

Composition and seasonality of *Culicoides* in three host environments in Rabat region (Morocco)

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Summary

Morocco has suffered several outbreaks of *Culicoides*-borne viruses in recent decades and most studies have focused on *Culicoides imicola*, considered for a long time as the only important vector. The change in bluetongue (BT) epidemiology in the Mediterranean Basin and Europe over the past two decades has highlighted the role of other *Culicoides* species in BT virus transmission. The objective of this study was to provide new insights on the *Culicoides* species composition and seasonality in three different host environments (a horse-riding center, a goat farm and a cattle farm) around Rabat, the capital of Morocco, where BT has been endemic since 2004. Light / suction trap collections were carried out on two consecutive nights at fortnight intervals from May 2016 to May 2017. *Culicoides* were identified morphologically at the species level when possible. Multivariate analyses were used to compare the impact of the site / vertebrate species, and the collection month on the species communities. In addition, statistical modeling was used to identify environmental drivers of the *Culicoides* seasonality. A total of 12,460 *Culicoides* individuals belonging to at least 15 different species were collected during the survey. *Culicoides imicola* was by far the most abundant species (71.4% of total catches). The site location, and thus the vertebrate species, did not influence the species composition, which was mainly impacted by the month of collection. Surprisingly, the atmospheric pressure was the environmental parameter the most frequently selected in seasonal models. The potential impact of this meteorological parameter along with the other selected variables is discussed. Identifying the environmental parameters driving *Culicoides* seasonal abundance is the first step to implementing robust *Culicoides* dynamic models that could later be used in transmission risk modeling.

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■ INTRODUCTION

The *Culicoides* Latreille biting midges (Diptera: Ceratopogonidae) are small hematophagous insects, which are biological vectors of viruses responsible for major livestock diseases, such as bluetongue (BT), African horse sickness (AHS), epizootic hemorrhagic disease (EHD) or Schmallenberg disease (SB) (Purse et al., 2015). Several incursions of AHS and EHD have occurred in recent decades in the Mediterranean region, though outbreaks were limited geographically and lasted only a few years. In contrast, after sporadic incursions, the Mediterranean BT epidemiology changed radically in the last two decades. What happened in Morocco illustrates this epidemiological drift in the BT situation compared to other *Culicoides*-borne viruses.

Morocco experienced AHS outbreaks in the 1960s (serotype 9) and in the late 1980s (serotype 4) (Mellor and Hamblin, 2004), and EHD outbreaks (serotype 6) in 2006 (Savini et al., 2011). During the 1980s AHS outbreak, observed circulation of AHS virus (AHSV) was reported for five years before returning to an absence of transmission (Mellor and Hamblin, 2004). No EHD clinical cases were reported after 2006. Bluetongue (serotype 10, BTV-10) was described for the first time in Morocco in 1956. The virus spread was controlled thanks to the implementation of a vaccination campaign and a temperature drop in the fall (Lhor, 2016). Meanwhile, other BTV serotypes were extensively transmitted in the Mediterranean Basin from 1998, but the disease had apparently remained absent in Morocco until BTV-4 emergence in 2004 (Lhor, 2016). In 2006, BTV-1 was reported in Algeria, then it spread to Morocco in 2006–2007 (Lhor, 2016). In 2008, sanitary policies implemented by veterinary services stopped these outbreaks, but the disease reappeared in 2009, with BTV-1 and BTV-4 co-circulating during *Culicoides* activity periods in both northern and southern parts of the country (Lhor, 2016). The last census reported 305 BT outbreaks across the country with 1456 cases leading to 449 livestock deaths in 2017. The BT endemic situation is weighing on the sheep industry, which increased from 1.6 billion to 2.4 billion euros between 2008 and 2017. The national herd is currently estimated at 20.6 million head, including 2.5 million of the Sardi breed, which are reared for the Eid al-Adha sacrifice fest. The economic impacts of BT include i) direct losses due to mortality, weight loss, abortion, reduction in the fertility rate, and decreased meat production efficiency, and ii) indirect costs due to vaccination, monitoring, surveillance measures, and trade restrictions limiting the access to higher-value markets (Garros and Balenghien, 2017).

A total of 54 *Culicoides* species have been recorded in Morocco. This list has been mainly established in the 1970s after the 1965–1967 AHS outbreaks and has been comprehensively reviewed and updated recently (Bourquia et al., 2019).

Culicoides distributions were investigated in Morocco in 1994–1995 after the 1989–1991 AHS outbreaks (Baylis et al., 1997), and in 2000–2003 just before the 2004 BT emergence (Lhor, 2016). These studies mainly focused on *Culicoides imicola* Kieffer, as a proven AHSV and BTV vector, which was, at that time, considered the only significant vector in the Mediterranean region. The rest of the individuals have been identified at group level, mainly *Obsoletus* and *Pulicaris* groups (Bourquia et al., 2019). *C. imicola* abundance (i.e. the annually-averaged mean daily trap catch) has been found to be positively correlated with the Normalized Difference Vegetation Index (NDVI), and negatively with the wind speed (Baylis et al., 1998). NDVI was suggested to be related to soil moisture and thus to the suitability for *C. imicola* breeding sites (Baylis et al., 1998). Wind speed is suggested to affect the abundance of *C. imicola* by increasing adult mortality rates through desiccation or, more likely according to authors, through the loss of adults due to wind dispersal (Baylis et al., 1998). The seasonal pattern of *C. imicola* populations was described in Morocco during the 1994–1995 and 2009–2010 surveys (Baylis et al., 1997; Lhor, 2016) but was not analyzed to identify climatic drivers. *C. imicola* dynamic is described as mainly unimodal, and the catch of *C. imicola* peaks in general in late summer and fall, with a smaller peak in spring. In Senegal and South Africa, this species is associated with rainfall, NDVI, temperature, wind speed and percentage cover of water bodies (Diarra et al., 2015). Other studies including environmental parameters (related to land use, landscape and climate) conducted in the Mediterranean Basin suggest that these parameters improve the prediction of habitat suitability occupied by *C. imicola* as well as the accuracy of abundance estimates at local scale (Ippoliti et al., 2013).

The species composition and seasonality of *Culicoides* in Rabat region were thus investigated to provide new insights on the role of

Culicoides in BTV transmission by highlighting the environmental drivers of the seasonality.

