.6	42.9	100	48	39	14.3
	30.1<	7.5	6.1	2.8	9.5	0	0	4.9	14.3
Depth of habitat (cm)	0–20	87	87.8	85.7	95.2	100	96	87.8	85.7
	20.1–50	13	12.2	14.3	4.8	0	4	12.2	14.3
Vegetation situation	Without vegetation	61.1	59.2	65.7	61.9	50	56	61	71.4
	With vegetation	7.4	8.2	5.7	9.5	0	4	7.3	0
	With algae	31.5	32.6	28.6	28.6	50	40	31.7	28.6
Substrate type	Mud	31.5	24.5	25.7	14.3	0	16	26.8	21.4
	Sand	53.7	67.3	65.7	71.4	100	64	61	57.2
	Rock	14.8	8.2	8.6	14.3	0	20	12.2	21.4
Water situation	Turbid	22.2	20.4	20	23.8	0	0	21.9	50
	Clear	72.2	73.5	71.4	76.2	100	96	73.2	35.7
	Algal	5.6	6.1	8.6	0	0	4	4.9	14.3
Sunlight situation	Full sunlight	79.6	77.6	88.6	85.7	50	76	68.3	92.9
	Partial sunlight	14.8	18.3	8.6	9.5	50	20	24.4	7.1
	Shaded	5.6	4.1	2.8	4.8	0	4	7.3	0
Habitat type (Natural)	River edge	20.4	22.4	22.9	33.3	50	32	29.3	21.4
	River bed	53.7	48.9	57.1	57.1	0	48	39	71.4
	Stream edge	11.1	12.2	2.8	0	0	16	17.1	0
	Marsh	1.8	4.1	2.8	4.8	0	0	4.8	0
Habitat type (Artificial)	Palm irrigation channel	3.7	4.1	2.9	0	50	4	4.9	0
	Water leakage	9.3	8.1	11.4	4.8	0	0	4.8	7.2

