

Original Article

Larval Habitat Characteristics of the Genus *Anopheles* (Diptera: Culicidae) and a Checklist of Mosquitoes in Guilan Province, Northern Iran

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Abstract

Background: Ecological data are important in the vector control management of mosquitoes. There is scattered published information about the larval habitat characteristics and ecology of the genus *Anopheles* (Diptera: Culicidae) in Iran and most of available data is in relation to malaria vectors in southern Iran.

Methods: This cross sectional investigation was carried out to study the mosquito fauna and ecology in Guilan Province, northern Iran, during April–December 2000. Larvae were collected using the standard dipping technique. Larval habitat characteristics were recorded according to water situation (clear or turbid), vegetation, substrate type, sunlight situation, habitat situation (transient or permanent, running or stagnant), habitat type (natural or artificial), and water temperature.

Results: In total, 1547 third- and fourth-instar larvae of *Anopheles* from 90 habitats were collected and morphologically identified. Five species; *Anopheles claviger*, *An. 'hyrcanus'*, *An. maculipennis s.l.*, *An. plumbeus*, and *An. superpictus* were identified and respectively comprised 6.3%, 22.4%, 54.4%, 13.0%, and 3.9% of the samples. The mean and range temperatures of the larval habitat water were 19.6°C (n=14) (16–25°C), 22.6°C (n=53) (12–33°C), 23.8°C (n=52) (10–33°C), 11.5°C (n=12) (9–21°C), and 20.4°C (n=7) (12–26°C), respectively. There was a significant difference in the mean water temperatures (11.5–23.5°C) of the larval habitats of different species (P=0.000). Most of the genus larvae were collected from natural habitats (86.9%) such as river bed pools (46.4%) and rain pools (33.1%) with transient (98.3%), stagnant (99.5%) and clear (95.3%) water, with vegetation (69.9%), mud (42.0%) or gravel (39.7%) substrate in full sunlight (69.6%) or shaded (22.7%) area. A checklist of the province mosquitoes including 30 species and seven genera has been provided.

Conclusion: The main larval habitats of the most abundant species, *An. 'hyrcanus'* and *An. maculipennis s.l.*, in Guilan Province are: river bed pools, rain pools, and rice fields.

Keywords: Anophelinae, Ecology, Iran, larvae

Introduction

According to the most recent classification of mosquitoes, the family Culicidae (Diptera) includes two subfamilies, 11 tribes, 113 genera, and 3531 species in the world fauna and the genus *Anopheles* Meigen includes seven subgenera and at least 465 species (Harbach 2007). Certain species of *Anopheles* are involved in the transmission of various arboviral and filarial diseases to humans and domestic animals and/or are im-

portant for their biting in different parts of the world, but the most important disease transmitted by them is malaria. About 70 *Anopheles* species are malaria vectors in which about 40 are important vectors (Service 1993).

Sindbis virus was reported in Iran as well as West Nile virus in Iran and Guilan Province of the Caspian Sea littoral, northern Iran (Naficy and Saidi 1970, Saidi et al. 1976);

however there is no information about their vectors in the country. The mosquito-borne filariae; *Dirofilaria* Railliet and Henry (dirofilariasis) and *Setaria* Viborg (setariasis) (Spirurida: Onchocercidae) have been reported in Iran and Guilan Province (Eslami 1997, Azari-Hamidian et al. 2007). *Anopheles maculipennis* Meigen and *Culex theileri* Theobald are known vectors of *Setaria labi-atopapillosa* (Alessandrini) and *Dirofilaria immitis* (Leidy) respectively, in Ardebil Province, northwestern Iran (Azari-Hamidian et al. 2009). Malaria is the most important mosquito-borne disease in Iran, especially in southeastern areas, and seven species have been assumed to play role as malaria vectors: *An. sacharovi* Favre, *An. maculipennis sensu lato* (s.l.), *An. fluviatilis* James s.l., *An. stephensi* Liston, *An. superpictus* Grassi, *An. dthali* Patton, and *An. culicifacies* Giles s.l. (Edrissian 2006). Also, Zaim et al. (1993) reported *An. pulcherrimus* Theobald as a potential vector in southeastern Iran. Eshghy (1977) observed *Plasmodium* oocysts in *An. multicolor* Cambouliu, but sporozoites have not been detected in this species and it is not considered a vector in Iran. Recently, Djadid et al. (2009) reported *An. hyrcanus* (Pallas) as a potential vector of malaria based on nested Polymerase Chain Reaction (PCR) in Guilan Province.

The most recent checklist of Iranian mosquitoes includes 64 species and three subspecies belonging to seven genera (Azari-Hamidian 2007a). Almost half of the Iranian mosquito species (31 species) and seven genera have been previously recorded in Guilan Province (Zolotarev 1945, Dow 1953, Lotfi 1973, Danilov 1975, Saebi 1987, Zaim 1987b, Harbach 1988, Momeni et al. 1992, Azari-Hamidian et al. 2002b, Sedaghat et al. 2003, Gholizadeh et al. 2004, Gholizadeh et al. 2005).

Despite of the importance of ecological data in the vector control management of mosquitoes, there is scattered published information about the larval habitat character-

istics and ecology of *Anopheles* in Iran and most of available data is in relation to malaria vectors in southern Iran. Marsh (1933) showed some larval breeding places of *An. apoci* Marsh larvae when described it for the first time in Iran. Macan (1950) studied the different aspects of some *Anopheles* species in northern and western Iran. Dow (1953) and Yaghoobi-Ershadi et al. (1986) stressed the larval breeding sites and associate species of some anophelines in the different areas of the country and southern Iran, respectively. Manouchehri and Rohani (1975) studied the larval habitats of *An. dthali* in southern Iran. Zaini et al. (1975) and Manouchehri et al. (1976) mentioned the larval breeding place characteristics of *An. stephensi* in southern Iran. Eshghi et al. (1976) stressed some larval breeding places of *An. fluviatilis* s.l. in southern Iran. Eshghy (1977) noted the adult and larval ecology of *An. multicolor*. Zaim (1987a) studied the mosquito fauna of Kashan of Isfahan Province in central Iran with some notes on the larval breeding places and ecology of four anopheline species. Mousakazemi et al. (2000) studied the fauna and ecology of mosquitoes including *An. maculipennis* s.l. and *An. superpictus* in Zarrin-Shahr and Mobarakeh areas of Isfahan Province. Yaghoobi-Ershadi et al. (2001) stressed some information on the larval stage of *An. sacharovi* and *An. superpictus* in Ardebil Province of northwestern Iran. Azari-Hamidian et al. (2002a) showed some larval breeding places of mosquitoes including anophelines in Rasht County of Guilan Province in northern Iran. Ghanbari et al. (2005) studied some physical and chemical factors of the larval breeding places of the eight species of *Anopheles* in Iranshahr, southeastern Iran.

