Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.2014C186

The ecology and larval habitats characteristics of anopheline mosquitoes (Diptera: Culicidae) in Aligudarz County (Luristan province, western Iran)

Hamid Amani[1], Mohammad Reza Yaghoobi-Ershadi[2] , Hamid Kassiri[3*]

[1]*Azna Health Services Center, Luristan University of Medical Sciences, Azna, Iran*

[2]*Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran*

[3]*Department of Medical Entomology and Vector Control, School of Public Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

PEER REVIEW

Peer reviewer

Prof. Mohammad Reza Fakoorziba, Department of Medical Entomology, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran.
Tel: +987117251020
Fax: +987117260225
E-mail: fakoorziba@sums.ac.ir

Comments

It is a good research in which the authors found some aspects of ecology and characteristics of breeding places of anopheline mosquitoes in the western Iran.
Details on Page S240

ABSTRACT

Objective: To determine ecology and characteristics of the larval habitats of the genus *Anopheles* (Dipetra: Culicidae) in Aligudarz County, western Iran.

Methods: This descriptive cross-sectional research was carried out to study the anopheline larvae ecology in seven rural districts, Aligudarz County, from late April to late November 1997. Larvae were captured using the dipping method. Larval breeding places characteristics were noted according to water situation (turbid or clean, stagnant or running), substrate type, site type (man-made or natural), sunlight situation, site situation (transient or permanent, with or without vegetation).

Results: A total of 9620 3rd and 4th instar larvae of *Anopheles* from 115 breeding places in 22 villages were captured, which belonged to the following species: *Anopheles stephensi, Anopheles d'thali*, *Anopheles apoci*, *Anopheles superpictus* (forms A and B), *Anopheles marterii sogdianus, Anopheles turkhodi, Anopheles maculipennis* S.L and *Anopheles claviger. Anopheles stephensi, Anopheles maculipennis* S.L and *Anopheles apoci* were collected for the first time in this county. *Anopheles superpictus* (93.18%) was the most prevailed one and dispersed over the entire region. Larval habitats consisted of nine natural and three artificial larval habitats. The most important larval habitats were river edges (54.8%), rice fields (12.2%), and grassland (8.7%) with permanent or transient, stagnant or running and clean water, with or without vegetation, sand or mud substrate in full sunlight area.

Conclusions: Regarding this research, river edges and rice fields are the most important breeding places of malaria vectors in Aligudarz County. It is worthy of note in larvicidal programs.

KEYWORDS

Anopheles, Larval habitat, Ecology, Biology, Aligudarz County, Iran

1. Introduction

Currently, malaria in many countries especially in South Africa is one of the most important problems of the health. Unfortunately, many people die due to this disease around the world every year[1]. Malaria as the most important disease transmitted by mosquitoes was discussed in Iran. Prior to the implementation of malaria control programs to be launched in Iran, about 60 percent of people were living in endemic areas where morbidity rates were 30% to 40%[2]. Iran is categorized into four areas based on the disease epidemiology[3]. The south of the country is the most important area in which malaria is a

*Corresponding author: Dr. Hamid Kassiri, Assistant Professor, Department of Medical Entomology and Vector Control, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
Tel: 0098-611-3738269
Fax: 0098-611-3738282
E-mail: Hamid.Kassiri@yahoo.com
Foundation Project: Supported by Luristan University of Medical Sciences, Iran (Grant No: 2501).

Article history:
Received 13 Dec 2013
Received in revised form 20 Dec, 2nd revised form 23 Dec, 3rd revised form 29 Dec 2013
Accepted 28 Jan 2014
Available online 5 Apr 2014

problem. More than 90% of malaria cases are reported from the provinces of Sistan-Baluchestan, Kerman and Hormozgan[4]. Of 11460 cases of malaria reported in Iran in 2008, 9.7% was due to *Plasmodium falciparum* and 90.3% was due to *Plasmodium vivax*[5]. According to the report of World Health Organization (WHO), gradual reduction of disease cases is seen due to the widespread implementation of malaria control programs in the country. Thus, Iran is classified in the pre-elimination phase of the disease[1].

Luristan province was previously one of the malarious zones; and now is divided into two zones: the malaria-free zone (sensitive to the transmission) and low transmission zone (vulnerable). In the malaria-free zone, the most cases observed are imported from the malarious zones of Iran; and the disease transmission does not occur. But in low transmission zone, some cases of malaria are seen each year that some of them may have been infected through local transmission. The rural districts of Eastern Zalaghi, Western Zalaghi and Mahroo in Aligudarz County are in low transmission zone; and due to technical and administrative problems, and unlike the first zone, these regions cannot easily respond to the malaria control activities. Aligudarz County has the potential for further transmission; and the tribal and rural residents are more at risk of infection. Given the importance of malaria in Iran including Luristan Province and potential possibility for transmitting other diseases such as West Nile fever, Sindbis, Dirofilariasis, Rift Valley fever, Japanese Encephalitis, *etc*. by Culicidae mosquitoes[6], the necessity of this study is highlighted. In addition to the above mentioned problems, the nuisance caused by mosquito bites is another part of the problems of people in this county.

Based on the common classification, the Culicidae family includes three subfamilies, 10 tribes, 39 genera, 135 subgenera and more than 3450 species and subspecies[7]. The genus *Anopheles* consists of six subgenera and at least 484 species identified in the world[8]. According to the latest classification of mosquitoes, the Culicidae family has been introduced in such a way that includes two subfamilies, 11 tribes, 113 genera and 3531 species in the world. Also, the genus *Anopheles* is divided into 7 subgenera and at least 465 species[9].

