**Original Article**

# Larval Habitat Characteristics of Mosquitoes of the Genus *Culex* (Diptera: Culicidae) in Guilan Province, Iran

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## ABSTRACT

An investigation was carried out during April to December 2000 to study mosquito fauna and ecology in Guilan Province of northern Iran. The mosquito larvae were collected by dipping method and larval habitat characteristics recorded according to hydro-ecological features. In total, 3937 larvae of the genus *Culex* from 92 larval breeding sites were collected. Six species of the genus *Culex*; *Cx. mimeticus*, *Cx. pipiens*, *Cx. theileri*, *Cx. tritaeniorhynchus*, *Cx. hortensis*, and *Cx. territans* were identified in the province and respectively comprised 10.3%, 47.2%, 2.2%, 31%, 6.5%, and 2.8% of the samples. Most of the larvae were collected from the natural habitats (75.6%) such as river edges (6.5%), riverbed pools (28.2%), rain pools (47.8%), stream edges (9.4%), grasslands (1.9%), marshes (2.8%), and hoof-prints (3.4%) and others from artificial habitats (24.4%) including rice fields (32.1%), irrigation channels (7.1%), wells (16.4%), discarded concrete tubes (33.1%), discarded tires (11.0%), and agricultural water-storage pools (0.3%). The ecology of *Cx. pipiens* and *Cx. tritaeniorhynchus*, which are the most prevalent species and potentially involved in the transmission of many pathogens to humans and domesticated animals, must be extensively studied.

**Keywords:** *Culex,* Ecology, Iran, Larva

## INTRODUCTION

The genus *Culex* L. includes 25 subgenera and at least 751 species in the world fauna (Service 1993, Reinert 2001). Certain species of the genus *Culex* are involved in the transmission of the various arboviral and filarial diseases to humans and domesticated animals and/or are important for their biting nuisance in different parts of the world (Cranston et al. 1987, Service 1993). Naficy and Saidi (1970) reported Sindbis virus in Iran as well as West Nile virus in Iran and Guilan Province of northern Iran. Siavashi and Massoud (1995) reported human cutaneous dirofilariasis in Guilan Province. There is no information about the vectors of these diseases in Iran. The possibility of some culicine borne arboviral outbreaks like Japanese encephalitis (JE) and Rift Valley fever in the WHO Eastern Mediterranean Region, including Iran, is noteworthy (WHO 2004). The species *Cx. tritaeniorhynchus* Giles was mentioned as the vector of JE in Iran and Iraq (Harbach 1988).

Based on the last classification of the tribe Aedini (Reinert et al. 2004) and the genus *Anopheles* Meigen (Harbach 2004), Iranian mosquitoes include nine genera and near to seventy species (Zaim and Cranston 1986, Azari-Hamidian et al. 2002b, Sedaghat and Harbach 2005, Azari-Hamidian et al. 2006, Doosti et al. 2006). While providing exact Iranian mosquito checklist needs more investigations, 24 species of the genus *Culex* have been recorded so far in Iran (Gutsevich 1943, Lotfi 1970, 1973, 1976, Danilov 1975, Zaim and Cranston 1986, Harbach 1988). The old records of *Cx. apicalis* Adams in the Palaearctic Region by different investigators,

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including Iran by Gutsevich (1943), were misidentifications. This species is very similar to *Cx. territans* Walker and occurs only in the Nearctic Region (Cranston et al. 1987). *Cx. impudicus* Ficalbi was recorded in Iran only by Lotfi (1970, 1973, 1976) and was not mentioned in the checklist of Iranian *Culex* by Zaim and Cranston (1986). Zaim and Cranston (1984) noted that the records of *Cx. vishnui* Theobald in Iran were misidentifications and the correct name is *Cx. pseuodovishnui* Colless. Also, *Culex vagans* Wiedemann and *Cx. torrentium* Martini are hard differentiated from *Cx. pipiens* L. in the larval stage (Zaim and Cranston 1986, Harbach 1988), so the records of these species based on the larval stage (Lotfi 1970, 1976) are doubtful. Of course, Danilov (1975) recorded *Cx. torrentium* in Rasht, northern Iran. *Cx. univittatus* Theobald in southwestern Asia and Iran was considered to refer to *Cx. perexiguus* Theobald. The former species is an Afrotropical one that occurs only in Yemen Republic in south-western Asia (Harbach 1988). At last, Zaim and Cranston (1986) mentioned 19 species of the genus *Culex* in Iran. Almost half of the Iranian mosquito species (31 species) and seven genera have been reported in Guilan Province (Danilov 1975, Harbach 1988, Azari-Hamidian et al. 2002b, Sedaghat et al. 2003, Gholizadeh et al. 2004).

There are more faunistic data about the genus *Culex* than other culicine mosquitoes in Iran, but the information on their ecology is not great. Dow (1953) presented some notes on mosquitoes including six species of the genus *Culex* in different areas of Iran such as larval breeding places and associated species. Golestani (1967) studied *Cx. pipiens* (as form *molestus* Forskal) in the sewage wells of Tehran. Lotfi (1970, 1973, 1976) briefly noted some ecological aspects of the *Culex* species that she found in Iran including larval breeding places and water temperature and pH. Yaghoobi-Ershadi et al. (1986) showed the characteristics of the larval breeding sites and associated species of mosquitoes including eight species of the genus *Culex* in Minab, Hormozgan Province (southern Iran). Zaim (1987a) found six species of this genus in Kashan, Isfahan Province (central Iran), and noted some aspects of their adult and larval ecology. Zaim (1987b) reviewed the distribution and larval habitat characteristics of the Iranian Culicinae including 19 species of the genus *Culex*. Mousakazemi et al. (2000) stressed the fauna and ecology of mosquitoes including *Cx. pipiens*, *Cx. theileri* Theobald, and *Cx. perexiguus* in Zarrin-Shahr and Mobarakeh area of Isfahan Province. Azari-Hamidian et al. (2002a) studied mosquito fauna in Rasht County of Guilan Province, northern Iran, and presented some breeding places and associated species of *Cx. mimeticus* Noe, *Cx. pipiens*, *Cx. theileri*, and *Cx. tritaeniorhynchus*.