Investigation on the mosquito ecology and larval habitats is important in different aspects including source reduction in vector control through modifications of these habitats. This helps us to decrease, even if not very much, vector population and density

then vector-reservoir and vector-pathogen contacts, and biting nuisance. This may decrease the transmission of different mosquito-borne diseases and the burdens of these diseases. The present article is focused on some aspects of the larval ecology of *Anopheles* species, such as the larval habitat characteristics, water temperature, associated species, and provides a checklist of mosquitoes in Guilan Province.

Materials and Methods

Study area

Guilan Province in the Caspian Sea littoral of northern Iran, between Caspian Sea and Alborz Mountain range, has coastal, plain, foothill, and mountainous areas with an area of approximately 14700 square kilometers. The province with temperate climate and relatively warm-humid summer is located between 36°34'–38°27' N latitude and 48°34'–50°36' E longitude and formally includes 16 counties: Amlash, Astaneh-e-Ashrafiyeh, Astara, Bandar-e-Anzali, Fuman (Fooman), Lahijan, Langrud (Langroud), Masal, Rasht, Rezvan-shahr, Rudbar, Rudsar (Roudsar), Shaft, Siahkal, Some'e Sara, and Talysh (Talesh). Guilan Province with about 1200 mm annual rainfall has the greatest rainfall in Iran and the main agricultural crop is rice. This province with vast deciduous forests of Hyrcania and temperate climate is a great location to breed mosquitoes.

Specimen and data collection and analysis

In this cross sectional investigation, in each county (in total 16) one fixed and three variable sites (including different topographical areas) randomly selected and larval specimen collection was carried out for 15–20 minutes during spring, summer, and autumn seasons in 2000. Thus, larval collection was carried out at least three times in fixed sites and 9 times in variable sites during three seasons in each county. Larvae were col-

lected from natural breeding sites such as river edges, river bed pools, rain pools, marshes, grasslands, and tree holes and from artificial breeding sites such as rice fields, irrigation channels, wells, discarded tires, and buckets using the standard dipping technique (350 mL dipper) and also by means of pipette (for small habitats) and bucket (for wells) (WHO 1975). Physical and biological characteristics of larval habitats including habitat situation (permanent or transient, stagnant or running), habitat type (natural or artificial), vegetation situation (with or without vegetation), substrate type, sunlight situation (full or partial sunlight or shaded), water situation (clear or turbid), and water temperature were recorded. The continuous variable of the temperatures of larval habitats was analyzed by One-Way ANOVA analysis using SPSS (Version 11.5 for windows, SPSS Inc., Chicago, IL) software. The third- and fourth-instar larvae and adult specimens were identified using the keys of Shahgudian (1960), Zaim and Cranston (1986), and Harbach (1988). The mosquito name genera and subgenera abbreviations followed Reinert (2001).

Taxonomic note

Two close species *Anopheles hyrcanus* and *An. pseudopictus* Grassi of the Hyrcanus Group are identified based on the characters of adult in Guilan Province (Dow 1953, Azari-Hamidian et al. 2002b, 2003a, 2006), these species have not been identified in larval stage in this investigation and mentioned as *An. 'hyrcanus'* (*An. hyrcanus* group). Dar-sie and Samanidou-Voyadjoglou (1997) mentioned just one character of seta 2-C (inner clypeal seta) to distinguish the larvae of *An. hyrcanus* from those of *An. pseudopictus*. This seta is simple in *An. pseudopictus* and bears some short apical branches in *An. hyrcanus*. This character needs to be studied carefully in Iran. Seven species of *An. maculipennis* complex have been recorded in

the province based on morphological characters including egg pattern and PCR technique (Dow 1953, Azari-Hamidian et al. 2002b, Sedaghat et al. 2003, Gholizadeh et al. 2004, Gholizadeh et al. 2005). Only *An. sacharovi* is reliably distinguishable from other species in adult and larval stage. In larvae, the mean number of seta 2 branches of the fourth and fifth abdominal segments in *An. sacharovi* is 36.8 whereas it is 16.5 for *An. maculipennis s.l.* (Doosti et al. 2006). It seems the use of PCR technique is the only reliable way to distinguish other species of the group, so the Maculipennis Group larvae cited as *An. maculipennis s.l.* herein.

Results

Larval habitat characteristics and ecology

In these investigations, totally 6656 larvae including 1547 anopheline larvae (23.2%) and 5109 culicine larvae (76.8%) were collected from 127 larval breeding sites during 55 occasions. Among 127 larval breeding sites 14 (11.0%) contained only subfamily Anophelinae, 37 (29.2%) included only subfamily Culicinae, and 76 (59.8%) contained both subfamilies. In total, anopheline larvae were found in 90 breeding sites (70.8%) and culicine larvae in 113 ones (88.9%). In the present study, 1547 larvae of the genus *Anopheles* were collected during 11 occasions (20.0% of the total). Five species of *Anopheles*; *An. claviger* (Meigen) (6.3%), *An. 'hyrcanus'* (22.4%), *An. maculipennis s.l.* (54.4%), *An. plumbeus* Stephens (13.0%), and *An. superpictus* (3.9%) were found among the samples (Table 1). The association occasions of *Anopheles* species with other collected species are shown in Table 2. Most of the genus larvae were collected from natural habitats (86.9%) such as river bed pools (46.4%) and rain pools (33.1%) with transient (98.3%), stagnant (99.5%) and clear (95.3%) water, with vegetation (69.9%), mud (42.0%) or gravel (39.7%) substrate in

full sunlight (69.6%) or shaded (22.7%) area (Table 3). The association percentages of *Anopheles* species with other collected species are shown in Table 4. There is a significant difference in the mean water temperatures of the larval habitats of different species ($P=0.000$).