■ MATERIALS AND METHODS

Study sites and entomological collections

This study was conducted in the region of the Moroccan capital, Rabat. The city is located on the Atlantic coast in northwestern Morocco, with a Mediterranean climate (characterized by hot, dry summers, and mild, wet winters). The average annual precipitation reaches 492 mm and the average annual temperature is 17.1°C (1970–2000 seasonal averages, WorldClim, <http://worldclim.org/version2>). The presence of the Atlantic Ocean is conducive to a high relative humidity, which fluctuates depending on the distance from the sea and time of the year.

Culicoides midges were collected around Rabat at two-week intervals from May to October 2016 in two sites, a cattle farm (33° 57' 44.1" N; 6° 48' 11.7" W) and a goat farm (33° 51' 36.0" N; 6° 51' 00.0" W), and from May 2016 to April 2017 in a horse-riding center (33° 53' 46.0" N; 6° 49' 53.3" W), using a UV light / suction trap (OVI type) manufactured by the Onderstepoort Veterinary Institute (Venter et al., 2009). The traps were placed near animals and were operated from dusk to dawn for two consecutive nights fortnightly. The *Culicoides* collected were transferred to 70% ethanol, then, for most individuals, identified morphologically to species-level under a stereomicroscope using the key of Mathieu et al. (2012). Additionally, molecular identification was performed i) to differentiate morphologically close species (*Culicoides obsoletus* Meigen, *Culicoides scoticus* Downes and Kettle, or *Culicoides montanus* Shakirzjanova), ii) to confirm morphological identifications if specimens were damaged, and iii) to confirm new species records (Bourquia et al., 2019). Females of all species were age-graded as nulliparous, parous, gravid or freshly blood-fed after abdominal examination of pigmentation.

Environmental data

Daily rainfall estimates were obtained from 1983 to 2017 from the database of Tropical Applications of Meteorology using SATellite data and ground-based observations (TAMSAT, www.tamsat.org.uk/data/archive) (Maidment et al., 2014). Day and night land surface temperatures (LSTday and LSTnight), vegetation indices (Enhanced Vegetation Index [EVI] and NDVI) from 2000 to 2017 were extracted from version 6 MODIS MOD11A1 product (Wan et al., 2015) and from version 6 MODIS MOD13A1 (Didan, 2015) for each site location and sample date. Hourly climatic data, including 2-m temperature, 2-m dewpoint temperature, surface pressure, 10-m u-component of wind, and 10-m v-component of wind were extracted for 2016 and 2017 from the fifth generation of the European Centre for Medium-range Weather Forecasts (ECMWF) atmospheric reanalysis (ERA5) of the global climate (Dee et al., 2011). The saturated vapor pressure and the environmental vapor pressure, then the relative and the absolute humidity (see Shaman and Kohn [2009] for calculation details and definitions) were calculated from the 2-m temperature and the 2-m dewpoint temperature. Then, meteorological parameters from ECMWF-ERA5 were rescaled from hourly data to daily data (corresponding to 24 hours which included night collection) by computing the minimum, maximum and mean of each parameter.

Statistical analyses

Meteorological conditions during the collection years (2016 and 2017) were described by comparing 2016 and 2017 monthly minimum and maximum temperatures (MODIS LSTday and LSTnight), and 2016 and 2017 total rainfall (TAMSAT database) to the average of temperatures from 2000 to 2015 and rainfall from 1986 to 2015.

To determine broadly the diversity per site, species communities were described using a principal component analysis (PCA) on the log-transformed abundances. Then, a between-class analysis was carried out on this PCA to assess the importance of both the site and the collection month. Between-class analysis is a particular case of a PCA, where the variability between groups is optimized.

A correlation matrix was produced for all environmental variables to select non-correlated variables to be included in the modeling of *Culicoides* seasonal abundance. Then, cross correlation maps (CCM) were used to assess the correlation between the *Culicoides* abundance averaged between sites and the selected environmental variables at different time lags. CCM were used to assess average or accumulated meteorological quantities over a period beginning at a first time lag and ending at a second time lag (Brugger and Rubel, 2013). Analyzing CCM determined which time lags of environmental variables may have had an impact on *Culicoides* abundance.

Finally, abundance was modeled with these environmental parameters and time lags (after centring and scaling) using a generalized linear model (GLM) or a negative-binomial log linear model to account for any over-dispersion of insect collection data. The model selection was based on corrected Akaike's (cAIC) and Bayesian information criteria (BIC). The validity of the selected GLM was assessed by i) plotting the observed versus the fitted values and testing Pearson's product moment correlation coefficient, ii) graphically checking the normality of the residuals, iii) graphically testing the linearity hypothesis (random distribution of residuals around 0 after they had been plotted by fitted values), and iv) graphically confirming the homogeneity of residuals.

All statistical analyses and graphs were implemented with version 3.4.2 of R software, using *ade4* package for PCA and between class analysis, *fields* for CCM, *MuMIn* for calculating the corrected Akaike and Bayesian information criteria, and *aod* for analysis of over-dispersed data.

RESULTS

Culicoides collections

The goat and cattle farms were sampled fortnightly over a period of 22 weeks (12 and 11 collections, respectively, the last sampling was not

considered due to electrical failure) during most of the *Culicoides* activity period (from mid-May 2016 to mid-October 2016). The horse-riding center was sampled fortnightly for 48 weeks (22 collections) to measure the population seasonality over an entire year (from mid-May 2016 to the end of April 2017).

During sampling periods, the temperature conditions were similar to the seasonal normal, with an average annual temperature of 20.5°C in 2016 and 20.6°C in 2017, compared to 20.1°C for the 2000–2015 period (Figure 1). The 2016 collections began with a relatively dry period with a total of 125 mm rainfall from January to May 2016, compared to 193 mm on average for the same months in the 1986–2015 period (Figure 1). In contrast, the winter of 2016–2017 was wetter than normal with 440 mm rainfall from September 2016 to May 2017 compared to 389 mm on average for the same months in the 1986–2015 period (Figure 1).

Culicoides diversity and seasonality in the three collection sites

A total of 12,460 *Culicoides* individuals belonging to at least 15 different species were collected during the survey, including 12,053 (96.7%) females and 407 (3.3%) males (Table I). From May to October 2016, during the 11 collections common to the three sites, the most abundant species were *C. imicola* (71.4% of total catches), *Culicoides newsteadi* Austen (6.4%), *Culicoides circumscriptus* Kieffer (6.4%), *Culicoides kingi* Austen (4.1%), *Culicoides cataneii* Clastrier / *Culicoides geigelensis* Dzhafarov (3.8%), *Culicoides puncticollis* (Becker) (3.6%), and *C. obsoletus* / *C. scoticus* (2.2%). Altogether these species represented 97.9% of the collected individuals (Table I).

