<u>Original Article</u> Fauna and Larval Habitat Characteristics of Mosquitoes in Neka County, Northern Iran

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Abstract

Background: Ecological studies on mosquitoes are very important in vector control programs. There are a few studies about the ecology of mosquitoes in northern Iran. This study was carried out to detect fauna and larval habitat characteristics of mosquitoes.

Methods: This study aimed to determine fauna and the ecology of mosquitoes in Neka County, Mazandaran Province, northern Iran from April to December, 2009. The larval collection was conducted using standard dipper, and the characteristics of larval habitat were investigated based on degree of transparency of water, type of water (stagnant or running), plant vegetation, sunny or shady, temperature and altitude of the natural or artificial breeding places.

Results: The mosquito larvae were collected from 72 habitats and identified using systematic keys. Nine species of mosquitoes were identified: *Anopheles claviger* (0.31%), *An. maculipennis* (0.54%), *An. plumbeus* (10.28%), *An. superpictus* (0.01%), *Culiseta annulata* (1.07%), *Cs. longiareolata* (8.91%), *Culex mimeticus* (0.03%), *Cx. pipiens* (63.99%), and *Ochlerotatus geniculatus* (14.85%). The range of temperature in the larval habitats was 19.6–22.5 °C. Significant difference was observed in the rate of temperature among the species in the larval habitats (P< 0.05). A checklist of mosquitoes including seven genera and 32 species has been provided for Mazandaran Province.

Conclusion: The most dominant species were *Cx. pipiens*. They were collected from the larval habitats like Border Rivers, ponds, rain water pools, discarded tires and tree holes. *Culiseta annulata* was included to the checklist of mosquitoes in Mazandaran Province.

Keywords: Larval habitat, Culicidae, Ecology, Iran

Introduction

According to the latest classification of Culicidae, the family includes two subfamilies, 11 tribes, 112 genera and 3537 species. The subfamily Anophelinae has three genera and Culicinae has 109 genera (Harbach 2007).

Many of the world's major diseases such as Malaria, West Nile, Sindbis, Dirofilariasis, Dengue fever, Yellow fever, Rift valley fever are transmitted by mosquitoes. West Nile and Sindbis viruses have been reported in northern parts of Iran (Naficy and Saidi 1970, Saidi et al. 1976). The mosquito borne filarial worms, *Dirofilaria immitis* and *D. repens, Setaria (S. labiatopapillosa, S. digitata* and *S. equina)* and *Diptalonema evansi* (camel filariasis) have been reported in Iran (Siavashi and Massoud 1995, Eslami 1997, Azari-Hamidian et al. 2007, Oryan et al. 2008). *Anopheles maculipennis* and *Culex theileri* were reported as vectors of *Setaria labiatopapillosa* and *Dirofilaria immitis* respectively, in Ardebil Province (Azari-Hamidian et al. 2009). Dengue fever cases were reported in Tehran, imported from Malaysia, India and Thailand. In addition, six DF cases were reported from Sistan and Baluchistan Province in southeast Iran (Chinikar et al. 2010, 2012).

The systematic key of Anopheles species in Iran was reported by Shahgudian (1960). Many studies have been carried out about the genus of Culex and its medical importance (Ghaffary 1956, Lotfi 1970, Lotfi 1973, Lotfi 1976, Zaim et al. 1984a, 1984b, 1985, 1986). The results of these studies have led to the publishing of the Iranian Culicinae checklist (Zaim and Cranston 1986). The role of mosquitoes in malaria transmission and its geographical distribution have been studied by Saebi (1987). The fauna and ecological characteristics of Culicidae with emphasis on their medical importance were conducted in Isfahan and Hormozgan Provinces as well as Chabahar County (Mousakazemi et al. 2000, Moosa-Kazemi et al. 2005, 2009) and Guilan Province (Azari-Hamidian et al. 2002a). Moreover, the study on the genus of Aedes is very scattered in the northern parts of the Iran. Azari-Hamidian et al. (2002a) stressed the occurrence of Oc. caspius, Oc. echinus, Ae. vexans and Oc. geniculatus in Guilan Province, northern Iran. More than 30 culicidae species reported from Guilan province (Azari-Hamidian et al. 2003a).

