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Species composition and abundance of mosquito larvae in relation with their habitat characteristics in Mazandaran Province, northern Iran

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Abstract

Mosquitoes transmit a variety of diseases to humans. Their abundance and distribution are related to the characteristics of larval habitats. Mosquito larvae were collected from 120 natural and artificial habitats in 30 villages of 16 counties using standard 350 ml dippers and pipette, on a monthly basis from May–December 2014 in Mazandaran Province, northern Iran. Larval habitat characteristics were recorded separately, based on the conditions of the habitats (permanent or temporary, stagnant or running), type of habitats (natural or artificial), vegetation, exposure to sun, type of bed, water condition (clear or turbid), expanse (m), depth (cm, m) and temperature (°C) of habitats. The relationship between larval density and environmental variables was assessed by Chi-square tests. Totally, 19,840 larvae from three genera and 16 species were collected and identified. *Anopheles maculipennis s.l.* and *Culex pipiens* were the dominant species and collected with the highest density in plain areas. The highest number of larvae were collected from natural habitats (60.34%), including; river edge, marsh, pit and wetlands; with temporary and stagnant water, expanse of 0–5 m, depth of 1–25 cm, without plant, shadow-sun, muddy floor, turbid water, temperature 20–25°C and in sunny conditions. River edge and rice fields for *An. maculipennis s.l* and, wetlands and discarded tires for *Cx. pipiens*

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were the main larval habitats in the province. Statistical analysis revealed significant relation between occurrence of *An. maculipennis* s.l., *Cx. pipiens*, *Culex torrentium*, *Culex mimeticus* and *Cs. annulata* with each of the environmental variables ($P < 0.001$). These findings are essential in expanding our knowledge of the vectors ecology specially the type of habitat preference and will be beneficial in larval control programs.

Keywords: *Anopheles*, *Culex*, *Culiseta*, species composition, abundance, ecology, larval habitats, Mazandaran, Iran

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Introduction

Based on the recent classification, Culicidae comprises two subfamilies, 112 genera and 3549 species (Harbach, 2016). Over 100 species of mosquitoes are able to transmit different diseases to humans and other animals, the most important of which being malaria (Ndenga *et al.*, 2012). Seven species of *Anopheles* are present as vectors of malaria in Iran, among which *An. maculipennis*, *An. sacharovi* and *An. superpictus* are the main malaria vectors in western, northwestern and central plateau of the country (Hanafi-Bojd *et al.*, 2011). According to the latest malaria report, 1109 and 134 cases of malaria caused by *P. vivax* and *P. falciparum* respectively were reported from Iran in 2014 (WHO, 2015). Twenty-two cases of imported malaria were reported from Mazandaran Province during 2008–2012 (Ghaffari *et al.*, 2012).

A number of mosquito-borne viruses, including West Nile, Sindbis (Naficy & Saidi, 1970; Saidi *et al.*, 1976; Ahmadnejad *et al.*, 2011) and Dengue fever (Chinikar *et al.*, 2010, 2013; Mardani *et al.*, 2013) were reported from Iran. The mosquito-borne filarial nematodes, including *Dirofilaria* (dirofilariasis) and *Setaria* (setariasis) were reported from Iran (Oryan *et al.*, 2008; Azari-Hamidian *et al.*, 2009*b*).

Mosquitoes are extensively distributed throughout the world and their breeding sites are different in terms of quantity and quality of water and natural vegetation (Dehghan *et al.*, 2010). In some habitats, more than one species of mosquito can lay eggs, where they share food (Marcondes & Paterno, 2005; Nikookar *et al.*, 2016). Therefore, selection of suitable habitats by mosquitoes is important in their survival and population dynamics. Various studies have shown that mosquitoes are completely distinctive in choosing breeding places (Adeleke *et al.*, 2008; Liu *et al.*, 2012; Baak-Baak *et al.*, 2014). There are many resemblances and differences between mosquitoes oviposition behavior in artificial and nature habitats. *Anopheles*, *Sabethes*, *Toxorhynchites*, and *Wyeomyia* oviposit individually on the water surface, usually without touching it. *Coquillettidia*, *Culex* and *Culiseta* put their egg rafts directly on the water surface. *Aedes* deposit individual eggs on a layer above the water line and some species can also attach their eggs to vegetation under the water surface (Surendran & Ramasamy, 2005). Therefore, vegetation of mosquito larval habitats is important in the process of egg-laying and density.

Climate fluctuation accompanied by environmental changes in ecosystems such as agricultural activities and urbanization may cause destruction of certain mosquitoes main habitats and observing sylvatic species in urban areas with temperate climate. This phenomenon may occur for a mosquito such as *Ae. albopictus* that resided in many Mediterranean countries of Europe during the past decade and now has begun to transmit dengue viruses (Adeleke *et al.*, 2008; Baak-Baak *et al.*, 2014).

In Iran, preliminary studies were conducted on larval habitats of *An. apoci* by Marsh (1933), *An. dthali* by Manouchehri & Rohani (1975), *An. stephensi* by Zaini *et al.* (1975) and Manouchehri *et al.* (1976), *An. fluviatilis* by Eshghi *et al.* (1976). All of these studies were conducted on malaria vectors in the southern parts of Iran, followed by an investigation by Zaim (1987) on larval habitat characteristics of Culicinae in 24 provinces of Iran. In recent years, studies on larval habitats of *Anopheles* were followed by Vatandoost *et al.* (2004); Hanafi-Bojd *et al.* (2012) and Soleimani-Ahmadi *et al.* (2013, 2014) in malarious areas in Hormozgan Province and by Amani *et al.* (2014) in Luristan Province. Most of these studies are again limited to the larval habitats of *Anopheles* in the southern regions of the country.

In the northern parts of Iran, Larval habitat characteristics of *Anopheles*, *Culex* and *Culiseta* were investigated by Azari-Hamidian *et al.* (2005, 2007, 2011) in Guilan Province, Khoshdel-Nezamiha *et al.* (2014) in West Azerbaijan Province and Nikookar *et al.* (2015) in Neka County. There is no comprehensive study on larval habitat characteristics of Culicidae across the province; this is the first extensive investigation in this regard in Mazandaran Province.

As environmental management is a major intervention in larvae control programs (Hanafi-Bojd *et al.*, 2012), identification of active larval habitats with the vegetation therein will be essential in planning adequate mosquito control programs. Therefore, this study aimed to determine the species composition, distribution and some aspects of the larval ecology, including habitats characteristics and their relationship with larval density.

Material and methods

Study area

The study was conducted in Mazandaran Province, in the Caspian Sea littoral of northern Iran located between latitude 35°47′–36°35′N and longitude 50°34′–54°10′E. The study area has a population of approximately 3073, 943 and an area of 23,756 km^2, according to Mazandaran Census Report in 2011. The main occupations of the people are agriculture especially rice cultivation, horticulture and animal husbandry. Maximum and minimum temperatures and rainfall were 1.2–29.2°C and 0–755.6 mm in 2014, respectively. The moderate weather and Hyrcanian forests provide favorable conditions for the development of mosquitoes. Sari is the capital of the province and is located at an altitude of 123 m above sea level.

Specimen collection and identification

In total, 120 sentinel sites in 30 villages of 16 counties throughout the province were checked once a month during

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May–December 2014. The villages in each county were randomly selected based on ecological zones of woodland and plain. In each village, one fixed and three variable habitats within a radius of 1 km were chosen for sampling. The different environmental characteristics of the habitats were equally represented in each village.

Larvae were collected from natural and artificial sites using standard 350 ml dipper and pipette, they were kept in separate glass vials and transported to the laboratory for morphological identification. Approximately 10–30 dips were done in each larval habitat depending on their size. Samplings of the same breeding places were always performed by the same individual (100 members of staff of the Mazandaran Health Centers were recruited and adequately educated for the project) in the morning (09.00–12.00 h) or afternoon (14.00–17.00 h) for about 30 min at each larval habitat. Larval densities were calculated as the average number of larvae per ten dips (WHO, 1975).

