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Communication

Seasonal and Nocturnal Activity of *Culicoides* spp. (Diptera: Ceratopogonidae) Adapted to Different Environments in the Balearic Islands

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Abstract: Several pathogens are known to be transmitted by arthropods. One of the most relevant, in economic terms, affecting animals is bluetongue virus. Its known vectors are several species of *Culicoides* midges. In Europe, the considered main vector species are *Culicoides imicola* and *Culicoides obsoletus*, though other species may be implicated at different levels. In the present work, the activity of these vector species between sunset and sunrise from May to November is analyzed according to their captures in a rotator bottle collector adapted to a light trap. Additionally, the *Culicoides* populations of two areas in Mallorca (Spain) with different characteristics (rural vs. urban) are compared. The results indicated that June is the month of higher abundance of *C. obsoletus* in our climatic conditions—being active during the first three hours after sunset. Conversely, *C. imicola* reached the maximum level of captures during October, and captures were more evenly distributed during the night. Collections from the two areas revealed that *Culicoides* populations were composed by the same species; however, abundance and sex ratio presented marked differences. These results add valuable insight into the ecology of *Culicoides* and may be used to design more accurate strategies to control diseases associated with *Culicoides*.

Keywords: Bluetongue; bottle rotator; *Culicoides imicola*; *Culicoides obsoletus*; sex ratio; Spain

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1. Introduction

Culicoides Latreille (Diptera: Ceratopogonidae) midges are small (1–3 mm) Nematocera insects known to act as vectors of several pathogens. The most important, from an economical point of view, are bluetongue (BT) and African horse sickness [1]. However, a significant number of other pathogens –ranging from nematodes to several types of virus—may be transmitted to animals and humans by the bites of some species of *Culicoides* [2–4]. In Europe, the Southern species *Culicoides imicola* Kieffer 1913 and the Northern species from the Obsoletus complex ((mainly *C. obsoletus* (Meigen 1818) and *Culicoides scoticus* Downes and Kettle 1952)) are considered to play a major role in the transmission of BT disease [5–9]. An large number of BT outbreaks have occurred in Europe since 2000, when it was detected for the first time in the Balearic Islands (Spain) [10,11]. Nowadays, restrictive and prophylactic measures have succeeded in reducing the incidence of BT disease in Europe [12,13]. However, a certain degree of entomological and serological surveillance should always be maintained since one of the main entry pathways of the disease to Europe is considered to be via infected *Culicoides* transported by warm air currents from North Africa to southern Europe [14–16].

Due to their small size and, as a result, the difficulty in rearing the vector species of *Culicoides* in laboratory conditions, knowledge of the ecological and behavioral aspects of this genus is still quite limited. Flight patterns and optimal environmental conditions for the different *Culicoides* species have been determined in previous studies [17–20];

however, sub-populations of the same species may exhibit different behavioral patterns depending on their suitable environment. The most important environmental drivers for *Culicoides* vector species are related to temperature and precipitation [21]. In Spain, the species *C. obsoletus*/*C. scoticus* are active almost the whole year in Northern provinces, while *C. imicola* is absent in the North, with peaks of abundance between September and November in Southeastern areas [22]. The activity period of adult *C. obsoletus*/*C. scoticus* is longest in low elevation sites with warmer springs and high livestock abundance; meanwhile, *C. imicola* adult females prefer not only lower elevation regions but also sites with broad-leaved vegetation [23].

For the present study, our aim was to improve the knowledge of the above mentioned ecological aspects of *Culicoides* midges, focusing on the vector species *C. imicola* and *C. obsoletus*/*C. scoticus*. Seasonal and nocturnal flight activity patterns were determined for several *Culicoides* in two different habitats (rural vs. urban), and the composition and abundance of midges between the two study areas were compared.

