International Journal of Epidemiologic Research
doi:10.34172/ijer.2020.15 2020 Spring;7(2):74-91 http://ijer.skums.ac.ir

Original Article

Vertical Distribution, Biodiversity, and Some Selective Aspects of the Physicochemical Characteristics of the Larval Habitats of Mosquitoes (Diptera: Culicidae) in Chaharmahal and Bakhtiari Province, Iran

Seyed-Mohammad Omrani[1*], **Shahyad Azari-Hamidian**[2]

[1]Medical Parasitology Department, Shahrekord University of Medical Sciences, Shahrekord, Iran.
[2]Department of Health Education, Research Center of Health and Environment, School of Health, Guilan University of Medical Sciences, Rasht, Iran.

Abstract

Background and aims: Mosquitoes (Diptera: Culicidae) are still a focus of research because of their role in the transmission of diseases and annoying biting behavior. Source reduction is an effective measure to control mosquito populations, which is based on good knowledge of larval habitats. This study was conducted to obtain that basic knowledge in Chaharmahal and Bakhtiari province.

Methods: This study was carried out in 2011 and 2012. Geographical coordinates, altitude, pH, temperature, and the dissolved oxygen level of larval habitats were recorded by relevant devices, followed by documenting physical attributes by direct observation. In addition, the indices of biodiversity were calculated to analyze the vertical biodiversity of species. Finally, the affinity index was calculated to elucidate species co-occurrence.

Results: Eighteen species were recovered from 92 larval habitats. Low- (≤ 1400 m), mid- (1401–2000 m), and high- (≥ 2001 m) altitudes lodged 7, 17, and 14 species, respectively. Further, the indices of the species richness and biodiversity for these altitudinal categories were 0.93, 1.94, and 1.58, as well as 1.54, 2.13, and 1.96, respectively. Larval habitats were mostly natural, temporary, with standing but clear water, muddy substrate, sunlit, and with vegetation. Other physicochemical characteristics and affinity of species were described and discussed as well.

Conclusion: To the best of our knowledge, this is the first report of vertical distribution and biodiversity of mosquito larvae in Iran. The relative uniformity of physicochemical characteristics of larval habitats was attributed to prevailing water resources in the studied area and sampling design. The oviposition site selection of gravid mosquitoes is still an unresolved problem which needs further investigations.

Keywords: Elevation diversity gradient, Breeding place, Oviposition site

*Corresponding Author: Dr. Seyed-Mohammad Omrani, Assistant Professor, Medical Parasitology Department, Shahrekord University of Medical Sciences, Shahrekord, Iran, P.O. Box: 3391, Shahrekord, Iran, Tel: 0098 383 33335635, E-mail: omranism@skums.ac.ir

Received: 17 Mar. 2020
Accepted: 18 May 2020
ePublished: 28 June 2020

Introduction

Mosquitoes (Diptera: Culicidae) are of major importance not only because of their role in the transmission of the causal agents of many diseases but also because of their blood-sucking behavior which imposes considerable amounts of stress and discomfort to human beings and domesticated livestock.[1,2] Therefore, lots of resources are spent yearly to combat these annoying insects, even in the absence of any diseases they transmit.

Source reduction through environmental management is an effective supplementary tool in controlling mosquitoes operating at their potential larval habitats. The advantage of this method is that it not only affects endophagic and endophilic mosquitoes but also exophagic and exophilic populations that are harder to be eliminated by other control measures. However, the success of this intervention depends on the type, number, and accessibility of available larval habitats in the targeted area.[3]

Mosquitoes do not lay their eggs in a random manner; rather they discriminatively select where to oviposit.[4,5] While environmental factors such as relative humidity, ambient temperature and wind speed are involved in the flight orientation of mosquitoes to locate a potential oviposition site; long- to short-range visual, olfactory and tactile cues are consecutively engaged to choose a suitable place for laying eggs. Once contact with the substrate is made, the right chemical profile originating from previously laid eggs, growing larvae, pupae, and even the substrate itself stimulate the female gravid mosquito to oviposit. Otherwise, the female mosquito flies away to find another place to lay her eggs.[6,7] Interestingly, it is showed that arboviral infection could affect the mosquito associate olfactory learning and results in the loss of oviposition site preference.[8]

On the other hand, the growth and development of mosquito larvae in a given aquatic habitat is under the influence of biotic and abiotic surrounding environment.[9] Water movement, suspended or dissolved organic and inorganic materials, temperature, hydrogen ion concentration, dissolved oxygen, and the presence of food particles could be listed as abiotic factors. Vegetation type and the presence of predators or competitors are also considered as biotic parameters.[4-7] Although some studies report that there is a correlation between larval abundance and some sets of these variables,[10-13] others do not prove it.[14,15]

In spite of considerable advances in the behavioral and chemical ecology of oviposition in mosquitoes, contradictory evidence on the role of environmental variables in the life history of immature stages demands further research both under controlled and field conditions.

Regardless of numerous reports on the larval habitat characteristics of mosquitoes in other countries, there are few studies characterizing the larval breeding places of mosquitoes in Iran. While early researchers have mostly concentrated on the general description of larval habitats,[16,17] late researchers have rather paid attention to the physicochemical characteristics of these places in detail. Several examples of the latter type of studies are those conducted by Yaghoobi-Ershadi et al[18] in Ardebil, Azari-Hamidian[19-21] in Guilan, Dehghan et al[22] in Hamedan, Hanafi-Bojd et al[23,24] and Soleimani-Ahmadi et al[13,25] in Hormozgan, Banafshi et al[26] in Kurdistan, Amani et al[27] in Lorestan, Khoshdel-Nezamiha et al[28] in West Azerbaijan, Ghanbari et al[29] in Sistan and Baluchistan, Ladonni et al[30] in Isfahan, Nikookar et al[12,31,32] in Mazandaran, Abai et al[14] in Qom, Sofizadeh et al[33-35] in Golestan, and Paksa et al[36] in East Azerbaijan. In a number of these studies, the biodiversity of mosquitoes[23,24,36,37] and associate species[12,19-24,26,30,31,38,39] are considered as well. The biodiversity of the mosquitoes of Iran is relatively a young and an intriguing field for mosquito ecologists, which has demonstrated a growing trend in recent years.[23,24,37] Although evidence on the horizontal biodiversity of mosquitoes is increasingly accumulated, the vertical biodiversity of mosquitoes as a closely related subject has not received enough attention of entomologists. This need may be reinforced by the fact that it is newly showed that the prevalence of some mosquito-borne diseases is dramatically decresed at a certain level of elevation.[40]

In a recent faunistic larval survey, all previously reported culicine mosquitoes (11 species) and six anopheline species were collected from Chaharmahal and Bakhtiari province.[41] The distribution maps of the recovered species were reported, and *Culex territans* was introduced as a new species to the fauna of this province. Briefly, while *Cx. theileri* (25.1%) and *Anopheles superpictus* (15%) were the most abundant and widespread species, *Cx. territans*, *Cx. tritaeniorhynchus*, and *Ochlerotatus caspius* s.l. (<0.01% each) were the least frequent and the most limited species in the province. There are some controversial debates on the classification of the tribe Aedini. Based on Reinert et al. [42-45] there are 82 genera in the tribe and based on Wilkerson et al. [46] just ten genera with numerous subgenera. Debates continue in this regard.[1,47] This study listed the species of the tribe based on the first classification. As far as we know, there is little or no information on the physicochemical characteristics of the larval habitats of mosquitoes in Chaharmahal and Bakhtiari province. There is also no information on the biodiversity and inter-specific association of this taxon in this region. Considering the above-mentioned explanations, the present study addressed some selective aspects of the larval habitat characteristics of mosquitoes in this province, followed by studying the biodiversity and inter-specific association of the identified species.

Materials and Methods

Study Area

This descriptive-analytical cross-sectional study was carried out in Chaharmahal and Bakhtiari province. The province with an area of 16 532 km^2 is located between 31º 9′ to 32º 48′ N and 49º 28′ to 51º 25′ E at the western part of Iran (Figure 1). It borders with Isfahan province in the north and east, Kohgylooyeh and Boyerahmad province in the south and southeast in addition to Khuzistan province in the west and southwest and Lorestan in the northwest. Moreover, its population is 947 763 and the province officially includes 10 counties. The province with 2153 meters altitude above the sea level on average is mostly a highland area in which few plains are stretched between hilly and mountainous parts.[48]

Additionally, the climate is the Mediterranean based on Köppen's classification so that winters are cold and humid and summers are relatively temperate.[48,49] The average annual rainfall is 1152 mm, and the minimum and maximum temperatures are 17.98ºC and 33.68ºC, respectively. Rainfall generally starts from October each year and peaks around December. Then, it gradually decreases until the next April. Water bodies are so rich that about 10% of the total resources of the country are ensured by Chaharmahal and Bakhtiari province. Regardless of special climate, landfilling and agricultural activities are the major determinants of the distribution of temporary

Figure 1. Map of the Governmental Provinces of Iran.
Note. Chaharmahal and Bakhtiari province is highlighted with red color

surface waters in the province. It should be mentioned that July and December are the warmest and coldest months of the year, respectively. The plant coverage is relatively rich, and human settlements more or less follow the water course.[48] There are many livestock in the province and animal husbandry and agriculture are two common occupations of people.

Larval Collection

In this study, 92 larval habitats in 10 counties of Chaharmahal and Bakhtiari Province were sampled during the June-September periods of 2010 and 2011 (Table 1). The sampling sites (villages and cities) were selected based on their geographical location and altitude from the sea level. In each locality, various potential larval breeding places were checked for the presence of mosquito larvae. In addition, the geographical attributes of the collecting sites were recorded by a hand-held GPS device (Etrex, Garmin, Taiwan). Then, field surveys were carried out from the early morning up to late afternoon by the 30-minute dipping method in each sampling site. Next, the collected larvae were preserved in lactophenol and Berlese's medium for temporary and permanent fixation purposes, respectively. All specimens were morphologically identified at the species level by a valid local key [50.] No attempts were made on the differentiation of sibling species.

Larval Habitats and Physicochemical Characteristics

Mosquito larval breeding places were generally classified into river edge, river bed, stream bed, spring bed, pond, rice field, drainage, agricultural irrigation pool, agricultural irrigation channel, and the man-made container. For each larval habitat, environmental variables including nature (natural/artificial), stability (temporary and permanent), water flow (slow running and standing), turbidity (clear and turbid), substrate type (muddy, sandy, and gravel), sunlight status (sunny, partially shaded, and shaded), and vegetation (with and without) were recorded separately. Vegetations included both aquatic and immersed terrestrial plants. It was assumed that all larval breeding places contain freshwater. Water temperature was measured by a hand thermometer and wherever possible, pH and dissolved oxygen levels were also recorded by a multimeter (Lutron, YK-2001 DO, Taiwan) equipped with special probes. The device was calibrated before each measurement to minimize the possibility of systematic errors.

