

Biodiversity of culicid mosquitoes in rural Neka township of Mazandaran province, northern Iran

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ABSTRACT

Background & objectives: This paper presents the results of a study on ecological parameters including diversity indices applied on populations of Culicidae mosquitoes (Diptera: Culicidae) from three rural areas (Darvishan, Chalmardi and Khairabad) of Neka township in Mazandaran province, northern Iran.

Methods: Adult and larval stages of mosquitoes were systematically sampled to obtain data required for measuring diversity of mosquitoes in these areas. Alfa and beta indices were analyzed to compare mosquito diversity. A total of 5270 specimens belonging to four genera and 14 different species were collected and identified.

Results: Evaluation of dominance structure of species showed that culicid mosquitoes of Neka rangelands include one eudominant species, two dominant species, two subdominant species, two rare species and eight subrare species when Heydemann classification was used.

Interpretation & conclusion: Data analysis revealed a higher diversity of mosquitoes in Khairabad with a low degree of interspecific dominance, while Darvishan and Chalmardi recorded the lowest observed diversity and a high degree of interspecific dominance. The cluster analysis based on Jaccard's index indicated the relationship between the categories and indicates that Darvishan and Khairabad are the closest categories in their specific composition.

Key words Alpha diversity; beta diversity; biodiversity; northern Iran; rarefaction

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are considered as most important groups of arthropods in public health and, like other organisms, show a direct relation to different factors such as environmental and habitat heterogeneity or host preferences¹. The family Culicidae is a diverse group of largely haematophagous insects, divided into two subfamilies, 11 tribes, 113 genera and 3526 species². Some mosquitoes that bite humans routinely, act as vectors of a number of infectious diseases affecting millions of people each year; such species usually are vectors of viruses, nematodes, protozoan parasites and due to their large geographical distribution and abundance can transmit various diseases to humans and animals like malaria, dengue, encephalitis, lymphatic filariasis and the West Nile fever³⁻⁴. The importance and dominance of invertebrates and their role in ecosystem is well documented and based on these data, it has been estimated that the

diversity of the mosquito species varies among different geographical regions of the world⁵. Many genera of mosquitoes are cosmopolitan, whereas some are endemic to certain areas so the importance of biodiversity of mosquitoes is very evident. For example, about 36% of the 42 known mosquito genera are endemic in four regions of the world⁶. This is the one of several reasons, which leads ecologists and biologists to measure the biological diversity of species in space and time in order to study the ecological and evolutionary processes related to a particular species or group of species⁷⁻⁹.

Mosquito biodiversity can be a major issue because of the risk associated with invasive species and the emergence and spread of mosquito-borne diseases. For instance, in the case of malaria, *Anopheles* mosquitoes transmit malaria from one human to another by biting, in another case *Culex* species act as vectors of a number of arboviruses (West Nile virus, Rift Valley, St. Louis encephalitis, Japanese encephalitis) and parasites

(Bancroftian and a large number of avian protozoans). So, it can be expressed that changes in biodiversity not only can create new ecological niches for proliferation of the vectors, but also can increasingly affect the spread of human diseases^{6,10-11}.

Comparison of mosquito diversity (alpha diversity) and structure of the populations in which they are integrated (beta diversity) can provide us with a powerful tool for the implementation of more effective and efficient vector population control programs^{8,12}. It is reported that because of the high adaptive capacity of mosquitoes, studies focusing on transitional environments become very important, such as those in rural areas, which are considered as bridges between wild diseases and human populations of urban areas¹³. Quantitative estimates of diversity and similarity were obtained using different indicators currently in use such as alpha (α) diversity as the specific richness of a community that is considered homogeneous and beta (β) diversity which refers to the replacement degree in a specific composition between different communities of a landscape¹².

In ecology, rarefaction is a technique to assess species richness from the results of sampling. Rarefaction allows the calculation of species richness for a given number of individual samples, based on the construction of so-called rarefaction curves. This curve is a plot of the number of species as a function of the number of samples. Slope of the curves provides information about the sampling frequencies and intensities required to establish the true composition of the species in a given environment⁷. These methods of biodiversity analysis are useful not only to explore the climatic, physical or biological influences on biodiversity, but also to study the effects of human pressure on biodiversity¹⁴⁻¹⁵. Moreover, identifying the mosquito and its habitats has a critical role in each control program¹⁶. This control cannot be effective without a better knowledge of the bioecology and the distribution of these insects, in space and time^{1,17}.

In Iran, studies regarding the biodiversity of mosquitoes are mainly limited to the southern parts of the country^{16,18}. However, there is no information on mosquito's diversity components and the factors that regulate changes in diversity components in northern Iran. In this study, we have collected mosquito samples (adults and larvae) in three rural areas of Neka township from Mazandaran province, northern Iran, at different times of the year, during a period of nine months (April–December 2009). Taking into account these considerations, the aim of this study was to investigate the structure of the culicid community, analyze the diversity of Culicidae family present in the natural areas considered, as well as the differences on the

faunistic composition of mosquito species in function of the climatic and ecological features of rural areas of Neka.

MATERIAL & METHODS

Study area

The study was carried out from April to December 2009. Three sites (Darvishan, Chalmardi and Khairabad) belonging to the rural Neka county of Mazandaran province, northern Iran (36°39' N, 53°17' E) were selected. The weather condition such as temperature, humidity and altitude recorded were on the standard forms. During the study period, maximum and minimum average temperatures recorded were 27.6 and 9.1°C in July and December, respectively. The altitude of these sites was 185, 210 and 290 m above the mean sea level in Darvishan, Khairabad and Chalmardi, respectively. The maximum annual rainfall reported was 204.6 mm in October, and the minimum 0.1 mm in July (average of annual rainfall is 265 mm) and relative humidity ranged between 60 and 85%.

Sampling methods and taxonomic identification

Larval collection: A simple random sampling method was carried out across the study areas by selecting all suitable biotopes to accommodate immature forms of mosquitoes. Sampling in larval habitats was performed biweekly using standard dipper. The mosquito larvae were collected in different oviposition sites such as discarded tyres, tree and rock holes, irrigation channels, rice fields, ponds, animal footprints, and marshes inside forest. Some mosquito larvae were collected from treeholes using dropper. These treeholes were also evaluated twice monthly. During each of the evaluations, pupae were collected and placed in bucket dishes covered with netting and transferred to the laboratory until they hatched to adults. Specimens were identified by using standard mosquito identification keys¹⁹⁻²¹.

