

Larval habitats characteristics of Culicinae subfamily in the south of Iran

S. DOOSTI^{1,2,*}, M.R. YAGHOOBI-ERSHADI², M.M. SEDAGHAT², K. AKBARZADEH²,
GIDIGLO NUTIFAFI GODWIN²

¹Center for Communicable Diseases Control, Ministry of Health, Tehran, Iran. Tel.: +98-81455028, *email: fania2008@gmail.com, sa-doosti@health.gov.ir

²Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Manuscript received: 19 May 2021. Revision accepted: 7 November 2021.

Abstract. Doosti S, Yaghoobi-Ershadi MR, Sedaghat MM, Akbarzadeh K, Godwin GN. 2021. Larval habitats characteristics of Culicinae subfamily in the south of Iran. *Nusantara Bioscience* 13: 194-201. Globally, mosquitoes (Diptera: Culicidae) are known vectors of many diseases such as Malaria, Dengue fever, Sindbis, West Nile virus, Rift Valley fever, Japanese encephalitis, and Filariasis. This study aimed to determine larval habitat characteristics of mosquitoes in the south of Iran between 2009 and 2013. The study was carried out in different larval habitats of mosquitoes, and samples were collected using dipping and pipette methods. The larvae were kept in lactophenol solution and transferred to the Medical Entomology of Tehran University of Medical Sciences. Then, microscopic slides were prepared, and species were identified using reliable morphological keys. Characteristics of larval breeding places were investigated based on habitat type, water conditions, vegetation type, and sunlight exposure. A total of 8,188 mosquito larvae were collected from 209 different breeding places. In all, 20 mosquito species belonging to 4 genera were identified. *Culex quinquefasciatus*, *Cx. pipiens*, *Culiseta longiareolata*, *Cx. tritaeniorhynchus*, *Cx. sitiens*, *Cx. theileri* and *Aedes caspius* were the most common species found in this study. The southern part of Iran has the most wharves and airports. Additionally, there is a high diversity of medically important mosquito species like *Cx. pipiens* and *Cx. tritaeniorhynchus*, *Ae. albopictus* and *Ae. vexans* from the Culicidae family. The species are capable of vectoring various diseases in the southern part of Iran; hence, there is a need for more investigations.

Keywords: Culicinae, ecology, mosquito genera

INTRODUCTION

Generally, mosquitoes act as vectors by transmitting diseases such as Malaria, Dengue fever, Sindbis, West Nile, Rift Valley fever, Japanese encephalitis, and Filariasis (Benelli and Duggan 2018; Suesdek 2019). Mosquitoes' life stages and population dynamics are affected by climatic conditions such as temperature, humidity, and larval habitats. Therefore, the biodiversity and variability of different species of mosquitoes in Iran may be attributed to various climatic conditions as major factors.

About 77 species of *Anopheles*, *Aedes*, *Culex*, *Coquillettidia*, *Mansonia*, *Psorophora*, and *Culiseta* play important roles in the transmission of dirofilariasis (Azari-Hamidian et al. 2009). Dirofilariasis disease has been studied in many parts of the world, and species such as *Cx. pipiens* and *Cx. theileri* are some of the vectors. In addition, West Nile and Sindbis viruses, as well as *Dirofilaria immitis* (dog heartworm) and *D. repens* (dirofilariasis), which are transmitted by mosquitoes, have been reported in Iran (Naficy and Saidi 1970; Saidi et al. 1976; Azari-Hamidian et al. 2007). In Iran, West Nile viruses are the most important mosquito-borne viruses, as reported using Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) from Golestan, Guilan, Isfahan, Kermanshah, Khuzistan, Qom, Razavi Khorasan, and Tehran Provinces (Chinikar et al. 2012, 2013a; Shah-Hosseini et al. 2014; Meshkat et al. 2015). Recently, Shah-

Hosseini et al. (2017) and Bagheri et al. (2015) showed that *Cx. pipiens* and *Ae. caspius* s.l. were infected with West Nile viruses in Guilan and West Azerbaijan provinces, respectively. Also, Azari-Hamidian et al. (2009) demonstrated that *An. maculipennis* and *Cx. theileri* are vectors of *Setaria* and *Dirofilaria immitis* in the Ardebil province.

Many studies have identified various species of mosquitos in Iran (Zaim 1987a; Vatandoost et al. 2004, 2006; Moosa-Kazemi et al. 2009; Azari-Hamidian 2011; Oshaghi et al. 2011; Hanafi-Bojd et al. 2017; Azari-Hamidian et al. 2019). The Culicidae family is divided into Anophelinae and Culicinae subfamilies, which include 11 tribes and 41 or 113 genera and 3,563 species (Harbach 2018). The Culicinae subfamily is the largest group of mosquitoes and includes more than 3,075 species in 110 genera (<http://mosquito-taxonomic-inventory.info>). The *Culex* genus has a worldwide distribution and is divided into 26 subgenera with 769 species. According to investigations by Zaim and Cranston (1986), the Culicinae subfamily includes 33 species (10 species of *Aedes*, 19 species of *Culex*, 3 species of *Culiseta*, and 1 species of *Uranotaenia*) in Iran. Also, in a recent study, 20 species of *Culex* were recorded in Iran (Azari-Hamidian and Harbach 2009). In addition to morphological studies, molecular studies can also solve many complex problems in the classification and taxonomy of mosquitoes (Oshaghi et al. 2008; Djadid et al. 2009). Mosquitos usually choose specific larval habitats for oviposition. Most information on Culicinae focused on

detecting the diversity and variety of species. However, there is little information about the ecology and characteristics of larval habitats. Many factors, such as temperature, pH, salinity, and organic matter, influence larval stage distribution (Muirhead 1951; Clements 1992).

