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Original Research Article

Biotypology of Culicidian Species in the Region of Fez, Central Morocco Using the Statistical Analytical Methods

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ABSTRACT

Controlling mosquitoes responsible for discomfort and serious contagious diseases, such as malaria, requires a better understanding of the vector-environment relationship. Therefore, the present study examined the biotypology of Culicidian species in the Fez Region, Central Morocco, using statistical analytical methods. Entomological investigations and measurements of the physicochemical parameters of the water breeding sites were carried out simultaneously from November 2015 to December 2016 in the region of Fez, Central Morocco. Multivariate analytical methods, including principal component analysis (PCA) and multiple correspondence analysis (MCA), were used to investigate the correlation between environmental factors and the bio-ecological characteristics of each species. The identification of 1,531 *Culicidae* larvae collected from the different breeding sites revealed the presence of 11 species belonging to five genera (*Anopheles*, *Culex*, *Culiseta*, *Aedes*, and *Uranotaenea*). The larvae of *Anopheles maculipennis* (the major malaria vector in Morocco) and the larvae of *A. sergentii* (the secondary vector), as well as *Culex pipiens* larvae, were collected among the species at the level of the prospected breeding sites. It was observed that the Shanon-Wiener diversity index (H') values were higher in areas with a high population of species compared to those with a low population of species. The multivariate analytical methods revealed many groups of species depending on many parameters. This research represents an original and rigorous approach to the vector-environment relationship in the Fez Region of Morocco. It has important implications for the control of mosquito vectors and the prevention of vector-borne diseases, thereby contributing to public health in the region.

Keywords: Biotypology, Central Morocco, Culicidian species, Disease vectors, PCA, MCA.

Introduction

Insect vectors are the leading cause of morbidity and mortality worldwide.[1,2] The diseases that spread between people via mosquitoes have caused numerous pandemics.[3,4] The distribution and transmission of this disease are closely linked to vector, ecological, and bioclimatic factors.[5–8] Morocco has achieved the goal of eliminating indigenous cases of malaria, as a health problem, for several years.[9–11]

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However, the risk of the emergence of new vector-borne diseases is still present and depends mainly on the introduction of new species and pathogens, as well as climate change. The latter has contributed to the emergence and resurgence of infectious diseases worldwide,[12] and the redistribution of their vectors. In addition, many parameters modified by socioeconomic development, urbanization, landscape transformation, immigration, and the globalization of travel and freight transport can affect vector distribution and increase the risk of transmission of vector-borne diseases. Temperature thresholds limit the geographical range of mosquitoes.[12] Habitat modification, increase, and movement of the human and animal population through migration and transport are also considered among the factors that favour the geographic distribution of mosquitoes.[13] Thus, the changing distribution of vectors and the appearance of many invasive species require improved knowledge of larval breeding sites, the biotope necessary for the survival of pre-imaginal stages, and the reproduction of adults.[14] Some authors have also suggested that the physicochemical characteristics of mosquito breeding sites may have some effect on the oviposition, survival, and spatial distribution of mosquito vectors.[15,16]
In the region of Fez, Central Morocco, studies are centered on entomological investigations and measurements of the physicochemical parameters of the water in the larval breeding habitats of Culicidae,

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which are conducted over one year. These studies are conducted with statistical factor analytical methods, such as principal component analysis (PCA) and multiple correspondence analysis (MCA). The PCA and the MCA allow the user to reduce the number of variables and make the information less redundant, especially when determining a correlation between a fairly large number of parameters and variables.[17] In Morocco, a few studies of the physicochemical characteristics of Culicidae larval breeding sites have been conducted by PCA.[18–21] However, these studies have always been limited in time and space and adapted to specific ecological parameters and local species in each study area.
The present study employed statistical analytical techniques to investigate the biotypology of Culicidian species in the Region of Fez, Central Morocco.

Materials and Methods

Study area
The Fez region (34°01'15.0"N, 5°00'34.1"W), is located at the point of convergence of four major natural regions: the Middle Atlas to the south, the Atlantic plains to the west, the Rif to the north, and the eastern highlands opening wide passages to the Tafilalet. It is well known for its epidemiological past. This region is characterized by various aspects of the hydraulic network, which primarily depends on the water table for the supply of water and irrigation of the surrounding countryside. Temperatures in the region are low when snow covers the surrounding mountains in winter, while the heat is very high in summer. However, there is sufficient rainfall with a relatively stable inter-annual regime and an average variable of monthly precipitation (Table 1).

