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Seasonal Frequency and Relative Density of Larval Populations of Mosquito Species (Diptera: Culicidae) in Şanlıurfa Province, Turkey*

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Abstract: This research was conducted between June 2000 and August 2002 in 2 regions of Şanlıurfa: one (IR) where there was substantial irrigated agriculture due to the Southeastern Anatolia Project and another (MR) where a high number of malaria cases were determined every year. Ten species, namely *Anopheles (Anopheles) claviger* Meigen, 1804, *An. (Anopheles) sacharovi* Favre, 1903, *An. (Cellia) superpictus* Grassi, 1899, *Culex (Neoculex) martinii* Medschid, 1930, *Cx. (Culex) pipiens* Linnaeus, 1758, *Cx. (Neoculex) territans* Walker, 1856, *Cx. (Culex) theileri* Theobald, 1903, *Culiseta (Allotheobaldia) longiareolata* Macquart, 1938, *Ochlerotatus (Ochlerotatus) caspius* Pallas, 1771 and *Uranotaenia (Pseudoficalbia) unguiculata* Edwards, 1903, were recorded in this study, and seasonal frequencies and relative densities of larval populations were determined separately for each region. Based on the frequencies, relative densities and vectorial significance of species found in this study, *Culex pipiens, Cx. theileri* and *Ochlerotatus caspius* in IR and *Anopheles claviger*, *An. sacharovi*, and *An. superpictus* in MR were of higher significance.

Key Words: Mosquitoes, larval population, frequency, relative density, malaria, Şanlıurfa

Şanlıurfa İli'nde Sivrisinek Türlerinin (Diptera: Culicidae) Larva Populasyonlarının Mevsimsel Sıklığı ve Nisbi Yoğunluğu

Özet: Bu araştırma, Şanlıurfa İli'nin, Güneydoğu Anadolu Projesi kapsamında sulu tarım uygulamalarının yapıldığı bölgesi (IR) ile her yıl çok sayıda sıtma vakasının tespit edildiği bölgesinde (MR) Haziran 2000-Ağustos 2002 tarihleri arasında yapılmıştır. Araştırmada, *Anopheles (Anopheles) claviger* Meigen, 1804, *An. (Anopheles) sacharovi* Favre, 1903, *An. (Cellia) superpictus* Grassi, 1899, *Culex (Neoculex) martinii* Medschid, 1930, *Cx. (Culex) pipiens* Linnaeus, 1758, *Cx. (Neoculex) territans* Walker, 1856, *Cx. (Culex) theileri* Theobald, 1903, *Culiseta (Allotheobaldia) longiareolata* Macquart, 1938, *Ochlerotatus (Ochlerotatus) caspius* Pallas, 1771 ve *Uranotaenia (Pseudoficalbia) unguiculata* Edwards, 1903 olmak üzere 10 sivrisinek türü tespit edilmiş ve bu türlerin larva populasyonlarının mevsimsel sıklıkları ve nisbi yoğunlukları bölgelere göre belirlenmiştir. Türlerin sıklıkları, nisbi yoğunlukları ve vektörel önemleri dikkate alındığında, IR'de, *Culex pipiens, Cx. theileri* ve *Ochlerotatus caspius*, MR'de, *Anopheles claviger*, *An. sacharovi* ve *An. superpictus* türlerinin diğer türlere göre daha önemli oldukları belirlenmiştir.

Anahtar Sözcükler: sivrisinekler, larva populasyonu, sıklık, nisbi yoğunluk, sıtma, Şanlıurfa

Introduction

Şanlıurfa province, in southeastern Turkey, is still one of the largest focuses of malaria in the country. Despite years of attempted eradication, malaria remains a major public health threat in Şanlıurfa, with 5447 cases reported between 1999 and 2002 (Yazgan, 2003). Generally, several factors such as climatic change, environmental corruption, progressive urbanization, population movement, irrigation development projects and bio-ecological properties of the mosquito species are responsible for the rate of transmission of mosquito-borne diseases like malaria. Although most of these factors are present in Şanlıurfa province, ecological properties such as breeding habitats, feeding habits, and larval and adult population densities of the mosquito species are the most important ones. In particular, the breeding habitat is crucial for mosquito dynamics, because it is the location where many important life cycle

