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Impact of environmental factors on *Anopheles maculipennis* complex (Diptera: Culicidae) populations in three localities of Turkey

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Abstract
The aim of this study is to investigate population dynamisms for the primary and potential malaria vector species (*Anopheles maculipennis* complex) during six years in three different regions (Birecik, Beyşehir, Çankırı) and their relationship with the climatic factors. Samples of the *Anopheles maculipennis* complex species were collected using 10 min/person standardized methods by a mouth aspirator. Data of temperature, precipitation and humidity were obtained by the Meteorological Service in the period from 2004 to 2009 searching for a relationship between population dynamics and climatic changes.
A gradual increase from 2004 to 2009 was determined in Çankırı populations. After a sharp increase in 2005, Beyşehir and Birecik population's have decreased in following years. Although observing a positive correlation between population size and temperature, in terms of humidity and precipitation parameters there was negative correlation with population size. As a result of research which has been conducted over six years, seasonal and annual fluctuations were observed of *Anopheles maculipennis* complex populations in all regions. Population dynamisms showed a bimodal structure in all areas except for Beyşehir populations. Those specie's density can reach serious levels due to the zoophilic tendencies.

Keywords: *Anopheles maculipennis* complex, population fluctuations, climatic factors, Turkey

Introduction

Turkey has been affected in many areas by Malaria since World War I. In 1950 the malaria eradication program was started with the World Health Organization. Malaria's incidence has decreased within a period of 40 years. Ministry of Health conducted a survey for parasite and mosquito control operations especially in the former endemic areas after the 1990s eradication program ended. Recently, malaria cases have only been reported by the Southeast Anatolian region of Turkey [1, 2, 3]. Incidence of malaria increased again from 1990s to 2000 and reached 80000-90000 confirmed cases and showed decreasing trend after 2000. Turkish Republic Ministry of Health reported to have no more Malaria cases in 2010 in Turkey [4]. Controversially by an observation in 2010, 13 local cases were found in 2011, 206 new confirmed (*Plasmodium vivax*) malaria cases appeared in 2012 in the Savur district of Mardin [3]. Many studies have been conducted to study main and potential malaria vectors in Turkey but these studies generally focused on systematics and insecticide resistance level [2, 5, 6, 7, 8].
Anopheles maculipennis complex species include malaria vector species in Northern hemisphere [9]. Although three of the *An. maculipennis* complex species are known important for malaria transmission (*Anopheles sacharovi, Anopheles labranchiae, Anopheles atroparvus*) in the continental Europe, many authors indicated that the other complex species can become a malaria vector because of increased population size, and changed vectorial capacity due to the climatic change [4, 10, 11, 12, 13]. According to the molecular studies, Turkey has three species (*An. sacharovi, An. maculipennis s.s, An. melanoon*) of this complex [2, 11]. *An. sacharovi* has distribution range from coastline to 1200 mt high altitude [14, 15]. The other two species are found above 500 mt altitude and extensively found in irrigated farming areas (cotton and rice fields, irrigated areas). *An. sacharovi* populations exist in small fresh water ponds which contain aquatic vegetation but this species also can live in brackish waters. In contrast to *An. sacharovi*, the other two species prefer fresh water ponds [14]. Although all the species showed zoo-anthropophilic behavior, they have a tendency to zoophilic behavior. The tendency of blood sucking from human increased in the absence of adequate animal resources [9]. Due to these reasons, malaria transmission potential can vary with the distribution and changeability of resources. Studying *An. maculipennis* complex in Turkey is important not only for exact

identification of species, and to find out distribution of *An. maculipennis* complex populations and but also to set up right control parameters. Despite significant contributions of these studies to knowledge about malaria vectors and vector control operations, still more detailed studies on population dynamics of vector species are needed.

The present study was aimed to determine temporal changes of population dynamics of the *An. maculipennis* complex species during six year in three different regions (Birecik, Beyşehir, Çankırı) and its relationship with the climatic factors. Besides, although there are many studies about malaria vectors in recent years, long term studies are still insufficient. This study makes a significant contribution to long term malaria vectors survey in Turkey.

Material Methods

Research areas

The investigation was conducted in Sanliurfa-Birecik (Doyduk village), Cankiri-Kizilirmak (Hacilar village), Konya-Beyşehir (Sugla and Afsar villages) from 2004 to 2009. Information of the study locations is shown in Figure 1.

Figure 1.

