Original Article

Larval Habitat Characteristics of Mosquitoes (Diptera: Culicidae) in Andimeshk County of Khuzistan Province, Southwestern Iran (2019–2020)

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Abstract

Background: Mosquitoes (Diptera: Culicidae), with their blood-sucking behavior, are known important vectors of certain pathogens. Since no research has been done on the habitat characteristics of mosquito larvae in Andimeshk County of Khuzistan Province, southwestern Iran, this study aimed to determine these features in the county during 2019–2020.

Methods: The third- and fourth-instar larvae were collected from natural and artificial habitats by the dipping method, from nine localities. Physical characteristics of larval habitats, including habitat situation (permanent/temporary, running/stagnant), sunlight situation, vegetation status, water turbidity, substrate type and habitat type (natural/artificial), were recorded. The collected specimens were kept in vials containing lactophenol and the microscope slides were prepared using Berlese's fluid. The larvae were identified using valid identification keys.

Results: In total, 1363 larvae, including four genera and 15 species, were identified. Eleven species were new to the county and one species, *Cx. bitaeniorhynchus*, was new to the province. The most common species were *Culex theileri* (17.2%) and *Cx. quinquefasciatus* (15.6 %), respectively, and the least species were *Anopheles dthali* (0.7%) and *An. multicolor* (0.4%). The most abundant mosquito larvae were in spring (44.2 %) and June (22.6%). Most of the larvae were caught from natural (75.6%), temporary (51.5%), without vegetation (57.5%) and full sunny (64.7%) habitats. Approximately, 85.2%, 56.5% and 78.1% of habitats had clear water, stagnant water with mud substrate, respectively. The affinity index of the species was calculated.

Conclusion: Regarding the medical importance of the abundant species, the investigation of adult ecology is suggested for future studies.

Keywords: Anophelinae; Culicinae; Fauna; Larval breeding places; Oviposition sites

Introduction

Mosquitoes (Diptera: Culicidae) are the most important insects in public health (1, 2). Causal agents of diseases transmitted by mosquitoes afflict millions of people in the world, which accounts for approximately 17% of the burden of parasitic and infectious diseases (3). The family includes two subfamilies: Anophelinae (with three genera) and Culicinae (with 38 genera across 11 tribes), encompassing 3727 described species (4). Among these, nearly 170 species are important in medicine and veterinary, with at least 43 species find in regions where they are not indigenous, including 12 considered invasive (2, 5).

In Iran, eight genera and 73 species of mosquitoes have been documented (6). The mosquito fauna of the country is of particular public health concern due to its association with a wide array of pathogens known to be transmitted, either biologically or mechanically, by these insects. These include two bacterial pathogens of anthrax and tularaemia, four helminthic parasites of dirofilariasis, setariasis, dipetalonemiasis and lymphatic filariasis, seven arboviruses of avian pox, bovine ephemeral fever, Chikungunya, dengue fever, Sindbis fever, Rift Valley fever and West Nile fever and two protozoal parasites of human and avian malaria (7, 8).

Seven *Anopheles* species are the proven vectors of malaria, including *An. culicifacies* s.l., *An. dthali*, *An. fluviatilis* s.l., *An. maculipennis* s.l., *An. sacharovi*, *An. stephensi* and *An. superpictus* s.l. (7). Meanwhile, *An. pulcherrimus* has been reported as a potential vector of malaria in southeastern Iran (9). Also, the oocysts of *Plasmodium* have been found in *An. multicolor*, but sporozoites have not been observed in this species (10). *Anopheles hyrcanus* has been introduced as a possible vector of malaria in northern Iran (11). Beyond malaria, arboviral activity is evidenced by the detection of Sindbis virus in *Culex pipiens* and *Cx. theileri* in northwestern Iran (12) and West Nile fever virus in five species of *Aedes caspius* s.l., *Cx. hortensis*, *Cx. pipiens*, *Cx. theileri* and *Culiseta longiareolata* in the north and northwest of the country (13–16). The Chikungunya fever virus was detected in *An. maculipennis* s.l., *Cx. tritaeniorhynchus* and *Cs. longiareolata* in northeastern Iran using primers de-

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signed for CHIKV Asian genotype; however, it failed to isolate the virus and whole genome sequencing was not performed (17). Moreover, *An. maculipennis* and *Cx. theileri* vector the parasitic helminths of setariasis and dirofilariasis in northwestern Iran, respectively (18).