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processes take place: oviposition, larval development, emergence and probably resting, swarming, and mating (Overgaard et al., 2002). Furthermore, a strong association exists between the density and distribution of mosquito larvae and that of the adult vectors. In addition, knowledge of larval population density and frequency in breeding habitats are critical when considering the control of mosquito-borne diseases. Therefore, vector control strategies aim at suppressing larval production densities, thereby limiting mosquito-borne disease transmission (Shililu et al., 2003). The present study is the first attempt to determine the density, frequency, distribution, and seasonal occurrence of the larval population of mosquito species in malarious and irrigated areas of Şanlıurfa province. The information gathered in this study should contribute to the understanding of the ecology of mosquito species of Şanlıurfa province and will make important contributions to the effectiveness of malaria and mosquito control programs.

Materials and Methods

Study area

Field research was carried out from June 2000 to August 2002 in malarious (MR) and irrigated (IR) regions of Şanlıurfa province (Figure 1).

Malarious area: Encompasses most parts of the Siverek and Viranşehir districts of Şanlıurfa. The region is made up of many high hills, the highest of which is Karacadağ. As we move south from Karacadağ elevation gradually decreases. Elevation in the region ranges from 500 to 1900 m. The region is a rural area rich in natural water reserves and harbors many seasonal creeks flowing from Karacadağ. The climate is characterized by a rainy season from October to May. Rainfall varies annually: 502 mm in 2000, 782 mm in 2001, and 374 mm in 2002. Between 2000 and 2002, the average temperature and relative humidity were 17.6 °C and 60%, respectively. The region economy is mainly dependent on livestock and agriculture. The main crops are wheat, lentil and barley. Rice cultivation is possible only in areas where irrigation water is abundant. In the region, malaria is still endemic and one of the important infectious diseases with 3476 cases reported between 1999 and 2002 (Yazgan, 2003).

Irrigated area: The region, which covers most parts of the Harran and Akçakale districts of Şanlıurfa, is a wide plain. The region is scarce in natural water reserves but has a large network of irrigation channels due to GAP. The climate of the region is relatively hot and dry. Rainfall varies annually: 387 mm in 2000, 546 mm in 2001, and 328 mm in 2002. The average temperature and relative

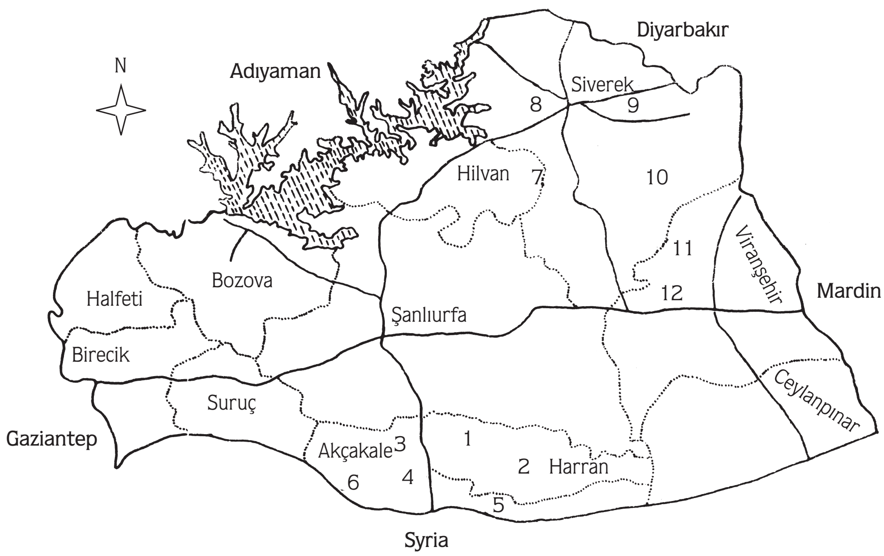

Figure 1. Map of the study area showing the locations of larval sampling quadrats (1-12).

humidity were 18.8 °C and 53%, respectively, between 2000 and 2002. The most important source of income in the region is agriculture. Cotton production is very common, with wheat and lentil also having an important share. Almost all areas where these products are grown are heavily irrigated. Therefore, this region is considered a potential site for future malaria epidemics; however, only 3 malaria cases were reported in the region from 1999 to 2002 (Yazgan, 2003).

