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DIVERSITY OF MOSQUITOES (DIPTERA, CULICIDAE) AND PHYSICO-CHEMICAL CHARACTERIZATION OF THEIR LARVAL HABITATS IN TIZI-OUZOU AREA, ALGERIA

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Diversity of Mosquitoes (Diptera, Culicidae) and Physico-Chemical Characterization of Their Larval Habitats in Tizi-Ouzou area, Algeria. Oussad, N., Lounaci-Ali BenAli, Z., Aouar-Sadli, M. — Many mosquito species are considered as vectors of several pathogens responsible of serious infectious diseases including Malaria, Dengue, Chikungunia and Rift Valley Fever. The characterization of the larval habitat of these arthropods is an important step for a nuisance/vector control program. In this study we aimed to identify mosquito larvae species in the Tizi-Ouzou area (north-central of Algeria) and to examine the physico-chemical parameters of their permanent breeding sites. Surveys are carried out during the dry and rainy seasons (2018/2019), the larval stages of the mosquitoes were sampled using the dipping technique and the physico-chemical parameters of the breeding sites were measured. The morpho-taxonomic examination of the Culicidae samples allowed the identification of 14 species belonging to five genera and two subfamilies. Interestingly, *Culex pipiens* was the most abundant species found in all entomological surveys. Moreover, *Culex pipiens* and *Culiseta longiareolata* species showed high ecological plasticity and were the best correlated species to the studied physico-chemical parameters. Temperature was positively correlated with the density of most inventoried species. Our data would be of great interest in the context of developing a nuisance control program and the prevention of vector-borne diseases.
Key words: Culicidae, physico-chemical parameters, breeding site, Tizi-Ouzou area, Algeria.

Introduction

Vector-borne diseases are a major threat to public health (Gillespie, Smith and Osbourn, 2004), transmitted to humans by blood-sucking arthropods (Rodhain and Perez, 1985), especially mosquitoes (Diptera, Culicidae). Mosquitoes affect millions of people worldwide by transmitting the disease-causing agent (pathogen) of several serious diseases (Ludwig et al., 2019) such as Malaria, Chikungunya, Lymphatic filariasis, Encephalitis, Rift Valley fever, Yellow fever (Marc et al., 2016)53 and 3,65 mg/l; BOD5 between 3 and 15 mg/l. In fact, more than 3.9 billion people in over 129 countries are at risk of contracting Dengue. Moreover, Malaria causes more than 400,000 deaths every year (WHO, 2020). The surveillance system (animal, human and vector) requires further support to prevent outbreaks (WHO, 2014 b). The World Health Organization (WHO, 2014 a) has noted the importance of the identification and monitoring various vector populations as part of global surveillance. The identification of vectors, where and when they appear, and their behavioral characteristics are basic steps in planning vector control interventions. To control mosquitoes effectively, it is important to understand their life cycle (Jackman and Olson, 2002). All mosquitoes need aquatic habitats for their development (Becker et al., 2010). Indeed, adult, larva and pupa mosquitoes have very different morphologies adapted to their lifestyle: aquatic for the pre-imaginal stages and aerial for the adult (Carnevale and Robert, 2009). Oviposition and pre-imaginal stages development are conditioned by the nature and the suitability of the breeding site (Liu et al., 2019). Of note, the larvae of some mosquito species tolerate a wide range of physico-chemical parameters that are important for their bioecology. Several studies have investigated the physico-chemical factors of habitats contributing to the selection of mosquito oviposition sites (Berchi, Aouati and Louadi, 2012; Gopalakrishnan et al., 2013; Waewwab et al., 2019). However, data regarding the physio-chemical characteristics of mosquito larval habitats in Algeria are limited. Moreover, diversity and distribution of mosquitoes in Algeria has not been fully investigated. In the present study, we aim to identify and monitor the larval density of mosquitoes and to highlight the parameters that have the greatest influence on the larval development of the mosquito species present or potentially present in the area.

