Original Article

Population Fluctuations and Abundance Indices of Mosquitoes (Diptera: Culicidae), as the Potential Bridge Vectors of Pathogens to Humans and Animals in Mazandaran Province, Northern Iran

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Abstract

Background: Seasonal activity patterns of mosquitoes are essential as baseline knowledge to understand the transmission dynamics of vector-borne diseases. This study was conducted to evaluate the monthly dynamics of the mosquito populations and their relation to meteorological factors in Mazandaran Province, north of Iran.
Methods: Mosquito adults and larvae were collected from 16 counties of Mazandaran Province using different sampling techniques, once a month from May to December 2014. "Index of Species Abundance" (ISA) along with "Standardized ISA" (SISA) was used for assessing the most abundant species of mosquitoes based on the explanations of Robert and Hsi. Pearson's correlation coefficient (R) was used to assess the relationships between the monthly population fluctuations and meteorological variables.
Results: Overall, 23750 mosquitoes belonging to four genera and nineteen species were collected and identified. The highest population density of mosquitoes was in July and the lowest in May. The ISA/SISA indices for *Culex pipiens* were both 1 for larvae and 1.25/0.973 for adults in total catch performed in human dwellings. For *Cx. tritaeniorhynchus*, the ISA/SISA were 1.68/0.938 in pit shelter method. A significant positive correlation was observed between population fluctuations of *Cx. tritaeniorhynchus* and mean temperature (R: 0.766, P< 0.027).
Conclusion: The results indicated that the mosquitoes are more active in July, and *Cx. pipiens* and *Cx. tritaeniorhynchus* were the most abundant species. Considering the potential of these species as vectors of numerous pathogens, control programs can be planed based on their monthly activity pattern in the area.

Keywords: Seasonal activity; Mosquitoes; Abundance indices; Mazandaran; Caspian Sea littoral

Introduction

Mosquitoes are distributed almost all over the world, except a few islands and the Antarctica (1). Since the earliest times, mosquito bites and habitats have been related with human diseases, and mosquitoes were the first arthropods formally convicted as intermediate hosts of vertebrate parasites in 1878 (2). They are the most important arthropod taxon in medical entomology because of their nuisance and transmission of malaria, arboviral diseases and microfilariae (3). Mosquito-borne infectious diseases are known as the most commonly transmitted diseases by vectors in terms of mortality and disability-adjusted life years (4). Malaria is one of the most important *Anopheles*-borne parasitic diseases in the world. The disease has a massive burden universally, an estimated number of cases of 229 million and 409000 deaths occurred in 2019 (5). There was also approximately 96 million cases and 1091 deaths related to dengue globally. Albeit, the global burden of Zika, chikungunya and West

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Nile is not as large as malaria and dengue (4), but their impact on health system is high, especially as several large outbreaks of the diseases occur every year and their transmission is extending to new areas (6-8). Iran is in the perspective of eliminating malaria by 2025. Although there were 1105 imported cases of malaria in 2019, no indigenous malaria cases were reported in Iran since 2018 (5). Thirty species of *Anopheles* have been documented from Iran (9), of which, seven species have been reported from Mazandaran Province (10). *Anopheles sacharovi*, *An. maculipennis* s.l., *An. fluviatilis* s.l., *An. stephensi*, *An. superpictus* s.l., *An. dthali*, and *An. culicifacies* s.l. are identified as the proven malaria vectors in Iran (11), while *An. pulcherrimus* is stated as a suspected vector (12). Recently, *An. hyrcanus* and *An. subpictus* s.l. were revealed to be infected with *Plasmodium* based on molecular analysis in northern and southern Iran (13, 14). *Anopheles maculipennis* s.l. and *An. sacharovi* are known to play an important role in transmission of malaria in the northern parts of the country (15).

Zika, dengue and chikungunya are arboviral diseases transmitted by *Aedes* mosquitoes, especially *Ae. aegypti* and *Ae. albopictus*. There are no reports of Zika virus in Iran, while imported cases of dengue (16-18) and chikungunya (19) have lately been reported from the country. *Aedes aegypti* and *Ae. albopictus* are the main vectors of dengue, chikungunya and Zika, worldwide. *Aedes aegypti* had been reported in southern parts of Iran in 1920–1953 (20-22), however, it retreated to Arabian Peninsula and northern Africa since for no known reason. Recently, only a few adults and larvae of *Ae. albopictus* has been observed in Sistan and Baluchistan Province, southeastern Iran (23).

Knowledge about the behavior of mosquitoes is very important in the epidemiology of disease transmission and vector control. Data on fluctuations of seasonal abundance of species can describe their relative risk in the transmission of diseases in human populations, and can also help in the planning and implementation of proper control programs (24). Environmental changes greatly affect the habitat of mosquito species (25). Meteorological factors also affect the population of mosquitoes by quantitative and qualitative changes on the larval habitats (25, 26). The combination of mosquito behavior pattern (circadian rhythmicity) with climate factors makes a foundation for determining the timing/month/season of mosquito activities (27, 28). Therefore, determining the seasonal prevalence of mosquito fauna in an area is crucial for the development of effective vector control programs and updating of ecological information related to vectors of diseases in the area (25, 29).

There were little published data on the seasonal abundance of mosquitoes in Iran (30-32). Up to now, fauna, checklist, physicochemical factors of larval habitats, co-occurrence, association, affinity and biodiversity of mosquitoes have been studied in Mazandaran Province (10, 33-35), however, there is no recent comprehensive study on the seasonal activity of mosquitoes in the province. Therefore, this study was conducted to evaluate the monthly dynamics of the mosquito populations and their relation to climatic factors in Mazandaran Province.