Sedaghat and Harbach reported 25 species of the *Anopheles* fauna in Iran[10]; but in the checklist of Iranian mosquitoes, Azari-Hamidian has pointed to 64 species and 3 subspecies in the Culicidae family; and in the meantime, he introduced two subgenera (*Anopheles* and *Celia*) and 28 species in the genus *Anopheles*[11]. Some species of *Anopheles* transmit some of filariasis and arboviral diseases to humans and domestic animals; however, the most important disease transmitted by them is malaria. About 70 species of *Anopheles* have been reported as vectors of malaria in the world from which about 40 species are considered as more important vectors[12]. Seven species, including *Anopheles culicifacies, Anopheles d'thali* (*An. d'thali*), *Anopheles fluviatilis* (*An. fluviatilis*), *Anopheles maculipennis* (*An. maculipennis*), *Anopheles sacharovi* (*An. sacharovi*), *Anopheles stephensi* (*An. stephensi*) and *Anopheles superpictus* (*An. superpictus*) are well known vectors for malaria in Iran. *Anopheles pulcherrimus* is a potential vector for this disease in the southeastern of Iran[2]. Also, *Plasmodium* oocytes have been observed in *Anopheles multicolor* in Sabzevar County[6]. Recently, *Plasmodium* parasites were identified in *Anopheles hyrcanus* (*An. hyrcanus*) by molecular genetics methods in Guilan Province; and thus, this species was introduced as a potential vector for malaria[13].

An. superpictus is an important vector in the Palaearctic region; and distributes across Afghanistan, Pakistan, India, Middle East, North Africa, newly independent countries of the former Soviet Union, and central and southern Europe. This species is widely distributed in the whole country; and it has been reported across the plateau of Iran, Alborz Mountains and southern regions of Zagros Mountains; it has been found rarely in the plains adjoining the Persian Gulf and the Caspian Sea. Sporozoite indices of this *Anopheles* in Masjed-Soleiman, Tabas and Kazeroun were 1.20%, 6.40% and 0.65%, respectively. The blood feeding of this species is done on human and animals, although this species does prefer animal's blood. Also, it has exophagic and exophilic-endophilic behaviors. *An. superpictus* is the dominant species and the main vector for malaria in the Luristan Province[14]. Recently, a molecular study on different populations of *An. superpictus* throughout Iran has revealed three different genotypes in this species; and therefore, this species has been proposed as a sibling vector (composed of species X, Y and Z)[15].

One of the important properties for breeding the mosquitoes is appropriate biological conditions. Since the developmental stages of mosquitoes are in the water, the specific ecological conditions, such as abundant larval habitats with appropriate chemical and physical conditions to increase their density are very important. Despite the importance of ecological and biological studies in the mosquitoes control programs, there is scattered and limited information about the characteristics of the larval nests and ecology of the species of *Anopheles* in Iran; and the most of the information are in connection with vectors of malaria in south of the country. Marsh noted a number of larval nests of the *Anopheles apoci* (*An. apoci*) (with description of this species for the first time from Iran)[16]. Yaghobi-Ershadi studied larval nests of some species of *Anopheles* in the south of the country. Manouchehri and Rouhani studied larval nests of *An. d'thali* and *An. stephensi* in southern Iran. Eshghy described some larval nests of *An. fluviatilis*[17]. Moosa-Kazemi et al. determined mosquitoes' fauna and ecology (including species such as *An. superpictus* and *An. maculipennis*) in the counties of Zarin-Shahr and Mobarakeh[18]. Also, Azari-Hamidian *et al*. published distribution and characteristics of larval nests

of mosquitoes in the County of Rasht[19]. In another study, Azari-Hamidian has noted characteristics of *Anopheles'* larval habitats in the Guilan Province[17]. In a study, Ghanbari has noted the physical and chemical properties of the *Anopheles* mosquitoes' larval habitats in Iran-Shahr County[17]. Despite the researches that some of them were mentioned, there is no information about the distribution and characteristics of the larval nests of *Anopheles* (especially *An. superpictus*) in the west of Iran. Therefore, the aim of this study was to investigate the distribution and characteristics of larval nests of the malaria vectors in the Aligudarz County during April-December 1997.

2. Materials and methods

Aligudarz County in the east of Luristan Province and in the west of Iran is based on the Zagros Mountains. Its area is 5338 km^2 with a population of 145000 people. Average rainfall in this county has fluctuated 450-800 mm per year. The climate is temperate mountains and its altitude is 2000 meters above sea level. In terms of geographical location, it is between 49°42′ E longitude and 33°24′ N latitude. This descriptive and cross-sectional study was aimed to determine the distribution and features of the larval nests of malaria vectors, from late April to late November 1997. A total of 115 larval habitats were visited and identified in seven rural districts: Eastern Zalaghi, Eastern Zez, Farsesh, Pishkoh-Zalaghi, Mahroo, Eastern Pachelak and Eastern Berberud. Sampling stations were selected randomly. Two rural districts of Eastern Zalaghi and Eastern Zez were selected as fixed villages; and sampling was done every 15 d. The other rural districts were selected as variable villages, larvae hunting were done for at least three occasions in the spring, summer and autumn.

Anopheline larvae were collected by the ladle or with a dropper from natural and artificial larval nests and kept in lactophenol solution. Larvae of each type of larval nests were kept in separate test tubes; and all physical-chemical properties of the larval nests such as status of habitat (permanent or temporary, stagnant or running), type of habitat (natural or artificial), vegetation status (with or without plants), type of bed, position of sun (sun or shade) and water condition (clean or turbid) were recorded. After at least 2 d, microscopic slides of 3rd and 4th larval instars were prepared with the help of liquid iodophor solution and were identified with key of Iranian *Anopheles* identification[20].

3. Results

During this study, 9620 of *Anopheles* larvae were collected from 115 larval habitats. In this study, totally 8 species of *Anopheles* larvae and 2 morphological forms of *Anopheles* larvae were collected, identified and reported including *An. superpictus* (form A), *An. superpictus* (form B), *Anopheles turkhodi* (*An. turkhodi*), *An. maculipennis* complex, *Anopheles marterii* (*An. marterii*), *An. d'thali*, *Anopheles claviger* (*An. claviger*), *An. stephensi* and *An. apoci*. For the first time in Aligudarz County, species of *An. maculipennis* complex, *An. stephensi* and *An. apoci* were captured. Also in this study, for the first time, morphological form B of *An. superpictus* was reported that its larvae were with collar, two pairs of frontal hairs, two pairs of palmate hair in each of abdominal segments and oval form accessory tergal plates.

