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Short Communication: Species diversity, habitat and abundance of Culicid mosquitoes in Bushehr Province, South of Iran

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Abstract. *Khoobdel M, Keshavarzi D, Sobat H, Akbari M. 2020. Short Communication: Species diversity, habitat and abundance of Culicid mosquitoes in Bushehr Province, South of Iran. Biodiversitas 21: 1401-1406.* Culicid-borne diseases are causes of substantial illness worldwide. Effective culicid control depends on up-to-date knowledge of ecology and dispersal of species. Hence, this entomological study was conducted from January - December 2017 to provide baseline data on the distribution and ecology of mosquitoes in the Southwest of Iran. In total, 2530 specimens belonging to 14 different species were collected and identified, as follows: *Culex hortensis, Cx. laticinctus, Cx. mimeticus, Cx. perexiguus, Cx. pipiens, Cx. modestus, Cx. sinaiticus, Cx. theileri, Cx. torrentium, Cx. tritaeniorhynchus, Anopheles stephensi, An. superpictus, An.dthali*, and *An.subpictus*. Diversity analysis indicated higher species richness for uninhabited areas (Margalef 1/86). The average of Shannon diversity index ranged from 1.98 to 2.10. Differences between urban and uninhabited areas were significant according to the diversity t-test (P = 0.024, t = 2.26). The analysis of beta biodiversity showed that rural and uninhabited areas were the closest environments in their specific composition (Whittaker index = 0.130). These findings could be useful in vector control program.

Keywords: Larval habitat, mosquitoes, species diversity, vector control

INTRODUCTION

Culicidae are main vectors for a wide range of diseases such as malaria, lymphatic filariasis, West Nile, zika, sindbis, dengue and yellow fever (Azarihamidian et al. 2009; Bagheri et al. 2015; Lebl et al. 2015; Foster and Walker 2019). Malaria, West Nile, and dirofilariasis are the most important Culicid-borne diseases in Iran (Naficy and Saidi 1970; Khoobdel 2008; Azarihamidian et al. 2009; Bagheri et al. 2015). According to WHO report, Japanese encephalitis virus and Rift Valley virus, are two culicid-borne pathogens with the potential to invade Iran (WHO 2004). *Cx. tritaeniorhynchus* was declared as the potential vector of Japanese encephalitis in Iran (Harbach 1988). In addition, zika and dengue fever threatening the Middle East countries (Khoobdel and JonaidiJafari 2016; Khoobdel et al. 2016). Third stage of *D. immitis* larvae was reported in *Culex theileri* in Iran, so this species could be the vector of that parasite (Azarihamidian et al. 2009). Sero-survey of West Nile virus in the equine populations in Iran indicated circulation of virus in southwestern provinces (Ahmadnejad et al. 2011). Culex pipiens reported as a bridge vector for West Nile virus (Hamer et al. 2008). Recently, there is evidence for Zika virus transmission by *Culex quinquefasciatus* (Guedes et al. 2017). Therefore, due to the possibility of culicid mosquitoes to transmit vector-borne diseases, the necessity of the present study is highlighted.

Survival, behavior, and reproduction of mosquitoes can be affected by rainfall, temperature and humidity changes in different seasons. Those factors can strongly affect pathogen development within vectors and also influence the availability of breeding sites for culicid vectors (Rogers and Randolph 2006).

There is very little information about the fauna and ecology of mosquitoes in the coastal regions of the Persian Gulf. Therefore, the main objective of the present study was to determine the species diversity, habitat and monthly abundance of culicid mosquitoes in the coastal Iran section of the Persian Gulf.

MATERIALS AND METHODS

Study area

To develop the study, 6 areas were selected in different ecosystems (urban areas (UA), rural areas (RA) and uninhabited area (UNA) of the north of Bushehr Province, Iran (50° 31' 1E, 29° 34' 45N) (Figure 1). Two sites were chosen for each of those areas.

Sampling methods and taxonomic identification

In order to study the ecology of mosquitoes, sampling was carried out by dipping technique with a metal dipper for collecting larvae. Four dips were taken from each breeding site (350 mL capacity each dip). Larval investigation was conducted two times a month during the study period.