The structure of the PCA on log-transformed abundances was driven by the seven most abundant species (or pair of species), leading to a cumulative projected inertia of 70.4% on the first three axes. The site (or the dominant domestic vertebrate species) explained 17.1% of PCA inertia ($p = 0.001$, with a permutation test), whereas the collection month explained 26.7% of PCA inertia ($p = 0.001$). The 'site/host effect' was mainly due to a higher abundance of *C. circumscriptus* in the cattle farm than in the other two sites (Figure 2A), and, but less significantly, to a higher abundance of *C. imicola* in the horse-riding center and of *C. puncticollis* and *C. paolae* in the goat farm (see

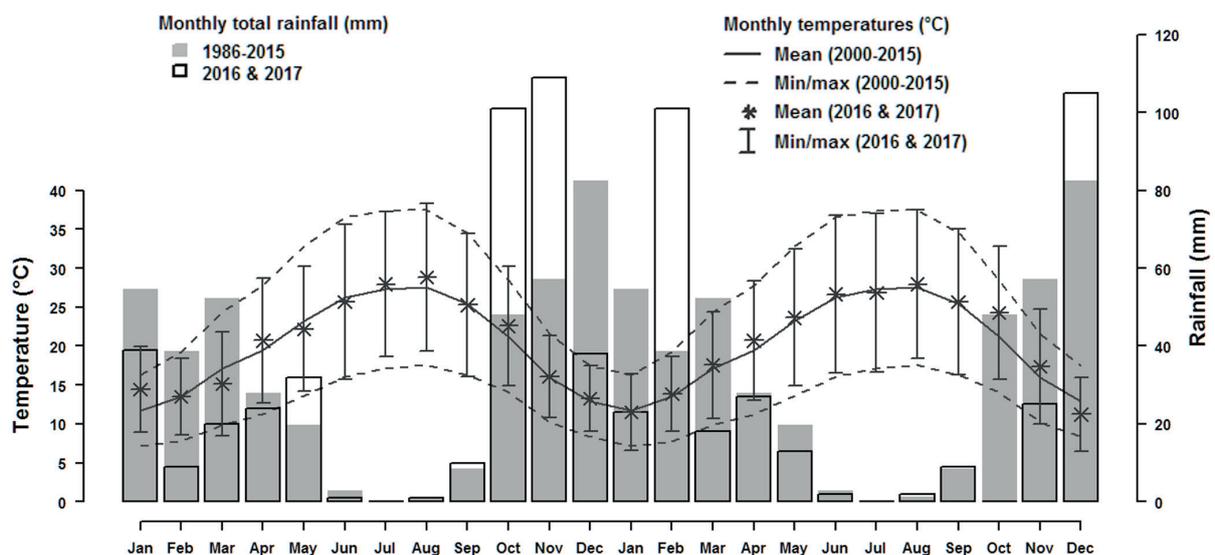


Figure 1: 2016 and 2017 monthly average meteorological conditions (temperature and precipitations) compared to seasonal normal at Rabat, Morocco.

Temperature data (day and night land surface temperatures) were extracted from MOD11A1 version 6 (Wan et al., 2015) using a 4-km² area from 2000 to 2017. Rainfall data were extracted from TAMSAT database (Maidment et al., 2014) from 1986 to 2017.

Table 1

Total number of *Culicoides* collected per species every two weeks using a UV light / suction trap (OVI type) in two farms from May to October 2016, and in a horse-riding center from May 2016 to April 2017, in Rabat region (Morocco)

| Species | Riding center (n = 25 collections) | | | Goat farm (n = 12 collections) | | | Cattle farm (n = 11 collections) | | | Total common collections* | | | | | |
|--|------------------------------------|--------|------|--------------------------------|--------|------|----------------------------------|--------|------|---------------------------|--------|------|------|-----|----|
| | Indiv. | Female | Male | Indiv. | Female | Male | Indiv. | Female | Male | Indiv. | Female | Male | Rank | | |
| <i>C. imicola</i> | 5469 | 5348 | 121 | 2027 | 1944 | 83 | 1 | 1432 | 1392 | 40 | 1 | 5844 | 5688 | 156 | 1 |
| <i>C. newsteadi</i> | 276 | 274 | 2 | 131 | 130 | 1 | 3 | 270 | 237 | 33 | 3 | 524 | 489 | 35 | 2 |
| <i>C. circumscriptus</i> | 68 | 60 | 8 | 79 | 75 | 4 | 5 | 434 | 399 | 35 | 2 | 522 | 480 | 42 | 3 |
| <i>C. kingi</i> | 1010 | 966 | 44 | 107 | 105 | 2 | 4 | 71 | 69 | 2 | 6 | 335 | 315 | 20 | 4 |
| <i>C. cataneii</i> / <i>C. geigelensis</i> | 84 | 83 | 1 | 47 | 44 | 3 | 6 | 222 | 221 | 1 | 4 | 314 | 309 | 5 | 5 |
| <i>C. cataneii</i> / <i>C. geigelensis</i> | 55 | 54 | 1 | 47 | 44 | 3 | | 222 | 221 | 1 | | | | | |
| <i>C. cataneii</i> | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>C. geigelensis</i> | 27 | 27 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>C. puncticollis</i> | 20 | 19 | 1 | 212 | 197 | 15 | 2 | 64 | 56 | 8 | 7 | 293 | 269 | 24 | 6 |
| Obsoletus group | 87 | 87 | 0 | 30 | 30 | 0 | 8 | 96 | 96 | 0 | 5 | 181 | 181 | 0 | 7 |
| <i>C. obsoletus</i> / <i>C. scoticus</i> | 40 | 40 | 0 | 4 | 4 | 0 | 0 | 8 | 8 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>C. obsoletus</i> | 41 | 41 | 0 | 23 | 23 | 0 | 0 | 55 | 55 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>C. scoticus</i> | 6 | 6 | 0 | 2 | 2 | 0 | 0 | 33 | 33 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>C. montanus</i> | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>C. paolae</i> | 11 | 11 | 0 | 35 | 33 | 2 | 7 | 9 | 9 | 0 | 9 | 46 | 44 | 2 | 8 |
| <i>C. subfagineus</i> | 22 | 21 | 1 | 13 | 13 | 0 | 9 | 6 | 6 | 0 | 10 | 41 | 40 | 1 | 9 |
| <i>C. longipennis</i> | 8 | 8 | 0 | 4 | 4 | 0 | 10 | 23 | 23 | 0 | 8 | 30 | 30 | 0 | 10 |
| <i>C. fagineus</i> | 53 | 53 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 14 | 27 | 27 | 0 | 11 |
| <i>C. juminei</i> | 4 | 4 | 0 | 0 | 0 | 0 | 0 | 6 | 6 | 0 | 10 | 9 | 9 | 0 | 12 |
| Unknown species | 7 | 7 | 0 | | | | 13 | | | | | 7 | 7 | 0 | 13 |
| <i>C. univittatus</i> | 0 | 0 | 0 | 2 | 2 | 0 | 12 | 4 | 4 | 0 | 12 | 6 | 6 | 0 | 14 |
| <i>C. parroti</i> | 9 | 9 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 15 |
| <i>C. sahariensis</i> | 0 | 0 | 0 | 4 | 4 | 0 | 10 | 0 | 0 | 0 | 0 | 4 | 4 | 0 | 15 |
| <i>C. festivipennis</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0 | 13 | 2 | 2 | 0 | 17 |
| <i>Culicoides</i> sp. | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