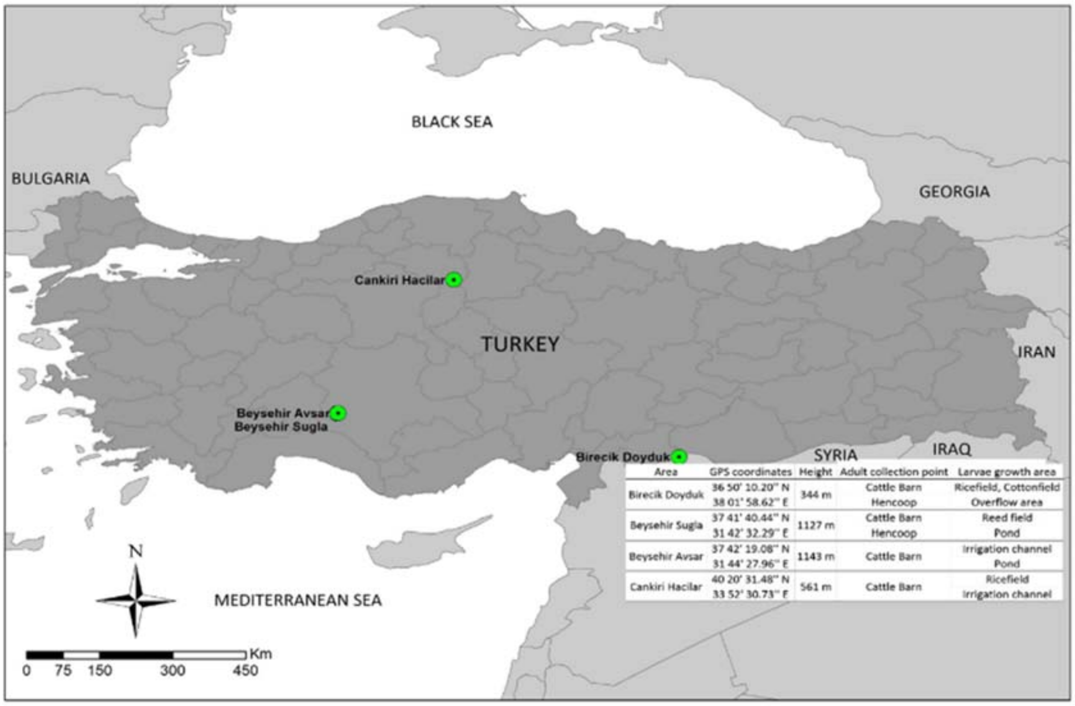

Figure 1: Study locations and information about the collection points

Determination of seasonal dynamics of the adult populations

Mouth aspirator was used for collecting adults and method was standardized 10mins/person. Adults were collected in animal barns during their resting period with mouth aspirator between April and October from 2004 to 2009 at four localities in southeastern and central Anatolia. Adults were collected during 1st, 2nd and 3rd subsequent days in the same month for four different collection points. Any kind of animal or human were not used as a trap for collecting adults. Three subsequent resting barns were selected to collect adults, and mean number of collected adults of barns and days was used for population amount. All samples were collected as an adult and any other type of lifestage was not collected during the study. *An. maculipennis* complex was identified by their morphology according to the descriptions of Glick [16] and Schaffner *et al.* [17]. Monthly and annual dynamics were observed as a result of the mean number of the identified adults. One way Anova and Tukey HSD tests were used for comparison of the differences between years. 200 samples (50 Birecik, 50 Beysehir Afsar, 50 Beysehir Sugla, 50 Cankiri) of *An. maculipennis* complex were identified with molecular markers (ITS2) to describe exact complex species that dispersed in the study areas. All molecular studies for description were conducted according to the Proft *et al.* 1999 [18].

Comparison of the seasonal dynamics and climatic parameters

Temperature, humidity and precipitation data were obtained as a monthly average between 2004 and 2009 years from Turkish State Meteorological Service. Pearson correlation analysis was applied to investigate annual change of adult populations and climatic parameters for the relation. Monthly average of the population densities and climatic parameters were used for this purpose.

