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## Original Article

# A Rapid Assessment of Larval Habitats of Mosquitoes (Diptera: Culicidae) in Aqqala County of Golestan Province, Northeastern Iran, Following the 2019 Flood

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## Abstract

**Background:** Mosquitoes (Diptera: Culicidae) are the most important arthropods in public health. Larval habitat features play an important role in their selection by female mosquitoes for oviposition**.** The purpose of this study was to determine the oviposition sites of mosquitoes in Aqqala County of Golestan Province following the flood of 2019.

**Methods:** Two categories of larval habitats were defined: those that naturally occurred and those that were a result of flooding. Samples were collected using dippers with a 350 ml capacity. The collected larvae were preserved in lactophenol solution. Microscope slides were prepared using Berlese medium**.** The specimens were morphologically identified. Larval habitat characteristics were recorded according to the habitat situation (permanent/temporary, running/stagnant), vegetation situation, sunlight situation, substrate type, water situation (turbid/clear) and habitat type (natural/artificial).

**Results:** In total, 774 third- and fourth-instar mosquito larvae were collected from different larval habitats not affected by the flood, including seven species representing four genera. *Culex pusillus* was the dominant species (41.2%). The larvae of *Aedes caspius*, *Cx. pipiens*, *Cx. theileri*, *Culiseta longiareolata* and *Uranotaenia unguiculata* were collected only from artificial habitats, 96.9% of *Cx. tritaeniorhynchus* larvae from artificial habitats and 78.7% of *Cx. pusillus* larvae from natural habitats. No larvae were collected from the larval habitats affected by the flood.

**Conclusion:** Field observations suggested that the flushing of natural larval habitats may cause such a decrease in larval population in the habitats affected by the flood. The presence of the larvivorous fish *Gambusia holbrooki* may also be taken into consideration.

**Keywords:** Culicinae; Flushing; Larval habitat characteristics; Larval breeding places

## Introduction

Mosquitoes (Diptera: Culicidae) are the most important arthropods in public health, because malaria, filarial and arboviral infections are among the most significant diseases transmitted by mosquitoes (1). Two bacteria (agents of anthrax and tularemia), four helminths (agents of dirofilariasis, setariasis, dipetalonemiasis and lymphatic filariasis), two protozoa (agents of human and bird malaria) and seven viruses (agents of avian or fowl pox, bovine ephemeral fever, chikungunya, dengue, Rift Valley fever, Sindbis and West Nile fever) have been recorded in Iran, which are biologically or mechanically known or assumed to be transmitted by


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mosquitoes (2, 3).

According to the most recent classification, the family Culicidae comprises two extant subfamilies, Anophelinae and Culicinae, 11 tribes, 41 genera, and 3727 species (4, 5). Eight genera and 73 species have been recorded in Iran (6). At least 27 species representing six genera of the family have been recorded in Golestan Province of northeastern Iran, Caspian Sea littoral, as follows: *Aedes vexans*, *Ae. echinus*, *Ae. geniculatus*, *Ae. caspius*, *Ae. pulcritarsis*, *Anopheles algeriensis*, *An. claviger*, *An. hyrcanus*, *An. maculipennis* s.s., *An. melanoon*, *An. plumbeus*, *An. pseudopictus*, *An. pulcherrimus*, *An. sacharovi*, *An. superpictus* s.l., *Coquillettidia richiardii*, *Culex hortensis*, *Cx. mimeticus*, *Cx. perexiguus*, *Cx. pipiens*, *Cx. pusillus*, *Cx. territans*, *Cx. theileri*, *Cx. tritaeniorhynchus*, *Culiseta longiareolata*, *Cs. subochrea* and *Uranotaenia unguiculata* (7–13). There are few studies on mosquitoes in Aqqala County; only one has mentioned *Cx. pipiens* and *Cx. tritaeniorhynchus* in this county (13).