Female mosquitoes usually select specific and suitable breeding places for oviposition to develop their larval and pupal stages. Water is essential to develop their aquatic stages. These larval habitats are not selected randomly. Each species has its own special biology. Key physical factors, such as water flow velocity, kind of habitat substrate, habitat access to light, temperature, electric conductivity (EC), turbidity, salinity and acidity (pH), define suitable larval breeding sites. Biological factors, including the presence of predators, vegetation, algae, zooplankton, microbial communities and phytoplankton, further influence habitat selection and larval development (19–28). Moreover, various organic and inorganic chemical compounds may influence the selection of larval habitat by female mosquitoes (29). Understanding these ecological determinants is fundamental for predicting species distribution and implementing targeted vector control.

By now, five genera and at least 34 mosquito species have been recorded in Khuzistan Province (11, 19, 28, 30–42). This region is endemic or receptive for several mosquito-borne diseases, including avian pox, bovine ephemeral fever, Sindbis fever, West Nile fever, anthrax, tularaemia, dirofilariasis, setariasis and malaria (for more details, see 7, 8, 43–45). Recent climatic shifts, particularly altered precipitation patterns in Khuzistan Province, have likely modified larval breeding site availability and disease transmission dynamics, underscoring the need for updated entomological surveillance. The data for the mosquitoes of Andimeshk County within the province remain exceptionally scarce, with only four species, *An. dthali*, *An. superpictus*, *An. turkhudi* and *Cx. pereximguus*, have been previously recorded (33, 35, 36). Given this significant knowledge gap and the region's vulnerability to vector-borne disease, this study was conducted to determine the mosquito fauna and characterize the physical properties of larval habitats in Andimeshk County from 2019 to 2020. The findings establish a crucial ecological baseline for informed vector management and risk assessment.

Materials and Methods

Study area

This cross-sectional study was conducted from April 2019 to February 2020 in Andimeshk County, located in the northern part of Khuzistan Province, southwestern Iran (center coordinates: latitude 32° 29'N, longitude 48° 22'E) (Fig. 1). The county features a semi-arid climate characterized by extremely hot summers and mild winters. Annual precipitation, which is comparatively higher than in much of southern Iran, falls almost exclusively between November and April. Monthly rainfall can exceed 250 mm during this period, with annual totals occasionally surpassing 600 mm. Sampling was performed across nine localities to capture diverse ecological settings: seven rural villages and two urban areas. These sites were selected to represent distinct topographic zones: mountainous area (the villages of Shirin-Ab, Ghalam-Ab, Mongreh, Ghaleh-Roze, Varan-Toshmal) and plain area (the villages of Sabz-Ab and Ali-Abad, along with Azadi City and Andimeshk City). The topography, geographic coordinates and altitude of each sampling locality are detailed in Table 1.

Sampling, identification and larval habitat characteristics

Third- and fourth-instar mosquito larvae were collected from aquatic habitats using the standard dipping method. At each larval breeding site, 10 dips were taken from various parts of the habitat to ensure a representative sample. The following physical and ecological characteristics of each larval habitat were recorded on standardized field forms: habitat persistence (temporary or permanent), water flow (slow-running or stagnant), sunlight exposure (full sun, partial shade or full shade), presence of vegetation (with or without vegetation), water turbidity (clear or turbid), substrate type (mud, sand or other) and habitat type (natural or artificial). Following collection, the larvae from each site were rinsed with distilled water and conserved in vials containing lactophenol solution and transported to the laboratory. The larvae were kept in this solution for at least two days to become clear. Finally, the microscope slides of the third- and fourth-instar larvae were prepared using Berlese's solution and specimens were studied by means of the microscope. The authors morphologically identified the specimens using taxonomic keys (6, 46).

Dominance structure and Fager's index of affinity

The dominance structure for each species was defined as the percentage of the species specimens in the total collected sample. The following five categories are used as measures (percentage) of eudominat species (>30%), dominant (10–30%), subdominant (5–10%), recedent (1–5%) and subrecedent (<1%) (47, 48). Interspecific habitat associations were quantified using Fager's index of affinity. The index (I_{AB}) for species pairs A and B was calculated using the formula $I_{AB}=2J/n_A+n_B$, where J= number of joint occurrences (that is, the number of

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habitats where both species were found together), n_A= total number of occurrences of species A and n_B= total number of occurrences of species B (49). A threshold value of $I_{AB} \geq 0.50$ was used to identify significant species associations within larval habitats. This criterion aligns with the recommendation of Fager and McGown (49) who stated, "This breakpoint was chosen because it was felt that species should be found together in somewhat more than 'half' their recorded occurrences if they are to be grouped".