Larval sampling

The most important breeding habitats of both regions were determined, prior to periodic (monthly) larval sampling. After that, 12 of those habitats (6 in each area) were selected to represent the general breeding habitats of the mosquitoes in both regions and long-lasting enough to make periodic sampling possible. In each selected breeding habitat, sampling quadrats of 30 m^2 were determined for the evaluation of the seasonal larval population frequencies and relative densities of mosquitoes in the regions (Figure 1). In every sampling quadrat, one-dip samples were taken randomly for every 1 m^2 on a monthly basis with a standard long-handled dipper (WHO, 1975). Samples were always taken by the same individual at the same time in the morning (07:30-10:30). Every dip sample taken from each quadrat was put into a different pot and labeled before transportation to the Harran University Research Laboratory of Department of Biology (BL). Third and fourth instar larvae were immediately put into MacGregor solution (Snow, 1990) in the laboratory, whereas first and second instars were allowed to develop before putting into MacGregor solution. At the end of the sampling period, all samples were transported from BL to the Hacettepe University Ecological Sciences Research Laboratories (ESRLs). Identification was performed using several keys (Dubose and Curtin, 1965; Merdivenci, 1984; Şahin, 1984; Harbach, 1985, 1988; Snow, 1990; Glick, 1990; Darsie and Samanidou-Voyadjoglou, 1997; Schaffner et al., 2001) under the microscope in the ESRLs. Finally, the number of larvae of each species, identified in every quadrat, was recorded.

Seasonal frequency

Frequency was calculated monthly by using sampling number of each species and total sampling number, according to the following formula (Kocataş, 1992; Şişli, 1996):

$$F = Na/N \times 100$$

where F is frequency, Na is sampling number of species a, and N is total sampling number.

The following frequency classes were adopted (Greig-Smith, 1964; Kocataş, 1992; Alten and Boşgelmez, 1996; Şişli, 1996):

F1- sporadic (0%-20%)

F2- infrequent (20.1%-40%)

F3- moderate (40.1%-60%)

F4- frequent (60.1%-80%)

F5- constant (80.1%-100%)

Seasonal relative density

Relative density was calculated using the number of specimens of each species and the number of all specimens, according to the following formula (Kocataş, 1992):

$$D = NA/N \times 100$$

where D is relative density, NA is the number of specimens of species A, and N is the number of all specimens.

Results

Mosquito species

At the end of the monthly sampling studies performed in the 12 sampling quadrats, *Anopheles claviger*, *An. sacharovi*, *An. superpictus*, *Culex martinii*, *Cx. pipiens*, *Cx. territans*, *Cx. theileri*, *Culiseta longiareolata*, *Ochlerotatus caspius* and *Uranotaenia unguiculata* were found. Distribution and total number of mosquito larvae in the breeding habitats are shown in Table 1.

Seasonal frequency

Seasonal frequencies of species in IR and MR calculated from data obtained from larval sampling quadrats are presented in Table 2.

Monthly frequencies indicated that the frequency rates of *Oc. caspius*, *Cx. theileri* and *Cx. pipiens* were higher than those of *Cx. martinii*, *An. sacharovi* and *An. superpictus* in IR. When species are evaluated based on frequency classes, *Oc. caspius*, having the highest frequency rates, can be regarded as moderate (F3) or frequent (F4) species in most of the months in which this species was sampled, and *Cx. pipiens* as a moderate species in general. *Culex theileri*, being generally

Table 1. Distribution and total number of the mosquito larvae in the breeding habitats.

