

# Spatio-temporal variation in larval densities of disease vector *Culex* species (Diptera: Culicidae) in the northeast province of Sidi Slimane (Morocco)

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**Abstract.** It is crucial to understand the spatio-temporal distribution of Culicidae in the study region, in order to minimize the risk of vector-borne diseases and to effectively control sources of nuisance. The objectives of the present study were to survey four mosquitoes breeding sites (Laabiat, Sidi Haggouch, Soualem and Kariat el Hasnaoui), to make an inventory of *Culex* species, to monitor their distribution and to explore their spatio-temporal fluctuations in order to develop an effective control strategy that will reduce the costs of the various treatments currently underway. This research collected 889 Culicidae larvae belonging to five *Culex* species during the 2016-2018 hydrological cycle: *Culex pipiens*, *Culex hortensis*, *Culex modestus*, *Culex theileri*, and *Culex laticinctus*. The results show that *C. pipiens* predominated in the various habitats surveyed, with a proportion of 61% of the total larvae collected in the four habitats over the entire hydrological cycle concerned. It is a ubiquitous species. *C. hortensis*, which accounts for 15% of all species at the four larval sites, is classified as a spring species. On the other hand, *C. laticinctus* and *C. modestus*, with 9% and 7% respectively of the total larval density distributed across the four habitats, are known for their specific preferences in terms of habitats to colonize. *C. theileri*, represents 8% of the total larval density in the four habitats. These results highlight the importance of spatio-temporal monitoring of *Culex* populations to prevent the emergence of new cases of vector-borne diseases and significantly reduce nuisances.

**Key Words:** *Culex*, fluctuation, Sidi Slimane, time and space.

**Introduction.** Morocco continues to be exposed to the risk of spreading vector-borne diseases, mainly caused by Culicid fauna. The Culicidae family comprises around 3,200 species worldwide, capable of adapting to different environments and climatic and environmental conditions. It colonizes vast and highly varied ecosystems (Clements 1992; Takken & Knols 2007; Alencar et al 2021). Vector-borne diseases, including those transmitted by mosquitoes, have recently experienced a sharp upsurge (Morens et al 2004) and accounted for 14% of infectious diseases and 28% of emerging diseases (Jones et al 2008). They have a major impact on public health and the global economy (Morens et al 2004; Jones et al 2008; Suaya et al 2009). In fact, these mosquitoes are responsible for transmitting numerous pathogens of medical and veterinary importance (viruses, bacteria, protozoa or nematodes) (Takken & Knols 2007; Guidez et al 2024), and their bites can cause nuisance and severe irritation for humans or animals (Takken & Knols 2007; Becker et al 2010). In this context, it is crucial to know and monitor

seasonal fluctuations in the densities of each species inventoried, in the larval biotopes of the study region. Among these mosquitoes species, the *Culex* are of particular economic and health interest. Their development in the study area may be harmful to the health of humans and animals. To this end, the adoption of an effective control and eradication strategy ostensibly and reasonably requires a relevant ecological study and adequate knowledge of larval breeding sites and the spatio-temporal variations in their development in natural or anthropised biotopes. These mosquitoes require monitoring and knowledge of the optimum development periods for each species inventoried during the 2016-2018 hydrological cycles, to better guide control activities. This Culicidae fauna requires innovative and sustainable pest control that does not pollute the environment, is adapted to each species and is carried out at the right time, without compromising terrestrial ecosystems by polluting the environment, water, larval biotopes and soil through the random and abusive use of insecticides. It is in this context that this study of Culicidae populations in the North-eastern province of Sidi Slimane in Morocco took place. It highlights the spatio-temporal fluctuations in larval densities of *Culex* species.

## Material and Method

**Presentation of the study area.** The Province of Sidi Slimane (Figure 1), located between 34°16' North and 5°55' West, is bordered to the north and east by the Province of Sidi Kacem (Rabat-Salé-Kénitra Region), to the south-east by the Prefecture of Meknes (Fès-Meknes Region), to the south by the Province of Khémisset (Rabat-Salé-Kénitra Region) and to the west by the Province of Kénitra (Rabat-Salé-Kénitra Region). It covers an area of 1492 km<sup>2</sup>, or almost 16.95% of the Region's surface area. It has an estimated population of 292,877 (15.75% of the Region's population), with an average density of 192 inhabitants per km<sup>2</sup>.