Anopheles claviger

Anopheles claviger larvae were collected during 11 occasions (20% of the total) from 14 larval breeding sites (11.0% of the total and 7.9% of the anopheline larval habitats) in April, May, June, and September 2000. The maximum and minimum water temperatures of larval habitats were 25° C and 16° C respectively, and mean temperature was 19.6° C (for 14 larval breeding sites) (SD= 3.0).

Anopheles 'hyrcanus'

Anopheles 'hyrcanus' larvae were collected during 33 occasions (60% of the total) from 53 larval breeding sites (41.7% of the total and 4.4% of the anopheline larval habitats) during April–November 2000. The maximum and minimum water temperatures of larval habitats were 33° C and 12° C respectively, and mean temperature was 22.9° C (for 53 larval breeding sites) (SD= 4.7).

Anopheles maculipennis s.l.

Anopheles maculipennis s.l. larvae were collected during 30 occasions (54.5% of the total) from 53 larval breeding sites (41.7% of the total and 3.5% of the anopheline larval habitats) during April–November 2000. The maximum and minimum water temperatures of larval habitats were 33° C and 10° C respectively, and mean temperature was 23.8° C (for 52 larval breeding sites) (SD= 4.0).

Anopheles plumbeus

Anopheles plumbeus larvae were collected during 9 occasions (16.3% of the total) from 12 larval breeding sites (9.4% of the total and 3.5% of the anopheline larval

habitats) in October and November 2000. The maximum and minimum water temperatures of larval habitats were 21°C and 9°C respectively, and mean temperature was 11.5°C (for 12 larval breeding sites) (SD= 3.2). The mean temperature of the larval habitat water of this species showed significant difference from other species ($P= 0.000$).

Anopheles superpictus

Anopheles superpictus larvae were collected during four occasions (7.2% of the total) from 7 larval breeding sites (9.4% of the total and 3.5% of the anopheline larval habitats) in May, August, and October 2000. The maximum and minimum water temperatures of larval habitats were respectively 26°C and 12°C, and mean temperature was 20.4°C (for 7 larval breeding sites) (SD= 6.3).

Checklist of the mosquitoes of Guilan Province

The checklist of 30 species of mosquitoes in seven genera represented in Guilan Province is given below. The species which were recorded in the province by other authors and/or using PCR technique and were not found or identified in this investigation are shown by asterisks (*): *Anopheles algeriensis* Theobald*, *An. atroparvus* van Thiel*, *An. claviger* (Meigen), *An. hyrcanus* (Pallas), *An.*

labranchiae Falleroni*, *An. maculipennis* Meigen, *An. melanoon* Hackett, *An. messeae* Falleroni, *An. persiensis* Linton, Sedaghat and Harbach*, *An. plumbeus* Stephens, *An. pseudopictus* Grassi, *An. sacharovi* Favre*, *An. superpictus* Grassi, *Aedes vexans* (Meigen), *Coquillettidia richiardii* (Ficalbi), *Culex hortensis* Ficalbi, *Cx. mimeticus* Noe, *Cx. pipiens* Linnaeus, *Cx. territans* Walker, *Cx. torrentium* Martini, *Cx. theileri* Theobald, *Cx. tritaeniorhynchus* Giles, *Culiseta annulata* (Schrank), *Cs. longiareolata* (Macquart), *Cs. morsitans* (Theobald), *Cs. subochrea* (Edwards)*, *Ochlerotatus caspius* (Pallas) s.l., *Oc. echinus* (Edwards), *Oc. geniculatus* (Olivier), *Uranotaenia unguiculata* Edwards.

Table 1. The composition and abundance of *Anopheles* larvae in Guilan Province, Iran, April–December 2000

Taxon	n	Percentage of family (%)	Percentage of genus (%)
<i>An. claviger</i>	97	1.46	6.27
<i>An. 'hyrcanus'</i>	346	5.20	22.37
<i>An. maculipennis s.l.</i>	841	12.63	54.36
<i>An. plumbeus</i>	202	3.03	13.06
<i>An. superpictus</i>	61	0.92	3.94
Total Anophelinae	1547	23.24	100
Total Culicidae	6656	-	-

Table 2. The association occasions of *Anopheles* larvae with different mosquito larvae in Guilan Province, Iran, April–December 2000

Species	Total occasions	<i>An. claviger</i>	<i>An. 'hyrcanus'</i>	<i>An. maculipennis s.l.</i>	<i>An. superpictus</i>	<i>Ae. vexans</i>	<i>Cx. mimeticus</i>	<i>Cx. pipiens</i>	<i>Cx. theileri</i>	<i>Cx. tritaeniorhynchus</i>	<i>Cx. hortensis</i>	<i>Cx. territans</i>	<i>Cs. longiareolata</i>	<i>Cs. morsitans</i>	<i>Cs. annulata</i>	<i>Oc. echinus</i>	<i>Oc. geniculatus</i>	<i>Ur. unguiculata</i>
<i>An. claviger</i>	14	-	4	4	1	3	-	2	-	1	4	4	3	-	1	-	-	-
<i>An. 'hyrcanus'</i>	53	4	-	39	4	6	12	13	7	31	4	5	1	1	2	-	-	1
<i>An. maculipennis s.l.</i>	53	4	39	-	3	1	10	14	8	30	2	5	1	1	-	-	-	-
<i>An. plumbeus</i>	12	-	-	-	-	-	-	1	-	-	-	-	-	-	-	4	1	-
<i>An. superpictus</i>	7	1	4	3	-	-	2	1	2	5	2	-	1	-	-	-	-	1

Table 3. The larval habitat characteristics and occurrence percentages of *Anopheles* larvae in Guilan Province, Iran, April–December 2000