Adult collection: Adult mosquitoes were collected on a monthly basis by one human bait net trap using mouth aspirator in randomly selected forest areas in the vicinity of trees with holes. Sampling was performed in consideration of ethical issues and personal satisfaction. The mosquitoes were placed into plastic cups with a stainless screen and transferred to laboratory, where they were identified to species using the keys of Shahgudian¹⁹, Zaim and Cranston²⁰.

Species dominance structure

The Heydemann's classification was used to evaluate the dominance structure²². This classification has five

degrees of dominance: Eudominant species—those making up <30% of all the specimens caught, dominant (10–30%), subdominant (5–10%), rare (1–5%) and subrare (<1%).

Biodiversity of species and statistical analyses

Alpha diversity: Alpha diversity for larval and adult stages, and for the combination of both stages were estimated by calculating classic diversity indices including Margalef, Simpson and Shannon indices¹². The procedures employed to calculate Margalef index in the studied area is described as follows:

$$\text{Margalef index: } D_{Mg} = \frac{S-1}{\ln N}$$

To perform this spatial analysis, the Simpson and Shannon diversity indices were first estimated for each larval, adult and larval+adult stage distributed in each rural area.

Simpson index is calculated by the equation:

$$D = \lambda = \sum_{i=1}^S P_i^2$$

Where, $P_i = \frac{n_i}{N}$; n_i , relative abundance of the species calculated as the proportion of individuals of a given species against the total number of individuals of a community, N . In essence, it captures the variance of the species abundance distribution. Thus, when expressed as the complement ($1-D$) or reciprocal ($1/D$) of D , the value of the measure will rise as the assemblage becomes more even⁷. So, finally the Simpson index is estimated by the following equation:

$$1 - D = 1 - \sum_{i=1}^S P_i^2$$

Shannon diversity index is commonly used to characterize species diversity in a community, accounting for both abundance and evenness of the species present²³ and has probably been the most widely used index in community ecology²⁴. The Shannon index can be calculated as follows:

$$\text{Shannon index: } H' = -\sum p_i \times \ln p_i$$

Where, p_i is the proportional abundance of the i^{th} species.

Species richness (S) is the number of species present in a community while species evenness (J') indicates the distribution of individuals within the species and it is calculated by using Pielou's index formula¹²

$$\text{Pielou's } J \text{ evenness index: } J' = \frac{H'}{H'_{\max}}$$

Where, $H'_{\max} = \ln(S)$ so, Pielou's J' evenness index

is given by:

$$J' = H' / \log(S)$$

Where, H' is the Shannon-Wiener function and S is the total number of species observed⁷.

Beta diversity: Beta diversity index is a statistic used to compare the similarity of two samples to calculate beta diversity (similarity index); a variety of similarity/dissimilarity indices were used like both qualitative (Jaccard's index) and quantitative (Sorensen's index), as well as Whittaker's (calculates the species replacement) and Complementarily index^{7, 12}. The following β diversity indices were used according to the following formula:

$$\text{Jaccard's Index } (S_J): I_J = \frac{a}{a+b+c}$$

$$\text{Sorensen's Index } (S_J): I_s = \frac{2pN}{aN+bN} \text{ or } \frac{2a}{2a+b+c}$$

The Sorensen's index (Sorensen's similarity coefficient) is a statistic used to compare the similarity of two samples. The Sorensen coefficient is mainly useful for ecological community data⁷.

$$\text{Whittaker's index: } \beta_W = \frac{S}{(2a+b+c)-1}$$

Where, a is the common species in the regions A and B; b is the number of species in the region A that do not exist in the region B; c is the number of species in the region B that do not exist in region A. This index will be equal to one, when the sum of the species of two habitats is quite similar^{7, 12}.

$$\text{Complementarily index: } C_{AB} = \frac{SA+SB-2V_{AB}}{SA+SB-V_{AB}}$$

Where, V_{AB} represents the number of common species to both sites A and B^{12, 25}.

Rarefaction standardization method

During rarefaction, the information provided by all of the species that were collected is used to estimate the richness of a smaller sample and species richness of two assemblages with different abundance pattern⁷. Rarefaction curves are used to assess species richness from the results of sampling. On the left, the steep slope indicates that a large fraction of the species diversity remains to be discovered. If the curve becomes flatter to the right, a reasonable number of individual samples have been taken and more intensive sampling is likely to yield only few additional species⁷. Thus, rarefaction generates and calculates the expected number of species and allows mean-

ingful standardization and comparison of samples data sets that differ in terms of individual size or plot size²⁶⁻²⁷. The expected number of species $E(S_n)$ in a sample of size n and the variance $V(S_n)$ are then given by²⁸.

$$E(S_n) = \sum_{i=1}^S [1 - \frac{\binom{N-N_i}{n}}{\binom{N}{n}}]$$

Where, N is the total number of individuals in the sample, s the total number of species, and N_i the number of individuals of species number i .

Finally, to calculate the ecological distance between different environments, a cluster (based on Jaccard's index) was made, offering the cophenetic correlation value for the Jaccard cluster to calculate the degree of reliability of the classification system used. PAST-V.3 software (Paleontological Statistics Software Package) was used to carry out all calculations developed^{25, 29}.

RESULTS

Faunistic and systematic results

A total of 5,270 mosquito larvae and adults were collected from sampling points. The systematic study showed a total of 14 species belonging to four different genera. Evaluation of dominance structure of species composition by use of Heydemann's classification showed that Culicid mosquitoes of Neka rangelands include one eudominant species, one dominant species, two subdominant species, two rare species and eight subrare species (Table 1). Also the complete catalogue of species collected in each area and its species composition is listed in Table 1. Study on seasonal activity of *Culex pipiens* Linnaeus, 1758; as eudominant species in each area showed that the first appearance of the species was in April in all of areas (Darvishan, Chalmardi and Khairabad) with an increasing trend continued in the next months, as the populations reached their peaks in June, July and August in Khairabad, Chalmardi and Darvishan, respectively. Afterwards, the state of appearance declined and continued till December (Fig. 1).