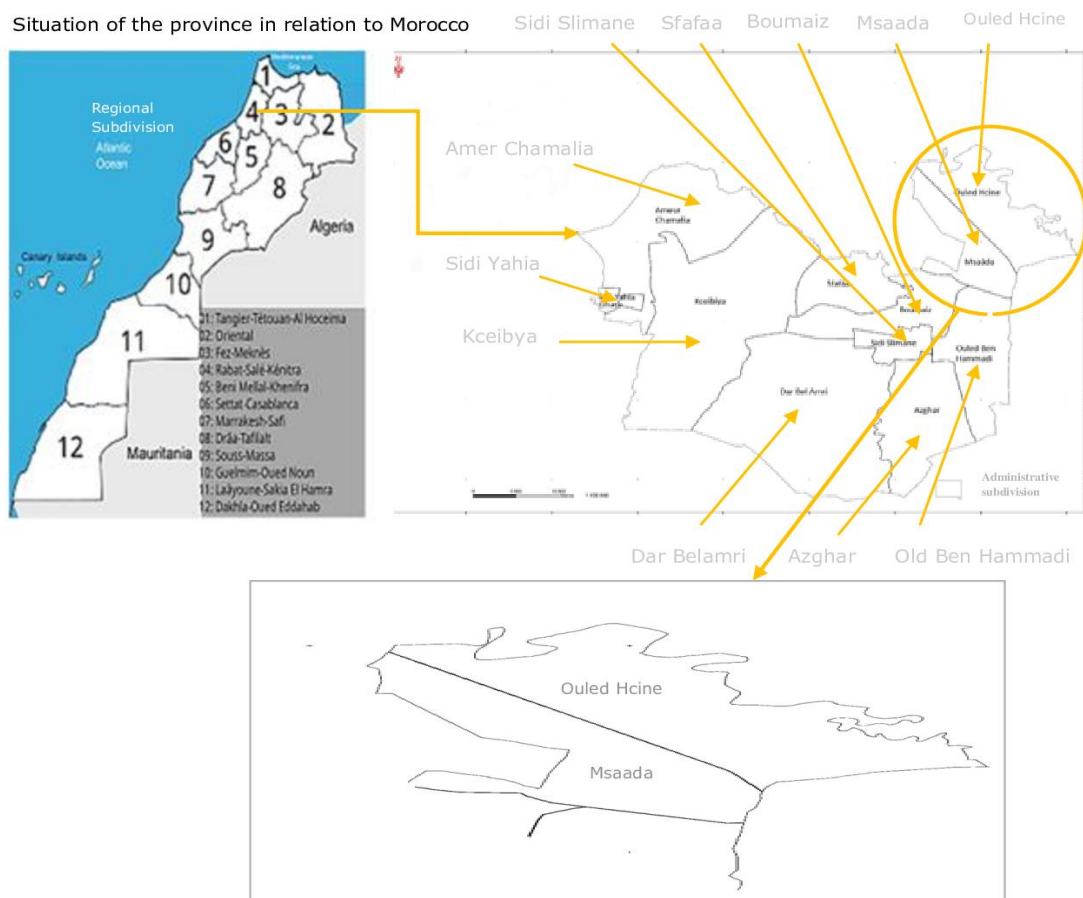


Figure 1. Map of study region in the Province of Sidi Slimane and its location in the map of Morocco.

According to the administrative division of June 2009, the Province of Sidi Slimane is made up of 11 communes, including two urban communes (or municipalities): Sidi Slimane, its administrative centre, and Sidi Yahya du Gharb. Fertile land, a temperate, humid climate and abundant water resources make the province of Sidi Slimane a prime agricultural area. This province is a natural collector of surface water. Its flat morphology (most of the plain has a slope of less than 12 m) makes it difficult for floodwaters from the wadis to reach the sea. This makes the area highly vulnerable to the risk of flooding. The Sidi Slimane station records less rainfall than other stations in the Rabat-Salé-Kénitra region, due to the combined effect of the altimetric gradient and the E-W continental gradient, which reflects the effect of the ocean. The wettest months are November, December and January, and the driest are June, July and August. July and August are the hottest months, while December, January and February are the coolest. The dry period is from May to September.

**Survey for larval breeding sites.** Entomological surveys of the four breeding sites in the study region: Laabiat, Sidi Haggouch, Soualem and Kariat el Hasnaoui were carried out during the hydrological cycles from 2016 to 2018 (Figure 2).