Indices of Biodiversity and Species Co-occurrence

The species richness index was calculated based on Margalef index as

$D_{mg} = (S-1)/ln(n)$

where S is the total number of species and n equals the total number of individuals in the sample.[51] Then, the Shannon-Wiener index of biodiversity, $H' = -\Sigma p_i \times \ln p_i$, in which p_i denotes the proportional abundance of the *ith* species, was calculated for each stratum to compare species richness and evenness between the strata.[52] Evenness (J' or E or Pielou's index) was calculated by $J' = H'/ H'max$. H'max is the maximum possible Shannon's diversity that is computed by $H'max = Log10k$

where k indicates the number of species collected in the sample.[53] The affinity index of species co-occurrence

Table 1. Locality and Geographical Attributes of the Sampled Larval Habitats of Mosquitoes in Chaharmahal and Bakhtiari Province in 2010 and 2011

Counties	Larval Habitat Coordinates	Altitude (m)	Locality	Code Number
Ardal	N 32° 01' 26'' E 50° 37' 36''	1700	Ardal	01
	N 31° 57' 58'' E 50° 24' 30''	1955	Aziz-abad	02
	N 31° 58' 01'' E 50° 24' 30''	1954	Aziz-abad	03
	N 32° 01' 27'' E 50° 37' 36''	1683	Behesht-abad	04
	N 32° 01' 27'' E 50° 37' 36''	1678	Behesht-abad	05
	N 32° 08' 57'' E 50° 26' 02''	1808	Dashtak	06
	N 31° 48' 09'' E 50° 33' 19''	1870	Gandom-kar	07
	N 32° 04' 54'' E 50° 31' 57''	1700	Sardaab	08
	N 31° 44' 39'' E 50° 32' 49''	1486	Sarkhon	09
Boroujen	N 31° 55' 22'' E 50° 26' 26''	2281	Avergan	10
	N 31° 56' 16'' E 51° 01' 19''	2263	Boldaji	11
	N 31° 51' 42'' E 51° 03' 06''	2589	Chaleh-tar	12
	N 31° 39' 07'' E 51° 11' 44''	2100	Dorahan	13
	N 31° 52' 24'' E 51° 09' 08''	2238	Gandoman	14
	N 31° 52' 23'' E 51° 09' 07''	2242	Gandoman	15
	N 31° 52' 23'' E 51° 09' 07''	2242	Gandoman	16
	N 31° 52' 04'' E 51° 08' 26''	2230	Gandoman	17
	N 31° 33' 42'' E 51° 12' 39''	2586	Gerd-e-bisheh	18
	N 31° 53' 01'' E 51° 09' 00''	2242	Mamooreh	19
	N 31° 52' 28'' E 51° 04' 55''	2290	Sanaagan	20
	N 31° 47' 25'' E 51° 05' 34''	2241	Vastegan	21
Farsan	N 32° 11' 11'' E 50° 37' 08''	1998	Chogha-hast	22
	N 32° 11' 09'' E 50° 37' 12''	2007	Chogha-hast	23
	N 32° 21' 35'' E 50° 26' 00''	2501	Ghaleh-jahan-gholi	24
	N 32° 17' 08'' E 50° 31' 10''	2075	Isa-abad	25
Khan-mirza	N 31° 33' 00'' E 51° 02' 51''	1848	Alooni	26
Kiar	N 31° 49' 42'' E 50° 40' 16''	1370	Berenjegan	27
	N 31° 50' 03'' E 50° 50' 15''	2092	Chaar-tagh	28
	N 31° 45' 34'' E 50° 49' 08''	1285	Darreh-bid	29
	N 31° 54' 29'' E 50° 42' 16''	1721	Deh-e-no (Naghan)	30
	N 32° 05' 34'' E 50° 58' 21''	2050	Dezak	31
	N 32° 09' 46'' E 50° 19' 30''	1982	Dezak	32
	N 31° 55' 03'' E 50° 36' 10''	1997	Do-polan	33
	N 31° 30' 40'' E 50° 43' 32''	1517	Goosheh	34
	N 31° 51' 54'' E 50° 46' 53''	2285	Heidar-abad	35
	N 31° 43' 16'' E 50° 52' 38''	1820	Kol-koleh	36
	N 31° 41' 26'' E 50° 52' 51''	1155	Kol-koleh	37
	N 31° 43' 30'' E 50° 52' 24''	1182	Sar-rok	38
Koohrang	N 32° 23' 14'' E 50° 20' 04''	2326	Abolghasem-abad	39
	N 32° 10' 47'' E 50° 04' 12''	1711	Bazoft (Chaman-goli)	40
	N 32° 10' 46'' E 50° 04' 12''	1711	Bazoft (Chaman-goli)	41
	N 32° 27' 30'' E 49° 47' 19''	1730	Chebd	42
	N 32° 27' 30'' E 49° 47' 19''	1710	Chebd	43
	N 32° 28' 57'' E 50° 18' 25''	2376	Choobin-rahman	44
	N 32° 26' 20'' E 50° 11' 35''	2294	Deh-e-no (Chelgerd)	45
	N 32° 26' 20'' E 50° 11' 36''	2299	Deh-e-no (Chelgerd)	46
	N 32° 10' 22'' E 50° 16' 50''	2011	Do-ab Samsami	47
	N 32° 20' 58'' E 49° 53' 14''	1620	Garaab	48
	N 32° 06' 41'' E 50° 06' 38''	1623	Gazestan	49
	N 32° 17' 37'' E 50° 14' 08''	2221	Herbekool	50
Koohrang	N 32° 42' 42'' E 49° 49' 34''	2556	Khoyeh	51
	N 32° 42' 30'' E 49° 49' 29''	1987	Khoyeh	52
	N 32° 27' 11'' E 49° 56' 38''	2312	Kiars	53
	N 32° 27' 07'' E 49° 56' 41''	2323	Kiars	54
	N 32° 38' 08'' E 49° 35' 45''	1921	Lebd	55
	N 32° 37' 49'' E 49° 35' 16''	1830	Lebd	56
	N 32° 38' 58'' E 49° 34' 59''	1704	Lebd	57
	N 32° 38' 58'' E 49° 34' 59''	1704	Lebd	58
	N 32° 34' 01'' E 49° 33' 14''	2008	Lebd	59
	N 32° 33' 59'' E 49° 38' 16''	2000	Lebd	60
	N 32° 09' 35'' E 50° 05' 52''	1480	Mavarz	61
	N 32° 12' 17'' E 50° 15' 49''	2346	Razgah	62
	N 32° 08' 49'' E 50° 24' 46''	1814	Safa-abad	63
	N 32° 38' 48'' E 49° 53' 23''	2036	Sar Agha-seyed	64
	N 32° 38' 44'' E 49° 53' 24''	2227	Sar Agha-seyed	65
	N 32° 38' 43'' E 49° 53' 25''	2254	Sar Agha-seyed	66
	N 32° 28' 49'' E 50° 17' 02''	2213	Seil-gah	67
	N 32° 29' 12'' E 50° 17' 01''	2190	Seil-gah	68
	N 32° 29' 11'' E 50° 16' 60''	2190	Seil-gah	69
	N 32° 30' 41'' E 50° 02' 21''	2514	Sheikh Ali-khan	70
Lordegan	N 31° 14' 22'' E 51° 13' 06''	1542	Ab-garmak	71
	N 31° 18' 57'' E 51° 17' 10''	2163	Aboo Eshagh	72
	N 31° 31' 07'' E 50° 24' 15''	911	Barz	73
	N 31° 31' 07'' E 50° 24' 15''	913	Barz	74
	N 31° 15' 53'' E 51° 15' 51''	1731	Dasht Pagerd	75
	N 31° 24' 52'' E 50° 39' 57''	1044	Gargar	76
	N 31° 32' 02'' E 50° 37' 49''	1406	Monj	77
	N 31° 26' 21'' E 50° 39' 21''	1280	Patoveh	78
	N 31° 25' 36'' E 50° 39' 47''	1220	Sar-ghaaleh	79
	N 31° 18' 54'' E 50° 51' 25''	1563	Tallineh	80
Saman	N 32° 28' 13'' E 50° 56' 57''	1833	Cham Khorram	81
	N 32° 27' 48'' E 50° 57' 33''	1815	Cham Nar	82
	N 32° 38' 46'' E 50° 50' 01''	1852	Mar-kadeh	83
	N 32° 38' 51'' E 50° 50' 37''	1936	Mar-kadeh	84
	N 32° 27' 47'' E 50° 55' 15''	1906	Saman	85
Shahrekord	N 32° 29' 03'' E 50° 36' 19''	1669	Marghmalek	86
	N 32° 28' 34'' E 50° 30' 17''	2497	Marghmalek	87
	N 32° 12' 34'' E 50° 48' 40''	2044	Nou-abad	88
	N 32° 03' 53'' E 51° 03' 02''	2087	Soork	89
	N 32° 12' 52'' E 50° 49' 44''	2036	Taghanak	90
	N 32° 08' 34'' E 50° 50' 39''	2015	Vaght-o-Saat	91
	N 32° 20' 52'' E 50° 37' 26''	2155	Vanan	92

Table 2. Species Occurrence of Collected Mosquito Larvae Based on the Altitude of Larval Habitats in Chaharmahal and Bakhtiari Province in 2010 and 2011