Species richness and evenness

Analysis of α biodiversity indices (Table 2) shows that Khairabad environment is the most diverse ($S = 13$; $D_{Mg} = 1.51$), while Chalmardi is the least diverse ($S = 9$; $D_{Mg} = 1.12$). Simpson and Shannon indices highlights that in Darvishan ($\lambda = 0.665$; $H' = 1.379$) and Chalmardi ($\lambda = 0.665$; $H' = 1.379$) *Cx. pipiens* (85.1 and 88.2%, respectively) strongly dominate the rest of the species present

Table 1. Number of specimens captured for each environmental category and total area. Abundance and dominance structure of culicid mosquito

Taxa	Darvishan	Relative abundance	Dominance structure	Chalmardi	Relative abundance	Dominance structure	Khairabad	Relative abundance	Dominance structure	Total area	Relative abundance	Dominance structure
<i>Anopheles</i>												
<i>An. claviger</i>	6	0.48	Subrare	14	1.1	Rare	6	0.22	Subrare	26	0.49	Subrare
<i>An. hyrcanus</i>	5	0.40	Subrare	2	0.16	Subrare	7	0.26	Subrare	14	0.27	Subrare
<i>An. maculipennis</i>	61	4.84	Rare	36	2.8	Rare	28	1.02	Rare	105	2	Rare
<i>An. plumbeus</i>	3	0.24	Subrare	0	0	0	333	12.2	Dominant	336	6.4	Subdominant
<i>An. pseudopictus</i>	1	0.08	Subrare	3	0.24	Subrare	15	0.55	Subrare	19	0.36	Subrare
<i>An. superpictus</i>	0	0	0	1	0.08	Subrare	0	0	0	1	0.02	Subrare
<i>Culex</i>												
<i>Cx. mimeticus</i>	0	0	0	1	0.08	Subrare	2	0.07	Subrare	3	0.06	Subrare
<i>Cx. pipiens</i>	1072	85.1	Eudominant	1123	88.2	Eudominant	1411	51.55	Eudominant	3606	68.43	Eudominant
<i>Cx. theileri</i>	0	0	0	0	0	0	3	0.11	Subrare	3	0.06	Subrare
<i>Cx. tritaeniorhynchus</i>	1	0.08	Subrare	0	0	0	23	0.84	Subrare	24	0.46	Subrare
<i>Culiseta</i>												
<i>Cu. annulata</i>	7	0.56	Subrare	50	3.9	Rare	17	0.62	Subrare	74	1.40	Rare
<i>Cu. longiareolata</i>	102	8.1	Subdominant	43	3.4	Rare	408	14.9	Dominant	553	10.50	Dominant
<i>Ochlerotatus</i>												
<i>Oc. geniculatus</i>	0	0	0	0	0	0	483	17.64	Dominant	483	9.2	Subdominant
<i>Oc. spulcritarsis</i>	2	0.16	Subrare	0	0	0	1	0.04	Subrare	3	0.06	Subrare
Total count	1260	24%		1273	24%		2737	53%		5270		

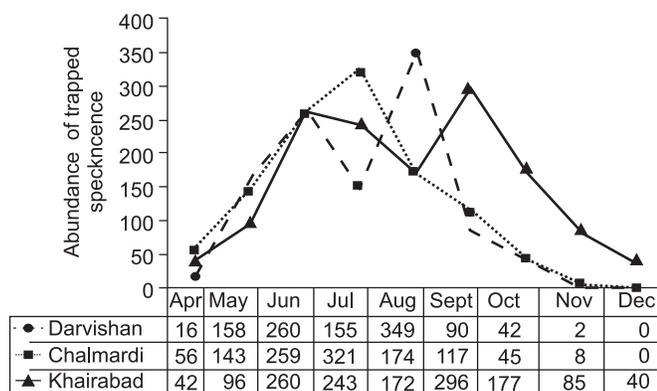


Fig. 1: Monthly dynamics of the eudominant taxon of *Cx. pipiens*

Table 2. Alpha biodiversity estimates for each environmental category

Alpha diversity indices	Darvishan	Chalmardi	Khairabad
<i>Larva</i>			
Abundance	1143	1106	2546
Specific richness (S)	6	7	8
Margalef index (D_{Mg})	0.71	0.86	0.9
Simpson index (λ)	0.217	0.198	0.656
Shannon index (H')	0.471	0.474	1.268
Evenness of Pielou index (J')	0.267	0.229	0.444
<i>Adult</i>			
Abundance	117	167	191
Specific richness (S)	7	4	10
Margalef index (D_{Mg})	1.26	0.586	1.752
Simpson index (λ)	0.56	0.319	0.674
Shannon index (H')	1.021	0.591	1.535
Evenness of Pielou index (J')	0.396	0.451	0.464
<i>Larva+Adult</i>			
Abundance	1260	1273	2737
Specific richness (S)	10	9	13
Margalef index (D_{Mg})	1.26	1.12	1.516
Simpson index (λ)	0.27	0.218	0.665
Shannon index (H')	0.599	0.538	1.379
Evenness of Pielou index (J')	0.182	0.190	0.305

in the community, whereas in the area of Khairabad ($\lambda = 0.665$; $H' = 1.379$), *Cx. pipiens* (51.55%), *Oc. geniculatus* (17.64%), *Cs. longiareolata* (14.9%) and *An. plumbeus* (12.27%) develop a strong influence. Khairabad environment is the area of Neka township where a greater degree of evenness can be observed, because the most dominant species do not show such a strong influence as in the two other areas.

The results of diversity survey of each site and the whole study area by using the three indices, Shannon, Dominance and Abundance, species showed that when species emergence started in April, Shannon index and species abundance were low, but reached to the highest value in September in Darvishan (0.52) and in October in Chalmardi and Khairabad (0.8 and 1.34, respectively) and species evenness was the lowest in August in all areas.

These values were the highest levels of the index during appearance of culicid mosquitoes and were related inversely to species dominance that reached its lowest value. Therefore, Khairabad had the most diversity compared to the rest of areas (Fig. 2).

