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Fauna and larval habitat characteristics of mosquitoes (Diptera: Culicidae) in Setif Region, North-East of Algeria

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Abstract

Mosquitoes are blood-sucking flying arthropods, known for their critical role as vectors of several infectious diseases such as dengue, chikungunya, malaria, and lymphatic filariasis. Understanding the entomological fauna in a given area is not only essential for defining potential health risks but also to plan and assess mosquito control actions. Our study aimed to identify mosquito larvae species in the province of Setif in Algeria, and also to examine some physical parameters of their breeding sites. Surveys were carried out over a 19-month period (May 2022—November 2023), the larval stages of the mosquitoes were sampled using the dipping technique and the physical parameters (Type and nature of larval habitat, type and situation of water, status of vegetation and sunlight) of the breeding sites were measured. Collected larvae were then morphologically identified using reference identification keys. In total, 4098 mosquito larvae were collected and further identified and distributed among four genera: *Culex* (76.61%), *Anopheles* (6.71%), *Culiseta* (16.42%), and *Uranotaenia* (0.24%). Twelve species of Culicidae were identified, with *Cx. pipiens* (69.42%) and *Cs. longiareolata* (16.37%) being the most abundant. The abundance of the inventoried species was influenced by the physical nature of breeding sites as well as climatic factors, including rainfall and temperature. The highest number of larvae was collected from natural habitats (70.79%), including grasslands, river edges, and ponds, with permanent and turbid water, vegetation out of water, and in sunny conditions. River edge for *Cs. longiareolata* and *An. maculipennis* s.l. and, ponds for *Cx. pipiens* were the main larval habitats in the province. The present study sheds light on the entomological diversity of the Culicidae fauna in the study area. This result will aid in directing the fight against mosquito vectors, taking into consideration existing species and various types of breeding sites.

Keywords: Culicidae, Larval ecology, Breeding sites, Physical parameters, Setif.

Introduction

Mosquitoes (Diptera: Culicidae) are dipteran insects of great global importance, not only for ecological roles but also for issues related to human and animal health. Currently, over 3500 species of mosquitoes are recognised all over the world, but only a small handful can carry the pathogens that cause human or animal disease and it is these species which have been studied most thoroughly (Hawkes and Hopkins, 2022). Chikungunya, yellow fever, Zika, dengue, Japanese encephalitis, West Nile, lymphatic filariasis, and malaria are among the most common mosquito-borne diseases, while three main genera of mosquitoes from the Culicidae family (*Aedes*, *Culex*, and *Anopheles*) are the main vectors incriminated in their transmission (Nebbak et al., 2022). In recent years, several natural and manmade factors, such as those related to climate change and human activities, have allowed the emergence and establishment of numerous sylvatic mosquito species—which are usually found only in tropical regions—in urban environments of temperate territories (Mordecai et al., 2019; Khezzani et al., 2023). The Asian tiger mosquito, *Aedes albopictus* (Skuse, 1894), is one example of this concerning trend. It has invaded the majority of the Mediterranean countries in recent decades and is now starting to disseminate several tropical arboviruses over the Old Continent, such as agents of dengue or chikungunya (Venturi et al., 2017; Ducheyne et al., 2018; Brem et al., 2024; Garrido et al., 2024).

For oviposition, mosquitoes utilize a range of aquatic habitats, with many mosquito species prefer to use both natural and artificial containers as their breeding sites resulting in complex larval communities (Aigbodion and Anyiwe, 2005; Aditya et al., 2006). Larvae of Culicidae mosquitoes have been observed thriving in a variety of aquatic environments, including stream and river edges, riverbeds, fresh or salt water marshes, and small, temporary rain pools. Larval habitats are of great interest because they are important determinants of both the distribution and abundance of adult

mosquitoes (Piyaratnea et al., 2005; Mwangangi et al., 2012). Some research studies have shown that mosquito density is influenced by the physicochemical properties of the water in breeding habitats, as well as by various ecological factors, including climate-related ones (Abai et al., 2016; Ferraccioli et al., 2023). To this end, understanding how habitat factors affect larval production is essential for comprehending the temporal and spatial distribution patterns of mosquito vector species. In addition, assessing the composition and quality of larval mosquito habitats also contributes to understanding the bio-ecology of these insects and aids in developing effective larval control strategies (Nikookar et al., 2017).

In Algeria, the largest country in Africa, the culicidian fauna has been the focus of a great deal of research since the early twentieth century. So far, a total of 53 mosquito species have been recorded in Algeria, 44 of which belong to the genera *Culex*, *Aedes*, and *Anopheles* (Merabti et al., 2021). In their study, Nebbak et al. (2022) indicated that 26 mosquito species are involved in the circulation of seven vector-borne diseases (VBDs) in North Africa, including two parasitic (malaria, filariasis) and five viral infections (West Nile virus, Rift Valley Fever virus, Dengue virus, Sindbis and Usutu viruses). The continuous shifts in the geographical distribution of several mosquito species, along with the appearance or disappearance of others driven by local or global changes, expose the country to the re-emergence or emergence of multiple VBDs. Therefore, it is intriguing to regularly monitor mosquito populations in vulnerable regions like northern Algeria (Arsevska et al., 2016) to improve efforts in preventing the potential spread and transmission of VBDs.

The present study aimed to identify the mosquito fauna present in the Setif region and to determine the physical factors influencing their proliferation in the area's aquatic habitats. This study also planned to gain a deeper understanding of Culicidae diversity in the region and produce

valuable data that can assist the vector control program in implementing targeted and cost-effective interventions.

Materials and methods

Study area description

The study was carried out from May 2022 to November 2023 in Setif Province, located in the northeast of Algeria at a latitude of 36°11' N and a longitude of 5°24' E (Figure 1). The province is administratively divided into 60 municipalities and covers an area of 6.549 km² with a population of 2.021.687 according to the 2021 census. Setif is situated 1.064 meters above sea level and is bordered by Mila Province to the east, Bordj Bou Arreridj Province to the west, Bejaia and Jijel Provinces to the north, and Batna and M'sila Provinces to the south. The study area comprises 50% high plains, 40% mountainous regions (in the northern and southern parts), and 10% depression zones (in the southern part). The inhabitants are mainly involved in agriculture, commerce, administration, and industry (ANIREF, 2022).

The climate of the study area is classified as semi-arid, characterised by hot, dry summers. The average annual precipitation is 402 mm, although it is irregular both in time and space. Monthly average temperatures range from 5.8 to 24.8 °C, while relative humidity varies between 37% and 79.1% (Gherbi et al., 2020). Data on monthly rainfall, temperature, and relative humidity for Setif during the study period were sourced from the Meteorological Station of Ain Sfiha.