Results

This study was carried out at 12 animal shelters in four different collection sites from three different parts of Anatolia. Population densities were calculated by using three animal shelters according to monthly and yearly average of samples. Totally 11238 individuals were collected during six years (2293 *An. sacharovi*, 1503 *An. maculipennis* s.l in Cankiri-Hacilar, 1558 *An. maculipennis* s.l individuals from Sugla-Beysehir, 2447 *An. maculipennis* s.l individuals from Afsar-Beysehir, and 3437 *An. sacharovi* individuals from Birecik). Annual abundance of four different locations is summarized in table 1 and monthly change is summarized in Figure 2-4. The highest density of *An. sacharovi* population in Cankiri was detected in 2007 (418) and the lowest density was detected in 2004 (317). In contrast to this situation, the highest density of

An. maculipennis s.l population was detected in 2009 (265) in Cankiri (Figure 2). Population densities in Cankiri region increased gradually from 2004 to 2009 (Figure 5). Although increasing trend was identified for Cankiri populations, we couldn't find significant differences between yearly changes (p>0,05) (Figure 5).

Table 1: Number of *Anopheles sacharovi* and *Anopheles maculipennis* s.l. individuals at three sites from 2004-2009

Area	**Cankiri**		**Beyşehir**		**Birecik**
Species	***An. sacharovi***	***An. maculipennis* s.l**	***An. maculipennis* s.l**		***An. sacharovi***
Year/Locations	***Hacılar***		***Suğla***	***Afşar***	***Doyduk***
2004	317	230	322	483	612
2005	328	255	424	538	630
2006	404	256	191	323	605
2007	418	247	175	359	543
2008	414	250	229	368	535
2009	412	265	217	376	512
Sum	2293	1503	1558	2447	3437

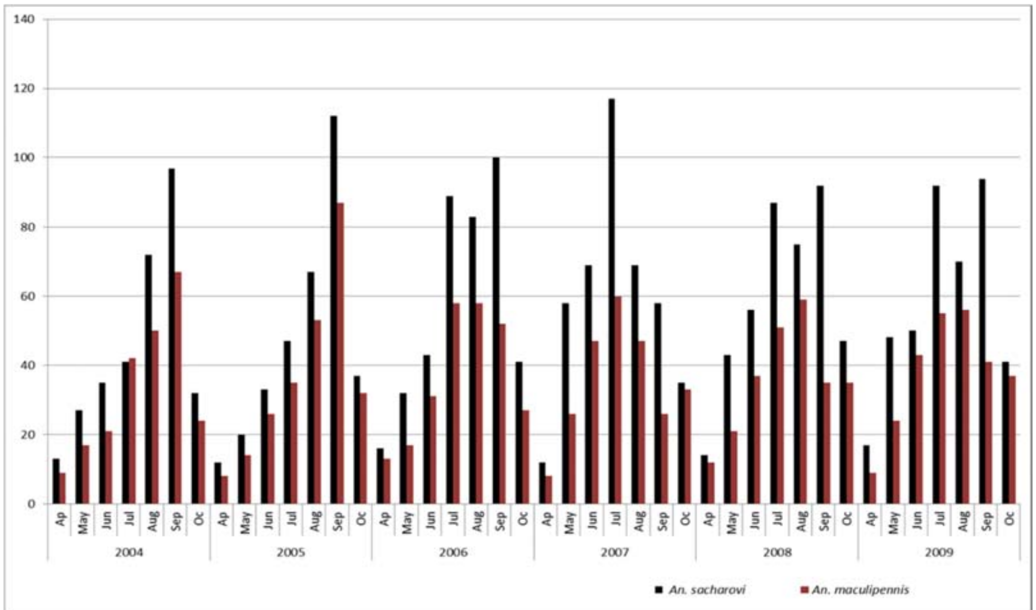

Figure 2: Monthly fluctuations of the *Anopheles maculipennis* complex species in Çankırı from 2004 to 2009