Sampling of mosquito larvae
Twenty-seven (27) potential mosquito breeding sites were selected, as shown in Figure 1. From November 2015 to December 2016, a total of 44 trips were made, during which 225 examinations were carried out. The parameters were recorded, taking into account the type of site, its origin, depth, and altitude. The sampling of larvae was carried out with the immersion method, which was conducted almost every two months in the different existing biotopes and with biweekly collections in four representative breeding sites. The identification of the morphological characteristics of the mosquito larvae was determined using the Moroccan *Culicidae* identification key,[22] and the Mediterranean Africa Mosquito Identification Software.[23]

Collection and analysis of water samples
Two hundred and seventeen water samples were taken from possible breeding sites. Parameters studied in the field were water flow (m^3/s), temperature (°C), dissolved oxygen (ppm), pH, total dissolved solids (mg/l), salinity (‰), electrical conductivity (µs/cm), and turbidity (NTU). These parameters were studied using portable instruments (Consort Multiparameter Analyzer C561, YSI Scientific Water Quality Instrument, and Lovibond® Turbidirect Turbidimeter).

Analyses of data by ecological indices
The mosquito community structure at each breeding site was analyzed using different indexes. One of these indexes included the Shannon-Weiner index (H').[24] This index (H') provides information about species diversity in each environment. A low H' value suggests that species richness in the ecosystem is considered to be low, while a high H' value implies that the environment is densely populated with species or favorable to the development of species. The Shannon-Weiner index (H') was calculated as follows:

$$H' = -\sum_{i=1}^{S} pi\log_2 pi$$

Where H' denotes the Shannon-Weiner index, pi denotes the relative abundance, which is the percentage of individuals of a species (ni) relative to the total number of individuals (N).

Another index employed in the study was the Shannon equitability index.[25,26] It allows the appreciation of the distribution of the various taxa in sampled sites and was calculated using the formula:

$$E = \frac{H'}{\log_2 S}$$

Where E represents the Shannon equitability index, H' denotes the Shannon-Weiner index; and S denotes the species richness, represented by the total number of species recorded per unit area.

Statistical analysis
Multivariate analysis was performed using the statistical package for the social sciences (SPSS; version 23.0) and Unscrambler software (version 9.0). The analysis of the biotypology of Culicidian species was carried out using a matrix that relates the values of nine physicochemical parameters of the environment to the appearance of the taxa, which were treated by PCA. The choice of this analysis is justified by the quantitative nature of the variables studied.[27] To determine the distribution of species as a function of altitude, nature, and the type of breeding sites, the association between species and factors of altitude, nature, and origin of the breeding site was processed with MCA.

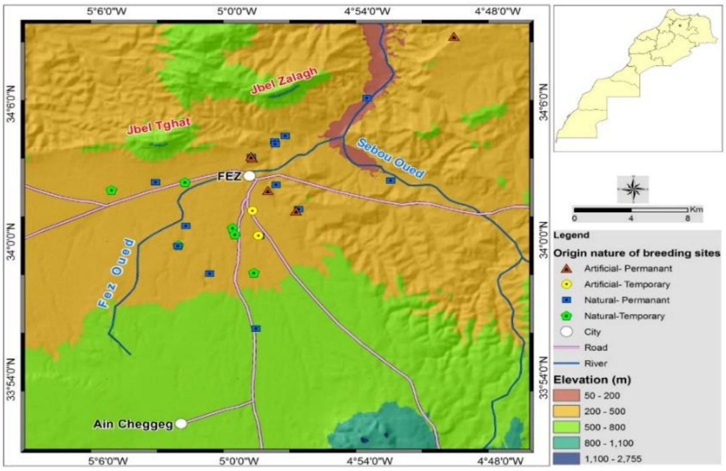

Figure 1: Location of the studied sites in Fez region, Morocco.

Table 1: Average monthly rainfall in the region of Fez, Morocco, during the study period.