Additionally, water is essential for breeding mosquitoes and affects the growth and development of larval stages. Some compounds such as sodium, potassium, calcium, and dissolved oxygen significantly correlate with the density of specific species (Sasikumar et al. 1986; Piyaatne et al. 2005; Surendran and Ramasamy 2005). Lotfi (1970, 1973, 1976) studied ecological aspects like water temperature and pH of the breeding places of *Culex* species. Also, Yaghoobi-Ershadi et al. (1986) studied the characteristics of larval breeding sites in the Minab and Hormozgan provinces. Zaim (1987b) reviewed the larval breeding places and distribution of Iranian Culicinae. Azari-Hamidian et al. (2002) investigated mosquito species, characteristics of larval breeding places, and species diversity of *Culex* in Rasht province. Also, in several studies by Nikookar et al. (2015a,b, and 2017), mosquitoes' larval habitat characteristics and biodiversity in Mazandaran province were investigated. Currently, there is no updated information on Culicinae and larval habitats in Iran. Hence, this article aimed to investigate the characteristics of mosquitoes' larval habitat in southern Iran.

MATERIALS AND METHODS

Study area

The selected study areas included Sistan and Baluchestan, Hormozgan, Khuzistan, Ilam, South Khorasan, Kerman, and

Fars provinces. These areas are bounded by Iraq, Saudia Arabia, Pakistan, and Afghanistan and are located in arid and semi-arid regions. Due to the existence of different climatic conditions in the areas, different larval habitats were found. The field study was conducted in different parts of rural and urban areas between May and September of 2009-2013, with 18 rounds of sampling.

Sampling method

Sampling was carried out using dipping and pipette methods. Mosquito larvae were collected from natural breeding sites such as river edges, river bed pools, rain pools, marshes, grasslands, tree holes, and artificial breeding sites such as rice fields, irrigation channels, wells, discarded tires, buckets and old tires (Figure 1). For each habitat, sampling was done about 2 to 3 times.

Characteristics of the larval habitats such as the kind of habitat (natural or artificial), habitat situation (permanent or transient, standing or running), vegetation type (with or without vegetation), bottom type, water temperature, water situation (clear or turbid) and sunlight situation (full or partial sunlight or shaded) were recorded. The larvae were preserved in 100% lactophenol solution. The details (collector's name, date of collection, name of location, type of habitat) were recorded on tubes and then transferred to the Medical Entomology laboratory of Tehran University of Medical Sciences. Larvae were then identified morphologically using reliable keys of Harbach (1985), Zaim and Cranston (1986), and Azari-Hamidian and Harbach (2009). Then, slides of the third and fourth instars larvae were prepared using Berlese's Fluid medium, and specimens were studied under the microscope using 10X and 40X magnifications.



Figure 1. Some larval breeding places for sampling in southern Iran, 2009-2013. A. Old tire, B. Man-made container, C. Irrigation channel, D. Rain pool (Hootak), E. Rice field, F. River edge

RESULTS AND DISCUSSION

Results

This study was conducted from 2009 to 2013 in the southern provinces of Iran (Figure 2), with 8,188 mosquito larvae belonging to four genera: *Culex*, *Aedes*, *Culiseta*, and *Uranotaeniya* were collected. Larvae identified using morphological keys included 13 species of the *Culex* genus, 5 species of the *Aedes* genus, one *Culiseta* genus, and one *Uranotaeniya* genus (Table 1).

Among the mosquito species found, *Culex quinquefasciatus* had the highest distribution (2010, 24.55%). This species was the most abundant specimen collected alongside other species from natural and artificial larval habitats. Also *Cu. longiareolata* were the second-highest in distribution and were also collected from most larval habitats. Overall, 4,997 specimens from natural larval habitats and 3,191 from artificial habitats were collected (Table 2). During this study, 56.05% of the larvae were collected from temporal larval habitats, compared to those from the permanent habitats (38.8%); 4.67% of them were collected from stagnant water, compared to running water (0.46%). In our study, most of the larvae were collected from natural habitats of river edges (33.58%) and artificial larval habitats of pools (18.05%) (Tables 3 and 4).



Figure 2. Provinces that were studied for larval collection of the Culicinae subfamily in the southern part of Iran, 2009-2013

Table 1. Frequency of Culicinae subfamily larvae in the south of Iran, 2009-2013

Species	Provinces								Total	%
	Sistan & Baluchistan	Khuzestan	Hormozgan	Fars	Kerman	South Khorasan	Bushehr	Ilam		
<i>Cx. arbieeni</i>	0	0	2	0	0	0	0	0	2	0.02
<i>Cx. hortensis</i>	0	0	0	0	0	40	1	0	41	0.50
<i>Cx. pusillus</i>	0	28	0	1	0	0	63	0	92	1.12
<i>Cx. perexiguus</i>	121	1	4	180	56	2	51	5	420	5.13
<i>Cx. pipiens</i>	55	184	235	19	0	1	522	39	1055	12.88
<i>Cx. theileri</i>	148	9	157	64	0	28	17	21	444	5.42
<i>Cx. mimiticus</i>	75	0	81	105	19	1	22	0	303	3.70
<i>Cx. sinaiticus</i>	12	0	52	0	0	0	0	0	64	0.78
<i>Cx. laticinctus</i>	0	0	0	0	4	0	2	0	6	0.07
<i>Cx. sitiens</i>	438	6	0	0	71	0	56	0	571	6.97
<i>Cx. tritaeniorhynchus</i>	311	157	142	9	203	2	5	0	829	10.12
<i>Cx. quinquefasciatus</i>	1145	58	135	60	23	0	80	509	2010	24.55
<i>Cx. bitaeniorhynchus</i>	18	0	0	42	35	0	0	0	95	1.16
<i>Cs. longiareolata</i>	159	0	648	0	45	825	62	24	1763	21.53
<i>Ur. unguiculata</i>	0	6	0	0	0	0	8	0	14	0.17
<i>Ae. caspius</i>	113	101	0	0	56	0	165	0	435	5.31
<i>Ae. vexans</i>	2	0	26	0	0	0	7	0	35	0.43
<i>Ae. flavescens</i>	2	0	0	0	0	0	0	0	2	0.02
<i>Ae. caballus</i>	1	0	1	0	0	0	0	0	2	0.02
<i>Ae. albopictus</i>	5	0	0	0	0	0	0	0	5	0.06
Total	2605	550	1483	480	512	899	1061	598	8188	100