The ecological data about *Culex* larvae in Iran are mostly based on the little information of faunal records, only Yaghoobi-Ershadi et al. (1986) and Zaim (1987b) exactly studied the larval habitat characteristics of the genus, especially based on the specimens of southern Iran. There is little information about the ecology of this genus in northern Iran. This article is the first that specializes in some aspects of the ecology of *Culex* larvae such as their larval habitat characteristics, water temperature, and associated species in northern Iran.

## MATERIALS AND METHODS

### Study area

Guilan Province in the Caspian Sea littoral of northern Iran, between Caspian Sea and Alborz mountain chains has coastal, plain, foothill, and mountainous areas with an area of approximately 14700 square kilometers. The province has a temperate climate and relatively warm-humid summer, and is located between 36°34′-38°27′ N latitudes and 48°34′-50°36′ E longitudes. With about 1200 mm annual rainfall, Guilan Province has the highest rainfall in Iran. The vast forests of Hyrcania and temperate climate of this province provide ideal conditions for mosquitoes to breed.

**Specimen and data collection**

Guilan Province includes 16 counties. In each county one fixed and three variable sites (including different topographical areas) were randomly selected and larval collection was carried out for 15-20 minutes in the sites during the spring, summer, and autumn seasons in 2000. Larvae were collected from natural breeding sites such as river edges, riverbed pools, rain pools, marshes, grasslands, and tree holes and from artificial breeding sites such as rice fields, irrigation channels, wells, discarded tires, and buckets by means of dipper, pipette, and bucket. Physical and biological characteristics of the larval habitats such as habitat situation (permanent or transient, standing or running), habitat kind (natural or artificial), vegetation situation (with or without vegetation), bottom type, sunlight situation (full or partial sunlight or shaded), water situation (clear or turbid), and water temperature were recorded.

**Taxonomic note**

Despite collecting and identifying *Anopheles hyrcanus* (Pallas) and *An. pseudopictus* Grassi in the adult stage in Guilan Province (Dow 1953, Azari-Hamidian et al. 2002b), these species have not been differentiated from each other in the larval stage in the present investigations and mentioned only as *An. 'hyrcanus'* in this article. Different species of *An. maculipennis* complex have been recorded in the province according to egg pattern and Polymerase Chain Reaction (PCR) (Dow 1953, Azari-Hamidian et al. 2002b, Sedaghat et al. 2003, Gholizadeh et al. 2004), but they have not been differentiated in the larval stage either and are cited as *An. maculipennis* Meigen *sensu lato*. The abbreviations for mosquito genera and subgenera follow Reinert (2001) and Reinert et al. (2004).

## RESULTS

A total of 6656 larvae including 1547 anopheline larvae (23.2%) and 5109 culicine larvae (76.8%) were collected from 127 larval breeding sites on 55 occasions. Among 127 larval breeding sites 14 (11.0%) included only the subfamily Anophelinae, 37 (29.2%) included only the subfamily Culicinae, and 76 (59.8%) included both subfamilies Anophelinae and Culicinae. Anopheline larvae were found in 90 breeding sites (70.8%) and culicine larvae in 113 (88.9%). During this study, 3937 larvae of the genus *Culex* were collected from 92 larval breeding sites (72.4% of the total breeding sites and 81.4% of the culicine breeding sites) on 44 occasions (80.0% of the total). Six species of the genus *Culex*; *Cx.* (*Culex*) *mimeticus*, *Cx.* (*Cux.*) *pipiens*, *Cx.* (*Cux.*) *theileri*, *Cx.* (*Cux.*) *tritaeniorhynchus*, *Cx.* (*Maillotia*) *hortensis* Ficalbi, and *Cx.* (*Neoculex*) *territans* were found among the samples. The larvae were collected from many different natural habitats (75.6%) such as river edges (6.5%), riverbed pools (28.2%), rain pools (47.8%), stream edges (9.4%), grasslands (1.9%), marshes (2.8%), and hoof-prints (3.4%) and artificial habitats (24.4%) including rice fields (32.1%), irrigation channels (7.1%), wells (16.4%), discarded concrete tubes (33.1%), discarded tires (11.0%), and agricultural water-storage pools (0.3%). Abundance percentages of these species are shown in Table 1. Details of the larval habitat characteristics of the six species are shown in Table 2. The association occasions of the *Culex* species with other collected species are shown in Table 3. The association percentages of the species with other mosquito larvae are shown in Table 4.

***Culex* (*Culex*) *mimeticus* Noe**

*Cx. mimeticus* larvae were collected on 12 occasions (21.8% of the total) from 14 larval breeding sites (11.0% of the total and 12.3% of the culicine breeding sites) in June, July, August, September, October, and November 2000. Maximum and minimum breeding site water temperatures were respectively 28 $^{o}$C and 12 $^{o}$C, and the mean temperature was 21.6 $^{o}$C (for 13 larval breeding sites).