Mosquito Species	Counties	Low-altitude (≤1400 m)	Mid-altitude (1401-2000 m)	High-altitude (≥2001 m)	Min. (m)	Max. (m)	Mean (m)	SD (m)
Anopheles claviger	Ardal, Koohrang, and Shahrekord	-	3	53, 87, 92	1954	2497	2229.5	230.8
Anopheles dthali	Ardal, Kiar, Koohrang, and Lordegan	29*, 37, 38, 74, 76, 79	2, 36, 40, 41, 42, 48, 61, 63, 71, 80	-	913	1955	1484.1	312.4
Anopheles maculipennis s.l.	Ardal, Boroujen, Farsan, Kiar, Khanmirza, Koohr3ang, Lordegan, Saman, and Shahrekord	27, 73	1, 2, 4, 8, 9, 22, 26, 30, 32, 33, 34, 71, 75, 77, 81, 82, 84, 85	10, 23, 25, 31, 35, 39, 47, 50, 62, 89, 90, 91, 92	911	2346	1876.7	313.7
Anopheles marteri	Ardal, Kiar, and Koohrang	-	3, 52, 56, 58, 60	28, 51	1704	2556	2017.6	269.0
Anopheles superpictus s.l.	Ardal, Boroujen, Farsan, Kiar, Lordegan, Saman, and Shahrekord	27, 29, 37, 38, 73, 74, 76, 78, 79	1, 2, 4, 6, 8, 9, 32, 34, 36, 40, 41, 42, 48, 49, 52, 55, 56, 58, 60, 61, 63, 71, 77, 80, 81, 82	10, 13, 18, 25, 28, 31, 35, 39, 46, 47, 50, 51,53, 54, 62, 65, 89	911	2586	1797.7	411.9
Anopheles turkhudi	Koohrang and Lordegan	74	48, 80	-	913	1620	1365.3	392.8
	Subtotal No. (%) of occasions	18 (50.0)	62 (39.7)	35 (26.5)	911	2586	1793.8	392.8
Culex arbieeni	Koohrang	-	41, 42, 48, 52, 57, 58, 60	51, 53, 59	1620	2556	1933.2	305.2
Culex hortensis	Ardal, Boroujen, Koohrang, Lordegan, Saman, and Shahrekord	-	2, 9, 52, 55, 56, 60, 83, 84	12, 13, 15, 17, 18, 19, 20, 21, 28, 31, 35, 39, 45, 46, 51, 54, 59, 62, 65, 66, 68, 69, 70, 89, 92	1486	2589	2172.2	234.1
Culex laticinctus	Koohrang	-	49, 56	54	1623	2323	1925.3	359.6
Culex mimeticus	Ardal, Boroujen, Farsan, Kiar, Koohrang, Lordegan, and Saman	29, 38, 78, 79	2, 6, 8, 30, 32, 33, 34, 36, 41, 42, 43, 48, 49, 52, 55, 57, 60, 61, 75, 84	18, 21, 23, 28, 51, 53, 59, 70	1182	2586	1811.4	342.5
Culex perexiguus	Ardal, Boroujen, Kiar, Koohrang, Lordegan, Saman, and Shahrekord	27, 29, 37, 38, 73, 74, 78, 79	1, 2, 3, 4, 8, 9, 26, 30, 33, 34, 49, 56, 61, 63, 71, 75, 77, 81, 82, 86	10, 11, 18, 28, 35, 47, 50, 92	911	2586	1708.7	398.3
Culex pipiens	Koohrang, Saman, Shahrekord, and Lordegan	-	75, 81, 82, 83, 86	68, 88, 90	1669	2190	1896.3	176.9
Culex territans	Kiar	-	30	-	1721	1721	1721.0	0
Culex theileri	Ardal, Boroujen, Farsan, Lordegan, Khanmirza, Kiar, Koohrang, Saman, and Shahrekord	27, 29, 38, 73, 74, 78	1, 4, 5, 8, 9, 26, 30, 32, 33, 34, 49, 60, 61, 63, 71, 75, 77, 81, 82, 85	10, 11, 12, 13, 14, 16, 17, 20, 23, 25, 28, 31, 35, 39, 47, 50, 62, 68, 69, 72, 89, 90, 91, 92	911	2589	1877.7	381.0
Culex tritaeniorhynchus	Koohrang	-	55	-	1921	1921	1921.0	0
Culiseta longiareolata	Ardal, Boroujen, Farsan, Koohrang, Saman, and Shahrekord	-	7, 56, 58, 60, 82, 83, 85	12, 14,15, 16,17, 19, 20, 21,24, 31, 35, 44, 45, 46, 51, 54, 59, 64, 65, 66, 68, 72	1704	2589	2167.4	224.1
Culiseta subochrea	Boroujen and Koohrang, Shahrekord	-	83, 86	17, 67, 68	1669	2230	2030.8	255.5
Ochlerotatus caspius s.l.	Shahrekord	-	-	90	2036	2036	2036.0	0
	Subtotal No. (%) of occasions	18 (50.0)	94 (60.3)	97 (73.5)	911	2586	1933.1	360.7
	Total No. (%, %) of occasions	36 (100, 11.1)	156 (100, 48.2)	132 (100, 40.7)	911	2589	1883.7	377.8

Note. * Code number of the larval habitat; Min: Minimum; Max: Maximum; SD: Standard deviation was also calculated based on Fager and McGowan test as follows.

$[J/(N_A N_B)^{½}] – 1/[2(N_B)^{½}]$

where J and N_A are the number of joint occurrences and the total number of the occurrences of species A, respectively. Further, N_B is the total number of the occurrences of species B. The species were assigned to the letters so that $N_A<N_B$. This index ranges from -1.0 to +1.0. Eventually, values equal to or more than 0.5 were considered as significantly associated species in larval habitats.[54]

Data Analysis

Data were entered into SPSS software, version 20.0, and the indices of central tendency or proportions were calculated for the physicochemical parameters of each type of larval breeding place, separately. Furthermore, independent samples t-test was used to compare the means of pH, dissolved oxygen, and temperature between anopheline and culicine larval habitats at $P=0.05$ level of significance. The differences in the physicochemical properties of the larval habitats of mosquito species were determined using ANOVA. The association between altitudinal categories and the subfamily or species of mosquitoes was examined by chi-square and logistic regression tests, respectively. Additionally, the indices of species richness and biodiversity were calculated by a free package of Biodiversity Calculator on the web, followed by computing the affinity index of species by Microsoft Office Excel software, version 10.0. In this report, altitudinal data were arbitrarily classified into three relatively equal range strata to figure out the vertical distribution of mosquito species.

Results

Vertical Distribution and Biodiversity

In total, 8335 mosquito larvae representing four genera and 18 species were collected in this study. Mosquito larvae were collected from a wide range of altitudes from 911 to 2589 meters above the sea level. Table 2 presents collected mosquito larvae, the location and the altitude of their larval breeding places, and the total occasion of larval habitats according to the altitudinal categories. For more convenience, henceforward, low-, mid-, and high altitudes are used instead of ≤1400 m, 1401-2000 m, and ≥2001 m to short writings. In general, 9.8%, 43.5%, and 46.7% of the total larval breeding places were placed in low-, mid-, and high-altitudes, respectively. These altitudinal ranges were represented by 7, 17, and 14 mosquito species, respectively (Table 2). There was a significant association between the altitudinal strata of larval habitats and mosquitoes at the subfamily level ($X^2 = 9.19$, df = 2, $P=0.010$) except for *Cx. territans*, *Cx. tritaeniorhynchus*, and *Oc. caspius* s.l. Table 3 provides the selective alpha biodiversity indices of the collected mosquito larvae based on the altitudes of larval habitats in the province.

Physicochemical Characteristics

In general, spring bed (32.3%), rice field (20.2%), and river edge (19.9%) were the most prevalent types of larval habitats for both anopheline and culicine mosquitoes while drainage (0.9%) and man-made container (1.6%) were the least ones. The agricultural irrigation channel was a bit more frequent to contain culicine compared to anopheline larvae (13% versus 4.3%). In addition, the single culicine larva of *Cx. territans*, *Cx. tritaeniorhynchus*, and *Oc. caspius* s.l. was collected from the rice field, man-made container, and stream bed, respectively (Table 4 and Figure 2).

All mosquito larvae were collected from freshwater habitats (Table 5). Regardless of few inter-specific differences, most anopheline and culicine larval breeding places were natural (67.6%) and temporary (70.7%) in nature with standing (60.5%) but clear water (94.4%), a muddy substrate (69.4%), sunlit (84.0%), and with some sorts of vegetations (96.6%). Few exceptions to this generalization are *Cx. pipiens* which was equally collected from both natural (50%) and artificial (50%) larval habitats. The larval breeding places of *An. claviger* (100%), *An. turkhudi* (66.7%), and *Cx. laticinctus*

Table 3. Some Alpha Biodiversity Indices of the Collected Mosquito Larvae Based on the Altitudes of Larval Habitats in Chaharmahal and Bakhtiari Province in 2010 and 2011

Index	Low Altitude (≤ 1400 m) N = 637 S = 7 n = 9	Mid Altitude (1401–2000 m) N = 3905 S = 17 n = 39	High Altitude (≥ 2001 m) N = 3793 S = 14 n = 44	The Whole Province N = 8335 S = 18 n = 92
Margalef richness index (D_{Mg})	0.929	1.9	1.6	1.9
Simpson index (C or D)	0.24	0.15	0.19	0.14
Shannon index (H') (ln)	1.54	2.1	2	2.2
Shannon index (H') (log)	0.668	0.92	0.85	0.95
Pielou's evenness index (J') (ln)	0.791	0.741	0.757	0.761
Pielou's evenness index (J') (log)	0.790	0.747	0.741	0.756

Note. N: Number of specimens; S: Number of species; n: Number of larval habitats.

Table 4. Mosquito Larval Habitat Types in Chaharmahal and Bakhtiari Province in 2010 and 2011

Mosquito Species	River Edge (%)	River Bed (%)	Stream Bed (%)	Spring Bed (%)	Pond (%)	Rice Field (%)	Drainage (%)	Agricultural Irrigation Pool (%)	Agricultural Irrigation Channel (%)	Man-made Container (%)
Anopheles claviger	25.0	-	25.0	50.0	-	-	-	-	-	-
Anopheles dthali	18.8	6.3	-	50.0	-	25.0	-	-	-	-
Anopheles maculipennis s.l.	3.0	3.0	9.1	24.2	12.1	33.3	-	6.1	9.1	-
Anopheles marteri	71.4	-	-	28.6	-	-	-	-	-	-
Anopheles superpictus s.l.	21.2	3.8	1.9	36.5	5.8	23.1	-	1.9	3.8	1.9
Anopheles turkhudi	-	-	-	66.7	-	33.3	-	-	-	-
	18.3	3.5	4.3	35.7	6.1	24.3	-	2.6	4.3	0.9
Culex arbieeni	40.0	-	-	60.0	-	-	-	-	-	-
Culex hortensis	31.3	-	-	21.9	12.5	9.4	-	3.1	18.8	3.1
Culex laticinctus	33.3	-	-	66.7	-	-	-	-	-	-
Culex mimeticus	21.9	6.3	-	46.9	3.1	12.5	-	-	6.3	3.1
Culex perexiguus	16.7	2.8	2.8	30.6	-	38.9	2.8	-	5.6	-
Culex pipiens	-	-	12.5	12.5	12.5	12.5	12.5	-	37.5	-
Culex territans	-	-	-	-	-	100	-	-	-	-
Culex theileri	12.2	2.0	4.1	30.6	12.2	24.5	-	4.1	10.2	-
Culex tritaeniorhynchus	-	-	-	-	-	-	-	-	-	100
Culiseta longiareolata	31.0	3.4	-	17.2	6.9	6.9	-	3.4	27.6	3.4
Culiseta subochrea	-	-	-	20.0	40.0	-	20.0	-	20.0	-
Ochlerotatus caspius s.l.	-	-	100	-	-	-	-	-	-	-
	20.8	2.4	2.4	30.4	7.7	17.9	1.4	1.9	13.0	1.9
	19.9	2.8	3.1	32.3	7.1	20.2	0.9	2.2	9.9	1.6

Figure 2. Typical Mosquito Larval Habitats in Chaharmahal and Bakhtiari Province.
Note. A: River edge; B: River bed; C: Stream bed; D: Spring bed (arrows are aimed at three batches of culicine eggs); E: Pond; F: Drainage; G: Agricultural irrigation pool; H: Agricultural irrigation channel.