Determination of sampling sites

Sampling sites for this study were selected based on criteria such as the type of larval habitats, water availability, flow and transparency, and sunlight penetration, to account for the potential impact of these physical conditions on mosquito diversity and richness. Based on these

data, 21 larval habitats across 12 municipalities in Setif, representing a range of environmental and ecological conditions, were selected for sampling to provide an accurate depiction of the mosquito species potentially present in the study area. During the study, we used a Global Positioning System (GPS) to record the coordinates of each larval habitat. The locations of these sites are shown in Figure 1.

Physical parameters recorded for each larval habitat include habitat type and condition (natural or artificial), water availability (temporary or permanent), water transparency (clear, muddy, or turbid), vegetation status (out of water, water surface, out and water surface, without plant), and sunlight status (shadow, semi-shade, or sunny). These parameters are detailed in Table 1.

Mosquito collection and identification

Field surveys aimed to collect mosquito larvae were carried out from the early morning up to late afternoon using a standard dipping method (dips of 500 mL dipper) in each larval habitat, as described by Coffinet et al. (2009). In the small areas where the dipping method was impractical, a 1 mL transfer pipette was employed to pick up mosquito larvae. A variety of larval habitats (Figure 2) were used to collect mosquito larvae across the twelve selected municipalities, including grasslands, river edges, ponds, sewages, and fountains.

All collected samples were placed in plastic bottles before being transported to the Improvement and Development of Animal and Plant Production laboratory at the Faculty of Nature and Life Sciences, Setif University, where they were kept to allow the development of eggs, as well as first, second, and third instar larvae (14:10 light/dark cycle, 25 °C and a mixture of biscuit (75%) and dry yeast (25%)) (Rehimi et Soltani, 1999; Bendali et al., 2001). Both sampled and reared fourth-instar larvae were stored in glass tubes containing 70% ethanol, each labelled with

the date, sampling location, breeding site number, and number of collected larvae (Duvallet et al., 2017). In the laboratory, specimens were mounted on microscope slides using Hoyer's solution (Matile, 1993), and the larvae were then identified based on their morphological characteristics using relevant taxonomic keys (Himmi et al., 1995; Becker et al., 2020; Robert et al., 2022). Larvae identification was subsequently verified using the latest version of MoskeyTool software 2.2, an interactive key for identifying Europe-Mediterranean mosquitoes, as described by Günay et al. (2020).

Ecological indices and statistical analysis of data

The mosquito inventory data were analysed using ecological indices to evaluate both composition and structure. Composition was assessed through relative abundance and frequency of occurrence, while structural analysis involved maximum diversity, equidistribution, and the Shannon-Weaver diversity index. For the frequency of occurrence, the species were qualifying as constant when $C > 50\%$, as accidental when $C < 25\%$, as accessory when $25\% \leq C < 50\%$, and as rare when $C \leq 4\%$. These indices provide insights into the distribution of the larval population and the diversity of mosquito species.

In order to highlight the relationship between mosquito larval abundance and climatic factors (rainfall and temperature) in the study area, the Spearman correlation coefficient, indicating r-squared values, was used. The distribution of mosquito species within the study region was done using Correspondence Factorial Analysis (CFA). In addition, Chi-square (χ^2) analysis was employed to assess the relationship between environmental variables and larval occurrence across different habitats. Statistical analyses were executed using IBM SPSS version 26 software (IBM Corporation, Armonk, NY), and results were considered significant at $P < 0.05$.

Results

Culicidae inventory

A total of 4,098 specimens were collected from various habitats around the province of Setif. The inventoried culicid fauna in this region included 12 species from two subfamilies: Culicinae, with three genera (*Culex*, *Culiseta*, and *Uranotaenia*), and Anophelinae, with a single genus, *Anopheles*. The Culicinae subfamily was the most diverse, with ten species recorded: *Cx. deserticola*, *Cx. hortensis*, *Cx. laticinctus*, *Cx. modestus*, *Cx. pipiens*, *Cx. tritaeniorhynchus*, *Cx. theileri*, *Cs. annulata*, *Cs. longiareolata*, and *Ur. unguiculata* (Table 2).

Ecological index of composition

The relative abundance and frequency of occurrence of Culicidae species are shown in Table 2 and Figure 3.

Data analysis reveals that *Cx. pipiens* is the most abundant species, constituting 69.42% of all identified specimens and exhibiting the highest frequency of occurrence at 38.66% (accessory species). It is followed by *Cs. longiareolata*, which has a relative abundance of 16.37% and a frequency of occurrence of 25% (accessory species). Other species, such as *Cx. modestus*, *Cx. theileri*, and *Anopheles maculipennis*, show much lower abundances and frequencies of occurrence, ranging from 0.02% to 5.81% for relative abundance and 0.33% to 10.67% for frequency of occurrence.

Ecological index of structure

Table 2 displays the maximum diversity, equidistribution, and Shannon-Weaver diversity index values for Culicidae species collected in the region of Setif.

The Shannon-Weaver index (H') for specific diversity is 1.51 bits, while the maximum diversity ($H'_{\max}$) is 3.58, indicating that this population is moderately diverse. Moreover, the

equitability (E) is approximately 0.42, or 42%, meaning that the Culicidae species identified in the deposits of Setif region are moderately balanced.

Distribution of Culicidian species based on station

The chi-square test of independence calculated for the Setif region yields a p-value of 0.001 which is lower than the significance level of 0.05. This indicates a significant relationship between the distribution of species and the study stations.

A factorial correspondence analysis (FCA) was used to better understand the distribution of culicids in the Setif region. According to Figure 4, the contribution of first dimension axis to the total inertia is 46.3%, while the second-dimension axis contributes 23.4%, resulting in a combined total of 69.7 %. This percentage exceeds 50%, indicating that the results of the FCA could only be used for dimensions 1 and 2.

The results obtained indicate that species in group A are more common at the Oued El Barad station, while species in group B are mainly found at the Oued El Dehab, Machtat Lahnache, Machtat Boudhiaf, Ain Roua, Ain Oulmene, Setif, Ras Isly, and El Hamam stations. Species in group C are more widely distributed across El Karia, Bir Abdellah, and Dehamcha.

Physical characteristics of the larval habitats

As summarized in Table 3, most larvae were collected from natural habitats (n=2901, 70.79%), including ponds (n=2051, 50.05%), river edges (n=767, 18.72%), and grasslands (n=83, 2.03%). The frequency of larvae was 53.15% in permanent sites compared to 46.85% in temporary ones. Moreover, 2000 (48.8%), 1450 (35.38%), and 648 (15.81%) larvae were collected from sun-lighted, shady, and semi-shaded sites, respectively. Most larvae were found in turbid (38.97%) and clear water (31.5%), and in sites with vegetation out of water (60.40%). Further details are

presented in Table 3. A statistically significant association ($p < 0.05$) was observed between larval abundance and all studied variables.