Water is necessary for female mosquitoes to lay their eggs as well as for the development of their larval and pupal stages. Females select appropriate larval habitats for the development of their aquatic stages. These habitats are not chosen by chance; rather, each species of mosquito has its own unique biology and traits (14). Different biological factors, such as the presence of phytoplanktons, zooplanktons, algae, bacteria, vegetation and invertebrates and vertebrate predators, such as larvivorous fish, are some of the most crucial factors in the selection of these habitats (14–18). The aforementioned factors are also impacted by different physical and chemical factors such as the water chemical composition, the habitat accessibility to light, temperature, acidity, kind of habitat bed and flow rate (11–14). One physical factor that primarily influences larval habitat characteristics is the speed at which water flows, and it is usually categorized as still or stagnant versus slow-running (14).

Floods are one type of natural disaster that can disrupt and alter the environment, which can have an impact on larval habitats. Floods have the power to alter the amount of nutrients in larval environments, which can either raise or decrease the number of mosquitoes. Additionally, floods can change the diversity of mosquitoes by changing the competitors of aquatic stages in larval environments (19, 20). Floods may lead to outbreaks of several mosquito-borne infections due to their impact on the mosquito population. Barmah Forest virus infection, dengue fever, Japanese encephalitis, malaria, Murray Valley encephalitis, Rift Valley fever, Ross River virus infection and West Nile fever may emerge after floods and occasionally outbreaks have been documented in certain areas (19–25).

Golestan Province and some other areas of Iran experience moderate temperatures, rainy winters and scorching, dry summers. These regions are vulnerable to unexpected floods and overflowing rivers (26). West Nile virus is present in Golestan Province (2, 3, 27). The known vectors of this virus, such as *Ae. caspius* s.l., *Cx. pipiens* and *Cx. theileri* are found in the province (2, 3, 7–13). This province was one of the most significant malaria foci in the past (28). Malaria vectors, *An. maculipennis* s.l., *An. pulcherrimus*, *An. superpictus* s.l. and *An. sacharovi*, have also been collected in the province (2, 7–13). Also, dirofilariasis, caused by the dog heartworm *Dirofilaria immitis*, and its known vector *Cx. theileri* is found in the province (2, 12, 13). Because a flood devastated roughly 18 cities and villages of Golestan Province in 2019 due to heavy rainfall and the overflow of the Gorgan River, this study was carried out to determine the mosquito larval habitats and fauna during the post-flood period in Aqqala County.

## Materials and Methods

### Study area

Aqqala County (37.00958567°N, 54.46138267°E), which translates to "White

Fortress," is situated in the north of the province of Golestan and borders Turkmenistan to the north, Bandar Turkmen and Gamishan to the west, Gorgan and Aliabad Katul to the south, and Gonbad Qaboos to the east. There are two cities, two divisions, five districts and ninety villages in this County. The primary industries that provide revenue for the residents of this city are trade, agriculture and animal husbandry, followed by the selling of handicrafts. The city of Aqqala is situated on the banks of the Gorgan River and serves as its capital. One of the most significant rivers in northeastern Iran is the Gorgan River. This river rises in the Bojnord Aladagh mountain range and travels 200 kilometers across the Turkmen desert regions before entering the Gorgan plain and emptying into the Caspian Sea near the Turkmen port in Khaja Nafs area. The Gorgan River is formed by the confluence of twelve major and minor branches (Fig. 1) (29).

