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Environmental Drivers of Adult Seasonality and Abundance of Biting Midges *Culicoides* (Diptera: Ceratopogonidae), Bluetongue Vector Species in Spain

Carlos Barceló,[1,5] Bethan V. Purse,[2] Rosa Estrada,[3] Javier Lucientes,[3] Miguel Á. Miranda,[1] and Kate R. Searle[4]

[1]Applied Zoology and Animal Conservation Research Group, Department of Biology, University of the Balearic Islands (UIB), Ctra. Valldemossa Km 7.5, 07122 Palma de Mallorca, Spain, [2]Centre for Ecology and Hydrology, Oxfordshire, OX10 8BB, United Kingdom, [3]Department of Animal Pathology, Faculty of Veterinary, University of Zaragoza, Zaragoza, Spain, [4]Centre for Ecology and Hydrology, Bush Estate, EH26 0QB Edinburgh, Scotland, and [5]Corresponding author, e-mail: carlos.barcelo@uib.es

Abstract

Bluetongue is a viral disease affecting wild and domestic ruminants transmitted by several species of biting midges *Culicoides* Latreille. The phenology of these insects were analyzed in relation to potential environmental drivers. Data from 329 sites in Spain were analyzed using Bayesian Generalized Linear Mixed Model (GLMM) approaches. The effects of environmental factors on adult female seasonality were contrasted. Obsoletus complex species (Diptera: Ceratopogonidae) were the most prevalent across sites, followed by *Culicoides newsteadi* Austen (Diptera: Ceratopogonidae). Activity of female Obsoletus complex species was longest in sites at low elevation, with warmer spring average temperatures and precipitation, as well as in sites with high abundance of cattle. The length of the *Culicoides imicola* Kieffer (Diptera: Ceratopogonidae) female adult season was also longest in sites at low elevation with higher coverage of broad-leaved vegetation. Long adult seasons of *C. newsteadi* were found in sites with warmer autumns and higher precipitation, high abundance of sheep. *Culicoides pulicaris* (Linnaeus) (Diptera: Ceratopogonidae) had longer adult periods in sites with a greater number of accumulated degree days over 10°C during winter. These results demonstrate the eco-climatic and seasonal differences among these four taxa in Spain, which may contribute to determining sites with suitable environmental circumstances for each particular species to inform assessments of the risk of Bluetongue virus outbreaks in this region.

Key words: Bluetongue, *Culicoides*, model, overwinter, seasonality

Bluetongue, Schmallenberg, and African Horse Sickness are viral infectious diseases of ruminants (Theiler 1921, Hoffmann et al. 2012, Carpenter et al. 2017, Wernike and Beer 2017) transmitted by several species of biting midges *Culicoides* (Diptera; Ceratopogonidae) (Mellor et al. 2000, Purse et al. 2015).

Bluetongue virus first appeared in Spain when serotype 10 arrived in 1956 (Sellers et al. 1978). Since then, the circulation of five different serotypes (Bluetongue virus-1, 2, 4, 8, and 10) has been detected during different time periods. The major source of incursions of Bluetongue virus strains is considered to be via infected *Culicoides* transported on the wind from North Africa (Zientara and Sánchez-Vizcaíno 2013, Pérez de Diego et al. 2014). Another potential introduction method of this virus is animal movements across Europe (de Koeijer 2011). Since 1956, more than 150,000 domestic animals have been lost during Bluetongue virus outbreaks in Spain, with devastating impacts on farmers' livelihoods (Alba et al. 2004, Pérez de Diego et al. 2014). Currently, the outbreaks are restricted to serotype 4 in the center of Iberian Peninsula and serotypes 1 and 4 in central and south areas (EU 2019).