The results demonstrated that all species were dispersed in temporary sites, except for *Ur. unguiculata* (100%), which was recorded exclusively in permanent oviposition sites. Three *Culex* species—*Cx. laticinctus*, *Cx. tritaeniorhynchus*, and *Cx. modestus*—were absent from permanent sites. All twelve identified species (100%) were collected from natural sites, particularly ponds, making them the most species-diverse and the primary habitat for *Cx. pipiens*. *Culiseta longiareolata* was predominantly found at river edges (39.19%), followed by ponds (34.38%). *Cx. pipiens* was the most frequent species in permanent (66.89%) and shady sites (48.82%), in turbid water (51%), and in sites with vegetation out of the water (77.40%). In contrast, *Cs. longiareolata* was mainly found in clear water (61.25%), sun-lighted (78.39%) and temporary (79.88%) sites lacking vegetation (54.40%). *Anopheles maculipennis* was exclusively found in natural sites (100%), primarily represented by river edges (90.75%). This species was also predominantly found in clear water (100%), sun-lighted (97.9%), and temporary (88.65%) sites, where vegetation was restricted to the outer and surface of the water (85.72%). The number and percentage of the remaining species in natural and artificial habitats, with different characteristics, are listed in Table 3.

Phenology and impact of environmental factors on larval abundances

The relationship between temperature, rainfall, and mosquito abundance is depicted in Figure 5A.

During our study period, temperatures ranged from 4.1 °C in January to 30.1 °C in July. Additionally, the highest monthly rainfall was recorded in May 2023 at 708.08 mm, while the

lowest rainfall was observed in June 2022, July 2023, and August 2023, with 0.25 mm recorded for each of these months.

Monthly data analysis revealed that the number of larvae for all species combined was highest during the hot seasons, particularly in June 2022 (1022 larvae) and July 2023 (433 larvae). Temperatures during these months ranged from 27.1°C to 30.1°C, while rainfall was minimal, with 0.25 mm recorded for each month. In contrast, larval density was low during the colder months, notably in February and November, with 4 and 10 larvae, respectively. Temperatures and rainfall during these two months were 5.8°C and 10.8°C, and 16.01 mm and 37.34 mm, respectively. Overall, larval abundance peaked in June 2022 (1,022 larvae), declined to its lowest value in February (4 larvae), then reached a second peak in July 2023 (433 larvae) before declining again to a low of 10 larvae in November (Figure 5A).

Monthly mosquito counts are highly significantly correlated with temperature ($R_s = 0.89$, $n = 19$, $p = 0.001$) and significantly correlated with precipitation ($R_s = -0.45$, $n = 19$, $p = 0.049$), according to the Spearman correlation test.

For combined species, a similar trend was observed for individual species as well (Figure 5B). *Culex pipiens* abundance peaks in June (560 larvae), then declines to its lowest value in February (3 larvae), before increasing again to a second peak in July (348 larvae). In contrast, *Cs. longiareolata* abundance also peaks in June (252 larvae in 2022 and 50 larvae in 2023) during the hot season but is at its lowest during the cold season.

Discussion

The present study reveals that the Culicidae fauna in the Setif area is rich and diverse, with 12 species identified. Previous studies in Algeria (Lafri et al., 2014; Asloum et al., 2021; Oussad

et al., 2021; Chahed et al., 2022; Halimi et al., 2022) have already reported these species. However, our study made it possible to update the list of culicidian species reported at the level of the studied area since it allowed the addition of four species—*An. maculipennis*, *An. multicolor*, *Cx. tritaeniorhynchus*, and *Ur. unguiculata*—to the last inventories that were previously made (Nabti and Bounechada, 2020; Bouaoud et al., 2024). Nevertheless, some species reported in earlier research studies, such as *Cx. univitattus*, *Ae. caspius* and *Ae. vexans*, (Nabti and Bounechada, 2020; Bouaoud et al., 2024) were not detected in our inventory. The discrepancy in the results may have arisen from differences in habitat types, sampling techniques, or the larval deposits surveyed.

The genus *Culex* accounted for the bulk of mosquitoes collected in this study, likely because *Culex* species can colonize a wide range of breeding sites (Faraj et al., 2006). This suggests that *Culex* has fewer environmental requirements for development compared to *Anopheles*, *Culiseta*, or *Uranotaenia*. A similar observation was made in the Khenchela province (northeast) by Halimi et al. (2022), who revealed 57.89% *Culex*. Seven species of *Culex* were identified, with *Cx. pipiens* being the most abundant. This finding is in line with what has been reported in other studies, such as those of Oussad et al. (2021) in Tizi Ouzou (north-centre) and Halimi et al. (2022) in Khenchela (north-east), where *Cx. pipiens* was the most frequently collected species. *Cx. pipiens* is renowned for its high ecological plasticity and adaptability, which may explain these results (Meegan et al., 1980). In contrast, inventories by Hamaidia and Berchi (2018) in Tebessa, Belkhir et al. (2021) in Batna, and Asloum et al. (2021) in M'sila indicated a predominance of *Cs. longiareolata*. In our study, this species ranked second after *Cx. pipiens*. This could be attributed to the bio-ecology of this species and its spatial and temporal adaptations. *Cs. longiareolata* is widely distributed in the Mediterranean region (Brunhes et al., 2001).

For the remaining identified species, we observed low relative abundance and frequency of occurrence. This result may be attributed to several factors, including water quality, reduced egg laying (possibly due to fewer female mosquitoes emerging), limited nutrient availability (either in quantity or quality), drying of larval sites during dry seasons, site washout due to rainfall, slowed larval development from temperature drops, and mortality from predation by invertebrates (Berchi, 2000).

The species identified in this study are known to transmit significant pathogens with notable medical and veterinary importance. In Algeria, *Cx. pipiens* is an efficient vector of the West Nile virus and, to a lesser extent, the Rift Valley fever virus under experimental conditions (Amraoui et al., 2012). Recently, this species was also found to be infected with the Sindbis virus in Timimoun, southern Algeria (Ayhan et al., 2022). While other Culicidae species are recognized as common or potential vectors of human and animal diseases worldwide, their vectorial roles in Algeria have not yet been studied.