2. Materials and Methods

Culicoides midges for this trial were collected from two different areas in Mallorca (Balearic Islands, Spain). The areas separated 31.4 km from each other. One of them was a rural farm named Ca's Boter, located 6 km away from the closest urban area (Felanitx; 39°31′27′′ N; 3°7′36′′ E), dedicated to cattle raising and milking (≈30 dairy cattle and 20 veal calves), and 18.4 km from the nearest city (Manacor). The farm had an area of 7413 m^2, of which 5620 m^2 (75.8%) was intended for the animals and surrounded by several areas of different sizes dedicated mainly to agricultural or farming practices (sheep flocks of different sizes were scattered in some of the surrounding areas). In this area, no light sources were available during the night. The other was a non-farming property in a residential area named Rialema (s'Aranjassa; 39°32′22′′ N, 2°47′28′′ E) and located 10 km away from the capital city of Mallorca island (Palma). The area covers 7530 m^2 of Mediterranean scrub and vegetation (pine grove area). The area has streetlights, and the property was surrounded by other residences with similar sizes and characteristics. Animals commonly found in the area mainly include pet dogs and cats.

Insects were collected from the two areas during 2012 using a collection bottle rotator (model 1512, John W. Hock Company, Gainesville, FL, USA) adapted to a downdraft blacklight (UV) trap (CDC miniature light trap model 912, John W. Hock Company, Gainesville, FL, USA). The rotator traps were set at 1.8 m from the ground. In Ca's Boter, the trap was placed 1 m close to the livestock premises, while in Rialema, it was set in the backyard. Rotator traps operated during the main *Culicoides* activity period in the area (May–November). A number of nights were sampled per month (Table 1), avoiding rain and strong winds, and bottles were rotated every 1:15 h to 2 h depending on the scotoperiod. Trappings were conducted during the same nights between both places. Eight bottles were rotated during the sampling nights, the first one starting soon after sunset (between 19:00 h to 21:00 h depending on the month) and the last one collecting insects before sunrise (between 7:00 h to 8:00 h) (GTM + 1).

Collected midges were taken to the laboratory of zoology of the University of the Balearic Islands to identify their wing pattern according to the method described by Rawlings [24], and were separated by sex and gonotrophic condition according to Dyce [25]. Composition and activity patterns of the *Culicoides* populations from the two areas (rustic vs. urban) were compared. Flight activity of vector species *C. imicola* and *C. obsoletus*/*C. scoticus* were analyzed. Two flight activity patterns were observed: the activity pattern (from May to November) and the nocturnal activity pattern (from sunset to sunrise). Temperatures during the trial were obtained from a nearby climatic station of AEMET [26].

Species from Obsoletus complex were sent to CIRAD (Centre International de Recherche de l'Agriculture et du Dévéloppement) and identified via PCR assay according to the procedures of Nolan et al. [8].

Table 1. Collected *C. obsoletus*/*C. scoticus* and *C. imicola* species from two areas in Mallorca with different environmental conditions—Ca's Boter (rural) and Rialema (urban). Letters denote significant differences between months (ANOVA test, $p < 0.011$ for Obsoletus complex and $p < 0.006$ for *C. imicola*).

Species	Month	Ca's Boter Total Captures	Ca's Boter Nights Sampled	Ca's Boter Captures/Night	Rialema Total Captures	Rialema Nights Sampled	Rialema Captures/Night
C. obsoletus/ *C. scoticus*	May	1993	3	664.3 ± 11.2 [a]			
	June	5514	7	853.2 ± 9.2 [a]	6	1	6.0 ± 0.0
	July	272	3	90.7 ± 4.7 [a]	12	5	2.4 ± 0.3
	August	47	3	15.7 ± 2.0 [b]	2	2	1.0 ± 0.0
	September	28	6	4.7 ± 1.3 [b]	6	4	1.5 ± 0.7
	October	43	10	4.3 ± 0.6 [b]	6	4	1.5 ± 1.0
	November	7	3	2.3 ± 1.0 [b]	2	5	0.4 ± 0.4
	Total	**7904**	**35**	**225.8 ± 21.9**	**34**	**21**	**1.6 ± 0.3**
C. imicola	May	7	3	2.3 ± 0.2 [b]			
	June	36	7	5.1 ± 0.9 [b]	0	1	0.0
	July	9	3	3.0 ± 1.2 [b]	2	5	0.4 ± 0.4
	August	7	3	2.3 ± 1.2 [b]	1	2	0.5 ± 0.7
	September	56	6	9.7 ± 1.9 [b]	3	4	0.8 ± 0.9
	October	259	10	28.8 ± 1.3 [a]	5	4	1.3 ± 0,4
	November	41	3	13.7 ± 3.7 [b]	1	5	0.2 ± 0,4
	Total	**415**	**35**	**9.3 ± 0.5**	**12**	**21**	**1.0 ± 0.6**