* Data from the weeks when all the sites were sampled so as to compute a global abundance rank for each species; Indiv.: individuals

Supplementary Material I for abundance per species and site). The between class analysis on the collection month highlighted the higher abundances of *C. imicola* and *C. kingi* in August/September, of *C. obsoletus* / *C. scoticus* and *C. newsteadi* in May/June and October, and the lesser abundance of *C. puncticolis* in June/July (Figure 2B).

C. imicola populations increased progressively from spring to peak in September (Suppl. Mat. II), whereas *C. kingi* was abundant only in August/September. Population densities of *C. circumscriptus*, *C. cataneii* / *C. gejjelensis* and *C. puncticolis* were unimodal with a maximal abundance in August for the first species and in July for the latter two (Suppl. Mat. II). Finally, both *C. obsoletus* / *C. scoticus* and *C. newsteadi* populations showed two peaks of abundance, the first in June, the second in October (Suppl. Mat. II).

Influence of environmental parameters on Culicoides abundance

Supplementary Material III details the correlation matrices produced with all environmental parameters, including EVI and NDVI MODIS parameters, TAMSAT daily rainfall and ECMWF-ERA5 meteorological parameters. Comparison of the day and night land surface temperatures from MODIS products and the daily 2-m temperature from ECMWF-ERA5 showed high correlations (Suppl. Mat. III). Therefore, only the temperature ECMWF-ERA5 data was used for the rest of the analysis. The temperature, which was highly correlated with the environmental vapor pressure, the relative and the absolute humidity, the atmospheric pressure, the wind speed, the rainfall and EVI, which was highly correlated with NDVI, were also retained.

Bivariate correlations between *C. imicola* abundance and environmental variables highlighted a positive impact of the temperature and absolute humidity for a large range of time lags (Figure 3). The time lags that had the highest correlation coefficients with abundance were selected (Pearson's product moment correlation coefficient $\rho = 0.80$ for 25–27 days as time lags for temperature and $\rho = 0.75$ for 23–26 days for absolute humidity). In contrast, the atmospheric pressure was negatively correlated with *C. imicola* abundance (Figure 3, $\rho = 0.83$ for 27–43 days). CCM did not indicate any specific relation between the wind speed and the number of *C. imicola* collected. The best correlation was

positive and obtained with the wind speed 36 days before collection, which seemed incidental and without any biological sense (the loss of adults is generally due to wind dispersal the day of collection). Thus, only the wind speed on the day of collection was included as a variable for modeling procedure. Finally, the EVI of the week of collection was negatively correlated with *C. imicola* abundances (Figure 3).

Table II shows the environmental parameters with the optimum time lags selected for the modeling procedure after having analyzed CCM for *C. newsteadi* (Suppl. Mat. IV), *C. circumscriptus* (Suppl. Mat. V), *C. kingi* (Suppl. Mat. VI), *C. cataneii* / *C. gejjelensis* (Suppl. Mat. VII), *C. puncticolis* (Suppl. Mat. VIII), and *C. obsoletus* / *C. scoticus* (Suppl. Mat. IX).

The selected GLM included atmospheric pressures (27-to-43-day average before collections) and mean temperatures (25-to-27-day average before collections). It correctly predicted the seasonal pattern of *C. imicola* populations ($R^2 = 0.778$, Table III), i.e. a slow increase in population from March to September when it reached a maximum, then a rapid decrease from October to February (Figure 4).

The selected models (Table III) correctly predicted the seasonal pattern of *C. circumscriptus* ($R^2 = 0.752$), *C. cataneii* / *C. gejjelensis* ($R^2 = 0.754$) and *C. puncticolis* ($R^2 = 0.670$), with the lowest accuracy for the latter (see Suppl. Mat. X for details on model selection, validation and prediction for all species). The selected models did not successfully predict the bimodal seasonal patterns of *C. newsteadi* ($R^2 = 0.463$), and *C. obsoletus* / *C. scoticus* ($R^2 = 0.700$). GLM was able to predict the abundance peaks of *C. newsteadi* populations in fall 2016 and spring 2017, but not in spring 2016 (Suppl. Mat. X). Similarly, GLM was able to predict abundance peaks of *C. obsoletus* / *C. scoticus* populations in spring 2016 and 2017, but not fully in fall 2016 (Suppl. Mat. X). Finally, the selected model predicted the general seasonal pattern of *C. kingi* populations, but failed to reproduce the November peak of abundance (Suppl. Mat. X). The atmospheric pressure was selected as a significant predictor in 5 of 7 models, always with a negative correlation, rainfall in 4 models but only once with a significant positive effect, humidity in 2 models, wind speed in 2 models with a positive correlation, and EVI and temperature only in 1 each (Table III).