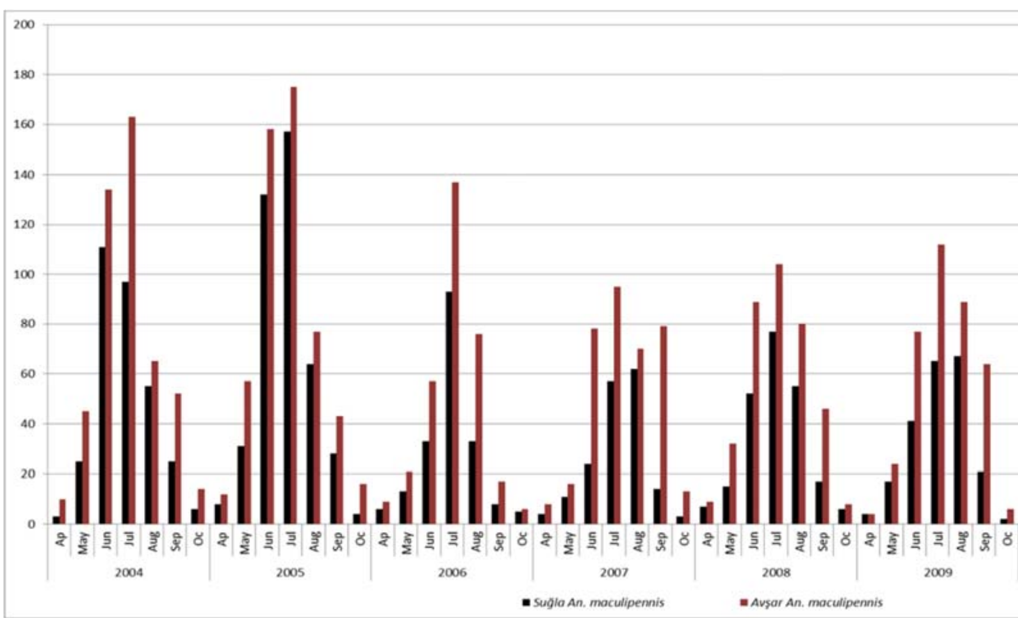

Figure 3: Monthly fluctuations of the *Anopheles maculipennis* complex species in Beyşehir from 2004 to 2009

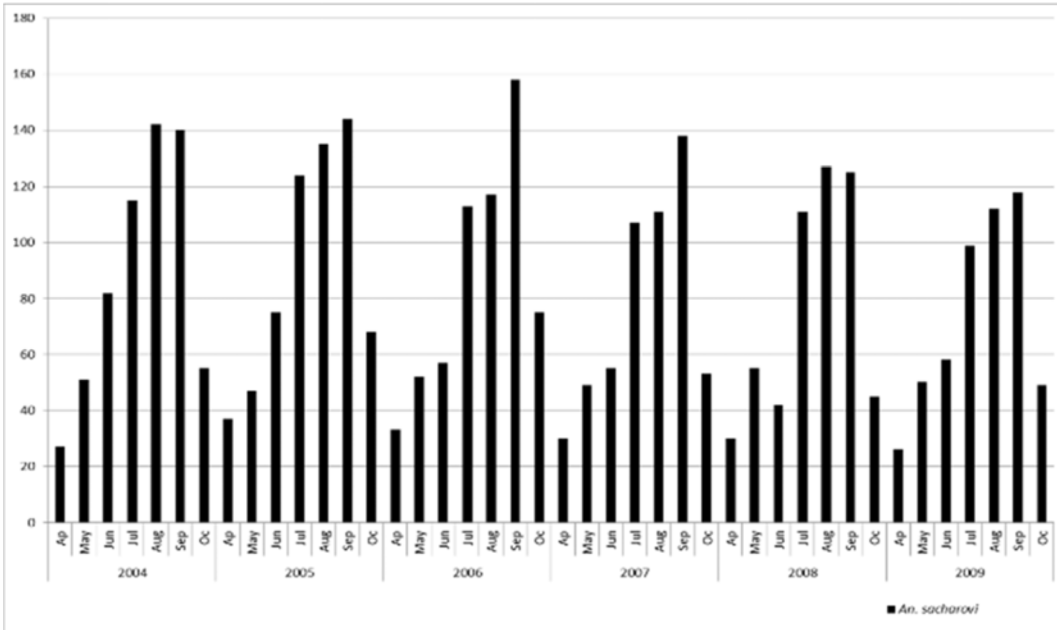

Figure 4: Monthly fluctuations of the *Anopheles sacharovi* species in Birecik from 2004 to 2009