***Culex* (*Culex*) *pipiens* L.**

*Cx. pipiens* larvae were collected on 28 occasions (50.9% of the total) from 40 larval breeding sites (31.4% of the total and 35.3% of

the culicine breeding sites) in April, May, June, July, August, September, October, and November 2000. Maximum and minimum breeding site water temperatures were respectively 32 °C and 12 °C, and the mean temperature was 20.1 °C (for 39 larval breeding sites).

***Culex* (*Culex*) *theileri* Theobald**

*Cx. theileri* larvae were collected on 7 occasions (12.7% of the total) from 10 breeding sites (7.8% of the total and 8.8% of the culicine breeding sites) in May, June, July, and August 2000. Maximum and minimum breeding site water temperatures were respectively 33 °C and 20 °C, and the mean temperature was 25.2 °C (for 10 larval breeding sites).

***Culex* (*Culex*) *tritaeniorhynchus* Giles**

*Cx. tritaeniorhynchus* larvae were collected on 25 occasions (45.4% of the total) from 44 breeding sites (34.6% of the total and 38.9% of the culicine breeding sites) in June, July, August, September, October, and November 2000. Maximum and minimum breeding site water temperatures were respectively 33 °C and 12 °C, and the mean temperature was 23.5 °C (for 44 larval breeding sites).

***Culex* (*Maillotia*) *hortensis* Ficalbi**

*Cx. hortensis* larvae were collected on 7 occasions (12.7% of the total) from 15 breeding sites (11.8% of the total and 13.2% of the culicine breeding sites) in May, August, and October 2000. Maximum and minimum breeding site water temperatures were respectively 31 °C and 12 °C, and the mean temperature was 19.6 °C (for 15 larval breeding sites).

***Culex* (*Neoculex*) *territans* Walker**

*Cx. territans* larvae were collected on 8 occasions (14.5% of the total) from 11 breeding sites (8.6% of the total and 9.7% of the culicine breeding sites) in April, June, July, August, September, and October 2000. Maximum and minimum breeding site water temperatures were respectively 32 °C and 16 °C, and the mean temperature was 23.0 °C (for 11 larval breeding sites).

**Table 1.** Composition and abundance of the genus *Culex* larvae in Guilan Province, Iran, Apr to Dec 2000

| Category | No. | Percentage in family (%) | Percentage in subfamily (%) | Percentage in genus (%) |
|---|---|---|---|---|
| *Cx. mimeticus* | 405 | 6.08 | 7.93 | 10.29 |
| *Cx. pipiens* | 1858 | 27.92 | 36.37 | 47.19 |
| *Cx. theileri* | 88 | 1.32 | 1.72 | 2.24 |
| *Cx. tritaeniorhynchus* | 1220 | 18.33 | 23.88 | 30.99 |
| *Cx. hortensis* | 256 | 3.85 | 5.01 | 6.50 |
| *Cx. territans* | 110 | 1.65 | 2.15 | 2.79 |
| Total | 3937 | 59.15 | 77.06 | 100 |
| Culicinae | 5109 | 76.76 | - | - |
| Culicidae | 6656 | - | - | - |

**Table 2.** Larval habitat characteristics and occurrence percentages of larvae of the genus *Culex* in Guilan Province, Iran, Apr to Dec 2000

| Larval breeding site-characteristics and habitats | *Cx. mimeticus* (%) | *Cx. pipiens* (%) | *Cx. theileri* (%) | *Cx. tritaeniorhynchus* (%) | *Cx. hortensis* (%) | *Cx. territans* (%) | *Culex* (%) |
|---|---|---|---|---|---|---|---|
| A) Habitat situation | | | | | | | |
| 1. Permanent | 0.49 | 19.05 | - | 0.74 | 4.3 | 3.64 | 9.67 |
| 2. Transient | 99.51 | 80.95 | 100 | 99.26 | 95.7 | 96.36 | 90.33 |
| 3. Slow-running water | 0.25 | 9.31 | - | 0.49 | 1.17 | 2.73 | 4.73 |
| 4. Standing water | 99.75 | 90.69 | 100 | 99.51 | 98.83 | 97.27 | 96.27 |
| B) Vegetation situation | | | | | | | |
| 1. Without vegetation | 7.40 | 68.14 | 1.14 | 24.18 | 76.95 | 51.82 | 46.89 |
| 2. With vegetation | 92.60 | 31.86 | 98.86 | 75.82 | 23.05 | 48.18 | 53.11 |
| 2a) Out of water | 14.40 | 98.64 | 83.90 | 72.21 | 35.59 | 13.20 | 67.28 |
| 2b) Water surface | - | - | - | 1.51 | - | - | 0.66 |
| 2c) Beneath of water surface | 86.13 | 35.97 | 19.54 | 38.48 | 66.10 | 86.80 | 47.53 |
| C) Bottom type | | | | | | | |
| 1. Mud | 22.47 | 82.19 | 84.09 | 55.24 | 59.45 | 57.8 | 62.73 |
| 2. Sand | 15.06 | - | - | 16.57 | 39.37 | 15.6 | 11.32 |
| 3. Gravel | 62.47 | 17.81 | 15.91 | 28.19 | 1.18 | 26.6 | 25.95 |
| D) Water situation | | | | | | | |
| 1. Turbid | - | 2.83 | - | 0.25 | - | - | 1.35 |
| 2. Clear | 100 | 97.17 | 100 | 99.75 | 100 | 100 | 98.65 |
| E) Sunlight situation | | | | | | | |
| 1. Full sunlight | 99.75 | 35.67 | 95.45 | 84.09 | 36.33 | 6.36 | 57.79 |
| 2. Partial sunlight | 0.25 | 12.43 | - | 3.3 | 28.12 | 35.46 | 9.74 |
| 3. Shaded | - | 51.19 | 4.55 | 12.61 | 35.55 | 58.18 | 32.47 |
| F) Habitat kind | | | | | | | |
| 1. Natural habitat | 88.15 | 67.30 | 78.62 | 80.59 | 84.77 | 98.18 | 75.57 |
| 1a. River edge | 14.85 | 0.85 | - | 8.95 | 17.98 | 12.04 | 6.53 |
| 1b. Riverbed pool | 83.19 | 8.29 | 66.40 | 26.15 | 30.41 | 53.70 | 28.25 |
| 1c. Stream edge | 0.28 | 13.40 | - | 11.81 | - | 2.78 | 9.43 |
| 1d. Grassland | - | - | 13.60 | 4.36 | - | - | 1.85 |
| 1e. Marsh | - | 6.35 | - | - | - | - | 2.76 |
| 1f. Rain pool | 1.68 | 63.36 | 20 | 48.62 | 51.61 | 31.48 | 47.78 |
| 1g. Hoof-print | - | 7.75 | - | 0.11 | - | - | 3.40 |
| 2. Artificial (man-made) habitat | 11.85 | 32.70 | 21.38 | 19.41 | 15.23 | 1.82 | 24.43 |
| 2a. Rice field | 100 | 7.81 | 100 | 66.19 | 94.87 | 50 | 32.08 |
| 2b. Rice irrigation channel | - | - | - | 32.38 | - | - | 7.08 |
| 2c. Well | - | 24.56 | - | - | 5.13 | 50 | 16.36 |
| 2d. Discarded concrete tube | - | 50.72 | - | - | - | - | 33.13 |
| 2e. Discarded tire | - | 16.91 | - | - | - | - | 11.04 |
| 2f. Water-storage pool | - | - | - | 1.43 | - | - | 0.31 |