Parameter	Nov. 2015	Dec. 2015	Jan. 2016	Feb. 2016	Mar. 2016	Apr. 2016	May. 2016	Jun. 2016	Jul. 2016	Aug. 2016	Sep. 2016	Oct. 2016	Nov. 2016
Men P	6	0	14.7	69.0	57.0	19.2	40.6	0.9	0	0	0	71.9	65.6
Min	2		0.6	0.2	0.5	0.3	0.2	0.3				0.9	0.3
Max	4		7.4	30.1	26.4	12.9	12.9	0.6				37.0	21.6
NRD	2	0	4.0	8.0	6.0	10.0	10.0	2.0	0	0	0	3.0	8.0

Men P: Monthly precipitation; Min: Minimum; Max: Maximum; NRD: Number of rainy days

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Results and Discussion

A total of 1,531 larvae belonging to 11 species were collected; five species of the genus *Culex* (*Cx*), three of the genus *Anopheles* (*An*), two of the genus *Uranotaenia* (*Ur*), one of the genus *Culiseta* (*Cs*), and one of the genus *Aedes* (*Ae*) (Table 2). The qualitative results obtained revealed the existence of species richness of 11 species similar to those reported in the same region by Mouatassem *et al.* (2019),[28] of which were cited by Lalami *et al.* (2010).[29] Most of the *Culex* mosquitoes collected pose a significant risk to human and animal health.[3,30,31] The entomological information collected is presented in Tables 3-5. The species inventory allowed the determination of the distribution of immature mosquitoes in the breeding sites and the identification of the areas at risk (Table 3). The Shanon-Wiener diversity index (H') values ranged from 0 to 1.94. This index was higher in areas with a high number of species (H' = 1.94 at the Awinat Elhajaj puddles with 7 species and H' = 1.74 at the Douar lhandiya spring with 8 species) compared to areas with a low number of species where its value was 0.18 at the Wislane Oued and 0.22 at the Montfleurie II sheepfold, both deposits containing two species each. It was noted that the values of H' were different in the deposits where the number of species (S) was the same. These values remained different at the theoretical maximum diversity value (H_{max}). Furthermore, the equitability index (E) varied between 0 and 1 and tends to 0 when a single species dominated the whole stand, which was the case at Lmsefer (E = 0.12), Wislane Oued (E = 0.18), Lmzah Oued (E = 0.24), Sebou Oued (E = 0.26), Grand Canal (E = 0.29), and the Sheepfold of Montfleurie II (E = 0.24).

In the present study, the presence of *Anopheles* species in different collections of sunny, vegetation-rich waters, such as dams, springs, and puddles, confirms that *Anopheles* larval breeding sites are diverse.[32] These results also indicated a potential health risk in terms of possible transmission of the parasite if an infected person is present. The significant effect of conductivity and TDS at the breeding site on *Anopheles* larval production has already been noted by several authors.[33–35] The high electrical conductivity and turbidity of the water in the breeding sites have been reported to have a negative influence on *Anopheles* density.[32,36] However, in the present study, larvae of *An. cinereus* were observed in saline environments and at high electrical conductivity. Although high TDS appeared to play a role in the presence of *An. maculipennis*, which was not the case for *An. cinereus.* The same observation has been reported in other investigations.[18,19,21] Previous studies have shown that *Anopheles* larvae prefer fresh water with an acidic pH and a high temperature.[19,37,38] Meanwhile, the results of the present study showed that *An. maculipennis* tolerates high temperatures and pH environments. Conversely, other studies have confirmed that *Anopheles* prefer low saline and alkaline water where temperatures are particularly low,[39] which is consistent with the observations in the present study. In the present study, *An. sergentii* was found in oxygenated and low-turbidity waters. This is an indication that Anopheles can colonize different types of sites depending on the species. This observation is consistent with other analyses showing a positive correlation between the number of *Anopheles* larvae collected and the parameters, such as TDS, pH, electrical conductivity, and temperature.[40] These parameters, as well as salinity, have a significant influence on the larval abundance of mosquitoes.[15,41]

Principal component analysis was used to investigate the correlations between physicochemical parameters and species (Figure 2). Table 6 shows the eigenvalues and the explained variability of each of the nine components and their cumulative explained variability. To decide on the number of components to be retained, the Kaiser criterion was applied. The Kaiser criterion states that in a standardized ACP, components with eigenvalues higher than 1 must be retained.[27] The same table shows that the first three components have eigenvalues greater than 1 and explain 85% of the variability. The results in Table 6 also showed that the first two components, which explain 67% of the total data variability, meet this criterion. Therefore, they can be used to explain correlations. The loading plot indicates the presence of some correlations between the physicochemical parameters, especially the correlation between salinity and conductivity; the correlation of pH with temperature and total dissolved solids; and the correlation between discharged and dissolved oxygen.