Results

Overall, 1363 larvae representing four genera and 15 species were collected from 25 larval habitats across the nine sampling localities. Most larval habitats (91%) were located in rural areas, with the remaining 9% in urban settings. The collected fauna comprised six species of *Anopheles*, one of *Aedes*, seven of *Culex* and one of *Culiseta*. The most abundant species were *Culex theileri* (n=235, 17.2%) and *Cx quinquefasciatus* (n=213, 15.6%), both classified as dominant species. The least abundant were *An. dthali* (n=9, 0.7%) and *An. multicolor* (n=6, 0.4%), categorized as subrecedent species. Among culicine species, *Ae. caspius* s.l. was the least abundant (n=34, 2.5%), while among anophelines, *An. stephensi* (n=92, 6.7%) and *An. pulcherrimus* (n=84, 6.2%) were the most abundant. *Culex theileri* (Culicinae) and *An. stephensi* (Anophelinae) were the most widely distributed species across sampling sites. The maximum prevalence of larvae was obtained in spring (n=603, 44.2%), followed by summer (n=426, 31.3%), autumn (n=319, 23.4%) and winter (n=15, 1.1%). The relative abundance and dominance structure by season and species distribution by locality were presented in Tables 2 and 3.

Analysis of co-occurrence in larval habitats revealed that *Cx. theileri* was present in 15 habitats and co-collected with all other species, whereas *An. multicolor* was collected from only two sites associated with eight other species. Using Fager's Index of Affinity (threshold ≥ 0.50), significant species associations were identified for the following 27 species pairs (Table 4): *Cx. theileri*/*Cs. longiareolata*, *Cx. theileri*/*Cx. perexiguus*, *Cx. theileri*/*Cx. hortensis*, *Cx. quinquefasciatus*/*Cx. pusillus*, *Cx. quinquefasciatus*/*Cx. tritaeniorhynchus*, *Cx. quinquefasciatus*/*Cx. perexiguus*, *Cx. quinquefasciatus*/*Cx. hortensis*, *Cx. quinquefasciatus*/*An. stephensi*, *Cx. pusillus*/*Cx. tritaeniorhynchus*, *Cx. pusillus*/*Cx. perexiguus*, *Cx. pusillus*/*Cx. hortensis*, *Cx. pusillus*/*Ae. caspius* s.l., *Cs. longiareolata*/*Cx. tritaeniorhynchus*, *Cx. tritaeniorhynchus*/*Cx. bitaeniorhynchus*, *Cx. tritaeniorhynchus*/*An. pulcherrimus*, *Cx. tritaeniorhynchus*/*An. superpictus*, *Cx. bitaeniorhynchus*/*Ae. caspius* s.l., *Cx. hortensis*/*An. stephensi*, *Cx. hortensis*/*An. superpictus*, *Cx. hortensis*/*An. dthali*, *Ae. caspius* s.l./*An. stephensi*, *Ae. caspius* s.l./*An. multicolor*, *An. stephensi*/*An. pulcherrimus*, *An. stephensi*/*An. superpictus*, *An. stephensi*/*An. multicolor*, *An. superpictus*/*An. turkhudi* and *An. superpictus*/*An. dthali*. All co-occurrence frequencies and calculated Fager's indices were presented in Table 4.

Most of the mosquito larvae (75.6%) were collected from natural oviposition sites, including rain pools, grasslands, river edges, and river bed pools. The remaining 24.7% were from artificial sites, including waters beside the palm trees, irrigation channels, rice fields, discarded tires and domestic containers (such as buckets and ponds inside houses).

Habitat characterization revealed a strong preference for specific larval breeding conditions. Most mosquito larvae were collected in natural larval habitats (75.6%), with temporary water (51.5%), still water (56.5%), exposed to sunlight (64.7%), without vegetation (57.5%), clear water (85.2%) and muddy substrate (78.1%). The larval habitat features of different species were displayed in Table 5. Some representative larval habitats are shown in Fig. 2. Larval activity of the species began in April and ended in February. Mosquito larvae had two peaks of activity during June and September. No larvae were collected during February and March (Fig. 3).