Table 2. larval habitats characteristics of Culicinae subfamily collected in the southern part of Iran, 2009-2013

Species																					
Larval habitats characteristic	<i>Cx. arbieeni</i>	<i>Cx. hortensis</i>	<i>Cx. pusillus</i>	<i>Cx. perexiguus</i>	<i>Cx. pipiens</i>	<i>Cx. theileri</i>	<i>Cx. mimiticus</i>	<i>Cx. sinaiticus</i>	<i>Cx. laticinctus</i>	<i>Cx. sitiens</i>	<i>Cx. tritaeniorhynchus</i>	<i>Cx. quinquefasciatus</i>	<i>Cx. bitaeniorhynchus</i>	<i>Cs. longiareolata</i>	<i>Ur. unguiculata</i>	<i>Ae. caspius</i>	<i>Ae. vexans</i>	<i>Ae. flavescens</i>	<i>Ae. caballus</i>	<i>Ae. albopictus</i>	Total
Habitat situation																					
Permanent	0	36	83	249	331	173	109	8	2	164	388	644	42	825	14	109	0	0	0	0	3177
Temporary	2	5	8	124	642	250	102	56	4	407	342	1338	18	938	0	312	35	0	2	5	4590
Running water	0	0	1	43	68	21	88	0	0	0	89	27	35	0	0	9	0	2	0	0	383
Stagnant water	0	0	0	4	14	0	4	0	0	0	10	1	0	0	0	5	0	0	0	0	38
Vegetation situation																					
Without	2	25	8	32	728	174	59	17	4	471	89	1326	0	920	0	31	26	0	0	3	3915
Out of water	0	0	8	23	19	0	21	0	0	0	131	81	0	270	2	72	0	0	0	0	627
In water level	0	1	12	16	63	10	29	2	0	1	90	173	0	150	6	215	2	0	1	0	771
Underwater	0	0	0	106	97	29	64	45	0	0	107	284	37	210	0	26	0	0	1	2	1008
All types	0	15	64	243	148	231	130	0	2	99	412	146	58	213	6	91	7	2	0	0	1867
Sunlight situation																					
Full sunlight	0	41	67	231	521	365	255	56	6	458	440	940	58	1421	6	289	33	2	1	3	5192
Partial sunlight	2	0	18	105	285	52	43	8	0	56	295	495	19	306	6	36	0	0	0	2	1728
Shaded	0	0	7	84	249	27	5	0	0	57	94	575	18	36	2	110	2	0	1	0	1267
Substrate																					
Muddy	0	40	29	274	635	250	103	27	0	301	614	1033	41	1074	14	359	35	2	1	0	4832
Sandy	2	1	0	62	10	85	96	35	2	108	74	323	38	337	0	76	0	0	1	2	1252
Rocky	0	0	0	26	143	91	11	2	4	140	22	643	0	172	0	0	0	0	0	0	1254
Others	0	0	0	0	267	0	0	0	0	22	0	10	0	51	0	0	0	0	0	3	353
All types	0	0	63	58	0	18	93	0	0	0	119	2	16	128	0	0	0	0	0	0	497
Water situation																					
Turbid	0	5	20	6	334	37	20	24	0	351	176	394	0	264	6	106	26	0	0	0	1769
Clear	2	36	72	414	721	407	283	40	6	220	653	1616	95	1499	8	329	9	2	2	5	6419
Fresh	2	0	29	402	1045	387	303	60	6	386	812	1364	95	1275	14	333	35	2	1	0	6551
Salty	0	41	63	18	10	57	0	4	0	185	17	646	0	488	0	102	0	0	1	5	1637
Habitat kind																					
Artificial	2	20	20	85	620	302	13	0	4	377	244	835	23	338	6	290	9	0	0	3	3191
Natural	0	21	72	335	425	142	290	64	2	194	585	1175	72	1425	8	145	26	2	2	2	4997

Table 3. Frequency of larval species of Culicinae subfamily in natural habitats in the southern part of Iran, 2009-2013

Species	Natural habitats type							Total
	River edge	Marsh	Water leakage	Pit	Creek	River bed	Fountain	
<i>Cx. hortensis</i>	0	0	0	1	0	20	0	21
<i>Cx. pusillus</i>	0	3	66	0	2	0	1	72
<i>Cx. perexiguus</i>	68	73	86	6	17	85	0	335
<i>Cx. pipiens</i>	55	97	7	185	77	14	0	435
<i>Cx. theileri</i>	3	12	33	8	28	58	0	142
<i>Cx. miniticus</i>	139	17	23	31	27	53	0	290
<i>Cx. sinaiticus</i>	41	14	0	9	0	0	0	64
<i>Cx. laticinctus</i>	0	1	0	0	0	1	0	2
<i>Cx. sitiens</i>	85	0	108	1	0	0	0	194
<i>Cx. tritaeniorhynchus</i>	266	38	160	75	42	4	0	585
<i>Cx. quinquefasciatus</i>	412	325	249	61	93	35	0	1175
<i>Cx. bitaeniorhynchus</i>	35	0	18	0	19	0	0	72
<i>Cs. longiareolata</i>	537	195	83	143	228	202	37	1425
<i>Ur. unguiculata</i>	0	0	0	0	8	0	0	8
<i>Ae. caspius</i>	35	0	0	110	0	0	0	145
<i>Ae. vexans</i>	0	0	0	26	0	0	0	26
<i>Ae. flavescens</i>	2	0	0	0	0	0	0	2
<i>Ae. caballus</i>	0	0	0	2	0	0	0	2
<i>Ae. albopictus</i>	0	0	0	2	0	0	0	2
Total	1678	775	833	660	541	472	38	4997