Materials and Methods

Study area

Mazandaran Province is located on the coast of the Caspian Sea in northern Iran, in coordinate latitude 35°47′–36°35′N and longitude 50°34′–54°10′E between the Caspian Sea and the Alborz mountain range. The province is enclosed by Golestan Province in the east, Guilan Province in the west and Tehran and Semnan Provinces in the south and the Caspian Sea to the North. The diverse nature of the province included plains, grassland, forests and rainforest with an area of approximately 23,842 square kilometers and a population of approximately 3,073,943. In the study area, the main agricultural products are rice, followed by wheat, barley, beans, fruits and vegetables. Vast Hyrcanian forests, temperate climate, abundant wetlands and rice fields in the province, provide enormous adequate habitats for the development of mosquitoes.

Specimen and data collection

Mosquitoes were collected from 30 villages in 16 counties from Mazandaran Province, in a uniform method, once a month from May to December 2014. In each county, two villages were randomly designated for sampling mosquitoes according to topography of areas. In each village, one fixed habitat was selected for collection of larvae. Larval collection was conducted by a standard 350ml dipper for 15–20min per natural and artificial breeding sites in fixed habitats. In the larval habitat, 10–30 dips were taken based on the size of the breeding site. Samples were always taken by the same individual in the morning (0800–1200 hours) or afternoon (1400–1800 hours).

Adult mosquitoes were collected in eight places (three human and three animal fixed and one human and one animal variable places) between 0500 and 0800 hours in selected villages by methods of total catch. Before spraying, all the eaves, windows, doors and other exit points were closed and white cloth sheets were spread on the floor. After Pyrethrum spraying, the room was kept closed for 10min and the knocked-down mosquitoes were then collected from the floor sheet. Pit shelters (90× 150× 150cm W× L× D) were dug in shady places in each village, then small cavities, about 30cm deep were dug horizontally at each side, about 50cm above the bottom of the pit. The collections of resting mosquitoes were carried out by aspirator from the walls of the pits between 0500 to 0600 hours. CDC light traps (John W. Hock Company, Gainesville, FL) were set about 1.5–2m above the ground in human and animal dwellings in each village. Light traps were switched on at 1900 and switched off at 0600 hours local time. The mosquitoes were attracted to the light at night, and were arrived to funnel nets screen using the airflow generated by the fan motor. Human-landing collections (two human baits and one collector) using mouth aspirators were performed for sampling of *Aedes*, during the daytime between 0900 and 2100 hours, in each village. Sampling teams were stationed mostly between human and animal sites near appropriate larval habitats. The mosquitoes were collected at 1h intervals and were placed in paper cups relevant to the same hour. The third and fourth instar larvae were preserved in lactophenol solution and adults were transferred to cups container with moist cotton, labeled and mounted (10). The specimens were identified by direct observation of morphological characters using valid taxonomic keys (36). The specimens are deposited at the Museum of Medical Entomology, School of Health, Mazandaran University of Medical Sciences, Sari, Iran. The abbreviations of mosquito genera and subgenera are cited by Reinert (37).

Meteorological data

Monthly meteorological variables containing mean temperature and rainfall were obtained from the synoptic stations of Mazandaran Province Meteorological Organization in 2014.

Abundance Indices

"Index of Species Abundance" (ISA) was used to assess the most abundant species of mosquitoes in the province based on the explanations of Robert and Hsi (38) with a minor modification in the formula. ISA is calculated by the following formula:

$$ISA = \frac{(a \times c) + Rj}{K}$$

Where 'a' is the number of sampling sites that the species not present in it, 'c' is the highest rank of the species in sampling sites plus 1, and 'Rj' is the sum of the rankings of each species in all sampling sites, 'K' is the number of sampling sites.

"Standardized Index of Species Abundance" (SISA) was applied as a standardized formula for ISA by converting to a scale from zero to one with the following changes:

$$\mathrm{SISA} = \frac{c - \mathrm{ISA}}{c - 1}$$

When SISA is closer to 1, it represents the most abundant species (39).

Statistical analysis

Statistical analysis was performed on data obtained from mosquito population density during the study to understand whether meteorological variables could be the cause of population fluctuations of mosquitoes in the study area. The data were calculated using SPSS Ver. 23. The relationships between monthly population fluctuations of mosquitoes with meteorological variables were evaluated by statistical test of "Pearson's correlation coefficient (R)".

Results

Overall, 23,750 mosquitoes (7,566 larvae and 16,184 adults) belonging to two subfamilies, four genera and nineteen species were collected and identified throughout the year during this study. The subfamily Anophelinae was represented by one genus and 7 species, while the subfamily Culicinae was in 3 genera and 12 species. Monthly fluctuations in population dynamic of mosquitoes are displayed in (Fig. 1, Table 1 and 2). The highest total number of larvae (2,383) and adult (4,723) mosquitoes were observed in July and the lowest in May (Fig. 1). Among the larvae, *An. maculipennis* s.l. and *An. pseudopictus* were collected during each month except May and December, respectively, while *An. hyrcanus* was observed only during June–October. The population density of *An. maculipennis* s.l. reached its peak in June (n= 238). After June, the population density of the species with an irregular fluctuation decreased rapidly and the lowest density was in September (n= 5). The highest population peak of *An. hyrcanus* was quite similar to that of *An. maculipennis* s.l, while it was in July for *An. pseudopictus*. *Culex pipiens* and *Cx. torrentium* was found from May to December. The largest population density of this species was observed in July (n= 1670) and June (n= 287) respectively. *Culex tritaeniorhynchus* was collected from May to November, with a major peak in July. *Culex perexiguus* and *Cx. mimeticus* were almost non-active in the first and last seasons of the year and had the maximum number in July. *Culiseta annulata* and *Cs. longiareolata* were almost absent during the warm seasons of the year and their highest activity peaks were recorded in October and November, respectively.