Table 1. Data collection of mosquito larvae for the localities in Andimeshk County of Khuzistan Province, southwestern Iran, 2019–2020

Locality/Station	Topography	Coordinates	Altitude (m)
Shirin-Ab Village	mountainous	N 32º 49' 7.774", E 48º 30' 32.663"	493
Ghalam-Ab Village	mountainous	N 32º 44' 2.197", E 48º 30' 36.391"	443
Mongreh Village	mountainous	N 32º 48' 32.269", E 48º 18' 4.762"	533
Ghaleh-Roze Village	mountainous	N 32º 47' 13.555", E 48º 8' 48.079"	399
Varan-Toshmal Village	mountainous	N 32º 43' 58.109", E 48º 13' 42.258"	435
Sabz-Ab Village	Plain	N 32º 20' 8.298", E 48º 16' 49.896"	102
Ali-Abad Village	Plain	N 32º 17' 38.158", E 48º 16' 27.792"	91
Andimeshk City	Plain	N 32º 27' 33.557", E 48º 21' 20.622"	146
Azadi City	Plain	N 32º 23' 56.984", E 48º 15' 8.674"	111

Table 2. Fauna, relative abundance and dominance structure of mosquito larvae according to various seasons in Andimeshk County of Khuzistan Province, southwestern Iran, 2019–2020 (The species were arranged based on total numbers, percentages and dominance structure in Culicinae and Anophelinae, respectively)

Seasons Species	Spring No (%)	Summer No (%)	Autumn No (%)	Winter No (%)	Total No (%)	Dominance structure
Culex theileri	111 (18.4)	69 (16.1)	51 (15.9)	4 (26.7)	235 (17.2)	Dominant
Cx. quinquefasciatus	80 (13.3)	63 (14.8)	68 (21.3)	2 (13.3)	213 (15.6)	Dominant
Cx. pusillus	64 (10.6)	36 (8.4)	29 (9.1)	-	129 (9.5)	Subdominant
Culiseta longiareolata	70 (11.6)	35 (8.2)	10 (3.1)	-	115 (8.4)	Subdominant
Cx. tritaeniorhynchus	46 (7.6)	39 (9.1)	21 (6.6)	-	106 (7.8)	Subdominant
Cx. bitaeniorhynchus	48 (8.0)	32 (7.5)	21 (6.6)	2 (13.3)	103 (7.5)	Subdominant
Cx. perexiguus	46 (7.6)	26 (6.1)	30 (9.4)	-	102 (7.5)	Subdominant
Cx. hortensis	24 (4.0)	21 (5.0)	12 (3.8)	-	57 (4.2)	Recedent
***Aedes caspius* s.l.**	18 (3.0)	11 (2.6)	5 (1.6)	-	34 (2.5)	Recedent
Anopheles stephensi	30 (5.0)	27 (6.3)	31 (9.7)	4 (26.7)	92 (6.7)	Recedent
An. pulcherrimus	26 (4.3)	34 (8.0)	22 (7.0)	2 (13.3)	84 (6.2)	Recedent
An. superpictus	33 (5.5)	21 (5.0)	9 (2.8)	1 (6.7)	64 (4.7)	Recedent
An. turkhudi	5 (0.8)	5 (1.2)	4 (1.2)	-	14 (1.1)	Recedent
An. dthali	-	4 (1.0)	5 (1.6)	-	9 (0.7)	Subrecedent
An. multicolor	2 (0.3)	3 (0.7)	1 (0.3)	-	6 (0.4)	Subrecedent
Total	603 (44.2)	426 (31.3)	319 (23.4)	15 (1.1)	1363 (100)	

Table 3. The distribution of mosquito larvae collected in different regions of Andimeshk County of Khuzistan Province, southwestern Iran, 2019–2020 (+ present, - absent)

Locality Species	Shirin-Ab	Ghalam-Ab	Mongreh	Ghaleh-Roze	Varan-Toshmal	Sabz-Ab	Ali-Abad	Andimeshk	Azadi
Culex theileri	+	+	+	+	+	-	-	+	+
Cx quinquefasciatus	-	+	+	-	-	+	-	+	+
Cx. pusillus	-	-	-	-	-	+	+	-	+
Culiseta longiareolata	+	+	+	+	+	-	-	-	-
Cx. tritaeniorhynchus	-	-	-	+	-	-	+	+	-
Cx. bitaeniorhynchus	-	-	-	-	-	+	+	-	+
Cx. perexiguus	+	-	+	-	-	+	+	+	-
Cx. hortensis	+	-	-	-	+	+	-	-	-
***Aedes caspius* s.l.**	-	-	-	-	-	+	+	-	-
Anopheles stephensi	-	-	+	-	+	+	+	-	-
An. pulcherrimus	-	-	-	-	-	+	+	-	-
An. superpictus	+	-	+	-	-	+	-	-	-
An. turkhudi	+	-	+	-	+	-	-	-	-
An. dethali	-	-	+	-	+	-	-	-	-
An. multicolor	+	-	-	+	-	-	-	-	-

Table 4. Co-occurrences (Lower part) and Fager's index of affinity (Upper part) of mosquito larvae in Andimeshk County of Khuzistan Province, southwestern Iran, 2019–2020 (Dash [-] means the pair of species did not occur together, bold number means significant affinity)