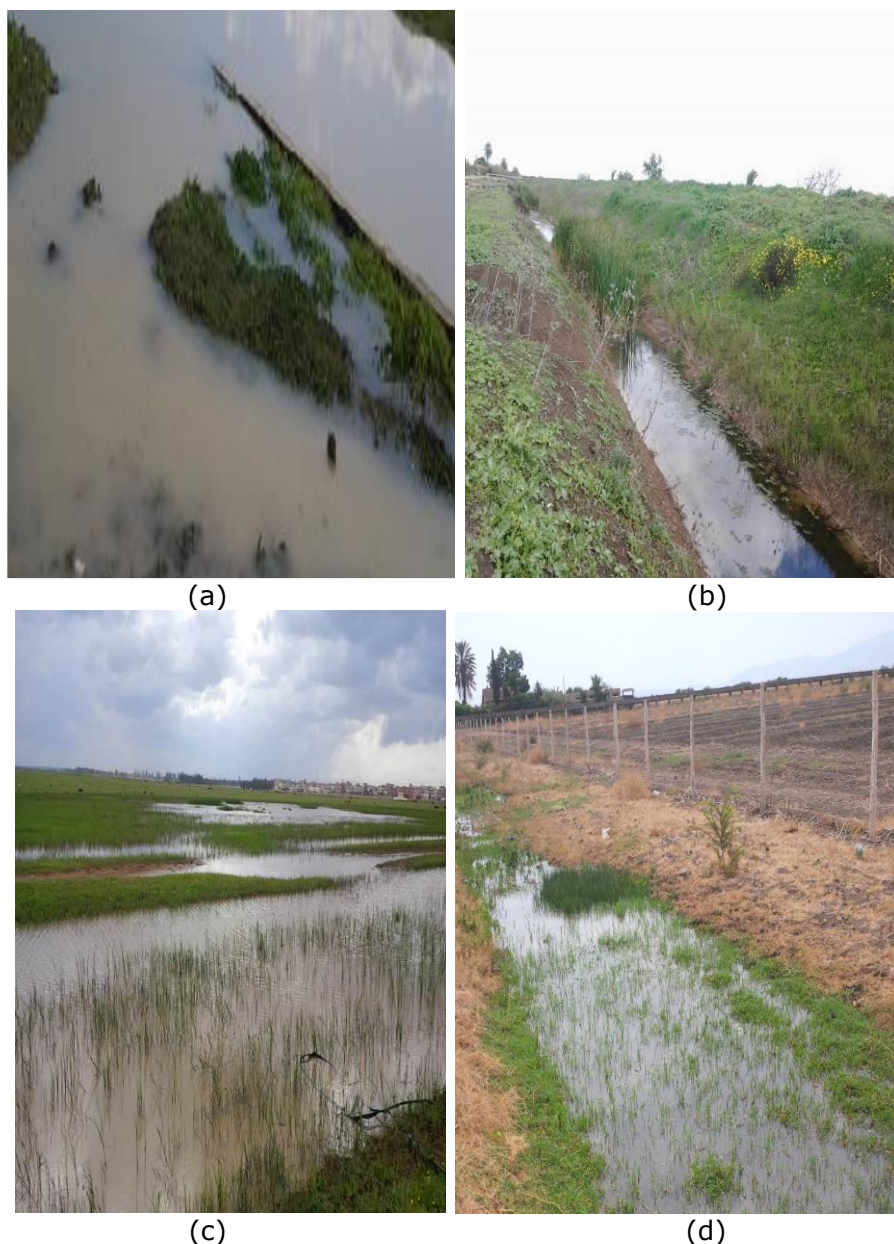


Figure 2. Prospected breeding sites: (a) Laabiat, (b) Sidi Hagouch, (c) Soualem,(d) Kariat El Hasnaoui.

Samples were taken at precise intervals, once a month for each larval biotope, during weather conditions that are favourable for sampling the species. Larvae fishing was carried out using a troubleau which consists of an iron circle 30 cm in diameter onto which a tulle cloth bag is fitted to allow the water to drain away while retaining the larvae. A 2 m handle made of wood or iron cane is fitted to the circle using a socket (Bameul 1990). For small water collections, larvae are collected using ladles with a capacity of 500 mL (Bendali al 2001). The larvae were collected in a tray filled with water and then sampled using an entomological pipette. They were preserved in a bottle (Ministry of Health, Morocco 1996), containing 70° alcohol and labelled with the name of the site, the date of sampling, the locality and the commune (Figure 3).



Figure 3. Material used for mosquitoes larval fishing.

The surface area of the breeding sites was calculated from direct measurements of the dimensions of the breeding sites in the field. The species collected were identified at the Biology and Health Laboratory: Environment and Parasitology Team at the Faculty of Science in Kénitra. We used a binocular magnifying glass, an optical microscope and software for identifying mosquitoes from Mediterranean Africa (Brunhes et al 1999), which enables identification based on a set of very precise microscopic criteria and descriptors. The identification of Culicidae larvae was also based on the works of Rioux (1958) and Himmi et al (1995).