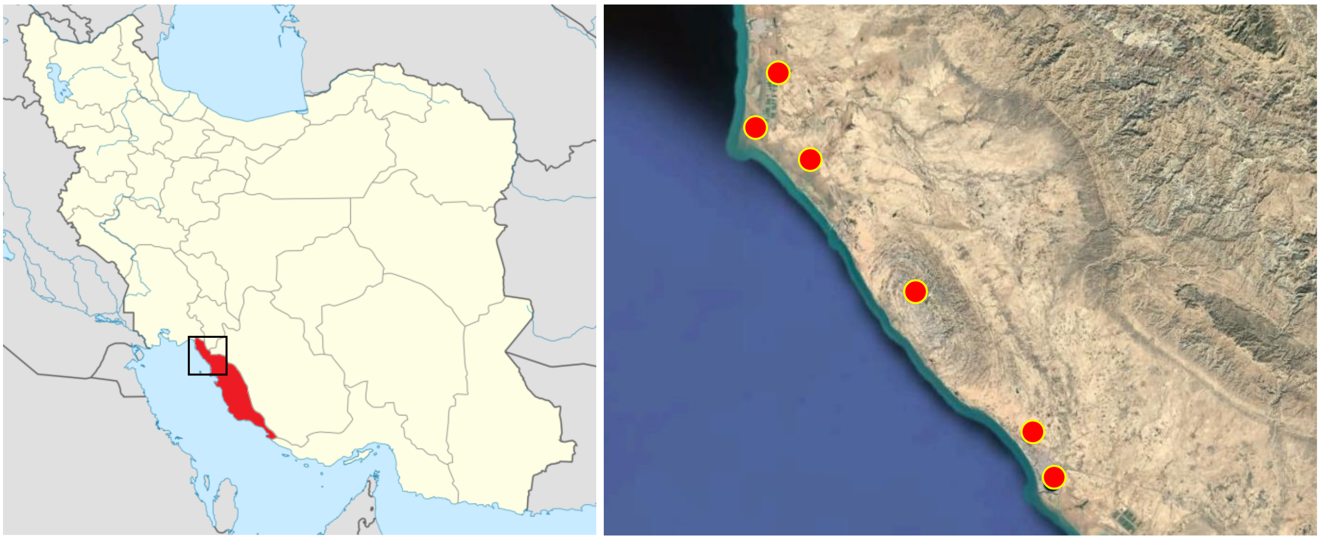

Figure 1. Map of Iran, highlighting the position of Bushehr Province, showing distribution of sampling sites in the study area

All the samples were brought to the laboratory of the Entomology department, Tehran University of Medical Sciences, Iran. The mosquito larvae were preserved in 75% ethanol and the microscopic slides were prepared using the chloral gum mounting, this is a medium of high refractive index, very good for mounting small insects. Microscope was used for the taxonomic study and identification, up to the species level using taxonomic keys available in literature (Azari-Hamidian and Harbach 2009).

Biodiversity and statistical analysis

Diversity studies (alpha diversity) were conducted separately for each category (rural, urban and uninhabited areas) by calculating classic diversity indices: (i) Margalef richness index, is a measure for the total number of the species in a community: $S-1 / \ln N$ (S = total number of species and N = total number of individuals), higher values represents greater richness. (ii) Simpson index: $1-\Sigma pi2$, where $pi = ni/N$ (ni is number of individuals of taxon i, N = total number of individuals), with this index, 0 represents infinite diversity and 1, no diversity (Magurran 1988; Simpson 1949). (iii) Dominance = 1-Simpson index. Ranges from 0 (all taxa are equally present) to 1 (one taxon dominates the community completely). (iv) Shannon diversity index: $-\Sigma$ (pi lnpi), where "ln" is the natural logarithm, $pi = ni/N$ (ni is number of individuals of taxon i, N = total number of individuals). This index accounts for both abundance and evenness of the species present. Values usually ranging from 1.5 to 3.5 and lower values indicate less diversity. (Shannon and Weaver 1949). (v) Jaccard index was used for comparison of similarities between categories: $M / (M+N+O)$, where M = number of species common to (shared by) categories, N = number of species unique to the first category, O = number of species unique to the second category. The Jaccard index ranges from 0-1 showing complete dissimilarity to perfectly match the sampling intervals for any insect species (Magurran 1988).

Data were analyzed using PAST software version 3.14 (Paleontological Statistics Software Package). The assumption of normality checked by P-P plots and Shapiro-Wilk normality test. Kruskal-Wallis test was used to evaluate differences between the variables of temperature, month, and species abundances. Pearson's correlation test was used to measure the strength of the relationship between the variables.