Larval breeding site-characteristics and habitats	<i>An. claviger</i> (%)	<i>An. hyrcanus</i> (%)	<i>An. maculipennis s.l.</i> (%)	<i>An. plumbeus</i> (%)	<i>An. superpictus</i> (%)	<i>Anopheles</i> (%)
A) Habitat situation						
1. Permanent	7.2	2.6	1.2	-	-	1.7
2. Transient	92.8	97.4	98.8	100	100	98.3
3. Slow-running water	5.2	0.6	0.1	-	-	0.5
4. Stagnant water	94.8	99.4	99.9	100	100	99.5
B) Vegetation situation						
1. Without vegetation	33.0	14.7	16.8	100	65.6	30.1
2. With vegetation	67.0	85.3	83.2	-	34.4	69.9
2a) Emergent	78.9	51.4	30.2	-	66.7	39.8
2b) Floating	-	0.6	0.4	-	-	0.4
2c) Submerged	21.1	48.0	69.4	-	33.3	59.8
C) Substrate type						
1. Mud	73.7	56.1	30.7	-	67.8	42.0
2. Sand	8.4	20.8	19.7	-	-	18.3
3. Gravel	17.9	23.1	49.6	-	32.2	39.7
D) Water situation						
1. Turbid	-	1.7	8.0	-	-	4.7
2. Clear	100	98.3	92.0	100	100	95.3
E) Sunlight situation						
1. Full sunlight	22.7	71.7	89.4	-	88.5	69.6
2. Partial sunlight	11.3	21.7	4.1	-	-	7.7
3. Shaded	66.0	6.6	6.5	100	11.5	22.7
F) Habitat type						
1. Natural habitat	97.9	83.2	83.6	98.0	100	86.9
1a. River edge	-	-	0.1	-	-	0.1
1b. River bed pool	28.4	37.2	67.0	-	31.1	46.4
1c. Stream edge	5.3	7.6	0.3	-	-	2.2
1d. Grassland	-	1.0	0.7	-	1.7	0.7
1e. Marsh	-	13.2	-	-	-	2.8
1f. Rain pool	66.3	41.0	31.9	-	67.2	33.1
1g. Tree hole	-	-	-	100	-	14.7
2. Artificial habitat	2.1	16.8	16.4	2.0	-	13.1
2a. Rice field	-	79.4	61.6	-	-	64.8
2b. Rice irrigation channel	-	10.3	26.8	-	-	21.3
2c. Well	100	-	-	-	-	1.0
2d. Discarded concrete tube	-	-	5.1	-	-	3.5
2e. Discarded tire	-	-	-	100	-	2.0
2f. Water storage pool	-	10.3	6.5	-	-	7.4

Table 4. The association percentages of *Anopheles* larvae with different mosquito species larvae in Guilan Province, Iran, April–December 2000

Species association	Abundance (%)
<i>An. claviger</i>	
Alone	19.59
<i>Cx. hortensis</i> , <i>Cs. longiareolata</i>	17.53
<i>Cx. territans</i>	12.37
<i>Ae. vexans</i>	9.28
<i>An. 'hyrcanus'</i> , <i>An. maculipennis s.l.</i> , <i>Ae. vexans</i> , <i>Cx. pipiens</i>	9.28
<i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cs. longiareolata</i>	8.25
<i>An. 'hyrcanus'</i> , <i>An. maculipennis s.l.</i>	7.22
<i>Cx. hortensis</i> , <i>Cs. annulata</i> , <i>Cs. longiareolata</i>	5.15
<i>An. maculipennis s.l.</i> , <i>Cx. territans</i> , <i>Cx. tritaeniorhynchus</i>	4.12
<i>Cx. hortensis</i>	3.09
<i>An. 'hyrcanus'</i> , <i>Ae. vexans</i> , <i>Cx. territans</i>	2.06
<i>Cx. pipiens</i> , <i>Cx. territans</i>	2.06
Total	100
<i>An. 'hyrcanus'</i>	
<i>An. maculipennis s.l.</i> , <i>Cx. tritaeniorhynchus</i>	27.75
<i>Ae. vexans</i> , <i>Cx. pipiens</i> , <i>Cs. annulata</i>	10.98
<i>An. maculipennis s.l.</i> , <i>Cx. mimeticus</i>	9.83
<i>An. maculipennis s.l.</i>	7.80
<i>An. maculipennis s.l.</i> , <i>Cx. mimeticus</i> , <i>Cx. tritaeniorhynchus</i>	7.51
<i>An. maculipennis s.l.</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i> , <i>Cx. tritaeniorhynchus</i>	3.18
<i>An. maculipennis s.l.</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i>	2.90
<i>Cx. tritaeniorhynchus</i>	2.89
Alone	2.60
<i>Cx. hortensis</i> , <i>Cx. theileri</i> , <i>Cx. tritaeniorhynchus</i>	2.60
<i>An. maculipennis s.l.</i> , <i>Cx. theileri</i> , <i>Cx. tritaeniorhynchus</i>	2.31
<i>Ae. vexans</i> , <i>Cx. tritaeniorhynchus</i>	2.02
<i>An. claviger</i> , <i>Ae. vexans</i> , <i>Cx. territans</i>	1.73
<i>An. claviger</i> , <i>An. maculipennis s.l.</i>	1.73
<i>An. maculipennis s.l.</i> , <i>Cx. territans</i>	1.73
<i>An. claviger</i> , <i>An. maculipennis s.l.</i> , <i>Ae. vexans</i> , <i>Cx. pipiens</i>	1.44
<i>An. maculipennis s.l.</i> , <i>Cx. hortensis</i> , <i>Cx. tritaeniorhynchus</i>	1.44
<i>An. maculipennis s.l.</i> , <i>Cx. pipiens</i> , <i>Cx. theileri</i> , <i>Cx. tritaeniorhynchus</i> , <i>Cs. longiareolata</i>	1.44
<i>An. maculipennis s.l.</i> , <i>Cx. pipiens</i> , <i>Cx. tritaeniorhynchus</i>	1.16
<i>An. maculipennis s.l.</i> , <i>An. superpictus</i> , <i>Cx. mimeticus</i> , <i>Cx. tritaeniorhynchus</i>	1.16
<i>An. maculipennis s.l.</i> , <i>Cx. pipiens</i> , <i>Cx. territans</i>	0.87
<i>An. maculipennis s.l.</i> , <i>An. superpictus</i> , <i>Cx. theileri</i> , <i>Cx. tritaeniorhynchus</i>	0.87
<i>An. maculipennis s.l.</i> , <i>Cx. territans</i> , <i>Cx. tritaeniorhynchus</i>	0.87
<i>Cx. pipiens</i>	0.87
<i>An. maculipennis s.l.</i> , <i>Cx. mimeticus</i> , <i>Cx. pipiens</i>	0.58
<i>Ae. vexans</i>	0.29
<i>Cx. hortensis</i> , <i>Cx. pipiens</i> , <i>Cx. tritaeniorhynchus</i>	0.29
<i>Cx. mimeticus</i> , <i>Cx. pipiens</i> , <i>Cx. territans</i> , <i>Cx. tritaeniorhynchus</i>	0.29
<i>An. superpictus</i> , <i>Cx. hortensis</i> , <i>Cx. pipiens</i> , <i>Cx. tritaeniorhynchus</i>	0.29
<i>Ae. vexans</i> , <i>Cx. mimeticus</i> , <i>Cx. pipiens</i> , <i>Cs. annulata</i> , <i>Cs. morsitans</i>	0.29
<i>An. superpictus</i> , <i>Cx. mimeticus</i> , <i>Ur. unguiculata</i>	0.29
Total	100

Table 4. Continued....