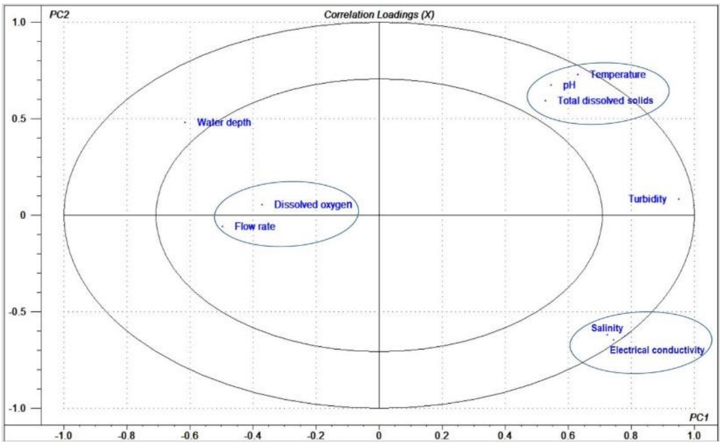

Figure 2: Loading plot of physicochemical parameters according to the first two components.

Table 2: Repertory of Culicidae identified in the region of Fez, Morocco during the period of study

Subfamily Anophelinae	Subfamily Culicinae			
Anopheles	**Aedes**	**Culex**	**Culiseta**	**Uranotaenia**
Anopeles . maculipennis s. l.	*Aedes flavescens*	*Culex. perexiguus* (Theobald, 1903)	*Culiseta longiareolata* (Macquart, 1838).	*Uranotaenia unguiculata* (Edwards, 1913)
Anopheles sergentii (Theobald, 1907)		*Culex pipiens* (Linné, 1758)		*Uranotaenia. balfouri* (Theobald, 1904)
Anopheles cinereus (Theobald, 1901)		*Culex. hortensis* (Ficalbi, 1889)		
		Culex theileri (Theobald, 1903)		

Table 3: Species found in different study locations in the region of Fez, Morocco

Location	***Ae.fla***	***An.cin***	***An.mac***	***An.ser***	***Cx.hor***	***Cx.per***	***Cx.pip***	***Cx.the***	***Cu.lon***	***Ur.bal***	***Ur.ung***
Sais Airport	-	-	-	-	-	-	+	-	-	-	-
Aîn Amier	-	-	-	-	-	+	-	+	-	+	-
Awinat Elhajaj	-	+	+	+	+	+	+	+	-	-	-
Lgaâda dam	-	-	+	-	-	+	-	+	-	-	-
Diamant Vert	+	-	-	-	-	+	+	+	-	-	-
Douwar Lhandiya	-	+	-	+	+	+	+	+	+	-	+
Marjat Lwazani	-	-	-	-	-	-	+	-	-	-	-
Jnan Sbil Fountain	-	-	-	-	-	-	-	-	+	-	-
Grand canal	-	-	-	-	-	-	+	+	+	-	-
Jnan EL Alami	-	-	-	-	-	+	+	-	-	-	-
Lmsefer	+	-	-	-	-	-	+	+	-	-	-
Al mamounia	-	-	-	-	-	-	+	-	+	-	-
Montfleurie II Sheepfold	-	-	-	-	-	-	+	-	+	-	-
Lmzah Oued	-	-	-	-	-	+	+	-	+	-	-
Sebou Oued	-	-	-	-	-	+	+	+	-	-	-
Wislane Oued	-	-	-	-	-	-	+	+	-	-	-

Ae.fla: *Aedes flavescens* ; An.cin: *Anopheles cinereus ;* An.mac: *Anopheles maculipennis ;* An.ser*: Anopheles sergentii ;* Cx.hor: *Culex hortensis ;* Cx.per: *Culex perexiguus ;* Cx.pip: *Culex pipens* ; Cx.the: *Culex theileri ;* Cs.lon: *Culiseta longiareolata* ; Ur.bal: *Uranotaenia balfouri* ; Ur.ung: *Uranotaenia unguiculata* ; +: Presence of species; -: Absence of species.