Larval habitat characteristics, including conditions of habitat (permanent or temporary, stagnant or running), type of habitat (natural or artificial), vegetation (with or without plants), situation of sun (sunny or shade), type of bed (soil, stone, concrete), water condition (clear or turbid), expanse (0–5, 5.1–10, 10.1–15 m), depth (1–25 cm, 25.1–50 cm, 50.1–75 cm, 75.1 cm–1 m, 1.1–1.5 m, 1.51–2 m) and water temperature (5–10, 10.1–15, 15.1–20, 20.1–25, 25.1–30°C) (Gimnig *et al.*, 2001; Hanafi-Bojd *et al.*, 2012) were recorded separately in specific forms. Water temperature of each type of larval habitat was measured by a thermometer and the expanse and depth of each habitat by using a metal ruler on site. Third- and fourth-instar larvae were mounted by de Faure's medium and morphologically identified using the key for the mosquitoes of Iran (Shahgudian, 1960; Azari-Hamidian and Harbach, 2009*a*). Collected larvae were not reared up to adults.

Statistical analysis

Statistical analyses were executed by the IBM SPSS version 19 software (IBM Corporation, Armonk, NY). Chi-square (χ^2) analysis was used to determine the relationship between environmental variables and the occurrence of larvae in different habitats, and results were considered significant when $P < 0.05$.

Results

A total of 19,840 Culicidae larvae belonging to 16 species and three genera were collected and morphologically identified. Of these, 1267 (6.38% of total larvae) anopheline larvae and 18,573 (93.62% of total larvae) culicine larvae were collected from 120 larval habitats.

The highest number and percentage of *Anopheles* were collected in Sari ($n = 285$, 22.49% of total *Anopheles*) followed by Ramsar ($n = 255$, 20.12% of total *Anopheles*), while the lowest was in Noshahr County ($n = 35$, 2.18% of total *Anopheles*). Surprisingly, no *Anopheles* mosquitoes were caught in the Counties of Ghaemshahr, Juybar, Babolsar, Fereydunkenar and Mahmudabad.

Anopheles maculipennis s.l. was the dominant species of anopheline larvae in the province. The lowest and highest densities of this species were found in the counties of Behshahr ($n = 3$, 0.14% of total larvae in this county) and Ramsar ($n = 172$, 17.4% of total larvae in this county) (table 1).

The maximum number of culicine larvae were collected from Behshahr ($n = 2055$, 11.06% of total Culicinae) followed by Galugah ($n = 1993$, 10.73% of total Culicinae) and the minimum from Chalus ($n = 383$, 2.06% of total Culicinae) and Mahmudabad ($n = 703$, 3.79% of total Culicinae) Counties.

Culex pipiens was the dominant species, its least number occurred in Chalus ($n = 273$, 46% of total larvae in this County) while Sari recorded the highest number ($n = 1667$, 80.3% of total larvae in this County) respectively. *Culex hortensis* and *Culiseta morsitans* were observed with the lowest distribution in Counties of Fereydunkenar, Behshahr, Neka, Sari and Noor (table 1).

Density of larvae collected in the woodland and plain regions were 45.5 and 54.5%, respectively. *An. maculipennis s.l.* and *Cx. pipiens* were collected with the highest density of 417 (3.85%) and 8165 (75.44%), in plain areas, respectively. Data on other species in woodland and plain areas are presented in table 2.

As summarized in table 3, most larvae were collected from natural habitats ($n = 11972$, 60.34%), including river edge ($n = 1865$, 9.4%), wetlands ($n = 4989$, 25.1%), pit ($n = 2488$, 12.5%) and marsh ($n = 1349$, 6.8%). The same was true with temporary habitats ($n = 10978$, 55.3%), stagnant ($n = 16870$, 85%), expanse of 0–5 m ($n = 15662$, 78.9%), depth of 1–25 cm ($n = 11651$, 58.7%), without plant ($n = 8540$, 43%), shadow-sun ($n = 9740$, 49.1%), muddy floor ($n = 8108$, 49.9%), turbid water ($n = 8667$, 43.7%), temperature 20–25°C ($n = 9985$, 50.3%) and in sunny conditions ($n = 15508$, 78.2%). The highest number and percentage of species in natural and artificial habitats with different characteristics are listed in table 3.

All of the different types of habitats were occupied with more than one species mosquito larvae that it is deducible from table 3.

Chi-square analysis indicated that each of the environmental variables is significantly associated with occurrence of *An. maculipennis s.l.*, *Cx. pipiens*, *Cx. torrentium*, *Cx. mimeticus* and *Cs. annulata* ($P < 0.001$). *Anopheles plumbeus*, *Cx. pipiens*, *Cx. tritaeniorhynchus*, *Cx. territans*, *Cx. mimeticus*, *Cs. annulata* and *Cs. morsitans* showed the highest interest to occur in natural habitats compared with other species in the province (table 4).

The rainfall and temperatures fluctuations in study area are shown in fig. 1.

Discussion

This is the first comprehensive study on the density of species in different habitats of Mazandaran Province, north of Iran, during which three genera and 16 species of mosquitoes were recorded. In contrast, five species of mosquito larvae were collected by Azari-Hamidian (2011) from Guilan Province, 14 species by Saghafipour *et al.* (2012) from Qom Province, 11 species by Banafshi *et al.* (2013) from Kurdistan Province and by Vatandoost *et al.* (2004) from Hormozgan Province.

In agreement with other studies, *An. maculipennis s.l.* and *An. pseudopictus* showed the highest geographical distribution in almost all sectors of the study area and the highest density especially in the plain. In contrast, *An. marteri* showed the lowest distribution and density in woodland and plain regions (Dow, 1953; Nicolescu *et al.*, 2002; Azari-Hamidian *et al.*, 2003; Azari-Hamidian *et al.*, 2009*b*; Amani *et al.*, 2014). To our surprise, despite the through sampling effort, no *Anopheles* were found in the counties of Ghaemshahr, Juybar, Babolsar, Fereydunkenar and Mahmudabad, an

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Table 1. Numbers and percentage of the distribution of larvae species caught from larval habitats in different counties of Mazandaran Province, May–December 2014.