Mosquito species	Irrigated Region						Malarious Region					
	Breeding habitats (quadrats)											
	1	2	3	4	5	6	7	8	9	10	11	12
	Total number of mosquito larvae sampled											
Anopheles claviger							1108	980	1208	1337		
Anopheles sacharovi	182				559		1154		1730	1738	1871	
Anopheles superpictus					620				589	642	1956	
Culiseta longiareolata							393					
Culex martinii	471							717	799			
Culex pipiens		2259		3455		1959					1390	
Culex territans							949	882				
Culex theileri	2152	1790	1865			828	1388	1278	1313	638		1748
Ochlerotatus caspius	5100	4842	3558			4266		1011			1423	2894
Uranotaenia ungiuculata							885					

infrequent (F2) in 2000, turned out to be a generally moderate species in 2001 and 2002. *An. sacharovi* (except September 2000-2002 and July 2001), *An. superpictus*, and *Culex martinii* had lower frequency rates compared to the other species and can be regarded as sporadic species in the region. The frequency rates of *An. claviger*, *An. sacharovi*, *An. superpictus*, *Ochlerotatus caspius* and *Culex theileri* in MR were higher when compared to the frequency rates of other species in MR. When the monthly frequency rates of species in MR were evaluated in general according to the frequency classes, it was determined that in this region species mostly belong to the frequent class. *Anopheles sacharovi*, having a high frequency rate, was within the frequent class only during June and July of 2001 but was determined to be in the moderate class in most of the sampled months. *Culex theileri* was in the moderate class in most of the sampled months in 2000 and 2001. In 2002 frequency rates of *Cx. theileri* increased enough to classify the species in the frequent class. In contrast to *Anopheles superpictus* being in the sporadic class in all sampled months in 2000, frequency rates increased in 2001, becoming a moderate species in July and September, and *An. superpictus* remained in the moderate class during most of 2002. *Anopheles claviger*, which was sampled in all months of the study except December 2001, generally remained in the sporadic class during November-April (due to lack of reproduction) when all other species were absent. However, this species was mostly determined to be in the moderate class during May-October (with the beginning of reproduction activities) when other species were also present. When the monthly frequency rates of other species sampled in MR were evaluated, *Ochlerotatus caspius* was mostly observed to be an infrequent species, *Culex martinii* and *Cx. territans* to be sporadic or infrequent species depending on the months in which they were sampled, and *Cx. pipiens*, *Culiseta longiareolata* and *Uranotaenia unguiculata* to be in the sporadic class in all of the sampled months (Table 2).

Seasonal relative density

Results of seasonal relative densities of IR and MR are shown in Figures 2-4, and Figures 5-7, respectively. Relative density results of both regions were parallel with frequency rates, and it was determined that the species with higher frequency rates had also higher density rates. It was determined that *Culex pipiens*, *Cx. theileri* (Figure 3) and *Ochlerotatus caspius* (Figure 4) species in IR; and *Anopheles claviger*, *An. sacharovi, An. superpictus* (Figure 5), *Culex theileri* (Figure 6) and *Ochlerotatus caspius* (Figure 7) species in MR have high relative densities.

Table 2. Seasonal frequency results from sampling quadrats obtained between June 2000 and August 2002.