**Table 3.** The association occasions of *Culex* larvae with different mosquito larvae in Guilan Province, Iran, Apr to Dec 2000

| Species | Total occasions | *An. claviger* | *An. 'hyrcanus'* | *An. maculipennis* s.l. | *An. plumbeus* | *An. superpictus* | *Ae. vexans* | *Cx. mimeticus* | *Cx. pipiens* | *Cx. theileri* | *Cx. tritaeniorhynchus* | *Cx. hortensis* | *Cx. territans* | *Cs. longiareolata* | *Cs. morsitans* | *Cs. annulata* | *Ur. unguiculata* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cx. mimeticus* | 14 | - | 12 | 10 | - | 2 | 3 | - | 4 | - | 5 | 1 | 1 | - | 1 | 1 | 1 |
| *Cx. pipiens* | 40 | 2 | 13 | 14 | 1 | 1 | 4 | 4 | 9 | 3 | 12 | 4 | 5 | 3 | 1 | 3 | - |
| *Cx. theileri* | 10 | - | 6 | 8 | - | 2 | - | - | 2 | - | 8 | 1 | - | 1 | - | - | - |
| *Cx. tritaeniorhynchus* | 44 | 1 | 31 | 30 | - | 5 | 1 | 5 | 12 | 8 | 3 | 4 | 3 | 2 | - | - | - |
| *Cx. hortensis* | 15 | 4 | 4 | 2 | - | 2 | 1 | 1 | 4 | 1 | 4 | 1 | - | 5 | - | 2 | - |
| *Cx. territans* | 11 | 4 | 5 | 5 | - | - | 2 | 1 | 5 | - | 3 | - | - | - | 1 | - | - |

**Table 4.** Association percentages of *Culex* larvae with different mosquito species larvae in Guilan Province, Iran, Apr to Dec 2000

| Species association | Frequency (%) |
|---|---|
| ***Cx. mimeticus*** | |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 41.98 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l. | 16.05 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *An. superpictus*, *Cx. tritaeniorhynchus* | 12.34 |
| *An. maculipennis* s.l. | 11.85 |
| *Cx. hortensis*, *Cx. pipiens* | 9.38 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens* | 7.16 |
| *An. 'hyrcanus'*, *An. superpictus*, *Ur. unguiculata* | 0.74 |
| *An. 'hyrcanus'*, *Cx. pipiens*, *Cx. territans*, *Cx. tritaeniorhynchus* | 0.25 |
| *An. 'hyrcanus'*, *Ae. vexans*, *Cx. pipiens*, *Cs. annulata*, *Cs. morsitans* | 0.25 |
| **Total** | **100** |
| ***Cx. pipiens*** | |
| Alone | 31.16 |
| *An. maculipennis* s.l. | 11.95 |
| *An. 'hyrcanus'*, *Cx. hortensis*, *Cx. tritaeniorhynchus* | 10.55 |
| *Cx. tritaeniorhynchus* | 6.08 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. theileri*, *Cx. tritaeniorhynchus* | 5.49 |
| *An. 'hyrcanus'*, *Ae. vexans*, *Cs. annulata* | 4.41 |
| *An. claviger*, *An. 'hyrcanus'*, *An. maculipennis* s.l., *Ae. vexans* | 3.93 |
| *Cx. tritaeniorhynchus*, *Cs. longiareolata* | 3.40 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. mimeticus* | 3.34 |
| *An. plumbeus* | 2.91 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. territans* | 2.31 |
| *Cx. hortensis*, *Cs. annulata* | 2.26 |
| *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 2.26 |
| *Ae. vexans* | 1.72 |
| *An. 'hyrcanus'* | 1.61 |
| *An. 'hyrcanus'*, *Ae. vexans*, *Cx. mimeticus*, *Cs. annulata*, *Cs. morsitans* | 1.45 |
| *An. claviger*, *Cx. territans* | 0.97 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 0.97 |
| *An. maculipennis* s.l., *Cx. territans* | 0.86 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. theileri*, *Cx. tritaeniorhynchus*, *Cs. longiareolata* | 0.7 |
| *Cx. territans* | 0.59 |
| *An. 'hyrcanus'*, *Cx. mimeticus*, *Cx. territans*, *Cx. tritaeniorhynchus* | 0.38 |
| *Cs. longiareolata* | 0.32 |
| *An. 'hyrcanus'*, *An. maculipennis* s.l., *Cx. theileri* | 0.16 |
| *An. 'hyrcanus'*, *An. superpictus*, *Cx. hortensis*, *Cx. tritaeniorhynchus* | 0.11 |
| *Cx. mimeticus*, *Cx. hortensis* | 0.11 |
| **Total** | **100** |