A Shapiro–Wilk test for checking normal distribution of the data followed by an Analysis of Variance (ANOVA) was performed with the STATGRAPHICS plus V 3.0 program to determine the statistical differences between *Culicoides* species abundance among sites, months, and sex ratio. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Species Composition, Sex Ratio, and Gonothrophic Condition

During the complete trial, 12,376 *Culicoides* midges were collected—12,050 from Ca's Boter and 326 from Rialema. At least 10 species were collected from Ca's Boter, namely the following: Obsoletus complex (7904 specimens; 66.1%), *C. imicola* (415 specimens; 3.5%), *Culicoides cataneii* Clastrier 1957 (494 specimens; 4.1%), *Culicoides circumscriptus* Kieffer 1918 (1683 specimens; 14.1%), *Culicoides jumineri* (Callot and Kremer 1969) (345 specimens; 2.9%), *Culicoides longipennis* (Khalaf 1957) (26 specimens; 0.2%), *Culicoides maritimus* (Kieffer 1924) (36 specimens; 0.3%), *Culicoides newsteadi* (Austen 1921) (527 specimens; 4.4%), *Culicoides paolae* (Boorman 1996) (436 specimens; 3.6%), *Culicoides puncticollis* (Becker 1903) (86 specimens; 0.7%). The remaining 98 specimens had missing wings or were unidentifiable. A molecular analysis of the Obsoletus complex population revealed that 0.6% of the samples belonged to *C. scoticus*, while the remaining (99.4%) belonged to *C. obsoletus*.

Nine out of the ten species encountered in Ca's Boter were also present in Rialema. However, the predominant species in the latter area were *C. circumscriptus* and *C. paolae* (instead of *C. obsoletus*), which represented more than 45% of the *Culicoides* collected. The species composition of the *Culicoides* collected from Rialema was as follows: Obsoletus complex (34 specimens; 10.4%), *C. imicola* (12 specimens; 3.7%), *C. cataneii* (47 specimens; 14.4%), *C. circumscriptus* (88 specimens; 27%), *C. jumineri* (9 specimens; 2.8%), *C. maritimus* (20 specimens; 6.1%), *C. newsteadi* (48 specimens; 14.7), *C. paolae* (67 specimens; 20.6%), and *C. puncticollis* (1 specimen; 0.3%).

Regarding the sex and gonotrophic condition of the *Culicoides* midges collected from Ca's Boter, 3.7% were males, while 95.3% were females, which included 34.6% nulliparous, 23.7% parous, 35.3% gravid, 1.3% blood-fed, and 0.5% specimens without an abdomen. The remaining 1% belonged to intersexed specimens of *C. circumscriptus*. For the *Culicoides* collected from Rialema, 48.2% were males, while the remaining females included 20.2% nulliparous, 17.5% parous, 13.2% gravid, and 0.9% blood-fed specimens. Differences in the abundance and sex ratio between the sampled areas were highly significant ($p < 0.016$).

3.2. Seasonal and Nocturnal Activity Pattern

The *Culicoides* collected from Ca's Boter farm indicated that June was the month in which the population of the *C. obsoletus*/*C. scoticus* reached its peak (Av. ± SE = 853.2 ± 9.2 *Culicoides*/night) (Table 1)—when the average temperature was 24.4 °C (max: 39.7 °C, min: 13.2 °C) (Table 2). Differences in the number of captures were not significant between June and May–July ($p > 0.011$), but they were with the rest of the months ($p < 0.011$). Regarding *C. imicola*, the highest number of captures were obtained also in Ca's Boter in October (Av. ± SE = 28.8 ± 1.3 *Culicoides*/night), recording an average temperature of 18.8 °C (max: 30.1 °C, min: 3.9 °C). Significant differences were observed between the collections of this species during this month and the rest of the season ($p < 0.006$). Populations of these two species reached their highest levels in Rialema during the same months.