Anopheles marteri, *An. claviger* and *Cs. morsitans* were not found in adequate numbers to draw their monthly activity patterns (Table 1). Among the adults, *An. maculipennis* s.l. was present almost throughout the year except for December, the population dynamics of this species starts in May, reaching a major peak in June and then decreases gradually. The population density of *An. hyrcanus* begins to increase in May, reaching its greatest peak in June and after that, decreased. The species disappeared from monthly sampling in August, and was observed with the lowest population density again in September. *Anopheles pseudopictus* was recorded during June to November, its highest and lowest population peak was in July (n= 914) and November (n= 40), respectively. The density of *An. claviger* and *An. sacharovi* was peaked during August (n= 28) and July (n= 87), respectively. *Culex pipiens* was successively found throughout the monthly sampling period with major peak in July. Since July, the population of the species decreased gradually in August and September, then the activity increases and reaches the smaller peak in December. The population density of *Cx. tritaeniorhynchus* among the collected mosquitoes was high in July (n= 1868), it decreased in August (n= 1288), increased again to the smaller population peak in September (n= 1820), finally decreased progressively to the end of the season. *Culex perexiguus, Ae. vexans* and *Cs. an-*

nulata had the most activity in June, October and November in the study area. The rest of the species were collected in low numbers, so it was not possible to predict a proper pattern of monthly population fluctuations for them in the study area (Table 2).

The highest mean temperature and rainfall was observed during the months of August and October, respectively (Fig. 2). Bivariate Pearson's correlation analyses of mosquito population density with meteorological variables exhibited that the population fluctuations of *Cx. territans* (R= 0.855, P= 0.007), *Cs. annulata* (R= 0.711, P= 0.0048), *Cs. longiareolata* (R= 0.826, P= 0.011) and *Ae. vexans* (R= 0.831, P= 0.011) have significant positive correlation with rainfall in the study area. However, no significant association was observed between other mosquito species and rainfall. The monthly temperature showed a significant positive correlation with the adult population fluctuations of *Cx. tritaeniorhynchus* (R= 0.766, P= 0.027). As for other species, the temperature was not seen as an important variable in population fluctuations in the study area (Table 3). The interaction between mosquito population fluctuations with mean monthly temperature and rainfall is shown in (Fig. 2). The highest number and percentage of mosquitoes were collected by total catch in animal places (n:8051, 49.74%) followed by 16.82% using light trap, 14.87% by total catch in human places, 11.15% using pit shelter and 7.42% by day biting. Among Anophelinae, *An. maculipennis* s.l. (n: 1555, 86.97%) and *An. pseudopictus* (n: 680, 52.84%) were the most common in total catch of animal places and the least in daily bites and pit shelter, respectively, while *An. claviger* and *An. sacharovi* were collected with the highest number and percentage using pit shelter sampling method. Among Culicinae, *Cx. tritaeniorhynchus* is more common than *Cx. pipiens* using total catch in animal places and light trap, whereas the species was observed with the lowest number and percentage by total catch in human places and pit shelter than *Cx. pipiens*. *Aedes vexans* were collected by all sampling methods, but the species was collected with the highest percentage up to 94.5% (n: 1200) in day biting collections. Further data on other species collected by each trap are shown in Table 4. Based on Table 5, larvae of *Cx. pipiens*, *Cx. torrentium* and *Cx. tritaeniorhynchus* showed values of SISA 1 (ISA= 1), 0.805 (ISA= 3.62) and 0.564 (ISA= 6.87), respectively, whereas it was 0.550 (ISA= 7.06) for *An. maculipennis* s.l. Among adult mosquitoes, the highest SISA (0.822, 0.637) and the lowest ISA (3.31, 5.71) were calculated for *An. pseudopictus* and *An. maculipennis* s.l. in total catch performed in animal shelters, respectively. SISA was 0.977 (ISA= 1.25), 0.946 (ISA= 1.594) and 0.933 (ISA= 1.87) in association with *Cx. pipiens* in total catch performed in human dwellings, pit shelter and total catch performed in animal shelters, respectively. It was 0.938 (ISA= 1.68) in pit shelter, 0.938 (ISA= 1.69) in light trap, 0.913 (ISA= 2.125) in total catch carried out in animal places and 0.886 (ISA= 2.25) in total catch performed in human dwellings for *Cx. tritaeniorhynchus.*

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Table 1. Monthly changes in population fluctuations of mosquito larvae in Mazandaran Province, northern Iran