Township	Galugah		Behshahr			Neka			Sari			Ghaemshahr			Savadkooh			Juybar			Babolsar	
Species	Nezammahale	Tileno	Total	Hossein Abad	Al Tappeh	Total	Chalmardi	Komishan	Total	QajarKhil	DallakKhil	Total	Rostam kola	Shahrud Kola	Total	Sorkh Kola	AndarKoli	Total	Astanesar	Pain Zarrin Kola	Total	KikhaMahalleh
An. claviger	–	–	–	–	–	–	–	–	–	–	8	8 (0.65)	–	–	–	–	1	1 (0.11)	–	–	–	–
An. hyrcanus	–	–	–	2	–	2 (0.1)	3	–	3 (0.19)	7	53	60 (4.9)	–	–	–	–	9	9 (0.96)	–	–	–	–
An. maculipennis s.l.	–	41	41(2)	–	3	3 (0.14)	53	29	82 (5.29)	6	29	35 (2.86)	–	–	–	1	11	12 (1.28)	–	–	–	–
An. pseudopictus	–	5	5 (0.24)	16	–	16 (0.77)	15	–	15 (0.97)	52	130	182 (14.9)	–	–	–	–	14	14 (1.49)	–	–	–	–
An. marteri	–	–	–	–	–	–	1	–	1 (0.06)	–	–	–	–	–	–	–	1	1 (0.11)	–	–	–	–
An. plumbeus	–	13	13 (0.63)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cx. pipiens	822	703	1525 (74.3)	624	1043	1667 (80.3)	413	630	1043 (67.3)	407	232	639 (52.2)	733	425	1158 (64)	231	304	535 (57.1)	448	467	915 (89)	1022 (82.4)
Cx. torrentium	47	51	98 (4.78)	21	12	274 (13.2)	51	4	55 (3.55)	25	60	85 (6.94)	21	2	23 (1.27)	188	33	221 (23.6)	10	19	29 (2.8)	14 (1.13)
Cx. tritaeniorhynchus	35	10	45 (2.19)	184	90	33 (1.59)	53	24	77 (4.97)	144	48	192 (15.7)	121	53	174 (9.62)	10	92	102 (10.9)	31	21	52 (5.1)	162 (13.1)
Cx. perexiguus	5	–	5 (0.24)	–	20	20 (0.96)	–	10	10 (0.65)	–	–	–	3	4	7 (0.39)	–	–	–	–	1	1 (0.1)	–
Cx. territans	–	8	8 (0.39)	–	–	–	1	4	5 (0.32)	–	–	–	10	–	10 (0.55)	–	14	14 (1.49)	–	–	–	–
Cx. mimeticus	–	–	–	–	–	–	27	22	49 (3.16)	–	–	–	–	–	–	–	8	8 (0.85)	3	–	3 (0.3)	–
Cx. hortensis	–	–	–	4	–	4 (0.19)	–	2	2 (0.13)	–	3	3 (0.25)	–	–	–	–	–	–	–	–	–	–
Cs. annulata	40	20	60 (2.92)	13	18	31 (1.49)	–	15	15 (0.97)	–	–	–	–	2	2 (0.11)	4	5	9 (0.96)	–	17	17 (1.7)	29 (2.34)
Cs. longiareolata	240	12	252 (12.3)	6	20	26 (1.25)	192	–	192 (12.4)	10	10	20 (1.63)	12	422	434 (24)	4	7	11 (1.17)	8	–	8 (0.8)	14 (1.13)
Cs. morsitans	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	1189	863	2052 (100)	870	1206	2076 (100)	809	740	1549 (100)	651	573	1224 (100)	900	908	1808 (100)	438	499	937 (100)	500	525	1025 (100)	1241 (100)

	Amol		Mahmudabad			Noor			Noshahr			Chalos			Tonekabon			Ramsar			Fereydunkenar	
	Qadi Mahalleh	Razakeh	Total	Galesh Pol	Bishe Kola	Total	Abbasa	Karat Koti	Total	Aliabad mir	Shofeskaj	Total	Sinava	Zavat	Total	Asadabad	Soleymanabad	Total	Shah Mansur mahale	Potak	Total	Firuzabad
An. claviger	–	–	–	–	–	–	–	–	–	6	15	21 (2.68)	–	–	–	1	–	1 (0.06)	–	–	–	–
An. hyrcanus	–	–	–	–	–	–	2	–	2 (0.23)	–	–	–	–	23	23 (3.88)	4	1	5 (0.32)	19	4	23 (2.32)	–
An. maculipennis s.l.	63	47	110 (13)	–	–	–	15	4	19 (2.17)	–	14	14 (1.79)	21	94	115 (19.4)	20	1	21 (1.36)	157	15	172 (17.4)	–
An. pseudopictus	–	–	–	–	–	–	41	10	51 (5.82)	–	–	–	–	72	72 (12.1)	8	1	9 (0.58)	46	14	60 (6.06)	–
An. marteri	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
An. plumbeus	–	–	–	–	–	–	–	1	1 (0.11)	–	–	–	–	–	–	19	26	45 (2.91)	–	–	–	–
Cx. pipiens	454	155	609 (70)	260	316	576 (81.9)	375	276	651 (74.3)	178	437	615 (78.5)	174	99	273 (46)	233	253	486 (31.4)	260	314	574 (58)	1415 (91)

Continued

Table 1. (*Cont.*)

	Amol			Mahmudabad			Noor			Noshahr			Chalos			Tonekabon			Ramsar			Fereydunkenar
	Qadi Mahalleh	Razakeh	Total	Galesh Pol	Bishe Kola	Total	Abbasa	Karat Koti	Total	Aliabad mir	Shofeskaj	Total	Sinava	Zavat	Total	Asadabad	Soleymanabad	Total	Shah Mansur mahale	Potak	Total	Firuzabad
Cx. torrentium	18	16	34 (3.9)	7	2	9 (1.28)	19	13	32 (3.65)	3	15	18 (2.3)	6	42	48 (8.09)	753	19	772 (49.8)	7	5	12 (1.21)	72 (4.6)
Cx. tritaeniorhynchus	49	49	98 (11)	54	47	101 (14.4)	–	17	17 (1.94)	49	34	83 (10.6)	12	25	37 (6.24)	7	17	24 (1.55)	12	42	54 (5.45)	57 (3.6)
Cx. perexiguus	–	–	–	–	–	–	–	–	–	5	4	9 (1.15)	2	–	2 (0.34)	6	–	6 (0.39)	–	13	13 (1.31)	–
Cx. territans	–	1	1 (0.1)	–	5	5 (0.71)	–	15	15 (1.71)	6	–	6 (0.77)	–	–	–	164	5	169 (10.9)	–	–	–	–
Cx. mimeticus	6	7	13 (1.5)	–	–	–	4	–	4 (0.46)	–	–	–	4	–	4 (0.67)	–	–	–	42	–	42 (4.24)	1 (0.1)
Cx. hortensis	–	0	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1 (0.1)
Cs. annulata	–	0	–	2	–	2 (0.28)	16	6	22 (2.51)	13	–	13 (1.66)	8	–	8 (1.35)	–	–	–	8	5	13 (1.31)	0 (0)
Cs. longiareolata	2	6	8 (0.9)	2	8	10 (1.42)	22	26	48 (5.48)	4	–	4 (0.51)	5	6	11 (1.85)	–	11	11 (0.71)	13	14	27 (2.73)	15 (1)
Cs. morsitans	–	–	–	–	–	–	6	8	14 (1.6)	–	–	–	–	–	–	–	–	–	–	–	–	–
Total	592	281	873 (100)	325	378	703 (100)	500	376	876 (100)	264	519	783 (100)	232	361	593 (100)	1215	334	1549 (100)	564	426	990 (100)	1561 (100)

important finding that should be revisited in future studies. Increasing urbanization may be at least part of the fact justifying the finding.

Culex pipiens, Cx. torrentium and *Cx. tritaeniorhynchus*had the highest distribution and dominance among culicine species in the province, especially in the plain and woodland areas. This is consistent with other studies in Iran (Azari-Hamidian, 2007; Khoshdel-Nezamiha *et al.*, 2014; Nikookar *et al.*, 2015; Nikookar *et al.*, 2016) and the world (Kim *et al.*, 2007; Gunduz *et al.*, 2009). It could be because of their compatibility with and very high diversity of habitats in the province.

Undoubtedly *An. maculipennis s.l.* is the main vector of malaria in the Caspian Sea shore area of Iran (Manouchehri *et al.*, 1992). This coupled with the malaria historical records and West Nile Virus cases in the Caspian Sea coast (Naficy & Saidi, 1970) plus the high density and distribution of this species recorded in the present study, poses a potential risk for reemergence of autochthonous transmission of malaria in the Mazandaran Province.

Cx. pipiens is a prevalent mosquito species in Europe, Asia, Africa, Australia, and North and South America (Harbach, 2012) as well as most parts of Iran (Zaim, 1987). It has been known mainly as the ornithophilic species and vector of West Nile Virus in different parts of the world (Zeller & Schuffenecker, 2004; Orshan *et al.*, 2008; Weitzel *et al.*, 2015). Blood meal analysis showed that this species bites both humans and animals; therefore, it can be as bridge vector between birds and humans (Fonseca *et al.*, 2004). Given the history of West Nile virus in the North of Iran (Naficy & Saidi, 1970) and availability of wetlands for migratory birds, there are great concerns about entry and spread of the virus in the province.