Table 2. Average temperatures during the sampling months.

Site	Month	Av. T (C°)	Max. T (C°)	Min. T (C°)
Ca's Boter	May	18.8	33.1	6.0
	June	24.4	39.7	13.2
	July	24.9	36.4	14.0
	August	27.0	39.4	16.8
	September	22.1	33.3	12.0
	October	18.8	30.1	3.9
	November	14.1	23.3	3.8
Rialema	May	18.9	32.0	7.4
	June	24.2	36.7	13.5
	July	25.3	37.5	14.9
	August	27.1	38.5	17.5
	September	22.5	32.6	12.2
	October	19.6	30.4	5.2
	November	15.0	23.6	4.5

The rates of collection regarding *Culicoides* midges from the Rialema area were too low to display a proper nocturnal activity pattern; hence, the results presented here are those obtained from Ca's Boter farm. More than 50% of the midges were collected during the first three hours after sunset, both for the *C. obsoletus*/*C. scoticus* and for the total population, while *C. imicola* was collected more evenly throughout the night (Figure 1). During the months wherein there was a higher abundance of *C. obsoletus*/*C. scoticus* (May, June, and July), the monthly distribution pattern of collection was well defined and in accordance with the seasonal pattern (Figure 2). However, the distribution pattern changed during the months wherein there was a lower abundance of these species (August, September, October, and November), and the rates of collection of individuals of these species were more uniform during the night or even before the sunset (Figure 3). In contrast, the nocturnal activity of *C. imicola* showed a different pattern, and collections of this species were obtained more evenly throughout the night, with peaks pre-sunset in October and November, as seen in Figure 4.

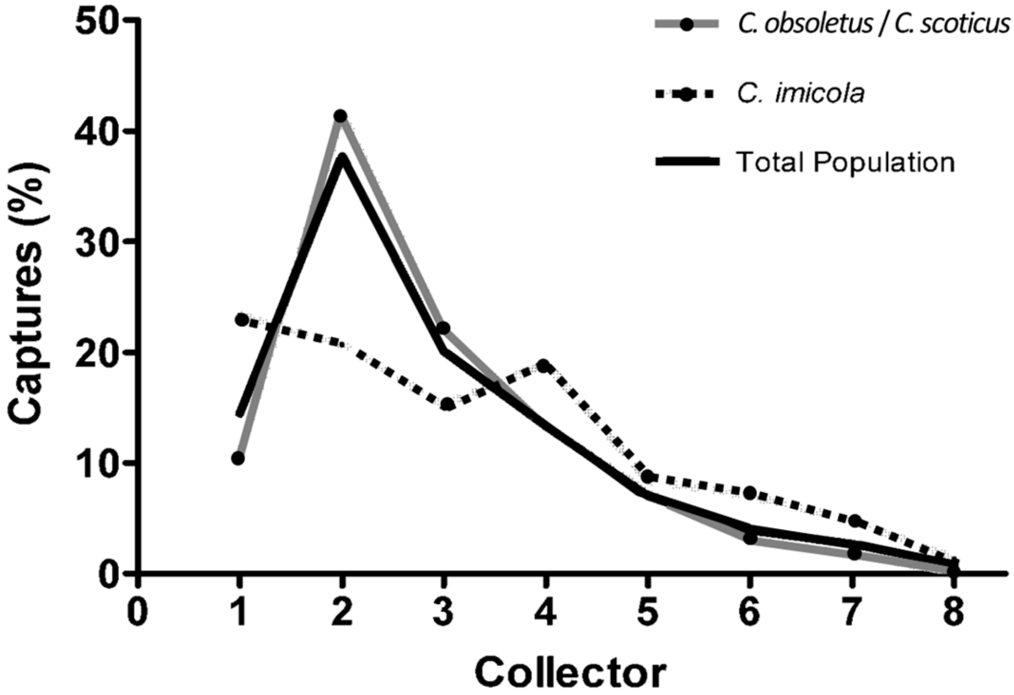

Figure 1. Percentage of different *Culicoides* species collected with a bottle rotator operated between sunset (collector 1) and sunrise (collector 8) in 2012 in Ca's Boter farm.