Seasonality and abundance of adult female *Culicoides* are key parameters governing the transmission of midge-borne viruses because a high ratio of adult vectors to hosts increases the basic reproduction ratio (R_0), i.e., the probability that disease transmission will establish following introduction (Diekmann and Heesterbeek 2000, Keeling and Grenfell 2000, Sanders et al. 2011). Moreover, the length of the adult vector-free period over winter determines whether Bluetongue virus will persist between years because the maximum length of viraemia in the vertebrate host is only 9 wk (in adult cattle) (OIE 2014). As a consequence, in temperate areas like Europe, disease management policy is governed by the knowledge of the timing

of this period (e.g., Seasonal Vector-Free Period, or SVFP, defined in Annexe V of Commission Regulation (EC) No. 1266/2007 by the European Union council). Both vaccination campaigns and animal movements between affected regions can be conducted within the SVFP. This period is also used as a parameter in models aiming to understand the behavior of virus outbreaks across geographical areas and, to assess the effectiveness of control measures to control the virus in Spain (Gubbins et al. 2008, Hartemink et al. 2009, Brugger and Rubel 2013, Brugger et al. 2016, Napp et al. 2016).

Larval development and reproduction of adult vectors can occur year round in tropical areas, and adults may be active during the whole year (Sellers 1980, Verhoef et al. 2014). However, in temperate and cool regions, *Culicoides* overwinter at larval stage and some adult species enter into diapause, thought to be triggered when the number of daylight hours or the temperature declines below a threshold level (Rawlings and Mellor 1994, Lysyk and Dank 2007).

The transmission of Bluetongue virus in Spain is mainly attributed to *Culicoides imicola* Kieffer and the Obsoletus complex (Braverman et al. 1985, Mellor and Wittmann 2002, Purse et al. 2007, Wilson and Mellor 2009) (Table 2). The Obsoletus complex includes the species *Culicoides obsoletus* (Meigen), *Culicoides scoticus* Downes and Kettle, *Culicoides chiopterus* (Meigen), *Culicoides montanus* (Shakirzjanova), and *Culicoides dewulfi* Goetghebuer (Boorman et al. 1995, Monaco et al. 2010, Garros et al. 2014); however, *C. obsoletus* and *C. scoticus* are two of the most common species across Spain and central Europe (Pagès and Sarto I Monteys 2005, Hoffmann et al. 2008, Meiswinkel et al. 2008). In Spain, this group is found mainly in the North (Calvete et al. 2008, Lucientes et al. 2008, Calvete et al. 2009b, Talavera et al. 2015, Alarcón-Elbal et al. 2016), whereas the Afrotropical species *C. imicola* may have expanded its range northwards in recent decades due to climate change (Purse et al. 2005, Cagienard et al. 2006, Purse and Rogers 2009, Guichard et al. 2014, Niedbalski and Fitzner 2018). In Spain, this species is still restricted to south-western parts, including the Mediterranean coast (Ortega et al. 1998; Tatem et al. 2003; Calvete et al. 2008, 2009a; Acevedo et al. 2010; Talavera et al. 2015; Cuéllar et al. 2018b).

Other species, namely *Culicoides newsteadi* Austen and *Culicoides pulicaris* (L.), have also been incriminated as vectors following the isolation of Bluetongue virus from field specimens (Vanbinst et al. 2009, Goffredo et al. 2015, Foxi et al. 2016). *Culicoides pulicaris* species is less prevalent and is not closely linked to livestock than the others (Harrup et al. 2013, 2016); however, this species shares a similar overall distribution to the Obsoletus complex in Spain (Lucientes et al. 2008, Ducheyne et al. 2013, Talavera et al. 2015). Conversely, *C. newsteadi* has a high population density in Spain and is distributed predominantly in the south (Ortega et al. 1999, Del Río et al. 2013, Ducheyne et al. 2013).

The objective of this study was to relate variability in the adult seasonality of the most important vector species of *Culicoides* present in Spain in different to concurrent host, landscape, and climate conditions with the aim of predicting variation in the SVFP in different environmental contexts.