**Table 4.** Continue…

| | |
|---|---|
| ***Cx. theileri*** | |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 37.50 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *An. superpictus*, *Cx. tritaeniorhynchus* | 19.32 |
| *An.'hyrcanus'*, *Cx. hortensis*, *Cx. tritaeniorhynchus* | 18.18 |
| *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 11.36 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens* | 3.41 |
| *An. superpictus*, *Cx. tritaeniorhynchus* | 3.41 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens*, *Cx. tritaeniorhynchus* | 3.41 |
| *An. maculipennis* s.l. | 2.27 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens*, *Cx. tritaeniorhynchus*, *Cs. longiareolata* | 1.14 |
| **Total** | **100** |
| ***Cx. tritaeniorhynchus*** | |
| *An.'hyrcanus'*, *An. maculipennis* s.l. | 30.90 |
| *An.'hyrcanus'* | 11.97 |
| *Cx. pipiens* | 9.51 |
| Alone | 6.64 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. theileri* | 5.90 |
| *An.'hyrcanus'*, *Cx. hortensis*, *Cx. theileri* | 5.24 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens*, *Cx. theileri*, *Cs. longiareolata* | 4.92 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *An. superpictus*, *Cx. mimeticus* | 3.61 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. mimeticus* | 3.36 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *An. superpictus*, *Cx. theileri* | 3.11 |
| *An. maculipennis* s.l., *An. superpictus* | 2.95 |
| *An.'hyrcanus'*, *An. superpictus*, *Cx. hortensis*, *Cx. pipiens* | 2.30 |
| *An. maculipennis* s.l., *Cx. pipiens* | 2.13 |
| *An. superpictus*, *Cx. theileri* | 1.89 |
| *An.'hyrcanus'*, *Ae. vexans* | 1.56 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. hortensis* | 1.15 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. territans* | 0.82 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens*, *Cx. theileri* | 0.74 |
| *An.'hyrcanus'*, *Cx. mimeticus*, *Cx. pipiens*, *Cx. territans* | 0.49 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens* | 0.41 |
| *An. claviger*, *An. maculipennis* s.l., *Cx. territans* | 0.16 |
| *An.'hyrcanus'*, *Cx. hortensis*, *Cx. pipiens* | 0.16 |
| *Cx. pipiens*, *Cs. longiareolata* | 0.08 |
| **Total** | **100** |
| ***Cx. hortensis*** | |
| *Cs. longiareolata* | 30.47 |
| *An. claviger* | 14.85 |
| *Ae. vexans* | 14.46 |
| *An. maculipennis* s.l. | 14.06 |
| *An. claviger*, *Cs. longiareolata*, *Cs. annulata* | 7.81 |
| *An. claviger*, *Cs. longiareolata* | 6.25 |
| *An. claviger*, *An. superpictus*, *Cs. longiareolata* | 3.52 |
| *An.'hyrcanus'*, *Cx. pipiens*, *Cx. tritaeniorhynchus* | 2.34 |
| *An.'hyrcanus'*, *Cx. theileri*, *Cx. tritaeniorhynchus* | 1.56 |
| *An.'hyrcanus'*, *An. superpictus*, *Cx. pipiens*, *Cx. tritaeniorhynchus* | 1.56 |
| Alone | 1.17 |
| *Cx. pipiens*, *Cs. annulata* | 0.78 |
| *Cx. mimeticus*, *Cx. pipiens* | 0.78 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 0.39 |
| **Total** | **100** |

**Table 4.** Continue…

| ***Cx. territans*** | |
|---|---|
| *An. claviger* | 26.36 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 26.36 |
| *An.'hyrcanus'*, *An. maculipennis* s.l. | 15.45 |
| *Cx. pipiens* | 11.82 |
| *An.'hyrcanus'*, *An. maculipennis* s.l., *Cx. pipiens* | 9.09 |
| *An.'hyrcanus'*, *Cx. mimeticus*, *Cx. pipiens*, *Cx. tritaeniorhynchus* | 2.73 |
| *An. claviger*, *An. maculipennis* s.l., *Cx. tritaeniorhynchus* | 2.73 |
| *Ae. vexans*, *Cs. morsitans* | 1.82 |
| *An. claviger*, *An.'hyrcanus'*, *Ae. vexans* | 1.82 |
| *An. maculipennis* s.l., *Cx. pipiens* | 0.91 |
| *An. claviger*, *Cx. pipiens* | 0.91 |
| **Total** | **100** |

# DISCUSSION

Results of this study revealed that the genus *Culex* species laid their eggs in many different natural and artificial habitats. In parallel to the present research, Service (1993) stressed this genus is, by far, the largest, most common, and most important genus of the tribe Culicini. In these investigations, the species of this genus were the most abundant species of mosquitoes in the studied area (Table 1).