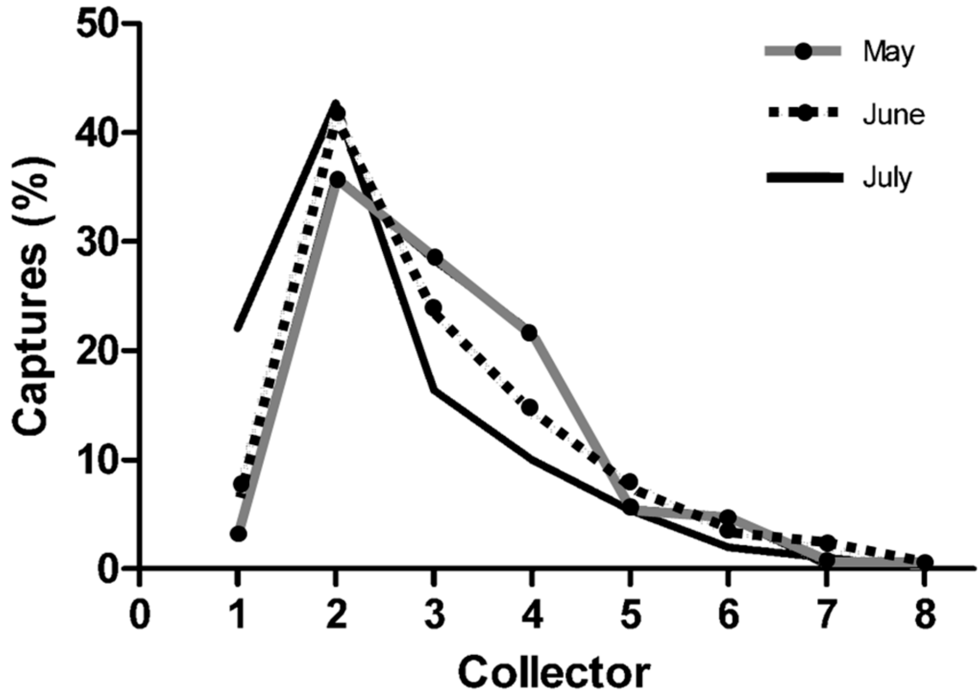

Figure 2. Percentage of *C. obsoletus*/*C. scoticus* collected with a bottle rotator operated between sunset (collector 1) and sunrise (8) during the months of highest abundance with regards to this species during 2012 in Ca's Boter farm.