Regions	Species	Months / 2000							Months / 20001												Months / 2002							
		J	J	A	S	O	N	D	J	F	M	A	M	J	J	A	S	O	N	D	J	F	M	A	M	J	J	A
Irrigated	*Anopheles sacharovi*	13.9	18.3	9.4	21.7	8.3	0	0	0	0	0	0	5.0	12.2	21.1	12.2	20.6	6.7	0	0	0	0	0	0	10.0	14.4	10.6	11.7
	Anopheles superpictus	13.3	12.2	11.1	8.3	5.0	0	0	0	0	0	0	6.1	11.1	11.7	9.4	10.0	4.4	0	0	0	0	0	0	7.2	9.4	10.6	9.4
	Culex martinii	0	0	0	10.0	10.6	3.3	0	0	0	0	0	0	0	12.2	9.4	12.2	8.9	0	0	0	0	0	0	0	10.6	12.8	10.0
	Culex pipiens	41.7	43.3	35.6	41.7	35.0	13.9	0	0	0	0	30.6	42.8	47.2	46.7	42.2	45.6	36.7	3.9	0	0	0	0	45.0	46.1	45.6	43.3	42.2
	Culex theileri	27.2	29.4	27.2	30.0	18.9	6.1	0	0	0	0	0	25.0	28.9	58.9	57.8	55.0	42.2	0	0	0	0	0	39.4	50.6	61.7	59.4	58.9
	Ochlerotatus caspius	61.1	58.9	50.0	52.2	43.9	8.3	0	0	0	57.2	64.4	61.1	65.6	61.1	57.8	54.4	40.0	0	0	0	0	59.4	58.9	55.0	47.2	51.1	55.6
Malarious	*Anopheles claviger*	42.2	31.1	28.3	47.8	40.0	26.7	24.4	16.7	13.9	6.7	15.6	40.0	55.6	46.1	44.4	41.7	29.4	8.3	0	1.1	1.7	3.3	7.2	18.9	50.0	46.1	41.7
	Anopheles sacharovi	56.1	58.3	46.7	58.3	32.2	0	0	0	0	0	0	49.4	61.7	61.1	56.7	58.9	21.7	0	0	0	0	0	0	54.4	60.0	54.4	57.2
	Anopheles superpictus	15.0	14.4	13.3	16.1	14.4	10.0	0	0	0	0	0	11.7	16.1	41.1	39.4	40.6	22.8	7.8	0	0	0	0	0	10.6	41.7	38.9	40.6
	Culiseta longiareolata	0	0	0	0	0	0	0	0	0	0	0	0	9.4	6.7	5.6	7.8	2.8	1.1	0	0	0	0	1.7	1.1	8.9	7.2	7.2
	Culex martinii	10.0	11.7	20.6	23.3	7.8	4.4	0	0	0	0	0	0	20.0	17.2	21.7	21.1	0	0	0	0	0	0	0	0	22.8	21.1	18.9
	Culex pipiens	10.0	13.9	11.7	10.6	10.6	0	0	0	0	0	0	8.9	11.7	12.8	11.7	13.9	8.3	0	0	0	0	0	0	13.9	14.4	16.1	14.4
	Culex territans	20.0	20.0	22.8	24.4	13.9	0	0	0	0	0	0	0	22.8	21.7	21.1	22.2	16.7	0	0	0	0	0	0	0	18.3	16.7	20.0
	Culex theileri	36.1	42.8	42.8	51.1	41.1	10.6	0	0	0	0	0	26.1	46.7	54.4	59.4	58.9	35.6	2.2	0	0	0	0	0	16.7	66.7	61.7	60.6
	Ochlerotatus caspius	31.7	33.3	25.0	36.6	35.6	0	0	0	0	0	8.9	27.8	37.2	41.1	30.0	39.4	21.1	0	0	0	0	0	8.9	26.7	38.9	35.6	33.3
	Uranotaenia ungiuculata	8.3	8.9	8.9	11.1	8.3	0	0	0	0	0	0	5.0	11.1	9.4	9.4	10.0	4.4	0	0	0	0	0	0	12.2	10.6	8.9	10.0

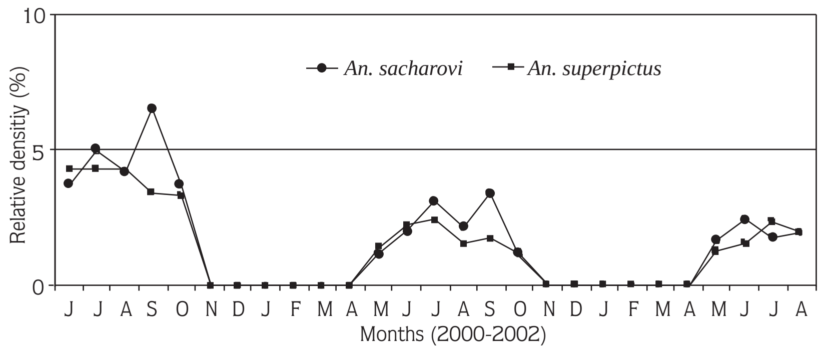

Figure 2. Seasonal fluctuations in relative densities of *Anophele sacharovi* and *An. superpictus* in irrigated region.