The most abundant of anopheline larvae caught were in the rural districts of Eastern Zalaghi (35.65%) and Eastern Zez (32.90%) and the lowest in the Mahroo (3.42%) and Eastern Berberud (3.45%). Meanwhile, *An. superpictus* form A (92.57%) was the dominant species anopheline larvae of the region. The most abundant *An. superpictus* form A larvae were obtained in the villages of Eastern Zalaghi (37.2%) and Eastern Zez (32.9%) and the lowest in Eastern Berberud (2.84%) and Mahroo (2.89%) (Table 1). Abundance of anopheline larvae hunted in the mountainous regions and plains were 87.9% and 12.1% respectively. *An. superpictus* (form B), *An. turkhodi*, *An. d'thali*, *An. marterii*, *An. stephensi* and *An. apoci* were collected only from the larval nests in mountainous regions. The most frequently anopheline larvae

Table 1

Abundance (numbers & percentage) of larvae of anopheline species caught from larval habitats separately on the rural districts, Aligudarz County, Iran.

Rural districts	*An. superpictus* (form A) *n* (%)	*An. superpictus* (form B) *n* (%)	*An. maculipennis* *n* (%)	*An. turkhodi* *n* (%)	*An. d'thali* *n* (%)	*An. claviger* *n* (%)	*An. marterii* *n* (%)	*An. stephensi* *n* (%)	*An. apoci* *n* (%)	Total *n* (%)
Eastern Zalaghi	3315 (95.80)	24 (0.70)	30 (0.90)	43 (1.20)	6 (0.17)	8 (0.23)	31 (0.90)	3 (0.10)	– (–)	3430 (100.00)
Eastern Zez	2932 (92.70)	30 (1.00)	4 (0.10)	151 (4.80)	7 (0.20)	3 (0.10)	27 (0.80)	9 (0.30)	– (–)	3163 (100.00)
Pishkuh-Zalaghi	831 (94.50)	– (–)	– (–)	21 (2.40)	27 (3.10)	– (–)	– (–)	– (–)	– (–)	879 (100.00)
Farsesh	558 (94.00)	4 (0.60)	26 (4.20)	2 (0.30)	2 (0.30)	2 (0.30)	– (–)	2 (0.30)	– (–)	626 (100.00)
Mahroo	257 (78.20)	1 (0.30)	– (–)	56 (17.00)	1 (0.30)	– (–)	– (–)	9 (2.70)	5 (1.50)	329 (100.00)
Eastern Berberud	253 (76.20)	– (–)	64 (19.30)	– (–)	– (–)	15 (4.50)	– (–)	– (–)	– (–)	332 (100.00)
Eastern Pachehlak	729 (87.70)	– (–)	102 (12.30)	– (–)	– (–)	– (–)	_ (–)	– (–)	– (–)	831 (100.00)
Total	8905 (92.57)	59 (0.61)	226 (2.34)	273 (2.84)	43 (0.45)	28 (0.29)	58 (0.61)	23 (0.24)	5 (0.05)	9620 (100.00)

collected in mountainous regions and plains was *An. superpictus* (form A) (Table 2).

Table 2

Abundance (numbers & percentage) of larvae of anopheline species caught from larval habitats separately on the mountains and plains, Aligudarz County, Iran.

Species	Mountain *n* (%)	Plain *n* (%)	Total *n* (%)
An. superpictus (form A)	7923 (89.0)	982 (11.0)	8905 (100.0)
An. superpictus (form B)	59 (100.0)	– (–)	59 (100.0)
An. maculipennis complex	60 (26.5)	166 (73.5)	226 (100.0)
An. turkhodi	273 (100.0)	– (–)	273 (100.0)
An. d'thali	43 (100.0)	– (–)	43 (100.0)
An. claviger	13 (46.4)	15 (53.6)	28 (100.0)
An. marterii	58 (100.0)	– (–)	58 (100.0)
An. stephensi	23 (100.0)	– (–)	23 (100.0)
An. apoci	5 (100.0)	– (–)	5 (100.0)
Total	8457 (87.9)	1163 (12.1)	9620 (100.0)

Overall, 12 types of the larval nests were observed in Aligudarz County that 9 types of them were natural larval nests and the other 3 types were artificial larval nests. Natural larval nests included the sides of rivers, grasslands, wetlands, pits of river beds, springs, rain water pits, edges of streams, marshes, and the footprints of animals that were respectively 54.8%, 8.7%, 6.9%, 3.5%, 3.5%, 2.6%, 2.6%, 1.7% and 0.9 % of the larval habitats. Also, artificial larval nests included plots of rice cultivations, sides of the concrete curbs and irrigation channels of farms that formed 12.2%, 1.7% and 0.9% of larval habitats, respectively. Overall, of 115 positive larval habitats, 85.2% were natural larval nests and the rest were artificial. Distribution and features of larval nests of identified species are as follows:

3.1. *An. superpictus (form A)*

Of 9620 *Anopheles* larvae hunted, 92.57% were *An. superpictus* that were found in all rural districts. Among the larval nests containing this species, 67.8% and 32.2% were permanent and temporary respectively, also, 62.6% of them were of stagnant water; and 37.4% were of the running water. The larval nests without plants, sunny, with a sandy bottom and clean water formed respectively 46.9%, 88.7%, 55.7% and 94.8% of the total larval habitats. Most of natural larval nests and most of the artificial larval nests were located respectively in the sides of the rivers (62.2%) and in the plots of rice cultivations (82.3%) (Table 3).

Table 3

Ecological characteristics of anopheline larval habitats and percentage of in Aligudarz County, Iran.