Studies have shown that factors such as temperature and precipitation influence the development and distribution of Culicidae species (Rodhain and Perez, 1985; Mouchet et al., 2004). Our findings revealed a diverse distribution of mosquito larvae throughout the study period, suggesting that temperature and rainfall likely have a critical impact on their abundance. The increase in mosquito numbers during the second peak, which occurred between June and July, was likely due to the high precipitation in the months preceding this peak, creating more ideal breeding sites for mosquito reproduction. Our results also showed a low abundance of mosquitoes during the cold months, likely due to unfavourable conditions that led to the destruction or instability of some breeding sites. The two studied parameters—rainfall and temperature—have been reported to significantly influence egg hatching, the creation of breeding sites, the speed of post-embryonic

development, and adult survival (Ntoumba et al., 2020). Our results are consistent with those of Poveda (2000) in Colombia, who demonstrated a correlation between temperature, precipitation, and mosquito development. In our study, the majority of abundant species (*Cx. pipiens* and *Cs. longiareolata*) were present almost year-round, with higher abundance observed in June and July.

The analysis of the relationship between Culicidae species and the physical parameters of breeding sites allowed us to identify those conditions that are favourable for the development of mosquito species, including those that pose a health risk to the region.

In the present study, five types of habitats were surveyed in Setif Province, two of which were artificial habitats. The highest abundance of larvae was observed in natural habitats including mainly ponds, and with characteristics such as permanent, turbid and sun-lighted, with vegetation out of water. Azari-Hamidian (2007) collected the highest number of larvae from natural habitats, including riverbed pools, rain pools, and artificial habitats such as rice fields with clean water, along with temporary water, out-of-water vegetation, and exposure to sunlight in Guilan Province.

Among the Culicinae, *Cx. pipiens* prefers natural habitats, including ponds, river edges and grasslands, respectively. It lays eggs in permanent water bodies under varying light conditions, including both shaded and sunny environments. This *Culex* species was predominantly found in turbid waters with vegetation extending above the surface. Our results are in line with those of other studies (Azari-Hamidian, 2007; Omrani et al., 2020), where *Cx. pipiens* is mainly observed in natural and shaded sites with temporary water. In contrast, Azari-Hamidian (2007), Nikookar et al. (2017), and Omrani et al. (2020) reported that this species is often collected from clear water. These findings indicate that *Cx. pipiens* exhibits a greater adaptability to a variety of physical conditions, including water transparency, site type, and sunlight exposure. Moreover, Ladonni et al. (2015) reported that 86.7% of *Cx. pipiens* larvae were found in artificial sites. This genus is

recognized as the largest, most common, and most significant genus within the Culicinae tribe (Service, 1993), and is the most densely populated species with a wide distribution throughout the province.

Cx. pipiens was collected year-round in various biotopes by Mouatassem et al. (2019) in Morocco. The species was found in both natural and artificial, temporary and permanent breeding sites, with and without vegetation, and in both clean and polluted waters. Notably, *Cx. pipiens* showed a strong preference for polluted environments (Mouatassem et al., 2019). In their study conducted in Mazandaran Province, northern Iran, Nikookar al. (2017) observed more *Cx. pipiens* larvae in river edges, marshes, pits, pools, discarded tires and wetlands. The same species was mainly found to invade artificial containers, stream margins, rice fields, and wells/ cisterns, in northern Gyeonggi Province, Korea (Kim et al., 2011). These investigations, combined with the present study, highlight the species' adaptability to various habitats, which may explain its high density and extensive distribution in Setif Province.

For *Cs. longiareolata*, the highest abundance was mainly observed in natural sites with temporary, fully sun-lighted, and clear water, devoid of vegetation. Interestingly, *Cs. longiareolata* was the only species recorded in artificial sites in our study. Similar findings were reported by Sofizadeh et al. (2018) and Omrani et al. (2020) in Iran, where specimens were predominantly collected from natural, temporary, sun-lighted sites with clear water. In contrast, other studies have reported that this species is mainly found in permanent (Zaim et al., 1987) and turbid water (Hanafi-Bojd et al., 2017). Additionally, Azari-Hamidian (2005) found that 100% of the specimens were sampled from natural sites. These differences may be attributed to the species' high adaptability to various aquatic habitats across different geographical conditions.

Anopheles is represented by two species, one of which is considered major potential vector of malaria (*An. maculipennis*) (Mouatasseem et al., 2019).

In this study, *An. maculipennis* larvae were mainly collected from river edges in temporary, natural environments. The key characteristics of their larval habitats included clear water, sun-lighted conditions, with vegetation both above and at the water surface. Our findings are similar to those of Nikookar et al. (2017) in Iran, where high abundance of this species' larvae was observed in natural habitats characterised by temporary, clear water, and vegetation both submerged and above the water surface. However, our results contrast with some studies that primarily reported *An. maculipennis* larvae in permanent and semi-shady natural larval habitats (Sofizadeh et al., 2017). In Morocco, Mouatasseem et al. (2019) noted that larvae of *An. maculipennis* s. l. were exclusively collected from clear waters with submerged vegetation. This species has been reported to inhabit a wide range of both artificial and natural environments, including river edges, river beds, marshes, grasslands, creeks, pits, wetlands, springs, rice fields, rice irrigation channels, ponds, pools, discarded tires, and concrete channels (Nikookar et al., 2017; Asgarian et al., 2021). *An. maculipennis* is also known to be a malaria vector (Nikookar et al., 2017; Mouatasseem et al., 2019).

Our findings revealed that larvae of *Cx. theileri*, *Cx. hortensis*, and *Cx. deserticola* were predominantly collected from natural sites, mainly in river edges. These mosquito species exhibited similar larval characteristics, thriving in clear water and sunny conditions. Notably, *Cx. hortensis* and *Cx. deserticola* were primarily found in temporary habitats, while *Cx. theileri* favoured permanent sites. Moreover, *Cx. hortensis* was commonly associated with vegetation at the water's surface, whereas *Cx. deserticola* and *Cx. theileri* were located in sites where vegetation was both submerged and above the water. Sofizadeh et al. (2017) noted that *Cx. theileri* and *Cx.*

hortensis were primarily collected from permanent, sunny sites; however, they reported that *Cx. theileri* prefers turbid, vegetated habitats, while *Cx. hortensis* favours clear water without vegetation. Similarly, Asgarian et al. (2021) found that *Cx. deserticola* and *Cx. hortensis* were predominantly located in natural, permanent sites with clear waters.