**Mosquito sampling**

Aqqala County experienced flooding in 18 of its cities and villages in April 2019. According to field assessments, three villages and one city from the flooded areas and the same number from the non-flooded areas of the county were randomly chosen to ascertain the larval habitats, fauna and abundance of mosquito larvae in the flooded and non-flooded areas. All sampling localities were in plain topographical areas with a semi-arid climate (Table 1). A month after the flood, in May 2019, every one of the chosen cities and villages was examined and the existing larval habitats were studied. First, larval habitat characteristics such as the habitat situation (permanent or temporary, running or stagnant), vegetation situation, sunlight situation, substrate type, water situation (turbid or clear) and habitat type (natural or artificial) data were recorded on standardized forms. After that, samples were collected using dippers with a 350 ml capacity. Ten dip samples were taken from each habitat at various locations within the larval habitats. All of the third and fourth-instar larvae were gathered in designated receptacles in lactophenol solution and brought to the Medical Entomology Laboratory of Kalaleh Health Center. Berlese medium was used in the preparation of the microscope slides. Morphological taxonomic keys were used to identify the specimens (30).

## Results

Of the 17 flood-created larval habitats studied, no larvae were collected. These habitats had the following characteristics: natural, temporary, stagnant, without vegetation, exposed to full sunlight, with mud substrate and clear water. Nevertheless, 774 larvae, including seven species across four genera, were captured and identified from 17 larval habitats, not affected by the flood. The most prevalent species was *Cx. pusillus* (41.2%) followed by *Cx. tritaeniorhynchus* (25.0%), *Cs. longiareolata* (21.5%), and *Cx. pipiens* (11.5%) (Table 2). *Culex pusillus*, along with *Cx. tritaeniorhynchus* had the broadest distribution and was collected from 11 out of 17 larval habitats. They were co-occurrent in six larval habitats. *Culex pipiens* and *Cs. longiareolata* were collected in five and four larval habitats, respectively. Few specimens of *Ae. caspius*, *Cx. theileri* and *Uranotaenia unguiculata* were found only in one larval habitat (Table 2). The majority of larvae (66.8%) were collected from artificial habitats (mainly from rice fields and rice irrigation channels: 67.5%) (Table 2). *Aedes caspius*, *Cx. pipiens*, *Cx. theileri*, *Cs. longiareolata* and *Ur. unguiculata* were collected only from artificial habitats (Table 2). *Culex tritaeniorhynchus* was mainly collected from artificial habitats (96.9%) (77.5% of those in rice fields) and *Cx. pusillus* mainly (78.7%) from natural habitats (55% of those in ponds) (Table 2). *Culiseta longiareolata* was collected in temporary, stagnant and artificial larval habitats with clear water and mud substrate. *Culex pipiens* was collected in an urban area from permanent running water with a cement substrate (sewage) (77.5%) (Table 2). All larvae were collected in larval habitats with sunlight exposure (Table 2).

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**Table 1.** Locality of villages and cities in Aqqala County, Golestan Province, northeastern Iran, for mosquito sampling, 2019 (All localities are in plain topographical areas with semi-arid climate)

| Flooded village/city | | Non-Flooded village/city | |
|---|---|---|---|
| **Locality** | **Coordinates** | **Locality** | **Coordinates** |
| Aqqala | 37.011916˚N, 54.460829˚E | Goog Tapeh | 37.180239˚N, 54.657471˚E |
| Sallaq Yilqi | 37.027761˚N, 54.527876˚E | Uch Tappeh | 37.045217˚N, 54.669038˚E |
| Mirzali | 37.034041˚N, 54.536320˚E | Qezli | 37.017225˚N, 54.609904˚E |
| Anbaralum | 37.129718˚N, 54.622960˚E | Voshmgir Dam | 37.210850˚N, 54.739837˚E |

**Table 2.** Larval habitat features of collected mosquitoes in Aqqala County, Golestan Province, northeastern Iran, 2019 (*Ae*= *Aedes*, *Cx*= *Culex*, *Cs*= *Culiseta*, *Ur*= *Uranotaenia*)