**Results.** Our study of the spatio-temporal variation in *Culex* species in the north-east of the province of Sidi Slimane identified several species in four breeding sites. With regard to the characteristics of the breeding sites of disease-carrying mosquitoes, the results show that Culicidae breeding sites are distributed as follows: 75% of the breeding sites are artificial (Laabiat, Sidi Hagouch, Soualem), while 25% are natural. Concerning the type of deposit, we surveyed 50% of the ditch-type deposits (Sidi Hagouch, Soualem), and 25% of the canal-type deposits (Laabiat). In addition, 75% of the sites surveyed were temporary (Laabiat, Soualem and Kariat El Hasnaoui), while 25% were permanent (Sidi Hagouch), as shown in Table 1.

Table 1  
Characteristics of the four breeding sites studied (Laabiat, Sidi Hagouch, Soualem, Kariat El Hasnaoui). Hagouch, Soualem, Kariat El Hasnaoui)

<i>Breeding sites</i>	<i>Type of breeding sites</i>	<i>Nature of breeding sites</i>	<i>Breeding sites character</i>
Laabiat	Canal	Artificial	Temporary
Sidi Hagouch	Ditch	Artificial	Permanent
Soualem	Ditch	Artificial	Temporary
Kariat El Hasnaoui	Water collection	Natural	Temporary

These larval biotopes vary in species richness. The Laabiat and Soualem breeding sites have a species richness of 2, the Kariat El Hasnaoui deposit has a species richness of 3, while Sidi Haggouch has the highest species richness with a score of 4 (Figure 4).

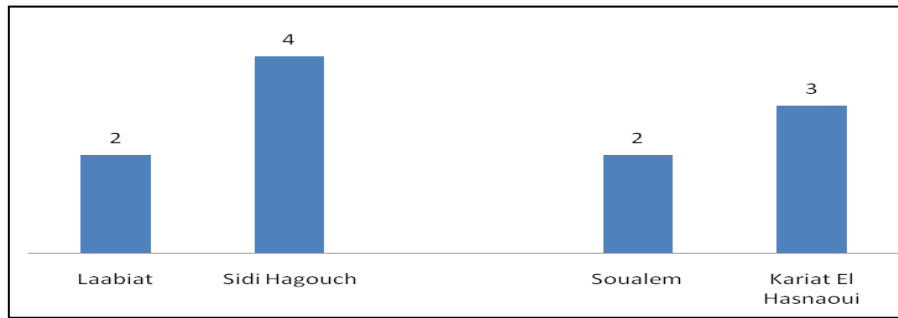


Figure 4. Distribution of species richness in the four breeding sites studied.

Entomological surveys of the four breeding sites resulted in the collection of 889 Culicidae larvae. This study enabled us to inventory five species of mosquitoes of the genus *Culex*: *Culex pipiens* with 61% of the total density in the four habitats, *Culex hortensis*, *Culex modestus*, *Culex theileri*, and *Culex laticinctus* with total densities in the four habitats of 15%, 7%, 8% and 9% respectively, as shown in Table 2 and Figure 5.

Table 2

Origin of mosquitoes larvae identified in the province of Sidi Slimane

Breeding sites	Species identified	Specific richness
Laabiat	<i>C. hortensis</i> , <i>C. pipiens</i>	2
Sidi Hagouch	<i>C. modestus</i> , <i>C. theileri</i> , <i>C. laticinctus</i> , <i>C. pipiens</i>	4
Soualem	<i>C. pipiens</i> , <i>C. hortensis</i>	2
Kariat El Hasnaoui	<i>C. pipiens</i> , <i>C. hortensis</i> , <i>C. modestus</i>	3

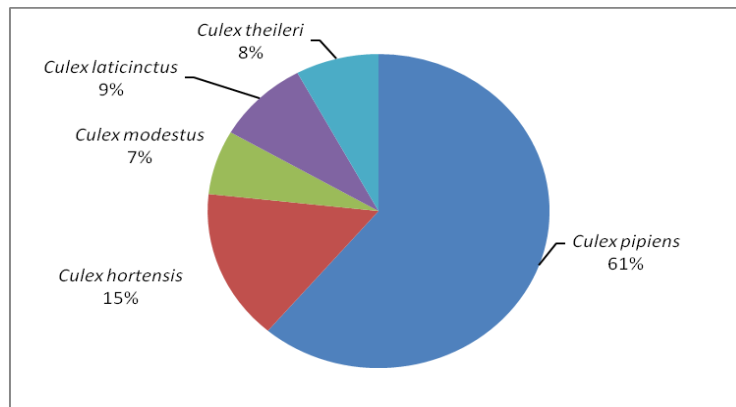


Figure 5. Percentage of larval densities of five culex species identified in the four breeding sites.