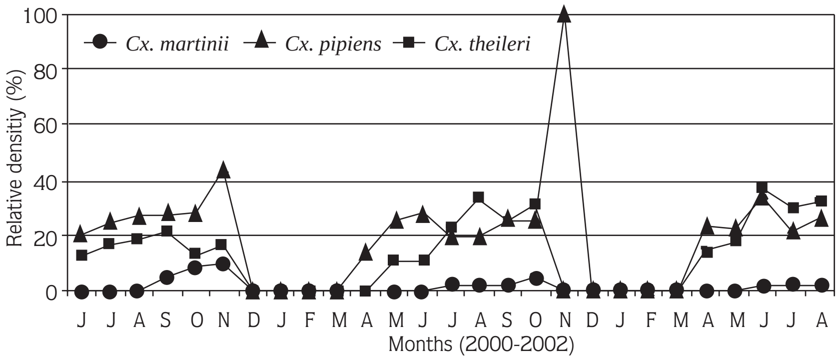

Figure 3. Seasonal fluctuations in relative densities of *Culex martinii*, *Cx. pipiens,* and *Cx. theileri* in irrigated region.

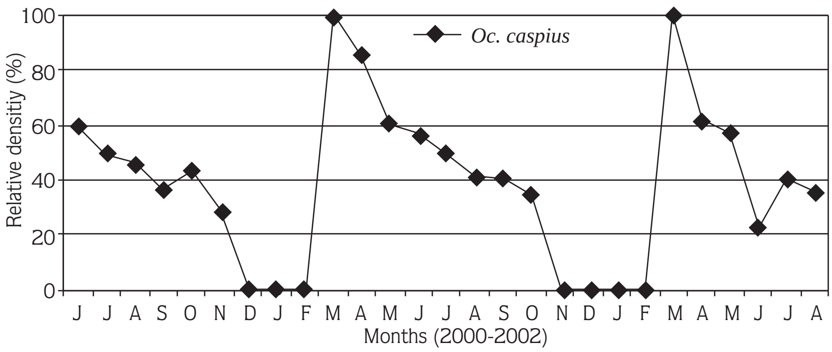

Figure 4. Seasonal fluctuations in relative densities of *Ochlerotatus caspius* in irrigated region.

It was also determined that *Anopheles sacharovi*, *An. superpictus* (Figure 2) and *Culex martinii* (Figure 3) species in IR, and *Cx. martinii*, *Cx. pipiens*, *Cx. territans* (Figure 6), *Culiseta longiareolata* and *Uranotaenia unguiculata* (Figure 7) species in MR have lower relative densities. The relative density evaluation of species found both in IR and MR shows that *Anopheles sacharovi* and *An. superpictus* had high relative density only in MR, and *Culex pipiens* had high relative density only in IR, while *Ochlerotatus caspius* and *Culex theileri* had high relative densities in both regions. *Cx. martinii,* on the other hand, had low relative densities in both regions.

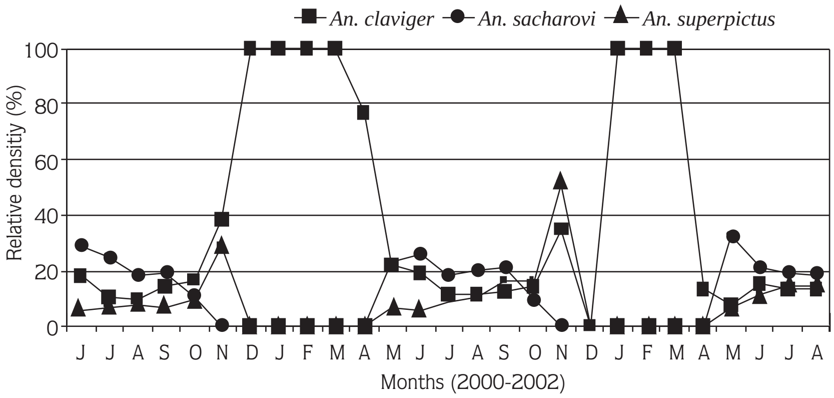

Figure 5. Seasonal fluctuations in relative densities of *Anopheles claviger*, *An. sacharovi,* and *An. superpictus* in malarious region.