Discussion

In this study, a total of 8,188 larvae belonging to 4 genera were identified from eight provinces (23 cities) in the southern part of Iran. Our investigations have revealed that of all species collected, larvae from the *Culex* genus, especially *Cx. quinquefasciatus* (24.55%) were the most frequent (Table 1). The *Culex* genus had 768 species, many of which are of biomedical importance (<https://www.wrbu.si.edu/vectorspecies/genera/culex>). *Cx. quinquefasciatus* is an established species worldwide and is usually found in areas with high human populations. Adults easily feed on blood from various vertebrates, including wild and domestic birds, mammals, and humans (Farajollahi et al. 2011). Females are generally active at night and feed both outdoors and indoors. This species is a known agent of diseases such as West Nile virus, Chikungunya virus, Rift Valley Fever, *Wuchereria bancrofti*, and Avian malaria. Also, *Cx. quinquefasciatus* was introduced as the main vector of West Nile Virus in Asia (Hubálek and Halouzka 1999). West Nile virus is probably one of the most important mosquito-borne arboviruses in Iran; the virus was identified in 26 out of the 31 provinces of Iran (Ahmadnejad et al. 2011; Chinikar et al. 2013a; Pourmahdi et al. 2013), using a serological technique (ELISA). In line with our study, Azari-Hamidian et al. (2010) also indicated that *Cx. quinquefasciatus* was the main species in the Persian Gulf islands (Qeshm, Lark, and Abu Musa). The breeding places of adults varied but larvae developed in typical habitats such as sewers, ground pools, ditches, agricultural seepage pits, and sewage treatment plant holding tanks. Larvae usually prefer water bodies with high organic content (<https://www.wrbu.si.edu/vectorspecies/genera/culex>).

Our results show *Culex* larvae mostly oviposited in natural and artificial habitats. Larvae of *Cx.*

quinquefasciatus were found in the pools (artificial habitats) and river edges (natural habitats). Some other breeding places of this species are dams, streams, and water leakages. Our studies collected this species from temporal water bodies with full sunlight without vegetation, muddy substrate, and clear and fresh water. This species was mostly reported in the Golestan province from temporal and stagnant water bodies with full sunlight and mud substrate (Sofizadeh et al. 2018). Still, Hanafi-Bojd et al. (2017) reported the presence of this species in permanent water bodies with full sunlight and sandy substrate habitats with clear and fresh water from the Hormozgan province.

In a previous study, *Cx. pipiens* were reported as the dominant species, but our study from North Khorasan has shown that the dominant species were *Cx. quinquefasciatus* (Azari-Hamidian et al. 2011). This species is widely distributed, and recent studies have demonstrated that *Cx. pipiens* is the most prevalent species in Iran (Khoshdel-Nezamiha et al. 2014; Nikookar et al. 2015a, 2016; Sofizadeh et al. 2017). *Cx. pipiens* is primarily an ornithophilic species but could feed on humans and other animals that act as vectors of WNV (Fonseca et al. 2004; Weitzel et al. 2015). This species was studied by Mousa-Kazemi et al. (2015) (16.5%), Nikookar et al. (2018) (69.7%), Moradi et al. (2018) (58.7%) and Azari-Hamidian (2011) (10.1%), and was reported to be the dominant species with a wide distribution in the study area.

Also, studies conducted by Navidpour et al. (2012) have shown that the most frequent mosquito species in the Shadegan (Khuzestan) province were *Cx. pipiens*. In Yaghoobi et al. (1986)'s investigations on the biology of mosquitoes in the Minab (Hormozgan) province, some species, such as *Cx. bitaeniorhynchus*, *Cx. pipiens*, *Cx. theileri*, *Cx. tritaeniorhynchus*, *Cx. perexiguus*, *Cx.*

quinquefasciatus, *Cx. sinaiticus*, *Cx. deserticola* and *Cs. longiareolata* were reported, but our studies in some districts of the Hormozgan and Minab provinces have revealed the presence of *Cx. tritaeniorhynchus*, *Cx. perexiguus*, *Cx. quinquefasciatus*, *Cx. pipiens* and *Cs. longiareolata*.

During our study, larvae of the *Cx. pipiens* were collected from natural and artificial breeding places such as pits, marshes, pools, and ovitrap. Previous studies have revealed the existence of *Cx. pipiens* in flooded cellars drains, wells, septic tanks, underground drainage systems, abandoned mine tunnels, and coal mines (Horsfall 1955; Cranston et al. 1987; Zaim 1987b; Harbach 1988; Service 1993), whereas in our study, larval habitats of *Cx. pipiens* are mostly on a stagnant and artificial freshwater body. The characteristics of larval habitats have shown that *Cx. pipiens* usually inhabit different breeding places with various ecological conditions. The situation led to high larval dispersal and distribution from Iran (Azari-Hamidian 2005). In Isfahan, *Cx. pipiens* were mostly found in rice fields and natural breeding places (Mousakazemi 2000). This species has been reported in almost all provinces in Iran (Azari-Hamidian et al. 2005).

In the present study, *Culiseta longiareolata* had the greatest dispersal among the Culicinae subfamily. There is little information on the ecology of the *Culiseta* species in Iran. The large geographical distribution of *Cu. longiareolata* has been reported from the Palearctic region (Asia and Africa). This species was reported for the first time by Gutsevich in 1943 from the north of Iran. In Iran, five species, including *Cs. (Allotheobaldia) longiareolata*, *Cs. (Culicella) morsitans*, *Cs. (Culiseta) alaskensis*, *Cs. (Cul.) annulata* and *Cs. (Cul.) sabochrea* have been reported. This species was reported with a higher frequency of collection from different parts of Iran and various larval habitats (Mousakazem 2000; Azari-Hamidian 2005; Azari-Hamidian et al. 2011; Dehghan et al. 2011). In our study, this species was reported from Hormozgan and South Khorasan provinces but in Golestan provinces, *Cs. longiareolata* were collected from only larval habitats with cement substrate and without vegetation (Sofizadeh et al. 2018). Our studies have shown that *Cs. longiareolata* were collected more frequently from temporal and natural habitats (28.5%) (River edge, marsh, and water leakage) than from artificial habitats. The larval habitats are mostly in full sunlight, without vegetation, muddy substrate, and clear water.