	May		June		July		August		September		October		November		December		Total	
Species	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
An. claviger	0	0	0	0	0	0	15	100	0	0	0	0	0	0	0	0	15	100
An. hyrcanus	0	0	16	40	2	5	9	22.5	9	22.5	4	10	0	0	0	0	40	100
***An. maculipennis* s.l.**	0	0	181	47.14	80	20.83	30	7.81	5	1.30	14	3.64	64	16.67	10	2.60	384	100
An. pseudopictus	0	0	11	7.14	61	39.61	13	8.44	35	22.73	19	12.34	15	9.74	0	0	154	100
An. marteri	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	1	100
Cx. pipiens	75	1.52	1197	24.27	1670	33.87	527	10.69	818	16.59	261	5.29	277	5.61	106	2.15	4931	100
Cx. torrentium	1	0.16	287	46.22	154	24.8	20	3.22	17	2.74	76	12.24	53	8.53	13	2.09	621	100
Cx. tritaeniorhynchus	2	0.27	4	0.53	324	43.32	139	18.58	213	28.48	58	7.75	8	1.07	0	0	748	100
Cx. perexiguus	0	0	0	0	32	65.31	5	10.2	4	8.16	8	16.33	0	0	0	0	49	100
Cx. territans	0	0	0	0	1	1.59	0	0	0	0	44	69.84	18	28.57	0	0	63	100
Cx. mimeticus	1	1.19	0	0	54	64.29	22	26.19	1	1.19	6	7.14	0	0	0	0	84	100
Cx. hortensis	1	20	0	0	0	0	0	0	4	80	0	0	0	0	0	0	5	100
Cs. annulata	15	8.88	27	15.98	0	0	0	0	0	0	60	35.5	67	39.64	0	0	169	100
Cs. longiareolata	0	0	89	29.57	4	1.33	0	0	0	0	119	39.53	80	26.58	9	2.99	301	100
Cs. morsitans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	100	1	100
Total	95	1.26	1812	23.95	2383	31.5	780	10.31	1106	14.62	669	8.48	582	7.69	139	1.83	7566	100

Table 2. Monthly changes in population fluctuations of adult mosquitoes in Mazandaran Province, northern Iran

	May		June		July		August		September		October		November		December		Total	
Species	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**	**No**	**%**
An. claviger	8	14.55	0	0	2	3.63	28	50.91	14	25.45	3	5.46	0	0	0	0	55	100
An. hyrcanus	14	9.93	95	67.38	21	14.89	0	0	2	1.42	9	6.38	0	0	0	0	141	100
***An. maculipennis* s.l.**	20	1.12	889	49.72	652	36.47	148	8.28	44	2.46	31	1.73	4	0.22	0	0	1788	100
An. pseudopictus	0	0	54	4.19	914	71.02	109	8.47	90	6.99	80	6.22	40	3.11	0	0	127	100
An. marteri	2	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	100
An. sacharovi	0	0	1	0.89	87	77.68	22	19.65	1	0.89	0	0	1	0.89	0	0	112	100
An. superpictus	0	0	0	0	5	55.56	0	0	0	0	0	0	4	44.44	0	0	9	100
Cx. pipiens	426	8.26	918	17.8	1134	21.99	612	11.87	285	5.53	420	8.14	562	10.9	800	15.51	5157	100
Cx. tritaeniorhynchus	0	0	379	6.14	1868	30.26	1288	20.87	1820	29.48	607	9.84	210	3.40	1	0.01	6173	100
Cx. perexiguus	23	25.56	47	52.22	15	16.67	0	0	0	0	5	5.55	0	0	0	0	90	100
Cx. mimeticus	2	16.67	2	16.67	5	41.66	0	0	3	25	0	0	0	0	0	0	12	100
Cx. hortensis	0	0	0	0	1	100	0	0	0	0	0	0	0	0	0	0	1	100
Ae. vexans	5	0.39	0	0	11	0.87	9	0.71	4	0.31	1202	94.65	39	3.07	0	0	1270	100
Ae. caspius	7	18.42	21	55.26	8	21.05	0	0	0	0	1	2.63	0	0	1	2.63	38	100
Cs. annulata	1	2.04	0	0	0	0	2	4.08	0	0	2	4.08	44	89.8	0	0	49	100
Total	508	3.14	2406	14.87	4723	29.18	2218	13.70	2263	13.98	2360	14.58	904	5.59	802	4.96	16184	100

Table 3. Pearson's correlation coefficient (R) of mosquito population density with meteorological variables in Mazandaran Province, northern Iran

Species		Temperature		Rainfall	
		R	P	R	P
Larvae	*An. claviger*	0.394	0.335	-0.374	0.361
	An. hyrcanus	0.630	0.094	-0.143	0.735
	An. maculipennis s.l.	0.236	0.574	0.001	0.998
	An. pseudopictus	0.548	0.159	-0.263	0.529
	Cx. pipiens	0.666	0.710	-0.364	0.379
	Cx. torrentium	0.314	0.449	0.136	0.747
	Cx. tritaeniorhynchus	0.667	0.071	-0.478	0.231
	Cx. perexiguus	0.441	0.274	-0.179	0.672
	Cx. territans	-0.260	0.534	0.855	0.007
	Cx. mimeticus	0.496	0.211	-0.350	0.395
	Cs. annulata	-0.400	0.326	0.711	0.048
	Cs. longiareolata	-0.238	0.571	0.826	0.011
Adults	*An. claviger*	0.570	0.140	-0.546	0.162
	An. hyrcanus	0.295	0.478	0.051	0.904
	An. maculipennis s.l.	0.489	0.218	-0.166	0.694
	An. pseudopictus	0.426	0.293	-0.289	0.488
	An. sacharovi	0.444	0.270	-0.379	0.354
	Cx. pipiens	0.060	0.887	-0.115	0.786
	Cx. tritaeniorhynchus	0.766	0.027	-0.475	0.234
	Cx. perexiguus	0.292	0.483	-0.48	0.911
	Cx. mimeticus	0.584	0.128	-0.523	0.183
	Ae. vexans	-0.086	0.840	0.831	0.011
	Ae. caspius	0.298	0.474	-0.066	0.877
	Cs. annulata	-0.475	0.235	0.140	0.741