**Laabiat station.** We collected two species at this temporary station, which remains in water for seven months. The fauna identified at this breeding site is represented by *Culex pipiens* and *Culex hortensis*, with a total of 135 larvae. *C. pipiens* is clearly dominant at this site, reaching its maximum density in April and May (27 and 29 individuals  $m^{-2}$  respectively). This species accounts for 73% of the total number of specimens collected at this breeding site and persists throughout the impoundment period. *C. hortensis* is less represented in this environment, accounting for only 27% of the total number of specimens collected. *C. hortensis* was identified in this breeding site during four months (March, April, May, June), and reached its maximum density in May (14 individuals  $m^{-2}$ ), the Laabiat breeding site seems to be a favourable breeding site for *C. pipiens* and *C. hortensis*, particularly during periods of heat and humidity (spring). These results can be used to advise public health policies and to develop vector-borne disease control programmes in the region (Figures 6 and 7).

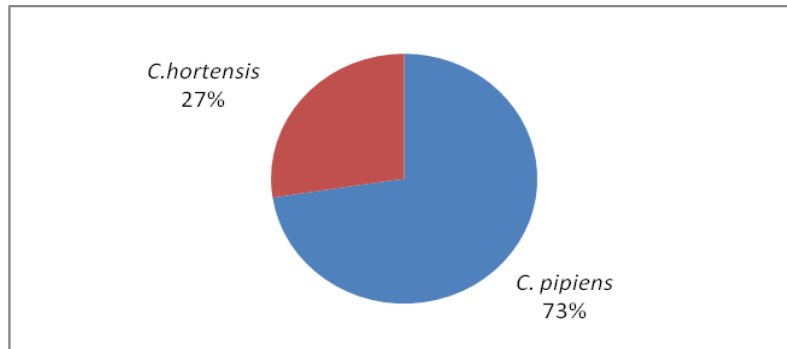


Figure 6. Percentage of species inventoried at the Laabiat station.

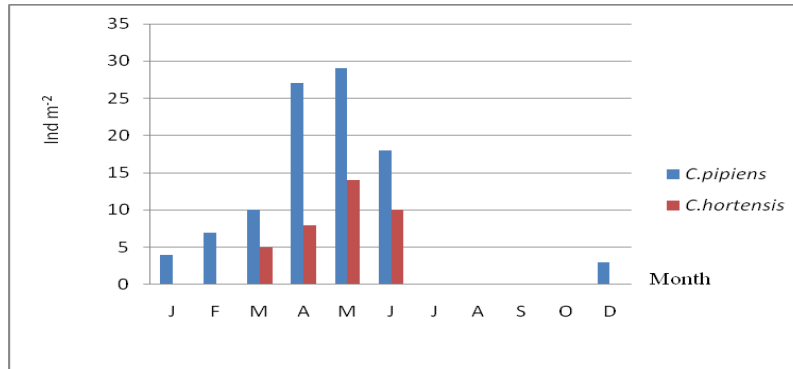


Figure 7. Temporal evolution of *Culex* larval densities at the Laabiat station.

**Sidi Haggouch station.** Four species were collected from this permanent breeding site: *C. pipiens*, *C. laticinctus*, *C. theileri* and *C. modestus*, totalling 391 larvae at various densities. With more than 57% of the species identified, *C. pipiens* predominates, colonizing the area throughout the year, with a maximum density in June and July (29 individuals m<sup>-2</sup>). Our results also indicate that *C. pipiens* exhibits continuous seasonal activity in this environment. *C. laticinctus* represents 20% of the population at this breeding site, and adapts to this environment almost all year round, with the exception of January and February, with a density peak in April (14 individuals m<sup>-2</sup>). *C. theileri* was identified at this habitat station, accounting for 18% of the total number of species collected. It occurs in this biotope during two distinct periods of the same hydrological cycle, in November and December with low densities (4 individuals m<sup>-2</sup>), and from February to June with maximum densities in June (14 individuals m<sup>-2</sup>). *C. modestus* develops in this habitat and accounts for only 5% of the total number of larvae collected in this environment. We recorded a maximum density in April (12 individuals m<sup>-2</sup>), over a very short period (March and April), as shown in Figure 8 and Figure 9.