Though the *Aedes* group is a large tribe and comprises 1,255 species belonging to 10 genera, there is little information about the *Aedes* in Iran, and till now, only 12 species have been reported. *Ae. Albopictus* and *Ae. unilineatus* were reported for the first time from southern Iran (Doosti et al. 2016). This species is a competent vector of 26 arboviruses, including Dengue fever, WNV, and Chikungunya (Paupy et al. 2009; Bonizzoni et al. 2013). In the present survey, *Ae. caspius*, *Ae. vexans*, *Ae. flavescens*, *Ae. caballus*, *Ae. unilineatus* and *Ae. albopictus* were collected from natural (177, 2.16%) and artificial (302, 3.68%) larval habitats. *Ae. caspius* is one of the most common mosquitoes distributed throughout the world. As exophagic species often bite indoors and outdoors during the

day (especially at dusk). It is a floodwater mosquito and has multiple generations per year. The mosquitoes act as vectors of West Nile, Tahyna, and Isfahan viruses and *Francisella tularensis*

(<https://wrbu.si.edu/vectorspecies/mosquitoes/caspius>).

Ae. caspius was mainly reported from most regions of Iran (Azari-Hamidian et al. 2010; Moosa Kazemi et al. 2010, 2015; Abai et al. 2016; Sofizadeh et al. 2016). Sofizadeh et al. (2018) reported only *Ae. caspius* from the Golestan province, but in another study in the Kalaleh county, 3 species (*Oc. caspius*, *Oc. echinus*, and *Oc. geniculatus*) were reported by Sofizadeh et al. (2016). In our study *Ae. caspius* had the highest frequency of distribution (435, 5.31%) and was found mostly in artificial habitats (290, 3.54%) than natural habitats (145, 1.77%). This species was typically collected from temporal, full sunlight and vegetation within water level, with muddy substrate and clear and fresh water breeding places.

In the Kurdistan province, *Ae. caspius* was mainly found in river edges with stagnant, clear water and partial sunlight habitats (Banafshi et al. 2013). In the Golestan province, the species preferred temporal, running water, muddy substrate, and full sunlight habitats, similar to our results. Although this species prefers larval habitats with clear and fresh water, it is sometimes collected from larval habitats with turbid and brackish water. Moradi et al. (2018) collected this species from the Ardebil province's natural, temporal and clayey larval habitats.

Many mosquito-borne viruses are endemic in some countries (Parhizgari et al. 2017). Many barriers, such as a change in the behavior of vectors, the landscape of disease epidemiology, climate change, the lifestyle of community members, illegal emigration, and war, have made preventing and controlling the diseases they transmit difficultly. Although there is information on malaria vectors in Iran, little is known about publications on the Culicinae family until now. Because of the emerging and remerging diseases caused by vectors, especially mosquito-borne pathogens, it is necessary to monitor mosquitoes with improved systems. Therefore, it is essential to study many different aspects of the ecology and behavior of vectors to plan and execute effective mosquito surveillance.

ACKNOWLEDGEMENTS

We are grateful to the Center of Health Services staff for their assistance in the fieldwork and to some community members for their cooperation during the survey. This research was financially supported by the Iran National Science Foundation (INSF) (project no 86052/18) and partly by the Tehran University of Medical Sciences (grant no 88-03-27-9200). We also appreciate the Tehran University of Medical Sciences for its unflinching support. The authors would like to thank Dr. S. Azari Hamidian for providing useful comments and reviewing the papers. The authors declare that there is no conflict of interest.