Distribution of larval habitat of *Cx. pipiens* was reported by Dehghan et al. (2011) in central Iran. Among the 467 species of *Anopheles* identified in the world (Harbach 2007), about 70 *Anopheles* species are malaria vectors in which about 40 species are important vectors (Service 1993). *Anopheles maculipennis* complex, one of the most important vectors of malaria, was reported in northern Iran (Eslami 1997, Azari-Hamidian 2007a). *Anopheles maculipennis*, and *Cx. pipiens* were reported as dominant species in

Isfahan Province, central Iran (Mousakazemi et al. 2000a) and in East Azarbaijan and Ardebil Provinces (Abai et al. 2007, Azari-Hamidian et al. 2009).

By now, seven species of malaria vectors have been reported in Iran (Vatandoost et al. 2009a). They include: An. culicifacies, An. dthali, An. fluviatilis, An. maculipennis, An. sacharovi, An. stephensi, and An. superpictus (Edrissian 2006, Vatandoost et al. 2008, Vatandoost et al. 2009a, Moosa-Kazemi et al. 2010). Anopheles pulcherrimus has been identified as potential vector in Iran using serological method (Zaim et al. 1993). Although oocyte of Plasmodium was found in the stomach of An. multicolor but sporozoite never found in its salivary gland and not considered as the malaria vector (Eshghy 1977). Anopheles hyrchanus was found as a malaria vector using the PCR method in northern Iran (Dinparast Djadid et al. 2009). At present, 64 species, three subspecies and seven genera of Culicidae have been reported from Iran (Azari-Hamidian 2007a).

The first study about the mosquitoes in Mazandaran Province was carried out by Gutzevich (1943) and Zolotarev (1945). The occurrence of the species of An. maculipennis, An. melanoon (An. subalpinus), An. Superpictus, An. sacharovi and An. messeae have been reported by Zolotarev (1945). However, Gutsevich (1943) reported the species of An. pulcherimus, Ae. vexans, Oc. geniculatus and Cq. richiardii in Mazandaran Province. Subsequently, Garrett Jones (1951) reported the species of An. claviger, An. maculipennis, An. plumbeus and An. hyrcanus in Klardasht. Institute of Parasitology and Malariology (1953) reported the species of An. marteri in Ramsar. Dow (1953) reported the species of An. maculipennis, An. melanoon, An. subalpinus (synonym of melanoon), An. hyrcanus, An. pseudopictus, An. superpictus, An. sacharovi, Cx. pipiens, Cx. mimeticus and Cx. tritaeniorhynchus. However, Faghih (1969) stressed the occurrence of the species

of An. melanoon, and An. messeae. Minar (1974) reported the species of An. Maculipennis, An. pseudopictus, Oc. pulcritarsis. However, Zaim et al. (1984a) cited the species of Ae. vexans, Oc. echinus, Oc. geniculatus and Oc. pulcritarsis. Zaim (1987) cited the species of Ae. vexans, Oc. echinus, Oc. geniculatus, Oc. pulcritarsis, Cx. perexiguss, Cx. theileri, Cx. mimeticus, Cx. Tritaeniorhynchus, Cx. hortensis, Cx. pipiens, Cs. longiareolata and Cs. subochrea. Saebi (1987) stated the species of An. plumbeus, An. hyrchanus, An. psudopictus, An. maculipennis and An. claviger. Momeni (1991) reported the species of An. messeae, An. melanoon, and An. maculipennis. Glick (1992) reported the species of An. psudopictus. Karami (2000) cited the presence of Cx. bitaeniorhynchus, Ur. unguiculata, Oc. caspius. Sedaghat et al. (2003) reported the species of An. Maculipennis, An. sacharovi, An. persiensis in Mazandaran Province. Sedaghat and Harbach (2005) stated the occurrence of the species of An. melanoon, An. persiensis, An. pseudopictus. Moreover, Dinparast et al. (2007) cited the species of An. atroparvous and An. persiensis. Dinparast et al. (2009) stressed the presence of the species of An. hyrchanus. Nikookar et al. (2010) reported the species of An. plumbeus, Oc. echinus, Oc. geniculatus, Oc. pulcritarsis, Cx. pipiens and Cs. annulata.