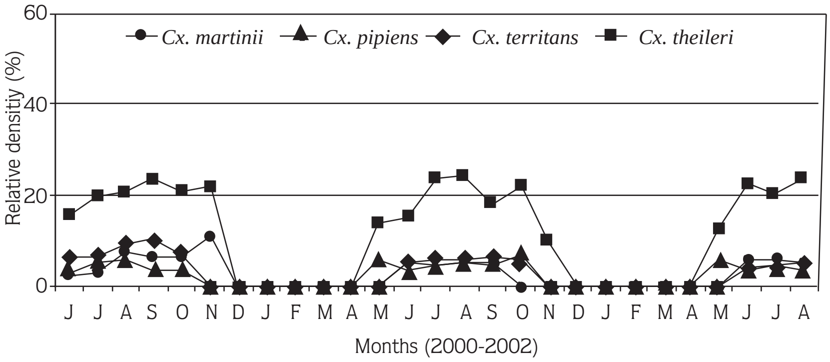

Figure 6. Seasonal fluctuations in relative densities of *Culex martinii, Cx. pipiens, Cx. territans* and *Cx.. theileri* in malarious region.

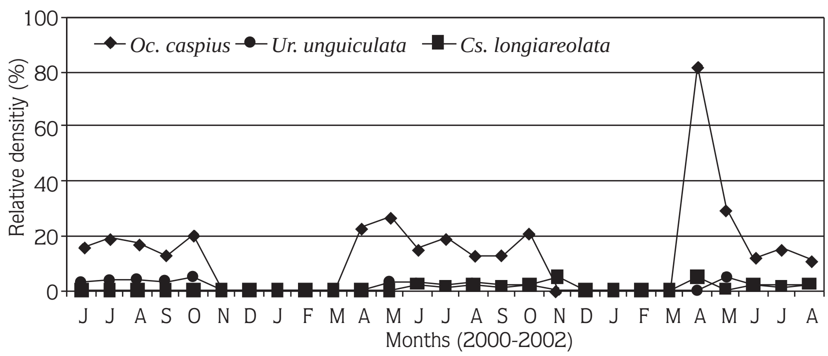

Figure 7. Seasonal fluctuations in relative densities of *Culiseta longiareolata, Ochlerotatus caspius,* and *Uranotaenia ungiucalata* in malarious region.

Discussion

Climatic changes, increases in population movements, and changes in agricultural culture as the result of large-scale irrigation initiated as part of the Southeastern Anatolia Project (abbreviated as GAP in Turkish) are expected to create a considerable increase in mosquito and malaria problems in Southeastern Anatolia (Boşgelmez et al., 1997; Alten and Çağlar, 1998; Özcel, 1999; Tabuk and Yıldırım, 1999; Özer et al., 2001).

The probability of increases in disorders mediated by and diseases originated from mosquitoes such as malaria as a result of irrigation and irrigated agriculture activities, which began in 1995 under GAP, has made Şanlıurfa the province facing the highest risk in the region. Primarily the bio-ecological properties of mosquito populations have to be established in order to define the magnitude and significance of this risk.

Ten mosquito species were found, consisting of 6 species in IR, namely *Anopheles sacharovi*, *An. superpictus*, *Culex martinii*, *Cx. pipiens*, *Cx. theileri*, and *Ochlerotatus caspius*, and 4 in MR, namely *Anopheles claviger*, *Culex territans*, *Culiseta longiareolata*, and *Uranotaenia unguiculata*. Although these species have been determined in previous studies (Şahin, 1984; Boşgelmez et al., 1994, 1995; Alten and Boşgelmez, 1996; Aldemir, 1997; Alptekin and Kasap, 1997; Şimşek, 1997) in various regions of Turkey, it was the first time in this study that *Culex martinii*, *Cx. territans*, *Cx. theileri*, and *Ochlerotatus caspius* were recorded in the research regions. According to Parrish (1959) and Merdivenci (1984), the species *Anopheles algeriensis* Theobald, 1903, *An. maculipennis* Meigen, 1818, *An. purcherrimus* Theobald, 1902, *Culex vagans* Wiedemann 1828, and *Culiseta morsitans* Theobald, 1901, reported to be prevailing in Southeastern Anatolia, were not found in the research regions. Studies (Abdelmalek, 1958; Muir and Keilay, 1972) performed in Syria revealed the existence of *Anopheles claviger*, *An. sacharovi*, and *An. superpictus*, and studies conducted in Iraq (Altikritiy, 1964; Jalil, 1967) added *Culex theileri* and *Ochlerotatus caspius* to those mentioned above.