As shown in Figure 3, the score plot showing the projection of the species according to the first two components allowed the extraction of three categories of Culicidian species. These groups of species are: *An. maculipennis* and *Ur. unguiculata* (Group 1); *Cx. hortensis*, *Cx. perexiguus*, *Cx. pipens*, *Cx. theileri*, *Ur. balfouri*, and *An. sergentii* (Group 2); and the two species *Ae. flavescens* and *Cs. longiareolata* (Group 3). The superposition of the two previous graphs gave the biplot of physicochemical parameters and species as depicted in Figure 4. From the graph, the following information can be deduced: The species group of *Cx. hortensis*, *Cx. perexiguus*, *Cx. pipens*, *Cx. theileri*, *Ur balfouri*, and *An. sergentii* is strongly influenced by dissolved oxygen and current. *Anopheles cinereus* is strongly influenced by salinity and electrical conductivity. Furthermore, both species of *An. maculipennis* and *Ur. unguiculata* predominate in high temperature, high pH, and high TDS environments.

To detect the influence of the parameters (altitude, type, and origin of the habitat) on the distribution of the species found, a multiple correspondence analysis was performed. The joint analysis of all the modalities by the MCA allowed the distinction of three groups, based on two dimensions, with a Cronbach alpha of 0.63 explaining 58.39% for the first dimension and 41.60% for the second (Table 7). The PCA conducted on many parameters studied, mainly salinity, temperature, and electrical conductivity, showed very disproportionate and differentiated groups of stations.

Anopheles mosquitoes prefer to breed in clear water.[42,43] A similar observation was made in the present study for Anopheles larvae, except for *An. sergentii* larvae, which were found in polluted waters. The presence of *Anopheles* larvae in polluted breeding sites has been demonstrated in a rural area of Tanzania.[6,44] The species *Cx. hortensis* has been linked with alkaline and very low saline waters in the Gharb.[45]

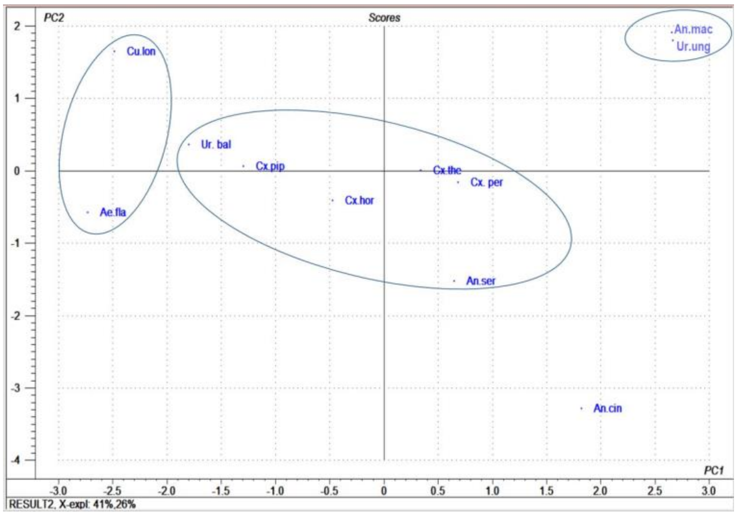

Figure 3: Score plot showing the distribution of culicidian species in the Fez Region, Morocco, according to the first two components.

Ae.fla: *Aedes flavescens;* An.cin: *Anopheles cinereus;* An.mac: *Anopheles maculipennis;* An.ser: *Anopheles sergentii;* Cx.hor: *Culex hortensis;* Cx.per: *Culex perexiguus;* Cx.pip: *Culex pipens;* Cx.the: *Culex theileri;* Cu.lon: *Culiseta longiareolata;* Ur.bal: *Uranotaenia balfouri*; Ur.ung: *Uranotaenia unguiculata*

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Table 4: Measurements of the means and standard deviations of the physicochemical parameters recorded as a function of the presence of each species