REFERENCES

- Abai MR, Saghaipour A, Ladonni H, Jesri N, Omid S, Azari-Hamidian S. 2016. Physicochemical characteristics of larval habitat waters of mosquitoes (Diptera: Culicidae) in Qom Province, Central Iran. *J Arthropod-Borne Dis* 10 (1): 65-77.
- Ahmadnejad F, Otarod V, Fallah MH, Lowenski S, Sedighi-Moghaddam R, Zavareh A, Durand B, Lecollinet S, Sabatier P. 2011. Spread of West Nile virus in Iran: A cross-sectional serosurvey in equines, 2008-2009. *Epidemiol Infect* 139: 1587-1593. DOI: 10.1017/S0950268811000173.
- Azari-Hamidian S, Abai MR, Arzamani K, Bakhshi H, Karami H, Ladonni H, Harbach RE. 2011. Mosquitoes (Diptera: Culicidae) of North Khorasan Province, Northeastern Iran and the zoogeographic affinities of the Iranian and Middle Asian Mosquito Fauna. *J Entomol* 8 (3): 204-217. DOI: 10.3923/j.e.2011.204.217.
- Azari-Hamidian S, Behzad N, Ralph EH. 2019. A detailed review of the mosquitoes (Diptera: Culicidae) of Iran and their medical and veterinary importance. *Acta Tropica* 194: 106-122. DOI: 10.1016/j.actatropica.2019.03.019.
- Azari-Hamidian S, Harbach RE. 2009. Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa* 2078: 1-33. DOI: 10.11646/zootaxa.2078.1.1.
- Azari-Hamidian S, Joeifshani MA, Moss-lem M. 2005. Mosquitoes fauna and distribution of the genus *Culex* (Diptera: Culicidae) in Guilan Province. *Pajouhesh Sazandegh* 68: 39-45. [Persian]
- Azari-Hamidian S, Linton YM, Abai MR, Ladonni H, Oshaghi MA, Hanafi-Bojd AA, Moosa-Kazemi SH, Shabkhiz H, Pakari A, Harbach RE. 2010. Mosquito (Diptera: Culicidae) fauna of the Iranian islands in the Persian Gulf. *J Nat His* 44: 913-925. DOI: 10.1080/00222930903437358.
- Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E, Mobedi I, Abai MR, Linton YM, Harbach RE. 2009. Distribution and ecology of mosquitoes in a focus of dirofilariasis in northwestern Iran, with the first finding of filarial larvae in naturally infected local mosquitoes. *Med Vet Entomol* 23: 111-121. DOI: 0.1111/j.1365-2915.2009.00802.x.
- Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E. 2002. The distribution and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Rasht County (Guilan Province, Iran). *Modarres J Med Sci* 4: 87-96.
- Azari-Hamidian S. 2005. Larval habitat characteristics of mosquitoes of the ge-nus *Culiseta* Felt, 1904 (Diptera: Cu-licidae) in the Caspian Sea littoral, Iran. *Zool Middle East* 36 (1): 59-66. DOI: 10.1080/09397140.2005.10638128.
- Azari-Hamidian S. 2007. Checklist of Iranian mosquitoes (Diptera: Culicidae). *J Vect Ecol* 32 (2): 235-242. DOI: 10.3376/1081-1710 (2007) 32 [235: coimdc] 2.0.co; 2.
- Azari-Hamidian S. 2011. Larval habitat characteristics of the genus *Anopheles* (Diptera: culicidae) and a checklist of mosquitoes in Guilan Province, northern Iran. *Iran J Arthropod Borne Dis* 5 (1): 37-53.
- Bagheri M, Terenius O, Oshaghi MA, Motazakker M, Asgari S, Dabiri F, Vatandoost H, Mohammadi BM, Chavshin AR. 2015. West Nile virus in mosquitoes of Iranian wetlands. *Vector-Borne Zoonotic Dis* 15: 750-754. DOI: 10.1089/vbz.2015.1778.
- Banafshi O, Abai MR, Ladonni H, Bakh-shi H, Karami H, Azari-Hamidian S. 2013. The fauna and ecology of mosquito larvae (Diptera: Culicidae) in western Iran. *Turk J Zool* 37: 298-307.
- Benelli G, Duggan MF. 2018. Management of arthropod vector data - social and ecological dynamics facing the One Health perspective. *Acta Trop* 182: 80-91. DOI: 10.1016/j.actatropica.2018.02.015.
- Bonizzoni M, Gasperi G, Chen X, James AA. 2013. The invasive mosquito species *Aedes albopictus*: Current knowledge and future pers-pectives. *Trends Parasitol* 29 (9): 460-468. DOI: 10.1016/j.pt.2013.07.003.
- Chinikar S, Javadi A, Ataei B, Shakeri H, Moradi M, Mostafavi E, Ghiasi SM. 2012. Detection of West Nile virus genome and specific antibodies in Iranian encephalitis patients. *Epidemiol Infect* 140: 1525-1529. DOI: 10.1017/S0950268811002056.
- Chinikar S, Shah-Hosseini N, Mostafavi E, Moradi M, Khakifirouz S, Jalali T, Goya MM, Shirzadi MR, Zainali M, Fooks AR. 2013a. Seroprevalence of West Nile virus in Iran. *Vector-Borne Zoonotic Dis* 13: 586-589. DOI: 10.1089/vbz.2012.1207.
- Clements AN. 1992. *The Biology of Mosquitoes Volume 1. Development, Nutrition and Reproduction*. Chapman and Hall, London.
- Cranston PS, Ramsdale CD, Snow KR, White GB. 1987. Keys to the Adults, Male Hypopygia, Fourth-Instar Larvae and Pupae of the British Mosquitoes (Culicidae) with Notes on their Ecology and Medical Importance. Fresh Water Biological Association Scientific Publication, Cumbria.