| Larval habitat characteristics | *Ae. caspius* | *Cx. pipiens* | *Cx. pusillus* | *Cx. theileri* | *Cx. tritaeniorhynchus* | *Cs. longiareolata* | *Ur. unguiculata* | Total Number | Total % |
|---|---|---|---|---|---|---|---|---|---|
| **Habitat situation** | | | | | | | | | |
| **Permanent** | - | 69 | - | - | 1 | 6 | - | 76 | 9.9 |
| **Temporary** | 2 | 20 | 319 | 2 | 192 | 160 | 3 | 698 | 90.1 |
| **Running water** | - | 69 | - | - | 1 | 6 | - | 76 | 9.9 |
| **Stagnant water** | 2 | 20 | 319 | 2 | 192 | 160 | 3 | 698 | 90.1 |
| **Vegetation situation** | | | | | | | | | |
| **With** | 2 | 4 | 154 | 2 | 163 | 48 | 3 | 376 | 48.6 |
| **Without** | - | 85 | 165 | - | 30 | 118 | - | 398 | 51.4 |
| **Sunlight situation** | | | | | | | | | |
| **Full sunlight** | 2 | 89 | 319 | 2 | 193 | 166 | 3 | 774 | 100 |
| **Shaded** | - | - | - | - | - | - | - | - | - |
| **Substrate type** | | | | | | | | | |
| **Mud** | 2 | 20 | 319 | 2 | 192 | 160 | 3 | 698 | 90.1 |
| **Cement** | - | 69 | - | - | 1 | 6 | - | 76 | 9.9 |
| **Water Situation** | | | | | | | | | |
| **Turbid** | - | - | 1 | - | 35 | - | - | 36 | 4.7 |
| **Clear** | 2 | 89 | 318 | 2 | 158 | 166 | 3 | 738 | 95.3 |
| **Habitat Type** | | | | | | | | | |
| **Natural** | - | - | 251 | - | 6 | - | - | 257 | 33.2 |
| **Stream edge** | - | - | 113 | - | 6 | - | - | 119 | 46.3 |
| **Pond** | - | - | 138 | - | - | - | - | 138 | 53.7 |
| **Artificial** | 2 | 89 | 68 | 2 | 187 | 166 | 3 | 517 | 66.8 |
| **Rice field** | - | 19 | 19 | - | 145 | - | - | 183 | 35.4 |
| **Water-storage pool** | 2 | 1 | 3 | - | 38 | - | - | 44 | 8.5 |
| **Dam** | - | - | 8 | 2 | 3 | 32 | 3 | 48 | 9.3 |
| **Sewage** | - | 69 | - | - | 1 | 6 | - | 76 | 14.7 |
| **Rice irrigation channel** | - | - | 38 | - | - | 128 | - | 166 | 32.1 |
| **Total Number** | 2 | 89 | 319 | 2 | 193 | 166 | 3 | 774 | |
| **%** | 0.2 | 11.5 | 41.2 | 0.2 | 25 | 21.5 | 0.4 | 100 | |


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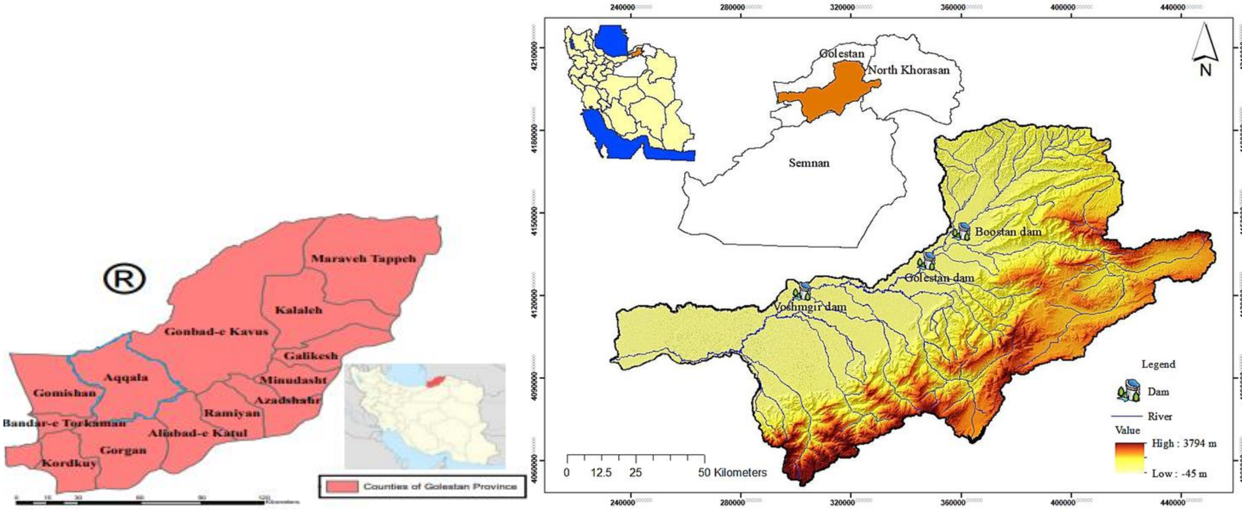