Similarity and dissimilarity analysis

The analysis of β biodiversity (Table 3), indicates that Darvishan and Khairabad are the closest categories in their specific composition (larvae: $I_j = 0.75$; $I_{Squant} = 0.86$; adult: $I_j = 0.70$; $I_{Squant} = 0.82$; larvae+adult: $I_j = 0.77$; $I_{Squant} = 0.87$) and showing the lower replacement degree (larvae: $\beta_W = 0.14$; adult: $\beta_W = 0.18$; larvae+adult: $\beta_W = 0.22$) between pairs analyzed; observations also supported by the complementarily index ($C_{Darvishan-Khairabad} = 25, 30$ and 23% for larva, adult and larva+adult, respectively).

With the aim of representing the information provided by the Jaccard's index, a cluster analysis based on Jaccard's distance was carried out (Fig. 3). The high value of Jaccard distance cophenetic correlation ($r_c = 0.9986$) indicates a high correlation level between the ecological distance observed in the study and the distance predicted by the hierarchical configuration of the cluster.

Rarefaction analysis

Rarefaction curves were obtained with the aim of observing the asymptotic trends of the number of species and an evaluation of the similarity in the rural areas (Fig. 4). The rarefaction curves obtained gave an indication of the stability of the number of species in each sample. In Chalmardi, the curve has a tendency to stabilize with the number of nine species, but in Khairabad an increase in the sampling effort causes the number of species in the curve to increase, without an observable limit.

DISCUSSION

This is the study on the biodiversity components of culicidae mosquitoes in north of Iran. The abundance of species and each temporal sample has shown the largest number of specimens in the collections made in August, July and October in Darvishan, Chalmardi and Khairabad, respectively. Based on the Shannon index the temporal samples of November had the highest diversity in Darvishan and October had the highest diversity in Chalmardi and Khairabad. During these times dominance had the lowest levels. These observations can be explained according to the bioclimatic characteristics of each area. The Khairabad record varieties of environmental conditions that enable mosquitoes to colonize compared to the other categories of Darvishan and Chalmardi. For ex-

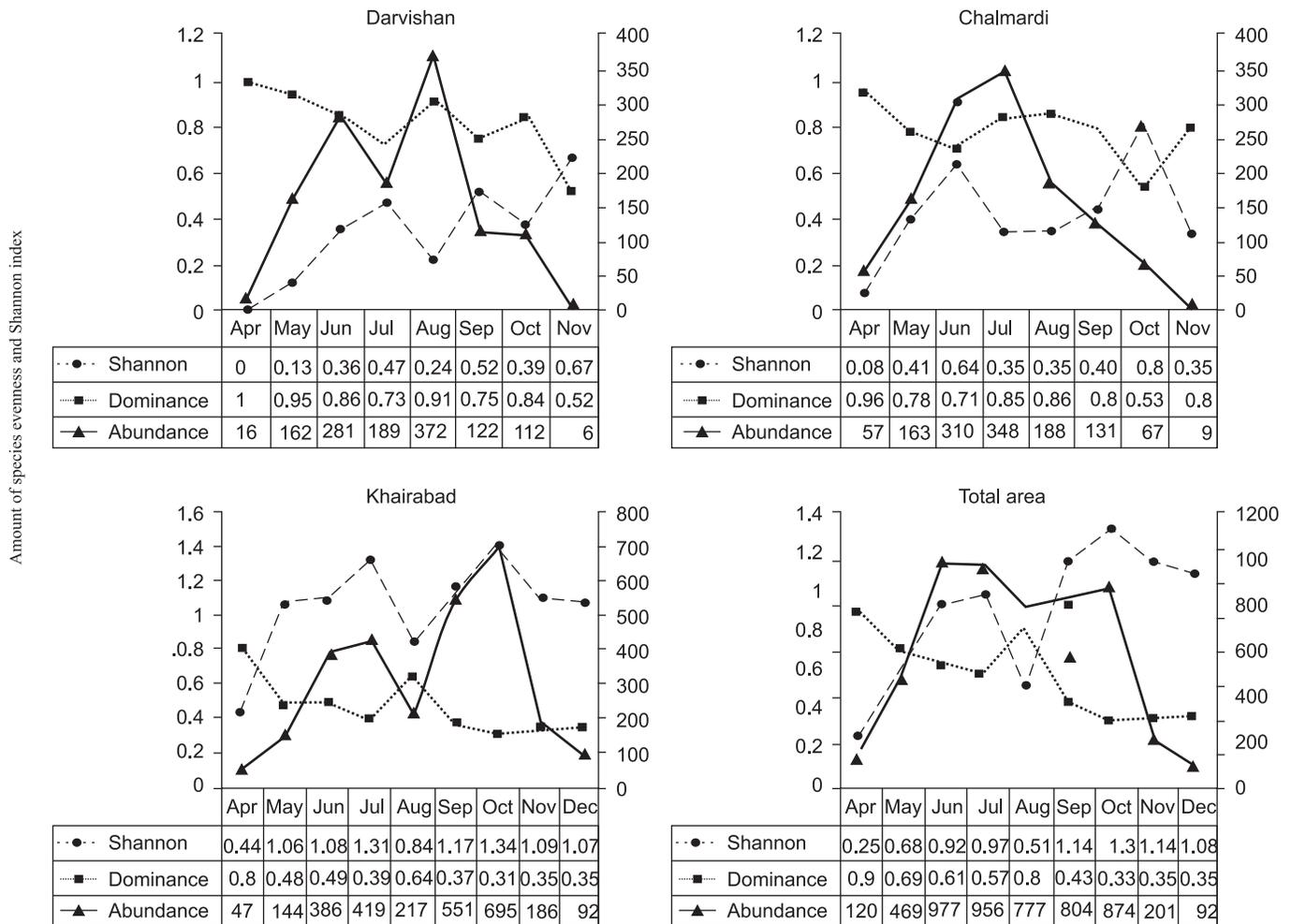


Fig. 2: Evaluation of diversity of culicid mosquito species using three indices (species Evenness, Shannon index and species abundance).

ample, the existence of holes in tree trunks as aquatic habitats for larvae means that a greater amount of larval

biotopes are available to be exploited by different species of culicids throughout the year in Khairabad, that may be an important reason for increase in diversity. This factor, in combination with severe drought during the summer months, determines a population dynamics fea-

