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Fauna and some biological characteristics of *Anopheles* mosquitoes (Diptera: Culicidae) in Kalaleh County, Golestan Province, northeast of Iran

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ABSTRACT

Objective: To determine fauna and some ecological aspects of *Anopheles* mosquitoes in northeast of Iran.

Methods: In this descriptive study, 3 villages in Kalaleh County were selected in different geographical zones. *Anopheles* mosquitoes were collected biweekly from May to October using standard dipping method for larvae, and hand catch, total catch, artificial pit shelter as well as night-biting collections on human and animal baits for adults.

Results: Totally 399 larvae and 2 602 adults of *Anopheles* mosquitoes were collected and identified as 2 species: *Anopheles superpictus* s.l. (*An. superpictus* s.l.) and *Anopheles maculipennis* s.l. The dominant species was *An. superpictus* s.l. (92.1%). Activity of these mosquitoes found to be started from middle of May and extended till September with two peaks of activity in July and August.

Conclusions: *An. superpictus* s.l. as one of the main malaria vectors in Iran as well as some other parts of the world is the dominant species in the study area. This species has high potential for transmission and possibility of establishing a transmission cycle with low abundance. Other species, *Anopheles maculipennis* s.l. also has introduced as a malaria vector in northern parts of Iran. As this *Anopheles* is a complex species, genetic studies are recommended to determine the members of this complex in the study area.

1. Introduction

Malaria is one of the most important vector-borne diseases caused by *Plasmodium* spp. This disease is the 5th cause of mortality in children under 5 years and 6th cause of disability in children under 4 years in developing countries. Also about half of the mortality of infectious diseases in the world is due to AIDS, tuberculosis and malaria [1]. In Iran, malaria is one of the important infectious diseases. The country has two completely distinct eco-epidemiological zones for malaria: temperate and oriental zones. Oriental zone includes Sistan va Baluchestan, south of Kerman and Hormozgan Provinces with meso-endemicity of malaria infection. Temperate zone including other provinces and malaria transmission is very restricted in this area [1].

Malaria campaign started after 1950 has dramatically decreased the incidence of the disease as well as its geographical distribution across the country, so that Iran is classified in pre-elimination phase of malaria control program [1].

Anopheles mosquitoes are vector and main reservoir of *Plasmodium* spp. More than 422 species for this genus are identified in the world. Out of them 70 species are malaria vectors and 40 species can be considered as main vectors of the disease. In Iran, there are 31 *Anopheles* species including siblings and biological forms, while 7 species are the main vectors of the disease in different parts of the country including *Anopheles culicifacies* Giles s.l., *Anopheles dthali* Patton, *Anopheles fluviatilis* James s.l., *Anopheles maculipennis* Meigen s.l. (*An. maculipennis* s.l.), *Anopheles sacharovi* Favre (*An. sacharovi*), *Anopheles stephensi* Liston, and *Anopheles*

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superpictus s.l. (*An. superpictus* s.l.) [2]. Among these species, *An. maculipennis* s.l. and *An. sacharovi* are mostly distributed in northern parts of the country and so considered as the malaria vectors in those areas based on distribution pattern and entomological studies [2].

Previous studies in Golestan Province show that 8 *Anopheles* species are active in this part of Iran, including: *Anopheles algeriensis*, *Anopheles claviger* (*An. claviger*), *Anopheles hyrcanus* (*An. hyrcanus*), *An. maculipennis* s.l., *Anopheles multicolor*, *Anopheles plumbeus* (*An. plumbeus*), *Anopheles pulcherrimus* and *Anopheles turkhodi* [2]. Appropriate environmental conditions are the most important factors for breeding and increasing the mosquito population. The best water temperature for mosquito breeding and rearing is 25–28 °C. When the ambient temperature is between 20 and 30 °C and the relative humidity is more than 60%, *Plasmodium* spp. has the best opportunity for developing their life cycle in malaria vectors [1]. These conditions exist in the most parts of Golestan Province, so the risk of malaria transmission should be considered in this province.

In the past, malaria has been one of the main health problems in Golestan Province and Kalaleh was one of the main foci of the disease in the past. Large rivers such as: Atrak, Gorgan, Aghso and Zav make the area suitable for agriculture, and rice fields are favorable breeding sites for mosquitoes. During the pre-elimination phase of malaria, it is very important to update the transmission potential in areas with population movements. Therefore, this study was aimed to identify anopheline species and to determine their bioecology in Kalaleh County.