Characteristics of larval habitats		ASU (form A)	ASU (form B)	AM complex	AT	AD	AC	AM	AS	AA
Type of water	1. Permanent	67.8	50.0	51.6	68.0	75.0	75.0	80.0	43.7	75.0
	2. Temporary	32.2	50.0	48.4	32.0	25.0	25.0	20.0	56.3	25.0
Stream of water	1. Current	37.4	25.0	29.0	34.0	58.3	37.5	33.3	43.7	75.0
	2. Stagnant	62.6	75.0	71.0	66.0	41.7	62.5	66.7	56.3	25.0
Status of plant	1. Out of water	36.5	50.0	61.3	27.7	25.0	37.5	13.3	12.5	25.0
	2. The water level	12.2	4.0	6.4	14.9	16.7	–	26.7	12.5	25.0
	3. Under water	4.4	–	–	8.5	–	–	20.0	25.0	–
	4. Without plants	46.9	46.0	32.3	48.9	58.3	62.5	40.0	50.0	50.0
Sunlight status	1. Sunny	88.7	87.5	93.5	95.7	91.7	87.5	93.3	87.5	100.0
	2. Shadow	0.9	–	–	–	–	–	–	–	–
	3. Semi-Shade	6.9	8.3	6.5	4.3	8.3	12.5	–	6.2	–
	4. Shadow-Sun	3.5	4.2	–	–	–	–	6.7	6.3	–
	1. Muddy	40.8	50.0	54.8	36.2	41.7	37.5	33.3	43.7	75.0
Bottom of nest	2. Sandy	55.7	44.8	45.2	63.8	58.3	62.5	66.7	56.3	25.0
	3. Concrete	3.5	5.2	–	–	–	–	–	–	–
Status of water	1. Turbid	5.2	4.2	9.7	2.1	–	–	6.7	6.2	–
	2. Clean	94.8	95.8	90.3	97.9	100.0	100.0	93.3	93.8	100.0
The natural larval habitats	1. Edge of river	62.2	53.2	76.1	75.6	70.0	42.8	80.0	43.7	100.0
	2. Pit of river bed	6.1	6.7	–	7.3	10.0	14.3	–	12.5	–
	3. Spring	4.1	6.7	–	2.4	–	–	13.3	12.5	–
	4. Wetland	8.1	6.7	4.8	4.9	–	–	–	18.8	–
	5. Grassland	9.2	13.3	14.3	7.4	10.0	–	–	12.5	–
	6. Pit of rain water	3.1	6.7	–	–	–	14.3	–	–	–
	7. Edge of stream	3.1	6.7	–	–	10.0	14.3	–	–	–
	8. Marsh	3.1	–	4.8	2.4	–	14.3	6.7	–	–
	9. Footprint of animal	1.0	–	–	–	–	–	–	–	–
Artificial larval habitat	1. Plot of rice cultivation	82.3	87.5	90.0	100.0	100.0	–	–	–	–
	2. Irrigation channel of farm	5.9	–	10.0	–	–	100.0	–	–	–
	3. Side of curb	11.8	12.5	–	–	–	–	–	–	–

ASU: *An. superpictus*, AM: *An. maculipennis* , AT: *An. turkhodi*, AD: *An. d'thali*, AC: *An. claviger*, AM: *An.* marterii, AS: *An. stephensi*, AA: *An. apoci*.

An. superpictus (form A) that is dominant species in Aligudarz County caught in all larval nests. This species was obtained alone in 32.1% of larval nests. This *Anopheles* has been obtained 14.8% along with *An. maculipennis* complex, 13.9% with *An. turkhodi*, 5.2% with *An. turkhodi* and *An. marterii*, 2.6% with *An. d'thali*, 2.6% with *An. maculipennis* complex and *An. turkhodi*, and finally along with other species (including *An. apoci*) between 0.87%–1.74% of larval habitats. *An. superpictus* is a vector of malaria in the central plateau and southern parts of the country[14].

3.2. An. superpictus (form B)

The larvae of this species were hunted from the larval nests in the rural districts of Farsesh, Eastern Zalaghi, Eastern Zez and Mahroo that all are mountainous areas of Aligudarz County during June to September. The abundance of this species was 0.61%; and was caught in the 24 larval habitats. Larvae of this *Anopheles* usually were collected from permanent and temporary waters, and the most from stagnant waters. This species has been active in the waters where plants were outside or on the surface of waters; 46% of larvae were caught from the larval nests without plants; it mostly lays eggs in the sunny larval nests (87.5%). It often chooses larval habitats with sandy and muddy bottom and prefers more clean water. Natural larval nests of this species is often in the rivers' sides (53.2%) and larval artificial nests is often plots of rice cultivations (87.5%) and the others are sides of the concrete curbs (Table 3). Larvae of this *Anopheles* have been gathered along with larvae of *An. superpictus* (form A), *An. turkhodi*, *An. Stephensi*, *An. d'thali*, *An. maculipennis* complex, *An. marterii* and *An. claviger* in the areas under its distribution.

3.3. An. maculipennis complex

These species included 2.34% of all larvae caught from the area. They were hunted from all the villages under investigation but Pishkuh-Zalaghi and Mahroo. These species are seen almost equally in the permanent and temporary waters. More than 93.0% of larval habitats of these species had been exposed to the sun. More than 61.0% of their larval nests situated in the plant out of the water. This complex often breeds in stagnant waters (71.0%) and is inclined to egg-laying on the clean water (90.3%). They select more than 76.0% of their natural larval nests on the river sides and also, 90.0% of artificial larval nests in the plots of rice cultivations (Table 3). Other species that their larvae were collected with larvae of this complex are *An. superpictus* (form A), *An. superpictus* (form B), *An. turkhodi*, *An. marterii*, *An. stephensi*, *An. claviger and An. d'thali*.

An. maculipennis complex includes several species of sibling that are distinguishable based on morphology and designs on eggs. Eleven species of *An. maculipennis* complex are in the Palearctic region. So far species of *An. maculipennis*, *An. sacharovi* and *Anopheles persiensis* have been identified based on the molecular methods in Iran. Also, *Anopheles melanoon* and *Anopheles messeae* have been confirmed in Iran according to the golden standard of the egg morphology that is for identifying the members of this complex[10]. According to various reports, eight species from the above complex have been reported in Iran, including *An. maculipennis*, *An. sacharovi*, *Anopheles persiensis*, *An. subalbinus*, *Anopheles melanoon*, *Anopheles messeae*, *Anopheles atroparvus* and *Anopheles martinus*[10,11]. Some members of this complex are the main vectors of malaria in the central plateau (north of the Alborz and Zagros mountain ranges). *Dirofilaria immitis* and Calvo arbovirus are transmitted by it[11,19]. The stable of domestic animals have been mentioned as the most suitable habitats and a resting places for adults of mentioned complex in the provinces of Guilan and Mazandaran; but, this complex has been caught from human dwellings, and even outdoors, too[21].