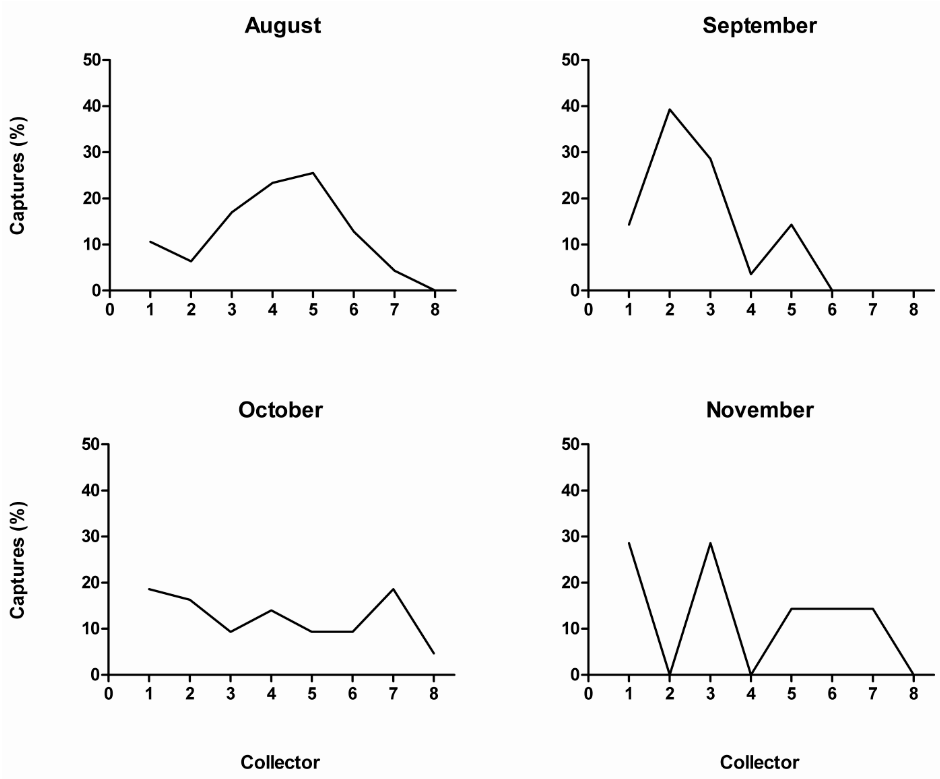

Figure 3. Percentage of *C. obsoletus*/*C. scoticus* collected with a bottle rotator operated between sunset (collector 1) and sunrise (collector 8) during the months of lowest abundance with regards to this species during 2012 in Ca's Boter farm.

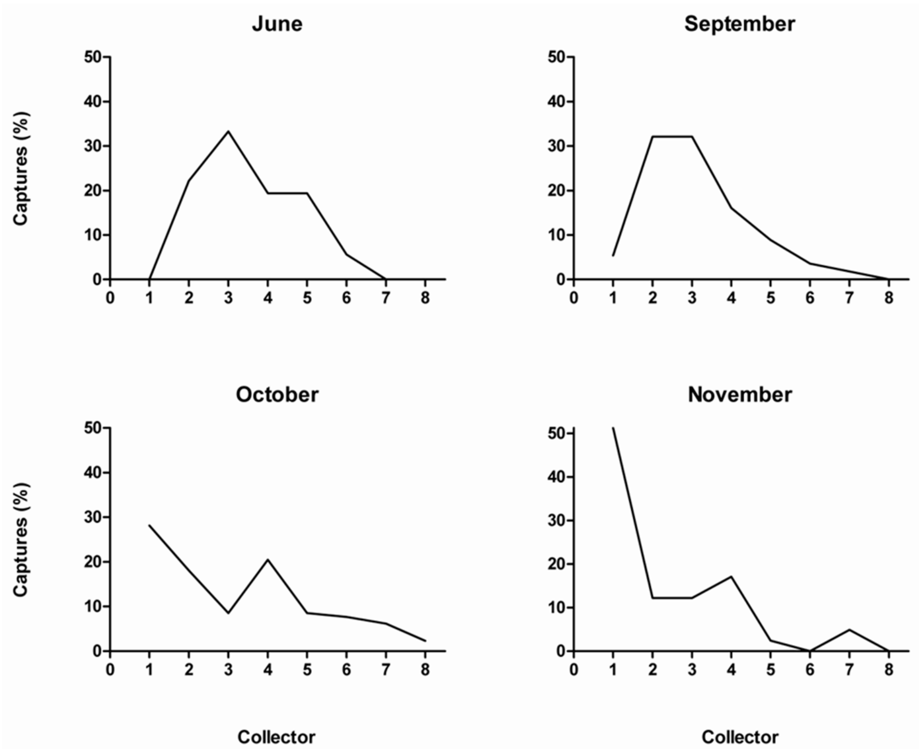

Figure 4. Percentage of *Culicoides imicola* collected with a bottle rotator operated between sunset (collector 1) and sunrise (collector 8) during the months of highest abundance with regards to this species during 2012 in Ca's Boter farm.