Iran is in the malaria pre elimination stage (WHO 2008), thus the bionomic study of mosquitoes in this country is very important. There are scattered studies on the fauna and ecological properties of mosquitoes in Mazandaran Province. Many travelers may travel to Mazandaran Province and Neka County during visiting Iran. This part of Iran holds specific location due to access to the Caspian Sea and to other aspects such as trade, commerce and navigation.

This study aimed to determine fauna and the ecology of mosquitoes in Neka County, Mazandaran Province, northern Iran from April to December, 2009. The data obtain in this study will be valuable to develop programs for future planning of mosquito control in this area.

Materials and Methods

The study area

Mazandaran Province is located in the northern Iran between the Caspian Sea and the Alborz Mountains (36°39 N, 53°17 E) (Fig. 1). This area located in the north with Caspian Sea, in the south with Tehran and Semnan Provinces, in the east with Golestan Province and in the west Guilan Province, including 19 Counties (Geography of Mazandaran Province 2013). Maximum and minimum temperatures were 27.6 and 9.1 °C in July and December 2009, respectively. The maximum annual rainfall has been reported 204.6 mm in October, and the least was 0.1 mm in July 2009. The most important crop of the region is rice, and Hyrcanian forest area and mild and rainy weather caused this Province to be a suitable mosquito habitat (Mazandaran Local Meteorological Office 2009).

Three fixed villages, namely Darvishan, Chalmardi, and Khairabad, were randomly selected for field survey. Moreover, six variable villages (Golbestan, Elmiyeh, Baghearab, Nimchah, Chalehpol, and Khorshid) were selected with regard to existing facilities in Neka County. In each fixed village, six fixed and two variable locations were selected in plain, slope and mountain conditions. Larvae sampling was collected from the natural larval habitats of fixed villages including river margins, ponds and water craters, craters rain water, marsh, tree holes, rice fields, worn tires, irrigation canals, wells, and sewage, using the standard dipper rate of 350 mm capacity every 15 days (WHO 1963). In addition, larval collection carried out in variable villages randomly.

We attempted to collect the data about the biological and physical characteristics of the larval habitats, including running water, residents (permanent or temporary), the type of larval habitats natural or artificial, presence or absence of the plant, type of substrate (sand, clay, or rocky), to be sunny (sunny or shady), water status (clear or opaque) and water temperature.

The larval stages III and IV were preserved in 100 % lactophenol solution and later identified morphologically using the keys of Shahgudian (1960), Zaim and Cranston (1986a), Harbach (1985, 1988), and Azari-Hamidian and Harbach (2009). The results were analyzed by SPSS 11.5 software. The abbreviations of the genus names were regulated based on Reinert (2009).

Results

A total of 32530 larvae were collected: 3625 larvae of anophelinae and 28905 culicinae. Table 1 shows the results of anophelinae and culicinae species in the fixed and variable breeding places and tree holes. Four species of *Anopheles* were collected: *An. claviger* (0.31%), *An. maculipennis* (0.54%), *An. plumbeus* (10.28%), and *An. superpictus* (0.01%).

Anopheles claviger collected 0.31 %, 2.78 % of total culicidae and Anopheles collection, respectively (Table 1). The characteristics of the habitats under different temperature and altitude conditions are shown in Table 2. The minimum and maximum temperature of water of the larval habitats was calculated as 19 °C and 22 °C, respectively (average 20.5 °C). On the other hand, the maximum and minimum altitude of the habitats was 300 and 200 meters, respectively (average 250 meters).

Anopheles maculipennis was collected 0.54 %, 4.82 % of the total and Anopheles collection, respectively. Maximum and mini-

mum water temperature was reported as 26 °C and 17 °C, respectively (average 22.5 °C). Maximum and minimum altitude was 300 and 180 meters respectively, (average 240 meters).

Anopheles plumbeus was collected 10.28 %, 92.27 % of total and Anopheles collection, respectively. Moreover, this species was collected in tree holes and the artificial habitats such as pond water. Maximum and minimum temperature was found as16 °C and 14 °C, respectively (average 15 °C). The minimum and maximum sea level of larval habitat was calculated at least 180 and 250 meters, respectively and the average were 215 meters.