Conclusion

The results of this study led to an updated list of mosquito species recorded in the Setif region and provided the first data on mosquito larval habitats. Twelve species from four different genera were identified: *Anopheles*, *Culex*, *Culiseta*, and *Uranotaenia*. *Cx. pipiens* is abundant in ponds, while *Cs. longiareolata* and *An. maculipennis* s.l. are found in high numbers along river edges, each associated with distinct habitat characteristics. Among the species inventoried, two play key roles in transmitting important diseases: *An. maculipennis* s.l. in malaria transmission and *Cx. pipiens* in the transmission of West Nile virus fever. These findings will be critical for developing entomological surveillance and vector control strategies in the region.

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Conflict of Interests

The authors do not have any conflict of interest to declare.

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Figures:

Figure 1: Map shows sampling places in various municipalities of Setif Province. These comprise: Beni Fouda municipality (s1, s2, s3), Tachouda (s4, s5, s6, s7), Ouled Saber (s8), Beni Aziz (s9), Dehamcha (s10), Oued El Barad (s11, s12, s13), Ain Roua (s14), El Ouricia (s15), Setif (s16), Ain Oulmane (s17), Rosfa (s18, s19, s20), and Ouled Tebben (s21).

Figure 2: A variety of fixed habitats of mosquito larvae in Setif Province: A: River edge in Ouled Saber, B: River edge in Dehamcha, C: pond in Oued El Bered, D: Sewage in Rasfa, E: pond in Tachouda, F: grassland in Ain Oulmene, G : fountain in Setif, H : sewage in El Ouricia.

Figure 3: Relative abundance and frequency of occurrence of mosquitoes inventoried in Setif province.

Figure 4: The factorial correspondence analysis between mosquito species and the studied areas.

Figure 5: Monthly relationships between temperature, rainfall and abundance of Culicidae (A), Monthly abundance distribution of *Cx. pipiens* and *Cs. longiareolata* (B).

Tables:

Table 1: Natural characteristics of the 21 breeding sites from the 12 study municipalities, Setif province.

Table 2: Numbers, percentage, maximum diversity, equidistribution, and Shannon-Weaver diversity index values for Culicidae species collected in Setif region.

Table 3: Abundance and percentage of larvae collected in habitats with different ecological characteristics, Setif Province, Algeria.