2. Materials and methods

2.1. Study area

Kalaleh County is located in a semi-temperate mountainous area in the eastern part of Golestan Province at coordinates of 37.380 8° N and 55.491 7° E. The average of annual rainfall is about 500 mm and the range of daily temperature average is 8.8–29.1 °C. With due attention to suitable climatology, geographical and topographical conditions of Kalaleh County for breeding and development of anopheline mosquitoes [3], it is selected for this descriptive study. For this reason, 3 villages *i.e.* Azizabad (55.23° E, 37.53° N), Gharankijangal (55.78° E, 37.57° N) and Binal (55.45° E, 37.35° N) were nominated based on their natural ecosystems. Sampling was carried out biweekly to collect larvae and adult mosquitoes.

2.2. Sampling

For this purpose, all natural and artificial breeding places in and around the selected villages were visited and recorded. Sampling was conducted by standard dipping method [4]. Samples were conserved in lactophenol, and in the laboratory they were mounted in likidophor medium and then identified at the species level by the key for Iranian anopheline [5].

Adult mosquitoes were collected by hand catch, spray sheet collection, landing catch at night on human/animal baits and artificial outdoor resting places (pit shelter) methods during the year. In the laboratory, all anopheline specimens were identified at the species level [5] and their physiological status was recorded.

2.3. Data analysis

Obtained data from the field study were recorded in an Excel sheet and tables/charts were prepared using this software. Statistical analysis was conducted using IBM SPSS statistics version 23 and χ^2 test to find correlation between species density and types of breeding places, resting places, physiological stages of adults, as well as their seasonal activity ($P < 0.05$).

3. Results

During the study period, a total of 2 602 adults and 399 larvae of anopheline mosquitoes were collected and identified, representing two species, *i.e. An. superpictus* s.l. and *An. maculipennis* s.l. All anopheline specimens were collected during spring and summer, while their abundance during autumn and winter months was zero. *An. superpictus* s.l. was the dominant species in this survey (Table 1). There was no significant difference between type of breeding place and the species ($P > 0.05$).

Pits and water leaks were caused by traditional irrigation of farms. These types of water bodies were the favorite breeding places for anopheline in the study area and we collected most of specimens from them. Water leakages were in margins of the rivers and water bodies in riverbeds. Characteristics of breeding places in rice fields were as follows: average of water temperature: 25 °C, pH: 7, still water or with laminar flow, with vegetation in and out of water, clay bed, clear and fresh water and average depth of about 10 cm.

Breeding places in river margins had running water, with vegetation in and out of water, and a depth of 10–30 cm. They had also clear and fresh water with rocky bed.

Study on adult mosquitoes by spray sheet collection method during spring (4 times) and summer (6 times) resulted in capturing 637 *Anopheles* from human resting places comprising two species: *An. superpictus* s.l. (90.1%) and *An. maculipennis* s.l. (9.9%). Density per human was calculated as 13.7 and 1.5 for *An. superpictus* s.l. and *An. maculipennis* s.l., respectively. Monthly activity of anopheline mosquitoes was started in early June in human resting places and ended in October with a peak during July–August (Figure 1). In animal resting places, a total of 1 530 *Anopheles* were collected, while density per shelter for *An. superpictus* s.l. (35.6) was higher than *An. maculipennis* s.l. (3.6). Overall, density of mosquitoes was higher in summer, although it was not significant ($P > 0.05$) (Table 2). Females (78.5%) were collected more than males (20.4%) using this method. Table 3 shows that most of female anopheline mosquitoes collected from indoor resting places were freshly blood-fed. Physiological stage of the collected mosquitoes was significantly different both in human resting place and animal shelters ($P < 0.05$).

Table 1
Fauna, number of collected and breeding places of anopheline larvae in Kalaleh County, Golestan Province, northeast of Iran, 2012–2013.

Species	Breeding place			Percent
	Rice field	River margins	Total	
An. superpictus s.l.	309	14	323	81
An. maculipennis s.l.	76	0	76	19
Total	385	14	399	100