An. maculipennis s.l.	
<i>An. 'hyrcanus', Cx. tritaeniorhynchus</i>	27.82
<i>An. 'hyrcanus', Cx. mimeticus</i>	14.74
<i>Cx. pipiens</i>	10.46
<i>An. 'hyrcanus', Cx. mimeticus, Cx. tritaeniorhynchus</i>	10.23
<i>An. 'hyrcanus', Cx. theileri, Cx. tritaeniorhynchus</i>	9.16
<i>An. 'hyrcanus', An. superpictus, Cx. mimeticus, Cx. tritaeniorhynchus</i>	3.69
<i>An. 'hyrcanus'</i>	3.33
<i>Cx. mimeticus</i>	2.62
Alone	2.02
<i>An. 'hyrcanus', Cx. hortensis, Cx. tritaeniorhynchus</i>	1.90
<i>An. claviger, An. 'hyrcanus'</i>	1.78
<i>Cx. theileri</i>	1.78
<i>Cx. theileri, Cx. tritaeniorhynchus</i>	1.43
<i>An. 'hyrcanus', Cx. pipiens, Cx. theileri</i>	1.43
<i>An. 'hyrcanus', Cx. territans, Cx. tritaeniorhynchus</i>	1.19
<i>An. claviger, Cx. territans, Cx. tritaeniorhynchus</i>	1.07
<i>An. 'hyrcanus', Cx. mimeticus, Cx. pipiens</i>	0.71
<i>An. 'hyrcanus', Cx. pipiens, Cx. territans</i>	0.71
<i>Cx. pipiens, Cx. tritaeniorhynchus</i>	0.71
<i>An. 'hyrcanus', Cx. pipiens, Cx. theileri, Cx. tritaeniorhynchus, Cs. longiareolata</i>	0.59
<i>An. 'hyrcanus', An. superpictus, Cx. theileri, Cx. tritaeniorhynchus</i>	0.59
<i>An. 'hyrcanus', Cx. territans</i>	0.48
<i>An. claviger, An. 'hyrcanus', Ae. vexans, Cx. pipiens</i>	0.36
<i>An. superpictus, Cx. tritaeniorhynchus</i>	0.36
<i>Cx. pipiens, Cx. territans</i>	0.36
<i>An. 'hyrcanus', Cx. pipiens, Cx. theileri, Cx. tritaeniorhynchus</i>	0.12
<i>Cx. hortensis</i>	0.12
<i>An. 'hyrcanus', Cx. pipiens, Cx. tritaeniorhynchus</i>	0.12
<i>Cs. morsitans</i>	0.12
Total	100
An. plumbeus	
Alone	62.87
<i>Oc. echinus, Oc. geniculatus</i>	18.81
<i>Oc. echinus</i>	16.34
<i>Cx. pipiens</i>	1.98
Total	100
An. superpictus	
<i>An. 'hyrcanus', Cx. hortensis, Cx. pipiens, Cx. tritaeniorhynchus</i>	26.23
<i>An. claviger, Cx. hortensis, Cs. longiareolata</i>	19.67
<i>An. maculipennis s.l., Cx. tritaeniorhynchus</i>	19.67
<i>Cx. theileri, Cx. tritaeniorhynchus</i>	11.48
<i>An. 'hyrcanus', An. maculipennis s.l., Cx. mimeticus, Cx. tritaeniorhynchus</i>	11.48
<i>An. 'hyrcanus', Cx. mimeticus, Ur. unguiculata</i>	9.84
<i>An. 'hyrcanus', An. maculipennis s.l., Cx. theileri, Cx. tritaeniorhynchus</i>	1.63
Total	100

Discussion

Larval habitat characteristics and ecology

In the present investigation, five species of the genus *Anopheles* were found and iden-

tified based on the morphological characters of larvae in different aquatic habitats in Guilan Province. While the tree-hole *An. plum-*

beus has its own special habitat and way of adaptation, other species have shared some common larval habitat characteristics such as temporary, stagnant, and clear water. However every species shows some preferred characteristics in comparison with others as follow: *An. superpictus* prefers the habitats without vegetation (65.6%); *An. claviger* and *An. superpictus* more adapt the habitats with mud (73.7% and 67.8%, respectively) or gravel (17.9% and 32.2%) substrate, respectively, whereas *An. 'hyrcanus'* and *An. maculipennis s.l.* are less conservative in this regard; *An. claviger* lays the eggs often (66.0%) in shaded area; *An. claviger* and *An. superpictus* choose mostly natural habitats (97.9% and 100%), especially rain pools (66.3% and 67.2%), however *An. 'hyrcanus'* and *An. maculipennis s.l.* show more diverse habitats and were also collected from artificial habitats (16.8% and 16.4%), especially rice fields (79.4% and 61.6%) (Table 3). These explain why *An. maculipennis s.l.* and *An. 'hyrcanus'* are the most abundant species in the studied area where rice fields and irrigation channels are numerous and vast (Table 1).