Table 5. Physical Characteristics of Mosquito Larval Habitats in Chaharmahal and Bakhtiari Province in 2010 and 2011

Mosquito Species	Nature		Stability		Water Flow		Turbidity		Substrate Type				Sunlight Status			Vegetation	
	Natural (%)	Artificial (%)	Permanent (%)	Temporary (%)	Slow-running water (%)	Standing water (%)	Turbid (%)	Clear (%)	Muddy (%)	Sandy (%)	Gravel (%)	Other (%)	Sunlit (%)	Shaded (%)	Partially Shaded (%)	Without Vegetation (%)	With Vegetation (%)
Anopheles claviger	100	-	100	-	75.0	25.0	-	100	50.0	-	50.0	-	25.0	-	75.0	-	100
Anopheles dthali	75.0	25.0	31.3	68.8	37.5	62.5	6.2	93.8	62.5	18.8	18.8	-	87.5	-	12.5	-	100
Anopheles maculipennis s.l.	57.6	42.4	27.3	72.7	33.3	66.7	3.0	97.0	84.8	6.1	6.1	3.0	94.0	3.0	3.0	-	100
Anopheles marteri	100	-	28.6	71.4	57.1	42.9	-	100	14.3	14.3	71.4	-	71.4	-	28.6	-	100
Anopheles superpictus s.l.	73.1	26.9	30.8	69.2	42.3	57.7	5.8	94.2	65.4	13.5	19.2	1.9	90.4	1.9	7.7	1.9	98.1
Anopheles turkhudi	66.7	33.3	66.7	33.3	33.3	66.7	-	100	33.3	33.3	33.3	-	100	-	-	-	100
	71.3	28.7	33.0	67.0	40.9	59.1	4.3	95.7	66.1	12.2	20.0	1.7	87.8	1.7	10.4	0.9	99.1
Culex arbieeni	100	-	20.0	80.0	40.0	60.0	-	100	20.0	20.0	60.0	-	80.0	-	20.0	-	100
Culex hortensis	63.6	36.4	24.2	75.8	42.4	57.6	6.1	93.9	66.7	6.1	21.2	6.1	81.8	3.0	15.2	9.1	90.9
Culex laticinctus	100	-	66.7	33.3	66.7	33.3	-	100	66.7	33.3	-	-	66.7	-	33.3	33.3	66.7
Culex mimeticus	78.1	21.9	31.3	68.8	40.6	59.4	-	100	59.4	12.5	25.0	3.1	84.4	-	15.6	-	100
Culex perexiguus	58.3	41.7	30.6	69.4	41.7	58.3	8.3	91.7	86.1	5.6	8.3	-	86.1	5.6	8.3	-	100
Culex pipiens	50	50	25.0	75.0	37.5	62.5	25.0	75.0	100	-	-	-	50.0	25.0	25.0	12.5	87.5
Culex territans	-	100	-	100	-	100	-	100	100	-	-	-	100	-	-	-	100
Culex theileri	64.0	36.0	32.0	68.0	38.0	62.0	4.0	96.0	80.0	6.0	10.0	4.0	94.0	4.0	2.0	-	100
Culex tritaeniorhynchus	-	100	100	-	-	100	-	100	--	-	100	-	100	-	-	-	100
Culiseta longiareolata	58.6	41.4	10.3	89.7	31.0	69.0	10.3	89.7	62.1	6.9	20.7	10.3	72.4	3.4	24.1	13.8	86.2
Culiseta subochrea	60	40	20.0	80.0	20.0	80.0	20	80	100	-	-	-	20.0	40.0	40.0	20.0	80.0
Ochlerotatus caspius s.l.	100	-	100	-	100	-	-	100	100	-	-	-	100	-	-	-	100
	65.6	34.4	27.3	72.7	38.8	61.2	6.2	93.8	71.3	7.7	17.2	3.8	81.8	4.8	13.4	4.8	95.2
	67.6	32.4	29.3	70.7	39.5	60.5	5.6	94.4	69.4	9.3	18.2	3.1	84.0	3.7	12.3	3.4	96.6

(66.7%) were mostly permanent. The larval habitats of *An. claviger* (75%) and *Cx. laticinctus* (66.7%) had mostly slow running water. The substrate of the larval breeding places of *An. claviger* was equally muddy (50%) or gravel (50%). Further, *An. marteri* (71.4%) and *Cx. arbieeni* (60%) were mostly collected from larval habitats with gravel substrate and 75% of the oviposition sites of *An. claviger* were shaded partially. Finally, 40% and 40% of *Cs. subochrea* larval habitats were shaded and partially shaded, respectively.

The chi-square test did not show any significant association between larval habitat types and mosquitoes at the subfamily level.

Table 6 presents the mean of water temperature, pH, and dissolved oxygen in anopheline and culicine larval breeding places. Based on the findings, a significant difference was observed between the mean temperature of anopheline and culicine larval habitats ($P=0.006$). Excluding *Cx. territans*, *Cx. tritaeniorhynchus*, and *Oc. caspius* s.l. because of their single occurrence, acidity ($P=0.010$), dissolved oxygen ($P=0.031$), and temperature ($P=0.001$) significantly differed between the larval habitats of different mosquito species.

Species Occurrence, Co-occurrence, and Affinity

Species occurrence, association, and affinity are shown in Table 7. The highest species occurrences were 52 (*An. superpictus* s.l.) and 50 (*Cx. theileri*) whereas the lowest ones were one for species *Cx. territans*, *Cx. tritaeniorhynchus*, and *Oc. caspius* s.l. based on the results, the highest co-occurrences belonged to *Cx. theileri*/*An. superpictus* s.l. (31), *Cx. theileri*/*An. maculipennis* s.l. (30), *Cx. theileri*/*Cx. perexiguus* (29), and *An. superpictus* s.l. /*Cx. perexiguus* (28) pairs. However, only *Cx. theileri*/*An. maculipennis* s.l. (0.67), *Cx. theileri*/*Cx. perexiguus* (0.61), *An. superpictus* s.l. /*Cx. perexiguus* (0.56), *An. superpictus* s.l. /*Cx. theileri* (0.54), *Cx. perexiguus*/*An. maculipennis* s.l. (0.53), and *Cs. longiareolata*/*Cx. hortensis* (0.52) pairs showed significant affinity.

Discussion

This study described the selective aspects of the larval habitats of mosquitoes in Chaharmahal and Bakhtiari province, including vertical biodiversity and distribution, species occurrence, species affinity, and the physicochemical characteristics of the larval habitats of 18 recovered mosquito species.

Vertical Distribution and Biodiversity

Referring to the species occurrence provides further

Table 6. Acidity (pH), Dissolved Oxygen, and Water Temperature of Mosquito Larval Habitats in Chaharmahal and Bakhtiari Province in 2010 and 2011

Mosquito Species	pH				Dissolved Oxygen (mg/l)				Water Temperature (ºC)			
	N	Mean ± SD	Minimum	Maximum	N	Mean ± SD	Minimum	Maximum	N	Mean ± SD	Minimum	Maximum
Anopheles claviger	3	7.3 ± 0.3	7.0	7.5	4	7.4 ± 1.8	5.6	9.8	4	15.8 ± 1.0	15.0	17.0
Anopheles dthali	14	8.2 ± 0.7	7.1	9.8	16	9.0 ± 4.0	3.5	21.1	13	22.7 ± 3.4	15.0	28.0
Anopheles maculipennis s.l.	28	8.0 ± 0.9	7.1	10.9	33	9.1 ± 3.7	2.2	18.3	32	20.7 ± 3.7	12.0	27.0
Anopheles marteri	7	7.7 ± 0.5	7.2	8.6	7	6.6 ± 2.0	3.3	8.5	6	18.2 ± 5.3	12.0	27.0
Anopheles superpictus s.l.	46	8.0 ± 0.8	6.8	10.9	52	8.3 ± 3.4	1.8	21.1	48	20.8 ± 4.2	12.0	28.0
Anopheles turkhudi	2	8.4 ± 0.2	8.3	8.6	3	9.4 ± 2.0	7.2	11.2	3	24.0 ± 4.0	20.0	28.0
	100	8.0 ± 0.8	6.8	10.9	115	8.5 ± 3.4	1.8	21.1	106	20.7 ± 4.1	12.0	28.0
Culex arbieeni	9	7.6 ± 0.4	7.1	8.2	10	6.4 ± 2.7	3.3	11.5	7	19.6 ± 4.4	15.0	27.0
Culex hortensis	30	7.6 ± 0.5	6.8	8.8	33	7.1 ± 2.7	3.3	17.5	32	17.9 ± 3.8	10.0	25.0
Culex laticinctus	2	7.7 ± 0.3	7.4	7.9	3	8.4 ± 0.8	7.5	9.0	3	19.7 ± 7.1	12.0	26.0
Culex mimeticus	28	7.9 ± 0.9	6.9	10.9	32	7.7 ± 3.6	1.8	18.3	29	19.9 ± 4.1	12.0	26.0
Culex perexiguus	31	8.1 ± 0.9	7.0	10.9	36	8.9 ± 4.1	1.5	21.1	35	20.5 ± 4.6	10.0	28.0
Culex pipiens	7	7.6 ± 0.8	7.0	9.3	7	7.0 ± 4.8	1.5	16.5	8	17.9 ± 4.5	10.0	24.0
Culex territans	1	7.8	7.8	7.8	1	6.3	6.3	6.3	1	20.0	20.0	20.0
Culex theileri	43	8.0 ± 0.8	7.1	10.9	50	8.9 ± 4.0	2.2	21.1	49	20.2 ± 3.9	12.0	28.0
Culex tritaeniorhynchus	1	7.1	7.1	7.1	1	6.1	6.1	6.1	1	15.0	15.0	15.0
Culiseta longiareolata	29	7.5 ± 0.5	6.8	8.8	29	6.4 ± 2.9	2.2	17.5	28	18.2 ± 3.9	12.0	27.0
Culiseta subochrea	4	7.2 ± 0.2	7.0	7.4	5	5.2 ± 3.6	1.5	10.4	5	15.6 ± 3.6	10.0	20.0
Ochlerotatus caspius s.l.	1	7.7	7.7	7.7	1	5.2	5.2	5.2	1	24.0	24.0	24.0
	186	7.8 ± 0.7	6.8	10.9	208	7.7 ± 3.6	1.5	21.1	199	19.3 ± 4.2	10.0	28.0
	286	7.9 ± 0.8	6.8	10.9	323	8.0 ± 3.6	1.5	21.1	305	19.8 ± 4.2	10.0	28.0

Table 7. Species Occurrence, Co-occurrence, and Affinity Index of Collected Mosquito Larvae in Chaharmahal and Bakhtiari Province in 2010 and 2011

Mosquito Species	Total Occasions	*An. claviger*	*An. dthali*	*An. maculipennis* s.l.	*An. marteri*	*An. superpictus* s.l.	*An. turkhudi*	*Cx. arbieeni*	*Cx. hortensis*	*Cx. laticinctus*	*Cx. mimeticus*	*Cx. perexiguus*	*Cx. pipiens*	*Cx. territans*	*Cx. theileri*	*Cx. tritaeniorhynchus*	*Cs. longiareolata*	*Cs. subochrea*	*Oc. caspius* s.l.
Anopheles claviger	4	*	0	1	1	1	0	1	1	0	1	2	0	0	1	0	0	0	0
Anopheles dthali	16	-0.13	*	2	0	16	3	3	1	0	10	9	0	0	6	0	0	0	0
Anopheles maculipennis s.l.	33	0.00	0.00	*	0	22	0	0	9	0	10	21	4	1	30	0	4	0	1
Anopheles marteri	7	0.00	-0.19	-0.19	*	6	0	4	5	1	4	3	0	0	2	0	4	0	0
Anopheles superpictus s.l.	52	0.00	0.49	0.46	0.25	*	3	8	18	3	23	28	2	0	31	1	10	0	0
Anopheles turkhudi	3	-0.29	0.14	-0.29	-0.29	-0.05	*	1	0	0	1	1	0	0	1	0	0	0	0
Culex arbieeni	10	0.00	0.08	-0.16	0.32	0.19	0.02	*	4	0	9	0	0	0	1	0	4	0	0
Culex hortensis	33	0.00	-0.04	0.19	0.24	0.35	-0.09	0.13	*	2	10	7	2	0	15	1	19	3	0
Culex laticinctus	3	0.00	-0.29	-0.29	-0.07	-0.05	-0.29	-0.29	-0.09	*	1	2	0	0	1	0	2	0	0
Culex mimeticus	32	0.00	0.35	0.22	0.18	0.48	0.01	0.41	0.22	0.01	*	15	1	1	15	1	4	0	0
Culex perexiguus	36	0.08	0.29	**0.53**	0.11	**0.56**	0.01	-0.08	0.12	0.11	0.36	*	4	1	29	0	3	1	0
Culex pipiens	8	-0.18	-0.18	0.07	-0.18	-0.08	-0.18	-0.18	-0.05	-0.18	-0.11	0.06	*	0	5	0	3	3	1
Culex territans	1	-0.50	-0.50	-0.33	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.32	-0.33	-0.50	*	1	0	0	0	0
Culex theileri	50	0.00	0.14	**0.67**	0.04	**0.54**	0.01	-0.03	0.30	0.01	0.30	**0.61**	0.18	0.07	*	0	12	2	1
Culex tritaeniorhynchus	1	-0.50	-0.50	-0.50	-0.50	-0.36	-0.50	-0.50	-0.33	-0.50	-0.32	-0.50	-0.50	-0.50	-0.50	*	0	0	0
Culiseta longiareolata	29	-0.09	-0.09	0.04	0.19	0.16	-0.09	0.14	**0.52**	0.12	0.04	0.00	0.10	-0.09	0.22	-0.09	*	3	0
Culiseta subochrea	5	-0.22	-0.22	-0.22	-0.22	-0.22	-0.22	-0.22	0.01	-0.22	-0.22	-0.15	0.25	-0.22	-0.10	-0.22	0.03	*	0
Ochlerotatus caspius s.l.	1	-0.50	-0.50	-0.33	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.15	-0.50	-0.36	-0.50	-0.50	-0.50	*