Materials and methods

Study area

The district of Tizi-Ouzou is a part of the northern Algeria, it is limited by the Mediterranean Sea from the north, Bouira district from the south, Boumerdes from the west and Bejaia from the east. Its steep relief, strongly dissected by an important hydrographic network, gives rise to an alternation of landscapes and geosystems: coastal plains, coastal massifs, valleys and inland depressions, low, medium and high mountains (Medour, 2010). The area has a mediterranean hot summer climate, the dry season (May–September) proceeds the rainy season (October–April). During the study period, the average rainfall varied from 0 to 187 mm and the temperature from 9.2 to 27.8 °C (fig. 1).

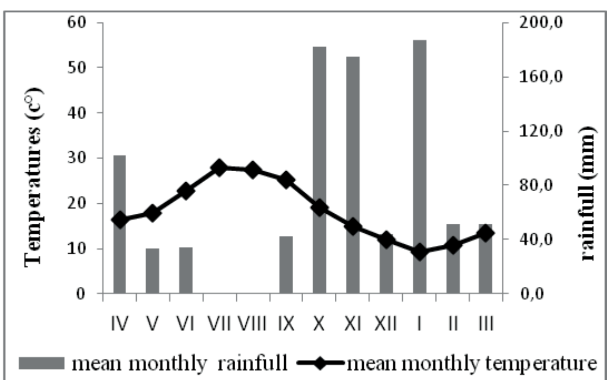

Fig. 1. Temperature fluctuations and average rainfall in Tizi-Ouzou area (2018–2019) (NMO, 2019).

Description of the prospected larval sites

In order to have a representative picture of the mosquito species potentially present in the study area (fig. 2), seven permanent sites, which differ ecologically from one another, were selected (fig. 3).

Site 01 (36°32.5800´ N, 4°5.3400´ E; 470 m a .s. l.): is an open water ditch, fed by spring and rainwater. It is situated next to a dump in Ouadhia Municipality, sunny and rich in vegetation. The water of this ditch is rather clear and deep.

Site 02 (36°33.4800´ N, 4°11.8200´E; 382 m a. s. l.): is a shallow cemented basin without vegetation, located on the road to the Ath Yanni region. This site is fed by spring water as well as rainwater.

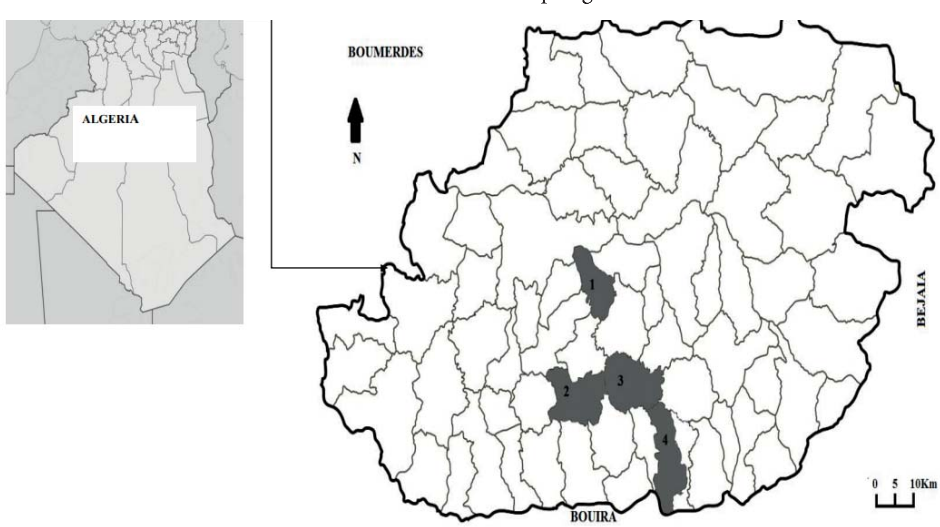

Fig. 2. Study sites location (1— Irdjen; 2 — Ouadhia; 3 — Beni Yenni; 4 — Iboudraren).