Table 3. Beta biodiversity estimates for each environmental category

Beta diversity indices	Darvishan-Chalmardi	Darvishan-Khairabad	Chalmardi-Khairabad
Larva			
Jaccard index (I_j)	0.63	0.75	0.67
Sorensen index (I_{Squant})	0.77	0.86	0.80
Whittaker index (β_w)	0.23	0.14	0.20
Complementarity (C_{AB})	38%	25%	40%
Adult			
Jaccard index (I_j)	0.57	0.70	0.40
Sorensen index (I_{Squant})	0.72	0.82	0.57
Whittaker index (β_w)	0.27	0.18	0.48
Complementarity (C_{AB})	43%	30%	60%
Larva+Adult			
Jaccard index (I_j)	0.58	0.77	0.57
Sorensen index (I_{Squant})	0.74	0.87	0.73
Whittaker index (β_w)	0.47	0.22	0.27
Complementarity (C_{AB})	42%	23%	43%

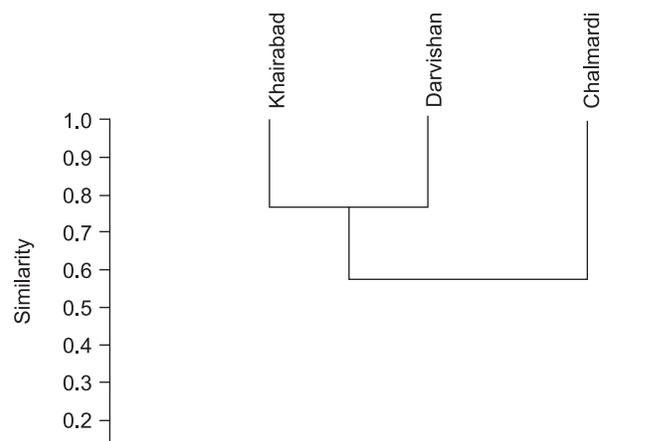


Fig. 3: Cluster analysis based on Jaccard's distance; cophenetic correlation, $r_c = 0.9975$.

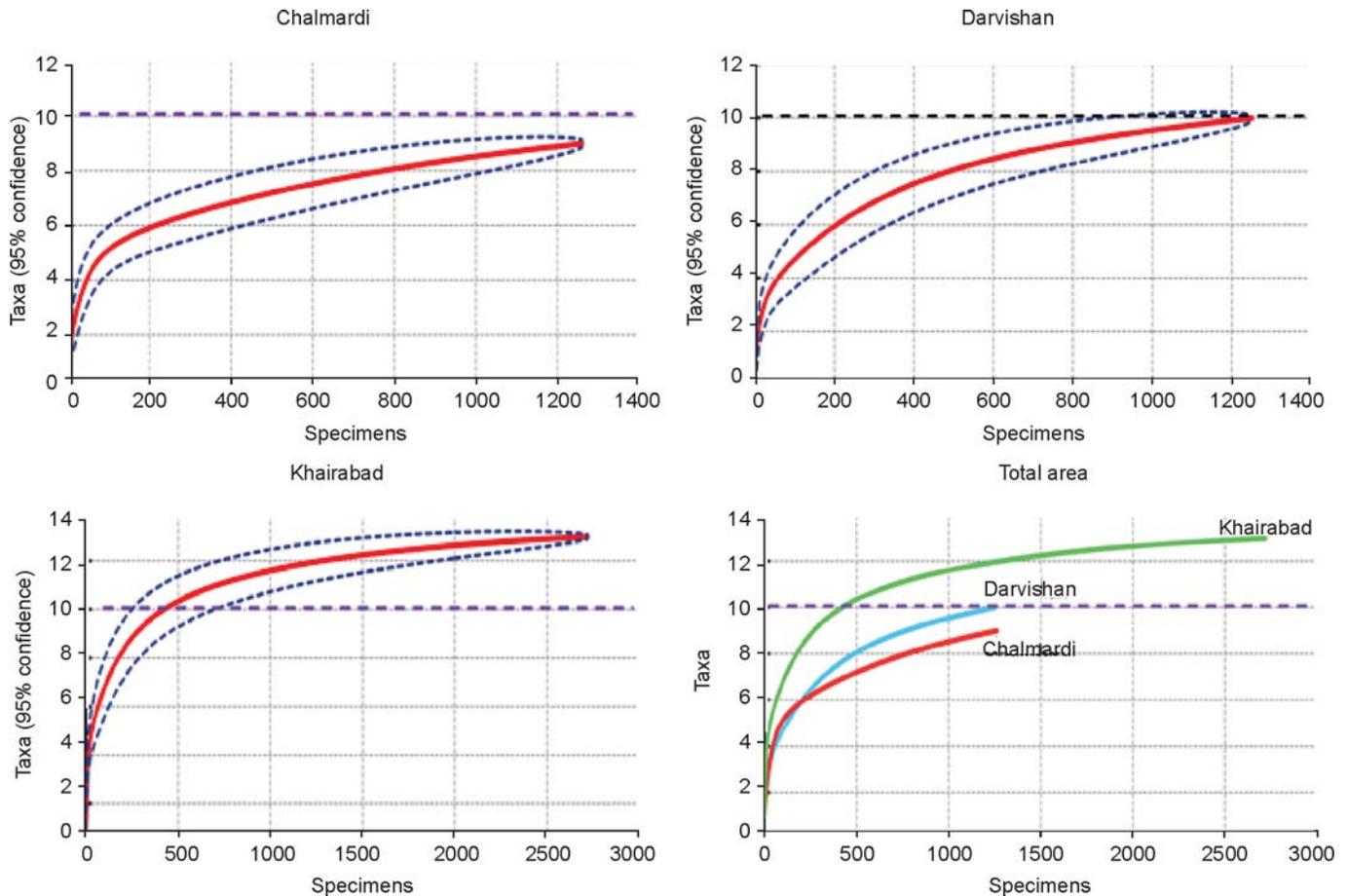


Fig. 4: Rarefaction figures of the result of the culicid samples. The figure also shows the 95% confidence limits for the species locality. This confirms that, for equivalent N , the Khairabad is the richest area.

ture which is reflected in the diversity observed in this category. Also environmental changes such as reduced rainfall may allow more tolerant species to thrive and gain dominance. For example, in August, because of low precipitation, many of these natural breeding sites may dry. Generally in rural areas, the breeding places are pools, streambeds and irrigation canals at the margin of streams, rivers and treeholes. *Culex pipiens* was the most frequent and dominant species in all areas. *Cx. pipiens* is one of important vector for arthropod borne viral infections affecting the health of humans, domestic and wild animals and transmit diseases like West Nile fever, St. Louis encephalitis, Japanese encephalitis, Western equine encephalitis, and Rift Valley fever³⁰⁻³². In general, *Cx. pipiens* comprised a total of 85, 88 and 51% of the specimens collected at Darvishan, Chalmardi and Khairabad, respectively, but when its abundance and dominance was decreased in October and November; Shannon indices have indicated a greater diversity (Fig. 2). Although, it should be noted that low abundance of *Cx. pipiens* in Khairabad led to decrease of dominance and on the other hand evenness index was increased.