Macan (1950) found *An. claviger* in "spring pools, sometimes exposed to the sun, and in slow-flowing reaches and isolated pools of the upper parts of streams where these were heavily shaded" with water temperature 14–16° C (except for one occasion 20° C) in Iraq and western Iran. Dow (1953) found *An. claviger* in "a small, shallow and shaded stream, with much emergent and partly submerged vegetation including some grass" with *Cx. pipiens* in Maragheh of northwestern Iran. Horsfall (1955) mentioned that *An. claviger* is an associate of *An. cinereus* Theobald (as *An. hispaniola*), *An. maculipennis*, *An. marteri* Senevet and Prunelle, *An. superpictus*, *Cs. longiarolata*, *Cs. morsitans*, *Cx. laticinctus* Edwards, *Cx. mimeticus*, *Cx. pipiens*, *Cx. territans* (as *Cx. apicalis*), and *Cx. tritaeniorhynchus* and noted that the larvae have been

collected from water with temperature 12–15° C and even beneath the ice during winter and larval sites include: small ponds, flowing stream margins, springs, cisterns, fountains, coastal areas and marshes, shaded rain barrels, roadside ditches, even sewages, and especially wells in different areas of its distribution. Zaim (1987a) collected *An. claviger* from stream edge in Kashan. In the present study, *An. marteri* and *Cx. laticinctus* were not found in Guilan Province (Azari-Hamidian et al. 2002b, 2004a, 2005, Azari-Hamidian 2007b) and *An. cinereus* has not been collected in Iran (Azari-Hamidian 2007a). *Culiseta morsitans* was found in the province, not in association with *An. claviger*, however other mentioned species were collected with this species (Tables 2 and 4). As Horsfall (1955) expressed wells are the important habitats of *An. claviger* larvae, in this study among man-made habitats it was found in wells only, although the species was collected mostly from natural habitats (97.9%) (Table 3).

Unfortunately, there is little information about the larval habitat characteristics and ecology of *An. hyrcanus* and *An. pseudopictus* in details because of their problematic differentiation in larval stage (see Taxonomic note). Dow (1953) collected only *An. pseudopictus* (as *An. hyrcanus* var. *pseudopictus*) in northern Iran based on rearing immature stages 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" with *An. maculipennis*, *An. melanoon* (as *An. subalpinus*) and *Cx. tritaeniorhynchus*. Horsfall (1955) stressed *An. algeriensis*, *An. coustani* Laveran, *An. sacharovi*, *An. sergentii* (Theobald) and rarely *An. claviger*, *An. superpictus*, *Cs. annulata*, *Cx. perexiguus* Theobald, *Oc. caspius s.l.*, and *Oc. detritus* as the associated species of *An. 'hyrcanus'* in Palestine. Azari-Hamidian et al. (2002a) found *An. 'hyr-*

canus' larvae with those of *An. maculipennis s.l.*, *Ae. vexans*, *Cx. mimeticus*, *Cx. pipiens*, *Cx. theileri*, and *Cx. tritaeniorhynchus* often in rice fields and also ground pools in Rasht County. Among mentioned species, *Anopheles coustani* is not recorded in Iran (Azari-Hamidian 2007a) and *An. algeriensis*, *An. sacharovi*, *An. sergentii*, *Cx. perexiguus*, and *Oc. detritus* were not found in Guilan Province in this study (Azari-Hamidian et al. 2002b, 2002c, 2004a, 2005, Azari-Hamidian 2007b) and *Oc. caspius s.l.* was collected only as adult (Azari-Hamidian et al. 2002c). Other species larvae were found with *An. 'hyrcanus*' larvae. In the present study, *An. hyrcanus*, with dark hindtarsomere 4, except at tip, and completely dark hindtarsomere 5, reared from pupae was an associate of *An. maculipennis s.l.*, *An. pseudopictus*, *Ae. vexans*, *Cx. mimeticus*, *Cx. pipiens*, and *Cx. tritaeniorhynchus*, which were reared from pupae or collected as larvae from the same larval habitats in ground pools with emergent vegetation and mud or sand substrate and river bed pools with submerged vegetation and gravel substrate both habitats were exposed to sunlight. Also, reared *An. pseudopictus*, with completely pale hindtarsomere 4 and completely dark hindtarsomere 5, was with *An. claviger*, *An. maculipennis s.l.*, *An. hyrcanus*, *Ae. vexans*, *Cx. mimeticus*, *Cx. pipiens*, *Cx. territans*, *Cx. theileri*, *Cx. tritaeniorhynchus* in river edge without vegetation, with mud substrate in shaded area, river bed pool with submerged vegetation and gravel substrate in full sunlight situation, rice irrigation channel without vegetation with sand substrate in shaded area, and ground pool with emergent vegetation and mud substrate in full sunlight situation. Both species were collected from habitats with clear, transient, and stagnant water. In general, two species occur sympatrically in northern Iran; however *An. pseudopictus* is much more abundant than *An. hyrcanus* (Dow 1953, Azari-Hamidian et al. 2001, 2006) and the main larval habitats

are: river bed pools, rain pools, and rice fields (Table 3). Ghanbari et al. (2005) mentioned some physical and chemical factors of the larval habitats of *Anopheles* including *An. hyrcanus* in Iranshahr, southeastern Iran, however most probably their identification is not correct and needs to be verified. Because, the Oriental species of the Hyrcanus Group, *An. peditaeniatus*, has been found in southeastern Iran and old records of the Oriental *An. nigerrimus* Giles in southern and southeastern Iran using old keys and the record of the Palaearctic *An. hyrcanus* in southeastern Iran might be misidentifications and need to be verified (Azari-Hamidian et al. 2006).

There is little data about the larval breeding places and ecology of the Maculipennis Group species in details in Iran, because the species were not identified carefully in larval stage or using egg pattern in the most of ecological investigations and most of recorded species based on PCR technique were collected as adult (see Taxonomic note). Dow (1953) identified *An. maculipennis* based on egg collected in "rice field", "small spring in ditch", "shady irrigation reservoir", "shady irrigation ditch", "wide irrigation ditch with floating plants, debris and algae, also emergent grass and sedge", "puddle in small irrigation ditch beside road", and "small stream with much floating and emergent vegetation" in northwestern Iran and the Caspian Sea littoral (Mazandaran Province) and the associated species were *An. 'hyrcanus*', *An. sacharovi*, *An. superpictus*, *Cx. hortensis*, and *Cx. theileri*. Also he identified *An. melanoon* (as *An. melanoon* subspecies *subalpinus*) collected in "quiet river channel", "canal with emergent grass along bank", "small brook below spring with mats of glove-like alga", "rain barrel", and "shallow pond" in Guilan Province and the associated species were *An. 'hyrcanus*' and *Cx. tritaeniorhynchus*. *Anopheles sacharovi* was collected in a "shady irrigation reservoir" and in a "pasture" in northwestern Iran. Horsfall (1955) mentioned *An. macu-*