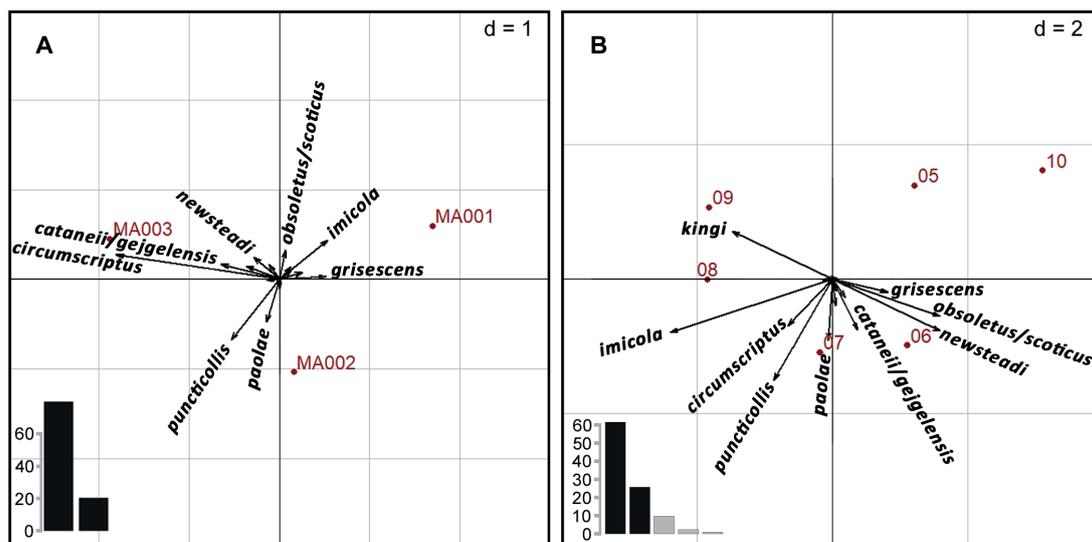


Figure 2: Between-class analysis (particular case of a principal component analysis [PCA] with optimization of the variability between groups) with the site (A) and the collection month (B) as group applied on the PCA carried out of the log-transformed 2016–2017 abundances. Bart chart: percentage of inertia explained by the axes. Culicoides collections were carried out twice a month with UV light / suction trap (OVI type) in three sites around Rabat (Morocco): a horse-riding center (MA001), a goat farm (MA002) and a cattle farm (MA003) from May (05) to October (10).

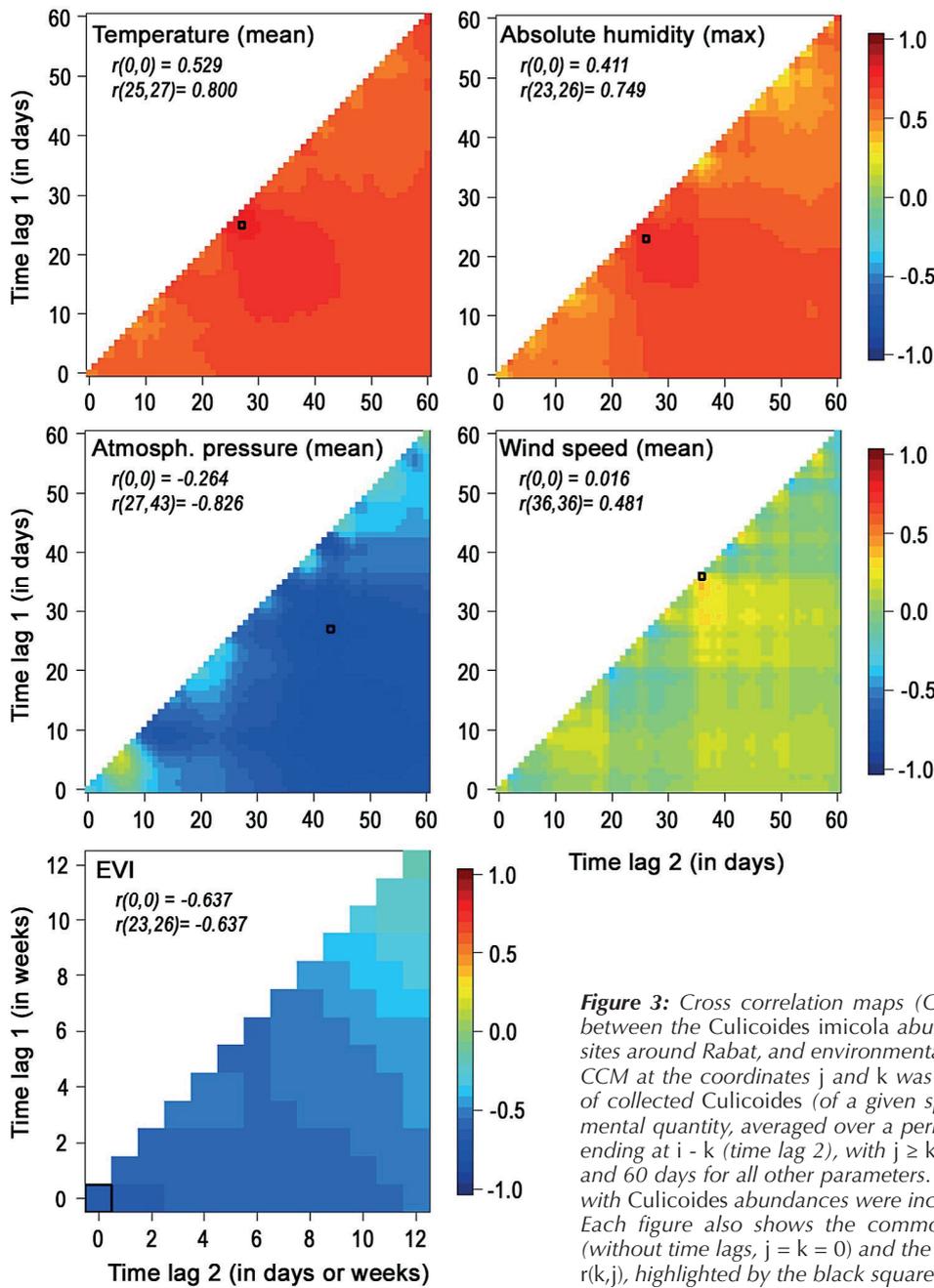


Figure 3: Cross correlation maps (CCM) investigating bivariate correlations between the *Culicoides imicola* abundance averaged at the three collection sites around Rabat, and environmental variables at different time lags. CCM at the coordinates j and k was $CCM_{j,k} = \text{cor}(Y_i, X_{i,j-i-k})$, Y_i the number of collected *Culicoides* (of a given species) at time i , and $X_{i,j-i-k}$ an environmental quantity, averaged over a period starting at time $i - j$ (time lag 1) and ending at $i - k$ (time lag 2), with $j \geq k$. Tested time lags were 12 weeks for EVI and 60 days for all other parameters. Only environmental variables correlated with *Culicoides* abundances were included in the figure. Each figure also shows the commonly used correlation coefficient $r(0,0)$ (without time lags, $j = k = 0$) and the maximum correlation coefficient of map $r(k,j)$, highlighted by the black square symbol.