Fager's index of affinity Species co-occurrences	No. of Larval habitat	*Culex theileri*	*Cx. quinquefasciatus*	*Cx. pusillus*	*Culiseta longiareolata*	*Cx. tritaeniorhynchus*	*Cx. bitaeniorhynchus*	*Cx. perexiguus*	*Cx. hortensis*	*Aedes caspius* s.l.	*Anopehles stephensi*	*An. pulcherrimus*	*An. superpictus*	*An. turkhudi*	*An. dthali*	*An. multicolor*
Culex theileri	15	*	0.43	0.24	**0.58**	0.20	0.10	**0.69**	**0.57**	0.28	0.47	0.33	0.28	0.11	0.31	0.23
Cx. quinquefasciatus	8	5	*	**0.77**	0.47	**0.61**	0.33	**0.75**	**0.71**	0.42	**0.85**	0.47	-	0.18	-	0.40
Cx. pusillus	10	3	7	*	0.42	**0.66**	0.42	**0.88**	**0.50**	**0.75**	0.37	-	0.37	0.30	-	-
Culiseta longiareolata	9	7	4	4	*	**0.71**	0.46	0.47	0.40	-	0.26	0.33	0.26	0.16	-	0.18
Cx. tritaeniorhynchus	5	2	4	5	5	*	**0.88**	0.46	0.36	0.18	0.36	**0.57**	**0.72**	-	0.18	-
Cx. bitaeniorhynchus	4	1	2	3	3	4	*	0.33	0.40	**0.60**	-	-	0.40	0.28	-	0.33
Cx. perexiguus	8	8	6	8	4	3	2	*	0.42	0.28	0.42	0.23	0.28	-	0.16	-
Cx. hortensis	6	6	5	4	3	2	2	3	*	-	**0.66**	0.26	**0.50**	0.44	**0.60**	0.25
***Aedes caspius* s.l.**	6	3	3	6	-	1	3	2	-	*	**0.50**	0.40	0.33	-	0.20	**0.50**
Anopheles stephensi	6	5	6	3	2	2	-	3	4	3	*	**0.80**	**1.0**	-	0.40	**0.50**
An. pulcherrimus	9	4	4	-	3	4	-	2	2	3	6	*	0.26	0.33	-	0.18
An. superpictus	6	3	-	3	2	4	2	2	3	2	6	2	*	**0.66**	**0.80**	-
An. turkhudi	3	1	1	2	1	-	1	-	2	-	-	2	3	*	0.28	-
An. dthali	4	3	-	-	1	1	-	1	3	1	2	-	4	1	*	-
An. multicolor	2	2	2	-	1	-	1	-	1	2	2	1	-	-	-	*

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Fig. 1. Location of Andimeshk County of Khuzistan Province, southwestern Iran, for mosquito sampling, 2019–2020

Fig. 2. Some active larval habitats of mosquitoes in Andimeshk County of Khuzistan Province, southwestern Iran, 2019–2020

Table 5. Larval habitat characteristics and occurrence percentages (%) of mosquito larvae in Andimeshk County of Khuzistan Province, southwestern Iran, 2019–2020