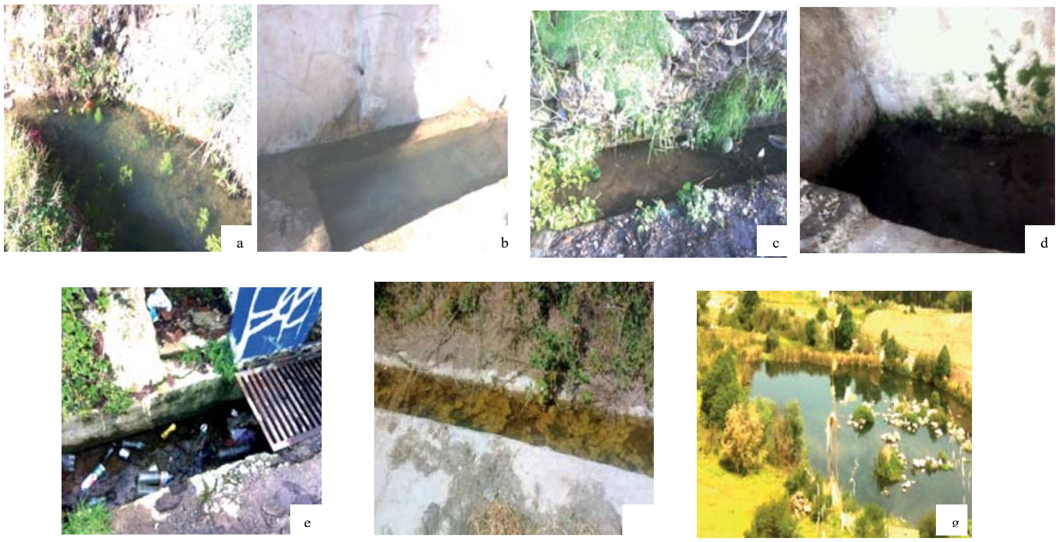

Fig. 3. Mosquito breeding sites (site 01, a; site 02, b; site 03, c; site 04, d; site 05, e; site 06, f); site 07, g).

Site 03 (36°30.1200' N, 4°14.4000´ E; 790 m a. s. l.): is rather a shallow shady channel with clear water, vegetation and a layer of dead leaves on the surface.

Site 04 (36°30.6600´N, 4°14.6400´ E; 923 m a. s. l.): represents two drinking troughs located in Tassaft, a village in the municipality of Iboudraren and supplied with spring water during the summer. It is a shaded, medium deep site with a sandy bottom and without vegetation in the surrounding area. The water in this breeding site is rather clear.

Site 05 (36°30.3000´N, 4°15.4200´ E; 1000 m a. s. l.): is a water reservoir, which receives rainwater and spring water. Located at the entrance of Yatafen village in the commune of Iboudraren. The water in this gîte is turbid, shallow and polluted by domestic waste.

Site 06 (36°39.4200´ N, 4°4.8060´ E; 85 m a. s. l.): is a relatively large, shallow swamp in the Irdjen area, with extensive vegetation cover characterized by duckweed and filamentous algae, the water is rather clear.

Site 07 (36°40.6800´N, 4°6.9600´ E; 95 m a. s. l.): is a shallow water channel fed by dam waters coming from the Irdjen area and rainwater. The water in this sunny breeding site is clear and covered with filamentous algae.

Larval harvesting

The monitoring of the breeding sites was carried out once every two weeks from April 2018 to May 2019. Dipping technique was used to collect the larval stages as described by (Papierok, Croset and Rioux, 1973). Several samples were taken from the same site to form a single homogeneous sample. The larvae are then sorted, counted, placed in small-aerated bottles and transported to the laboratory.

Identification of Culicidae

Captured larvae were mounted using protocol (Matile, 1993) and identified using dichotomous keys (Becker et al., 2010; Himmi et al., 1995) and the Mediterranean African mosquito identification software (Brunhes et al., 2000).

Physical and chemical parameter recordings

The physico-chemical parameters used to study the mosquito larval breeding sites are: pH, conductivity, dissolved oxygen, salinity and temperature. These were measured directly in situ using a multi-parameter analyzer PCE-PHD-1-KIT1.