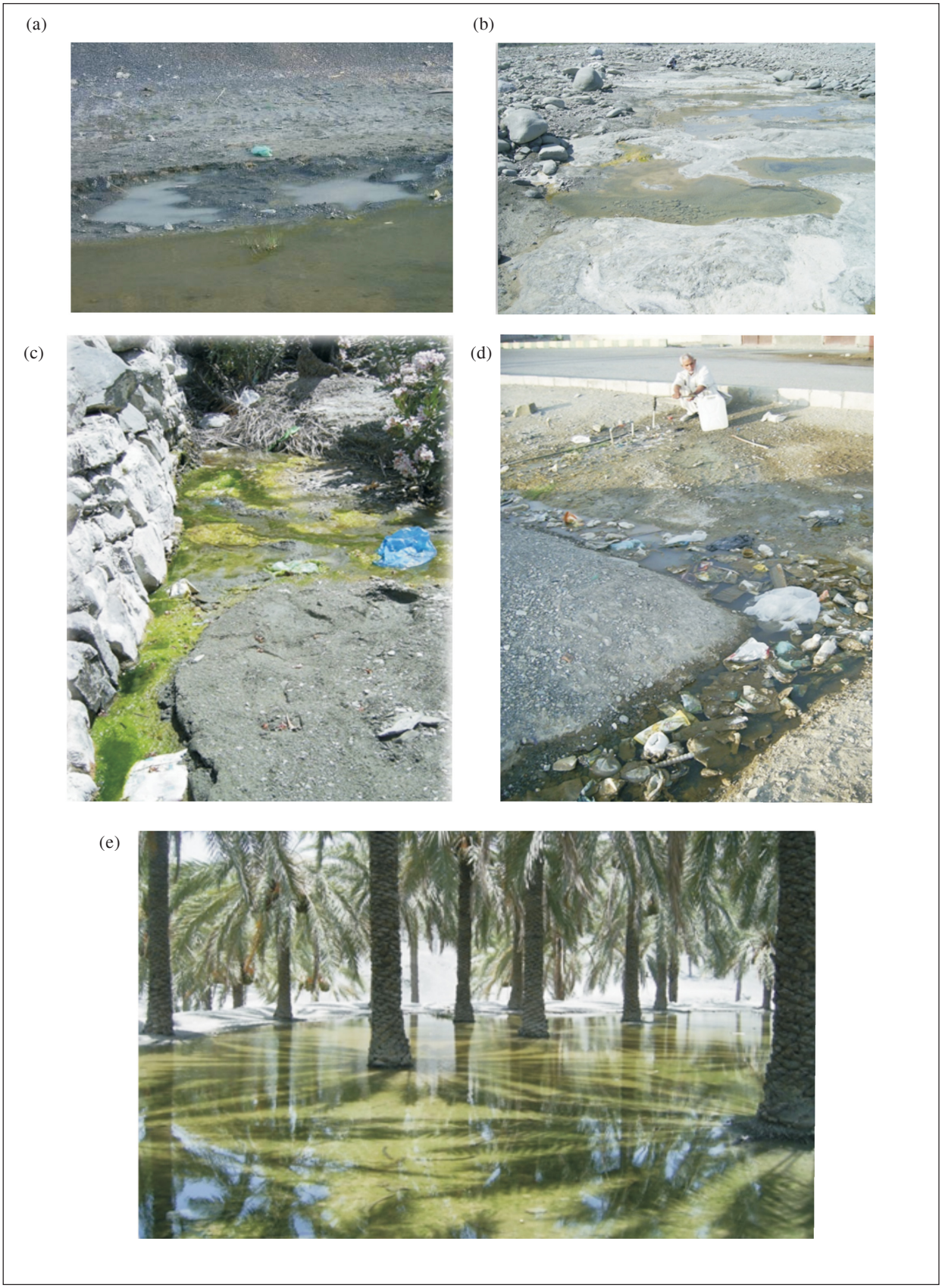

Fig. 2 (a–e): Different types of anopheline breeding places in Bashagard district, southern Iran during 2009–10. (a) River edges; (b) Riverbeds; (c) Stream edges; and (d) marshes; (e) palam irrigation channels and water leakages.

Table 2. Results of chemical analysis of anopheline breeding places in Bashagard district, southern Iran in 2010

Sampling place	Sardasht	Chowkhoon1	Chowkhoon2	Dargazan	Molkan1	Molkan2
Ph	8.20	7.32	7.14	7.30	8.90	8
Total hardness (mg/l)	180	392	368	164	200	240
Electrical conductivity (mho/cm)	964	1892	2000	961	1064	1412
Dry residue at 180°C (mg/l)	578.4	1135.2	1200	576.6	638.4	847.2
An. culicifacies	+	+	+	+	+	+
An. dthali	+	+	+	+	–	+
An. stephensi	+	–	+	–	+	+
An. superpictus	–	+	+	+	–	–
An. fluviatilis	–	–	–	–	–	–
An. turkhudi	–	–	–	+	–	–
An. moghulensis	–	+	+	+	–	+
An. apoci	+	+	+	+	+	+

Table 3. Percent of co-occurrence and affinity index between pairs of anopheline species in visited breeding places of the study area, Bashagard district, southern Iran during 2009–10

Species	*An. culicifacies*	*An. dthali*	*An. stephensi*	*An. fluviatilis*	*An. superpictus*	*An. turkhudi*	*An. moghulensis*	*An. apoci*
An. culicifacies		58.9	41.1	1.4	23.3	28.8	48.8	19.2
An. dthali	0.769		35.6	2.7	26	26	47.9	16.4
An. stephensi	0.619	0.557		1.4	17.8	15.1	28.8	12.3
An. fluviatilis	0.028	0.030	0.034		0	1.4	1.4	0
An. superpictus	0.437	0.521	0.394	–0.109		13.7	20.5	5.5
An. turkhudi	0.504	0.472	0.285	0.041	0.337		20.5	4.1
An. moghulensis	0.675	0.710	0.472	0.032	0.548	0.390		13.7
An. apoci	0.441	0.387	0.322	–0.134	0.125	0.060	0.339	

eles stephensi was found in 47.9% of all visited habitats described physicochemically in Tables 1 and 2. *Anopheles superpictus* was distributed in the study area and found in 28.8% of breeding sites. Characteristics of the breeding place water are listed in Tables 1 and 2. *Anopheles fluviatilis* James *s.l.* was found in 2 occasions from slow running water of one small branch of the Sardasht river and the water channel of a spring in Bolbolabad. *Anopheles moghulensis*, a non-vector species, was collected from 56.1% of visited habitats. Its breeding sites are described in Tables 1 and 2. *Anopheles turkhudi*, another non-vector species, was found in 34.2% of breeding sites. Physical and chemical characteristics of breeding sites for *An. turkhudi* are listed in Tables 1 and 2. *Anopheles apoci*, which has no role in malaria transmission, was mainly found in 19.2% of visited waters. Breeding sites of this species are characterized in Table 1 for physical conditions and Table 2 for chemical factors.