The measures of diversity and richness revealed different results when the data of different months were considered. The Shannon index indicated a minimum diversity in all regions in August while in the months before and after August, Shannon index was bigger. This trend caused by declined rainfall led to drying of mosquito larval habitats (Fig. 2). According to Margalef index and species richness, Khairabad shows the highest diversity observed in Neka; probably there are various factors that could be taken into account such as landscape heterogeneity and larval biotopes diversity that in turn help their colonization and increase the fitness of the suitable host on which they feed. For example, in Khairabad more treeholes containing water suitable for mosquito breeding were found than in two other villages, a phenomenon that strongly influences the abundance and biodiversity indices and therefore, the species richness and density of mosquitoes in this village. Lower levels of diversity in Darvishan and Chalmardi can then be related to decrease in turnover rate and retention of water and long dry season¹. In other words, a lower level of replacement and water permanence are ecological reasons for lesser

biodiversity in different areas²⁵. This supports the presence of heavily adapted species and led to an increase in Dominance and decrease in Evenness of species in Darvishan and Chalmardi compared to Khairabad (Fig. 5).

Rarefaction curves are necessary for estimating species richness. Raw species richness counts, which are used to create accumulation curves, can only be compared when the species richness has reached a clear asymptote. Rarefaction curves produce smoother lines that facilitate point-to-point or full dataset comparisons²⁴. The purpose of rarefaction is to make direct comparisons amongst species on the basis of number of individuals in the smallest sample. This permits comparisons amongst species where sampling effort has been unequal and it is a technique that reduces sample data to a common abundance level (typically the same number of individuals) so that direct comparisons of the species richness of an area can be made. The comparison of species richness by rarefaction curves in Fig. 4 illustrated that species richness in Khairabad is higher than to other areas leading to sampling of more species at low sampling intensity in this rural area. This confirms that the mosquito community in Khairabad is richer.

As measured by the Jaccard's index, similarity was found to be high between Darvishan and Khairabad. In accord with the fact that there is an inverse relationship between insect species diversity and distribution, and altitude above sea level; wider distribution and higher diversity and probability of finding similar species were observed in Darvishan and Khairabad, where altitude above the sea level is the lowest^{9,33}. It should be noted that the rain fed and irrigated cultivation in Darvishan and Khairabad (220 and 230 ha, respectively) is more than that in Chalmardi (65 ha) and consequently such similar cultivations could result in similarity of species composition between the former areas. In other words, Darvishan and Khairabad had more rural texture (10,000 and 5,000 ha respectively) than Chalmardi (2,000 ha) and it seems to increase the likelihood of having similar habitats in Darvishan and Khairabad (Fig. 3).

Several studies suggest that the lower the biodiversity, the higher the potential of transmission of diseases⁶. The decline of biodiversity might lead to a faster rate of emergence and re-emergence of infectious diseases and, therefore, the infection of a greater proportion of the human population^{5, 34-35}. In other words, there are links between high biodiversity and reduced risk of vector-borne dis-