Anopheles superpictus was collected 0.01 %, 0.13 % of the total and Anopheles collection, respectively. Minimum and maximum temperature of the larval habitat was reported as 17 °C and 19 °C, respectively and average was 180 °C. The minimum altitude was 280 meters, and the maximum was 300 (average 290 meters). The optimum of temperature and favorable altitudes of other species are presented in Tables 1 and 2.

The majority of An. plumbeus was collected in natural, permanent, and stagnant water with vegetation, turbid and transparent water and semi-shade tree holes are shown in Table 3. An. superpictus larvae were collected from natural, permanent, stagnant water with vegetation, clear and transparent breeding places. On the other hand, An. claviger larvae were collected mostly in permanent, and stagnant, transparent water with semi-shade and vegetation. Culex mimeticus was found in the natural habitats such as river and ponds from Permanent River, and stagnant pools with vegetation. The larvae of Cx. pipiens, the most abundant species, were found in artificial habitats and sewage breeding places in agricultural lands and low abundance along the river. Moreover, Oc. geniculatus, the most dominant species, was found in permanent water with vegetation in slow running with clay substrate water, semishade in natural breeding places. The larvae of *Cs. longiareolata* were found in most the larval habitats. The larval habitat characteristics of the other species are shown in Table 3.

Checklist of Culicidae in Mazandaran Province

Family culicidae

Subfamily Anophelinae Subgenus Anopheles Meigen 1- An. (Ano.) algeriensis 2- An. (Ano.) claviger 3- An. (Ano.) marteri 4- An. (Ano.) maculipennis 5- An. (Ano.) melanoon 7- An. (Ano.) melanoon 7- An. (Ano.) messeae 8- An. (Ano.) persiensis 9- An. (Ano.) persiensis 9- An. (Ano.) plumbeus 11- An. (Ano.) hyrcanus 12- An. (Ano.) pseudopictus

Subfamily Culicinae Subgenus Cellia Theobald 13- An. (Cel.) pulcherrimus 14- An. (Cel.) superpictus

Subgenus *Aedimorphus* **Theobald** 15- *Ae.* (*Adm.*) *vexans*

Subgenus Finlaya

16- Oc. (Fin.) echinus 17- Oc. (Fin.) geniculatus

Subgenus *Ochlerotatus* 18- *Oc.* (*Och.*) *caspius* 19- *Oc.* (*Och.*) *pulcritarsis*

Subgenus Culex

20- Cx. (Cux.) pipiens
21- Cx. (Cux.) perexiguss
22- Cx. (Cux.) territans
23- Cx. (Cux.) theileri
24- Cx. (Cux.) mimeticus
25- Cx. (Cux.) tritaeniorhynchus
26- Cx. (Cux.) hortensis

Subgenus Allotheobaldia

27- Cs. (All.) longiareolata

Subgenus Culiseta

28- Cs. (Cus.) annulata 29- Cs. (Cus.) subochrea

Subgenus Coquillettidia

30- Cq. (Coq.) richiardii

Subgenus Pseudoficalbia

31- Ur. (Pfc.) unguiculata

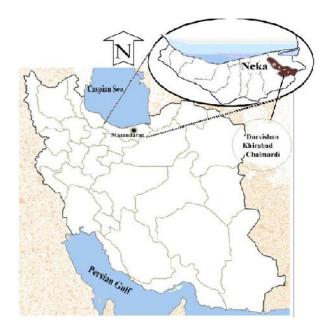


Fig. 1. Map of Iran and Neka County, northern Iran

Species						
	Darvishan	rishan Chalmardi Khairabad Variab		Variable	n	%
An. claviger	2	6	2	0	100	0.31
An. maculipennis	10	4	3.5	0	175	0.54
An. plumbeus	1.5	0	333	0	3345	10.28
An. superpictus	0	0.5	0	0	5	0.01
Cs. annulata	2	25	8	0	350	1.07
Cs. longiareolata	50.5	21.5	202	16	2900	8.91
Cx. mimeticus	0	0.5	0.5	0	10	0.03
Cx. pipiens	527.5	494	991	69	20815	63.99
Oc. geniculatus	0	0	483	0	4830	14.85