Spatial distribution of larval habitats

The study area has a sandy and porous soil texture and, therefore, it is not able to preserve the rain water for a long-time. Thus, these cannot provide permanent breeding places for mosquitoes. Potential breeding places in the study area are classified in Fig. 3 where 11 villages were visited for larval collection. Most of the villages of Bashagard district have a slope of 0–8%. Ten out of 11 villages in our study classified in this group, although one was classified in 8–15% slope. Based on the aspect there are classes: north (337.5–22.5), northeast (22.5–67.5), east (67.5–112.5), southeast (112.5–157.5), south (157.5–202.5), southwest (202.5–247.5), west (247.5–292.5), and northwest (292.5–337.5). Four studied villages were located in south, 3 in east, 1 in west, 1 in southeast and 2 in southwest aspects. Spatial distribution of the collected larvae was also mapped in the study area (Fig. 4).

Temporal distribution of species

The temporal distribution of anopheline larvae was also noted. During monthly collections for one year *An. dthali*, *An. stephensi* and *An. moghulensis* were collected throughout the year, while *An. culicifacies* was not found only in February, *An. turkhudi* was collected during the year except for March, *An. superpictus* was not found in February, and September, *An. apoci* was found in April & September to November, and finally *An. fluviatilis* was

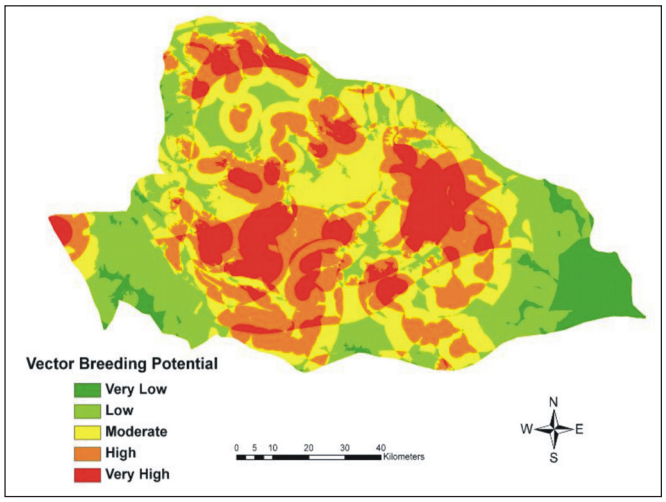

Fig. 3: Potential mosquito breeding places in Bashagard district, southern Iran, 2010.

only collected in two occasions in July and September. Figure 1 shows the monthly prevalence of larvae of three malaria vectors in the study area.

Species biodiversity and evenness

The species diversity was calculated for 5 fixed villages, where the larval collection was conducted during the study period. Shannon's equitability is presented in Table 4, and the index ranged between 0.570 in Dargazan and 0.829 in Sardasht.

Affinity index between species

Affinity indices between pairs of species showed significant results for the following pairs (Table 3): *An. culicifacies* and *An. dthali* (0.769), *An. culicifacies* and *An. stephensi* (0.619), *An. culicifacies* and *An. turkhudi* (0.504), *An. culicifacies* and *An. moghulensis* (0.675), *An. dthali* and *An. stephensi* (0.557), *An. dthali* and *An. superpictus* (0.521), *An. dthali* and *An. moghulensis* (0.710), and *An. superpictus* and *An. moghulensis* (0.548).

DISCUSSION

Bashagard is a mountainous district with a dry and hot climate, and low precipitation. Therefore, the breeding places of mosquitoes in this area are mainly in river valleys close to rivers and streams. During this study we found 8 *Anopheles* species out of them 5 have been reported as malaria vectors in southern Iran[2]. These species were reported previously from the area[24].

Anopheles culicifacies s.l. occurs mainly in Sistan and Baluchistan, Kerman and Hormozgan provinces of Iran. It is the main malaria vector in southeastern corner of the country[3,29]. Rice-fields of Baluchistan area during April–September are the main larval breeding sources of *An. culicifacies s.l.*, along with irrigated palms and stream pools in other parts of its distribution area. During winter months, from October to April, stream pools serve as the predominant oviposition sites of this species in most areas[7, 30-32]. This *Anopheles* can survive in relatively high salinity degrees[32]. The spatial distribution of *An. culicifacies* is restricted to Bushehr, Hormozgan, Kerman, Sistan and Baluchistan, south Khorasan and Khuzistan provinces[4]. We found this species in its defined distribution range and the same breeding places as other authors have described.