Species / Larval habitats characteristics		*Culex theileri*	*Cx. quinquefasciatus*	*Cx. pusillus*	*Culiseta longiareolata*	*Cx. tritaeniorhynchus*	*Cx. bitaeniorhynchus*	*Cx. perexiguus*	*Cx. hortensis*	*Aedes caspius* s.l.	*Anopheles stephensi*	*An. pulcherrimus*	*An. superpictus*	*An. turkhudi*	*An. dthali*	*An. multicolor*	Total
Habitat persistence	**Permanent**	106 (45.1)	72 (33.8)	107 (82.9)	37 (32.2)	69 (65.1)	40 (38.8)	64 (62.7)	40 (70.1)	9 (26.5)	63 (68.5)	20 (23.8)	17 (26.6)	12 (85.7)	5 (55.5)	-	661 (48.5)
	Temporary	129 (54.9)	141 (66.2)	22 (17.1)	78 (67.8)	37 (34.9)	63 (61.2)	38 (37.3)	17 (29.9)	25 (73.5)	29 (31.5)	64 (76.2)	47 (73.4)	2 (14.3)	4 (44.5)	6 (100)	702 (51.5)
Water flow condition	**Running water**	68 (28.9)	176 (82.6)	87 (67.4)	20 (17.4)	17 (16.1)	86 (83.5)	38 (37.3)	18 (31.5)	-	57 (61.9)	7 (8.3)	16 (24.9)	1 (7.1)	2 (22.2)	-	593 (43.5)
	Stagnant water	167 (71.1)	37 (17.4)	42 (32.6)	95 (82.6)	89 (83.9)	17 (16.5)	64 (62.7)	39 (68.5)	34 (100)	35 (38.1)	77 (91.7)	48 (75.1)	13 (92.9)	7 (77.8)	6(100)	770 (56.5)
Sunlight situation	**Full sunlight**	167 (71.1)	125 (58.6)	94 (72.8)	85 (73.9)	61 (57.5)	62 (60.1)	63 (61.7)	15 (26.3)	27 (79.4)	57 (61.9)	55 (65.4)	51 (79.6)	10 (71.4)	7 (77.8)	3 (49.9)	882 (64.7)
	Partial sunlight	35 (14.8)	40 (18.7)	27 (20.9)	20 (17.3)	25 (23.6)	22 (21.3)	27 (26.4)	25 (43.8)	2 (5.8)	10 (10.8)	10 (11.9)	13 (20.4)	2 (14.3)	2 (22.2)	2 (33.3)	262 (19.2)
	Shaded	33 (14.1)	48 (22.7)	8 (6.3)	10 (8.8)	20 (18.9)	19 (18.6)	12 (11.9)	17 (29.9)	5 (14.8)	25 (27.3)	19 (22.7)	-	2 (14.3)	-	1 (16.8)	219 (16.1)
Vegetation situation	**With vegetation**	75 (31.9)	151 (70.8)	41 (31.7)	95 (82.6)	28 (26.4)	21 (20.3)	27 (26.5)	39 (68.4)	4 (11.7)	61 (66.4)	10 (11.9)	47 (73.4)	3 (21.4)	5 (55.5)	2 (33.3)	579 (42.5)
	Without vegetation	160 (68.1)	62 (29.2)	88 (68.3)	20 (17.4)	78 (73.6)	82 (79.7)	75 (73.5)	18 (31.6)	30 (88.3)	31 (33.6)	74 (88.1)	17 (26.6)	11 (78.6)	4 (44.5)	4 (66.7)	784 (57.5)
Water situation	**Clear**	206 (87.6)	159 (74.6)	119 (92.2)	101 (87.8)	90 (84.9)	97 (94.1)	93 (91.1)	42 (73.6)	29 (85.2)	75 (81.5)	70 (83.3)	53 (82.8)	14 (100)	7 (77.8)	6 (100)	1161 (85.2)
	Turbid	29 (12.4)	54 (25.4)	10 (7.8)	14 (12.2)	16 (15.1)	6 (5.9)	9 (8.9)	15 (26.4)	5 (14.8)	17 (18.5)	14 (16.7)	11 (17.2)	-	2 (22.2)	-	202 (14.8)
Substrate	**Mud**	166 (70.6)	159 (74.6)	104 (80.6)	94 (81.7)	88 (83.1)	71 (68.9)	85 (83.3)	44 (77.1)	26 (76.4)	75 (81.5)	74 (88.1)	53 (81.5)	14 (100)	6 (66.7)	6 (100)	1065 (78.1)
	Sand	51 (21.7)	38 (17.8)	25 (19.4)	15 (13.1)	18 (16.9)	32 (31.1)	17 (16.7)	13 (22.9)	8 (23.6)	5 (5.4)	10 (11.9)	7 (5.4)	-	3 (33.3)	-	242 (17.8)
	Others	18 (7.7)	16 (7.6)	-	6 (5.2)	-	-	-	-	-	12 (13.1)	-	4 (13.1)	-	-	-	56 (4.1)
Habitat type	**Natural**	159 (67.7)	142 (66.6)	109 (84.4)	97 (84.3)	80 (75.4)	83 (80.5)	74 (72.5)	50 (87.7)	24 (70.5)	72 (78.2)	62 (73.8)	44 (68.7)	14 (100)	8 (88.8)	6 (100)	1031 (75.6)
	Artificial	76 (32.3)	71 (33.4)	20 (15.6)	18 (15.7)	26 (24.6)	20 (19.5)	28 (27.5)	7 (12.3)	10 (29.5)	20 (21.8)	22 (26.2)	20 (31.3)	-	1 (11.2)	-	332 (24.4)