Diversity and statistical analysis

The results of the mosquito inventory are treated by ecological indices such as species richness (S), relative abundance ($F = Ni \times 100/N$), where Ni is the number of individuals of species i and N is the total number of individuals of all species present, As well as the frequency of occurrence and constancy of each study station ($C = \mathbf{Pi} \times \mathbf{100}/N$) where Pi represents the number of surveys containing the species studied (i) and N represents the total number of surveys carried out. The species is qualifies as omnipresent when $80\ \% \leq C < 100\ \%$, as constant when $60\ \% \leq C < 80\ \%$, as regular when $40\ \% \leq C < 60\ \%$, as infrequent when $20\ \% \leq C < 40\ \%$ and it is qualified as accidental when $C < 20\ \%$ (Scherrer, 1984; Silver, 2008). In order to highlight the relationship between the larval density of abundant species and the physico-chemical parameters of the studied sites, Pearson correlation coefficients indicating r-squared values were calculated using R software.

Results

Inventory

A number of 4968 mosquito larvae were collected from April 2018 to May 2019. Morphotaxonomic examination allowed the identification of 14 species of mosquitoes belonging to five genera: *Aedes, Anopheles*, *Culex, Culiseta* and *Uranoteania*. Culicidian larval production was higher in the dry season than in the rainy season. The most productive site was site 5, with a total of 1675 individuals in the dry season and 248 individuals in the rainy season (table.1).

Abundance

In terms of relative abundance, *Culex pipiens* L. seems to be the most abundant species with a total of 2,184 individuals during the dry period and 299 individuals during the rainy period where it was ubiquitous in three breeding sites (2, 3, 5). *Culiseta longiareolata* (Macquart, 1838) comes second with a total of 1,252 individuals during the dry period and 136 during the rainy period. This species is endowed with high ecological plasticity and occurs in various larval sites. Among the 14 listed species, a total of seven species are present throughout the year (table.1), including *Culex pipiens, Culiseta longiareolata, Culex hortensis* (Ficalbi, 1889), *Culex impudicus* (Ficalbi, 1890)*, Culex perexiguus* (Teobald, 1901)*, Anopheles labranchiae* (Meigen, 1818) *and Anopheles claviger* (Meigen, 18041). The

Table 1. Relative abundance (RA %); frequency occurrence (CO) and effectif of mosquito larvae per sampling station in dry and rainy season

Dry season	Site 1 RA% CO	Site 2 RA% CO	Site 3 RA% CO	Site 4 RA% CO	Site 5 RA% CO	Site 6 RA% CO	Site 7 RA% CO	Effectif
Cs. longiareolata	_	23.41 (C)	25.07 (C)	_	47.70 (O)	_	_	1252
Cx. pipiens	39.81(C)	72.93 (C)	65.03 (O)	_	52.18 (O)	0.30 (A)	_	2184
Cx. hortensis	47.39 (C)	1.95 (I)	8.78 (C)	1.57 (A)	_	38.91 (O)	8.96 (I)	369
Cx. impudicus	3.79 (I)	_	0.42 (I)	_	_	4.56 (R)	5.97 (I)	33
An. labranchiae	8.06 (I)	1.71 (I)	0.56 (R)	_	_	9.12 (I)	20.90 (I)	76
An. claviger	_	_	0.14 (A)	98.43 (C)	_	_	_	127
Ae. caspius	0.95 (A)	_	_	_	_	_	_	2
Ur. unguculata	_	_	_	_	_	0.61 (A)	_	2
Cx. mimeticus	_	_	_	_	0.12 (A)	_	5.97 (I)	6
Cx. theileri	_	_	_	_	_	0.61 (A)		2
Cx. perexiguus	_	_	_	_	_	45.90 (C)	58.21 (R)	190
Effectif /station	211	410	1424	127	1675	329	67	4243
			Rainy season					
Cs. longiareolata	_	13.09 (R)	20.45 (A)	_	41.11 (R)	_	_	136
Cx. pipiens	8.47 (I)	62.30 (O)	72.73 (I)	3.57 (A)	55.64 (R)	3.49 (A)	_	299
Cx. hortensis	72.8 (R)	22.51 (O)	_	_	0.81(A)	22.09 (R)	31.71 (C)	120
Cx. impudicus	3.39 (A)	_	_	_	_	11.63 (I)	14.63 (A)	18
An. labranchiae	_	_	_		_	1.16 (A)	31.71 (R)	14
An. claviger	13.56 (A)	_	6.82 (I)	96.43 (I)	0 .02 (A)	32.56 (R)	_	99
Cs. annulata	1.69 (A)	_	_	_	_	1.16 (A)	_	2
Cx. laticinctus	_	2.09 (A)	_	_	_	_	_	4
An. algeriensis	_	_	_	_	_	2.33 (A)	_	2
Cx. theileri	_	_	_	_	_	5.81 (A)	_	5
Cx. perexiguus	_	_	_	_	_	19.77 (R)	21.95 (I)	26
Effectif /station	59	191	44	56	248	86	41	725