Table II

Environmental variables and optimal time lags (in weeks for EVI and in days for the other parameters) selected for the modeling procedure after analyzing the cross correlation maps for each of the most abundant *Culicoides* sp. collected in 2016–2017 in Rabat region (Morocco)

| Species | Temperature | Absolute humidity | Relative humidity | Atmospheric pressure | Wind speed | Rainfall | EVI |
|--|--------------|-------------------|-------------------|----------------------|--------------|----------|-------|
| <i>C. imicola</i> | Mean [25.27] | Max [23.26] | – | Mean [27.43] | Mean [0.0] | – | [0,0] |
| <i>C. newsteadi</i> | – | – | Max [7.11] | Min [9.12] | – | [9. 17] | – |
| <i>C. circumscriptus</i> | Min [23.25] | Mean [6.25] | – | Min [27.40] | – | [37.40] | [0,0] |
| <i>C. kingi</i> | Mean [15.15] | Mean [20.30] | – | Max [29.39] | Mean [27.45] | [37.40] | [0,2] |
| <i>C. cataneii</i> / <i>C. geigelensis</i> | Min [14.14] | Mean [20.30] | Min [0.5] | Max [33.35] | Mean [27.45] | [9. 9] | [0,2] |
| <i>C. puncticollis</i> | Max [2.4] | Max [1.7] | Min [23.25] | Mean [15.15] | Min [5.38] | [2.60] | [0,0] |
| <i>C. obsoletus</i> / <i>C. scoticus</i> | – | – | Max [17.21] | Max [6.8] | Max [5.41] | [37.38] | – |

EVI: Enhanced Vegetation Index

Table III

Modeling *Culicoides* abundances assessed in 2016–2017 in Rabat region (Morocco) by environmental parameters using generalized linear or binomial model

| Species | Selected model | Selected variables and estimates | Pearson's correlation coefficient |
|--|----------------|--|--|
| <i>C. imicola</i> | GLM | Abun ~ 1.70 ^{***} - 0.77 × Pres_Mean [*] + 0.35 × Temp_Mean [°] | $\rho = 0.882$ ($p < 0.001$), $R^2 = 0.778$ |
| <i>C. newsteadi</i> | GLM | Abun ~ 0.87 ^{***} - 0.23 × Pres_Mean [*] + 0.59 × Rain [*] | $\rho = 0.680$ ($p < 0.001$), $R^2 = 0.463$ |
| <i>C. circumscriptus</i> | GLM.NB | Abun ~ 1.86 ^{***} - 1.49 × Pres_Mean ^{***} - 4.98 × Rain | $\rho = 0.867$ ($p < 0.001$), $R^2 = 0.752$ |
| <i>C. kingi</i> | GLM | Abun ~ 0.67 ^{***} - 0.53 × Pres_Max [*] - 0.35 × Rain | $\rho = 0.628$ ($p < 0.001$), $R^2 = 0.394$ |
| <i>C. cataneii</i> / <i>C. gejjelensis</i> | GLM | Abun ~ 0.51 ^{***} - 0.29 × EVI ^{***} + 0.14 × Rain + 0.87 × Wind_Mean ^{***} | $\rho = 0.868$ ($p < 0.001$), $R^2 = 0.754$ |
| <i>C. puncticollis</i> | GLM.NB | Abun ~ 1.56 ^{***} + 7.54 × AH_Max ^{***} - 5.06 × RH_Min [*] | $\rho = 0.818$ ($p < 0.001$), $R^2 = 0.670$ |
| <i>C. obsoletus</i> / <i>C. scoticus</i> | GLM.NB | Abun ~ 1.42 ^{***} - 2.69 × Pres_Max [*] - 5.85 × RH_Max ^{**} + 1.07 × Wind_Max ^{***} | $\rho = 0.837$ ($p < 0.001$), $R^2 = 0.700$ |

Asterisks denote significance: *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; ° $p < 0.1$

GLM: generalized linear model; GLM.NB: negative-binomial log linear model; Abun: log-transformed abundance for GLM or counts for NB; Pres: atmospheric pressure, Temp: temperature; Rain: total rainfall; EVI: Enhanced Vegetation Index; Wind: wind speed; AH: absolute humidity; RH: relative humidity

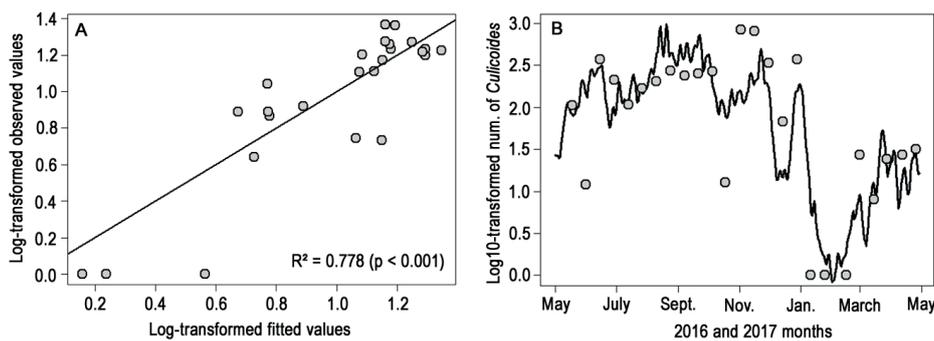


Figure 4: Comparison of fitted and observed values of the *Culicoides imicola* abundance averaged at the three collection sites around Rabat, at the collection days (A) and during the entire season (B).

DISCUSSION

Culicoides collections carried out in three different host environments around Rabat confirmed the presence of at least 15 *Culicoides* species, among which were several proven or probable vectors of arboviruses of veterinary interest. *C. imicola* is a proven BTV and AHSV vector and a suspected EHDV vector (Purse et al., 2015), *C. obsoletus* and *C. scoticus* are probable BTV vectors (Purse et al., 2015), whereas *C. newsteadi* and *C. paolae* are suspected BTV vectors (Foxi et al., 2016; Foxi et al., 2019).