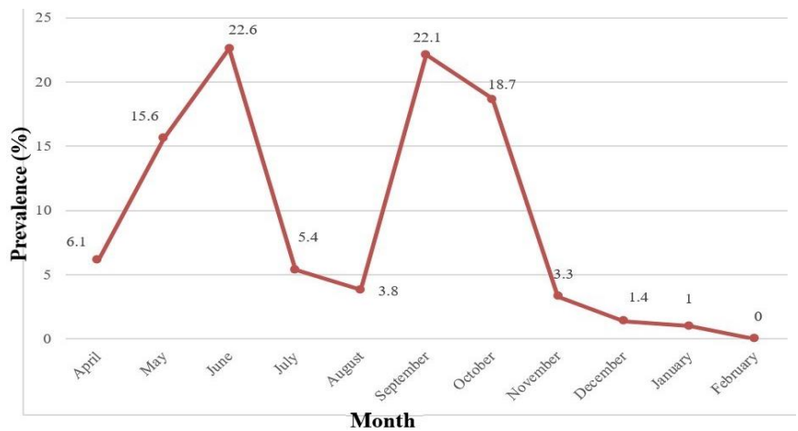

Fig. 3. Monthly prevalence (%) of collected mosquito larvae in Andimeshk County of Khuzistan Province, southwestern Iran, 2019–2020

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Discussion

This study documents the larval fauna of mosquitoes in Andimeshk County, identifying 15 species across four genera. Before this survey, only four species, *An. dthali*, *An. superpictus*, *An. turkhudi* and *Cx. perexiguus* had been recorded in the county (33, 35, 36). Thus, the remaining 11 species are new records for the mosquito fauna of the county. Moreover, *Cx. bitaeniorhynchus* was found for the first time in Khuzistan Province. The species was found in Bushehr, Fars, Hormozgan, Kerman, Kohgyluyeh and Boyerahmad and Sistan and Baluchistan Provinces before (19, 26, 28). Thus, the mosquito fauna of the province includes five genera and 35 species, including the old record of *Ae. aegypti* (30). There are some recent records of this invasive and important mosquito from the southern provinces of Iran (5, 6). It is noteworthy that the collection of the adult mosquitoes has not been a goal of the present study, which limits the results, especially for the species whose aquatic stages are rarely collected or choose very specific larval habitats. In addition to the dipping method for aquatic stages, using different methods of collection for the eggs (ovitraps) and for the adults (such as light trap, especially with Co_2-bait, hand catches, with or without baits and so on), with standardized protocols, will yield more results and new findings in the region. These data are basic knowledge on the fauna and ecology of different mosquito species, which is essential for a good control program (1).

Among the six anopheline species collected, three species of *An. dthali*, *An. stephensi* and *An. superpictus*, are proven vectors of malaria in Iran (7) while *An. pulcherrimus* is a probable vector (9). The culicine species collected include several species of known or suspected arboviral vectors: Sindbis virus is found in *Cx. pipiens* and *Cx. theileri* (12) and West Nile virus in *Ae. caspius* s.l., *Cx. hortensis*, *Cx. pipiens*, *Cx. theileri* and *Cs. longiareolata* (13–16). Also, *Cx. theileri* is a known vector of dirofilariasis (18). Regarding the vector potential of these species and other species recorded in this study, the established presence of the associated pathogens in Khuzistan Province and the regional threat of emerging arboviral diseases such as dengue and chikungunya, these findings underscore a tangible public and veterinary health risk (7, 8, 43–45). Therefore, it is strongly recommended that the health authorities pay serious attention to applying the intensive surveillance and control programs for the vectors and the related vector-borne diseases in the county and the province.

In this investigation, *Cx. theileri* and *Cx. quinquefasciatus* showed the most abundance among the whole collection and *An. stephensi* and *An. pulcherrimus* among anopheline larvae (Table 2). In two investigations on the larvae and females of mosquitoes in the Shadegan Wetland of Khuzistan Province, *Ae. caspius* s.l. was the most prevalent species (37, 39). This species was recedent in the present study (Table 2). In a study in Mahshahr County of the province (40), the larvae of *An. stephensi* and *Cx. quinquefasciatus* showed the most abundance. In the aforementioned study, *Cx. theileri* was not collected and *An. pulcherrimus* was found in a few numbers. In an investigation on anopheline mosquitoes in Izeh County of the province, the larvae of *An. superpictus* and *An. dthali* were the most prevalent species in the plain area and mountainous area, respectively, whereas *An. stephensi* was the second most abundant species in both plain and mountainous areas (41). In Luristan Province, north of Khuzistan Province, *An. superpictus* showed the most abundant among anopheline larvae (22–24). This variation is most probably because of different geographical locations, which are influenced by altitude and temperature and sampling. Data are not enough in this regard in Khuzistan Province.