Figure 1

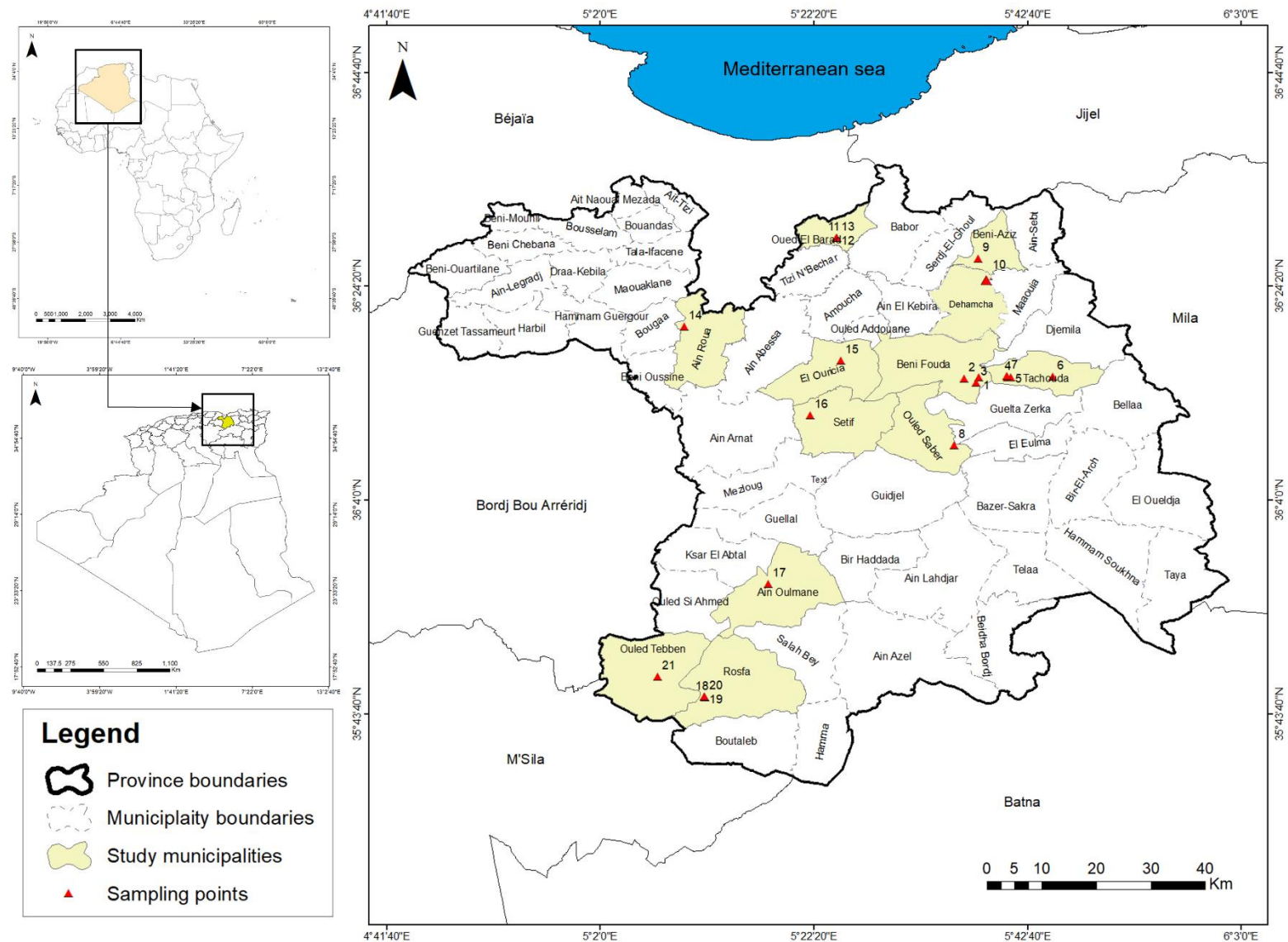


Figure 2



Figure 3

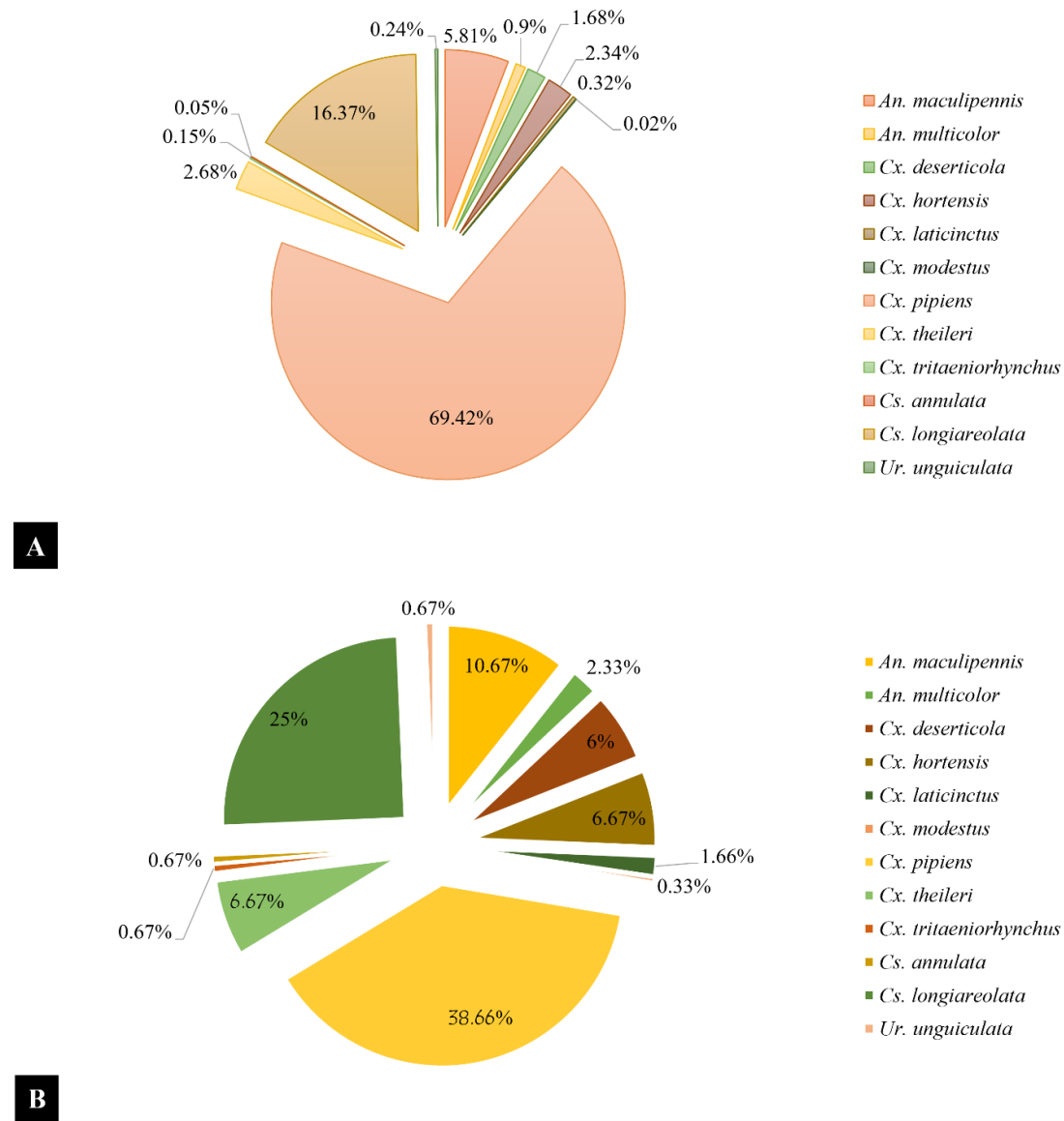


Figure 4

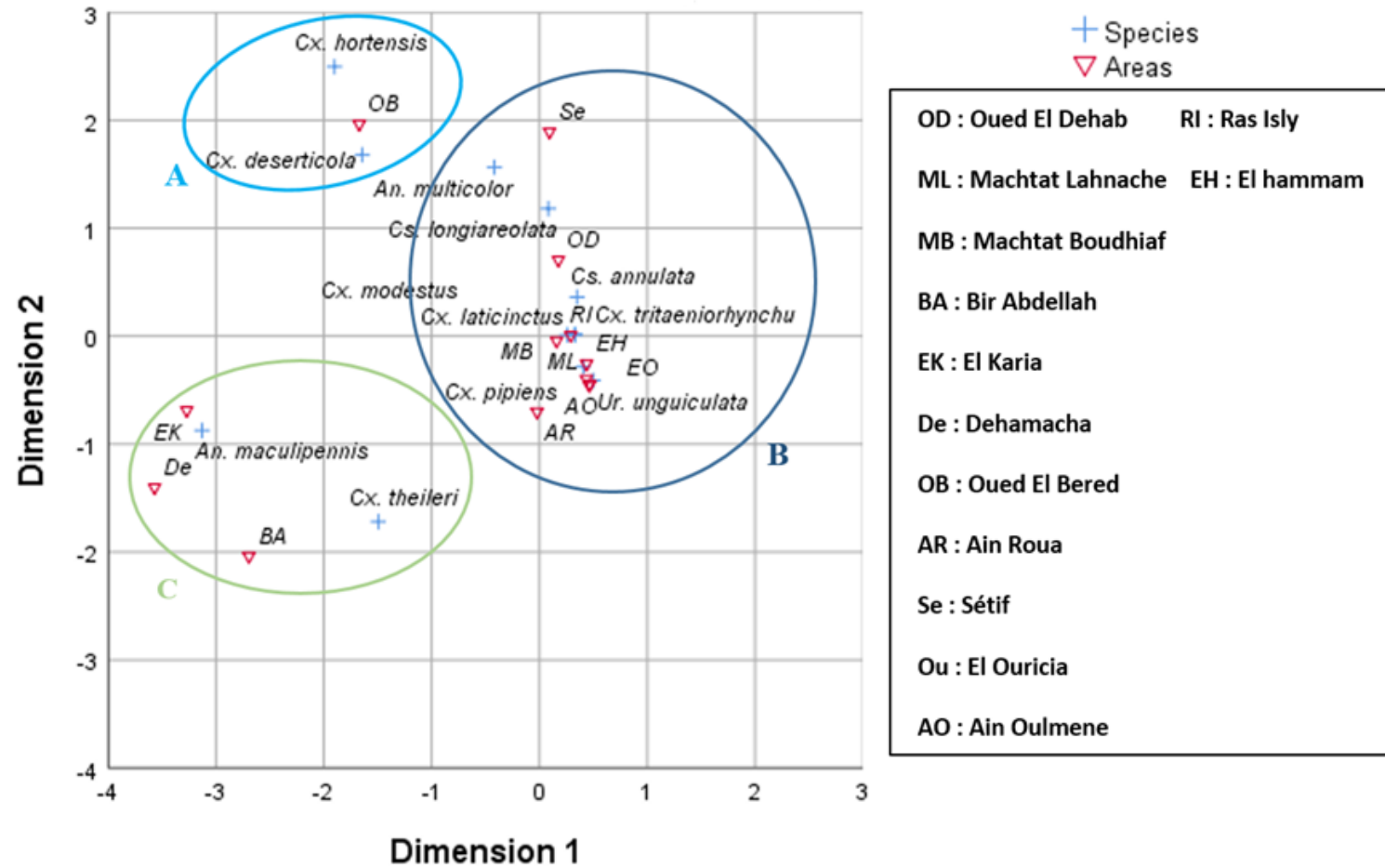


Figure 5

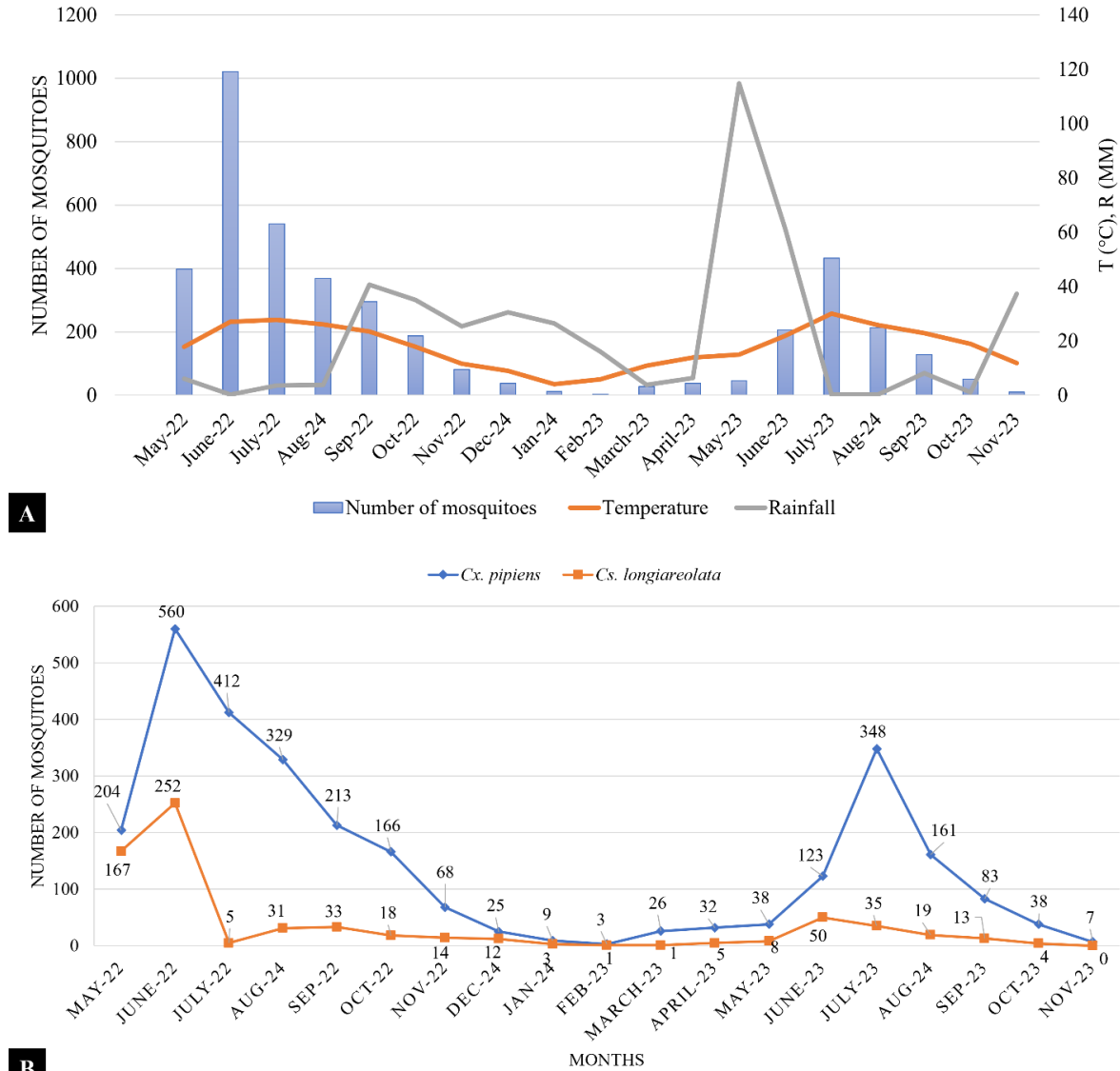


Table 1

Municipality	Locality	Site	Habitat types	Larval habitat coordinates	Altitude (m)	Nature of breeding site	Water appearance	Organic substance	Status of vegetation	Sunlight status	Origin of water
Beni Fouda	Oued El dahab	1	Rural	36°15'29.20680'' N 5°6'40.67860'' E	902	Temporary	Clean	Without	Out and Water surface	Full sunlight	River
		2		36°15'38.26200'' N 5°38'3.61600'' E	855	Temporary	Clean	Without	Without plant	Full sunlight	River
		3		36°15'8.52690'' N 5°37'47.75730'' E	874	Temporary	Muddy	With	Without plant	Full sunlight	Wastewater
Tachouda	Machtat Lahnache	4	Rural	36°15'40.01480'' N 5°40'50.76050'' E	949	Permanent	Turbid	Without	Out of water	Shaded	Wastewater
		5		36°15'38.13550'' N 5°41'5.28070'' E	968	Permanent	Muddy	With	Out of water	Full sunlight	Wastewater
		6		36°15'45.84730'' N 5°40'43.03820'' E	945	Permanent	Clean	Without	Water surface	Full sunlight	Tap water
	Machtat Boudhiaf	7	Rural	36°15'41.85320'' N 5°45'6.49120'' E	1254	Permanent	Clean	Without	Water surface	Partial sunlight	River
Ouled Saber	Bir Abdellah	8	Rural	36°9'12.14900'' N 5°35'40.53770'' E	1075	Temporary	Clean	Without	Water surface	Full sunlight	Rainwater
Beni Aziz	El Karia	9	Rural	36°26'55.93810'' N 5°37'58.12540'' E	627	Temporary	Clean	Without	Water surface	Full sunlight	River