We assume that major environmental factors affecting development, activity, and distribution of *Culicoides* populations in Europe are temperature, humidity and rainfall due to the use of moist/soil breeding sites (Harrup et al. 2013; Zimmer et al. 2013, 2014b; Purse et al. 2015). In addition, prior studies established a direct relationship between *Culicoides* abundance measured in traps and the probability of Bluetongue virus occurrences (Calvete et al. 2008, Guis et al. 2012). In agreement with the

Table 1. Summary of the variables used in the literature for the presence (pres.), phenology (phen.), abundance (abund.) and distribution (distr.) of Obsoletus complex (OBS), *C. imicola* (IMI), *C. newsteadi* (NEW) and *C. pulicaris* (PUL)

Source reference	OBS	IMI	NEW	PUL	Variables included	*Culicoides* association
Braverman et al. (1974)	X				Breeding sites	Pres.
Mellor and Pitzolis (1979)	X	X			Breeding sites	Pres.
Rawlings and Mellor (1994)	X	X			Spa. loc.	Phen.
Ortega *et al* (1998)	X	X		X	-	Phen., distr., abund
Ortega et al. (1999)		X	X	X	Clim.	Phen., abund.
Conte et al. (2003)		X			Clim., top.	Pres., distr.
Capela et al. (1993)		X			Breeding sites	Abund., distr.
Miranda et al. (2004)	X	X			Hosts	Phen., abund.
Tatem et al. (2003)		X			Soil	Pres.
Purse et al. (2004)	X	X	X	X	Soil, top.	Pres.
Torina et al. (2004)	X	X	X	X	Bluetongue virus Outbreaks	Pres., distr.
Purse et al. (2007)	X	X		X	Clim.	Pres., abund.
Conte et al. (2007)	X	X			Clim., top., soil, land-cov.	Abund., distr.
Carpenter et al. (2008b)	X			X	Clim.	Phen., abund.
Takken et al. (2008)	X			X	Climate, spa. loc.	Abund., distr.
Calvete et al. (2009a)	X	X		X	PPT, soil, hosts	Presence
Acevedo et al. (2010)		X			Spa.loc., top., Clim., hosts, soil.	
Foxi and Delrio (2010)	X	X	X	X	Breeding sites	Phen., abund.
Garros et al. (2011)	X		X	X	Hosts	Blood source
Sanders et al. (2011)	X			X	Hosts, top.	Phen., abund.
Calvo et al. 2012	X		X	X	Hosts	Blood source
Purse et al. (2012)	X			X	Hosts, top., soil	Abund.
Searle et al. (2012)				X	Clim., Phot., land-cov.	Phen., abund.

Clim., Climate; land-cov., land cover; Phot., photoperiod; spa. loc., spatial location; top., topography.

literature summarized in Table 1, *C. imicola* is highly abundant in south-western regions of Spain during summer; has longer adult activity seasons and prefers higher temperatures, probably related with its Afrotropical preferences (Ortega et al. 1999; Miranda et al. 2004, 2007; Talavera et al. 2015; Ramilo et al. 2017). Its peaks of adult activity occur between 16 and 18°C, and the species is absent in sites with cold winters (Rawlings and Mellor 1994, Venter et al. 2019). According to its heliophilic behavior, *C. imicola* breeds in grassland areas with high vegetation temperature including moist coastal areas (Braverman et al. 1974; Mellor and Pitzolis 1979; Conte et al. 2007; Peters et al. 2014a, b) and those with lower altitudes (Conte et al. 2003, 2007; Torina et al. 2004).

Obsoletus complex is favored by lower average temperatures (Miranda et al. 2004, Conte et al. 2007, Talavera et al. 2015, Cuéllar et al. 2018a) and arid sites with high elevation (Torina et al. 2004, Conte et al. 2007). This species breeds in a wide diversity of habitats (Carpenter et al. 2008; Takken et al. 2008; Foxi and Delrio 2010; González et al. 2013b; Zimmer et al. 2014a, b). In the North of Europe, this species occurs in areas with dense vegetation, including forests, presumably linked to breeding preferences for leaf-litter in some group members (Harrup et al. 2013, Zimmer et al. 2013). However, in Mediterranean areas, this species, along with *C. imicola*, usually breed in habitats enriched with organic matter near livestock animals (Capela et al. 1993, Conte et al. 2007, Foxi and Delrio 2010, González et al. 2013b).