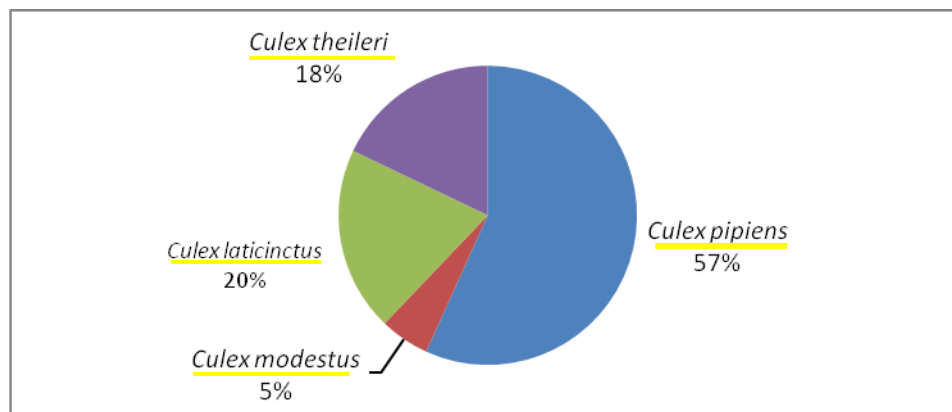


Figure 8. Percentage of species identified at the Sidi Haggouch station.

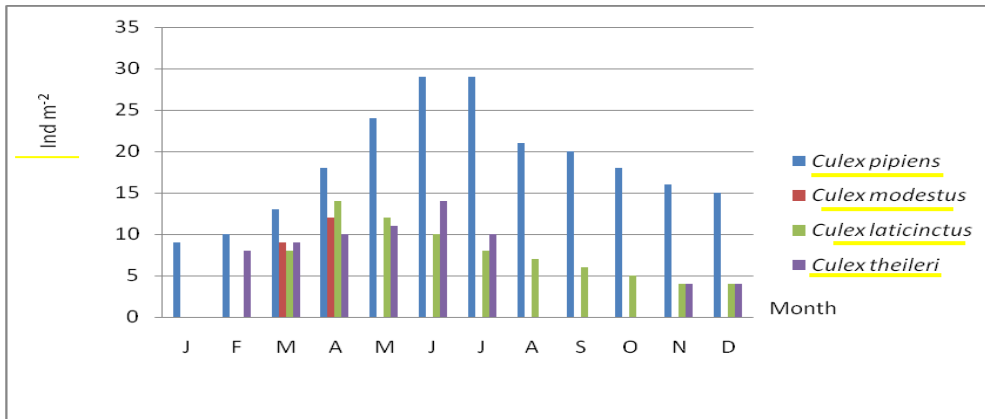


Figure 9. Temporal evolution of *Culex* larval densities at the Sidi Haggouch station.

**Soulem station.** We collected 152 mosquitoes larvae, with two species inventoried, *C. pipiens*, which is largely dominant in this breeding site and represents 69% of the species collected in this environment, and *C. hortensis*, which is less represented in this station, classified as a temporary breeding site, and represents only 31% of the total species identified in this biotope (Figure 10).

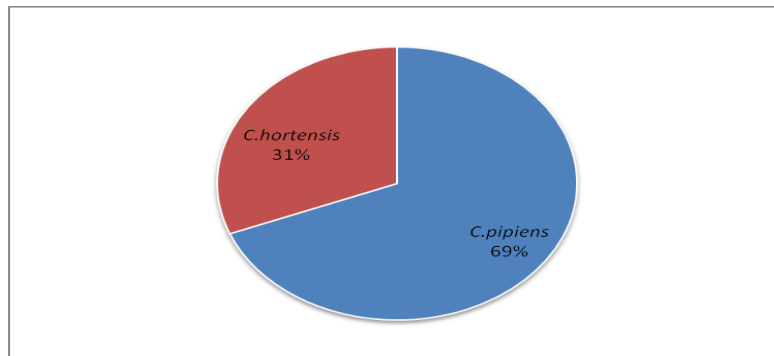


Figure 10. Percentage of species inventoried at the Soualem station.

We observed the presence of *Culex pipiens* in this biotope, with densities peaking in May (26 individuals m<sup>-2</sup>), while densities were significantly low in January (4 individuals m<sup>-2</sup>). *C. hortensis* also occupies this habitat for five months from March until the breeding site dries out at the end of July, reaching a maximum density in June (14 individuals m<sup>-2</sup>), as shown in Figure 11.