Note. (A) — accidental; (I) — infrequent; (C) —constant; (R) — regular; (O) — omnipresent.

remains of the species were recorded in small percentages and in only one station. Indeed, *Aedes caspius* was found only in site 1, *Culex laticinctus* (Edwards, 1913) in site 2 and *Uranotaenia unguculata* (Edwards, 1913), *Culex theileri* (Theobald, 1903) and *Anopheles algeriensis* (Theobald, 1903) in site 6.

Physico-chemical characterization of mosquito larvae breeding sites

The physicochemical properties of the studied sites are reported in table 2. The average water temperature of the studied deposits varies during the study period. Indeed, these temperatures are between 10.06 °C and 28.32 °C and were lower in the shaded deposits. This was the case for sites 3, 4, and 5 where the average temperature during the dry period was 19.67 °C, 17.05 °C and 18.76 °C respectively. The average pH values measured ranged from 6.81 to 7.97. A slightly acidic pH was observed during the dry season in both sites 1 and 6, and slightly alkaline in all the other studied sites. Average conductivity ranged from 0.2627 at deposit 02 to 1.19 mS/cm at deposit 03. The average dissolved oxygen content recorded at the study deposits is between 3.577 and 8.82 mg/L. It is higher in the rainy season than in the dry season, but in general, the dissolved oxygen level remains relatively low in all the monitored sites. The average salinity values recorded in the study sites are below 0.1 g/l. with a slight decrease during the rainy season.

Table 2. Seasonal variations of the physico-chemical parameters in the study sites

Sites	Dry season					Rainy season				
	salt (%)	water T (°C)	DO (mg/l)	CD (mS /cm)	Ph	salt (%)	water T (°C)	DO (mg/l)	CD (mS/cm)	pH
Site 1	0.026	23.35	5.21	0.4711	6.958	0.0235	15.72	5.93	0.427	7.95
Site 2	0.017	27.15	4.75	0.2972	7.641	0.01571	19.1	6.07	0.2627	7.97
Site 3	0.04	19.67	5.81	0.8423	7.372	0.0492	13.4	5.05	1.193	7.55
Site 4	0.022	17.05	8.56	0.5058	7.113	0.02571	13.02	7.38	0.4856	7.51
Site 5	0.035	18.76	7	0.7018	7.355	0.02101	10.06	4.78	0.457	7.8
Site 6	0.031	25.06	3.577	0.5085	6.813	0.02428	13.7	5.97	0.4551	7.82
Site 7	0.032	28.32	8.65	0.6334	7.341	0.03071	15.04	8.82	0.6378	7.75

Note. Salinity — salt; water temperature — water T; dissolved oxygen — DO; conductivity — CD; acidity — pH.

Table 3. Means and standard deviations of physicochemical characteristics along with occurrence of mosquito species in different larval habitats