Table 1. Abundance of larval mosquitoes (density per 10 dip) in Neka County, Mazandaran Province, northern Iran,2009

 Table 2. Species composition of mosquito larvae collected from different temperatures and altitudes in Neka

 County, northern Iran, 2009

	Ten	nperature C		Altitude			
Species	Minimum	Maximum	Mean	Minimum	Maximum	Mean	
An. claviger	19	22	20.5	200	300	250	
An. maculipennis	17	26	22.5	180	300	240	
An. plumbeus	14	16	15	180	250	215	
An. superpictus	17	19	18	280	300	290	
Cs. annulata	12	22	17	180	300	240	
Cs. longiareolata	12	25	18.5	180	300	240	
Cx. mimeticus	12	13	12.5	200	300	250	
Cx. pipiens	12	25	18.5	180	300	240	
Oc.geniculatus	11	14	12.5	230	250	240	

Table 3. Larval habitat characteristics in Neka County, Mazandaran Province, northern Iran, 2009

	An. claviger	An. maculipennis	An.plumbeus	An. superpictus	Cs.annulata	Cs. longiareolata	Cx. mimeticus	Cx.pipiens	Oc.geniculatus
Habitat status									
Permanent	91	95.3	91.3	66.2	82.5	75.1	100	71.6	99
Temporary	9	4.7	8.7	33.8	17.5	24.9	0	28.4	1
Water									
Slow running	10	16	20	29.5	10	2	3	30	0
Stagnant	90	84	80	70.5	90	98	87	70	100
Vegetation									
Without vegetation	2	2	7.6	20	5	29	19.6	13	0
With vegetation	98	98	92.4	80	95	71	80.4	87	100
Type of bed									
Clay	47	1	84.5	50	98	88	66	56	94
Sand	3	17	15.5	20	2	5	30	33	6
Stone or cement	50	82	0	30	0	5	4	11	0

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Water status									
Opaque	1	40	50	9.1	50	40	40	45	50
Transparent	99	60	50	90.9	50	60	60	55	50
Status Light									·
Sunny	1	33.2	0	14.7	0	2.26	26.7	37.4	0
Shaded	34	28.4	5	26.1	5	26.9	19.4	18.9	5
Semi-shade	65	38.4	95	59.2	95	70.5	54.9	43.7	95
Habitat type									·
Natural	99	55	98	89	98	97	70	41	100
Artificial	1	45	2	11	2	3	30	59	0

Discussion

In the present study, *An. plumbeus* larvae were collected from tree holes and artificial habitats such as permanent and stagnant water. However, the maximum and minimum of temperature of the larval habitat was as 16 °C and 14 °C, respectively. In parallel, Azari-Hamidian (2011a) found this species in tree holes and discarded tire. The most favorable temperature is 15–18 °C; however, the species has tolerated to freeze (up to -6° C to -8 °C) in the laboratory (Horsfall 1955). However, Garrett Jones reported the occurrence of this species in Kelardasht of Mazandaran Province (Garrett Jones 1951).

In our investigation, Cs. annulata was found in natural, permanent, stagnant, semishade habitats with vegetation. This species has been reported in Guilan Province, Alborz and Zagros Mountains, East Bushehr and Robat Tork of Isfahan Province (Azari-Hamidian et al. 2003b). Zaim and Cranston (1986) included Cs. subochrea in their systematic keys and Cs. annulata in their checklist. The taxonomy and distribution of Cs. annulata, Cs. subochrea and Cs. alaskaensis need to be more investigated in Iran (Azari-Hamidian 2005). Record of Cs. annulata in our research was based on adults, whereas Cs. subochrea were not found in our collection, and neither were the adults of this species (Table1). Culiseta subochrea and Cs. annulata were reported in Ardebil Province (Azari-Hamidian et al. 2009).

The larval habitats of *Cs. annulata* have also been reported from various places except tree holes (Azari-Hamidian et al. 2003b). However, *An. plumbeus* and *Oc. geniculatus* species have been reported in tree holes (Nikookar et al. 2010).