RESULTS AND DISCUSSION

In total, 2530 specimens belonging to 14 different species were collected and identified. Diversity analysis indicated higher species richness for UNA (*Margalef* 1/86). The average of Shannon diversity index for the three environment types ranged from 1.98 (RA) to 2.10 (UA). According to the diversity t-tests, difference in species diversity was significant only among UA and UNA communities ($P = 0.024$, $t = 2.26$). The analysis of beta biodiversity shows that RA and UNA were the closest environments in their specific composition (Jaccard index = 0.130). More ecological information's about the genus of *culex* and *anopheles* are provided bellow.

***Culex* mosquitoes**

A total of 1369 specimens belonging to 10 different mosquito species were collected and identified viz.: *Cx. hortensis* Ficalbi, *Cx. laticinctus* Edwards, *Cx. mimeticus* Noe, *Cx. perexiguus* Theobald, *Cx. pipiens* Linnaeus, *Cx. modestus* Ficalbi, *Cx. sinaiticus* Kirkpatrick, *Cx. theileri* Theobald *Cx. torrentium* Martini and *Cx. tritaeniorhynchus* Giles. In the dipping collection, *Cx. theileri* (28.0%), *Cx. pipiens* (26.1%), *Cx. tritaeniorhynchus* (23.1%) were predominated, respectively. The greatest number of mosquitoes were collected from RA (723 specimens) and the lowest in the UA (209 specimens). Monthly variation in abundance of mosquitoes is provided (Figure 2). The highest number of mosquitoes was collected in August (379) and September (370 specimens), while the lowest was in January (0 specimens) and February (3 specimens). Depending on the month, difference was not significant in species frequency according to Kruskal-Wallis test (P = 0.4). Analysis with Kruskal-Wallis showed that difference between environments (UA, RA, and UNA) and abundance of mosquitoes was not significant (P = 0.3). Overall, in the present study, there was a significant positive relationship between mean temperatures and abundance of culicid mosquitoes (r = 0.75, P = 0.005).

The larval habitat characteristics and occurrence percentages of different culicid larvae in this study are provided (Table 2). The most important larval habitats were shrimp pond (623n) and irrigation canals (436n).

Greater species richness was found in RA (M = 1/21), while UA has the lowest (M = 0.93). The average diversity indices for the three environment types ranged from 1.50 to 1.64 for Shannon index and from 0.730 to 0.738 for Simpson index. Shannon index was highest in UNA and lowest in UA. While, Simpson index in UA was highest. The t-test showed no statistically significant difference between Shannon index (t = 1.17, p = 0.24) and Simpson index (t = 0.39, p = 0.69) in RA and UA. Similarly, there was also no statistically significant difference between Shannon index (t = -1.36, p = 0.17) and Simpson index (t = 0.20, p = 0.83) in RA and UNA. But, there was a significant difference between Shannon index in UNA and UA (t = -2.26, p = 0.024). However, difference between Simpson index was not significant in these areas (t = -0.15, p = 0.87). Greater evenness was observed in UA, because the most dominant species do not show such a strong influence as in the two other environments. Where, *Cx. theileri* in UNA and *Cx. pipiens*, *Cx. theileri* and *Cx. tritaeniorhynchus* in RA were dominant and shows a strong influence. The analysis of β biodiversity showed that RA and UNA were the closest categories in their specific composition (W index = 0.17), while UNA and UA were the farthest categories (W index = 0.28).

Anopheles mosquitoes

During this investigation, 1161 anopheline mosquitoes were collected, of which 4 species were recognized, namely; *An.stephensi* (50.3%), *An. superpictus* (23.7%), *An.dthali* (21.8 %) and *An.subpictus* (4.2%). The highest number of mosquitoes were collected from RA (585 specimens) and the lowest in the UNA (279 specimens). *An.subpictus* was found only in the uninhabited area, but the rest of those species was caught in all areas.

Table 1. The larval habitat characteristics and occurrence percentages of *Culex* mosquitoes in southwest Iran, in 2017.