In the present study, 22 types of the habitats were visited in Mazandaran Province ten types of which were natural and 12 types were artificial habitats. The highest density of larvae were observed in wetlands with characteristics such as temporary and stagnant, expanse of 0–5 m, depth of 1–25 cm, without plant, shadow-sun, muddy floor, turbid water, temperature 20–25°C and in sunny days. Azari-Hamidian (2007, 2011) collected the highest number of larvae from natural habitats, including river bed pool, rain pool and artificial habitats like rice filed with clean waters, and temporary and stagnant water, out of water plants, muddy bed and exposure to sunlight in Guilan Province.

An. maculipennis s.l., *An. pseudopictus* and *An. hyrcanus* prefer more river edge and rice fields. These observations are consistent with the results of other investigations (Dow, 1953; Azari-Hamidian, 2011; Amani, *et al.*, 2014; Nikookar *et al.*, 2015).

An. maculipennis prefers river bed pool and rice field with the gravel bed, submerged plant, transient and stagnant water (Azari-Hamidian, 2011), which is consistent with the findings of our research. It was reported that this species lays eggs in habitats that are exposed to sunlight (Azari-Hamidian, 2011; Amani *et al.*, 2014), whereas, in the present study it prefers habitats with shadow–sun conditions.

Dow (1953) reported that *An. maculipennis* tends to occupy shady habitats with plant on water surface, whereas our study and another research in the study area (Nikookar *et al.*, 2015) showed that this species lays eggs in habitats with characteristics such as shadow–sun with underwater plants.

An. maculipennis were collected in temporary wetlands on the edge of rice fields and plot of rice field by Azari-Hamidian

Table 2. Frequency of larvae collected in woodland and plain areas of Mazandaran Province, May–December 2014.

Species	Woodland *N* (%)	Plain *N* (%)
An. claviger	24 (0.26%)	7 (0.06%)
An. hyrcanus	64 (0.7%)	63 (0.6%)
An. maculipennis s.l.	207 (2.3%)	417 (3.85%)
An. pseudopictus	177 (1.96%)	247 (2.28%)
An. marteri	1 (0.01%)	1 (0.01%)
An. plumbeus	20 (0.22%)	39 (0.36%)
Cx. pipiens	5538 (61.42%)	8165 (75.44%)
Cx. torrentium	1170 (13%)	385 (3.56%)
Cx. tritaeniorhynchus	497 (5.51%)	1052 (9.72%)
Cx. perexiguus	54 (0.6%)	19 (0.17%)
Cx. territans	186 (2.06%)	47 (0.44%)
Cx. mimeticus	38 (0.42%)	86 (0.8%)
Cx. hortensis	3 (0.03%)	7 (0.06%)
Cs. annulata	83 (0.92%)	138 (1.27%)
Cs. longiareolata	947 (10.5%)	144 (1.33%)
Cs. morsitans	8 (0.09%)	6 (0.05%)
Total	9017 (100)	10,823 (100)

et al., in Rasht County, Guilan Province (Azari Hamidian *et al.*, 2002), river edge and plot of rice field by Amani *et al.*, in Aligudarz County, western Iran (Amani *et al.*, 2014). Mousakazemi *et al.* (2000) found this species from rice fields in Zarrin-Shahr and Mobarakeh Counties, Isfahan Province (Mousakazemi *et al.*, 2000), which confirms the findings of this investigation.

An. maculipennis was observed in habitats with muddy bed in Counties of Rasht and Aligudarz, northern and western Iran (Azari Hamidian *et al.*, 2002; Amani *et al.*, 2014), whereas this species was collected from habitats with gravel and rocky bed by Azari-Hamidian in Guilan Province (Azari-Hamidian, 2011) and in the present study. The differences between the results of the present study with other studies could be due to the preference of this species to habitats such as rice fields and river edge.

Based on our observations, *An. pseudopictus* and *An. hyrcanus* prefer habitats with muddy bed unlike *An. maculipennis*. These *Anopheles* species lay eggs in temporary and stagnant waters with temperature of 15–20°C and are heliophilous, a finding that is consistent with the results of other studies (Azari-Hamidian 2011). Information is lacking on the larval habitat characteristics of *An. pseudopictus* in details due to difficulties in separating *An. pseudopictus* and *An. hyrcanus* in the larval stage. *Anopheles pseudopictus* (as *An. hyrcanus* var. *pseudopictus*) was collected from 'fairly deep channels in river bed with emergent vegetation and surface debris', 'rice fields', 'quiet river channel', 'canal with emergent grass along banks', 'small brook below spring with mats of glove-like alga' by Dow in northern Iran (Dow, 1953) which is consistent with the findings of this research.

An. maculipennis s.l., *An. pseudopictus* and *An. hyrcanus* were seen more in depth of 1–25 cm and expanse of 0–5 cm. There was no report associated with this feature in Iranian literature. These species of *Anopheles* show more habitats diversity than *An. claviger*, *An. plumbeus* and *An. marteri*, this could justify why the first three species have the highest density in the studied area where natural and artificial habitats including river edge and rice fields are widespread and various.

It should be noted that no *Anopheles* mosquitoes have been observed in natural (footprints of animal) and artificial habitats such as bog, cistern, sewage, dam, plastic dishes and tin can, with depth more than 1 m, temperatures higher than 25°C and metal bed in the present study. Only one *Anopheles* was collected from footprints of animals by Amani *et al.* (2014) in Aligudarz County, which is almost in accordance with the findings of our research.

Among Culicinae, *Cx. pipiens* and *Cx. torrentium* prefer natural and artificial habitats, including wetlands and discarded tire, respectively. These *Culex* species lay eggs in temporary and stagnant waters, with depth of 1–25 cm, expanse of 0–5 cm, without plant and shadow–sun conditions. These two species were found further in turbid waters with temperature of 20–25°C and muddy floor and similar to *Anopheles*, they are heliophilous. This genus was reported as the largest, most common, and most important genus of the tribe Culicinae (Service, 1993) and is the most densely populated species with vast distribution in the province.

Cx. pipiens was collected more in natural and artificial habitats such as stream bed pools and rice field, respectively, with vegetation that are exposed to sunlight by Zaim (1987) in 24 provinces, rain pool and discarded concrete tubes with presence of plant outside water, clear water and shadow by Azari-Hamidian (2007) in Guilan Province. Banafshi *et al.* (2013) found this species from river edge with vegetation that also is under sunlight in Kurdistan Province, northwestern Iran. Moosa-Kazemi *et al.* (2009) also collected this species from swamps, seepages, streams, river banks, drying river beds, pools and grasslands in Chabahar County, southeastern Iran. This observation is different with findings of our investigation. This species often chooses transient and standing water with muddy bottom (Zaim, 1987; Azari-Hamidian, 2007; Banafshi *et al.*, 2013; Nikookar *et al.*, 2015), which is in agreement with our study.

High abundance of *Cx. pipiens* was reported in natural habitats of tree holes (Nikookar *et al.*, 2010), but it should be noted that the tree holes are not the main habitat of this species (Horsfall, 1955; Zaim, 1987; Service, 1993; Azari-Hamidian, 2003, 2007; Moosa-Kazemi *et al.*, 2009; Banafshi *et al.*, 2013).

Cx. pipiens was collected from different habitats, including pool with semi-permanent and stagnant water by Ibrahim *et al.* (2011) in Qalyubiya Governorate, Egypt, artificial container, ground pool, marsh, rice field, stream margin, tire and well/cistern by Kim *et al.* (2007) in northern Gyeonggi Province, Korea. These investigations, along with the present study demonstrate high compatibility of this species in selection of different habitats, which can be a reason for their high density and vast distribution in Mazandaran Province.

In the present study, all environmental variables were statistically associated with the occurrence of *An. maculipennis s.l.*, *Cx. pipiens*, *Cx. torrentium*, *Cx. mimeticus* and *Cs. morsitans*. It is interesting that despite significant relationship, *Cx. mimeticus* and *Cs. morsitans* were collected with low density; this requires more studies in future. Tall and out of water plants can reduce the abundance of larvae by acting as barrier to spawning female and assist high diversity of predators in nests (Muturi *et al.*, 2008). Therefore, larval abundance decreases with increasing tall and out of the water vegetation (Mwangangi *et al.*, 2007; Fillinger *et al.*, 2009). Consequently, it can be suggested that density of *An. maculipennis*, *Cx. pipiens* and *Cx. torrentium* in the province, could be related with habitats without plant and mostly with underwater vegetation.