Parameters species	Temperature (°c)	pH	Salinity (‰)	Dissolved oxygen (PPM)	Electrical conductivity (µs/cm)	Water flow (m^3/s)	Total dissolved solids (g/l)	Turbidity (NTU)	Depth (cm)
An.cin	15.45 ± 1.34	8.2	1.25 ± 1.06	2.31	2250 ± 2192	0	0.273 ± 0.36	237 ± 66.5	5.56 ± 3.44
Cx.the	20.96 ± 6.46	8.31 ± 0.72	0.62 ± 0.67	3.04 ± 2.23	1304 ± 818	0.014 ± 0.05	0.80 ± 0.45	166.5 ± 180.8	21.12 ± 21
Cx.per	21.03 ± 5.20	8.11 ± 0.53	0.64 ± 0.7	2.748 ± 1.76	1492 ± 890	0.007 ± 0.024	0.84 ± 0.50	176.5 ± 156	23.56 ± 19.88
An.ser	17.34 ± 3.48	8 ± 0.30	0.86 ± 0.69	3.06 ± 0.71	1679 ± 959	0.0004 ± 0.001	0.71 ± 0.40	107.8 ± 100.34	13.02 ± 14.4
Ur. bal	17.6	8.8	0	8.08	900	0	0.4	13.4	19.1
Cx .pip	18.83 ± 4.85	8.009 ± 0.45	0.3 ± 0.54	2.69 ± 1.47	988 ± 476	0.018 ± 0.054	0.63 ± 0.33	130 ± 198	32.5 ± 25.6
Cs. lon	19.15 ± 4.95	8.35 ± 0.56	0.09 ± 0.3	3.26 ± 2.8	206.3 ± 243.8	0.003 ± 0.01	0.592 ± 0.472	26.02 ± 37.27	51.58 ± 24.82
Ae.fla	14.8 ± 4.24	8.03 ± 0.07	0	2.71 ± 0.03	600	0.02 ± 0.028	0.38 ± 0.03	20.9 ± 4.38	18.95 ± 1.5
Cx. hor	14.95 ± 4.31	8.3 ± 0.08	0.5 ± 0.7	6.69 ± 0.56	1100 ± 283	0	0.84 ± 0.4	91.95 ± 28.35	14.7 ± 9.42
An. mac	26.5 ± 8.45	9.44 ± 0.95	0.5 ± 0.5	2.61 ± 1.98	1260 ± 445	0	0.95 ± 0.32	284 ± 274	14.31 ± 0.79
Ur.ung	19 ± 1.98	7.8 ± 0.28	0	2.96 ± 0.7	1000	0	0.55	24.31 ± 22.9	34.07 ± 31.01

Ae.fla: *Aedes flavescens ;* An.cin: *Anopheles cinereus;* An.mac: *Anopheles maculipennis ;* An.ser: *Anopheles sergentii;* Cx.hor: *Culex hortensis ;* Cx.per: *Culex perexiguus ;* Cx.pip: *Culex pipens;* Cx.the: *Culex theileri;* Cs.lon: *Culiseta longiareolata ;* Ur.bal: *Uranotaenia balfouri* ; Ur.ung: *Uranotaenia unguiculata*

Table 5 : Faunal associations of mosquito species (Diptera Culicidae) in central Morocco

	A.fla	*An. cin*	*An.mac*	*An.ser*	*Cx.hor*	*Cx.per*	*Cx.pip*	*Cx.the*	*Cs.lon*	*Ur.bal*	Ur.ung
Ae.fla		-	-	-	-	+	+	+	-	-	-
An. cin	-		-	+	-	+	-	+	-	-	-
An.mac	-	-		-	-	+	+	+	-	-	-
An.ser	-	+	-		-	+	+	+	-	-	+
Cx.hor	-	-	-	-		-	-	+	+	-	-
Cx.per	+	+	+	+	-		+	+	-	+	+
Cx.pip	+	-	+	+	-	+		+	+	-	+
Cx.the	+	+	+	+	+	+	+		+	-	-
Cs.lon	-	-	-	-	+	-	+	+		-	-
Ur.bal	-	-	-	-	-	+	-	-	-		-
Ur.ung	-	-	-	+	-	+	+	-	-	-	

Ae.fla: *Aedes flavescens.* An.cin: *Anopheles cinereus.* An.mac: *Anopheles maculipennis s. l.* An.ser: *Anopheles sergentii* .Cx.hor: *Culex hortensis* .Cx.per: *Culex perexiguus.* Cx.pip: *Culex pipens.* Cx.the: *Culex theileri* .Cs.lon: *Culiseta longiareolata* .Ur.bal: *Uranotaenia balfouri* .Ur.ung: *Uranotaenia unguiculata*.
(-):no association. (+):presence of association