**Fig. 1.** Location of Aqqala County of Golestan Province for mosquito sampling in 2019, as well as the Gorgan River basin in northeastern Iran, adopted from Ghonchepour et al. (29), where the flood took place in 2019 (Scales in the two maps are different)

## Discussion

In the present investigation, mosquito larvae were collected from the habitats not affected by flooding, including seven species across four genera. All these species had been previously reported in Golestan Province; however, only *Cx. pipiens* and *Cx. tritaeniorhynchus* were found in Aqqala County before (13). In earlier studies in Golestan Province, *Cx. pipiens* was the dominant species and was collected in all counties of the province, mainly from artificial habitats (73%) (13). Various studies in Iran have shown that this species has a high adaptability to different larval habitats (12–14). *Culex tritaeniorhynchus* was the second most abundant species, collected in most counties, and 49.8% from artificial habitats (13). However, in this study, *Cx. pusillus* was the dominant species, with *Cx. tritaeniorhynchus* ranked second in Aqqala County (Table 2). Interestingly, whereas *Cx. pusillus* had been collected for the first time as just one larva from a natural habitat before (13). In this study, the species was captured much more abundantly due to the increase in natural habitats after the flood of 2019 (Table 2). It seems that a high increase in *Cx. pusillus* population in the region is the interesting observation of the present investigation. Little is known about the biology of the adults and the vector potential of *Cx. pusillus*. Females probably do not feed on humans (1).

In this study, it is generally found that more larvae were collected from temporary larval habitats than permanent ones and stagnant water habitats compared to slow-running water habitats; additionally, more larvae were collected from sunny habitats with muddy substrates and clear water and from artificial habitats than natural ones (Table 2). This is, to some extent in consistent with the previous studies in Golestan Province (11–13). Some variation may be seen due to sampling and geographical differences, for example, more larvae had been collected from permanent, natural habitats with slow-running water in Kalaleh County before (12). Also, there is some species-specific variation; for example, no anopheline mosquito was collected during this research; however, *Anopheles* larvae were found before in the province, not Aqqala County (11–13). It is noteworthy that despite the post-flood increase in natural habitats, more larvae were still collected from artificial habitats.

In the present study, no larvae were captured in the larval habitats formed by flooding. The absence of larvae could be attributed to various factors, including flushing larval habitats; however, since the larvivorous fish *Gambusia holbrooki* was abundantly observed in all these larval habitats, it can be inferred that this fish may have an impact on the population of larvae. *Gambusia holbrooki* is a non-native and invasive species in Iranian inland waters, first introduced from Italy to the northern provinces, including Golestan, to combat anopheline vectors of malaria. This fish inhabits streams, rivers, lakes, marshes, drainage channels, muddy and clean waters, and other nutrient-rich water sources (15, 18). They have survived over the years due to their high tolerance to temperature and salinity changes and their prolific reproduction (15, 18). The flood of Aqqala County flushed many larval habitats and at the same time created numerous larval habitats. The mosquito fish, which was already present in the region, entered these habitats, and due to favorable living conditions and high reproduction rates, their population increased.