Study on the physicochemical factors, pathogens, predators and nutritional factors which can play important role in

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Table 3. Number and percentage of larvae density in habitats with different ecological characteristics, Mazandaran Province, Iran.

	An. claviger	*An. hyrcanus*	*An. maculi-pennis s.l.*	*An. marteri*	*An. plumbeus*	*An. pseudopictus*	*Cx. pipiens*	*Cx. tritaeniorhynchus*	*Cx. torrentium*	*Cx. perexiguus*	*Cx. territans*	*Cx. mimeticus*	*Cx. hortensis*	*Cs. an-nulata*	*Cs. longiar-eolata*	*Cs. morsi-tans*	Total
Expanse of habitats (m)																	
0–5	11 (0.06%)	70 (0.35%)	450 (2.27%)	2 (0.01%)	52 (0.26%)	271 (1.37)	10806 (54.5%)	1135 (5.72)	1327 (6.69%)	72 (0.36%)	233 (1.17%)	65 (0.33%)	7 (0.04%)	221 (1.11%)	926 (4.67)	14 (0.07%)	15662 (78.9)
5.1–10	20 (0.1%)	–	3 (0.02%)	–	–	12 (0.06%)	481 (2.42%)	335 (1.69%)	159 (0.8%)	1 (0.01%)	–	6 (0.03%)	–	–	–	–	1017 (5.13%)
10.1–15	–	57 (0.29%)	171 (0.86%)	–	7 (0.04%)	141 (0.71%)	2416 (12.2%)	79 (0.4%)	69 (0.35%)	–	–	53 (0.27%)	3 (0.02%)	–	165 (0.83%)	–	3161 (15.9%)
Depth of habitats (cm, m)																	
1–25 cm	23 (012%)	99 (0.5%)	254 (1.28%)	1 (0.01%)	36 (0.18%)	274 (1.38)	8199 (41.3%)	988 (4.98%)	821 (4.14%)	50 (0.25%)	176 (0.89%)	25 (0.13%)	5 (0.03)	130 (0.66%)	562 (2.83%)	8 (0.04%)	11651 (58.7%)
25.1–50 cm	8 (0.04%)	15 (0.08%)	163 (0.82%)	–	23 (0.12%)	89 (0.45%)	3748 (18.9%)	419 (2.11%)	326 (1.64%)	16 (0.08%)	55 (0.28%)	23 (0.12%)	–	65 (0.33%)	458 (2.31%)	6 (0.03%)	5414 (27.3%)
50.1–75 cm		6 (0.03%)	90 (0.45%)	1 (0.01%)	–	41 (0.21%)	785 (3.96%)	57 (0.29%)	51 (0.26%)	–	1 (0.01%)	32 (0.16%)	4 (0.02%)	15 (0.08%)	22 (0.11%)	–	1105 (5.57%)
75.1 cm–1 m	–	7 (0.04%)	117 (0.59%)	–	–	19 0.1)	335 (1.69%)	44 (0.22%)	38 (0.19%)	3 (0.02%)	–	40 (0.2%)	1 (0.01%)	11 (0.06%)	49 (0.25%)	–	664 (3.35%)
1.1–1.5 m	–	–	–	–	–	1 (1.01%)	116 (0.58%)	10 (0.05%)	2 (0.01%)	–	–	4 (0.02%)	–	–	–	–	133 (0.67%)
1.51–2 m	–	–	–	–	–	–	520 (2.62%)	31 (0.16%)	317 (1.6%)	4 (0.02%)	1 (0.01)	–	–	–	–	–	873 (4.4%)
Weather conditions																	
Rainy	–	–	22 (0.11%)	–	–	7 (0.04%)	755 (3.81%)	146 (0.74%)	568 (2.86%)	20 (0.1%)	10 (0.05%)	14 (0.07%)	–	20 (0.1%)	28 (0.14%)	–	1590 (8.01%)
Cloudy	–	10 (0.05%)	51 (0.26%)	–	–	5 (0.03%)	1463 (7.37%)	64 (0.32%)	41 (0.21%)	7 (0.04%)	93 (0.47%)`	22 (0.11%)	2 (0.01%)	–	32 (0.16%)	–	1790 (9.02%)
Sunny	23 (0.12%)	95 (0.48%)	537 (2.71%)	2 (0.01%)	49 (0.25%)	319 (1.61%)	10763 (54.2%)	1298 (6.54%)	914 (4.61%)	46 (0.23%)	130 (0.66%)	86 (0.43%)	8 (0.04%)	201 (1.01%)	1023 (5.16%)	14 (0.07%)	15508 78.2
Sunny-wind	8 (0.04%)	22 (0.11%)	14 (0.07%)	–	10 (0.05%)	93 (0.47%)	722 (3.64%)	41 (0.21%)	32 (0.16%)	–	1 (0.01%)	2 (0.01%)	–	–	7 (0.04%)	–	952 (4.8%)
Type of water																	
Permanent	2 (0.01%)	62 (0.31%)	125 (0.63%)	1 (0.01%)	13 (0.07%)	183 (0.92%)	6317 (31.8%)	706 (3.56%)	760 (3.83%)	30 (0.15%)	96 (0.48%)	24 (0.12%)	1 (0.01%)	74 (0.37%)	471 (2.37%)	–	8865 (44.7%)
Temporary	29 (0.15%)	65 (0.33%)	499 (2.52%)	1 (0.01%)	46 (0.23%)	244 (1.23%)	7386 (37.2%)	843 (4.25%)	795 (4.01%)	43 (0.22%)	137 (0.69%)	100 (0.5%)	9 (0.05%)	147 (0.74%)	620 (3.13%)	14 (0.07%)	10978 (55.3%) 100%
Stream of water																	
Current	6 (0.03%)	37 (0.19%)	175 (0.88%)	–	10 (0.05%)	119 (0.6%)	1924 (9.7%)	197 (0.99%)	328 (1.65%)	23 (0.12%)	70 (0.35%)	40 (0.2%)	2 (0.01%)	12 (0.06%)	27 (0.14%)	–	2970 (15%)
Stagnant	25 (0.13%)	90 (0.45%)`	449 (2.26%)	2 (0.01%)	49 (0.25%)	307 (1.55%)	11779 (59.4%)	1352 (6.81%)	1222 (6.16%)	48 (0.24%)	163 (0.82%)	84 (0.42%)	8 (0.04%)	214 (1.08%)	1064 (5.36%)	14 (0.07%)	16870 (85%)
Status of vegetation																	
Out of water	–	15 (0.08%)	143 (0.72%)	–	13 (0.07%)	77 (0.39%)	3813 (19.2%)	476 (2.4%)	219 (1.1%)	8 (0.04%)	137 (0.69%)	13 (0.07%)	5 (0.03%)	93 (0.47%)	421 (2.12%)	14 (0.07%)	5447 (27.5%)
Water surface	–	14 (0.07%)	58 (0.29%)	–	45 (0.23%)	39 (0.2%)	1068 (5.38%)	76 (0.38%)	523 (2.64%)	–	75 (0.38%)	30 (0.15%)	–	49 (0.25%)	209 (1.05%)	–	2186 (11%)
Under water	9 (0.05%)	67 (0.34%)	285 (1.44%)	–	–	184 (0.93%)	1035 (5.22%)	147 (0.74%)	74 (0.37%)	3 (0.02%)	10 (0.05%)	67 (0.34%)	3 (0.02%)	29 (0.15%)	111 (0.56%)	–	2024 (10.2%)
Out, surface and under of water	21 (0.11%)	12 (0.06%)	100 (0.5%)	2 (0.01%)	–	64 (0.32%)	1047 (5.28%)	300 (1.51%)	84 (0.42%)	–	–	4 (0.02%)	–	–	11 (0.06%)	–	1645 (8.29%)
Without plant	1 (0.01%)	19 (0.1%)	40 (0.2%)	–	1 (0.01%)	60 (0.3%)	6740 (34%)	550 (2.77%)	655 (3.3%)	62 (0.31%)	11 (0.06%)	10 (0.05%)	2 (0.01%)	50 (0.25%)	339 (1.71%)	–	8540 (43%)
Sunlight status																	