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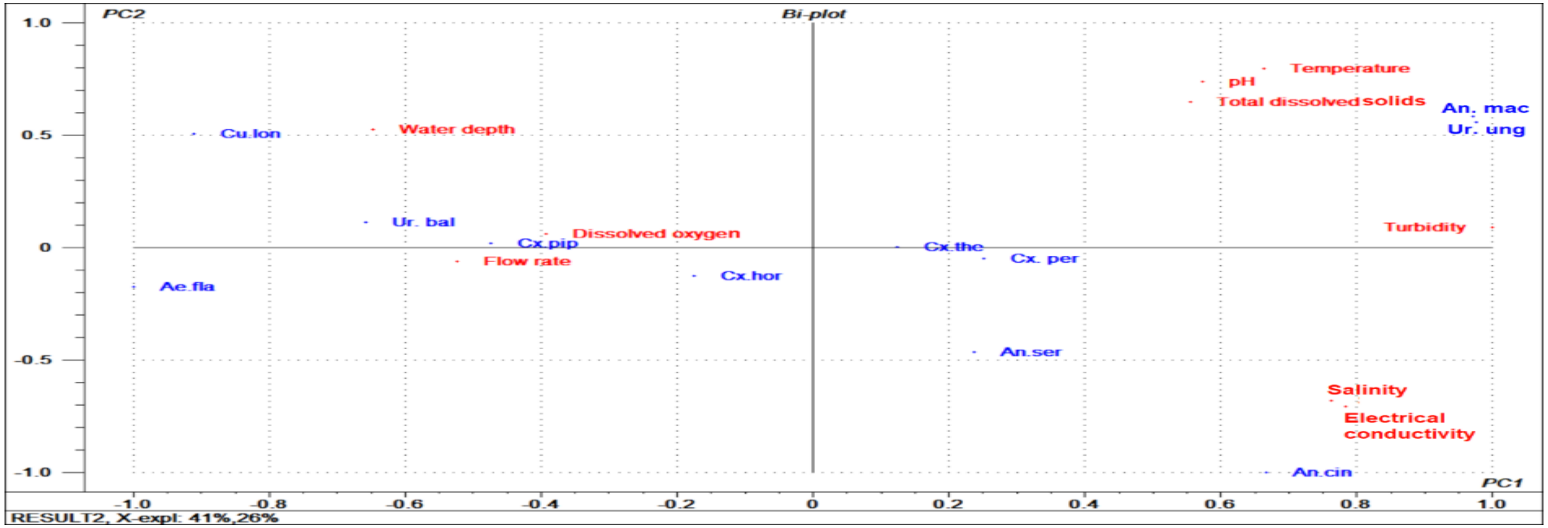

Figure 4: Biplot of loading and scores obtained by principal component analysis (PCA) showing the correlations between physicochemical parameters and species.
Ae.fla: *Aedes flavescens;* An.cin: *Anopheles cinereus;* An.mac: *Anopheles maculipennis;* An.ser: *Anopheles sergentii;* Cx.hor: *Culex hortensis*; Cx.per: *Culex perexiguus;* Cx.pip: *Culex pipens;* Cx.the: *Culex theileri;* Cs.lon: *Culiseta longiareolata;* Ur.bal: *Uranotaenia balfouri;* Ur.ung: *Uranotaenia unguiculata*.