lipennis s.l. an associate of *An. 'hyrcanus'*, *An. sergentii*, *An. superpictus*, *Cs. annulata*, *Cx. laticinctus*, *Cx. pipiens*, and *Ur. unguiculata* and added rarely two or more species of the group were found in the same habitat at the same time such as *An. atroparvus* with *An. messeae* and *An. maculipennis* with *An. melanoon* (as *An. subalpinus*). Mousakazemi et al. (2000) collected *An. maculipennis s.l.* larvae with those of *An. superpictus*, *Ae. vexans*, *Cs. longiareolata*, *Cx. pipiens*, *Cx. perexiguus*, *Cx. theileri*, *Oc. caspius s.l.* from rice fields in Isfahan Province. Yaghoobi-Ershadi et al. (2001) did not identify *An. sacharovi* based on egg pattern, larval chaetotaxy, or PCR in Parsabad of northern Ardebil Province; however they studied its larvae based on the high abundance of the adults in the studied area. They noted very high salinity of the larval habitat waters (263–414 mg/l) and *An. superpictus* as associated species. *Anopheles maculipennis* have been found in northern Ardebil Province (Parsabad and Bil-e-Savar Counties), however with very low density (Yaghoobi-Ershadi et al. 2001, Azari-Hamidian et al. 2009). Azari-Hamidian et al. (2002a) collected *An. maculipennis s.l.* larvae in rice fields, irrigation channels, ground pools, in sunlight or shaded situation, with or without vegetation, with mud substrate and clear water in Rasht County of Guilan Province. The associated larvae were *An. 'hyrcanus'*, *Ae. vexans*, *Cx. pipiens*, *Cx. theileri*, and *Cx. tritaeniorhynchus*. In the present investigation, among the mentioned species; *An. sacharovi*, *An. sergentii*, *Cx. laticinctus*, and *Cx. perexiguus* were not found in the province (Azari-Hamidian et al. 2002b, 2004a, 2005, Azari-Hamidian 2007b) and *Cs. annulata* and *Ur. unguiculata* larvae were not found with *An. maculipennis s.l.* (Azari-Hamidian et al. 2004b, Azari-Hamidian 2005), however other species were in association with this species (Tables 2 and 4). In general, *An. maculipennis* is the most abundant species of the Maculipennis Group in Guilan Prov-

ince (Zolotarev 1945, Dow 1953, de Zulueta et al. 1957, Azari-Hamidian et al. 2004a) and its main larval habitats, such as *An. 'hyrcanus'*, are: river bed pools, rain pools, and rice fields (Table 3).

The tree-hole mosquito *An. plumbeus* also has been found in different habitats other than those in soil including: wells, cisterns, vases and other domestic containers, even dung pits, and flowerpots. The most favorable temperature is 15-18° C; however the species has tolerated to freeze and -6° C to -8° C in the laboratory. Associated species are *Ae. aegypti* (Linnaeus), *Cs. annulata*, *Cx. pipiens*, *Ochlerotatus geniculatus*, *Oc. echinus*, and *Orthopodomyia pulcripalpis* (Rondani) (Horsfall 1955). There is no record of *Or. pulcripalpis* in Iran and *Ae. aegypti* has not been found in the country for more than fifty years (Azari-Hamidian 2007a). In the present investigation, *Cs. annulata* was collected in Guilan Province (Azari-Hamidian et al. 2003b, Azari-Hamidian 2005), however not in association with *An. plumbeus*. *Anopheles plumbeus* was associated with other three species (Tables 2 and 4). Most of *An. plumbeus* larvae were collected from the tree holes and only in one occasion four larvae were collected with *Cx. pipiens* in a discarded tire (Table 3). The larvae were only collected when rain season started, October and November, and tree-holes included water and the weather was cooler (Azari-Hamidian 2003, 2006). That is why the mean temperature of habitat water showed significant difference from other species.

Macan (1950) stressed "the edges of stony streams" as "the classic habitat" of *An. superpictus*, however he added larvae are found in "any available breeding places where the water is clean, exposed to sunlight and moderately shallow" in western Iran. Dow (1953) found *An. superpictus* larvae with *An. maculipennis*, and probably *An. sacharovi*, *Cx. hortensis*, *Cx. theileri*, and *Ur. unguiculata* in different kinds of habitats in-

cluding; rivers, streams, pools, pastures, springs, irrigation ditches in different areas of Iran. Horsfall (1955) said the associated species of the species are; *An. cinereus* (as *An. hispaniola*), *An. claviger*, *An. sacharovi*, *An. sergentii*, *Cx. laticinctus*, *Cx. mimeticus*, and *Ur. unguiculata* and mentioned that three main larval breeding places of *An. superpictus* are: "clear shallow water over rocky bottom", "rivers of clear water over sandy bottom", and "hill streams of shallow water over mud bottom". The larvae passed the winter in pit wells where the temperature was 5–15°C in Greece. Yaghoobi-Ershadi et al. (1986) found *An. superpictus* larvae in habitats such as river edges (80%), ground pools (15%), and pastures (5%) which were often transient (62.5%) and stagnant (100%), without (50%) or with submerged (22.8%) vegetation, in partial (56.3%) or full (37.4%) sunlight, with mud (56.2%) and sand (43.8%) substrate. Also they noted associated larvae: *An. dthali*, *An. fluviatilis s.l.*, *An. multicolor*, *An. stephensi*, and *An. turkhudi* in Minab. Mousakazemi et al. (2000) collected *An. superpictus* larvae with those of *An. maculipennis s.l.*, *Ae. vexans*, *Cs. longiareolata*, *Cx. pipiens*, *Cx. perexiguus*, *Cx. theileri*, *Oc. caspius s.l.* from rice fields in Isfahan Province. Yaghoobi-Ershadi et al. (2001) found *An. superpictus* larvae with *An. sacharovi* in Ardebil Province. Ghanbari et al. (2005) studied some physical and chemical factors of the larval habitats of *Anopheles* larvae in Iranshahr including *An. superpictus*. Among mentioned species *An. cinereus* has not been found in Iran (Azari-Hamidian 2007a) and *An. dthali*, *An. fluviatilis s.l.*, *An. multicolor*, *An. sergentii*, *An. stephensi*, *An. turkhudi*, *Cx. laticinctus*, and *Cx. perexiguus* in Guilan Province (Azari-Hamidian et al. 2002b, 2004a, 2005, Azari-Hamidian 2007b). In the present study, *Anopheles sacharovi* was not found in the province (Azari-Hamidian et al. 2002b, 2004a) and *Ae. vexans* larvae were not in association with *An. superpictus* larvae (Azari-