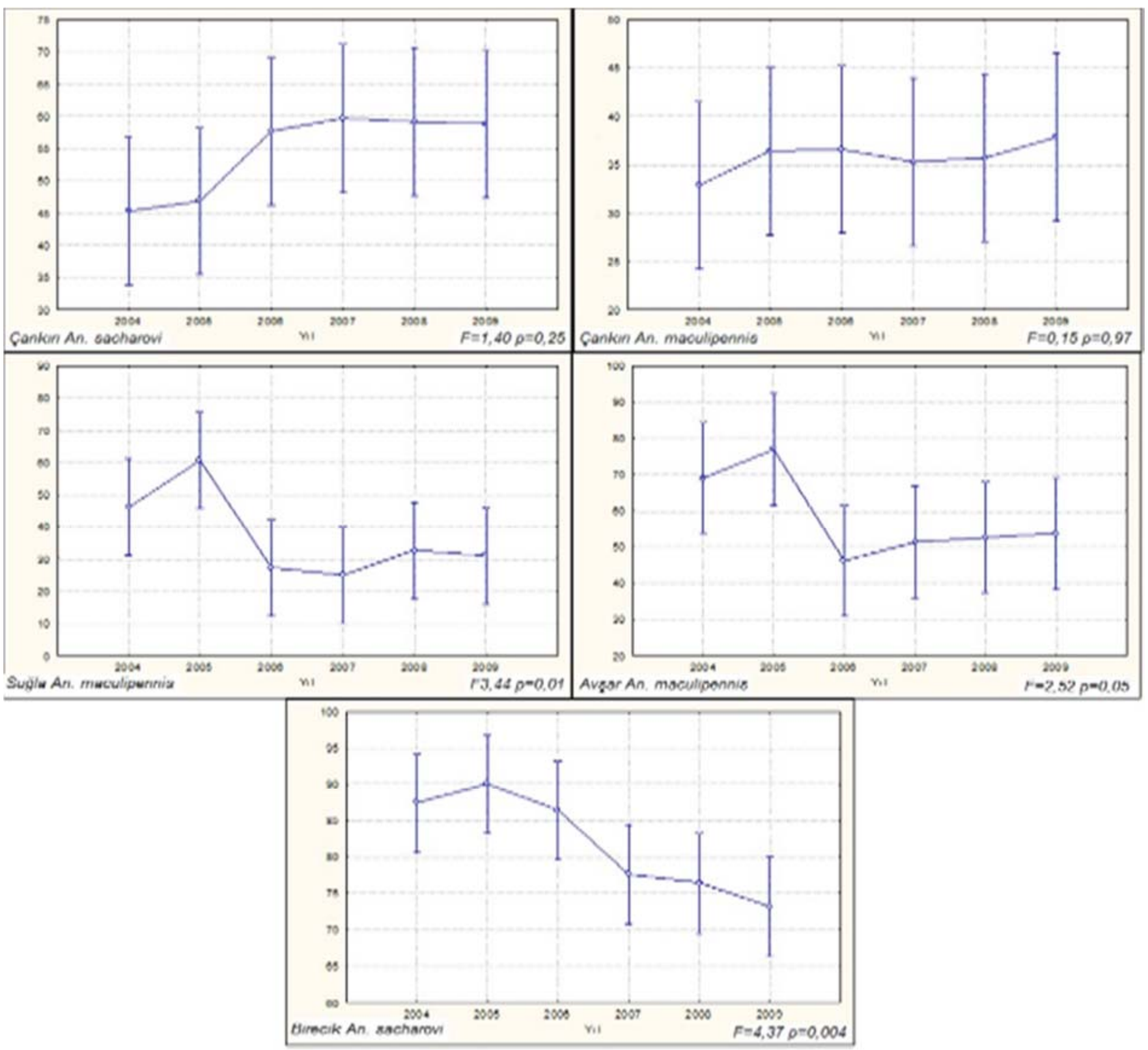

Figure 5: Comparison of yearly abundance of *Anopheles maculipennis* complex according to Anova and Tukey HSD tests

Contrary to results of Cankiri population, the study detected a gradual decrease in Beysehir and Birecik populations. Population densities showed variability for two collection sites in Beysehir region. Although the highest densities were detected in 2005 for two collection sites, the lowest density was found in 2007 (175) in Sugla collection sites and in 2006 (323) for Afsar collection sites (Figure 3). Population densities showed statistically significant differences between 2005, 2006 and 2007 (p<0,01) for Suğla collection sites (Figure 5). Comparably the same situation was found in Afsar collection sites. Population densities increased from 2004 to 2005, but in 2006 population densities decreased nearly 40% with respect to 2005. Statistically significant differences were not identified yearly but nearly obvious difference was identified between 2005 and 2006 (p=0,06) (Figure 5).
The highest density was found in 2005 (630) and the lowest in 2009 (512) in Birecik population. Population densities increased in small quantities from 2004 to 2005, but they gradually decreased in the other years (Figure 4, 5). Only significant differences were found between 2005 and 2009 (p<0,05) (Figure 5).
The average 6-year of temperature, precipitation and humidity data in study areas are given Table 2.
Table 2.