- Dehghan H, Sadraei J, Moosa-Kazemi SH, Davari B. 2011. Ecological study of *Culex pipiens*, the dominant species of Culicidae mosquitoes and its control solutions in Yazd Province. *Sci J Kurdistan Uni Med Sci* 15 (4): 81-88. [Persian]
- Djadjid ND, Jazayeri H, Gholizadeh S, Rad ShP, Zakeri S. 2009. First record of a new member of *Anopheles Hyrcanus* Group from Iran: Molecular identification, diagnosis, phylogeny, status of kdr resistance and *Plasmodium* infection. *J Med Entomol* 46: 1084-1093. DOI: 10.1603/033.046.0515.
- Doosti S, Yaghoobi-Ershadi MR, Schaffner F, Moosa-Kazemi SH, Akbarzadeh K, Gooya MM, Vatandoost H, Shirzadi MR, Mostafavi E. 2016. Mosquito surveillance and the first record of the invasive mosquito species *Aedes (Stegomyia) albopictus* (Skuse) (Diptera: Culicidae) in southern Iran. *Iran J Publ Health* 45 (6): 1064-1073.
- Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM. 2011. "Bird biting" mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect Genet Evol* 11 (7): 1577-1585. DOI: 10.1016/j.meegid.2011.08.013.
- Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, Fleischer RC, Wilkerson RC. 2004. Emerging vectors in the *Culex pipiens* complex. *Sci* 303: 1535-1538. DOI: 10.1126/science.1094247.
- Gutsevich AV. 1943. On the mosquitoes of North Iran. *Comtes Rendus Academic Science URSS*. XL (3): 123-125.
- Hanafi-Bojd AA, Soleimani-Ahmadi M, Doosti S, Azari-Hamidian S. 2017. Larval habitats, affinity and diversity indi-ces of Culicinae (Diptera: Culicidae) in southern Iran. *Intl J Mosq Res* 4 (2): 27-38.
- Harbach RE. 1985. Pictorial keys to the genera of mosquitoes, subgenera of *Culex* and the species of *Culex* (Culex) occurring in south-western Asia and Egypt, with a note on the subgeneric placement of *Culex deserticola* (Diptera: Culicidae). *Mosq Sys* 17 (2): 83-107.
- Harbach RE. 1988. The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: Culicidae). *Contrib Am Entomol Inst* 24: 1-240.
- Harbach RE. 2018. Mosquito Taxonomic Inventory. <http://mosquito-taxonomic-inventory.info/>. Accessed 29 July, 2018.
- Horsfall WR. 1955. *Mosquitoes. Their Bionomics and Relation to Disease*. Hafner Publishing, New York.
- Hubálek Z, Halouzka J. 1999. West Nile fever - a reemerging mosquito-borne viral disease in Europe. *Emerg Infect Dis* 5: 643-650. DOI: 10.3201/eid0505.990505.
- Khoshdel-Nezamih F, Vatandoost H, Azari-Hamidian S, Mohammadi Bavani M, Dabiri F, Entezar-Mahdi R, Chavshin AR. 2014. Fauna and larval habitats of mosquitoes (Diptera: Culicidae) of West Azerbaijan. *J Arthropod Borne Dis* 8 (2): 163-173.
- Lotfi MD. 1970. Iranian species of genus *Culex* ([Culinae]: Diptera). *Bull de la Soci de Patho Exoti* 63: 399-403.
- Lotfi MD. 1973. Iranian species of genus *Culex* (Culicinae: Diptera) II. Report of four species of larvae (including three new records) and 14 adult species. *Bull de la Soci de Patho Exoti* 66: 204-207.
- Lotfi MD. 1976. Key to Culicinae of Iran, genus *Culex* and their biology (Diptera: Culicidae). *Iranian J Publ Health* 5: 71-84.
- Meshkat Z, Chinikar S, Shakeri MT, Manavifar L, Moradi M, Mirshahabi H, Jalili T, Khakifirouz S, Shahhosseini N. 2015. Prevalence of West Nile virus in Mashhad, Iran: A population-based study. *Asian Pac J Trop Med* 8: 203-205. DOI: 10.1016/S1995-7645(14)60315-1.
- Moosa-Kazemi S, Vatandoost H, Nikookar H, Fathian M. 2009. Culicinae (Diptera: Culicidae) mosquitoes in chabahar County, Sistan and Baluchistan Province, Southeastern Iran. *Iran J Arthropod Borne Dis* 3 (1): 29-35.
- Moosa-Kazemi SH, Karimian F, Davari B. 2010. Culicinae mosquitoes in Sanan-daj County, Kurdistan Province, Eastern Iran. *J Vector Borne Dis* 47 (2): 103-107.
- Moosa-Kazemi SH, Zahirmia AH, Sharifi F, Davari B. 2015. The fauna and ecology of mosquitoes (Diptera: Culicidae) in Western Iran. *Arthropod Borne Dis* 9 (1): 49-59.
- Moradi-Asl E, Hazrati S, Vatandoost H, Emdadi D, Ghorbani E, Ghasemian A, Rafiee M, Panahi A, Shokri A. 2018. Fauna and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Ardabil Province, Northwestern Iran. *J Health* 9: 259-266.
- Mousakazemi S, Zaim M, Zahraei A. 2000. Fauna and ecology of Culicidae of the Zarrin- Shahr and Mobarakeh area in Isfahan Province. *Armaghan Danesh J Yasuj Univ Med Sci* 5: 46-54. [Persian]