In our study, the larvae of Cs. longiareolata were collected from all larval habitats except tree holes, which comprised 8.91 %, 10.03 % of the total and Culicinae collection, respectively. This species was reported first time in north of the country by Gutzevich (1943). The occurrence of this species was also reported by the previous study in Mazandaran Province (Zaim 1987). The larval habitat of this species has been reported from many parts of Iran (Zaim 1987). At the same study, the larval habitat has been reported as contaminated with organic materials, in old pit located in outdoor places and rice fields (Kitron and Pener 1986, Mousakazemi et al. 2000, Azari-Hamidian 2005). It has also been reported from the North Khorasan and Qom Provinces (Azari-Hamidian et al. 2011, Saghafipour et al. 2012).

There is scatter information about the distribution and taxonomy of the three species of *Cs. alaskaensis*, *Cs. annulata*, and *Cs. subochrea* in Iran (Zaim et al. 1986, Azari-Hamidian et al. 2003b, Azari-Hamidian 2005). Moosa Kazemi et al. (2010) found the species of *Cs. subochrea* and *Cs. longiareolata*

in Sanandaj County of Kurdistan Province, northwestern Iran.

In our research, An. claviger was found in permanent, stagnant water with vegetation habitat, as well as in the clay and stone substrate of water. In parallel, Macan (1950) has reported the existence of this species in the sunny/shadowy springs, and slow running pools water temperature of 14-16 °C in Iraq and western Iran. Dow (1953) reported the larval habitat of this species in shallow and small pools with little vegetation. It was also found within the larval habitats with temperature of 12-15 °C (Horsfall 1955). The occurrence of this species in Mazandaran was reported previously by Garrett Jones (Garrett Jones 1951). Zaim (1987) collected An. *claviger* larvae from Kashan, central Iran.

In our study, An. maculipennis was found in permanent, transparent, semi-shady natural breeding places with vegetation habitat, as well as in the cement or stone substrate of water. In parallel, Azari-Hamidian (2011) reported the presence of this species in the stagnant, gravel substrate, sunny springs, and pools with stagnant water. Anopheles maculipennis complex formally comprises 12 Palearctic members, including An. atroparvus, An. beklemishevi, An. labranchiae, An. maculipennis, An. martinius, An. melanoon, An. messeae, An. sacharovi, An. persiensis, An. daciae, An. lewisi and An. artemievi (Dinparast Djadid et al. 2007). Anopheles maculipennis species associated by An. hyrcanus, An. claviger from Mazandaran Province previously (Nikookar et al. 2010). This species has been identified in Guilan (Azari-Hamidian et al. 2004), Mazandaran and Golestan Provinces (Zaim et al. 1986). Anophelesmesseae and Anopheles melanon reported from Guilan Province, and An. sacharovi and An. hvrcanus from Golestan Province (Saebi 1987). Anopheles sacharovi has been reported from Mazandaran Province (Sedaghat et al. 2003). Dow reported the species of An. subalpinus in Sari, Babolsar,

260

Chalus and Astaneh cities in Mazandaran Province. He also reported *An. melanoon* in Astaneh city, Guilan Province (Dow 1983). *Anopheles subalpinus* is a Synonyms *An. melanoon*. Initial studies mainly focused on identification of the mosquitoes and their role in disease transmission (Gutsevich 1943, Macan 1950, Dow 1953, Minar 1974).

In our investigation, An. superpictus was found as 0.01 %, 0.13 % of the total and Anopheles collection, respectively. This species was found in permanent, stagnant, with clay substrate, transparent water, semi-shade, natural with vegetation habitat. Studies in different parts of Mazandaran Province confirmed the occurrence of this species (Zolotarev 1945, Dow 1953). Moreover, Azari-Hamidian (2011) stated its presence in stagnant, transient, mud substrate, fully sunlight places with vegetation in natural habitats. Further support for our results comes from a study by Mousakazemi et al. (2000) in Zarrin-Shahr and Mobarakeh areas of Isfahan Province. Some information on the larval stage of An. superpictus was reported by Yaghoobi-Ershadi et al. (2001) in Ardebil Province, northwestern Iran. Some ecological aspects of this species have been in Rasht County of Guilan Province (Azari-Hamidian et al. 2002b). Ghanbari et al. (2005) reported the larval breeding places of mosquitoes including anophelines in Iranshahr, southeastern Iran. However, the edges of stony streams with sunlight and semishallow have been mentioned as the main breeding places of this species in western Iran (Macan 1950). Three genotypes named X, Y, and Z within An. superpictus have been reported in Iran by Oshaghi et al. (2008). There are no reports about the genotypes of this species in Mazandaran Province, however, it needs to be studied by the future investigations.