Table 3. (*Cont.*)

	An. claviger	*An. hyrcanus*	*An. maculipennis s.l.*	*An. marteri*	*An. plumbeus*	*An. pseudopictus*	*Cx. pipiens*	*Cx. tritaeniorhynchus*	*Cx. torrentium*	*Cx. perexiguus*	*Cx. territans*	*Cx. mimeticus*	*Cx. hortensis*	*Cs. annulata*	*Cs. longiareolata*	*Cs. morsitans*	Total
Sunny	–	27 (0.14%)	145 (0.73%)	–	–	109 (0.55%)	3533 (17.8%)	291 (1.47%)	721 (3.63%)	4 (0.02%)	–	43 (0.22%)	–	2 (0.01%)	220 (1.11%)	–	5095 (25.7%)
Shadow	1 (0.01%)	2 (0.01%)	8 (0.04%)	–	19 (0.1%)	6 (0.03%)	1576 (7.94%)	127 (0.64%)	372 (1.88%)	14 (0.07%)	2 (0.01%)	28 (0.14%)	2 (0.01%)	75 (0.38%)	335 (1.69%)	–	2567 (12.9%)
Semi-Shade	22 (0.11%)	25 (0.13%)	51 (0.26%)	1 (0.01%)	–	213 (1.07%)	1905 (9.6%)	102 (0.51%)	89 (0.45%)	20 (0.1%)	–	–	–	4 (0.02%)	6 (0.03%)	–	2438 (12.3%)
Shadow-Sun	8 (0.04%)	73 (0.37%)	420 (2.12%)	1 (0.01%)	40 (0.2%)	96 (0.48%)	6689 (33.7%)	1029 (5.19%)	373 (1.88%)	35 (0.18%)	231 (1.16%)	53 (0.27%)	8 (0.04%)	140 (0.71%)	530 (2.67%)	14 (0.07%)	9740 (49.1%)
Floor of habitats																	
Muddy	22 (0.11%)	81 (0.41%)	203 (1.02%)	–	39 (0.2%)	284 (1.43%)	5104 (25.7%)	884 (4.46%)	845 (4.26%)	9 (0.05%)	133 (0.67%)	40 (0.2%)	8 (0.04%)	84 (0.42%)	358 (1.8%)	14 (0.07%)	8108 (49.9%)
Sandy	–	15 (0.08%)	131 (0.66%)	–	–	42 (0.21%)	856 (4.31%)	44 (0.22%)	245 (1.23%)	12 (0.06%)	1 (0.01%)	25 (0.13%)	–	–	173 (0.87%)	–	1544 (7.78%)
Rocky	9 (0.05%)	27 (0.14%)	267 (1.35%)	1 (0.01%)	10 (0.05%)	89 (0.45%)	3613 (18.2%)	136 (0.69%)	248 (1.25%)	10 (0.05%)	64 (0.32%)	37 (0.19%)	–	80 (0.4%)	211 (1.06%)	–	4802 (24.2%)
Plastic	–	4 (0.02%)	23 (0.12%)	1 (0.01%)	10 (0.05%)	9 (0.05%)	3999 (20.2%)	475 (2.39%)	187 (0.94%)	37 (0.19%)	35 (0.18%)	22 (0.11%)	2 (0.01%)	50 (0.25%)	334 (1.68%)	–	5188 (26.1%)
Metal	–	–	–	–	–	–	131 (0.66%)	10 (0.05%)	30 (0.15%)	5 (0.03%)	–	–	–	7 (0.04%)	15 (0.08%)	–	198 (1%)
Situation of water																	
Muddy	–	14 (0.07%)	27 (0.14%)	–	–	43 (0.22%)	2496 (12.6%)	173 (0.87%)	416 (2.1%)	17 (0.09%)	50 (0.25%)	27 (0.14%)	–	6 (0.03%)	89 (0.45%)	–	3358 (16.9%)
Clear	23 (0.12%)	104 (0.52%)	480 (2.42%)	2 (0.01%)	58 (0.29%)	253 (1.28%)	4586 (23.1%)	806 (4.06%)	441 (2.22%)	30 (0.15%)	143 (0.72%)	75 (0.38%)	7 (0.04%)	53 (0.27%)	740 (3.73%)	14 (0.07%)	7815 (39.4%)
Turbid	8 (0.04%)	9 (0.05%)	117 (0.59%)	–	1 (0.01%)	128 (0.65%)	6621 (33.4%)	570 (2.87%)	698 (3.52%)	26 (0.13%)	40 (0.2%)	22 (0.11%)	3 (0.02%)	162 (0.82%)	262 (1.32%)	–	8667 (43.7%)
Total																	100%
Type of water																	
Freshwater	31 (0.16%)	127 (0.64%)	624 (3.15%)	2 (0.01%)	59 (0.3%)	424 (2.14%)	13703 (69.1%)	1549 (7.81%)	1555 (7.84%)	73 (0.37%)	233 (1.17%)	124 (0.63%)	10 (0.05%)	221 (1.11%)	1091 (5.5%)	14 (0.07%)	19840 (100%)
Brackish	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Habitat temperature (°C)																	
5–10	–	–	24 (0.12%)	–	13 (0.07%)	4 (0.02%)	550 (2.77%)	2 (0.01%)	52 (0.26%)	–	26 (0.13%)	–	–	10 (0.05%)	39 (0.2%)	14 (0.07%)	734 (3.7%)
10.1–15	–	1 (0.01%)	84 (0.42%)	–	6 (0.03%)	29 (0.15%)	1209 (6.09%)	222 (1.12%)	211 (1.06%)	14 (0.07%)	45 (0.23%)	14 (0.07%)	1 (0.01%)	50 (0.25%)	144 (0.73%)	–	2030 (10.2%)
15.1–20	8 (0.04%)	98 (0.49%)	357 (1.8%)	2 (0.01%)	40 (0.2%)	209 (1.05%)	3980 (20.1%)	389 (1.96%)	920 (4.64%)	49 (0.25%)	147 (0.74%)	86 (0.43%)	5 (0.03%)	142 (0.72%)	533 (2.69%)	–	6965 (35.1%)
20.1–25	23 (0.12%)	28 (0.14%)	159 (0.8%)	–	–	80 (0.4%)	7940 (40%)	936 (4.72%)	372 (1.88%)	10 (0.05%)	15 (0.08%)	24 (0.12%)	4 (0.02%)	19 (0.1%)	375 (1.89%)	–	9985 (50.3%)
25.1–30	–	–	–	–	–	–	24 (0.12%)	–	–	–	–	–	–	–	–	–	24 (0.12%)
The natural larval habitats																	
Forest edge	–	–	–	–	10 (0.05%)	–	80 (0.4%)	–	–	–	–	–	–	–	–	14 (0.07%)	104 (0.52%)
River edge	15 (0.08%)	35 (0.18%)	195 (0.98%)	2 (0.01%)	–	112 (0.56%)	900 (4.54%)	240 (1.21%)	127 (0.64%)	2 (0.01%)	90 (0.45%)	65 (0.33%)	1 (0.01%)	60 (0.3%)	21 (0.11%)	–	1865 (9.4%)
River bed	5 (0.03%)	10 (0.05%)	51 (0.26%)	–	–	–	100 (0.5%)	–	16 (0.08%)	–	–	16 (0.08%)	–	2 (0.01%)	8 (0.04%)	–	208 (1.05%)
Marsh	–	–	2 (0.01%)	–	13 (0.07%)	–	1050 (5.29%)	100 (0.5%)	17 (0.09%)	1 (0.01)	52 (0.26%)	10 (0.05%)	–	4 (0.02%)	100 (0.5%)	–	1349 (6.8%)
Grassland	–	4 (0.02%)	20 (0.1%)	–	5 (0.03%)	1 (0.01%)	280 (1.41%)	89 (0.45%)	2 (0.01%)	–	–	2 (0.01%)	–	–	2 (0.01%)	–	405 (2.04%)