3.4. An. turkhodi

This species is 2.84% of the total larvae caught from the region and was collected from all the villages under study except Eastern Berberud, Eastern Pachelak. The majority of this species have been caught in permanent waters (68.0%) and 66.0% in the stagnant waters which have plants. About 48.9% of larval nests had no plants. This species lays egg in the larval nests that are exposed to the high sunlight (95.7%) and have clean water (97.9%). It chooses their larval nests mostly in sandy places (63.8%) and in plots of rice cultivations. Nearly 75.6% of its natural larval nests are on the sides of the rivers (Table 3). The larvae of this mosquito have been collected along with larvae of all species of *Anopheles* of the region.

3.5. An. d'thali

This species included 0.45% of the total larvae collected; and like *An. turkhodi*, it was collected exclusively from mountainous areas including the villages of Mahroo, Eastern Zez, Eastern Zalaghi, Pishkuh-Zalaghi and Fersesh. This *Anopheles* was found often in permanent waters (75%) and current waters (58.3%) with or without plants. This study includes 12 larval nests; and introduces *An. d'thali* as a mosquito that is heliophilia; and prefers clean waters for egg-laying. Seventy percent of natural larval nests were in the sides of rivers; but artificial larval nests were only plots of rice cultivations (Table 3). Larvae of this *Anopheles* were caught along with all other *Anopheles* species of the region except *An. apoci*.

3.6. An. claviger

This species included 0.29% of the total larvae caught; and was caught from the villages of Fersesh, Eastern Zalaghi, Eastern Zez (mountains) and Eastern Berberud (plains). This *Anopheles*

often was found in the larval nests with permanent waters (75%) and stagnant waters (62.5%). It is a heliophilia mosquito, too (Table 3). Larvae of this species were collected with larvae of other *Anopheles* species of region under study except *An. apoci*.

3.7. A. marterii

This species formed 0.61% of the total larvae collected in this study. Larvae of this species were collected only from the villages of Eastern Zez and Eastern Zalaghi (mountains). This species often egg-lays in the larval habitats containing permanent waters (80.0%), stagnant waters (66.7%) and clean waters (93.3%) where are exposed to sunlight (93.3%). This *Anopheles* was not found in the artificial larval nests; and 80.0% of its larval nests consisted of river sides (Table 3). Larvae of *An. marterii* were obtained along with larvae of *An. superpictus* (form A), *An. stephensi*, *An. claviger*, *An. turkhodi*, *An. maculipennis* complex and *An. d'thali* in the same larval habitats.

3.8. An. stephensi

Larvae of this species accounted for 0.24% of the *Anopheles* caught; and were obtained in the villages of Fersesh, Eastern Zalaghi, Eastern Zez and Mahroo which are of mountainous regions. This species lays eggs approximately in a same proportion in the permanent or temporary larval nests and so in stagnant or running waters; and this species is heliophilia. It often shows interest in egg-laying in the clean waters; however, it lays eggs in the larval nests with or without plants equally. Larvae of this species were not found in artificial larval nests (Table 3). Larvae of this species were caught with larvae of all the *Anopheles* species of the region except *An. apoci*.

3.9. An. apoci

This species was found only in the village of Mahroo where is completely mountainous and its climate is somewhat warm. And 0.05% of *Anopheles* caught was *An. apoci*. This species is a species completely heliophilia that preferres egg-laying on the clean, permanent and current waters and its natural larval nests was only river sides (Table 3). The larvae of this *Anopheles* were found only with larvae of *An. superpictus* (form A) and *An. turkhodi*.

4. Discussion

During this study, species of *An. superpictus*, *An. maculipennis* complex, *An. turkhodi* and *An. d'thali* with the highest geographical distribution were collected from all or most parts of the studied regions. *An. superpictus* was collected mainly from mountainous regions; *An. maculipennis* was collected mostly from plain regions; and two last species were collected only from mountainous areas. In contrast with the mentioned species, *An. claviger*, *An. marterii* and *An. stephensi* had a limited distribution. *An. claviger* was collected approximately in a same proportion from the plain and mountainous areas; and *An. marterii*, and *An. stephensi* were collected only from the highland areas. *An. claviger* had the lowest distribution in the regions and was only found in a mountainous rural district.

According to the results of this investigation, it is clear that the three species, *An. superpictus* (with 93.18%), *An. turkhodi* (2.84%) and *An. maculipennis* complex (2.34%) accounted for the highest percentage of the larvae caught. These species were collected from types of natural larval habitats (often the sides of rivers) and artificial larval habitats (mainly plots of rice cultivations); and they were anopheline dominant species. These three species of *Anopheles* selected the clear waters; and almost entirely are heliophilia. Also, these *Anopheles* lay eggs in the current or stagnant waters, permanent or temporary, with or without plants. It is worthy note that *An. marterii* (0.61%) and *An. claviger* (0.29%) in terms of percentage of the species composition are in the fourth and the fifth ranks respectively. Larval habitats of these two species have less diverse; and their natural larval nests were mainly in the rivers edges. *An. marterii* was not egg-laying in the artificial larval habitats, but *An. claviger* in artificial larval nests was only collected in the irrigation channels of the farms. These two species of *Anopheles* were mostly heliophilia and were egg-laying just in the clean and often in the stagnant waters; and they laid eggs equally in both larval nests, with or without the plants. In contrast, other species, *i.e. An. stephensi* (0.24%), *An. d'thali* (0.29%) and *An. apoci* (0.05%) had less abundance and less diversity of larval habitats. *An. apoci* and *An. stephensi* were not found in artificial larval nests. *An. d'thali* was collected in the artificial larval nests only from plots of rice cultivations. The dominant natural larval nests of *An. stephensi* and *An. d'thali* were on river sides. Also, *An. apoci* was caught in these larval habitats only on the river sides. As can be seen, larval nests of anopheline species were almost similar in terms of some characteristics such as being clean and sunny and to be situated by sides of the rivers. But in the study of Azari-Hamidian in Guilan Province, anopheline larval nests had a similarity in terms of characteristics such as having clear, stagnant and temporary water[17]. In this study, the most important habitats of larval nests were river sides (54.8 %), plots of rice cultivations (12.2%) and grasslands (8.7%) with permanent or temporary, stagnant or running and clean waters, with or without plants, sandy or muddy bottom and exposed to sunlight. But the most of the anopheline larvae (86.9%) in the Guilan Province were collected from the natural larval nests like pits of rivers' bed (46.4%) and pits of rain water (33.1%) with clean waters (95.3 %), temporary waters (98.3%), stagnant waters (99.5%), with plants (69.9%), muddy beds (42%) or gravel beds (39.7%), exposure to sunlight (69.6 %) or shade (22.7%). In the artificial larval nests, the majority of larvae were caught in the plots of the rice cultivations (64.8%),