Although 27 pairs of species showed significant affinity in this study (Table 4), this result should be carefully taken into consideration. Because some species have rarely been found and considered recedent and subrecedent, the affinity data probably are more accurate for dominant and subdominant species (Table 2). There are not many data about the affinity of mosquito species in larval habitats in southern Iran. In this regard, to the best of the authors' knowledge, there are no data in Khuzistan Province. In two studies in Hormozgan Province, the affinity of anopheline and culicine larvae was investigated (21, 26). In the study on anopheline larvae, the pairs of *An. dthali*/*An. stephensi* and *An. dthali*/*An. superpictus* showed significant affinity (21). In the present investigation, just *An. dthali*/*An. superpictus* showed significant affinity (0.80) (Table 4). It should be noticed that *An. superpictus* was recedent and *An. dthali* was subrecedent (Table 2). The significant affinity may show the similar biological requirements of two species and when there is no correlation, this may be due to different requirements of them (21, 26, 49).

There ara not many data about larval habitat characteristics in Khuzistan Province. In a study that recorded *Ae. vittatus* and *Cs. subochrea* new to the province fauna, some physical factors of the water of the Shadegan Wetland were presented, such as temperature, acidity, dissolved oxygen, salinity, hardness, total dissolved solids, total suspended solids, turbidity and electrical conductivity, but no cor-

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relation was calculated and analyzed among the collected species and these factors (39). In a study in Sistan and Baluchistan Province, significant correlations were observed between the following anopheline larvae with certain physicochemical features: *An. culicifacies* s.l. (Phosphate, EC and calcium), *An. stephensi* (Nitrate), *An. superpictus* s.l. (Total hardness, calcium), *An. turkhudi* (pH, total hardness, nitrate, calcium) and *An. multicolor* (pH, sulphate) (20). An investigation on anopheline larvae in Hormozgan Province showed that there was a significant correlation between temperature, pH, total alkalinity, conductivity, chloride, sulphate and larval density (25), whereas, in another study on culicine larvae in the province, there were no significant correlations between physicochemical factors and the presence of larvae (26). In a study in Chaharmahal and Bakhtiari Province, pH, dissolved oxygen and temperature significantly differed between the larval breeding sites of different mosquito species (27). According to a systematic review and meta-analysis, pH, turbidity, electrical conductivity, dissolved oxygen, nitrogen and phosphorus displayed a significant positive pooled correlation with mosquito presence and abundance, while alkalinity showed a nonsignificant null pooled correlation (29).

In an investigation on anopheline larvae in Izeh County of Khuzistan Province, the most prevalent species in the plain area, *An. superpictus*, was mostly collected from riversides (33%) and springs (20%), whereas in the mountainous area, the most abundant species, *An. dthali*, was mainly collected from stagnant waters such as waste water, drainage, borrow pits, as well as rice fields (32%) and springs (30%) (41). In this regard, much more data should be provided from Khuzistan Province to better analyze and compare with similar data from other parts of Iran, especially neighboring provinces with similar climate and species.

In the present study, larval activity began in April and ended in February, with two peaks of the larval collection during June and September (Fig. 3). There is not much information about the seasonal activity of mosquitoes in Khuzistan Province. In a study on anopheline females in Izeh County of the province (41), the activity of four species began in April and ended in January, with usually two peaks in May–July and August–October, depending on the species, indoor/outdoor collections, or mountainous/plain areas of collections. Interestingly, in Luristan Province, north of Khuzistan Province, anopheline larvae showed one peak of activity in the first or second half of August (22, 23). This variation is most probably because of geographical and climate differences, influenced by altitude and temperature.

Due to the diversity of climate in different areas of Adimeshk County, the mosquito fauna is rich compared to other counties. In the present study, the mosquito larvae were relatively abundant due to the lack of spraying and larviciding operations in the region, the presence of abundant and suitable larval habitats, the presence of abundant resting places for adult mosquitoes and the presence of livestock that provide sufficient blood sources for adult mosquitoes.

Conclusion

This study provides the first comprehensive survey of mosquito larval habitats and fauna in Andimeshk County, Khuzistan Province. The findings of this research confirm the presence of multiple medically and veterinary important vectors distributed in the county due to the existence of regions prone to rearing and growth of the larvae. The findings necessitate effective surveillance and control programs to mitigate public health risks. To inform these efforts, results should be expanded province-wide and future studies must include detailed bionomic, molecular and ecological research-such as species identification, insecticide resistance, pathogen detection and analysis of water chemistry and predators in larval habitats.

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Ethical considerations

This study was approved and registered by the Ethics Committee of Research at Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (IR.AJUMS.REC.1398.577).

Conflict of interest statement

The authors declare there is no conflict of interest.

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