Table 2: Study areas 6 years annual mean temperature, precipitation and humidity

Area	Çankırı			Beyşehir			Birecik		
Year/ Climatic parameters	Temp	Prec	Hum	Temp	Prec	Hum	Temp	Prec	Hum
2004	11,03	28,85	63,63	11,06	37,84	62,77	17,89	37,97	54,23
2005	11,78	33,37	62,9	11,2	39,79	62,74	17,92	24	55,3
2006	11,4	33,2	60,97	10,7	43,85	64,4	18,12	30,44	55,13
2007	11,92	21,37	59	11,05	45,21	61,32	18,2	33,61	55,44
2008	11,66	24,32	58,38	12,04	23,58	59,88	16,51	31,13	47,13
2009	11,95	43,77	60,83	12,22	33,67	64,05	16,22	31,87	53,26

Temperature data of Cankiri and Beysehir showed 1-1,5 ^{0}C annual fluctuations. Also an increasing temperature trend was observed to be very low. In contrast to this situation, temperature records of Birecik showed 0,5-1 ^{0}C an upward fluctuations, but temperature records of the last two years showed a decrease unlike the previous years. While temperature records showed such a structure for three areas, precipitation and moisture records showed significant reduction. Pearson correlation analysis was applied to detect whether there was a relationship or not between population fluctuations and climate parameters (Table 3). While a positive correlation between temperature and population size was observed, negative correlation was observed in terms of humidity and precipitation. Correlation between temperature and population size changed between 0,59 (Cankiri *An. maculipennis* s.l and *An. sacharovi*) and 0,76 (Beysehir Avsar *An. maculipennis* s.l). Correlation between precipitation and population size changed between -0,36 (Birecik *An. sacharovi*) and -0,51 (Cankiri Hacilar *An. maculipennis* s.l). Similarly, it was observed that the pattern for humidity and correlation changed between -0,44 (Çankırı Hacılar *An. maculipennis* s.l. population, Birecik Doyduk *An. sacharovi* population) and -0,56 (Beysehir Avsar *An. maculipennis* s.l). Although correlation between temperature and population size is the same for *An. sacharovi* and *An. maculipennis* s.l populations in Cankiri, correlation between humidity/precipitation and population size is different for these populations.
Table 3.

Table 3: Pearson correlation analysis results between population densities, temperature, precipitation and humidity

Area/climatic parameters	Temperature	Precipitation	Humidity
Beyşehir Suğla *An. maculipennis* s.l.	0,68	-0,39	-0,46
Beyşehir Afsar *An. maculipennis* s.l.	0,76	-0,45	-0,56
Çankırı *An. sacharovi*	0,59	-0,39	-0,52
Çankırı *An. maculipennis* s.l.	0,59	-0,51	-0,44
Birecik *An. sacharovi*	0,69	-0,36	-0,44

Results of molecular studies for species description are presented in Table 4. The descriptive PCR results displayed that 3 species of *An. maculipennis* complex. PCR products size was found 410 bp (*An. maculipennis* s.s), 224 bp (*An. melanoon*), 180 bp (*An. sacharovi*) and PCR results evaluated according to the Proft et al. [18]. While *An. maculipennis* s.s was found in Cankiri and Beysehir (Afsar, Sugla), *An melanoon* was found only Beysehir (Afsar, Sugla). *An. sacharovi* was observed all collection areas except Beysehir Afsar and existed pure population in Birecik. Table 4.

Table 4: Molecularly confirmed exact species of *Anopheles maculipennis* s.l. in the study areas

Area/Species	*An. sacharovi*	*An. maculipennis* s.s.	*An. melanoon*
Birecik Doyduk	50	0	0
Beysehir Sugla	6	42	2
Beysehir Avsar	0	45	5
Çankırı Hacılar	35	15	0

Discussion

It is known that population dynamics are influenced by many parameters such as temperature, humidity etc. Reisen and Reves [19] and Reisen *et al.* [20] indicated that the alteration of mosquito population dynamics is strongly correlated with the environmental conditions in habitat. Our observations revealed that bimodal temporal structure but peak months of *An. maculipennis* s.l. and *An. sacharovi* populations varied between the populations (Figure 2, 4). Bimodal temporal structure is not clearly observed in Beysehir area populations