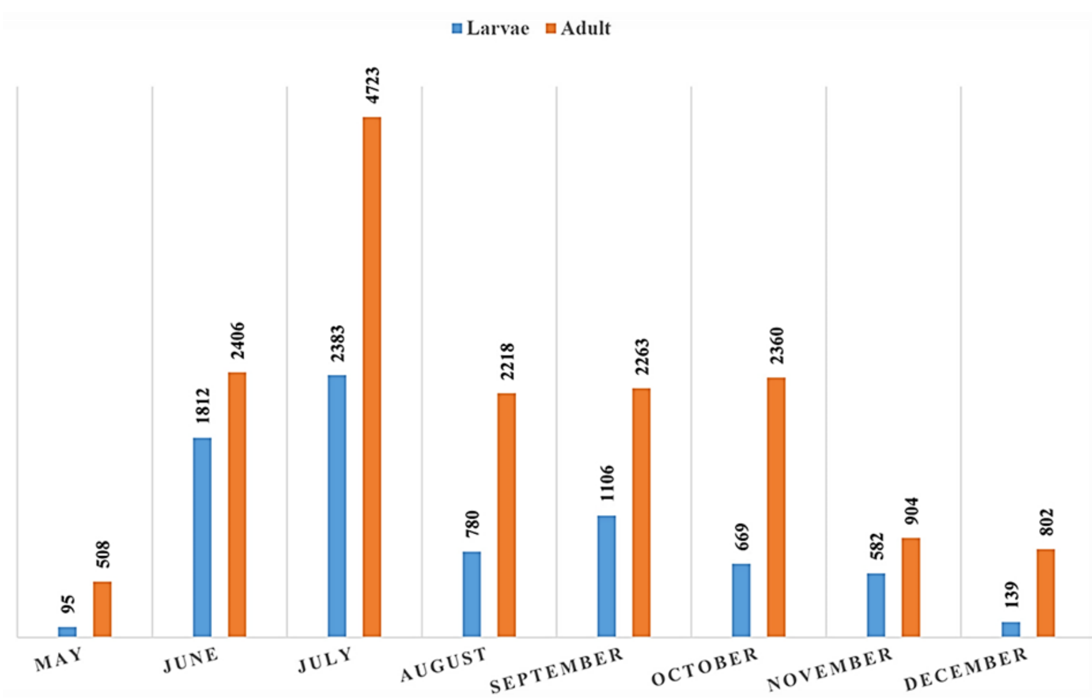

Fig. 1. The total number of mosquitoes collected by month in Mazandaran Province, northern Iran

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Table 4. Number and percentage of adult mosquitoes collected based on sampling method

Sampling method	Total catch in human places		Total catch in animal places		Pit shelter		Light trap		Daily biting		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
An. claviger	11	20	20	36.36	24	43.64	0	0	0	0	55	100
An. hyrcanus	7	4.97	89	63.12	2	1.42	44	31.21	0	0	141	100
***An. maculipennis* s.l.**	91	5.09	1555	86.97	41	2.29	101	5.65	0	0	1788	100
An. marteri	0	0	2	100	0	0	0	0	0	0	2	100
An. pseudopictus	88	6.84	680	52.84	81	6.30	438	34.03	0	0	1287	100
An. sacharovi	0	0	2	1.78	108	96.44	2	1.78	0	0	112	100
An. superpictus	0	0	9	100	0	0	0	0	0	0	9	100
Cx. pipiens	1437	27.87	2225	43.14	870	16.87	625	12.12	0	0	5157	100
Cx. tritaeniorhynchus	700	11.34	3371	54.60	621	10.06	1481	24	0	0	6173	100
Cx. perexiguus	27	30	25	27.77	18	20	20	22.22	0	0	90	100
Cx. mimeticus	1	8.33	10	83.33	0	0	1	8.33	0	0	12	100
Cx. hortensis	0	0	1	100	0	0	0	0	0	0	1	100
Ae. vexans	43	3.38	16	1.25	5	0.39	6	0.47	1200	9.54	1270	100
Ae. caspius	0	0	13	34.21	24	63.15	0	0	1	2.63	38	100
Cs. annulata	2	4.08	33	67.34	10	20.40	4	8.16	0	0	49	100
Total	2407	14.87	8051	49.74	1804	43.64	2722	16.82	1201	7.42	16184	

Table 5. Abundance indices (ISA/SISA)* of the most abundant species (lower ISA values and SISA closer to 1) of mosquitoes collected based on sampling method in Mazandaran Province, northern Iran