In our study, *Culex pipiens* was found as 63.99 % of the total and 72.01 % of Culicinae larvae collection in natural and

artificial larval habitats such as tree holes. In addition, hollow pipes, hot water, holes Hungarian sewage, stagnant water, and toilet field were reported as the breeding places of this species (Jupp 1970, Kulanin 1980, Zaim 1987, Vinogradova 2000, Mousakazemi et al. 2000). This species were reported in Natural habitat (rain pool, river edge) with Standing water Azari-Hamidian (2007b).

The species of Cx. *pipiens* and Cx. *quinquefasciatus* are very similar. There are no reports in regarding the existence of Cx. *quiquefasciatus* in Mazandaran Province. The shape of siphon of larvae in the tip, middle and the base has been mentioned to identification of two recent species (Zaim and Cranston 1986). However, Azari-Hamidian and Harbach (2009) mentiond the existence of two branches of seta 1 on abdominal segment III-VI for Cx. *pipiens* and one branch for Cx. *quinquefasciatus*.

The common characteristics among the species of *Cx. vagans*, *Cx. torrentium*, and *Cx. pipiens*, were reported as the presence of two pairs of long seta on the base of siphon (Zaim and Cranston 1986). The existence of seta 1 with 4–5 branches and seta 1-X with two branches is attributed to *Cx. torrentium* and two branches of seta 1 on abdominal segment III-V mentioned to *Cx. vagans* (Harbach 1985).

In our study, *Ochlerotatus geniculatus* was collected as comprising 14.85 %, 16.7 % of the total and Culicinae larvae collections, respectively. In addition, the most favorable temperature was 11–14 °C. Maximum and minimum altitude of the larval habitat was 250 meters and 230 meters, respectively and the average was 240 meters. Further support of this result comes from some previous studies (Gutsevich 1943, Zaim et al. 1984a, Zaim 1987, Azari-Hamidian et al. 2001, Nikookar et al. 2010). This species is very similar to *Oc. echinus* as reported in a recent study (Nikookar et al. 2010). *Ochlerotatus echinus* is distributed in Palaearctic, Europe,

north Africa, and south east Asia, and in Iran, it has been reported from Guilan Province in the north (Knight and Stone 1977, Azarai-Hamidian et al. 2002). In addition, this species were collected by janbakhsh in Sari County and reported by Zaim (1984a). Studies from some parts of Mazandaran Province showed the occurrence of this species (Zaim et al. 1984a, Zaim 1987, Nikookar et al. 2010). Moreover, the distribution of this species has been reported from both Guilan, Mazandaran Provinces (Azari-Hamidian et al. 2002a), as well as from Gulestan (Gutsevich 1943), and Ardebil (Azari-Hamidian et al. 2009).

In our study, *Culex mimeticus* was found as comprising 0.03 % of the total larvae collection in permanent, stagnant, transparent water, clay substrate, semi-shade natural breeding places. In parallel, Zaim (1987) stressed the permanent, slow running water, full sunlight, mud substrate, transient and fresh water with vegetation in natural stream bed pools and rice fields as the breeding places of this species. Studies from different parts of Mazandaran confirmed the occurrence of this species (Dow 1953, Zaim 1987).

Conclusion

Our results indicate that the presence of the dominant species of *Cx. pipiens* in larval habitat natural and artificial *Oc. geniculatus* and *An. plumbeus* in natural such as tree hole. These species dramatically increased and reach to peak in warm season. The recent species could be creating nuisance and health risks in this area.

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