(Figure 3). Merdic and Boca [21] indicated the same situation for *An. maculipennis* complex populations in Croatia. In contrast to this situation, Alkan and Aldemir [22] found one peak in August *An. maculipennis* complex populations in Igdir plain. Aldemir *et al.* [23] also observed that the peak months change year to year for *An. maculipennis* complex populations in the other study with *An. maculipennis* complex. Our study correlated with aforementioned studies and bimodal temporal structure changed monthly according to the habitat characteristics and height. It was observed bimodal temporal structure is seen clearly both *An. sacharovi* and *An. maculipennis* populations in Cankiri. Although Birecik population showed bimodal structure, population increased gradually July to September and also maximum population densities was seen in September in 2005, 2006, 2007 and 2009. The other years maximum population densities was seen in August in Birecik.

Temperature slightly increased but humidity and precipitation rates reduced severely during six years except Birecik precipitation rate. Precipitation rate slightly increased of Birecik in this period. It was determined that correlation with population size and temperature population of 59% (Cankiri) to 76% (Beyşehir Afşar). Although positive correlation was found between temperature and population size, precipitation and humidity showed negative correlation with population sizes. Study areas comprise permanent water ponds as larval habitats during the breeding season. Therefore precipitation and humidity could not be affected on the population size. It was also known number of rice field areas increased during the study in Cankiri. Although not precise correlation between climatic parameters and seasonal population fluctuations were found, there are various studies about the correlation between climatic change and *Anopheles* density and increased biting activity [24]. *Anopheles maculipennis* s.l species begin to appear active and intense in the areas as a result of the climatic suitability in fall season.

Adult density in the studied areas accumulated in the barns and density in barns higher than the other resting places (in house and inside the water discharge channel near the larval development areas). Reduced precipitation may seems like a negative factor for the population density, but agricultural activities were ensured suitable stable larval habitats in the areas. Closeness of the larval development areas and continuous blood feed source in resting places were provided high density in the barns. Poncon *et al.* [25, 26] has addressed to this situation and indicated to direct relation high population density with climatic parameters and area usage for malaria and West Nile virus vectors in the study area. Poncon *et al.* [25] showed two seasonal peaks for two different areas (Carbonniere, Marais du Vigueirat in France) for *An. melanoon* and one of them is june-august and the other is july-august in 2005.

Service [27] indicated that mosquito larval habitats are temporary, permanent, human made or natural. Larval habitats in all three study regions are predominantly man made and included agricultural areas. Suitable larval habitats in all three study areas are agricultural areas for *An. maculipennis* s.l. species which important malaria vectors in our country and palearctic. Alptekin and Kasap [28] indicated that mosquito larval habitats are occupied by various mosquito species but they noted that many species were found at specific habitat. *Anopheles maculipennis* s.l. species prefers fresh waters and generally be found dominant. Birecik region is 344 m height and it includes suitable larval habitats and adults feeding/resting for *An. sacharovi*. A pure population of the *An. sacharovi* was found in that area. Çankırı region, with 561 m height, comprises ricefield and watermelon production fields enable for larval development. Animal barns are enable for resting and blood feeding for *An. maculipennis* complex. *An. maculipennis* s.s. and *An. sacharovi* species were found in the area. Beysehir region is 1100 m height. Beysehir comprises vegetable and fruit production fields. Collection sites include suitable larval habitats for larvae (irrigation channel) and adults (animal barns). *An. maculipennis* s.s., *An. melanoon* and *An. sacharovi* species were found in the area. Akıner *et al.* [7] were confirmed species of all three area respectively of pure population of *An. sacharovi* in the Birecik region, *An. sacharovi*, *An. maculipennis* s.s. and *An. melanoon* species in Beysehir, *An. sacharovi* and *An. maculipennis* s.s. species in Cankiri region with molecular studies.

Conclusion

Determining time dependent changes of population density of predominant and crucial vector species is important both to understand their life cycle and develop right control strategies. *An. maculipennis* s.l. species were found with *Culex pipiens* complex species in many agricultural areas and both species were predominant. Densities can reach excessive amounts both animal barns and poultry house due to zoophilic tendencies. *An. maculipennis* s.l. species comprises main and potential vector species of Malaria. Therefore, studies of the population will provide important data both understand to population dynamics and step forward to necessary controlling practices. This long term study is essential for this respect, and more study is needed to understand both population dynamics and vector borne disease connection.

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