Characteristics		cx. theileri	cx. pipiens	cx. tritaeniorhynchus	cx. modestus	cx.sinaitcus	cx. perexigus	cx.mimeticus	cx.hortensis	cx.laticinctus
Water type	Permanent	6.3	40	0	0	63.4	22.7	3.2	11.7	12.5
	Temporary	93.7	60	100	100	36.6	77.3	96.8	88.3	87.5
Water flow	Running	4.6	21.8	12.6	30.2	32.7	26.8	8	5.8	0
	Stagnant	95.4	78.2	87.4	69.8	673	73	92	94.2	100
Water appearance turbid		35.2	36.3	12.3	0	3.6	14	15.4	0	37.5
	Clean	64.8	617	87.7	100	96.4	86.3	84.6	100	62.5
Vegetation	With	65.5	62.4	100	83.2	69.8	86.3	80	5.8	0
	Without	34.5	37.6	0	16.8	30.2	13.7	20	94.2	100
Substrate type	Muddy	84.3	69.3	100	81.9	72.3	77.5	81.5	52.9	75
	Sandy	15.1	30.7	0	18.1	27.7	22.4	18.5	47.1	25

Table 2. The larval habitat characteristics and occurrence percentages of *Anopheles* mosquitoes in southwest Iran, in 2017.

Characteristics		*An. stephensi*	*An. superpictus*	*An. dthali*	*An. subpictus*
Water type	Permanent	77.2	75.9	78.6	45.8
	Temporary	22.8	24.1	21.4	54.2
Water flow	Running	26.7	31.2	34.3	31.3
	Stagnant	73.3	68.8	65.7	68.7
Water appearance turbid		5.2	22.3	2.6	66.6
	Clean	94.8	77.7	97.4	33.4
Vegetation	With	22.8	37.6	26.8	27
	Without	77.2	62.4	73.2	73
Substrate type	Muddy	75	67.9	16.7	83.4
	Sandy	25	32.1	83.3	16.6

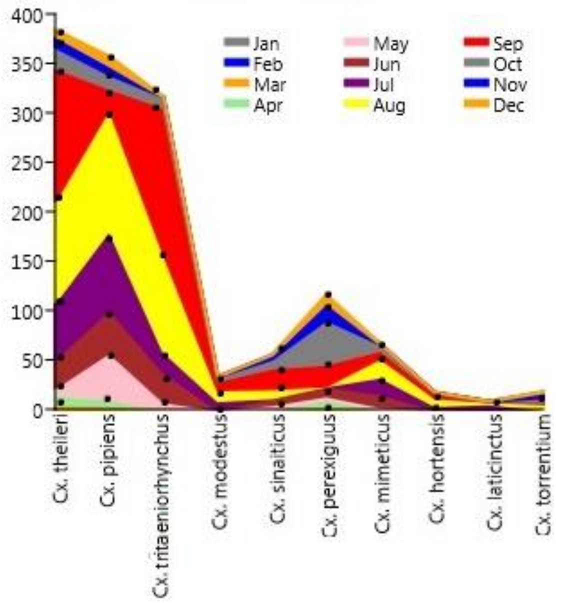

Figure 2. Monthly abundance of *Culex* mosquitoes in southwest Iran, in 2017

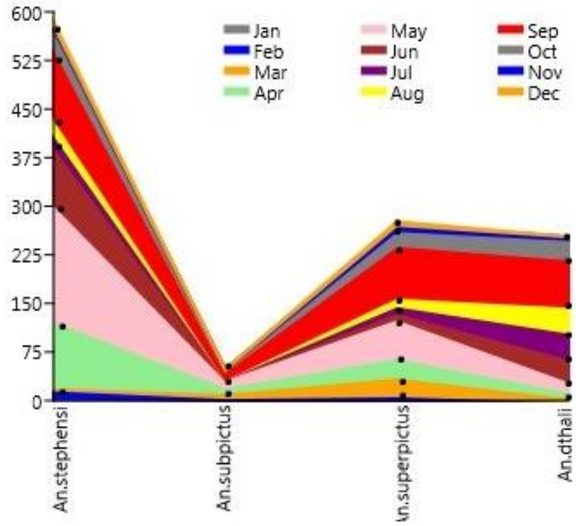

Figure 3. Monthly abundance of *Anopheles* mosquitoes in southwest Iran, in 2017

The highest number of mosquitoes were caught in September (274 specimens) and May (268 specimens), respectively, while the lowest was in January (4 specimens). During monthly collections for one year, *An. stephensi* were collected from January to December and reached its peak in May and September. *An. superpictus* was collected from February to December (Figure 3) and reached its peak in May and September, when mean temperature was lower than 34°C. However, relationships between mean temperatures and abundance of Anopheles mosquitoes were not significant (P = 0.07). The larval habitat characteristics and occurrence percentages of different species are provided (Table 2). The best breeding places for the most anopheline species were shrimp pond, river bed, and palm irrigation canal. We found *An. subpictus* species mostly in a marshy area.