Anopheles dthali has been found in southern parts of the Zagros chain, and coastal area of the Persian Gulf up to 1410 m of Iran[11]. Larvae of this *Anopheles* were found in water with high salinity (2.7 parts per 1000) in rural areas. *Anopheles dthali* breeds in pebbly margins of rivers, springs, pits around springs with or without vegetation, pools in dried-up riverbeds, and palm irrigation canals in Hormozgan province. In Bandar Abbas County, larvae were also found in mineral water. The water temperature of breeding places ranged between 13 and 28ºC, with a pH of 6.9–8. This species is a secondary vector in some parts of southern Iran especially in mountainous areas of Hormozgan province[11,19]. It is reported from Bushehr, Chaharmahal and Bakhtiari, Fars, Hamedan, Hormozgan, Ilam, Isfahan, Kerman, Kermanshah, Khuzistan, Kohgiluye and Buyerahmad, Lorestan, Sistan and Baluchistan, and Yazd provinces[5]. Our findings are the same with above described characteristics for this species, but the upper level of pH is increased to 8.2 in this study.

Anopheles fluviatilis s.l. is distributed on the southern slopes of the Zagros chain, from southwest of Kermanshah province to Sistan and Baluchistan province in the southeastern part of Iran. *Anopheles fluviatilis* breeds in fresh, slow flowing or stagnant waters, vast marshes, river banks, pits in the beds of stony or sandy rivers and rainfall pits[14]. This species is reported from Bushehr, Fars, Hormozgan, Ilam, Kerman, Kermanshah, Khuzistan, Kohgiluye and Buyerahmad, Lorestan and Sistan and Baluchistan provinces[5,33]. We found this species at two occasions and more search in the appropriate habitats is suggested to describe its breeding sites well.

Anopheles stephensi breeds in a wide range of both urban and rural habitats throughout its distribution region. In urban areas, this species breeds in all sources of water bodies, such as wells, cisterns, fountains, ornamental ponds, and in water pools used for building constructions. Larvae can be collected from ponds, pools, stream margins, catch basins, and seepage canals. It is found in water