*Cx. mimeticus* larvae are found in pooled streams, springs, and pools (Horsfall 1955). Dow (1953) collected the larvae in shallow pebbly pools in northern Iran. Lotfi (1970, 1976) found it in river edges and riverbed pools with water temperature 18-24 $^{o}$C and pH 6-7.5. Some other breeding places of this species are pools in swampy ground, irrigation ditches, and animal hoof-prints (Harbach 1988). Zaim (1987b) found this species mostly in natural habitats (88.7%), and in man-made ones such as overflow water and rice fields. Azari-Hamidian et al. (2002a) observed it only in natural habitats. This phenomenon was nearly seen in this study either, and a few *Cx. mimeticus* larvae (11.8%) found only in rice fields as artificial habitat (Table 2). Harbach (1988) mentioned *An. cinereus* Theobald, as *An. hispaniola* (Theobald), *An. sergentii* (Theobald), and *An. superpictus* larvae with *Cx. mimeticus*, and Azari-Hamidian et al. (2002a) collected it with *An. 'hyrcanus'*. *An. cinereus* does not exist in Iran and *An. sergentii* occurs only in southern Iran, but other species were collected with *Cx. mimeticus* in the current research (Table 4).

*Cx. pipiens* larvae have been found in many different natural and artificial habitats including underground ones such as flooded cellars, drains, wells, septic tanks, underground train systems, abandoned mine tunnels, and coal mines (Horsfall 1955, Cranston et al. 1987, Harbach 1988, Service 1993). The wide variety of the breeding places can explain why this species is one of the most abundant and common mosquito species in Iran as well as Guilan Province (Table 1 and 2). In Iran, Dow (1953), Lotfi (1970, 1976), and Yaghoobi-Ershadi et al. (1986) found this species mostly in natural habitats. Also, Zaim (1987b) collected it mainly in natural habitats (63.7%), but Mousakazemi et al. (2000) collected it only in rice fields (artificial habitat) and Azari-Hamidian et al. (2002a) found it mainly in man-made habitats (83.7%). In the present study, the species was collected mainly in natural larval sites (67.3%), but it was collected from man-made ones (32.7%) more than any other *Culex* species (Table 2). It seems that the most favorable breeding places of *Cx. pipiens* in cities are the house ponds, wells, and the sewage wells (Golestani 1967, Lotfi 1976, Zaim 1987a). Lotfi (1976) noted the water temperature and pH for *Cx. pipiens* 18-27 $^{o}$C and 7-8 (once 6.2), respectively. Dow (1953) found *Cx.*

*pipiens* larvae with *An. claviger* and *Cx. quinquefasciatus* Say, Yaghoobi-Ershadi et al. (1986) with *Cx. perexiguus*, as *Cx. univittatus*, *Cx. theileri*, *Cx. tritaeniorhynchus*, *Cx. deserticola* Kirkpatrick, and *Cs. longiareolata*, and Azari-Hamidian et al. (2002a) with *An. 'hyrcanus'*, *An. maculipennis* s.l., *Ae. vexans*, and *Cx. tritaeniorhynchus*. Among these species, *Cx. quiquefasciatus*, *Cx. perexiguus*, and *Cx. deserticola* were not found in Guilan Province in the current study, but *Cx. pipiens* was with other species (Table 4). *Cx. pipiens* may be associated with many species and share some larval sites with *Cx. torrentium* (Cranston et al. 1987). These species are difficult to be differentiated from each other and *Cx. vagans* in the larval stage (Zaim and Cranston 1986, Harbach 1988). There is little information about these two species in Iran.

*Cx. theileri* is found in a wide range of breeding sites such as streams, irrigation ditches, swamps, different pools, springs, disused wells, open cisterns, and seepage water (Harbach 1988). Lotfi (1970, 1976) found it in seepages, small ponds, grassy ponds, rice fields, and agriculture water-storage pools with water temperature 16-27 $^{o}$C and pH 7-8 (once 5.4) in Iran. Dow (1953) and Yaghoobi-Ershadi et al. (1986) found it in different natural and artificial habitats. Zaim (1987b) noted that the species was mainly in natural habitats (82.1%) and Mousakazemi et al. (2000) collected it from rice fields in Isfahan Province more than any other mosquito species. Azari-Hamidian et al. (2002a) found it only in natural habitats (pools). In the present study, *Cx. theileri* was often collected from natural breeding sites (78.6%) and a few larvae (21.4%) were found only in rice fields as man-made ones (Table 2). The larvae of *Cx. theileri* were collected with *An. 'hyrcanus'*, *An. maculipennis* s.l., *An. superpictus*, *Cx. hortensis*, *Cx. pipiens*, *Cx. tritaeniorhynchus*, *Cx. quiquefasciatus*, *Cx. perexiguus*, *Cx. deserticola*, *Cs. longiareolata*, *Oc. caspius* s.l., and *Ur. unguiculata* in different areas of Iran (Dow 1953, Yaghoobi-Ershadi et al. 1986, Azari-Hamidian et al. 2002a) and Harbach (1988) mentioned that the larvae often found alone or with *Cx. pipiens*, *Cx. perexiguus*, or *Cx. antennatus* (Becker). In the current investtigation, *Cx. theileri* larvae were not observed alone and among these species *Cx. perexiguus*, *Cx. quiquefasciatus*, *Cx. deserticola*, and *Cx. antennatus* were not found in Guilan Province and *Oc. caspius* s.l. was found only in the adult stage (Azari-Hamidian et al. 2002b), but the species was associated with other species except for *Ur. unguiculata* (Table 4).