Larvae			Adult											
				Total catch in human places		Total catch in animal places		Pit shelter		Light trap		Daily biting		
Species	**SISA**	**ISA**	**Species**	**SISA**	**ISA**	**SISA**	**ISA**	**SISA**	**ISA**	**SISA**	**ISA**	**SISA**	**ISA**
An. claviger	0.000	24.06	*An. claviger*	0.000	20.97	0.000	21.22	0.000	20.88	0.000	21.3	0.000	17.1
An. hyrcanus	0.000	14.5	*An. hyrcanus*	0.000	18.78	0.000	14.09	0.000	20	0.000	16.4	0.000	17.1
***An. maculipennis* s.l.**	0.550	7.06	*An. maculipennis* s.l.	0.369	7.93	0.637	5.719	0.000	12.78	0.000	16	0.000	17.1
An. pseudopictus	0.273	10.81	*An. pseudopictus*	0.454	7	0.822	3.313	0.46	6.938	0.668	4.66	0.000	17.1
An. marteri	0.000	24.5	*An. marteri*	0.000	22.19	0.000	23.97	0.000	20.75	0.000	22	0.000	17.1
Cx. pipiens	1	1	*An. sacharovi*	0.000	22.19	0.000	23.94	0.000	20.69	0.000	19.8	0.000	17.1
Cx. torrentium	0.805	3.62	*An. superpictus*	0.000	22.19	0.000	22.44	0.000	22.06	0.000	22	0.000	17.1
Cx. tritaeniorhynchus	0.564	6.87	*Cx. pipiens*	0.977	1.25	0.933	1.87	0.946	1.59	0.696	4.34	0.000	17.1
Cx. perexiguus	0.000	17	*Cx. tritaeniorhynchus*	0.886	2.25	0.913	2.12	0.938	1.68	0.938	1.69	0.000	17.1
Cx. territans	0.000	19.19	*Cx. perexiguus*	0.000	17.53	0.000	18.03	0.000	20.78	0.000	20.6	0.000	17.1
Cx. mimeticus	0.037	14	*Cx. mimeticus*	0.000	21.13	0.000	19.19	0.000	22.06	0.000	20.9	0.000	17.1
Cx. hortensis	0.000	21.69	*Cx. hortensis*	0.000	22.19	0.000	23.94	0.000	22.06	0.000	22	0.000	17.1
Cs. annulata	0.000	15.12	*Ae. vexans*	0.000	19.75	0.000	22.63	0.000	19.78	0.000	19.8	0.1	9
Cs. longiareolata	0.152	12.44	*Ae. caspius*	0.000	22.19	0.000	23.69	0.000	19.66	0.000	22	0.000	16.1
Cs. morsitans	0.000	22.69	*Cs. annulata*	0.000	19.97	0.000	16.34	0.000	16.47	0.000	18.6	0.000	17.1