Species (occurrence)	Salt, %	water T, °C	DO, mg/l	CD, mS/cm	pH
Aedes caspius (1)	0.02	20.2	4.4	0.454	7.2
Culex pipiens (36)	0.029 ± 0.018	19.93± 5.389	5.056 ± 3.043	0.572 ± 0.376	7.5 ± 0.533
Culex hortensis (36)	0.026 ± 0.009	19.81± 5.071	6.23 ± 3.15	0.49 ± 0.18	7.54 ± 0.593
Culex impudicus (17)	0.027 ± 0.007	22.43± 5.794	6.33 ± 3.527	0.493 ± 0.131	7.45 ± 0.554
Culex mimeticus (3)	0.028 ± 0.016	24.93 ± 10.18	7.63± 2.58	0.54± 0.232	7.21± 0.642
Culex theileri (3)	0.032 ± 0.007	17.15 ± 11.84	4.01± 0.275	0.467± 0.018	7.09 ± 1.481
Culex perexiguus (13)	0.032 ± 0.005	22.57 ± 6.563	5.42 ± 2.563	0.565± 0.105	7.23 ± 0.833
Culiseta longiareolata (21)	0.028 ± 0.019	20.44 ± 5.214	4.74 ± 2.953	0.588 ± 0.417	7.59 ± 0.417
Culiseta annulata (2)	0.02	14.3 ± 0.007	9.6 ± 2.828	0.369 ± 0.102	7.62 ± 0.603
Culex laticinctus (2)	0.02	23.33 ± 3.5	4.7± 0.141	0.297 ± 0.013	7.93 ± 0.035
Uranotania unguculata (1)	0.03	25.1	2.55	0.466	7.35
Anopheles algeriensis (1)	0.02	8.8	7.15	0.427	7.9
Anopheles labranchiae (17)	0.031 ± 0.009	23.21 ± 6.505	5.46 ± 2.822	0.588 ± 0.19	7.26 ± 0.641
Anopheles claviger (21)	0.0297 ± 0.015	14.18 ± 4.082	6.68 ± 3.461	0.588 ± 0.278	7.43 ± 0.558

Table 4. Correlation of physico-chemical parameters with the larval production of the most abundant species

Species	Salt	Water T	DO	CD	pH
Culiseta longiareolata	0.237± 0.02	0.148 ± 0.17	−0.084 ± 0.445	0.239 ± 0.028	-0.166 ± 0.12
Culex pipiens	0.242 ± 0.02	0.223 ± 0.04	−0.165 ± 0.133	0.239 ± 0.028	−0.183 ± 0.094
Culex hortensis	0.090 ± 0.41	0.347± 0.001	−0.232 ± 0.033	0.029 ± 0.786	−0.166 ± 0.131
Culex impudicus	1.95e-05±0.9	0.254± 0.019	−0.062 ± 0.569	−0.064 ± 0.562	−0.037± 0.736
Anopheles labranchiae	0.126 ± 0.25	0.42± 0,00	−0.131± 0.234	0.008 ± 0.940	−0.46± 9.6e-06
Culex perexiguus	0.121± 0.27	0.383 ± 0.00	−0.171 ± 0.121	−0.009 ± 0.934	−0.357± 0.001
Anopheles claviger	−0.052 ± 0.63	−0.062 ± 0.57	−0.019 ± 0.857	−0.003 ± 0.976	−0.124 ± 0.258

Note. Correlation coefficient (r) ± p values.

The means and standard deviations of the physico-chemical parameters collected at the study stations were calculated for each species (table. 3). *Culex perexiguus, Culex mimeticus, Culiseta annulata* species recorded the highest levels of salinity (0.032 ± 0.005), temperature (24.93°C ± 10.18), dissolved oxygen (9.6 ± 2.828) respectively, while *Culex theileri, Anopheles Algeriensis, Uranotania unguculata* and *Culex laticinctus* have the lowest rates in pH (7.09 ± 1.481), temperature (8.8 °C), dissolved oxygen (2.55) and conductivity (0.297 ± 0.013) each.

Table 4 indicates the correlation coefficients between physico-chemical parameters and larval productivity of the most frequent species. It shows that larval productivity is negatively correlated in an insignificant way with acidity and dissolved oxygen. However, larval productivity was positively correlated with salinity and temperature for most of the species, except for *Anopheles claviger*, which seems to be negatively correlated with all physico-chemical parameters. Moreover, the productivity of certain species such as *Culex pipiens, Culiseta longiareolata, Culex hortensis* and *Anopheles labranchiae* seems to be positively correlated to conductivity, whereas the productivity of *Culex perexiguus, Culex impudicus* and *Anopheles claviger* is negatively and insignificantly correlated to conductivity.