Hamidian 2006) and *Oc. caspius s.l.* was found only as adult (Azari-Hamidian et al. 2002c). Other mentioned species were associated with *An. superpictus* larvae in the province (Tables 2 and 4). In parallel to the results of many researchers (Macan 1950, Dow 1953, Horsfall 1955, Yaghoobi-Ershadi et al. 1986, Zaim 1987a), in this study *An. superpictus* was found in natural habitats (100%) and mostly exposed to sun (88.5%), with clear (100%) water, without vegetation (65.6%), and mud (67.8%) substrate (Table 3). Finding in rice fields (artificial habitat) by Mousakazemi et al. (2000) in Isfahan Province seems to be to some extent unusual and interesting.

Larval habitat water pH, electric conductivity (EC), turbidity, dissolved oxygen, and different organic and inorganic compounds, other aquatic associated animals especially predators, and the life tables of different mosquito species are items, which should be considered in future studies.

Checklist of the mosquitoes of Guilan Province

Azari-Hamidian et al. (2002b) found 23 species of mosquitoes representing seven genera based on morphological characters including egg pattern (for the *Maculipennis* Group) in Guilan Province. In this list three species *An. superpictus*, *Oc. echinus*, and *Ur. unguiculata* were recorded in the province and *Cs. morsitans* and its subgenus (*Culicella*) in Iran for the first time. Five species including *An. atroparvus* and *An. sacharovi* of the *Maculipennis* Group, *An. algeriensis*, *Cs. subochrea*, and *Cx. torrentium*, which were recorded in Guilan Province before (Danilov 1975, Saebi 1987, Zaim 1987b, Dinparast-Jadid et al. 2001), were not found by Azari-Hamidian et al. (2002b). After that, Sedaghat et al. (2003) and Gholi-zadeh et al. (2004) reported *An. persiensis* and *An. labranchiae* of the *Maculipennis* Group based on PCR technique in the prov-

ince, respectively. In general, all seven species of the Maculipennis Group recorded in Iran (Azari-Hamidian 2007a) have been found in Guilan Province. *Anopheles atroparvus*, *An. labranchiae*, *An. maculipennis*, *An. messeae*, *An. persiensis*, *An. sacharovi* are presented in the province and Iran based on DNA data and their sequences are available in GenBank (Sedaghat et al. 2003, Gholizadeh et al. 2004, Gholizadeh et al. 2005, Djadid et al. 2007), however *An. melanoon* (also as *An. subalpinus*) has been found only using egg pattern (Zolotarev 1945, Dow 1953, de Zulueta et al. 1957, Azari-Hamidian et al. 2002b). Dow (1953) and Djadid et al. (2007) found *An. sacharovi* in Guilan Province based on egg pattern (in Hassan Kiadeh) and DNA sequence, respectively, however it seems, as Dow (1953) noted, the species is quite rare in the province. Azari-Hamidian et al. (2002b, 2004a) identified three species *An. maculipennis*, *An. melanoon*, and *An. messeae* based on egg pattern in Guilan Province. During his studies, Saebi (1987) did not find *An. algeriensis* in Iran and mentioned it a very rare species which had been recorded in Lahijan and Fooman (Fuman) of Guilan Province before. This species was not found by Azari-Hamidian et al. (2002b, 2004b) either; however its occurrence in the province seems to be possible, because of the presence of its preferred habitats; reedy swamps (Horsfall 1955). Based on the second Internal Transcribed Spacer (ITS2) and Cytochrome c Oxidase subunit I (COI) sequence data, recently Oshaghi et al. (2007, 2008) reported three genotypes named X, Y, and Z within *An. superpictus* in Iran. Genotype X was found in all parts of the country except for the southeastern areas. It seems that the *An. superpictus* specimens of Guilan Province belong to genotype X, however it needs to be confirmed by more investigation. Zaim (1987b) reported *Cs. subochrea* in Guilan Province; however Azari-Hamidian et al.

(2002b, 2003b) found only *Cs. annulata*. These two close and rare species are very similar in larval stage; however they are easily distinguishable as adult. It seems the occurrence of *Cs. subochrea* in Guilan Province is possible, especially in view of the recent finding of both species in Ardebil Province, northwestern Iran (Azari-Hamidian et al. 2009). There is little information about taxonomy and distribution of three close species *Cs. alaskaensis* (Ludlow), *Cs. annulata*, and *Cs. subochrea* in Iran (Azari-Hamidian et al. 2003b, Azari-Hamidian 2005). Recently, *Cs. subochrea* and *Cs. longiareolata* were found in Sanandaj County of Kurdistan Province (Moosa Kazemi et al. 2010). Danilov (1975) was the unique reliable record of *Cx. torrentium* in Rasht of Guilan Province and Iran. Recently the larvae of this species was found in Ardebil Province (Azari-Hamidian et al. 2009) and the mountainous areas of the western part of Guilan Province (Heyran) (with *Cx. pipiens*; Azari-Hamidian, unpublished data), thus its occurrence in Iran, Guilan Province, and for the first time in Ardebil Province was verified. All 30 species, which have been discussed above, were mentioned in the checklist in the present article. There is a unique record of *Cx. quinquefasciatus* Say in Enzeli of Guilan Province by Harbach (1988). There is no more information about this record (Harbach, personal communication). This is out of the usual distribution of this species that occurs in southern Iran (Zaim 1987b), so it is not mentioned in the checklist. Taxonomy, distribution, and ecology of the Pipiens Subgroup of the Pipiens Group of *Culex* (*Culex*) need to be studied extensively in Iran. Beklemishev and Shipitzina (1947) found *An. marteri* in the valley of the river Yuzbashchai between Qazvin (Qazvin Province) and Rasht (Guilan Province); however there is no record of this species in Guilan Province. The species complexes and the mosquito fauna of the forest areas of the

province are important subjects to study in future taxonomic investigations.

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