In a study in India in 2006 (21), it was reported that heavy rains and dam overflow caused a massive flood, submerging parts of the city and causing significant damage to impoverished areas. Following this flood and the creation of larval habitats for mosquitoes, especially *Anopheles*, their abundance increased, leading to a malaria outbreak in the city. Most larval habitats for *Anopheles* were small water collections outside homes and small boats kept in yards due to the port nature of the city (21). Similar conditions were observed in Aqqala County, suggesting a potential increase in disease vectors. However, no larvae were observed in the flood-induced larval habitats for the reasons mentioned before.

Harrison et al. (22), in the USA in 2009, collected about 45 mosquito species to assess mosquitoes and arbovirus activity post-flood. A significant number of the dengue vector *Ae. albopictus* were collected post-flood. Among the West Nile virus vectors, species such as *Cx. pipiens*, *Cx. erraticus*, *Cx. tarsalis*, *Cx. salinarius* and *Cx. restuans* were collected, comprising 38.7% of the total mosquitoes. Three *Culex* species, including *Cx. erraticus*, *Cx. salinarius* and *Cx. pipiens* were reported to be infected with this virus. However, the increase in human infections was not observed. Given the presence of West Nile fever in Golestan Province and the records of *Ae. caspius, Cx. pipiens* and *Cx. theileri*, known vectors of West Nile virus in Iran, no increase in the larvae of these species was observed in the larval habitats following the flood.

In a study in Kenya in 2017 (23), several mosquitoes were notably higher in irrigated areas than in non-irrigated areas; additionally, the populations of *Ae. mcintoshi* and *Ae. ochraceous*, the primary vectors of Rift Valley fever, were higher in regions irrigated during dry and wet seasons than in non-irrigated areas. Similarly, the populations of *Cx. pipiens* and *Cx. univittatus*, secondary vectors of Rift Valley fever, exhibited similar trends. The authors concluded that rainfall, irrigation and humidity directly impacted mosquito abundance.

Vukmir et al. (24) analyzed West Nile infection in Bosnia and Herzegovina during the 2014–2018 period and concluded that 94% of the total number of patients were from flooded areas. However, Mourelatos et al. (25) did not observe any rise in West Nile virus circulation in mosquitoes or human cases after the flood in Thessaly of Greece, during the 2021–2023 period, whereas, *Culex* population increased post-flood. They concluded that this unexpected outcome may be because of various ecological factors, such as disruptions of avian host communities, human displacement, and the timing of the flood during the autumn bird migration period.

In a scoping review (20), it was concluded that dengue incidence decreased in a short-term view (< 1 month) and increased after that (1–4 months) post-flood. The results were mixed and less clear for malaria incidence, but the general conclusion was that the post-flood incidence increased. Also, other findings for Barmah Forest virus infection, Japanese encephalitis, Murray Valley encephalitis, Rift Valley fever and Ross River virus infection showed the possible human outbreaks following the flood. Although the study of the human incidence of mosquito-borne diseases was not among the goals of the present investigation, this should be taken into consideration for future studies.

## Conclusion

Based on the field observation, we suggest that flushing natural larval habitats as well as the presence of the larvivorous fish *G. holbrooki*, may be the reason for such a decrease in the larval population of habitats affected by the flood. Also, the high increase in non-vector *Cx. pusillus* population in natural habitats is a key finding of the present study. However, both active and inactive potential larval habitats with or without larvivorous fish, as well as other physicochemical and biological factors that influence larval populations, should be extensively investigated in the future. This may help us to better analyze the larval population and then the adult population before and after floods during a short-term or long-term period. Also, the study of the human incidence of mosquito-borne infections post-flood is suggested.

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## Ethical considerations

The ethical code IR.GOUMS.REC.1398.154 has been registered for this study.

## Conflict of interest statement

The authors declare there is no conflict of interest.

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