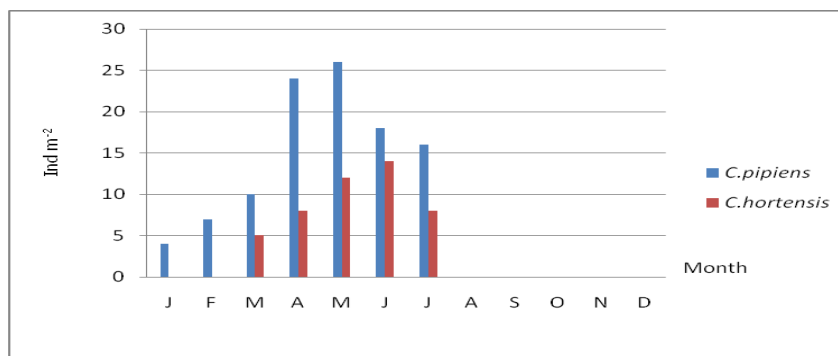


Figure 11. Temporal evolution of *Culex* larval densities at the Soualem station.

**Kariat El Hasnaoui station.** The entomological surveys carried out in this habitat, which is classified as a temporary refuge, where watering lasts a maximum of eight months, resulted in the collection of 211 mosquitoes larvae belonging to three species: *C. pipiens*, *C. modestus* and *C. hortensis*. This study showed that *C. pipiens* is highly responsive in this environment, accounting for 56% of all larvae collected. *C. hortensis* is

well represented, with an abundance of 26%, while *C. modestus* is less represented in this habitat, accounting for only 18% of the total number of larvae collected (Figure 12).

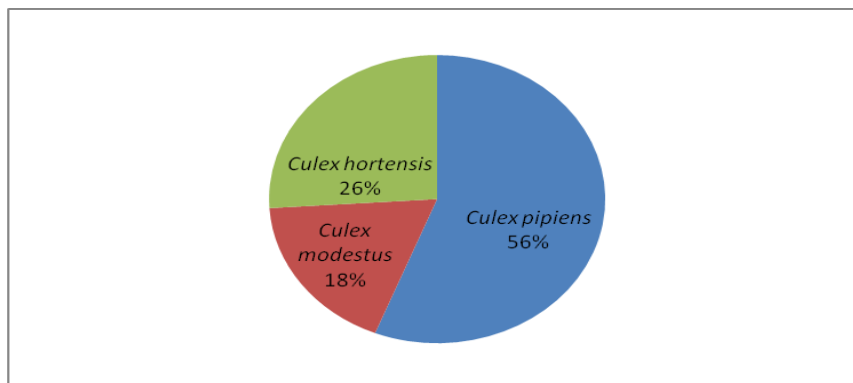


Figure 12. Percentage of species inventoried at the Kariat El Hasnaoui station.

*C. pipiens*, identified as a predominant species, attained maximum density in April (23 individuals  $m^{-2}$ ). As for *C. modestus*, this species occupies the habitat for a period of four months, with maximum density in May (13 individuals  $m^{-2}$ ). On the other hand, we identified *C. hortensis* in this environment during a period of five months, with a maximum density in May (15 individuals  $m^{-2}$ ) (Figure 13).

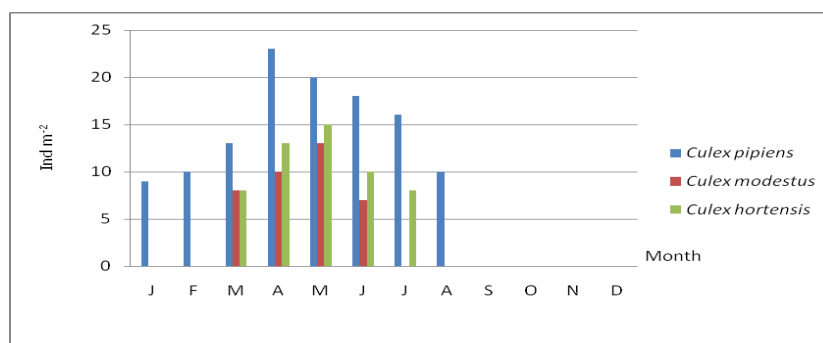


Figure 13. Temporal evolution of *Culex* larval densities in the Kariat El Hasnaoui station.