Continued

Table 3. (*Cont.*)

	An. claviger	*An. hyrcanus*	*An. maculipennis s.l.*	*An. marteri*	*An. plumbeus*	*An. pseudopictus*	*Cx. pipiens*	*Cx. tritaeniorhynchus*	*Cx. torrentium*	*Cx. perexiguus*	*Cx. territans*	*Cx. mimeticus*	*Cx. hortensis*	*Cs. annulata*	*Cs. longiareolata*	*Cs. morsitans*	Total
Creek	3 (0.02%)	6 (0.03%)	15 (0.08%)	–	–	11 (0.06%)	350 (1.76%)	21 (0.11%)	8 (0.04%)	1 (0.01%)	–	–	–	1 (0.01%)	5 (0.03%)	–	421 (2.12%)
Pit	–	–	12 (0.06%)	–	–	2 (0.01)	2060 (10.4%)	140 (0.71%)	104 (0.52%)	8 (0.04%)	25 (0.13%)	5 (0.03%)	4 (0.02%)	3 (0.02%)	125 (0.63%)	–	2488 (12.5%)
Wetlands	–	2 (0.01%)	13 (0.07%)	–	4 (0.02%)	32 (0.16%)	3620 (18.2%)	500 (2.52%)	455 (2.29%)	13 (0.07%)	–	2 (0.01%)	–	79 (0.4%)	269 (1.36%)	–	4989 (25.1%)
Springs	1 (0.01%)	8 (0.04%)	4 (0.02%)	–	8 (0.04%)	2 (0.01%)	55 (0.28%)	–	18 (0.09%)	–	–	3 (0.02%)	–	–	1 (0.01%)	–	100 (0.5%)
Footprint of animal	–	–	–	–	–	–	40 (0.2%)	–	3 (0.02%)	–	–	–	–	–	–	–	43 (0.22%)
The artificial larval habitats																	
Rice field	–	22 (0.11%)	89 (0.45%)	–	–	90 (0.45%)	430 (2.17%)	123 (0.62%)	–	23 (0.12%)	30 (0.15%)	15 (0.08%)	4 (0.02%)	–	122 (0.61%)	–	948 (4.78%)
Rice irrigation channel	6 (0.03%)	1 (0.01%)	70 (0.35%)	–	–	85 (0.43%)	100 (0.5)	95 (0.48%)	–	–	–	–	–	–	–	–	357 (1.8%)
Bog	–	–	–	–	–	–	65 (0.33%)	4 (0.02%)	2 (0.01%)	–	–	–	–	–	2 (0.01%)	–	73 (0.37%)
Cistern	–	–	–	–	–	–	13 (0.07%)	–	–	–	–	–	–	–	–	–	13 (0.07%)
Pond	–	12 (0.06%)	55 (0.28%)	–	–	–	42 (0.21%)	43 (0.22%	5 (0.03%)	–	22 (0.11%)	–	–	38 (0.19%)	150 (0.76%)	–	367 (1.85%)
Sewage	–	–	–	–	–	–	350 (1.76%)	–	20 (0.1%)	–	–	–	–	–	–	–	370 (1.86%)
Pool	–	8 (0.04%)	50 (0.25%)	–	5 (0.03%)	45 (0.23%)	1015 (5.12%)	100 (0.5%)	200 (1.01%)	13 (0.07%)	14 (0.07%)	6 (0.03%)	–	14 (0.07%)	75 (0.38%)	–	1545 (7.79%)
Dam	–	–	–	–	–	2 (0.01%)	263 (1.33%)	4 (0.02%)	7 (0.07%)	–	–	–	–	–	25 (0.13%)	–	301 (1.52%)
Discarded tire	–	8 (0.04%)	4 (0.04%)	–	10 (0.05%)	30 (0.15%)	1500 (7.56%)	15 (0.08%)	356 (1.79%)	2 (0.01%)	–	–	–	–	151 (0.76%)	–	2076 (10.5%)
Plastic dishes	–	–	–	–	–	2 (0.01%)	680 (3.43%)	15 (0.08%)	14 (0.07%)	2 (0.01%)	–	–	1 (0.01%)	8 (0.04%)	6 (0.03%)	–	728 (3.67%)
Tin dishes	–	–	–	–	–	–	110 (0.55%)	10 (0.05%)	21 (0.11%)	–	–	–	–	2 (0.01%)	9 (0.05%)	–	152 (0.77%)
Concrete channel	1 (0.01%)	11 (0.06%)	44 (0.22%)	–	4 (0.02%)	10 (0.05%)	600 (3.02%)	50 (0.25%)	180 (0.91%)	8 (0.04%)	–	–	–	10 (0.05%)	20 (0.1%)	–	938 (4.73%)

Table 4. Association between environmental variables and occurrences of larva in Mazandaran Province.

Environmental characteristics	*An. claviger*			*An. hyrcanus*			*An. maculipennis s.l.*			*An. pseudopictus*			*An. marteri*			*An. plumbeus*			*Cx. pipiens*			*Cx. torrentium*		
	K^2	*f*	*P*-value	K^2	*f*	*P*-value	K^2	*f*	*P*-value	K^2	*f*	*P*-value	K^2	*f*	*P*-value	K^2	*f*	*P*-value	K^2	*f*	*P*-value	K^2	*f*	*P*-value
Expanse of habitats (m)	226.01	2	<0.001	82.9	2	<0.001	84.13	2	<0.001	98.4	2	<0.001	0.53	2	0.76	4.2	2	0.11	305.91	2	<0.001	234.2	2	<0.001
Depth of habitats (cm, m)	5.63	5	0.34	27.7	5	<0.001	616.01	5	<0.001	43.9	5	<0.001	7.8	5	0.16	11.2	5	0.04	176.46	5	<0.001	1042.06	5	<0.001
Weather conditions	33.9	3	<0.001	52.4	3	<0.001	30.61	3	<0.001	317	3	<0.001	0.55	3	0.90	28.4	3	<0.001	502.32	3	<0.001	1895	3	<0.001
Type of water	18.3	1	<0.001	0.88	1	0.34	158.3	1	<0.001	0.94	1	0.33	0.002	1	0.088	12.27	1	<0.001	36.31	1	<0.001	12.03	1	0.001
Stream of water	0.46	1	0.49	20.1	1	<0.001	86.53	1	<0.001	57.4	1	<0.001	0.35	1	0.55	0.18	1	0.0.67	30.03	1	<0.001	50.6	1	<0.001
Status of vegetation	166.5	4	<0.001	261.9	4	<0.001	1049.21	4	<0.001	589.2	4	<0.001	4.5	4	0.45	263.7	4	<0.001	1135.78	4	<0.001	959.4	4	<0.001
Sunlight status	100.64	3	<0.001	21.2	3	<0.001	121.57	3	<0.001	613.9	3	<0.001	3.08	3	0.37	43.6	3	<0.001	165.50	3	<0.001	714.2	3	<0.001
Floor of habitats	18.02	4	0.001	46.6	4	<0.001	378.13	4	<0.001	176.6	4	<0.001	1.9	4	0.74	17.6	4	0.001	944.92	4	<0.001	403.6	4	<0.001
Situation of water	17.07	2	<0.001	100.4	2	<0.001	382.52	2	<0.001	75.1	2	<0.001	3.07	2	0.21	86	2	<0.001	655.78	2	<0.001	148.9	2	<0.001
Habitat temperature (°C)	8.5	4	0.007	100.2	4	<0.001	175.20	4	<0.001	130.5	4	<0.001	3.6	4	0.45	101.1	4	<0.001	1088.46	4	<0.001	530.3	4	<0.001
Natural and artificial larval habitats	3.78	2	0.052	4.48	2	0.03	28.8	1	<0.001	92.5	2	<0.001	1.31	2	0.25	1.37	2	0.24	69.8	1	<0.001	103.4	2	<0.001