Dehamcha	Dehamcha	10	Rural	36°25'19.72570'' N 5°38'55.65920'' E	607	Permanent	Clean	Without	Water surface	Partial sunlight	River
Oued El Bered	Oued El Bered	11	Rural	36°28'55.04390'' N 5°24'30.11040'' E	714	Permanent	Clean	Without	Out \ water surface	Full sunlight	River
		12		36°28'55.09340'' N 5°24'31.11710'' E	714	Temporary	Clean	Without	Water surface	Full sunlight	River
		13		36°28'55.75820'' N 5°24'33.53950'' E	714	Temporary	Clean	Without	Water surface	Full sunlight	River
Ain Roua	Ain Roua	14	Rural	36°19'36.27440'' N 5°7'17.08890'' E	968	Temporary	Clean	Without	Out of water	Full sunlight	Rainwater
El Ouricia	El Ouricia	15	Urban	36°17'13.31890'' N 5°24'54.46280'' E	1188	Permanent	Muddy	With	Out of water	Partial sunlight	Wastewater
Setif	Setif	16	Urban	36°12'3.45760'' N 5°21'59.87950'' E	1112	Temporary	Clean	Without	Without plant	Full sunlight	Rainwater
Ain Oulmene	Ain Oulmene	17	Urban	35°55'59.88240'' N 5°18'1.01060'' E	996	Temporary	Turbid	With	Water surface	Full sunlight	Rainwater
Rasfa	Ras Isly	18	Rural	35°45'14.90710'' N 5°12'0.78320'' E	1056	Permanent	Muddy	With	Out of water	Full sunlight	Wastewater
		19		35°45'15.25680'' N 5°11'56.44280'' E	1053	Temporary	Muddy	With	Water surface	Full sunlight	Wastewater
		20		35°45'15.06280'' N 5°11'56.13510'' E	1053	Temporary	Turbid	Without	Out of water	Partial sunlight	Rainwater
Ouled Tebben	El Hammam	21	Rural	35°47'11.17730'' N 5°7'27.54990'' E	1260	Permanent	Clean	Without	Out of water	Shaded	River