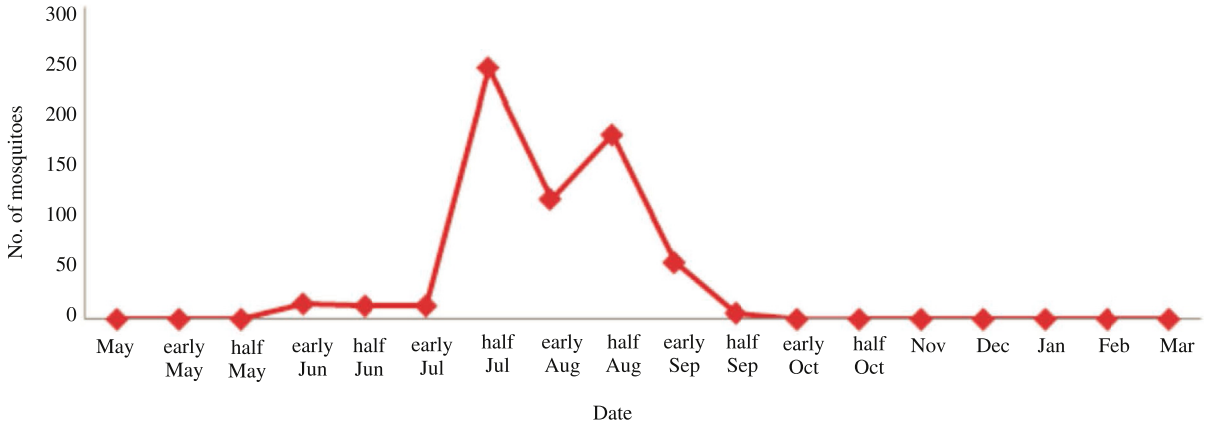

Figure 1. Monthly activity of anopheline collected from human resting places, Kalaleh County, 2012.

Table 2

Species composition and density of anopheline mosquitoes collected from indoor resting places, Kalaleh County, 2012.

Season	Human resting places				Animal resting place			
	An. superpictus s.l.		*An. maculipennis* s.l.		*An. superpictus* s.l.		*An. maculipennis* s.l.	
	No.	Density/human	No.	Density/human	No.	Density/shelter	No.	Density/shelter
Spring	30	10.0	0	0.0	58	4.8	13	1.1
Summer	544	13.9	63	1.6	1333	74.1	126	7.0
Fall	0	0.0	0	0.0	0	0.0	0	0.0
Autumn	0	0.0	0	0.0	0	0.0	0	0.0
Total	574	13.7	63	1.5	1391	35.6	139	3.6

Table 3

Physiological stages of female anopheline collected from indoor resting places, Kalaleh County, 2012. *n* (%).

Physiological stage		Un-fed	Freshly fed	Semi-gravid	Gravid
Animal shelters	*An. superpictus* s.l.	392 (35.9)	607 (55.6)	87 (8.0)	6 (0.5)
	An. maculipennis s.l.	27 (24.7)	66 (61.1)	14 (12.9)	2 (1.4)
	Total	419 (35.0)	673 (56.0)	101 (8.5)	8 (0.5)
Human resting places	*An. superpictus* s.l.	191 (42.3)	235 (52.1)	24 (5.4)	1 (0.2)
	An. maculipennis s.l.	12 (23.8)	33 (66.7)	4 (7.9)	1 (1.6)
	Total	203 (40.5)	268 (53.6)	28 (5.6)	2 (0.3)
Total		622 (36.5)	941 (55.3)	129 (7.6)	10 (0.6)

Mosquito collection was conducted from artificial outdoor resting places (pit shelters) and just 16 *An. superpictus* s.l. and 3 *An. maculipennis* s.l. were collected in summer. Density per pit shelter was calculated as 2.6 and 0.5 for these species, respectively.

Landing bait collection was conducted using human and animal (cow) baits and resulted in 409 *Anopheles* from both species. It had two peaks in 21–22 p.m. and 04–05 a.m., while most of specimens were collected using animal baits (Table 4).

Table 4

Species composition and density of anopheline mosquito using landing bait collection method, Kalaleh County, 2012.

Hour	Human bait				Animal bait				Environmental condition	
	An. superpictus s.l.		*An. maculipennis* s.l.		*An. superpictus* s.l.		*An. maculipennis* s.l.			
	No.	Density/bait/hour	No.	Density/bait/hour	No.	Density/bait/hour	No.	Density/bait/hour	Temperature (°C)	Relative humidity (%)
19–20	0	0	0	0	9	1.8	0	0.0	37	48
20–21	0	0	0	0	18	3.6	0	0.0	36	48
21–22	2	2	0	0	101	20.2	1	0.2	36	51
22–23	0	0	0	0	35	7.0	0	0.0	36	52
23–24	0	0	0	0	16	3.2	0	0.0	36	53
24–01	0	0	0	0	25	5.0	0	0.0	35	54
01–02	1	1	0	0	10	2.0	0	0.0	35	55
02–03	2	2	0	0	26	5.2	0	0.0	34	56
03–04	1	1	0	0	38	7.6	0	0.0	33	58
04–05	0	0	0	0	85	17.0	1	0.2	33	62
05–06	1	1	0	0	42	8.4	2	0.4	32	62