	Cx. tritaeniorhynchus			*Cx. perexiguus*			*Cx. territans*			*Cx. mimeticus*			*Cx. hortensis*			*Cs. annulata*			*Cs. longiareolata*			*Cs. morsitans*		
Expanse of habitats (m)	1023	2	<0.001	17.2	2	<0.001	62.8	2	<0.001	67.4	2	<0.001	1.8	2	0.39	59.6	2	<0.001	64.8	2	<0.001	3.7	2	0.15
Depth of habitats (cm, m)	41.4	5	<0.001	6.8	5	0.23	41.6	5	<0.001	456.1	5	<0.001	15.9	5	0.25	4.07	5	0.015	190.7	5	<0.001	3.2	5	0.66
Weather conditions	71.7	3	<0.001	40.04	3	<0.001	276.7	3	<0.001	16.1	3	0.001	2.6	3	0.45	35.8	3	<0.001	167.8	3	<0.001	3.9	3	0.27
Type of water	0.55	1	0.45	0.38	1	0.53	1.15	1	0.28	32.3	1	<0.001	4.8	1	0.029	11.3	1	<0.001	1.05	1	0.3	11.3	1	<0.001
Stream of water	6.69	1	0.01	16.9	1	<0.001	36.2	1	<0.001	29.3	1	<0.001	0.19	1	0.65	16.7	1	<0.001	141.6	1	<0.001	2.4	1	0.11
Status of vegetation	335.1	4	<0.001	53.9	4	<0.001	288.3	4	<0.001	307.1	4	<0.001	8.8	4	0.006	84.7	4	<0.001	233.8	4	<0.001	37	4	<0.001
Sunlight status	207.6	3	<0.001	27.4	3	<0.001	236.4	3	<0.001	29.2	3	<0.001	6.1	3	0.1	158.8	3	<0.001	424.8	3	<0.001	14.5	3	0.002
Floor of habitats	340.9	4	<0.001	67	4	<0.001	64.1	4	<0.001	33.1	4	<0.001	7.1	4	0.12	42.7	4	<0.001	136.7	4	<0.001	20.2	4	<0.001
Situation of water	119.3	2	<0.001	2.8	2	0.23	69.7	2	<0.001	35.1	2	<0.001	4.5	2	0.1	85	2	<0.001	391.7	2	<0.001	21.5	2	<0.001
Habitat temperature (°C)	168.5	4	<0.001	49.2	4	<0.001	196.5	4	<0.001	70	4	<0.001	1.2	4	0.85	164.7	4	<0.001	131.2	4	<0.001	362.7	4	<0.001
Natural and artificial larval habitats	70.5	2	<0.001	20.8	2	<0.001	12.6	2	<0.001	26.9	2	<0.001	0.44	2	0.5	4.6	2	0.031	65.7	2	<0.001	9.2	2	0.002

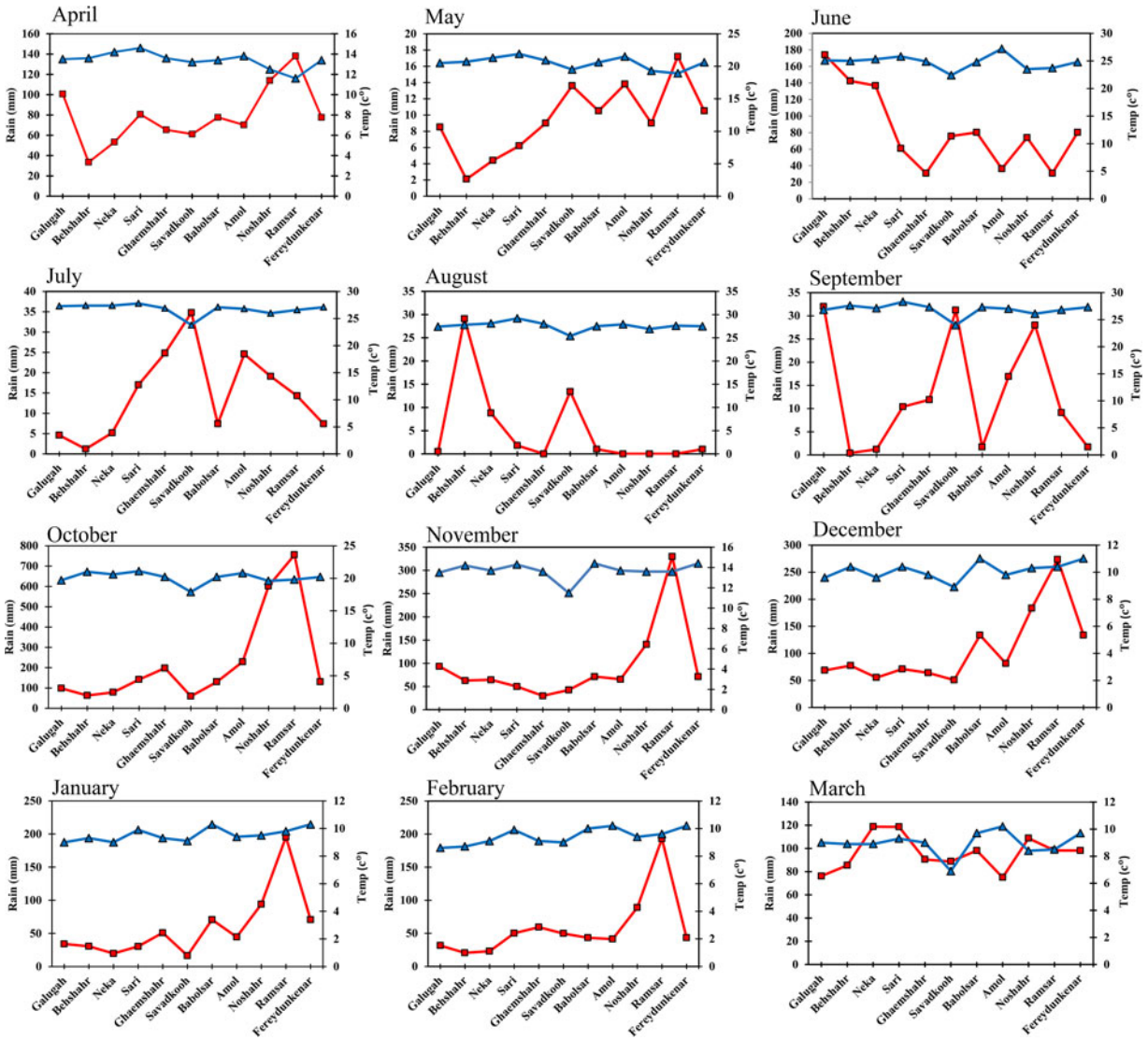

Fig. 1. The status of climate fluctuations in the study area of Mazandaran Province during April–March, 2014.

distribution, density and the presence of mosquito larvae could have improved the soundness of the results.

Conclusion

This study provided comprehensive data on larval habitats of mosquitoes for the first time in Mazandaran Province. There are high densities of *An. maculipennis s.l.* and *Cx. pipiens* in river edge and wetlands with characteristics, including underwater plants or without plants which can be important factors in determining the abundance of these species in the area. As *An. maculipennis* and *Cx. pipiens* are potential vectors of malaria and West Nile Virus, changes in the features of their habitats in line with other control programs could be operational strategies to reduce the abundance of these species in the province as well as in other parts of the world with similar ecological conditions.

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