**Discussion.** The results of this study have very important implications for understanding the ecology of the fauna inventoried, as well as the spatio-temporal fluctuations of mosquitoes in the north-east of the province of Sidi Slimane. The presence of these five species of *Culex* indicates that the study area is likely to be exposed to risks of vector-borne disease transmission and to major nuisance problems. The fauna inventoried in the study area represents 12.16% of Culicidae recorded in Morocco (Louah et al 1995). The presence of the species identified in certain localities and its absence in others are influenced by a large number of environmental factors as well as the specific requirements of each species with regard to the habitat to be colonized. *C. pipiens* is a mosquito species of medical and veterinary importance, and a recognized vector of West Nile virus (Farajollahi et al 2011; Jansen et al 2023). It is largely present in all larval biotopes, is a ubiquitous species that develops continuously, regardless of the type of environment (Himmi 2007), and occupies all types of breeding site surveyed. According to Hassaine (2002), *C. pipiens* is classified as a species with a very wide distribution in Mediterranean Africa. According to Bouallam-Tifnouti (1992), *C. hortensis* is a spring species, preferring mild temperatures. *C. modestus* evolves in the Sidi Haggouch deposit with a low density and during a very short period (March and April), this result is in agreement with the results of Hadji et al (2013). *C. laticinctus* is also present at the Sidi Haggouch station almost throughout the year, with the exception of January and February, and with a maximum density in April. This result is in line with those of Hadji et al (2013). *C. theileri* was observed at the Sidi Haggouch station during two distinct periods of the same hydrological cycle, as a winter and spring species. This result is

consistent with the work of Himmi (2007) and Hadji et al (2013). Climate change could induce transformations and modifications in the composition and spatio-temporal distribution of species, as well as in the dissemination dynamics of mosquito-borne diseases (Filali Mouatassef et al 2021; Rosenkrantz 2022). The results show that the mosquitoes surveyed in the Kariat El Hasnaoui biotope (*C. pipiens*, *C. modestus* and *C. hortensis*), reach their maximum density in April and May, and are present in variable numbers depending on the climatic factors prevailing at the time the specimens are collected, this result is in agreement with the studies of Merabeti & Ouakid (2011). The low densities of mosquitoes larvae surveyed in the various stations concerned by this study may be attributed to various causes, the most common of which are the physico-chemical parameters of the water (Mahamane et al 2020), climatic changes which explain the short periods of impoundment (the drying up of the breeding sites corresponding to the dry seasons), the small quantities of nutrients available, the leaching of breeding sites by precipitation, the slowdown in larval development following the drop in temperature and mortality by invertebrate or vertebrate predators (Berchi 2000). Some authors (Simard et al 2018; Onen et al 2023) stress the importance of implementing strategies to control Culicidae disease vectors, in order to reduce their density and development. With regard to the characteristics of mosquitoes breeding sites, the predominance of artificial breeding sites shows that human activities play an important role in the creation of breeding biotopes suitable for the development of mosquitoes. This work has important implications for the health of the population of the province of Sidi Slimane, and guides the health authorities in developing effective action plans for the control of these disease-carrying mosquitoes. It would be very useful to encourage studies into the spatio-temporal variations and ecology of Culicidae. This would enable us to gain a better understanding of the factors and elements that influence the distribution and abundance of mosquitoes in these larval habitats, and to develop effective and efficient control strategies to control mosquitoes populations and prevent the transmission of diseases (viral, parasitic) and nuisances.

**Conclusions.** This study highlighted the importance of monitoring the spatio-temporal variations of Culicidae vectors of diseases in the north-east of the province of Sidi Slimane. It provided valuable information on the dynamics of Culicidae disease vectors in the study region. The results of our study showed that an understanding of the distribution of Culicidae in space and time is essential if effective vector-borne disease control strategies are to be put in place. The data collected from this study has made it possible to identify localities at high risk of vector-borne disease transmission and to develop targeted control action plans to reduce the prevalence of these diseases in the region. In fact, these results have made it possible to undertake highly effective control measures and methods during specific periods and according to the high larval densities of mosquitoes, while avoiding the random and abusive use of insecticides. The results of this study enabled us to significantly reduce cases of vector-borne diseases and nuisance in the north-east of Sidi Slimane, thereby improving public health and the quality of life of residents living near the four breeding sites surveyed. The importance of this study resides in its contribution to understanding the ecology of Culicidae and to developing effective control strategies against vector-borne diseases. We hope that the results of this study will be useful in encouraging public health and local authority decision-makers to develop vector-borne disease control programmes in other regions of the province. This study demonstrated the importance of scientific research in understanding and controlling vector-borne diseases. This study demonstrated the importance of scientific research in understanding and controlling vector-borne diseases and in adopting effective control strategies against vector-borne diseases.

**Conflict of interest.** The authors declare that there is no conflict of interest.

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