and in the irrigation channels of fields (21.3%). It is worthy note that in the mentioned study, anopheline fauna included *An. maculipennis* complex (54.4%), *An. hyrcanus* (22.4%), *Anopheles plumbeus* (13%), *An. claviger* (6.3%) and *An. superpictus* (3.9%)[17].

In the present study, also any species had different characteristics compared to any other species, for example, *An. d'thali* and *An. claviger* preferred mostly larval nests without plants, but *An. superpictus*, *An. maculipennis* complex and *An. turkhodi* preferred mostly larval nests with plants. *An. superpictus*, *An. maculipennis* complex, *An. claviger* and *An. stephensi* had more adaptation with stagnant waters, but *An. d'thali* and *An. apoci* had more adaptation with running waters. Among the artificial larval nests, species of *An. superpictus*, *An. maculipennis* complex, *An. turkhodi* and *An. d'thali* select mostly plots of rice cultivations, but *An. claviger* selects irrigation channels of farms for egg laying. *An. superpictus*, *An. turkhodi*, *An. d'thali*, *An. claviger*, *An. marterii* and *An. stephensi* were collected mainly from larval habitats with sandy bottom; but *An. maculipennis* complex and *An. apoci* were collected mostly from larval habitats with muddy bottom.

In Azari-Hamidian's study, *An. superpictus* preferred larval habitats without plants (65.6%) but *An. maculipennis* complex (83.2%) and *An. claviger* (67%) preferred larval habitats with plants. This finding is similar with the present study only in terms of the *An. maculipennis* complex. Also in study of Azari-Hamidian, *An. superpictus* (100%), *An. maculipennis* complex (98.8%) and *An. claviger* (92.8%) showed interest in the current waters, which is not similar to the present study. Also in Guilan Province, regarding the artificial larval nests, *An. claviger* and *An. maculipennis* complex selected wells (100%) and the rice cultivation farms (61.6%) for egg-laying respectively. But *An. superpictus* was not found in the artificial larval habitats. The similarity of this finding with the present study was only about *An. maculipennis* complex. Horsefall (1955) believes that wells are the most important larval habitat of *An. claviger*[17]. Mossa-Kazemi *et al.* collected larvae of *An. superpictus* in rice fields in Esfahan[18]. This finding is identical with the present study. In researching the larval habitats of anopheline larvae in Guilan Province, larvae of *Anopheles plumbeus* was obtained only from cavities of the trees and the used tires of vehicles[17]. However, our study did not found this species. It is noted that in the present study, tree cavities and used tires were not searched in terms of catching the anopheline larvae. In research of Guilan Province, the main natural larval nests of *An. superpictus*, *An. maculipennis* complex and *An. claviger* were rain water pits (67.2%), the pits of the river bed (67%) and rain water pits (66.3%), respectively. Zaim collected larvae of *An. claviger* from the sides of the rivers in Kashan. Macan (1950) emphasized that classical larval habitats of *An. superpictus* in the west of Iran is edges of rocky rivers, although larvae of this species were collected in the sunny or semi-shade larval nests with clean water. Yaghoobi-Ershadi *et al.* in Minab County, reported the larval habitats of this *Anopheles* were on the sides of rivers (80%), with stagnant waters (62.5%), temporary waters (100%), without plants (50%), semi-shade (56.3%) or with muddy beds (56.2%)[17]. In the study of Azari-Hamidian in Guilan Province, *An. superpictus* (88.5%) and *An. maculipennis* complex (89.4%) were heliophilia, but *An. claviger* (66%) was sciophilia[17]. In the present paper, these three species were collected in sunny larval nests. In addition, in the study of Azari-Hamidian, *An. superpictus* (67.8%) and *An. claviger* (73.7%) showed their interest in the larval nests with the muddy bed; and *An. maculipennis* complex (49.6%) showed its interest in the stony bed[17]. In the present study, the first two species preferred larval habitats with sandy bottom (55.7% and 62.5% respectively) and the third species preferred larval nests with muddy bed (54.8%). But in the study of the ecology of mosquitoes in the County of Rasht[19], larval nests of *An. maculipennis* complex were reported with muddy bed, and often plot of rice cultivations and irrigation channels of the farms that confirms the findings of this study. Patton (1905) collected larvae of *An. d'thali* from springs and wells[22]. The larval nests of this species in Hormozgan Province were in a margin of rocky rivers, springs, holes around the springs with or without plants, pits of river bed and the irrigation channels of dates. Moreover, the larvae of this species were found in mineral water and the very salty water[22]. In this study, larval nests of *An. d'thali* were on sides of rivers, holes of river bed, grasslands, streams, and the plots of the rice cultivations.

Yaghoobi-Ershadi *et al.* collected larvae of *An. d'thali*, *An. fluviatilis*, *An. multicolor*, *An. stephensi* and *An. turkhodi* with each other in the same larval habitats in Minab County[17]. Mossa-Kazemi *et al.* collected larvae of *An. superpictus* and *An. maculipennis* complex together from plots of rice cultivations in Isfahan Province[18]. Larvae of *An. superpictus* along with *An. sacharovi* were caught in the same larval habitats in Ardabil Province[23]. Dow (1953) collected larvae of *An. superpictus* along with *An. maculipennis* complex (and perhaps *An. sacharovi*) in different regions of Iran. Horsfall (1955) reported larvae of *An. claviger* along with *An. maculipennis* complex, *An. marterii*, and *An. superpictus* in different areas of distribution. Dow (1953) collected larvae of *An. maculipennis* along with *An. hyrcanus*, *An. sacharovi* and *An. superpictus*. Manouchehri caught *An. d'thali* along with *An. stephensi* and *An. fluviatilis* in mountainous areas of Hormozgan Province. The larvae of dominant species in Guilan Province (*An. maculipennis* complex) were collected along with (and also, alone) all members of the anopheline fauna of the region *i.e. An. superpictus*, *An. hyrcanus* and *An. claviger*[17]. In this study, larvae of the dominant species of this region (*An. superpictus*) were found along with (and also, alone) all species of *Anopheles* of this county.