As well as *C. imicola*, the species *C. newsteadi* has also been found to prefer lower altitudes (Torina et al. 2004), and sites with warmer average temperatures (Ortega et al. 1999, Purse et al. 2004). *Culicoides pulicaris* has shown a preference for the soil of open pastures (Harrup et al. 2013, Zimmer et al. 2014b), and this species appears in the same sites as Obsoletus complex (Ortega et al. 1998, Ducheyne et al. 2013), and also in coastal zones (Ramilo et al. 2017). Obsoletus complex and *C. pulicaris* have been found to overwinter in stables during winter (Kameke et al. 2017), therefore, *C. pulicaris* is expected to be related to the same variables as the Obsoletus complex, such as lower temperatures and areas with livestock (Purse et al. 2004, Sanders et al. 2011, Searle et al. 2012).

In this study, we analyze the variation in phenology of adult females of populations of the following species: Obsoletus complex, *C. imicola*, *C. newsteadi*, and *C. pulicaris* according to different environmental variables. The objective is to facilitate understanding of how the environment could affect these species and to explore the contrast between the northern species Obsoletus complex/ *C. pulicaris* and the south-eastern species *C. imicola*/ *C. newsteadi*. The species from the Obsoletus complex were analyzed together.

Material and Methods

Entomological Data and Sampling Procedures

Data used for the study were samples from 329 trap sites distributed across the 50 provinces of Spain (Fig. 1) during the intensive National Entomosurveillance Program for Bluetongue virus sponsored by the Spanish Ministry of Rural and Marine Environment from the year 2005 to 2010. Data from this program have previously been used in several studies assessing various aspects of *Culicoides* ecology. Calvete et al. (2008, 2009a) analyzed data from 2004 to 2006 to model the spatial distribution of *C. imicola* and *C. obsoletus* and Bluetongue virus prevalence in Spain. The study of Acevedo et al. (2010) analyzed spatial relationships between environmental variables and *C. imicola* abundance between 2005 and 2008. The study of Ducheyne et al. (2013) used data from this dataset to model

spatial abundance patterns of the four Bluetongue virus vector species *C. imicola*, *C. obsoletus*, *C. pulicaris* and *C. newsteadi* in a single year (2007). Finally, the recent studies of Cuéllar et al.(2018a,b) used the same data as Acevedo et al. (2010), alongside similar datasets from eight other countries, to model the seasonal and spatial distribution of the *Culicoides* vector species across Europe.

Culicoides adults were collected by CDC-Miniature traps model 1212 (John W. Hock) with UV light and photoelectric cell considered one of the most effective traps for *Culicoides* collection (Del Río et al. 2013, Hope et al. 2015). The traps were set one night per week on farms at 1.7–2.0 meters from the floor and placed from dusk till dawn (at least 45 wk per year) between 1 and 30 meters from livestock (see detailed description in Calvete et al. (2008)). The traps used collector bottles containing antifreeze and alcohol to prevent the samples from decaying. The bottles were collected and transported to the laboratory where they were classified by species and gonotrophic status according to the taxonomic key of Mathieu et al. (2012) and Dyce (1969), respectively. The farms were located at altitudes from 2 to 1,456 meters above sea level and housed between 16 and 3,000 large livestock animals, including 'intensive' farm settings (50% of all sites), 27% 'extensive', and 23% 'semi-extensive' farms. Amongst the farms, 60% were bovine farms, 26% ovine farms, 6% equine farms, 5% caprine farms, and 3% porcine farms. An intensive setting consisted of industrialized livestock farms, where animals are housed under artificial conditions, with the objective to increase meat production and other animal derivatives such as eggs, milk, wool, etc. These farms included large facilities, often utilizing intensive technology. Conversely, an extensive setting employed traditional livestock farming methods in a natural environment, which improves the conditions for animal development. The objective of extensive farms is to use the land in a more sustainable and conservation orientated way. Finally, a semi-extensive setting is a mixture of extensive and intensive procedures.