information on the vertical and horizontal distribution of mosquito larvae in Chaharmahal and Bakhtiari province. To make these inferences, the rationale was taken that the higher occurrence leads to the wider distribution and the higher probability of an abundant population.

The larvae of 11 mosquito species such as *An. claviger*, *An. marteri*, *Cx. arbieeni*, *Cx. hortensis*, *Cx. laticinctus*, *Cx. pipiens*, *Cx. territans*, *Cx. tritaeniorhynchus*, *Cs. longiareolata*, *Cs. subochrea*, and *Oc. caspius* s.l. were not collected from low-altitudes. Similarly, the larvae of four mosquito species including *An. dthali*, *An. turkhudi*, *Cx. territans,* and *Cx. tritaeniorhynchus* were never collected from high-altitudes.

Anopheles dthali larvae were collected six times from low-altitudes. They were also collected 10 times from mid-altitudes while never from high-altitudes ($P<0.001$). These findings show that mid-altitude is more favorable for its low population.

Anopheles turkhudi larvae were collected only one time from low-altitudes. They were further collected two times from the mid-altitudes although they were never collected from high-altitudes ($P=0.025$). It implies that the population of this species is so low that it does not allow the species to spread horizontally and vertically.

The horizontal distribution maps of *An. dthali* and *An. turkhudi* larvae revealed that the populations of these species are limited to the western parts of Chaharmahal and Bakhtiari province where high elevations interfere with their dispersion into adjacent areas.[41] In their study, Amani et al[27] collected all *An. dthali* and *An. turkhudi* larvae from the mountainous parts of Aligudarz county in Loristan province, but in this study, these larvae were collected from the low- to mid-altitudes of Chaharmahal and Bakhtiari province. Unfortunately, they did not define mountainous and plain areas by altitude in their report.

In addition, *Cx. mimeticus* larvae were collected 4, 20, and 8 times from low-, mid-, and high-altitudes, respectively ($P=0.124$). The picture was taken 8, 20, and 8 times for *Cx. perexiguus*, respectively ($P<0.001$), indicating that the vertical distribution of *Cx. mimeticus* and *Cx. perexiguus* larvae was relatively similar. Although they both more horizontal distribution in mid-altitudes, the populations of *Cx. perexiguus* had more adaptive capacity to spread in low-altitudes.

Furthermore, *An. maculipennis* s.l. larvae were collected 2, 18, and 13 times from low-, mid-, and high-altitudes, respectively ($P=0.356$), implying that this species was well distributed in mid- to high- altitudes, and low-altitudes were also colonized by their medium size population.

In a study on the mosquito vectors of dirofilariasis in the northwest of Iran, the larvae of *An. maculipennis* s.l. and *An. sacharovi* were collected from higher (830-1650 m) and lower altitudes (40-80 m) of Ardebil province, respectively.[55] This finding is in contrast with those of studies in the low-altitude Caspian Sea littoral where *An. maculipennis* s.l. is much more abundant than *An. sacharovi* that is absent or rarely found in this area.[21,32,56] Probably, other factors such as rice fields, temperature, and humidity, instead of altitude, influence the composition of the species in the region. More accurate interpretation of the altitudinal data of *An. maculipennis* s.l. larvae in the present study needs further molecular identifications of the collected larvae.

It should be mentioned that *Cx. theileri* larvae were the most abundant and widely distributed species among all collected mosquito larvae in the province. They were collected 6, 20, and 24 times from low-, mid-, and high-altitudes, respectively ($P=0.189$), which implies that their high population confers an ability to spread everywhere in the province.

The larvae of *Cx. theileri* are collected infrequently from Guilan,[20] Qom,[14] Hormozgan,[24] and Golestan[33-35] provinces that are low land areas in Iran. On the other hand, it is a frequently encountered larval species in high-altitude provinces such as Ardebil,[55] Isfahan,[30,57] and Zanjan.[58] This evidence indicates that the larval habitats of *Cx. theileri* are located at higher altitudes. Conversely, some other reports may not comply with this outcome. For example, the larvae of this mosquito species were collected in low numbers from the high-altitude provinces of East[36,59] and West Azerbaijan.[28] The findings of the present study do not fit any of these studies. Perhaps, a reasonable explanation needs further research by controlling possible confounders to rectify these discrepancies.

Anopheles superpictus s.l. larvae were the second most abundant and widely distributed species next to *Cx. theileri*. They were collected 9, 26, and 17 times from low-, mid-, and high-altitudes ($P=0.001$), respectively, representing that this species is well adapted to mid-altitudes, but to some extent, breeds in low- or high-altitudes as well. However, the population size of *An. superpictus* s.l. larvae was 37% of *Cx. theileri.*[41] Given that the number of the occurrence of these two species is more or less the same, it means that the breeding places of *Cx. theileri* are more productive compared to *An. superpictus* s.l.

Nonetheless, *An. claviger*, *Cx. laticinctus,* and *Cs. subochrea* were never collected from low-altitudes. Although *An. claviger* was collected in one and three occasions ($P=0.107$), *Cx. laticinctus* was collected two and one time(s) from mid- and high-altitudes ($P=1.000$), respectively. Moreover, *Cs. subochrea* was similarly collected two and three times from mid- and high-altitudes ($P=0.520$), respectively. This pattern shows that these mosquito species breed in higher lands but their populations are quite low.

Anopheles marteri, *Cx. arbieeni,* and *Cx. pipiens* also showed relatively similar vertical and horizontal distributions although none of these species were collected from low altitudes. *Anopheles marteri* larvae were collected in 5 and 2 occasions ($P=0.500$) although *Cx. pipiens* and *Cx. arbieeni* larvae were collected 5 and 3 times ($P=0.819$), and 7 and 3 times ($P=0.943$) from mid- and high-altitudes, respectively. This indicates that these species breed in highlands but their populations are low.

A high number of *Cx. pipiens* larvae was collected from the low lands of the Caspian Sea littoral, including Guilan,[20] Mazandaran,[39] and Golestan[33,35] provinces. In comparison, the larvae of this species were collected in low numbers from the high land areas of Iran such as Ardebil,[55] Isfahan,[30] West Azerbaijan,[28] East Azerbaijan,[36,59] Kurdistan,[26] Kurdistan, and Kermanshah[38] provinces, implying that under suitable environmental conditions, *Cx. pipiens* tends to establish its population in low-altitudes. However, Dehghan et al[22] were able to collect a relatively high number of the larvae of this species from Hamadan province that is usually considered as a high land area in Iran. This report may be an indication of the influence of other factors in the establishment of the species (e.g., the adaptation for breeding and surviving around human settlements).[60] Based on the findings of the present study, the larvae of *Cx. pipiens* were found in man-made habitats more than other species (50%), which is in accordance with the findings of many previous investigations.[20,32,39,57] It seems that the most favorable larval habitats of the species in the cities are the house ponds, wells, septic tanks, and the sewage wells.[17,60-62] That is why *Cx. pipiens* is called the house mosquito, is found almost throughout Iran, and occurs most probably in cities with different altitudes more than any other mosquito species. Because of morphological similarities between female adults and the larvae of *Cx. pipiens* and *Cx. torrentium* in northern Iran where they may occur together, along with *Cx. pipiens* and *Cx. quinquefasciatus* in southern Iran for the same reason,[50] their compositions need to be investigated extensively with precise identification in the future.

Culex hortensis and *Cs. longiareolata* larvae were never collected from low-altitudes, but they were collected from mid- and high-altitudes 8 and 25 ($P<0.001$), as well as 7 and 22 times ($P<0.001$), respectively. The obtained data demonstrate that both species prefer higher, especially high-altitudes, to breed because their populations are well distributed in these areas.

Only one single specimen of *Cx. territans*, *Cx. tritaeniorhynchus* and *Oc. caspius* s.l., was collected in the present study. In addition, two species *Cx. territans* and *Oc. caspius* s.l. were collected in the central parts of the province. This may imply that some populations of these species were escaped from our sampling efforts. Therefore, it would be more logical to exclude these species from our explanations on the distributional patterns of the presented mosquito species.

Unfortunately, most literature regarding the mosquitoes of Iran either does not pertain to altitudinal information or contains so mixed data that cannot be easily retrieved and used in analytical comparisons. For example, no data are reported on the altitude of collected species in the studies conducted on the larval habitats of mosquitoes in Golestan,[33,35] Guilan,[19-21] Hormozgan,[13] Qom,[14] Hamedan,[22] Isfahan,[30] Kurdistan,[26] Kermanshah,[38] and Mazandaran[31] provinces. On the other hand, in studies carried out in Hormozgan[13,23,25] and East Azerbaijan[36] provinces, the altitudinal data were reported as a function of localities and sampling sites rather than mosquito species. Perhaps, in a number of these cases, such lack of data may actually come from the local ground features as there is little variation in elevations in low land areas. A few examples of this kind are found in studies conducted in Hormozgan,[24] Bushehr,[63] Mazandaran,[31] Golestan,[33,35] and Guilan[19-21] provinces. Similarly, data are presented by the topography of collection sites in few studies that paid attention to the vertical distribution of mosquito larval habitats.[27,64] In these investigations, there is no reference level to understand what they mean by mountainous or foothill areas.