*ISA: Index of Species Abundance; SISA: Standard Index of Species Abundance

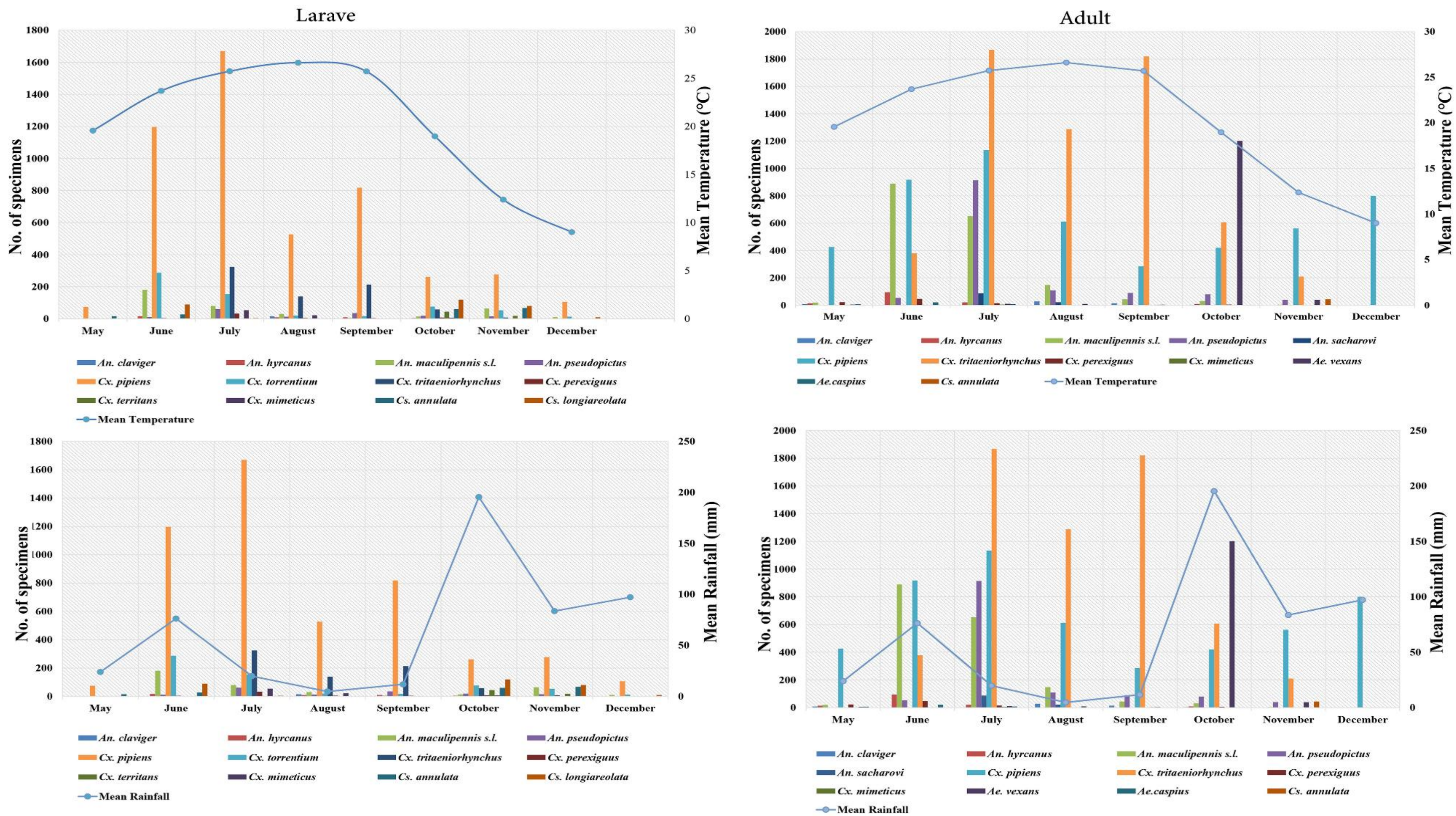

Fig. 2. Relation between population fluctuations of larvae/adult mosquitoes and meteorological variables in Mazandaran Province, northern Iran

Discussion

Demonstration of the monthly changes in the population abundance of mosquito species, along with meteorological parameters is crucial in understanding the biology of potential vector species for efficient mosquito control strategies. This is the first comprehensive study on this subject in Mazandaran Province, northern Iran using the ISA/SISA indices in order to identify the most abundant species. Vector abundance is a key determining factor that is often used as a risk indicator of vector-borne diseases. It is quantitatively more important than vector competence (although competence is a transmission requirement). It could explain the importance of abundant species in an area and why some vectors could contribute to epidemics without considering the competence for transmission (40-41). *Culex pipiens* is the most abundant species both in the larval and adult stages based on indices of SISA/ ISA in the present study. It was noted to be a common species in other studies in different parts of Iran (35, 42-45), however, it should be mentioned that none of these researches reported abundance of species based on the SISA/ISA indices. Although *Cx. pipiens* was collected most frequently by total catch in animal places, it was the predominant species (based on indices of SISA/ISA) in total catch in human places and pit shelter compared to other species, respectively. It indicates that the species tends to be attracted to humans, animals and also shows some extent of exophily in the study area. It is possible that humans come into contact with pathogens this species may carry. Such studies could establish baseline data for public health interventions in control programs, therefore, an assessment of different trapping methods is also required to adjust the design of future entomological and pathogen surveillance efforts (46). The monthly activity of *Cx. pipiens* (larvae and adult) begins in mid-spring after diapause termination and reaches its largest peak in July and then declines with irregular fluctuations until the species disappears. It seems that the spring rains are cause for the population of the species to peak in July, while no significant association was observed between *Cx. pipiens* and meteorological factors, probably high compatibility and access to diverse breeding places could help increasing population density of the species in early warm season. In contrast, *Cx. pipiens* was active from May to November and June to September (29, 47). The population density of this species increased in July (47) and May to August (48), reaching its largest peak in August (47-48). *Culex pipiens* showed an increase in population density from May to July in north-eastern Croatia, then sharp decline occurred toward the end of the season (49). In northern Italy, the species had maximum activity peak in July (50), that is almost consistent with the results of the present study. There is not much data about the seasonal activity of culicine mosquitoes, especially in adult stage, in Iran (30). The highest activity peak of *Cx. pipiens* was documented in mid-July in Guilan Province. There is no significant correlation between species population density and the meteorological data (30), which is in accordance with our research. Variations in the seasonal patterns of the species in this study and findings in other regions can probably be due to the differences in the topography and climates.

Culex pipiens with its ornithophilic and opportunistic feeding behavior that bites both humans and animals, can be as bridge vector between birds and humans so is believed to be the principal vector of West Nile Virus (51). It also shows an important role in transmission of several human pathogens including St. Louis encephalitis virus (SLEV), filarial worms as well as wildlife pathogens such as avian malaria (52). The existence of swamps for migrating birds and their active presence throughout the autumn and winter in Mazandaran Province and detection of virus in the species in Guilan Province (53) can cause a concern for

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entry and the spread of the virus in the region. *Culex tritaeniorhynchus*, the second most abundant species based on indices of SISA/ISA in the study area, was first collected in May (larval stage) and June (adult stage), increased sharply in July, becoming the most abundant species, and displayed a rapid decline in December. The abundant species shifted from *Cx. tritaeniorhynchus* to *Cx. pipiens* in November, probably due to high adaptability of *Cx. pipiens* to the environmental conditions in the area. In contrast, this species showed the highest peak of activity in August in Belek Region of Turkey (29). While the peak of activity was observed in July in Guilan Province (30), which is in agreement with our study. *Culex tritaeniorhynchus* is caught with the highest number by total catch in animal places which reflects the zoophilic tendencies of the species in the study area. Based on the results of a study in India, it is considered to be predominantly exophilic and normally zoophilic (54). It was recorded to be the most abundant species in numerous studies in Iran, without reporting any SISA/ ISA indices (33-34, 44-55). *Culex tritaeniorhynchus* is the primary vector of Japanese encephalitis (JE) in southern Asia. It has also been observed infected with dengue fever, Rift Valley fever, Sindbis, Getah and Tembusu viruses, and the filariae of both *Brugia malayi* and *Wuchereria bancrofti*, in many areas of eastern and southeastern Asia (55-56). The species is a principle vector of Rift Valley fever in Saudi Arabia (57), WNV in Asia (9) and a possible vector of Japanese encephalitis in Iraq (58), which indicates a high risk species for human health.