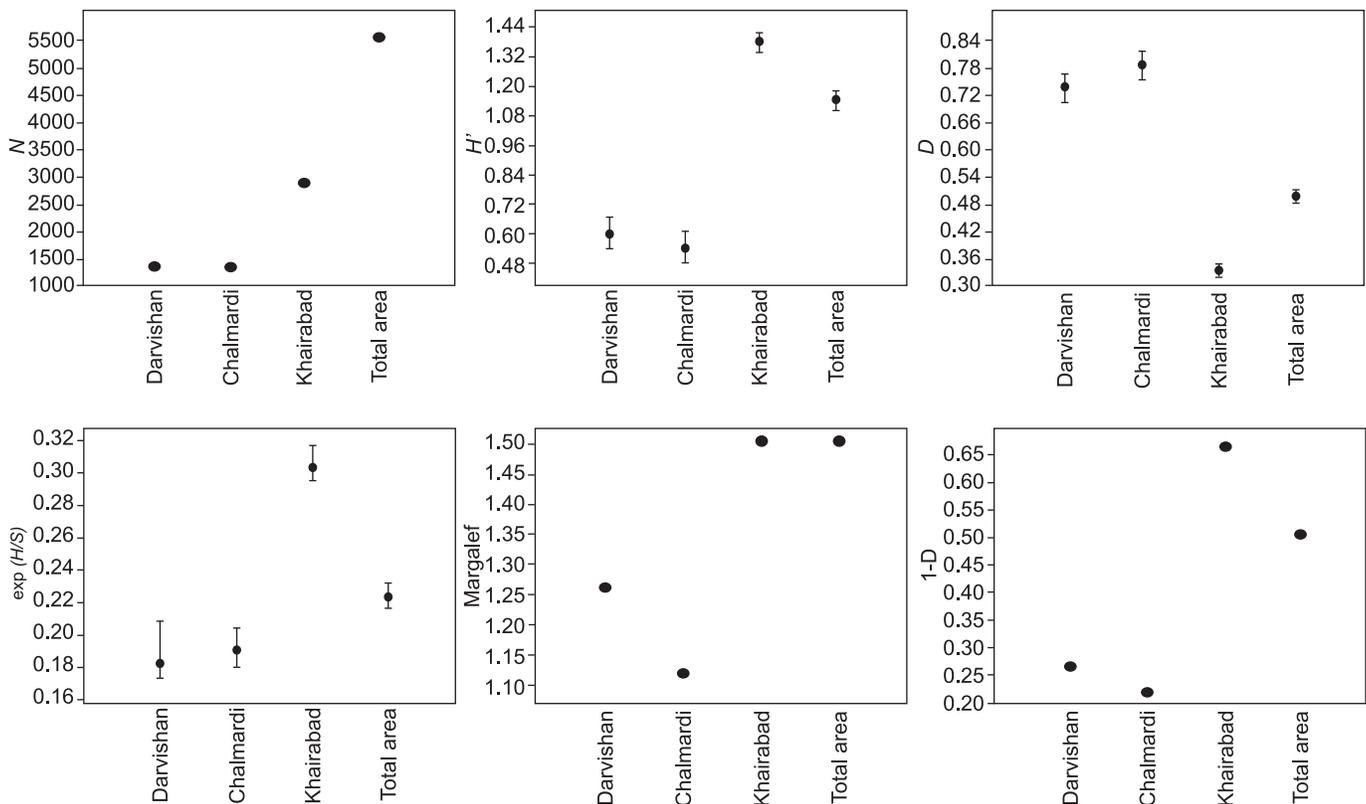


Fig. 5: Biodiversity indices [Abundance (N), Shannon (H'), Dominance (D), Evenness ($\exp(H/S)$), Margalef, and Simpson ($1-D$)] comparison between Darvishan, Chalmardi, Khairabad and total area (Darvishan + Chalmardi + Khairabad).

eases³⁶. Studies in the past few years showed an inverse relationship between the species richness and the increased risk of infections³⁷. Although, most of these studies did not include mosquito species, however, there are evidence that diversity, in the form of species richness can play an important role in determining diseases risk to humans (Dilution Effect model)³⁸⁻⁴⁰.

Mazandaran province with average annual rainfall of about 1000 mm has an environment perfectly fit for high biodiversity especially for arthropods and mosquitoes in particular. Although, malaria and other vector-borne diseases have a long history in this province, thanks to previous intensive malaria control programs, as only imported cases of the disease are reported. However, since mosquitoes' population is high, mosquito vigilance should be practiced, in coordination with Iranian and provincial centers for diseases control to maintain their success in managing vector-borne diseases. On the other hand, although no disease has been reported to be transmitted by *Cx. pipiens* in Mazandaran, this species is known as a biting nuisance. Also, the extent growth of economic activity, tourism, and human migration in Mazandaran province, northern Iran, can lead to even more cases of the movement of both disease vectors and the pathogens they carry, increasing the biodiversity of mosquitoes around northern Iran. Moreover, considering the increasing distribution of emerging diseases such as dengue fever in countries with common border with Iran, it is important to study the relationships between spatial and temporal changes of vector population composition and biodiversity to avert the risks of diseases⁴¹. Since, the changes in pathogens infection rates in *Culex* females is strongly associated with temporal or spatial changes of *Culex* population peak, the fact that *Cx. pipiens* is the dominant and most prevalent species, has potential human health implications in our study region⁴²⁻⁴³.

CONCLUSION

This study compared the species richness of vectors in different sites in three rural regions in Neka township, northern Iran. The notable dominance of one single species (*Cx. pipiens*, a carrier of viral infections) has certainly influenced the estimation of biodiversity parameters and the largest mosquito species diversity was found in Khairabad. In this area, a higher diversity of Culicidae with a lesser degree of dominance and a greater intraspecific evenness and Shannon index were determined. Darvishan and Chalmardi, on the other hand, represent a less diverse and uniform communities with a greater degree of interspecific dominance (Fig. 5). These commu-

nities are composed of a few abundant and a high number of rare species, establishing a clear relationship between Culicidae abundance and the prevailing climatic conditions. Considering the relationship between richness and diversity of vector populations and the risk of human infections; it is necessary to determine the structure of mosquito communities and its relationship to the risk of infectious diseases transmission in specific ecosystems. Therefore, it is recommended that further studies should be undertaken to assess the association between biodiversity of vectors and the risk of diseases transmission to humans.

ACKNOWLEDGEMENTS

The authors would like to sincerely thank Tehran University of Medical Sciences and Mazandaran University of Medical Sciences for financially supporting the projects of sampling mosquitoes and ecological analyses of the data. We also would like to extend our gratitude to anonymous field workers for their assistance in mosquito sampling.

REFERENCES

1. Becker N, Petric D, Zgomba M, Boase C, Dahl C, Madon N, *et al.* *Mosquitoes and their control*. II edn. Berlin, Germany: Springer Verlag 2010; p. 608.
2. Harbach RE. The Culicidae (Diptera): A review of taxonomy, classification and phylogeny. *Zootaxa* 2007; 1668: 591-638.
3. García-Rivera EJ, Rigau-Pérez JG. Encephalitis and dengue. *Lancet* 2002; 360(9328): 261.
4. Kofler RM, Hoenninger VM, Thurner C, Mandl CW. Functional analysis of the tick-borne encephalitis virus cyclization elements indicates major differences between mosquito-borne and tick-borne flaviviruses. *J Virol* 2006; 80(8): 4099-113.
5. Rueda LM. Global diversity of mosquitoes (Insecta: Diptera: Culicidae) in freshwater. *Freshwater Animal Diversity Assessment. Hydrobiologia* 2008; 595: 477-87. doi: 10.1007/s10750-007-9037-x.
6. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, *et al.* Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 2010; 468 (7324): 647-52.
7. Magurran AE. *Measuring biological diversity*. Oxford: Blackwell Publishing 2004; p. 264.
8. Whittaker RH. Evolution and measurement of species diversity. *Taxon* 1972; 21(2/3): 213-51.
9. Rohde K. Latitudinal gradients in species diversity: The search for the primary cause. *Oikos* 1992; 65(3): 514-27.
10. Keesing F, Holt RD, Ostfeld RS. Effects of species diversity on disease risk. *Ecol Lett* 2006; 9(4): 485-98.
11. Enayati A, Hemingway J. Malaria management: Past, present, and future. *Ann Rev Entomol* 2010; 55: 569-91.
12. Magurran AE. *Ecological diversity and its measurement*. London, UK: Croom Helm 1988; p. 179.
13. Piovezan R, Rosa SL, Rocha ML, de Azevedo TS, Von Zuben