In this study, larval habitats located in mid-altitudes showed the highest number of mosquito species, the highest value of the Margalef richness index, and the highest value of the Shannon-Weiner index of biodiversity while the least value of the Simpson index (Table 3). It is noteworthy that when Simpson index (D) decreases, the species diversity represents an increase, explaining why the Simpson index is sometimes shown as 1–D or 1/D. Interestingly, when the Pielou's evenness index was calculated for three altitudinal strata, the lowest altitude category (≤ 1400 m) displayed the highest value (Table 3). This shows the influence of the sample size of this stratum in which the numbers of collected larval specimens (637), species (7), and larval habitats (9) are the lowest among the strata. This is because Chaharmahal and Bakhtiari is a high altitude province and the most larval habitats are located in higher than 1400 m above the sea level. There is little information about the biodiversity of mosquitoes in Iran for comparison. For instance, Nikookar et al[37] mentioned some biodiversity indices of mosquitoes in three low-level sites (185–290 m) in Mazandaran province of the Caspian Sea littoral, northern Iran. Furthermore, Hanafi-Bojd et al compared the Shannon-Weiner index of biodiversity and the Pielous' richness index in five sites (450-1020 m) of Bashagard County of Hormozgan province, southern Iran.[23,24] However, none of the above-mentioned studies compared or discussed the biodiversity based on the altitudes of the sites.

The "latitudinal gradient of species richness" is a known and well-documented phenomenon in biogeography, which declares that biodiversity increases by decreasing the latitude.[65] In contrast, the "elevation diversity gradient" that is usually considered as a mirror of the previous phenomenon on a smaller scale is a more complex event and the scale and extent of the altitudinal gradient may be observed in different patterns depending on the targeted taxonomic group.[66] Our data are consistent with the "mid-elevation peak" pattern in which the species richness is higher in mid-altitudes.[67] To our knowledge, this is the first study to deal with the vertical biodiversity of mosquitoes in Iran.

Physicochemical Characteristics

Two sets of variables are commonly used in the description of the physico-chemical characteristics of mosquito larval habitats. The first set consists of qualitative nominal or ordinal variables that are normally employed to describe the physical attributes of mosquito larval habitats. These parameters are also referred to as environmental[9,13,25,68] or biological characteristics by some authors.[24,26,28,32,36]

In the present study, spring bed pools and, to some extent, river edge and rice fields were the most prevalent types of larval habitats for all six anopheline and 12 culicine mosquitoes. They were mostly natural and temporary in nature, with standing and clear water, muddy substrate, sunlit, and with vegetation. It is proposed that this relative uniformity of data is partly due to prevailing water resources for breeding mosquitoes in the studied area. Other possibilities are the insufficiency of criteria and the problem of the sampling design.

Nikookar et al[31] reported 6 anopheline and 10 culicine mosquito species in a larval survey in Mazandaran province located in northern Iran. The larval habitats were mostly temporary, stagnant, with plants, shadow-sun, muddy floors, and turbid or clear freshwater. In another study, Hanafi-Bojd et al[23] presented the larval habitats of 8 anopheline species from Hormozgan province in the south of the country. Natural larval breeding places, without vegetation, in full sunlight, with a sandy substrate and fresh and clear water, were the dominant physical characteristics of mosquito larval habitats. A couple of years later, they also characterized the oviposition sites of 12 culicine species from the same area.[24] Natural, temporary, or permanent larval breeding sites without vegetation, in full sunlight, with a sandy substrate and fresh and clear water, were the predominant physical features of mosquito larval habitats.

These findings of many other studies may be an indication of slight intra-provincial variation in the characteristics of mosquito larval habitats.

It is noteworthy that most exceptional cases in such reports are those species with low abundance and low occurrence. For instance, in the latter example, the larval habitats of 8 culicine species had a sandy substrate. However, 100% of the larvae of *Cx. arbieeni*, *Cx. theileri*, *Oc. caballus*, and *Oc. caspius* were exceptionally collected from larval breeding places with a muddy substrate. Looking at the companion data shows that all these four species were collected from only one larval habitat. This means that in the interpretation of the descriptive data of mosquito larval habitats, the population size and the number of occurrences should be taken into account in order to avoid unrealistic conclusions.

Probably, an example of mosquitoes with actually deviant physical characteristics of their larval habitats could be observed in the study of Azari-Hamidian,[21] who reported 5 anopheline species larvae from Guilan province although most other mosquito larvae (69.6%) were collected from sunlit oviposition sites. *Anopheles claviger* (66%) and *An. plumbeus* (100%) larvae were exceptionally collected from shaded habitats. These species constituted 6.3% and 13.1% of the total collected larvae with 14 and 12 occurrences, respectively. This validates that the obtained data about the sunlight status of the larval breeding places of these species in the studied context were not accidental.

We do not believe in the incompetence of currently used variables, but the sampling design could be arguable. Most studies on the larval habitats of mosquitoes, including the present investigation, do not intensively survey potential places for breeding mosquitoes. They neither report negative places nor characterize their attributes. Therefore, we have to admit that our knowledge on the oviposition sites of mosquitoes is highly preliminary, in a sense.

The second set of variables in the description of mosquito larval habitats is comprised of quantitative variables like pH, dissolved oxygen, along with the anions and cations of water resources. These are usually used in the description of the chemical characteristics of larval breeding places. Temperature is exceptionally classified in this group.

In this study, the larval habitats of anopheline and culicine larvae showed low levels of alkaline pH. Several studies in Iran reported the pH of mosquito oviposition sites. However, not all of them presented species-specific data.[13,23,25,36] In general, the mean pH level of mosquito larval habitats is reported to range from 7.0 to 8.0.[12-14,24] The minimum and maximum of the reported pH level are 6.9[12] and 8.9.[29] Other field and laboratory evidence shows that different mosquito species tolerate pH levels from lower than 4 up to 10.5.[69] Therefore, although there are some reports regarding the correlation between pH and the larval density of mosquitoes,[70,71] it is believed that it does not exert a direct effect on the distribution of mosquitoes.[69]

The present study reported the mean dissolved oxygen level for the larval habitats of 18 mosquito species in Chaharmahal and Bakhtiari province. To the best of our knowledge, no other report is available for comparison in this regard in the context of Iran. Mosquito larvae get oxygen directly from the air by breathing through their respiratory siphons or a pair of spiracles. Thus, the dissolved oxygen of water has not been before a focus of much research by mosquito ecologists[5]. Nevertheless, it is an indicator of water quality and productivity in aquatic habitats. A few recent works have reported that there is a strong correlation between habitat types,[72] larval abundance,[11,73] and mosquito species[74] with the dissolved oxygen level.

In this study, it was found that the mean of the temperature of anopheline larval breeding places is around 1.4 degrees of centigrade higher than that of culicine ones ($P = 0.006$). This difference might be attributed to the altitudinal level of oviposition sites as the larval habitats of anophelines were about 140 m lower than those of the culicine ones. Temperature is an imperative factor for the growth and development of anopheline and culicine larvae.[69] It is also shown that female mosquitoes do not lay eggs on waters with higher or lower levels of temperature than a certain one.[4] There is a great deal of information about the temperature of mosquito larval habitats from Iran. However, in the analysis of these data, it should be noted that the temperature of water resources is subject to a considerable change by the time of the day, season, size, depth, as well as the movement of water and the type of the substrate. Perhaps, most of these confounders could be managed with more realistic image obtained by the advent of remote sensing and the geographical information system.[68,75]

Several studies in Iran[12-14,25,29] and other countries[10,76] have explored the relationship between larval density and the physicochemical characteristics of mosquito larval breeding sites. Some of them reported a significant relationship between a variable and the larval density of a mosquito species although other studies did not find any relationship in this regard. Two points merit to be stressed in this respect. In these studies, it is not clear whether the effect of the number of mosquito species occurrence, as discussed above, is also incorporated or not. In addition, these relationships do not necessarily represent the preferred oviposition sites of a mosquito species. This is because the larval density is a post-oviposition phenomenon and is just an implication of the productivity of a habitat type.[5,7] The presence of even a single larva in a water body suggests that this place has been selected by a female mosquito for oviposition.

Mosquitoes adopt different strategies to lay eggs. Although some species do not touch the surface of the water, others make contact with it at least for some moments. It is believed that olfactory and chemical cues are the key elements of the oviposition behavior of these two mosquito groups, respectively.[7] The metabolites of decaying bacteria, plus other complex organic compounds released by predators and competitors, produce distinct odors and flavors that attract or repel a specific female mosquito to find a suitable place, and also stimulate or deter female mosquito to finally lay eggs there.[4,6,9] Despite remarkable progress in this regard, there are still many unanswered questions regarding this issue why two seemingly identical and adjacent water resources are differently selected by gravid mosquitoes. This knowledge would be quite useful in devising more efficient ovitraps.

Species Occurrence, Co-occurrence, and Affinity

In the present study, some mosquito species displayed shared larval habitats. Furthermore, the analysis of the species occurrence data, along with larval abundance, indicated that there is a positive correlation between these variables.[41] For example, *Cx. theileri* and *An. superpictus* s.l. were the most abundant and had the most frequent larval habitats. The same was true for the next abundant species *Cx. perexiguus*, *Cx. hortensis*, *An. maculipennis* s.l., *Cx. mimeticus*, and *Cs. longiareolata*, which is in agreement with the results of other studies in Guilan,[19-21] Kurdistan,[26] and Isfahan[30] provinces. However, in areas where the number of collected larvae is extremely low, this correlation loosens its strength. In these situations where larval breeding places are highly scattered (e.g., due to harsh conditions), it is not infrequent to collect a higher number of the larvae of a given species from just a single oviposition site.[14,24]

In this study, *An. superpictus* s.l., *An. maculipennis* s.l., and *Cx. perexiguus* larvae were frequently collected with *Cx. theileri*. Similarly, *Cx. perexiguus* larvae commonly occurred with *An. superpictus* s.l. Moreover, *Cx. theileri* highly occurred with *An. maculipennis* s.l., *An. superpictus* s.l., *Cs. longiareolata*, *Cx. perexiguus,* and *An. claviger* in Kalaleh of Golestan[34]; *Cx. perexiguus*, *Cx. hortensis*, *Cs. longiareolata*, and *An. maculipennis* in Isfahan[30]; *An. superpicus* and *An. maculipennis* in Kurdistan[26]; and *An. maculipennis* and *Cx. tritaeniorhynchus* in Guilan provinces.[20] Such co-occurrences could be the result of overlapping high populations of adults, common larval needs, enough nutritional resources, no interspecific competition, and the scarcity of available resources to deposit eggs.

It is reported that *Cs. longiareolata* larvae may be collected alone.[24,26] The predatory behavior of this mosquito species could be the reason. In this study, this species was collected with 3 anopheline and 7 culicine mosquito larvae. Others also reported its concurrent presence with other species.[19,22,28,30,34,38] This evidence signifies that the predatory behavior of *Cs. longiareolata* could not be all the reason. The general physical characteristics and the pH value of the larval habitats of this species overlap with others,[24,26] meaning that other factors must also be acted in those contexts.

The highest values of the affinity index in our study were recorded for the pairs of *Cx. theileri*/*An. maculipennis*, *Cx. theileri*/*Cx. perexiguus*, *An. superpictus* s.l./*Cx. perexiguus*, *An. superpictus* s.l./*Cx. theileri*, and *Cx. perexiguus*/*An. maculipennis* s.l. larvae in a descending order. Considering that around two-thirds of the occurrences of *Cs. longiareolata* happened with *Cx. hortensis*, it is not surprising that this pair exhibited a high value of the affinity index. There is little information on the affinity of mosquito larvae in Iran.[23,24,30] Ladonni et al[30] did not find any affinity between 15 mosquito species larvae in Isfahan province. On the other hand, Nikookar et al[12] calculated the inter-specific correlation coefficient for collected mosquito larvae in Mazandaran province. In another study, Hanafi-Bojd et al[23] reported a significant affinity between 8 pairs of anopheline mosquito species larvae in Hormozgan province. Of those pairs, *An. superpictus* s.l. and *An. dthali* showed a 0.521 affinity. However, the current study did not find such an affinity between these species. The reason could be related to the fact that only 31% of the total occurrences of *An. superpictus* s.l. were accompanied with *An. dthali*.