C. imicola was by far the dominant species in the three sites combined. It has been reported as the most abundant and frequent species in Morocco (Baylis et al., 1997; Lhor, 2016). The species composition was similar between sites, the month of collection having more impact than the site / host species. Even if the site and host effects were intertwined, it is likely that species composition is driven first by the site location because of the location of breeding sites. For instance, Larska et al. (2017) highlight no difference in species composition between cattle and horse farms. The main difference in species composition was due to higher abundance of *C. circumscriptus*, known as a coastal species (Garros and Balenghien, 2017), in the cattle farm, which was the closest to the coast. Other abundant species

are known to breed in specific site types. *C. imicola* larvae develop in moist clay mud rich in nutrients exposed to sunlight or in moist or water saturated soils rich in organic matter (Braverman et al., 1974). Species such as *C. newsteadi* and *C. puncticollis* are found near substrates rich in water-saturated organic matter (Braverman et al., 1974). *C. kingi* larvae are more likely to be found in sunny and very salty mud (Cornet and Brunhes, 1994), whereas *C. cataneii* and *C. gejjelensis* larvae may be found in rivers or pond edges in wet meadows (Garros and Balenghien, 2017). *C. obsoletus* and *C. scoticus* may be considered ubiquitous as they develop in forest litter, tree holes, corn silage residues or composting manure (Garros and Balenghien, 2017). Finally, *C. paolae* larvae are considered specific to decaying prickly pear trees, which are common in the Mediterranean Basin.

The seasonal pattern of *C. imicola* populations observed around Rabat is consistent with previous studies carried out in Morocco, with a peak in late summer / early fall (Baylis et al., 1997), which is associated with BTV transmission in Northwestern Morocco (Lhor., 2016). This seasonal pattern is usually observed in the Mediterranean Basin (Garros and Balenghien, 2017). The peak of abundance of *C. imicola* populations is observed at the same period (September/October) in Senegal with a tropical climate, where populations are highest during the rainy season (Diarra et al., 2014). Seasonal patterns of *C. obsoletus* and *C. scoticus* vary widely depending on the climate (Garros and

Balenghien, 2017). In the Mediterranean Basin, as observed in Spain or Sardinia, they exhibit a first main peak of abundance in May/June, and later a secondary peak in October (Lucientes and Alarcón-Elbal, 2016; Foxi et al., 2016). Our observations agreed with these previous reports. The seasonal pattern of *C. newsteadi* populations highlighted the same bimodal distribution. This species is abundant during spring in Morocco and Sardinia (Lhor, 2016; Foxi et al., 2016). Finally, *C. kingi* populations were mainly abundant around Rabat in November. On the contrary, in tropical regions of Sudan and Senegal, this species is most abundant in July/August during the rainy season, and also in April when temperature is highest (El Sinnary et al., 1985; Diarra et al., 2014).

Determining the environmental factors driving these seasonal patterns is useful not only to understand better the variations in abundance, but also to be able to predict abundance as a first step for transmission risk modeling. Other factors are of course involved. Adult population seasonality is also the consequence of the long-term impact of environmental factors on the different steps of the life cycle, e.g. the duration of larval development, the longevity of both adult and immature stages, the size and frequency of egg laying (Purse et al., 2015). The same meteorological parameters, such as temperature, rainfall, or wind, may also have a short-term impact on the *Culicoides* flight activity leading to important daily variation of the proportion of the *Culicoides* population which is active and can be collected. Moreover, a single meteorological variable, such as the temperature, may have a mainly positive impact on population dynamics for a given range of values, but mainly negative impacts for another range, leading to non-linear effects on population abundance. These complexities may explain why many models have been developed to predict the presence and distribution of *Culicoides* species, in particular *C. imicola* in Europe, using climatic factors (Wittmann et al., 2001), satellite imagery (Tatem et al., 2003) or a combination of both (Baylis and Rawlings, 1998), but only a few have described the influence of meteorological and environmental parameters on *Culicoides* populations using statistical (Sanders et al., 2011; Rigot et al., 2012; Searle et al., 2013; Brugger and Rubel, 2013; Scolamacchia et al., 2014; Diarra et al., 2015) or mechanistic (White et al., 2017) modeling.

The influence of environmental parameters on the abundance was explored for species collected in 2016–2017 around Rabat. The atmospheric pressure was negatively correlated with the abundance of *C. imicola*, *C. newsteadi*, *C. circumscriptus*, *C. kingi* and *C. obsoletus* / *C. scoticus* at different time scales. Although never assessed in *Culicoides* seasonality modeling before, the atmospheric pressure has long been known to impact insect populations (Wellington, 1946), especially mating or phototaxis behavior (Pellegriano et al., 2013; Zagvazdina et al., 2015). At this stage, it is not possible to conclude if the atmospheric pressure has a real impact on *Culicoides* population abundance or if this correlation is incidental (a seasonal pattern similar to *Culicoides* seasonal pattern) or indirect (through another meteorological parameter). This is the main limitation of statistical modeling. The rainfall was positively correlated to *C. newsteadi* and *C. cataneii* / *C. gejjelensis* abundances with time lags corresponding to two weeks before collections, but negatively to *C. circumscriptus* and *C. kingi* with longer time lags (about 40 days before collection). Rainfall may have a direct negative impact on *Culicoides* activity (Murray, 1991), and long-term effects on *Culicoides* abundance by increasing the availability of breeding sites or perhaps drowning nymphs (Nevill, 1967). Long-term positive effects have been highlighted in areas with temperate climates (Brugger and Rubel, 2013) and short-term negative effects in both temperate (Sanders et al., 2011) and tropical climates (Diarra et al., 2015). The wind speed was positively correlated with the abundance of *C. cataneii* / *C. gejjelensis* and *C. obsoletus* / *C. scoticus* with a large time lag (up to 45 days before collection). Wind speed on the day of collection has often been reported as having a negative

impact on abundance by reducing flight activity (Sanders et al., 2011; Scolamacchia et al., 2014). Baylis et al. (1998) add that wind speed negatively affects the abundance of *C. imicola* in distribution modeling, through the loss of adults caused by wind dispersal. The positive correlation shown in our study should thus be considered as incidental. It is worth noting that including wind speed on the day of collection in the model did not change the outcome (data not shown). Finally, the temperature, absolute humidity and EVI were rarely selected in the models. This result contrasts with those from other studies where temperature is considered a main positive driver of *Culicoides* seasonal abundance (Sanders et al., 2011; Rigot et al., 2012; Brugger and Rubel, 2013; Scolamacchia et al., 2014; Diarra et al., 2015). The positive impact of humidity has been less often highlighted in other studies (Diarra et al., 2015) though it is known to impact adult survival (Purse et al., 2015). NDVI, which is correlated to EVI, is associated with high abundance of *C. imicola* in distribution models (Baylis and Rawlings, 1998; Baylis et al., 1998; Tatem et al., 2003; Acevedo et al., 2010), but rarely to abundance in seasonality modeling (Diarra et al., 2015). In this latter study, NDVI was higher during the rainy season, and the association with *Culicoides* abundances may be coincidental.