Diversity analysis indicated higher species richness and species diversity for UNA (Margalef = 0/73; Shannon = 1.27). The average diversity indices for ecosystems ranged from 0.98 to 1.27 for Shannon index and from 0.57 to 0.69 for Simpson index. According to the diversity t-test, only the difference between UA and UNA ecosystems was significant (P = 0.0006).

Discussion

In the present investigation, 14 species of mosquitoes were identified. Among them, there are some major vectors of human and animal diseases such as *Cx. pipiens* (usutu and West Nile), *Cx. theileri* (dirofilariasis, Rift Valley fever), *Cx. perexiguus* (sindbis and West Nile), *Cx. tritaeniorhynchus* (Japanese encephalitis, Rift Valley fever, sindbis, West Nile) (Azari-hamidian et al. 2009; Clements 2012; Seufi and Galal 2010).

Culex theileri (28.0%) was the most abundant species in urban and uninhabited areas and appeared from March to December to make a peak with 135 individuals in September, when the mean temperature was 34.7 °C. This finding suggests that the oviposition activity of *Cx. theileri* is limited by the temperatures. According to a study in Turkey, *Cx. theileri* reached its peak in August (n = 101) (Gündüz et al. 2009). In another study in Fars Province, Iran, previously we found that *Cx. theileri* reached its peak in June and July (Soltani and Keshavarzi 2016). The present study does not provide similar findings. This species was the most dominant mosquito in Zanjan Province, Iran (Ghavami and Ladonni 2005).

Culex theileri is found in most parts of the world (Harbach 2007). This mosquito has been recorded in all provinces of Iran and has involvement in the transmission of *Dirofilaria immitis* nematode (Azarihamidian et al. 2009). In the study of ecology of mosquitoes in western Iran, *Cx. theileri* preferred habitats with permanent water and without plants (Moosa-Kazemi et al. 2015). This finding is not similar to the present study.

Culex pipiens was the most frequent species in rural areas, whereas *Cx. tritaeniorhynchus* came in the second order. In a study, *Cx. pipiens* in Lamerd County, Fars Province was predominant species and reached its peak in May and August (Keshavarzi et al. 2017). This is similar to our findings. This species in the north of Iran reached its peak in July (Nikookar et al. 2010). This species is reported in most provinces of Iran and in some areas reported as a dominant mosquito (Nikookar et al. 2010). Four anopheline species that were identified in this study are known as malaria vector in Iran.

Anopheles stephensi is a proven malaria vector in the south of the country. This species has an extensive dispersal in Iran and it has been recorded from Bushehr and neighboring provinces (Hanafi-Bojd et al. 2011). In the present study, the species of *An.stephensi* (49.9%) were the most frequent anopheline species and reached its peak in May and September. According to a study conducted in the southeast of Iran, *An. stephensi* has two main seasonal peaks during spring and autumn (Mehravaran et al. 2012). Similar findings have been reported from Fars province, Iran (Keshavarzi et al. 2017).

In the present study, *An. superpictus* (23.7%) was the second most abundant anopheline species and reached its peak in May and September (Figure 3). It is a secondary vector in the southern parts of Iran (Ladonni et al. 2015). The larval nests are the same as the An.stephensi species. But An. superpictus preferred the shade environment. The population dynamics of this species in our study had started from February to December. This species in the Isfahan province were reported from July to August (Ladonni et al. 2015).

According to Margalef index, UNA shows the highest diversity. The reason for this might be due to ecological and weather-related factors. Higher species diversity in UA can be due to permanent breeding places such as ponds in that area. According to a previous study in Iran, the higher species diversity found in rural areas (Hanafi-Bojd et al. 2017). Based on previous studies, the maximum reported value for Shannon's index is 1.7 (Keshavarzi et al. 2017; Nikookar et al. 2010).

In the present study, the maximum value for Shannon's index was 2.1. It seems that these differences in previous reports in Iran are due to the differences in the number of species, sample size, and data analysis method. Normally, the Shannon index in real ecological units ranges between 1.5 and 3.5, therefore, a smaller amount makes it difficult to interpret actual species diversity (MacDonald 2002).

To conclude, in the present research, some potential vectors of medical-veterinary importance were identified. Biodiversity analysis indicated that species diversity in urban areas is greater. Therefore, attention to these areas in vector control programs is essential. Paying attention to shrimp ponds and canals as larval nests is effective in controlling mosquitoes in this region.

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