Table 2

[illegible]

Table 3

	An. <i>maculipennis</i>	An. <i>multicolor</i>	Cx. <i>deserticola</i>	Cx. <i>hortensis</i>	Cx. <i>laticinctus</i>	Cx. <i>modestus</i>	Cx. <i>pipiens</i>	Cx. <i>theileri</i>	Cx. <i>tritaeniorhynchus</i>	Cs. <i>annulata</i>	Cs. <i>longiareolata</i>	Ur. <i>unquiculata</i>	Total
Situation of water: <i>p</i>-value = 0.001													
Muddy	-	-	-	-	6 (46.15%)	-	1045 (36.73%)	8 (7.27%)	1 (16.67%)	1 (50%)	149 (22.21%)	-	1210 (29.53%)
Clear	238 (100%)	37 (100%)	69 (100%)	96 (100%)	2 (15.38%)	-	349 (12.27%)	78 (70.91%)	-	1 (50%)	411 (61.25%)	10 (100%)	1291 (31.50%)
Turbid	-	-	-	-	5 (38.46%)	1 (100%)	1451 (51%)	24 (21.82%)	5 (83.33%)	-	111 (16.54%)	-	1597 (38.97%)
Sunlight status: <i>p</i>-value = 0.001													
Shadow	-	-	-	-	-	-	1389 (48.82%)	1 (0.91%)	-	1 (50%)	49 (7.30%)	10 (100%)	1450 (35.38%)
Sunny	233 (97.9%)	37 (100%)	66 (95.65%)	96 (100%)	8 (61.54%)	-	946 (33.25%)	86 (78.18%)	1 (16.67%)	1 (50%)	526 (78.39%)	-	2000 (48.80%)
Semi shade	5 (2.10%)	-	3 (4.35%)	-	5 (38.46%)	1 (100%)	510 (17.93%)	23 (20.91%)	5 (83.33%)	-	96 (14.31%)	-	648 (15.81%)
Type of water: <i>p</i>-value = 0.001													
Permanent	27 (11.35%)	5 (13.51%)	21 (30.43%)	20 (20.83%)	-	-	1 903 (66.89%)	56 (50.91%)	-	1 (50%)	135 (20.12%)	10 (100%)	2178 (53.15%)
Temporary	211 (88.65%)	32 (86.49%)	48 (69.57%)	76 (79.17%)	13 (100%)	1 (100%)	942 (33.11%)	54 (49.09%)	6 (100%)	1 (50%)	536 (79.88%)	-	1920 (46.85%)
Status of vegetation: <i>p</i>-value = 0.001													
Out of water	-	-	1 (1.45%)	1 (1.04%)	7 (53.85%)	1 (100%)	2202 (77.40%)	45 (40.91%)	5 (83.33%)	1 (50%)	202 (30.10%)	10 (100%)	2475 (60.40%)
Water surface	34 (14.28%)	6 (16.22%)	23 (33.33%)	57 (59.38%)	4 (30.77%)	-	259 (9.10%)	2 (1.82%)	1 (16.67%)	-	78 (11.62%)	-	464 (11.32%)
Out and Water surface	204 (85.72%)	31 (83.78%)	31 (44.92%)	30 (31.25%)	-	-	-	56 (50.91%)	-	-	26 (3.87%)	-	378 (9.22%)
Without plant	-	-	14 (20.29%)	8 (8.33%)	2 (15.38%)	-	384 (13.50%)	7 (6.36%)	-	1 (50%)	365 (54.40%)	-	781 (19.06%)
Type of breeding sites: <i>p</i>-value = 0.001													
Natural	238 (100%)	37 (100%)	69 (100%)	96 (100%)	7 (53.85%)	1 (100%)	1833 (64.43%)	102 (92.73%)	5 (83.33%)	1 (50%)	502 (74.81%)	10 (100%)	2901 (70.79%)
Artificial	-	-	-	-	6 (46.15%)	-	1012 (35.57%)	8 (7.27%)	1 (16.67%)	1 (50%)	169 (25.19)	-	1197 (29.21%)
The larval habitats: <i>p</i>-value = 0.001													
Grassland	6 (2.52%)	-	-	-	-	-	70 (2.46%)	-	-	-	7 (1.04%)	-	83 (2.03%)
River edge	216 (90.75%)	32 (86.49%)	50 (72.46%)	75 (78.13%)	-	-	73 (2.57%)	58 (52.72%)	-	-	263 (39.19%)	-	767 (18.72%)
Pond	16 (6.72%)	5 (13.51%)	19 (27.54%)	21 (21.87%)	7 (53.85%)	1 (100%)	1690 (59.40%)	44 (40%)	5 (83.33%)	1 (50%)	232 (34.58%)	10 (100%)	2051 (50.05%)
Sewage	-	-	-	-	6 (46.15%)	-	1012 (35.57%)	8 (7.27%)	1 (16.67%)	1 (50%)	66 (9.84%)	-	1094 (26.70%)
Fountain	-	-	-	-	-	-	-	-	-	-	103 (15.35%)	-	103 (2.51%)