- Muirhead-Thomson RC. 1951. Mosquito Behaviour in Relation to Malaria Transmission and Control in the Tropics. Edward Arnold and Co, London.
- Naficy K, Saidi S. 1970. Serological survey on viral antibodies in Iran. *Trop Geogr Med* 2: 183-188.
- Navidpour S, Vazirianzadeh B, Harbach R, Jahanifard E, Moravvej SA. 2012. The identification of culicine mosquitoes in the Shadegan wetland in southwestern Iran. *J Insect Sci* 12: 105. DOI: 10.1673/031.012.10501.
- Nikookar S, Fazeli-Dinan M, Azari-Hamidian S, Mousavinasab S, Arabi N, Ziapour S, Shojaei J, Enayati AA. 2017. Species composition and abundance of mosquito larvae in relation with their habitat characteristics in Mazandaran Province, northern Iran. *Bull Entomol Res*: 1-13. DOI: 10.1017/S0007485317000074.
- Nikookar SH, Azari-Hamidian S, Fazeli-Dinan M, Nasab SNM, Aarabi M, Ziapour SP, Enayati A. 2016. Species composition, co-occurrence, association and affinity indices of mosquito larvae (Diptera: Culicidae) in Mazandaran Province, northern Iran. *Acta trop* 157: 20-29. DOI: 10.1016/j.actatropica.2016.01.014.
- Nikookar SH, Fazeli-Dinan M, Azari-Hamidian S, Mousavi NSN, Aarabi M, Ziapour SP, Enayati AA, Hemingway J. 2018. Fauna, ecological characteristics, and checklist of the mosquitoes in Mazandaran Province, Northern Iran. *Med Entomol* 55: 634-645. DOI: 10.1093/jme/tjx228.
- Nikookar SH, Moosa-Kazemi SH, Oshaghi MA, Vatandoost H, Yaghoobi-Ershadi MR, Enayati AA, Motevali-Haghi M, Ziapour SP and Fazeli-Dinan M. 2015b. Biodiversity of culicid mosquitoes in rural Neka township of Mazandaran province, Northern Iran. *J Vector Borne Dis* 52 (1): 63-72.
- Nikookar SH, Moosa-Kazemi SH, Yaghoobi-Ershadi MR, Vatandoost H, Oshaghi MA, Ataei A, Anjamrooz M. 2015a. Fauna and larval habitat characteristics of mosquitoes in Neka county, Northern Iran. *J Vector Borne Dis* 9 (2): 253-266.
- Oshaghi MA, Vatandoost H, Gorouhi A, Abai MR, Madjidpour A, Arshi S, Sadeghi H, Nazari M, Mehravaran A. 2011. Anopheline species composition in borderline of Iran-Azerbaijan. *Acta Trop* 119 (1): 44-49. DOI: 10.1016/j.actatropica.2011.04.005.
- Oshaghi MA, Yaghoobi-Ershadi MR, Shemshad Kh, Pedram M, Amani H. 2008. The *Anopheles superpictus* complex: Introduction of a new malaria vector complex in Iran. *Bull Soc Pathol Exot* 101: 429-434.
- Parhizgari N, Gouya MM, Mostafavi E. 2017. Emerging and re-emerging infectious diseases in Iran. *Iran J Microbiol* 9: 122-142.
- Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D. 2009. *Aedes albopictus*, an arbovirus vector: from darkness to the light. *Microbes Infect* 11 (14-15): 1177-1185. DOI: 10.1016/j.micinf.2009.05.005.
- Piyaratne MK, Amerasinghe FP, Amerasinghe PH, Konradsen F. 2005. Physico-chemical characteristics of *Anopheles culicifacies* and *Anopheles varuna* breeding water in a dry zone stream in Sri Lanka. *J Vect Borne Dis* 42 (2): 61-67.
- Pourmahdi BM, Ghadrani MAR, Seif ASMR, Zeinvand M. 2013. A serological survey on antibodies against West Nile virus in horses of Khuzestan Province. *Iran J Vet Med* 7: 185-191.
- Province, Northwestern Iran. *J Arthropod Borne Dis* 8: 163-173. Res 114: 289-295.
- Saidi S, Tesh R, Javadian E, Nadim A. 1976. The prevalence of human infection with West Nile virus in Iran. *Iran J Publ Health* 5 (1): 8-13.
- Sasikumar PS, Suryanarayanan P, Thomas C, Kalyanaraman K, Prasad RS. 1986. Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum City, India. *Proc Indian Acad Sci* 95: 549-555.
- Service MW. 1993. Mosquitoes (Culicidae). In: Lane RP, Crosskey RW (eds). *Medical Insects and Arachnids*. Chapman and Hall, London.
- Shah-Hosseini N, Chinikar S, Ataei B, Fooks AR, Groschup MH. 2014. Phylogenetic analysis of West Nile virus genome, Iran. *Emerg Infect Dis* 20: 1419-1421. DOI: 10.3201/eid2008.131321.
- Shah-hosseini N, Chinikar S, Mossa-Kazemi SH, Sedaghat MM, Kayedi MH, Luh-ken R, Schmidt-Chanasit J. 2017. West Nile Virus lineage-2 in *Culex* specimens from Iran. *Trop Med Intl Health* 22 (10): 1343-1349. DOI: 10.1111/tmi.12935.
- Sofizadeh A, Edalat H, Abai MR, Hanafi-Bojd AA. 2016. Fauna and some biological characteristics of *Anopheles* mosquitoes (Diptera: Culicidae) in Kalaleh County, Golestan Province, northeast of Iran. *Asian Pac J Trop Biomed* 6 (9): 730-734. DOI: 10.1016/j.apjtb.2016.07.005.
- Sofizadeh A, Moosa-Kazemi SH, Dehghan H. 2017. Larval habitats characteristics of mosquitoes (Diptera: Culicidae) in north-east of Iran. *J Arthropod Borne Dis* 27 (2): 211-225.
- Sofizadeh A, Shoraka HR, Mesgarian F, Ozbaki GM, Gharaninia AS, Sahneh E, Dankoob R, Malaka A, Fallah S, Nemani SH. 2018. Fauna and larval habitats characteristics of mosquitoes (Diptera: Culicidae) in Golestan Province, Northeast of Iran, 2014-2015. *J Arthropod-Borne Dis* 12 (3): 240-251. DOI: 10.18502/jad.v12i3.76.
- Suesdek L. 2019. Microevolution of medically important mosquitoes - a review. *Acta Trop* 191: 162-171. DOI: 10.1016/j.actatropica.2018.12.013.
- Surendran SN, Ramasamy R. 2005. Some characteristics of the larval breeding sites of *Anopheles culicifacies* species B and E in Sri Lanka. *J Vect Borne Dis* 42: 39-44.
- Vatandoost H, Oshaghi MA, Abaie MR, Shahi M, Yaaghoobi F, Baghaei M, Hanafi-Bojd AA, Zamani G, Townson H. 2006. Bionomics of *Anopheles stephensi* Liston in the malarious area of Hormozgan Province, southern Iran, 2002. *Acta Trop* 97 (2): 196-203. DOI: 10.1016/j.actatropica.2005.11.002.
- Vatandoost H, Shahi H, Abai MR, Hanafi-Bojd AA, Oshaghi MA, Zamani G. 2004. Larval habitats of main malaria vectors in Hormozgan Province and their susceptibility to different larvicides. *Southeast Asian J Trop Med Public Health* 35 (2): 22-25.
- Weitzel T, Jawien P, Rydzanicz K, Lonc E, Becker N. 2015. *Culex pipiens* s.l. and *Culex torrentium* (Culicidae) in Wrocław area (Poland): Occurrence and breeding site preferences of mosquito vectors. *Parasitol Res* 114 (1): 289-295. DOI: 10.1007/s00436-014-4193-1.
- Yaghoobi-Ershadi MR, Zaim M, Manouchehri AV. 1986. Studies on the biology of the mosquitoes in the districts of Minab, Hormozgan province, Iran (1983-84). 1- Characteristics of the larval breeding sites. *J Environ* 13: 17-39. [Persian].
- Zaim M, Cranston PS. 1986. Checklist and keys to the Culicinae of Iran (Diptera: Culicidae). *Mosq Syst* 18: 233-245.
- Zaim M. 1987a. The mosquito fauna of Kashan, public health importance and control. *Desert Sci Res* 18: 1-41. [Persian].
- Zaim M. 1987b. The distribution and larval Habitat characteristics of Iranian Culicinae. *J Am Mosq Control Assoc* 3 (4): 573-588.