Comparison of the results of this study with Maleki's study in several years ago[24] shows that the species of *An. superpictus* (form B), *An. maculipennis* complex, *An. stephensi* and *An. apoci* that did not reported previously in Aligudarz County, were collected and identified in this study. Finding of new species in this study could be due to changing of weather conditions, changes in human ecology, and large sizes of sample, collecting larvae at all seasons of *Anopheles* activity and catching

the larvae in regions with different topography (plains and mountains).

In the study of species of *Anopheles* of Kurdistan Province, Vahabi (2001) has been reported, *An. superpictus*, *An. maculipennis* complex, *Anopheles sergenti*, *An. claviger*, *Anopheles algeriensis*, *An. sacharovi* and *An. marterii*. The most abundant species caught (more than 50%) was related to *An. superpictus*[25]. In the study on fauna mosquitoes in Bazoft division of Farsan County, some species were caught including *An. superpictus* (67.2%), *An. maculipennis* complex (27.2%), *An. d'thali* (2.4%), *An. marterii* (2.2%) and *An. claviger* (1%)[26]. In comparison with the present study, in the above mentioned two studies that were conducted in the west of the country, species of *An. stephensi*, *An. turkhodi* and *An. apoci* were not reported. Also, in the Kurdistan Province, species of *Anopheles sergenti* and *Anopheles algeriensis* were found, but they were not found in the present study. These differences are probably due to disparities in climatic conditions. Also in these studies, the most abundant species was related to *An. superpictus* that is identical with the result of this study.

In future studies, research on the physical and chemical characteristics of larval nests such as pH, dissolved oxygen, turbidity, organic and inorganic compounds, electrical conductivity, temperature, total hardness, nitrate, phosphate, calcium, sulphate, nitrite and chloride are recommended.

Considering species of *An. superpictus*, *An. maculipennis* complex, *An. d'thali* and *An. stephensi* are among the vectors of malaria in Iran, a careful study of the ecology and biology of them and molecular systematic of sibling or polymorphic species can be done for further researches.

Also, given the being zoophilic of the dominant species in this region (*An. superpictus*), the use of zooprophilaxis method (use of animals to attract vector mosquitoes,) can reduce the numbers of bloodsucking of *Anopheles* on human; and thus can prevent the risk of malaria transmission in areas that are potentially exposed to malaria.

Operational programs for controlling the *Anopheles* mosquitoes, if necessary, can only be concentrated on species of *An. superpictus*. Because of being exophilic of this species, operations of control against the adults are almost impractical. Also, because the larval habitats of this species is very diverse (the sides of rivers, grasslands, pits of river beds, springs, wetlands, rain water pits, rice cultivation plots, irrigation canals and sides of the concrete curbs) the effective larviciding with good temporal and spatial coverage is infeasible . Therefore, the personal protection methods such as insecticide impregnated bed nets should be emphasized.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

Hereby, the authors gratefully acknowledge the cooperation of Faculty of Public Health, Tehran University of Medical Sciences and Luristan University of Medical Sciences, Health-Medical Systems of Aligudarz County in executing this study. This work was funded for some logestic supports and physical facilities by Luristan University of Medical Sciences (Grant No. 2501).

Comments

Background

Malaria disease is a worldwide challenge that can be found in great parts of the globe. Malaria had being widely prevalent for a long time in Iran. Before starting any malarial control program in Iran about 60% of 13 millions populations on that time were living in malaria endemic regions. At present, about 80% of all malaria cases in the country occur in the provinces of the south-east. The vectors of the disease are *Anopheles* mosquitoes. For successful control in each focus of the disease, ecological studies should be performed. In this regard, the present investigation was done in Aligudarz County (Luristan Province, western Iran).

Research frontiers

This study found ecology and larval breeding places features of anopheline mosquitoes such as, water situation, substrate type, sunlight status, plant status, water stream, *etc*. These data are needed for effective control of malaria vectors in the mentioned area.

Related reports

In the study of species of *Anopheles* of Kurdistan Province (western Iran), the most abundant species was related to *An. superpictus* that is identical with the result of this study (Vahabi 2001). Comparison of the findings of this research with Maleki's survey (2003) displays that the species of *An. superpictus* (form B), *An. maculipennis* complex, *An. stephensi* and *An. apoci* that did not found previously in Aligudarz County, were hunted and identified in this project. Macan (1950) stressed that ordinary larval habitats of *An. superpictus* in the west of Iran is edges of rocky rivers which is in agreement with this study.

Innovations and breakthroughs

In this research, three species of *Anopheles* including *An. maculipennis* S.L , *An. stephensi* and *An. apoci* were reported for the first time in the study area. Also, *An. superpictus* was the most abundant *Anopheles* species and river sides were the most important larval habitats.

Applications

The results of present study on the ecology and larval places of anopheline mosquitoes could be applied in integrated vector management programs in the western Iran.

Peer review

It is a good research in which the authors found some aspects of ecology and characteristics of breeding places of anopheline mosquitoes in the western Iran .

References

[1] WHO. *World malaria report 2009*. Geneva: WHO Press; 2009, p. 66.

[2] Hanafi-Bojd AA, Vatandoost H, Philip E, Stepanova E, Abdi AI, Safari R, et al. Malaria situation analysis and stratification in Bandar Abbas county, southern Iran, 2004-2008. *Iran J Arthropod Borne Dis* 2010; **4**(1): 31-41.