Studies on the ecology of mosquito larvae like any other investigations are facing with a number of limitations. Banafshi et al[26] listed several limitations in this regard in their study conducted in Kurdistan province. It should be added that aquatic habitats do not stay constant but are subject to a change over time. Normally, most ecological studies take a lot of time, even longer than a season, to be conducted well. In the meantime, the quality and quantity of water bodies are considerably altered so that by a change in meteorological conditions, low nutritive and unsuitable water bodies convert to highly productive resources for breeding mosquitoes. Simultaneously, human activities like agriculture, substantially expand the potential site for mosquito breeding. Besides, the population of adult mosquitoes, which is, in turn, under the influence of the availability of preferred hosts, interferes with the distribution of larval habitats. A low number of collected specimens may come from really low populations of a mosquito species. This problem may not be resolved by intensive sampling and thus makes the generalization of the results difficult. Undoubtedly, all these constraints affect our assessments on the preferred oviposition sites of mosquitoes.

Conclusion

To the best of our knowledge, this is the first study to describe the selective aspects of the ecology of the larvae of mosquitoes in Chaharmahal and Bakhtiari province and to present species occurrence, species affinity, and physicochemical characteristics of the larval habitats of 18 recovered mosquito species. The data of the altitudinal distribution of these species are new to Iran and reveal higher vertical biodiversity in mid-altitudes. Additionally, the investigated physicochemical characteristics of larval habitats were indiscriminative. It is proposed that, regardless of the possibility of problems with sampling and the incompetence of criteria in characterizing larval habitats, this might be a reflection of typical water resources available for the breeding of mosquitoes in the studied area. On the other hand, the overlapping of the larval habitats of mosquitoes may be an indication of the generalist type behavior of some female mosquitoes in the selection of suitable places to lay eggs. The bottom line is oviposition site selection by female mosquitoes, like their host preference, is still an unresolved subject. More investigations, both under controlled and field conditions, are necessary to understand the basis of this vital behavior in mosquitoes.

Acknowledgements

The authors appreciate the corporation of our colleagues in the Health Care System of Chaharmahal and Bakhtiari province in supporting the first round of field visits in 2010. We would also like to thank Mr. Gholamreza Pourshahbazi, Shahrekord University of Medical Sciences, Shahrekord, Iran, for his contribution in a part of field and laboratory activities in 2011. We would also like to express our gratitude to Professor Soleiman Kheiri, Shahrekord University of Medical Sciences, for his invaluable comments on the statistical analysis of our data.

Conflict of Interest Disclosures

The authors declare that there are no conflicts of interest.

Ethical Approval

Not applicable.

Financial Support

This study was financially supported by the Deputy of Research and Technology of Shahrekord University of Medical Sciences under grant No. 1390-07-70-558.

References

1. Azari-Hamidian S, Norouzi B, Harbach RE. A detailed review of the mosquitoes (Diptera: Culicidae) of Iran and their medical and veterinary importance. Acta Trop. 2019;194:106-22. doi: 10.1016/j.actatropica.2019.03.019.
2. Mullen GR, Durden LA. Medical and Veterinary Entomology. 2nd ed. Burlington: Academic Press; 2009.
3. World Health Organization (WHO). Larval Source Management: A Supplementary Malaria Vector Control Measure: An Operational Manual. Geneva: WHO; 2013. p. 116.
4. Macan TT. Factors that limit the range of freshwater animals. Biol Rev Camb Philos Soc. 1961;36:151-98. doi: 10.1111/j.1469-185x.1961.tb01582.x.
5. Muirhead-Thomson RC. The ecology of vector snail habitants and mosquito breeding-places: the experimental approach to basic problems. Bull World Health Organ. 1958;19(4):637-59.
6. Bentley MD, Day JF. Chemical ecology and behavioral aspects of mosquito oviposition. Annu Rev Entomol. 1989;34:401-21. doi: 10.1146/annurev.en.34.010189.002153.
7. Day JF. Mosquito oviposition behavior and vector control. Insects. 2016;7(4). doi: 10.3390/insects7040065.
8. Gaburro J, Paradkar PN, Klein M, Bhatti A, Nahavandi S,

Duchemin JB. Dengue virus infection changes *Aedes aegypti* oviposition olfactory preferences. Sci Rep. 2018;8(1):13179. doi: 10.1038/s41598-018-31608-x.
9. Rejmánková E, Grieco J, Achee N, Roberts DR. Ecology of larval habitats. In: Manguin S, ed. *Anopheles* Mosquitoes: New Insights into Malaria Vectors. Rijeka: Intech; 2013. p. 397-446.
10. Ibrahim AEA, El-Monairy OM, El-Sayed YA, Baz MM. Mosquito breeding sources in Qalyubiya Governorate, Egypt. Egypt Acad J Biol Sci E Med Entomol Parasitol. 2011;3(1):25-39. doi: 10.21608/eajbse.2011.16454.
11. Kenawy MA, Ammar SE, Abdel-Rahman HA. Physico-chemical characteristics of the mosquito breeding water in two urban areas of Cairo Governorate, Egypt. J Entomol Acarol Res. 2013;45(3):96-100. doi: 10.4081/jear.2013.e17.
12. Nikookar SH, Fazeli-Dinan M, Azari-Hamidian S, Mousavinasab SN, Aarabi M, Ziapour SP, et al. Correlation between mosquito larval density and their habitat physicochemical characteristics in Mazandaran province, northern Iran. PLoS Negl Trop Dis. 2017;11(8):e0005835. doi: 10.1371/journal.pntd.0005835.
13. Soleimani-Ahmadi M, Vatandoost H, Zare M. Characterization of larval habitats for anopheline mosquitoes in a malarious area under elimination program in the southeast of Iran. Asian Pac J Trop Biomed. 2014;4(Suppl 1):S73-80. doi: 10.12980/apjtb.4.2014c899.
14. Abai MR, Saghafipour A, Ladonni H, Jesri N, Omidi S, Azari-Hamidian S. Physicochemical characteristics of larval habitat waters of mosquitoes (Diptera: Culicidae) in Qom province, central Iran. J Arthropod Borne Dis. 2016;10(1):65-77.
15. Liu X, Wu H, Guo Y, Ren D, Yang J, Li J, et al. Breeding Site characteristics and associated factors of *Culex pipiens* complex in Lhasa, Tibet, P. R. China. Int J Environ Res Public Health. 2019;16(8). doi: 10.3390/ijerph16081407.
16. Saebi ME. Morphological study on anopheline larvae and their distribution in Iran [dissertation]. Tehran, Iran: Tehran University of Medical Sciences; 1987.
17. Zaim M. The distribution and larval habitat characteristics of Iranian Culicinae. J Am Mosq Control Assoc. 1987;3(4):568-73.
18. Yaghoobi-Ershadi MR, Namazi J, Piazak N. Bionomics of *Anopheles sacharovi* in Ardebil province, northwestern Iran during a larval control program. Acta Trop. 2001;78(3):207-15. doi: 10.1016/s0001-706x(01)00080-8.
19. Azari-Hamidian S. Larval habitat characteristics of mosquitoes of the genus *Culiseta* Felt, 1904 (Diptera: Culicidae) in the Caspian Sea littoral, Iran. Zool Middle East. 2005;36(1):59-66. doi: 10.1080/09397140.2005.10638128.
20. Azari-Hamidian S. Larval habitat characteristics of mosquitoes of the genus *Culex* (Diptera: Culicidae) in Guilan province, Iran. J Arthropod Borne Dis 2007;1(1):9-20.
21. Azari-Hamidian S. Larval habitat characteristics of the genus *Anopheles* (Diptera: Culicidae) and a checklist of mosquitoes in Guilan province, northern Iran. J Arthropod Borne Dis. 2011;5(1):37-53.
22. Dehghan H, Moosa-Kazemi S, Zahirnia AH, Davari B, Sharifi F. Larval habitat diversity and species composition of mosquitoes (Diptera: Culicidae) in Hamadan province. Scientific Journal of Hamadan University of Medical Sciences and Health Services. 2011;18(3):50-8. [Persian].
23. 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.
24. Hanafi-Bojd AA, Soleimani-Ahmadi M, Doosti S, Azari-Hamidian S. Larval habitats, affinity and diversity indices of Culicinae (Diptera: Culicidae) in southern Iran. Int J Mosq Res 2017;4(2):27-38.
25. 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 Pac J Trop Med. 2013;6(7):510-5. doi: 10.1016/s1995-7645(13)60087-5.
26. Banafshi O, Abai MR, Ladonni H, Bakhshi H, Karami H, Azari-Hamidian H. The fauna and ecology of mosquito larvae (Diptera: Culicidae) in western Iran. Turk J Zool. 2013;37(3):298-307. doi: 10.3906/zoo-1206-12.
27. Amani H, Yaghoobi-Ershadi MR, Kassiri H. The ecology and larval habitats characteristics of anopheline mosquitoes (Diptera: Culicidae) in Aligudarz county (Luristan province, western Iran). Asian Pac J Trop Biomed. 2014;4(Suppl 1):S233-41. doi: 10.12980/apjtb.4.2014c186.
28. Khoshdel-Nezamiha F, Vatandoost H, Azari-Hamidian S, Bavani MM, Dabiri F, Entezar-Mahdi R, et al. Fauna and larval habitats of mosquitoes (Diptera: Culicidae) of west Azerbaijan province, northwestern Iran. J Arthropod Borne Dis. 2014;8(2):163-73.
29. Ghanbari MR, Rakhsh Khorshid A, Salehi M, Hasanzehi A. The study of physical and chemical factors affecting breeding places of *Anopheles* in Iranshahr. Zahedan Journal of Research in Medical Sciences. 2005;7(3):221-7. [Persian].
30. Ladonni H, Azari-Hamidian S, Alizadeh M, Abai MR, Bakhshi H. The fauna, habitats, and affinity indices of mosquito larvae (Diptera: Culicidae) in central Iran. North-West J Zool. 2015;11(1):76-85.
31. Nikookar SH, Fazeli-Dinan M, Azari-Hamidian S, Mousavinasab SN, Arabi M, Ziapour SP, et al. Species composition and abundance of mosquito larvae in relation with their habitat characteristics in Mazandaran province, northern Iran. Bull Entomol Res. 2017;107(5):598-610. doi: 10.1017/s0007485317000074.
32. Nikookar SH, Moosa-Kazemi SH, Yaghoobi-Ershadi MR, Vatandoost H, Oshaghi MA, Ataei A, et al. Fauna and larval habitat characteristics of mosquitoes in Neka county, northern Iran. J Arthropod Borne Dis. 2015;9(2):253-66.
33. Sofizadeh A, Eftekhari B, Pesaraklo AR, Mohammadnia A, Ajam F, Farrokhi Balajadeh M, et al. Species diversity and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Golestan province, 2016. Jorjani Biomed J. 2018;6(3):48-62. doi: 10.29252/jorjanibiomedj.6.3.48.
34. Sofizadeh A, Moosa-Kazemi SH, Dehghan H. Larval habitats characteristics of mosquitoes (Diptera: Culicidae) in north-east of Iran. J Arthropod Borne Dis 2017;11(2):211-25.
35. Sofizadeh A, Shoraka HR, Mesgarian F, Ozbaki GM, Gharaninia A, Sahneh E, et al. Fauna and larval habitats characteristics of mosquitoes (Diptera: Culicidae) in Golestan province, northeast of Iran, 2014-2015. J Arthropod Borne Dis. 2018;12(3):240-51. doi: 10.18502/jad.v12i3.76.
36. Paksa A, Sedaghat MM, Vatandoost H, Yaghoobi-Ershadi MR, Moosa-Kazemi SH, Hazratian T, et al. Biodiversity of mosquitoes (Diptera: Culicidae) with emphasis on potential arbovirus vectors in East Azerbaijan province, northwestern Iran. J Arthropod Borne Dis. 2019;13(1):62-75.
37. Nikookar SH, Moosa-Kazemi SH, Oshaghi MA, Vatandoost H, Yaghoobi-Ershadi MR, Enayati AA, et al. Biodiversity of culicid mosquitoes in rural Neka township of Mazandaran province, northern Iran. J Vector Borne Dis. 2015;52(1):63-72.