Among the *Anopheles*, *An. maculipennis* s.l. is numerically the most abundant species in the present study, mostly sampled by total catch method in animal places, displayed to be predominantly zoophilic. This is consistent with other studies, but there is no evidence of SISA/ISA indices to express the most abundant species in these studies (43, 59-61). What is interesting is that, despite having the highest numerical abundance of species, *An. pseudopictus* was calculated as the most abundant species in all sampling methods (except daily bites) based on ISA/SISA indices. In addition, *An. sacharovi* also had the highest numerical abundance in the pit shelter, but it was not considered as the most abundant species based on the indices of SISA/ISA. Moreover, *An. sacharovi* was the most abundant species in the pit shelter, but only with regards to its high numerical abundance. Therefore, this shows the importance of species distribution at different sites and computational value of the ISA/SISA indices. *Anopheles maculipennis* s.l. is known to be the most important malaria vector in northern and western parts and the central plateau of Iran (62). Moreover, there is a belief that this species can play a vector role in WNV transmission in various countries of the old world (63, 64). Recently, the myxoma virus genome was detected in wild caught *An. maculipennis* that fed on wild rabbits by polymerase chain reaction (PCR) in southern England, UK. Batai virus (BATV) and *Anopheles* associated C virus (AACV) was also identified and isolated from *An. maculipennis* complex in entomological surveys in Germany, Italy and France (65). With regards to the malaria historical records and WNV in the northern parts of Iran (53, 66), understanding the population dynamics and the maximum monthly peaks of the species is important in controlling the diseases in the study area. The highest monthly activity peak of *An. maculipennis* (larvae and adults) is in June. In contrary to our results, *An. maculipennis* s.l. showed the highest peak of activity in the mid-July in Guilan (30) and July–August in Kalaleh County of Golestan Province (31), northern Iran. In neighboring Turkey, the species peaked in August and July–August (67-68). These differences in population patterns could possibly be due to regional ecological differences. On the other hand, some researchers believe that these discrepancies might also be due to the low collection effort involved in the studies (27) or sampling regimes (29-30, 50, 67-68). Climate

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change and the environment affect the abundance and distribution of vectors and their intermediate hosts (69). In the present study, monthly population fluctuations of *Cs. annulata*, *Cs. longiareolata* and *Ae. vexans* are correlated with rainfall. Although these species showed irregular monthly population fluctuations, they were more active in spring and autumn. *Culiseta annulata* had a major peak in November (29), which is in accordance with our research. On the other hand, *Cs. longiareolata* was active in July and September (67), whereas in the present study, the species had the largest peak in October, which is quite similar to that of *Ae. vexans.* The largest peak of *Ae. vexans* was reported in June (67) and August (68). There are evidence that *Ae. vexans* and *Cs. annulata* are important vectors of WNV (70-71). Among the tribe Aedini, *Ae. vexans* is recorded to be the most abundant species based on ISA/SISA indices. It was caught in all traps and is the most abundant collected species in day biting method compared with other species, and shows anthropophilic tendencies in the study areas. This species demonstrated high prevalence and collected by day biting method in other studies performed in different parts of Iran (59, 72-74), this may be a reflection of the fact that this method has a high level of efficiency for collecting this species. However, to observe ethical values, this collection methods cannot be used freely and with extended time, and should in fact be restricted or modified in compliance with ethical standards (75). The species is known to be the main vector of Tahyna virus in central Europe (76), potential vector of the dog heartworm *Dirofilaria immitis* in Europe (77) and principal vectors of Rift Valley fever in Saudi Arabia (57). Recently, Zika virus is revealed in the salivary glands of the field-caught *Ae. vexans* (78). It is a flood water mosquito, so, their population abundance depends on the water dynamics of temporary pools (27). Rainfall as an important climatic factor may have a range of different correlation with the population of the species, from positive, negative and/or ineffective and sometimes with a lag phase (79), a significant positive correlation between its monthly activity and rainfall was observed in our study. The current study was not designed and aimed to address the analysis of lag time between climate factors and population density of the species, however, it seems that there is probably a lag time of at least 15 days after rain at the beginning of the rainy season before the *Ae. vexans* population jump start. In concordant with our findings, there is a lag time of at least 15 days between the peak of rainfall and abundance of the species (80-82). Moreover, other studies have revealed a correlation between rainfall and the abundance of the species with a 10 days lag in the early rainy season and 20 days after the end of the rainy season (83). In the study of Diallo et al. (82) rainfall patterns displayed that heavy rains between August and September 2005 had negatively impacted on the population density of *Ae. vexans*, whereas in 2006, population peak was observed following the rainfall peak. These findings show the complexity of the relationship between climate factors and population density of mosquitoes. Therefore, it is recommended to measure the seasonal activities of species over multiple years, to show a better understanding of the correlation between population frequency and climatic factors, and the impact of other variables. The highest number and population density of *Ae. vexans* was found during early autumn, especially in October. Vector-borne diseases show seasonal patterns with inter and intra-annual variability, which are partly described by climate and environmental factors (80). Therefore, these results are important for health authorities in controlling mosquitoes as well as in the tourism industry for nuisance control, but more importantly, it provides a detailed estimate of the timing of risk for human populations.

Conclusion

Based on ISA/SISA indices, July in which

Cx. pipiens and *Cx. tritaeniorhynchus* have the highest population peak, is the most crucial time for efficient mosquito control program in the area study.

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