[3] Ghaffrani S, Mahdavi S, Moulana Z, Mouodi S, Karimi-Nia H, Bayani M, et al. Malaria in Mazandaran, northern Iran: passive case finding during 1997-2012. *Iran J Parasitol* 2012; **7**(3): 82-88.

[4] Zoghi S, Mehrizi AA, Raeisi A, Haghdoost AA, Turki H, Safari R, et al. Survey for asymptomatic malaria cases in low transmission settings of Iran under elimination programme. *Malar J* 2012; **11**: 126.

[5] Sargolzaie N, Salehi M, Kiani M, Sakeni M, Hassanzehi A. Malaria epidemiology in Sistan and Balouchestan province during April 2008-March 2011, Iran. *Zahedan J Res Med Sci* 2014; **16**(4): 41-43.

[6] Azari-Hamidian S, Abai MR, Arzamani K, Bakhshi H, Karami H, Ludoni H, et al. Mosquitoes (Dipetra: Culicidae) of North Khorasan Province, northeastern Iran and the zoogeographic affinities of the Iranian and middle Asian mosquito fauna. *J Entomol* 2011; **8**(3): 204-217.

[7] Doosti S, Azari-Hamidian S, Vatoost H, Oshaghi MA, Hosseini M. Taxonomic differentiation of *Anopheles sacharovi* and *An. maculipennis* S.L. (Diptera: Culicidae) larvae by seta 2 (Antepalmate hair). *Acta Medica Iranica* 2006; **44**(1): 21-27.

[8] Harbach RE. The classification of genus *Anopheles* (Diptera: Culicidae): a working hypotheis of phylogenetic relations. *Bull Entomol Res* 2004; **94**(6): 537-553.

[9] Harbach RE. Mosquito taxonomic inventory. [Online] Available from: http://mosquito-taxonomic-inventory.info [Accessed on 23 September 2013].

[10] Sedaghat MM, Howard T, Harbach RE. Morphological study and description of *Anopheles* (anopheles) persiensis, a member of the maculipennis group (Diptera: Culicidae: Anophelinae) in Iran. *J Entomol Soc Iran* 2009; **28**(2): 25-35.

[11] Azari-Hamidian S. Checklist of Iranian mosquitoes (Diptera: Culicidae). *J Vector Ecol* 2007; **32**(2): 235-242.

[12] Hanafi-Bojd AA, Azari-Hamidian S, Vatandoost H, Charrahy Z. Spatio-temporal distribution of malaria vectors (Diptera: Culicidae) across different climatic zones of Iran. *Asian Pac J Trop Med* 2011; **4**(6) 498-504.

[13] Djadid ND, Jazayeri H, Gholizadeh S, Rad ShP, Zakeri S. First record of a new member of *Anopheles* hyrcanus group from Iran: molecular identification, diagnosis, phylogeny, status of Kdr resistance and *Plasmodium* infection. *J Med Entomol* 2009; **46**: 1084-1093.

[14] Shemshad K, Oshaghi MA, Yaghoobi-Ershadi MR, Vatandoost H, Abaie MR, Zarei Z, Faghih-Naini F, Jedari M. [Morphological and molecular characteristics of malaria vector *Anopheles* superpictus populations in Iran]. *Tehran Univ Med J* 2007; **65**(8): 6-13. Persian.

[15] Oshaghi MA, Yaghobi-Ershadi MR, Shemshad K, Pedram M, Amani H. The *Anopheles* superpictus complex: introduction of a new malaria vector complex in Iran. *Bull Soc Pathol Exot* 2008; **101**(5): 429-434.

[16] Hanafi-Bojd AA, Vatandoost H, Oshaghi MA, 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**: 91-100.

[17] Azari-Hamidian S. Larval habitat characteristics of the genus *Anopheles* (Diptera: Culicidae) and a checklist of mosquitoes in Guilan province, Northern Iran. *Iran J Arthropod Borne Dis* 2011; **5**(1): 37-53.

[18] Nikookar Sh, Moosa-Kazemi Sh, Oshaghi M, Yaghoobi-Ershadi M, Vatandoost H, Kianinasab A. Species composition and diversity of mosquitoes in Neka County, Mazandaran Province, Northern Iran. *Iran J Arthropod Borne Dis* 2010; **4**(2): 26-34.

[19] Azari-Hamidian S, Yaghoobi-Ershadi MR, Javadian E. [The distribution and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Rasht County (Guilan province, Iran)]. *Modarres J Med Sci* 2002; **4**: 87-96. Persian.

[20] Azari-Hamidisn S, Harbach RE. Keys to the adult females and fourth-instar larvae of the mosquitoes of Iran (Diptera: Culicidae). *Zootaxa* 2009; **2078**: 1-33.

[21] Azari-Hamidian S, Joeafshani MA, Mosslem M, Rassaei AR. [Adult mosquito habitats and resting-places in Guilan province (Diptera: Culicidae)]. *Hakim* 2003; **6**(3): 55-62. Persian.

[22] Vatandoost H, Shahi M, Hanafi-Bojd AA, Abai MR, Oshaghi MA, Rafii A. Ecology of *Anopheles* dthali Patton in Bandar Abbas district, Hormozgan province, Southern Iran. *Iran J Arthropod Borne Dis* 2007; **1**(1): 21-27.

[23] Yaghoobi-Ershadi MR, Namazi J, Piazak N. Bionomics of *Anopheles* sacharovi in Ardebil province, northwestern Iran diring a larval control program. *Acta Trop* 2001; **78**: 207-215.

[24] Maleki M. [The study of fauna and larval habitat characteristics of the genus *Anopheles* (Diptera: Culicidae) in five provinces, West and southwestern Iran] [dissertation]. Tehran: Tehran University of Medical Sciences; 2003. Persian.

[25] Kazemi SH, Karimian F, Davari B. Culicinae mosquitoes in Sanandaj County, Kurdistan Province, western Iran. *J Vector Borne Dis* 2010; **47**: 103-107.

[26] Kassiri H, Amani H. Bionomics and breeding places of the genus *Anopheles* (Diptera: Culicidae) in Mahroo and Sepid-Dasht districts, Luristan province, western Iran. *Zahedan J Res Med Sci* 2012; **14**(8): 11-17.