Discussion

Inventory

The present inventory done in Tizi-Ouzou area revealed the presence of 14 species belonging to five genera. A list of 62 species of mosquitoes found in Algeria was drawn up (Robert et al., 2019), including one extinct species (*Aedes aegypti*), one introduced species (*Aedes albopictus*), and one uncertain species (*Anopheles colluzi*). Some of our species have already been reported by (Lafri et al., 2014) including *Aedes albopictus* identified for the first time in Algeria, in Tizi-Ouzou area. *Culex territans, and Culex modestus* have also been recorded in the area (Lounaci, 2003). The absence of these species in our stations may be mainly related to differences between the types of habitats, as well as to the sampling technique adopted or the larval deposits prospected. Obviously, the use of multiple traps for sampling would have potentially allowed a broader study of the biodiversity of the region with a greater number of species. Among the most abundant species, *Culex pipiens* is widespread and has been recorded in various regions of Algeria (Amara Korba et al., 2016; Berchi, 2000; Bouabida, Djebbar and Soltani, 2012). This species has a high ecological plasticity and is a potential vector for West Nile virus and Rift Valley fever (Amraoui et al., 2012; Meegan et al., 1980), other species are also of medico-veterinary interest, such as *Culex perexiguus* reported by Benbetka et al. (2018) as a natural vector of West Nile virus in the Saharan oasis of Algeria. This species is dominant in sites 6 and 7 and fluctuates regularly.

Physico-chemical parameters

The study of the larval density of breeding sites and the physico-chemical parameters made it possible to deduce the preferences of certain species. *Anopheles algeriensis* seems to tolerate low salinity water (Becker et al., 2010). Indeed, it was recorded in fresh water with a salinity level not exceeding 0.02 mS/cm. *Culex laticinctus* and *Culex mimeticus* prefer high temperature sunny sites (Himmi, 2017), they are found in our sites at an average temperature of 23.33 °C and 24.93 °C each. On the other hand, *Culex theileri* was recorded at an average temperature of 17.15 °C, according to Hadji et al. (2013), this species cannot tolerate extreme temperatures. Described as a cold-loving species (Trari and Dakki, 2017), *Anopheles claviger* is negatively correlated with the temperature of our sites, it was recorded at an average temperature of 14.18 °C, and was rather frequent in shaded sites with cool water. The water temperature of breeding sites varies from 5 °C to 31 °C, and significantly correlates with the density of most of the identified species. It therefore seems to have a good influence on the larval development of mosquitoes (Muirhead-Thomson, 1951). The larval density of *Culex pipiens* and *Culiseta longiareolata* is best correlated with temperature, conductivity and salinity. Hence, these parameters appear to be important in the appearance and fluctuation of mosquito larval populations in Boussaâda area where *Culiseta longiareolata* and *Culex pipiens* species are the most abundant (Benhissen et al., 2018). While the density of most species was poorly or not at all correlated with the pH and dissolved oxygen of the selected sites, that of *Anopheles labranchiae* and *Culex perexiguus* were significantly correlated. According to the works of (Ahmed, Kuriji and Kheir, 2010; El-Naggar et al., 2018), pH and salinity have no significant effect on larval population dynamics. However, Rageau and Adam (1952) state that *Anopheles* larvae do prefer acidic pH waters. In Morocco, biotopes characterized by low dissolved oxygen levels, temperatures of around 27 °C and low conductivity varying between 595 and 1300 µs are home to the *Anopheles labranchiae* species (Lalami et al., 2010). In agreement with our results and according to (Hanafi-bojd et al., 2012; Ibrahim et al., 2011)diversity and affinity in the area, characterization of larval habitats, and mapping their potential distribution across the district.\nThe potential aquatic habitats for Anopheles larvae were extracted from Indian Remote Sensing Satellite (IRS, larval densities are not significantly related to certain physico-chemical parameters, this may indicate that other factors are involved in the variation of the abundance of mosquito species in the area In fact and according to (Bauer et al., 2011), other parameters such as sunshine, specific interaction, the size of the deposit and its vegetation can affect the mosquito assembly structure.

Conclusion

Despite the restricted area of the present study, our results revealed a significant diversity of 14 Culicidian species, belonging to five different genera. The *Culex* genus is the best represented and *Culex pipiens* appears to be the most abundant species. Our study is the first to associate the larval density of the species with the physico-chemical parameters in Tizi-Ouzou area. These data would be of great interest in the context of developing a program to control nuisances and prevent vector-borne diseases.

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