All specimens obtained from these sampling points were sent to the reference laboratory located in the faculty of veterinary of Zaragoza province for processing, and resulting data were sent to the Laboratory of Zoology (University of the Balearic Islands) for analysis.

Models

The *Culicoides* taxa considered in this analysis were Obsoletus complex species, *C. imicola*, *C. pulicaris*, and *C. newsteadi*. The species from Obsoletus complex are usually identified through molecular assays (Garros et al. 2014, Harrup et al. 2015); however, molecular methods were not used routinely during the surveillance program.

Phenology metrics were only calculated for a particular site-year combination when sufficient trapping effort had been employed year round to ensure robust detection of the start and end of seasonal activity. This was defined as site-years with at least 45 trapping weeks per year, and no more than 3 consecutive weeks with no-trapping. Site-by-year combinations were also only included in the analysis if at least 2 wk of trapping prior to the week in which seasonal activity was identified to start had been undertaken. Similarly, site-by-year combinations were only included in analyses if at least 2 wk of trapping had occurred after the week in which the end of the season was identified to occur. This was to ensure robust identification of the start and end of seasonal activity, avoiding errors arising from missing weeks in trapping time-series. Because sampled population dynamics are inherently difficult to detect and potentially biased in small populations, we further

excluded site-year combinations in which a species occurred in very low average abundance for one or more 2-mo periods of the year. These 2-mo periods were defined as January–February (Period 1), March–April (Period 2), May–June (Period 3), July–August (Period 4), September–October (Period 5), and November–December (Period 6). We analyzed *Culicoides* adult females only because only females take blood meals and lay eggs, making them the critical disease vector life-stage of these taxa.

To examine correlations between adult seasonality and environmental factors, we calculated the following metrics of annual abundance and seasonality for each site-by-year combination in which the above criteria had been met (sensu Searle et al. 2014):

Table 2. Number of total data points and data points after species abundance thresholds had been applied

Species	Total datapoints			Datapoints with threshold		
	N	Sites	Years	N	Sites	Years
Start and end of the season						
Obsoletus complex	106	61	6	61	42	6
C. imicola	92	48	6	57	37	6
C. newsteadi	93	50	6	58	38	5
C. pulicaris	81	44	6	40	25	5
Length of overwinter						
Obsoletus complex	47	31	5	27	21	5
C. imicola	46	26	5	29	18	5
C. newsteadi	44	26	5	28	19	4
C. pulicaris	42	27	4	22	17	4

For start and end of season, thresholds were as follows: Obsoletus complex, *C. newsteadi* and *C. pulicaris*: >5 females / trap catch and for *C. imicola* >20 females / trap catch. N: number of observations.

- Start of the season (Julian days): Defined as the first week of the year in which more than five (in case of Obsoletus complex, *C. newsteadi* and *C. pulicaris*) or 20 (in case of *C. imicola*) females were caught.
- End of the season (Julian days): Defined as the last week of the year in which more than five (in case of Obsoletus complex, *C. newsteadi* and *C. pulicaris*) or 20 (in case of *C. imicola*) females were caught.
- Length of the overwinter period (in weeks): Defined as the difference in weeks between the end of the season in one year and the start of the season of the following year.

The abundance thresholds that were used to define the start and end of seasonal activity were taxa-specific. For the Obsoletus complex, *C. newsteadi* and *C. pulicaris* we applied the SVFP criteria—using a number of 5 or less individuals per trap catch to define the start and end of adult activity; in contrast, for *C. imicola*, we used a number of 20 individuals or less per trap catch. This higher threshold for *C. imicola* better reflects the situation of this species in Spain because this species occurs in high abundance as adults in the south-western parts of the country. Therefore, using lower thresholds for *C. imicola* is problematic in Spain because this species numbers fluctuate widely over the year in Northern regions, resulting in multiple instances of apparent disappearance and reappearance throughout a single year (Barceló et al. 2020). Hence, using a threshold of 20 individuals to define the start and end of seasonal activity is a better reflection of the seasonal ecology of this species in this region of Spain. The numbers of site-year combinations available in total and the numbers that met the above criteria for each taxa are shown in Table 2.