*Cx. tritaeniorhynchus* is typically found in rice fields, flood waters, and marshy areas and some other natural or artificial habitats (Harbach 1988). Lotfi (1970, 1976) found this species in rice fields, ponds, river edges, and stream edges with water temperature 19-33 $^{o}$C and pH 6.4-7. Zaim (1987b) found it mostly (76%) in man-made breeding sites especially rice fields (68.5%), more than any other *Culex* species in Iran, but Azari-Hamidian et al.(2002a) found it mainly (83.9%) in natural habitats in Rasht County of northern Iran. This phenomenon was observed in this investigation either, and the species mainly found in natural habitats (80.6%) (Table 2). The reason probably is that there are many different natural breeding sites in Guilan Provine because of great annual rainfall, but other areas of Iran mainly have less annual rainfall and natural breeding sites are fewer. Horsfall (1955) mentioned many species with *Cx. tritaeniorhynchus* larvae. Some of them have been recorded in Iran but was not found in this study such as: *An. marteri* Senevet and Prunnelle, *An. sergentii*, *Cx. bitaeniorhynchus* Giles, and *Cx. vagans*. Dow (1953) found *Cx. tritaeniorhynchus* with *An. 'hyrcanus'* and *An. melanoon* Hackett (as *An. subalpinus* Hackett and Lewis) of the Maculipennis complex, and Yaghoobi-Ershadi et al. (1986) with *Cx. pipiens*, *Cx. theileri*, *Cx. sinaiticus* Kirkpatrick, *Cx. quiquefasciatus*, *Cx. perexiguus*, *Cx. deserticola*, *Oc. caspius* s.l., and *Ur. unguiculata* in southern Iran and Azari-Hamidian et al. (2002a) with *An. 'hyrcanus'*, *An. maculipennis* s.l., *Ae. vexans*, and *Cx. pipiens* in northern Iran. Among these species *Cx. sinaiticus*, *Cx. quiquefasciatus*, *Cx. perexiguus*, and *Cx. deserticola* were not found

in this study and *Oc. caspius* s.l. was found only in the adult stage (Azari-Hamidian et al. 2002b). *Cx. tritaeniorhynchus* was associated with other species except for *Ur. unguiculata* (Table 4).

*Cx. hortensis* is found in algal mats, seeps, brackish pools, domestic containers, and cement vats (Horsfall 1955). Dow (1953) found it in the irrigation ditches, small, spring like pools of the river banks and shallow pools in the river beds. Zaim (1987b) found the species mostly (93.5%) in natural habitats. In the present study, this species was mainly (84.8%) found in natural habitats (Table 2). Lotfi (1976) collected this species in seepages and agriculture water-storage pools with water temperature 15-26 $^{o}$C and pH 6-7.5 and associated species were *Cx. theileri* and *Cx. pipiens* in northern Iran and *Cx. theileri* and *Cx. quinquefasciatus* in southern Iran. Dow (1953) found *Cx. hortensis* with *An. maculipennis* s.l., *An. superpictus*, *Cx. theileri*, and *Cs. longiareolata* in northwestern Iran. Zaim (1987a) stressed that the species was found mainly with *An. maculipennis* s.l., *Cs. longiareolata*, and *Cs. annulata*. In this study, this species was with all of these species except for *Cx. quiquefasciatus* that it was not found in Guilan Province (Table 4).

*Cx. territans* is mostly found in natural swamps, ponds, and pools (Horsfall 1955). Lotfi (1973, 1976) found it in ponds with water temperature 23-27 $^{o}$C and pH 7-7.5. Zaim (1987b) found it in small permanent pools. There are few records of utilization of artificial habitats including containers such as barrel for this species (Cranston et al. 1987). It is noteworthy that this species was found in man-made habitats (1.8%) such as rice fields and wells as well as natural ones (98.2%), in this study (Table 2). Cranston et al.(1987) noted this species is often in company with *An. claviger*. This phenomenon was exactly observed in this investigation and most of *Cx. territans* larvae (26.3%) were associated with this species (Table 3 and 4). Horsfall (1955) listed many species with *Cx. territans* larvae. Among them, *An. claviger*, *Ae. vexans*, *Cx. pipiens*, *Cx. quinquefasciatus*, and *Cs. morsitans* are in Iran. In the present study, *Cx. quinquefasciatus* was not found in Guilan Province, but other species were associated with *Cx. territans* (Table 4).

During this investigation all reported species of the genus *Culex* in Guilan Provoince were collected (Zaim 1987b, Azari-Hamidian et al. 2002b), except for two records. Danilov (1975) reported *Cx. torrentium* from Rasht in Guilan province. The best character for identification of this species from *Cx. pipiens* complex is the characters of the male hypopygia (Cranston et al. 1987, Harbach 1988). There is a unique record of *Cx. quinquefasciatus* in Enzeli of Guilan Province by Harbach (1988). This is out of the usual distribution of this species that occurs in central and southern Iran (Zaim 1987b). There is no more information about this record (Professor RE Harbach, personal communication). The ecology of *Cx. pipiens* and *Cx. tritaeniorhynchus*, which are involved in the transmission of many pathogens to humans and domesticated animals and making nuisance, yet to be extensively studied. Larval habitat water temperature, pH, electric conductivity (EC), turbidity, and different organic and inorganic compounds are items which should be considered in forthcoming studies.

All specimens of this study are deposited in the Museum of Medical Entomology at the School of Public Health, Guilan University of Medical sciences.

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## REFERENCES

Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E (2002a) The distribution and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Rasht County (Guilan Province, Iran). Modarres J Med Sci. 4: 87-96 [In Persian with English abstract].