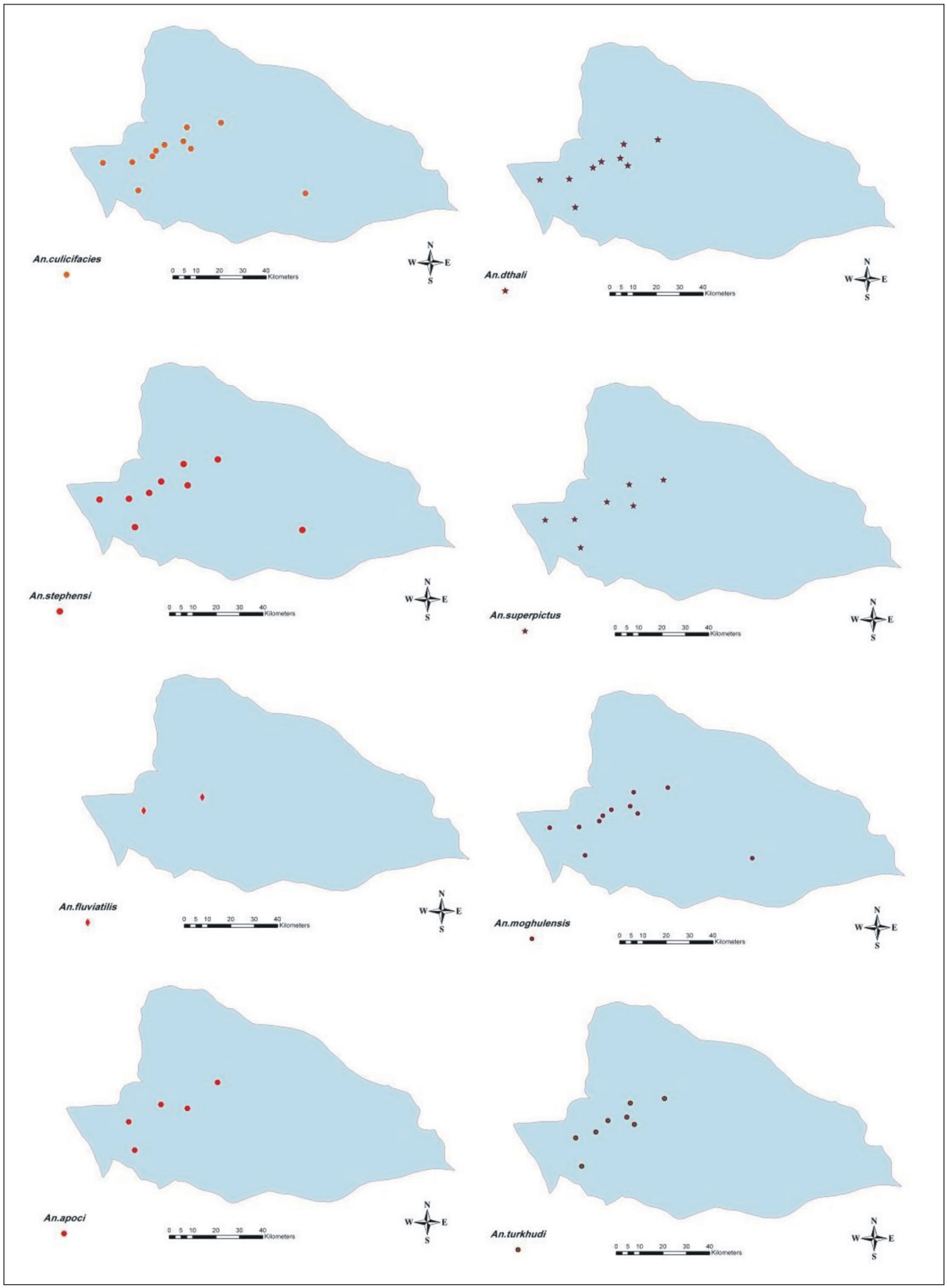

Fig. 4: Spatial distribution of *Anopheles* species in the study area of Bashagard district, southern Iran during 2009–10 (Dots show the villages where the *Anopheles* species were collected).

Table 4. Shannon diversity indices of anopheline mosquitoes in different villages of Bashagard district, southern Iran, 2009–10

Village	Species	Number	Proportion (Pi)	Loge Pi	Pi logePi	H'
Sardasht	*An. culicifacies*	539	0.358	–0.446	–0.159	
26º 27'N	*An. dthali*	198	0.131	–0.883	–0.116	
57º 54'E	*An. stephensi*	293	0.194	–1.639	–0.318	
710 m	*An. superpictus*	61	0.042	–1.377	–0.058	0.829
elevation	*An. fluviatilis*	2	0.001	–3	–0.003	
	An. moghulensis	175	0.116	–0.936	–0.011	
	An. turkhudi	179	0.119	–0.924	–0.109	
	An. apoci	59	0.039	–1.409	–0.055	
Molkan	*An. culicifacies*	594	0.408	–0.389	–0.158	
26º 29'N	*An. dthali*	140	0.096	–1.018	–0.098	
58º 02'E	*An. stephensi*	238	0.164	–0.785	–0.129	
920 m	*An. superpictus*	187	0.129	–0.889	–0.115	0.673
elevation	*An. moghulensis*	210	0.144	–0.842	–0.121	
	An. turkhudi	44	0.003	–2.523	–0.007	
	An. apoci	42	0.029	–1.538	–0.045	
Chowkhoon	*An. culicifacies*	64	0.125	–0.903	–0.113	
26º 41'N	*An. dthali*	260	0.506	–0.296	–0.150	
57º 41'E	*An. stephensi*	27	0.053	–1.276	–0.068	
450 m	*An. superpictus*	102	0.197	–0.706	–0.139	0.636
elevation	*An. moghulensis*	27	0.053	–1.275	–0.068	
	An. turkhudi	15	0.029	–1.538	–0.045	
	An. apoci	19	0.037	–1.432	–0.053	
Bolbolabad	*An. culicifacies*	32	0.241	–0.618	–0.149	
26º 25'N	*An. dthali*	26	0.195	–0.710	–0.138	
57º 39'E	*An. stephensi*	20	0.150	–0.824	–0.124	
680 m	*An. superpictus*	5	0.038	–1.420	–0.054	0.718
elevation	*An. fluviatilis*	1	0.008	–2.097	–0.017	
	An. moghulensis	42	0.315	–0.502	–0.158	
	An. turkhudi	6	0.045	–1.347	–0.061	
	An. apoci	1	0.008	–2.097	–0.017	
Dargazan	*An. culicifacies*	147	0.265	–0.577	–0.153	
26º 29'N	*An. dthali*	71	0.128	–0.893	–0.114	
57º 47'E	*An. stephensi*	1	0.002	–2.699	–0.005	
1020 m	*An. superpictus*	13	0.023	–1.638	–0.038	0.570
elevation	*An. moghulensis*	264	0.477	–0.321	–0.153	
	An. turkhudi	57	0.103	–0.987	–0.102	
	An. apoci	1	0.002	–2.699	–0.005	