- CJ. Entomological surveillance, spatial distribution, and diversity of Culicidae (Diptera) immatures in a rural area of the Atlantic Forest biome, State of São Paulo, Brazil. *J Vector Ecol* 2013; 38(2): 317–25.
14. Halffter G. A strategy for measuring landscape biodiversity. *Biol International* 1998; 38: 3–17.
 15. Colwell RK, Coddington JA. Estimating terrestrial biodiversity through extrapolation. *Philos Trans R Soc Lon Biol Sci* 1994; 345(1311): 101–18.
 16. Hanafi-Bojd A, Vatandoost H, Oshaghi M, Charrahy Z, Haghdoost AA, Sedaghat MM, *et al.* Larval habitats and biodiversity of anopheline mosquitoes (Diptera: Culicidae) in a malarious area of southern Iran. *J Vector Borne Dis* 2012; 49(2): 91–100.
 17. Scott TW, Harrington LC, Knols BG, Takken W. Applications of mosquito ecology for successful insect transgenesis-based disease prevention programs. New York: Landes Bioscience and Springer Science+Business Media 2008; p. 151–68.
 18. Shayeghi M, Vatandoost H, Gorouhi A, Sanei-Dehkordi AR, Salim-Abadi Y, Karami M, *et al.* Biodiversity of aquatic insects of Zayandeh Roud River and its branches, Isfahan Province, Iran. *J Arthropod Borne Dis* 2014; 8(2): 197–203.
 19. Shahgudian ER. A key to the anophelines of Iran. *Acta Med Iran* 1960; 3: 38–48.
 20. Zaim M, Cranston PS. Checklist and keys to the culicinae of Iran (Diptera: Culicidae). *Mosq Syst* 1986; 18: 233–45.
 21. Azari-Hamidian S, Harbach RE. Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa* 2009; 2078: 1–33.
 22. Weigmann G. Zur Ökologie der Collembolen und Oribatiden im Grenzbereich Land-Meer: (Collembola, Insecta-Oribatei, Acari): *Zeitschrift für wissenschaftliche Zoologie* 1973, 4: 295–391
 23. Shannon CE, Weaver W. *The mathematical theory of communication*. Urbana, IL: University of Illinois Press 1949; p. 117.
 24. Meerman J. Rapid ecological assessment Columbia River Forest Reserve Past Hurricane Iris. Report to Yaaxché Conservation Trust and Toledo Institute for Development and Environment 2004; p. 17.
 25. Bernués-Bañeres A, Jiménez-Peydró R. Diversity of mosquitoes (Diptera: Culicidae) in protected natural parks from Valencian Autonomous Region (Eastern Spain). *Biodiversity J* 2013; 4(2): 335–42.
 26. Gotelli NJ, Colwell RK. Quantifying biodiversity: Procedures and pitfalls in the measurement and comparison of species richness. *Ecol Lett* 2001; 4(4): 379–91.
 27. Koellner T, Hersperger AM, Wohlgemuth T. Rarefaction method for assessing plant species diversity on a regional scale. *Ecography* 2004; 27(4): 532–44.
 28. Newton AC. Forest ecology and conservation. *A handbook of techniques*. London: Oxford University Press 2007; p. 454.
 29. Hammer Ø, Harper D, Ryan P. Past: Paleontological statistics software package for education and data analysis. *Paleontología Electrónica* 2001; 4(1): 1–9.
 30. Kramer LD, Ebel GD. Dynamics of flavivirus infection in mosquitoes. *Advances Virus Res* 2003; 60: 187–232.
 31. Kilpatrick AM, Meola MA, Moudy RM, Kramer LD. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. *PLoS Pathogens* 2008; 4(6): 1–7.
 32. Farajollahi A, Fonseca DM, Kramer LD, Marm Kilpatrick A. “Bird biting” mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology. *Infect Genet Evol* 2011; 11(7): 1577–85.
 33. McCoy ED. The distribution of insects along elevational gradients. *Oikos* 1990; 58(3): 313–22.
 34. Peixoto ID, Abramson G. The effect of biodiversity on the hantavirus epizootic. *Ecology* 2006; 87(4): 873–9.
 35. Pongsiri MJ, Roman J, Ezenwa VO, Goldberg TL, Koren HS, Newbold SC, *et al.* Biodiversity loss affects global disease ecology. *Bioscience* 2009; 59(11): 945–54.
 36. Ezenwa VO, Godsey MS, King RJ, Guptill SC. Avian diversity and West Nile virus: Testing associations between biodiversity and infectious disease risk. *Proc R Soc Biol Sci* 2006; 273(1582): 109–17.
 37. Confalonieri UE, Costa Neto C. Diversity of mosquito vectors (Diptera: Culicidae) in Caxiuanã, Pará, Brazil. *Interdisciplin Perspect Infect Dis* 2012; 2012: 1–9.
 38. Ostfeld RS, Keesing F. Biodiversity and disease risk: The case of Lyme disease. *Conserv Biol* 2000; 14(3): 722–8.
 39. Ostfeld RS, LoGiudice K. Community disassembly, biodiversity loss, and the erosion of an ecosystem service. *Ecology* 2003; 84(6): 1421–7.
 40. LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F. The ecology of infectious disease: Effects of host diversity and community composition on Lyme disease risk. *Proc Nat Acad Sci USA* 2003; 100(2): 567–71.
 41. Rasheed SB, Boots M, Frantz AC, Butlin RK. Population structure of the mosquito *Aedes aegypti* (*Stegomyia aegypti*) in Pakistan. *Med Vet Entomol* 2013; 27(4): 430–40.
 42. Bolling BG, Barker CM, Moore CG, Pape WJ, Eisen L. Seasonal patterns for entomological measures of risk for exposure to *Culex* vectors and West Nile virus in relation to human disease cases in northeastern Colorado. *J Med Entomol* 2009; 46(6): 1519–31.
 43. Barker CM, Eldridge BF, Reisen WK. Seasonal abundance of *Culex tarsalis* and *Culex pipiens* complex mosquitoes (Diptera: Culicidae) in California. *J Med Entomol* 2010; 47(5): 759–68.

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Received: 4 May 2014

Accepted in revised form: 6 August 2014