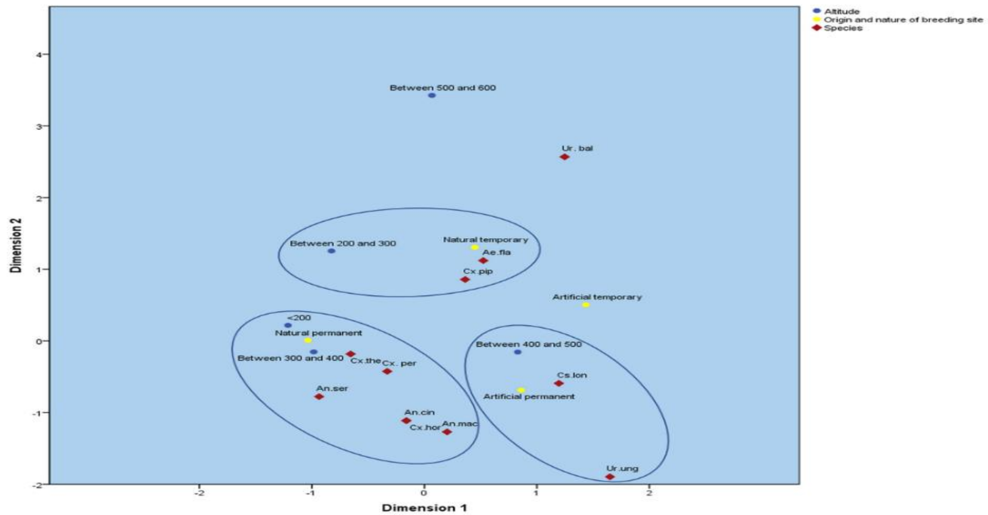

Figure 5: Projection of the variables studied according to the MCA.
Ae.fla: *Aedes flavescens;* An.cin: *Anopheles cinereus;* An.mac: *Anopheles maculipennis;* An.ser: *Anopheles sergentii;* Cx.hor: *Culex hortensis;* Cx.per: *Culex perexiguus*; Cx.pip: *Culex pipens;* Cx.the: *Culex theileri;* Cs.lon: *Culiseta longiareolata;* Ur.bal: *Uranotaenia balfouri;* Ur.ung: *Uranotaenia unguiculata*.

However, the development of this species was found to be correlated with dissolved oxygen and flow in the present study. *Culex pipiens* has been well represented and negatively correlated with dissolved oxygen and flow. Previous studies have confirmed its great capacity to colonize poorly oxygenated environments.[28,46] Another study on the characterization of breeding sites using the PCA method highlighted the affinity of *Cx. pipens* present in sites with high conductivity and low levels of dissolved oxygen.[47] *Culiseta longiareolata* was observed to grow in different types of environments.[40,48] In the present study, it was observed to be dominant in permanent and shallow breeding sites.

Figure 5 shows the correspondence between the explained and the explanatory variables. By analyzing the graph of correspondence between species and geographical parameters, three clusters of species that can coexist under similar conditions were detected. The five species, including *Cx. perexiguus*, *Cx. theileri*, *An. cinereus*, *Cx. hortensis*, and *An. maculipennis,* prefer permanent natural environments, as well as altitudes of less than 200 and between 300 and 400 feet. *Culiseta longiareolata* and *Ur. unguiculata* appear to prefer living in a permanent artificial environment at altitudes between 400 and 500 m. Meanwhile, the two species: *Ae. flavescens* and *Cx. pipiens* coexist in temporary natural environments at altitudes between 200 and 300 m. Mosquitoes have a great impact on humans and animals. Their emergence, disappearance, and appearance are related to altitude.[49–51] Altitude in mosquito habitats has a direct effect on the abundance, geographic distribution, vectorial capacity, epidemiology, and pathogenicity of mosquitoes.[52] The altitudinal distribution of mosquitoes varies in the region.[53] Thus, the maximum number of individuals of each mosquito species varies from one species to another. Furthermore, the number of mosquito species collected was higher at the lowest level of elevation. This finding confirmed the results of Devi and Jauhari (2004).[49]

Conclusion

The novel, interdisciplinary approach taken by this study to examine the mosquito-borne disease vectors in the Moroccan province of Fez makes it noteworthy. The findings of the study showed that PCA and MCA provide a means of defining the tolerance of each species detected by identifying the most important characteristics at the water level at which Culicidae are inhabited. Thus, it was discovered that mosquitoes colonize all the types of breeding sites studied, and the physicochemical parameters are determining factors in the proliferation of the species. These findings will be very useful in better understanding the correlation between environmental factors and the bio-ecological characteristics of each species to improve public health mosquito control strategies. Future studies may concentrate on the application of plant-based products in biological control, as this strategy is expected to be important to the long-term management of pests

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 6: Eigenvalues and percentages of explained inertia of the axes of the principal component analysis (PCA).

Component number	Eigenvalues	% of variance	Cumulative percentages
1	3.71097	41.233	41.233
2	2.37784	26.420	67.653
3	1.5918	17.687	85.340
4	0.595186	6.613	91.953
5	0.511853	5.687	97.641
6	0.135667	1.507	99.148
7	0.06137	0.682	99.830
8	0.0149571	0.166	99.996
9	0.000350632	0.004	100.000

Table 7: Cronbach's alpha score, eigenvalues, explained variability for the first two axis

		Variance represented		
Dimension	**Cronbach's alpha**	**Total (*eigenvalues*)**	**Inertia**	**% of variance**
1	759	2,025	675	58,391
2	460	1,443	481	41,609
Total		3,468	1,156	
Mean	635[a]	1,734	578	

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