with high salinity, sometimes reaching or even exceeding that of seawater. In rural areas, the breeding places are pools, streambeds, palm irrigation canals, at the margin of streams and rivers, seepages, and marshy areas with a gentle water flow[13,20,21]. This species is distributed in Bushehr, Fars, Hormozgan, Ilam, Kerman, Kermanshah, Khuzistan, Kohgiluye and Buyerahmad, Lorestan, Sistan and Baluchistan provinces[5]. We found this species mostly in full sunlight water bodies mainly located in riverbeds with sandy beds.

Anopheles superpictus has a widespread distribution in Iran, from 50 to 2000 m. Larvae of *An. superpictus* can be found in different water bodies with ground origin, especially sandy larval habitats of riverbeds with clear water. The species is recorded in all the provinces of the country, however, there is no formal report from Qazvin province[5]. We found the same conditions for larval habitats of this species as described previously.

Anopheles turkhudi has been collected mainly from the permanent water bodies, which often have vegetation. It breeds in fully sunlit habitats with sandy beds. It has also found from rice-fields, clean and sweet water. Most

of natural breeding places for this species are reported to be in riverside[34]. We found *An. turkhudi* in riverbed/riverside, from sandy beds with fully sunlight, but mostly from non-vegetation or algal waters.

There was no report for habitat characteristics of *An. moghulensis* in Iranian literature on mosquito fauna. We found this *Anopheles* as the main species in Dargazan village with elevation of 1021 m above the sea level. Generally, it was found from natural breeding sites including riverbed, riverside and stream edge; fully sunlight habitats without vegetation or with algae and pH range of 7.14–8, temperature of 17–30ºC and water depth <20 cm.

Anopheles apoci is distributed in southern Iran. Marsh[8] described this *Anopheles* for the first time from the southwest of the country. He found the larvae in stagnant brackish pools. Another study in west of Iran found this species mainly in permanent water bodies, with or without vegetation, fully sunlit, mud beds, clean water. It reported the natural breeding places of this species in riverside and mainly found *An. apoci* in the same breeding place along with *An. turkhudi* and *An. superpictus*[34]. The breeding sites of *An. apoci* in Iraq are described as streambed pools having heavily salt-encrusted margins, hollowed in the rock, exposed to direct sunlight, stagnant and contained no macroscopic vegetation[35]. We found this *Anopheles* in water pools of riverbeds accompanied mainly by *An. culicifacies*, *An. dthali* and *An. moghulensis*. The water pH of breeding places ranged from 7.14 to 8.9.

Shannon index was higher in Sardasht village (Table 4). This area has a permanent river that provides different breeding places in beds and margins, higher population and a variety of natural and artificial shelters for mosquito resting. Therefore, the species richness and density of mosquitoes is higher in this village. Nagm *et al*[36] found species diversity index that was higher for dry rather than rainy season. The affinity index showed significant correlation between *An. culicifacies* with *An. stephensi* and *An. dthali*, while *An. dthali* and *An. stephensi* had also the significant affinity (Index >0.5) with the highest value for pair of *An. culicifacies* and *An. dthali*. This may be due to the same biological requirements of these vector species. *Anopheles dthali* has also significant affinity with *An. superpictus* and *An. moghulensis* in habitats, while this affinity is significant in pair of *An. moghulensis* and *An. superpictus*. Based on the affinity index there was no correlation between *An. apoci* and other species. The result was the same for *An. fluviatilis* and other species. This may be due to different requirements of these species.

In conclusion, more knowledge generated on the ecology of malaria vectors will help us to manage the vector control programs better.

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Correspondence to: Dr H. Vatandoost, Professor, Department of Medical Entomology & Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.
E-mail: hvatandoost1@yahoo.com; vatando@tumo.ac.ir

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