38. Moosa-Kazemi SH, Zahirnia AH, Sharifi F, Davari B. The fauna and ecology of mosquitoes (Diptera: Culicidae) in western Iran. J Arthropod Borne Dis. 2015;9(1):49-59.
39. Nikookar SH, Azari-Hamidian S, Fazeli-Dinan M, Nasab SN, Aarabi M, Ziapour SP, et al. Species composition, co-occurrence, association and affinity indices of mosquito larvae (Diptera: Culicidae) in Mazandaran province, northern Iran. Acta Trop. 2016;157:20-9. doi: 10.1016/j.actatropica.2016.01.014.
40. Watts AG, Miniota J, Joseph HA, Brady OJ, Kraemer MUG, Grills AW, et al. Elevation as a proxy for mosquito-borne Zika virus transmission in the Americas. PLoS One. 2017;12(5):e0178211. doi: 10.1371/journal.pone.0178211.
41. Omrani SM, Azari-Hamidian S, Pourshahbazi G, Taghipour S. Fauna and the distribution of mosquitoes (Diptera: Culicidae) in Chaharmahal and Bakhtiari province, 2011-2012. Journal of Shahrekord University of Medical Sciences. 2015;16(6):127-38. [Persian].
42. Reinert JF, Harbach RE, Kitching IJ. Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. Zool J Linn Soc. 2004;142(3):289-368. doi: 10.1111/j.1096-3642.2004.00144.x.
43. Reinert JF, Harbach RE, Kitching IJ. Phylogeny and classification of *Finlaya* and allied taxa (Diptera: Culicidae: Aedini) based on morphological data from all life stages. Zool J Linn Soc. 2006;148(1):1-101. doi: 10.1111/j.1096-3642.2006.00254.x.
44. Reinert JF, Harbach RE, Kitching IJ. Phylogeny and classification of *Ochlerotatus* and allied taxa (Diptera: Culicidae: Aedini) based on morphological data from all life stages. Zool J Linn Soc. 2008;153(1):29-114. doi: 10.1111/j.1096-3642.2008.00382.x.
45. Reinert JF, Harbach RE, Kitching IJ. Phylogeny and classification of tribe Aedini (Diptera: Culicidae). Zool J Linn Soc. 2009;157(4):700-94. doi: 10.1111/j.1096-3642.2009.00570.x.
46. Wilkerson RC, Linton YM, Fonseca DM, Schultz TR, Price DC, Strickman DA. Making mosquito taxonomy useful: a stable classification of tribe Aedini that balances utility with current knowledge of evolutionary relationships. PLoS One. 2015;10(7):e0133602. doi: 10.1371/journal.pone.0133602.
47. Soghigian J, Andreadis TG, Livdahl TP. From ground pools to treeholes: convergent evolution of habitat and phenotype in *Aedes* mosquitoes. BMC Evol Biol. 2017;17(1):262. doi: 10.1186/s12862-017-1092-y.
48. Chaharmahal and Bakhtiari Meteorological Administration. Geographical position, climatological factors and climatological elements [16.03.2020]. Available from: http://www.chbmet.ir/c1.asp.
49. Soltani S, Yaghmaei L, Khodagholi M, Saboohi R. Bioclimatic classification of Chaharmahal and Bakhtiari province using multivariate statistical methods. Journal of Water and Soil Science. 2011;14(54):53-68. [Persian].
50. 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):1-33. doi: 10.5281/zenodo.187282.
51. Magurran AE. Measuring Biological Diversity. Malden, Mass: Blackwell, 2004.
52. Shannon CE. A mathematical theory of communication. Bell Syst Tech J. 1948;27(3):379-423. doi: 10.1002/j.1538-7305.1948.tb01338.x.
53. Pielou EC. The measurement of diversity in different types of biological collections. J Theor Biol. 1966;13:131-44. doi: 10.1016/0022-5193(66)90013-0.
54. Fager EW, McGowan JA. Zooplankton species groups in the North Pacific: co-occurrences of species can be used to derive groups whose members react similarly to water-mass types. Science. 1963;140(3566):453-60. doi: 10.1126/science.140.3566.453.
55. Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E, Abai MR, Mobedi I, Linton YM, et al. 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. 2009;23(2):111-21. doi: 10.1111/j.1365-2915.2009.00802.x.
56. Azari-Hamidian S, Norouzi B, Noorallahi A, Ali Hanafi-Bojd A. Seasonal activity of adult mosquitoes (Diptera: Culicidae) in a focus of dirofilariasis and West Nile Infection in northern Iran. J Arthropod Borne Dis. 2018;12(4):398-413.
57. Moosa-Kazemi SH, Zaim M, Zahraee A. Fauna and ecology of Culicidae of the Zarrin-Shahr and Mobarakeh area in Isfahan province. Armaghane Danesh. 2000;5(17-18):46-54. [Persian].
58. Ghavami MB, Ladonni H. The fauna and frequency of different mosquito species (Diptera: Culicidae) in Zanjan province. Journal of Zanjan University of Medical Sciences and Health Services. 2006;13(53):46-54. [Persian].
59. Abai MR, Azari-Hamidian S, Ladonni H, Hakimi M, Mashhadi-Esmail K, Sheikhzadeh K, et al. Fauna and checklist of mosquitoes (Diptera: Culicidae) of East Azerbaijan province, northwestern Iran. J Arthropod Borne Dis. 2007;1(2):27-33.
60. Harbach RE. The mosquitoes of the subgenus *Culex* in southwestern Asia and Egypt (Diptera: Culicidae). Contrib Am Entomol Inst. 1988;24(1):1-240.
61. Golestani J. The methods of the mosquito *Culex* control in Tehran city. J General Med Tehran Univ Med School. 1967;6:376-9. [Persian].
62. Lotfi M. Key to Culicinae of Iran, genus *Culex* and their biology (Diptera: Culicidae). Iran J Public Health. 1976;5:71-84.
63. Khoobdel M, Keshavarzi D, Moosa-Kazemi SH, Sobati H. Species diversity of mosquitoes of the Genus *Culex* (Diptera, Culicidae) in the coastal areas of the Persian Gulf. AIMS Public Health. 2019;6(2):99-106. doi: 10.3934/publichealth.2019.2.99.
64. Sanei-Dehkordi A, Soleimani-Ahmadi M, Jaberhashemi SA, Zare M. Species composition, seasonal abundance and distribution of potential anopheline vectors in a malaria endemic area of Iran: field assessment for malaria elimination. Malar J. 2019;18(1):157. doi: 10.1186/s12936-019-2795-x.
65. Willig MR, Presley SJ. Latitudinal gradients of biodiversity. In: Levin SA, ed. Encyclopedia of Biodiversity. 2nd ed. Elsevier Inc; 2013. p. 612-26.
66. Sanders NJ, Rahbek C. The patterns and causes of elevational diversity gradients. Ecography. 2012;35(1):1-3. doi: 10.1111/j.1600-0587.2011.07338.x.
67. Rahbek C. The elevational gradient of species richness: a uniform pattern? Ecography. 1995;18(2):200-5. doi: 10.1111/j.1600-0587.1995.tb00341.x.
68. El-Naggar AN, Elbanna SM, Kaiser MF, Gabre RM. Mosquito larval habitat mapping using remote sensing and GIS for monitoring the filarial infection regions in Alkorin village, Sharkia Governorate (Egypt). Int J Mosq Res. 2017;4(4):135-9.
69. Clements AN. The Biology of Mosquitoes: Development, Nutrition and Reproduction. London: Chapman & Hall; 1992.
70. Abdel-Hamid YM, Mostafa AA, Allam KM, Kenawy MA. Mosquitoes (Diptera: Culicidae) in El Gharbia Governorate, Egypt: their spatial distribution, abundance and factors affecting their breeding related to the situation of lymphatic filariasis. Egypt Acad J Biol Sci E Med Entomol Parasitol.

2011;3(1):9-16. doi: 10.21608/eajbse.2011.16452.

71. Sasikumar PS, Suryanarayanan P, Thomas C, Kalyanaraman K, Prasad RS. Influence of certain physico-chemical factors upon the larval population of *Mansonia* mosquitoes (Culicidae: Diptera) in Trivandrum city, India. Proc Indian Acad Sci. 1986;95(5):549-55. doi: 10.1007/bf03179417.
72. Olayemi IK, Omalu IC, Famotele OI, Shegna SP, Idris B. Distribution of mosquito larvae in relation to physico-chemical characteristics of breeding habitats in Minna, north central Nigeria. Rev Infect. 2010;1(1):49-53.
73. Dida GO, Anyona DN, Abuom PO, Akoko D, Adoka SO, Matano AS, et al. Spatial distribution and habitat characterization of mosquito species during the dry season along the Mara River and its tributaries, in Kenya and Tanzania. Infect Dis Poverty. 2018;7(1):2. doi: 10.1186/s40249-017-0385-0.
74. Grech MG, Manzo LM, Epele LB, Laurito M, Claverie A, Ludueña-Almeida FF, et al. Mosquito (Diptera: Culicidae) larval ecology in natural habitats in the cold temperate Patagonia region of Argentina. Parasit Vectors. 2019;12(1):214. doi: 10.1186/s13071-019-3459-y.
75. Nejati J, Bueno-Marí R, Collantes F, Hanafi-Bojd AA, Vatandoost H, Charrahy Z, et al. Potential risk areas of Aedes albopictus in south-eastern Iran: a vector of Dengue fever, Zika, and Chikungunya. Front Microbiol. 2017;8:1660. doi: 10.3389/fmicb.2017.01660.
76. Mahgoub MM, Kweka EJ, Himeidan YE. Characterisation of larval habitats, species composition and factors associated with the seasonal abundance of mosquito fauna in Gezira, Sudan. Infect Dis Poverty. 2017;6(1):23. doi: 10.1186/s40249-017-0242-1.