CONCLUSION

The primary objective of this study was to provide new insights on the *Culicoides* species composition and seasonality around Rabat, to understand better the role of *Culicoides* in BTV transmission in Morocco, where BT is currently endemic. Neither the site location (except for the cattle farm where the coastal species *C. circumscriptus* was more abundant than the other ones), nor the main vertebrate species influenced the global species composition. The seasonal pattern of *Culicoides* described was typical of the Mediterranean climate. Finally, the impact of environmental parameters which may drive *Culicoides* abundance was investigated, questioning the potential role of the atmospheric pressure.

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Author contributions statement

MB, KK and TBale were involved in the conception and planned the study. MB, IR, IB, MC, LG and WW collected the data. MB, CG, KH and TBale analyzed and interpreted the data. MB and TBale drafted the paper. CG, WW, TBald and KK revised and commented the manuscript.

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Résumé

Bourquia M., Garros C., Rakotoarivony I., Boukhari I., Chakrani M., Huber K., Gardès L., Wint W., Baldet T., Khallaayoune K., Balenghien T. Diversité et saisonnalité des *Culicoides* dans trois élevages aux hôtes différents dans la région de Rabat (Maroc)

Le Maroc a connu plusieurs épizooties liées à des virus transmis par les *Culicoides* au cours des dernières décennies, et la majorité des études associées ont porté sur *Culicoides imicola*, considéré depuis longtemps comme le seul vecteur d'importance. L'évolution de l'épidémiologie de la fièvre catarrhale du mouton (FCM) dans le bassin méditerranéen et en Europe au cours des vingt dernières années a souligné l'importance des autres espèces de *Culicoides* dans la transmission du virus de la FCM. L'objectif de cette étude était de fournir de nouvelles connaissances sur la composition d'espèces et la saisonnalité des *Culicoides* dans trois élevages aux hôtes différents (centre équestre, élevage de caprins et élevage de bovins) près de Rabat, capitale du Maroc, où la FCM est endémique depuis 2004. Des collectes ont été réalisées à l'aide de pièges lumineux et à aspiration pendant deux nuits consécutives, tous les 15 jours, de mai 2016 à mai 2017. Lorsque cela a été possible, les *Culicoides* ont été identifiés morphologiquement au niveau de l'espèce. Des analyses multivariées ont été utilisées pour comparer l'effet du site / de l'espèce animale à l'effet du mois de collecte sur la composition des espèces. En outre, des méthodes de modélisation statistique ont permis d'identifier les déterminants environnementaux de la saisonnalité des *Culicoides*. Un total de 12 460 *Culicoides*, appartenant à au moins 15 espèces différentes, ont été capturés pendant l'étude. *Culicoides imicola* a été de loin l'espèce la plus abondante (71,4 % du total des captures). La localisation du site, et donc l'espèce hôte, ont eu peu d'impact sur la composition des espèces, qui a été principalement influencée par le mois de collecte. De manière surprenante, la pression atmosphérique a été le paramètre environnemental le plus fréquemment sélectionné dans les modèles saisonniers. L'impact potentiel de ce paramètre météorologique et des autres variables sélectionnées est discuté. Identifier les paramètres environnementaux gouvernant l'abondance saisonnière des *Culicoides* est la première étape pour construire des modèles robustes de dynamique des populations, qui pourront être utilisés ultérieurement dans des modèles estimant le risque de transmission.

Mots-clés : bétail, cheval, *Culicoides*, virus bluetongue, conditions météorologiques, facteur du milieu, Maroc

Resumen

Bourquia M., Garros C., Rakotoarivony I., Boukhari I., Chakrani M., Huber K., Gardès L., Wint W., Baldet T., Khallaayoune K., Balenghien T. Composición y estacionalidad de *Culicoides* en tres medios ambientes de huéspedes en la región de Rabat (Marruecos)

En las últimas décadas, Marruecos ha sufrido varios brotes de virus transmitidos por *Culicoides* y la mayoría de los estudios se han centrado en *Culicoides imicola*, considerado durante mucho tiempo como el único vector importante. El cambio en la epidemiología de la lengua azul (BT) en la cuenca del Mediterráneo y Europa durante las últimas dos décadas ha puesto de relieve el papel de otras especies de *Culicoides* en la transmisión del virus de BT. El objetivo de este estudio fue proporcionar nuevos conocimientos sobre la composición y la estacionalidad de las especies de *Culicoides* en tres diferentes medios ambientes de huéspedes (centro de equitación, una cabra y una finca de ganado) cerca de Rabat, la capital de Marruecos, donde BT ha sido endémica desde 2004. Las colecciones con trampas de luz / succión se llevaron a cabo durante dos noches consecutivas, a intervalos de quince días, entre mayo de 2016 y mayo de 2017. Siempre que fue posible, los culicoides se identificaron morfológicamente a nivel de especie. Se utilizaron análisis multivariados para comparar el impacto del sitio/especies de vertebrados y el mes de recolección sobre las comunidades de especies. Además, se utilizó un modelo estadístico para identificar los gatillos ambientales de la estacionalidad de *Culicoides*. Un total de 12 460 individuos *Culicoides* pertenecientes a por lo menos 15 especies diferentes fueron recolectados durante la encuesta. *Culicoides imicola* fue ampliamente la especie más abundante (71,4% de las capturas totales). La ubicación del sitio, y por lo tanto las especies de vertebrados, no influyeron en la composición de la especie, que se vio afectada principalmente por el mes de recolección. Sorprendentemente, la presión atmosférica fue el parámetro ambiental más frecuentemente seleccionado en los modelos estacionales. Se discute el impacto potencial de este parámetro meteorológico junto con las otras variables seleccionadas. El primer paso es el de identificar los parámetros ambientales que favorecen la abundancia estacional de *Culicoides*, con el fin de implementar modelos robustos y dinámicos de *Culicoides*, que luego podrían usarse en modelos de riesgo de transmisión.

Palabras clave: ganado, caballos, *Culicoides*, virus lengua azul, condiciones atmosféricas, factores ambientales, Marruecos