4. Discussion

The collection of *Culicoides* from areas with different environmental conditions revealed that urban areas host a significantly lower abundance of *Culicoides* than rural areas. However, results showed that in spite of the lower rates of capture in Rialema, the species composition was mostly the same in both areas (nine out of ten) with significant differences in sex ratio (F/M ratio from Ca's Boter = 23.9 times higher than F/M ratio from Rialema). It has been suggested that males do not disperse as far as females, and the collection of high numbers of males are probably indicative of a proximal breeding site and larval habitat [27]. No identification of larval habitats was observed in Rialema, but the breeding conditions in that area did not seem optimal (i.e., absence of humid or standing water to lay eggs and lack of host animals to parasitically infect). Previous reports indicated that some species, such as *C. circumscriptus* or *C. puncticollis*, bred near the collection site of Ca's Boter [28], and the proportion of males collected in the last were extremely low compared to those obtained from Rialema. This fact suggests that most of the males of the different *Culicoides* species do not linger near the place wherein they spent time as larvae, as previously mentioned and supported by the results of Braverman [29]. Conversely, streetlights in the residential area of Rialema could interfere with the results. Further studies, including more locations and habitats (e.g., peri-urban sites) or even records of the moon phase [30], should be performed to compare *Culicoides* populations regarding breeding sites, available hosts, and anthropic presence.

The results obtained in the present trial indicated that, in our climatic conditions, June is the month in which the abundance of *C. obsoletus*/*C. scoticus* is at its peak (avg. temp = 24.4 °C), while, during October, environmental conditions are optimal for the development of *C. imicola* populations (avg. temp = 18.9 °C). These results are in accordance with those presented by Miranda et al. [31], indicating that, in our climatic conditions, the higher numbers of *C. obsoletus* are able to be observed and obtained between June–July, while, during October, the abundance of *C. imicola* reaches its peak. Hence, particular attention should be paid to the preventive measures against biting midges during these months due to the higher epidemiological risk associated with vector abundance. In fact, the BT outbreaks that occurred in Mallorca during 2000, 2004, and 2021 were detected during these months [12].

When *C. obsoletus*/*C. scoticus* populations are at their higher level, the species was most commonly captured during the first 3 h after sunset. However, *C. imicola* seemed to be active before sunset, with the collection of this species being more evenly distributed throughout the night. Peak nocturnal activity in *C. obsoletus* and *C. scoticus* during the first hours after sunset was also observed in mainland conditions by Viennet et al. [32]. Information concerning the most frequent hours of activity among *C. obsoletus* and *C. scoticus* indicates the period of time whereby the risk of disease transmission associated with this vector species is maximal; hence, the proper preventive measures (such as stabling during peak hours of vector activity) should be implemented during these time periods. However, as our results indicate, the activity of *C. imicola* is highest during the majority of the night; therefore, preventive measures should be maintained throughout the night during the period of maximal abundance of this vector species (October, in our conditions). The same *Culicoides* species, but in different numbers, were found in areas with very different environmental conditions. It should be considered that, in regions with temperate and cool climates, some *Culicoides* adult species enter into diapause or shift their diel activity patterns when the number of daylight hours or the temperature declines [33,34]. Hence, the lack of any daytime data, especially as overnight temperatures cool later in the year, could leave some uncertainty about *Culicoides* activity patterns and potential transmission dynamics.

The activity of *Culicoides* midges was measured following their capture in UV light traps. However, the composition of *Culicoides* populations may differ broadly depending on the collection method [35–37]. Traps with CO_2 and/or animal baited traps should be the preferable method to more accurately determine the epidemiologic risk depending on the hour of the night and the month of the season, and the results obtained from that trial could be compared with the *Culicoides* obtained with light traps to assess their validity. Additionally, environmental conditions may vary from one year to the next, and parameters such as temperature, rain, and relative humidity should be taken into consideration when conducting risks assessments for the area.

Future trials assessing the biting rate of *Culicoides* vector species among farm animals depending on the hour of the night and months of the season are encouraged. Light traps are easier to handle and less time-consuming than animal-baited traps; hence, if results derived from the use of UV light traps and animal baiting are found to be related, a gold standard to assess the risk of infection in a determined area could be adopted. This approach would provide information to more accurately determine the disease risk periods associated with *Culicoides* vectors and hence contribute to the optimization of available resources against potential disease outbreaks.

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