4. Discussion

In this study, two *Anopheles* species, *i.e. An. superpictus* s.l. and *An. maculipennis* s.l. complex were collected and identified morphologically. Both collected species are considered as malaria vectors in Iran [2]. Previous studies in northeast of Iran have reported 12 species of *Anopheles* including *An. plumbeus*, *An. claviger*, *An. hyrcanus*, *An. maculipennis*, *An. sacharovi*, *Anopheles persiensis*, *Anopheles atroparvus*, *Anopheles messeae*, *Anopheles labranchiae*, *Anopheles pseudopictus*, *An. superpictus* s.l. and *Anopheles pulcherrimus* [6–13]. Previous studies in Golestan Province also reported *An. maculipennis* s.l., *An. claviger*, *An. hyrcanus*, *Anopheles alrgeriensis*, *An. plumbeus*, *Anopheles turkhudi* and *Anopheles multicolor*. In northern part of Iran, *An. maculipennis* s.l. complex is reported from Khorassan-e-Shomali, Khorassan-e-Razavi, Gilan, Mazandaran, Ardabil, Azarbaijan-e Sharghi and Azarbaijan-e-Gharbi. Also *An. superpictus* s.l. is distributed in Gilan, Khorassan-e-Shomali, Azarbaijan-e Sharghi and Azarbaijan-e-Gharbi [2,6–13]. Our results are in accordance with these studies, although other reported species were not collected. It can be due to reduction in precipitation and limitation of suitable breeding places for other species, so that at present breeding places are restricted to margins of rice fields and rivers.

Study on breeding places of *An. maculipennis* s.l. in Azarbaijan-e-Gharbi reported this species from all slow running, transit, standing and permanent waters with vegetation both in and out of water [8,11,13]. About *An. superpictus* s.l., it was collected from stagnant and transit waters with or without vegetation in different parts of Iran [8,14–17]. Bed of breeding places for both species in these studies was clay and sandy. Our results were more or less the same as these studies. Dominant species in breeding places of our study was *An. superpictus* s.l. The same species was dominant in Khrassan-e-Shomali (located in eastern border of the study area), but dominant species in Gilan and Mazandaran (Neka County) were *An. maculipennis* s.l. and *An. plumbeus*, respectively [8,9,13].

An. maculipennis s.l. complex has 11 species in Palearctic region; out of them, *Anopheles atroparvus*, *Anopheles labranchiae*, *Anopheles maculipennis*, *Anopheles melanoon*, *Anopheles messeae*, *Anopheles persiensis* and *An. sacharovi* have been identified using molecular method and reported in Iran [14]. These species have a wide distribution in northern part of the country and found to be resistant to insecticides in this area [2,6,8–13,18–20]. *An. superpictus* s.l. is widely distributed in Iran and is considered to be a major and secondary malaria vector in the central plateau and southern areas of the country, respectively [2,14–17]. A previous study reported three genotypes for this species from different parts of Iran [21]. In this study, we used morphological characters to identify these species. Therefore, molecular studies are recommended to detect other probable species of *An. maculipennis* s.l. complex as well as genotypes of *An. superpictus* s.l. By the way, there is no study on the role of different species/genotypes of them in malaria transmission.

Results of different collection methods show that endophilic tendency of these species in the study area is more than their exophilicity. Also landing bait collection showed that both species have more tendency to animal bait (cow) rather than human. More studies on blood feeding habitats of both species are recommended. Previous studies have shown that *An. superpictus* s.l. prefers blood feeding from animal hosts and is generally known as a zoophilic species that also readily feeds on humans [22]. This was similar to our results, as we collected more mosquitoes from animal hosts than human.

Landing bait collection showed a peak of blood feeding activity for *An. superpictus* s.l. during 21–22 with a temperature of 36 °C and relative humidity of 51% and another peak in 04–05. Previous studies reported a peak of activity for this species during 03–05 [2] and 22–23 [14]. We also observed these peaks for *An. maculipennis* s.l. as well, but with smaller population. More accurate data on host seeking activity of these species will be obtained from a comprehensive study during the active seasons.

Adult mosquitoes had a peak of activity during half July–half August. This peak is different based on the environmental condition and weather variables, so that in southern coastal parts of the country mosquitoes (adults and larvae) usually have two peaks of activity [14,16,17,23].

The most important breeding places for anopheline mosquitoes in Kalaleh County are rice fields, so that with starting paddy there is an increasing trend in *Anopheles* population in the area. *An. superpictus* s.l. is the dominant species in this county, and a proven vector of malaria in Iran. Also *An. maculipennis* s.l. is considered as malaria vector in northern part of the country. Therefore, transmission of malaria can occur from imported cases, an important issue that should be noted by health authorities and national malaria elimination program.

Conflict of interest statement

We declare that we have no conflict of interest.

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