Azari-Hamidian S, Joeafshani MA, Mosslem M, Rassaei AR (2002b) Taxonomic survey of mosquitoes (Diptera: Culicidae) in Guilan Province with reporting of a subgenus new to Iranian mosquito fauna. The 15th Iranian Plant Protection Congress, 2002 September 7-11, Razi University of Kermanshah, pp. 319-320 [In Persian with English abstract].

Azari-Hamidian S, Abai MR, Ladonni H, Vatandoost H, Akbarzadeh K (2006) *Anopheles peditaeniatus* (Leicester) new to the Iranian mosquito fauna with notes on *Anopheles hyrcanus* group in Iran. J Am Mosq Cont Assoc. 22: 144-146.

Cranston PS, Ramsdale CD, Snow KR, White GB (1987) Keys to the Adults, Male Hypopygia, Fourth-Instar Larvae and Pupae of the British Mosquitoes (Culicidae) with Notes on their Ecology and Medical Importance. Fresh Water Biological Association Scientific Publication, No.48, Cumbria.

Danilov VN (1975) Mosquito fauna of northwestern Iran (Diptera: Culicidae). Med Parasitol and Parasitic Dis. 44: 732 [In Russian].

Doosti S, Azari-Hamidian S, Vatandoost H, Oshaghi MA, Hosseini M (2006) Taxonomic differentiation of *Anopheles sacharovi* and *An. maculipennis* s.l. (Diptera: Culicidae) larvae by seta 2 (antepalmate hair). Acta Med Iran. 44: 21-27.

Dow RP (1953) Notes on Iranian mosquitoes. Am J Trop Med Hyg. 2: 683-695.

Gholizadeh S, Tafsiri E, Zakeri S, Dinparast-Jadid N (2004) Molecular identification of a new species to Iranian anopheline fauna of *Anopheles maculipennis* complex (Diptera: Culicidae). The 13th Iranian Congress on Infectious Disease and Tropical Medicine, 2004 December 11-15, Tehran, p. 197 [In Persian].

Golestani J (1967) The methods of the mosquito *Culex* control in Tehran City. J General Med Tehran Univ Med School. 6: 376-379 [In Persian].

Gutsevich AV (1943) On the mosquitoes of north Iran. Compt Rend Acad Sci. 40: 123-125 [In Russian].

Harbach RE (1988) The Mosquitoes of the Subgenus *Culex* in Southwestern Asia and Egypt (Diptera: Culicidae). Contrib Am Entomol Inst. 24: 240-241.

Harbach RE (2004) The classification of genus *Anopheles* (Diptera: Culicidae): a working hypothesis of phylogenetic relationships. Bull Entomol Res. 94: 537-553.

Horsfall WR (1955) Mosquitoes. Their Bionomics and Relation to Disease. Hafner Publishing, New York.

Lotfi MD (1970) Iranian species of genus *Culex* (Diptera: Culicinae). Bull Soc Pathol Exot. 63: 399-403.

Lotfi MD (1973) Iranian species of genus *Culex* II Report of four species of larvae and 14 adult species. Bull Soc Pathol Exot. 66: 204-207.

Lotfi MD (1976) Key to Culicinae of Iran, genus *Culex* and their biology (Diptera: Culicidae). Iranian J Publ Health. 5: 71-84.

Mousakazemi S, Zaim M, Zahraii A (2000) Fauna and ecology of Culicidae of the Zarrin-Shahr and Mobarakeh area in Isfahan Province. Armaghan Danesh, J Yasuj Univ Med Sci. 5: 46-54 [In Persian].

Naficy K, Saidi S (1970) Serological survey on viral antibodies in Iran. Trop Geogr Med. 2: 183-188.

Reinert JF (2001) Revised list of abbreviation for genera and subgenera of Culicidae (Diptera) and the notes on generic and subgeneric changes. J Am Mosq Cont Assoc. 17: 51-55.

Reinert JF, Harbach RE, Kitching IJ (2004) Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. Zool J Linn Soc. 142: 289-368.

Sedaghat MM, Linton Y-M, Oshaghi MA, Vatandoost H, Harbach RE (2003) The *Anopheles maculipennis* complex (Diptera; Culicidae) in Iran: molecular characterization and recognition of a new species. Bull Entomol Res. 93: 527-535.

Sedaghat MM, Harbach RE (2005) An annotated checklist of the *Anopheles* mosquitoes (Diptera: Culicidae) in Iran. J Vect Ecol. 30: 272-276.

Service MW (1993) Mosquitoes (Culicidae). In: Lane RP, Crosskey RW (Eds) Medical Insects and Arachnids. Chapman and Hall. London, pp. 120-240.

Siavashi MR, Massoud J (1995) Human cutaneous dirofilariasis in Iran: A report of two cases. Iranian J Med Sci. 20: 85-86.

WHO (2004) Integrated Vector Management. WHO Regional Officer for Eastern Mediterranean, Cairo.

Yaghoobi-Ershadi MR, Zaim M, Manouchehri AV (1986) Studies on the biology of the mosquitoes in the district of Minab, Hormozgan Province, Iran, (1983-84)1-Characteristics of the larval breeding sites. J Envir Stud. 13: 17-39 [In Persian with English abstract].

Zaim M, Cranston PS (1984) On the occurrence of *Culex pseudovishnui* in Iran. Ann Trop Med Parasit. 78: 179-80.

Zaim M, Cranston PS (1986) Checklist and keys to the Culicinae of Iran (Diptera: Culicidae). Mosq Syst. 18: 233-245.

Zaim M (1987a) The mosquito fauna of Kashan, public health importance and control. Desert, Scientific Research. 18: 1-41 [In Persian].

Zaim M (1987b) The distribution and larval habitat characteristics of Iranian Culicinae. J Am Mosq Cont Assoc. 3: 568-573.