Based on frequencies, relative densities and vectorial significance of species found in this study, *Culex pipiens*, *Cx. theileri* and *Ochlerotatus caspius* in IR, and *Anopheles claviger*, *An. sacharovi*, and *An. superpictus* in MR were of higher significance.

Culex pipiens was sampled in quadrats 2, 4 and 6 in IR, but only in quadrat 11 in MR. The species has a wider distribution and a higher relative density in IR compared to MR. This is consistent with the cosmopolite nature of *Cx. pipiens*. Harbach (1988) reported that the species had high population densities generally in habitats in urban regions, rather than rural regions. Similarly, Alten (1993) and Şimşek (2003) sampled high densities of *Cx. pipiens* in urban breeding habitats. IR has more urban characteristics compared to MR and all of the breeding habitats in the region are man-made and quite close to areas of settlement. For example, quadrat 4, where the species was sampled at the highest levels, was formed by an artesian well, which is used for daily requirements such as watering animals and irrigating small gardens.

The high number of *Culex theileri* in the sampling quadrats and the capability of the species to form high relative densities show that it can easily adapt itself to various breeding habitats. Although in several studies (van der Linde et al., 1982; Harbach, 1988; Alten, 1993; Şimşek, 1997) prior to this the species was found to be capable of using various breeding habitats of different characteristics (springs, creeks, swamps, pastures, irrigation channels), this study revealed that it dominated particularly pasture breeding habitats (quadrats 1, 2, 8, and 12). The results of studies conducted by Alten and Boşgelmez (1996) in the Muğla region and by Şimşek (2004) in the Ankara Gölbaşı district showed that *Cx. theileri* similarly formed its highest population densities in pasture habitats.

Our study revealed that *Ochlerotatus caspius*, which has high relative densities, was the most common species in IR and this species is highly adaptable, like *Culex theileri*, to pasture (quadrats 1, 2 and 6) and irrigation channel (quadrat 3) breeding habitats. Similarly, the species was abundantly sampled in pasture habitats (quadrats 8 and 12) in MR although not as high as in IR. This is highly consistent with the characteristics of the species since it overwinters in the egg stage, lays its eggs in muddy areas, and the eggs hatch after several contacts with water (Roberts, 2001). Water does not exist continuously in these breeding habitats, which even sometimes dry out completely, since the pastures and agricultural areas (wheat and cotton fields) in this region are irrigated at certain intervals. In contrast, this species was not sampled in other breeding habitats (quadrats 4 and 5) in the region. This was attributed to the fact that the breeding habitats are permanent and never dry out. In their studies in the Çukurova region, Alptekin and Kasap (1997) found that *Ochlerotatus caspius* existed in high densities in vegetated breeding habitats formed by water diffusing in the area during irrigation of agricultural fields and which dry out in 10 to 15 days.

In IR, *Anopheles sacharovi* was sampled in 2 breeding habitats (quadrats 1 and 5) and *An. superpictus* only in 1 (quadrat 5) at low relative densities, whereas *An. claviger* was sampled in none of the quadrats. However, *An.*

claviger and *An. sacharovi* were sampled in 4, and *An. superpictus* in 3 of the 6 quadrats in MR and were found in high relative densities.

In conclusion, our study revealed that malaria vectors *An. sacharovi* and *An. superpictus* had limited distribution and low relative density in IR, where a considerable part of the region is used for irrigated agriculture within GAP, and the risk of increased malaria cases due to irrigation operations is not high under the given circumstances. On the other hand, the occurrence of a high number of malaria cases every year and the existence of 3 important vector species in high relative densities in MR shows that the risk of malaria is much higher in this region.

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