Environmental Parameters
Environmental data for each site were obtained from different sources, including CORINE, SRTM, ENSEMBLES, and Robinson

Fig. 1. Map with the location of sampling points during the Spanish National surveillance program from 2005 to 2010.

et al. (2012), and used within the analysis to detect correlations between *Culicoides* phenology metrics and environmental variables (Table 3). The environmental data used were: Topography (elevation and slope); the most predominant classes of CORINE land cover in Spain considered to be of relevance to *Culicoides* occurrence, including pastures, natural grassland zones, agro-forestry areas, schlerophyllous vegetation (type of vegetation adapted to cope with hot and dry summers), broad-leaved forest, and mixed forest; climate factors (accumulated degree days and rainfall for each season); photoperiod (in March, April, September, and November); host density (cattle and sheep in a 2-km grid square) and an estimate of the total *Culicoides* female abundance of each species at each site in each year (Table 3). This last variable was always included in statistical models because the timing of emergence and disappearance will be sampled more accurately in sites with higher abundances of *Culicoides* (Searle et al. 2012, 2014; Daoud 2017).

Statistical Methods

We used statistical models to analyze relationships between seasonal timing metrics and environmental drivers. Three different response variables were considered: length of the overwinter period (in weeks), start of the season (Julian day), and end of the season (Julian day). The relationship between the 'start' and the 'end' timings and the environmental drivers were quantified using Generalized Linear Mixed Models (GLMM) employing circular regression obtained by wrapping a normal distribution on the real line around a circle (Modlin et al. 2012, Searle et al. 2014). The Wrapped Normal distribution (WN) was used to model the timing of appearance and disappearance, to account for the circular nature of such phenological event data (Modlin et al. 2012, Searle et al. 2014). The weeks for the 'start' and 'end' of the season are circular variables because they may potentially range from week 1 to 52, and weeks at the end of one year and the beginning of the following year are close in time, so,

Table 3. Environmental variables included as potential predictors of patterns in *Culicoides* phenology in Spain (timing of start and end of season, and length of overwinter [OW]) extracted for each of the trapping site-by-year combinations

Environmental data	Notation	Definition	Start	End	OW
Temperature (source: ENSEMBLES.URL: http://ensembles-eu.metoffice.com/)	DDwin	Accumulated degree days greater than 10°C between Dec. and Mar.	X		X
	DDaut	Accumulated degree days greater than 10°C between Sept. and Dec.		X	X
	DDspr	Accumulated degree days greater than 10°C between Mar. and June.	X		
	DDsum	Accumulated degree days greater than 10°C between June and Sept.		X	
Precipitation (source: EU-FP6 project ENSEMBLES. URL: http://ensembles-eu.metoffice.com/)	Pwin	Summed daily precipitation (mm) over 1 Dec. to Feb. 28	X		X
	Paut	Summed daily precipitation (mm) over Sept. 1 to Nov. 31		X	X
	Pspr	Summed daily precipitation (mm) over Mar. 1 to May 31	X		
	Psum	Summed daily precipitation (mm) over June 1 to Aug. 31		X	
Topography (source: SRTM 90m Digital Elevation Database v4.1, CGIAR-CSI. URL: http://www.cgiar-csi.org/data/srtm-90m-digital-elevation-database-v4-1)	Elev	Elevation in meters above the sea level at 90-m grid square resolution	X	X	X
	Slope	Inclination of the land in degrees at 90-m grid square resolution	X	X	X
Host density (source: Robinson et al. 2012)	Cattle	Number of cattle in a 2-km grid square for each trapping site.	X	X	X
	Sheep	Number of sheep 2-km grid square for each sampling point.	X	X	X
Land cover (source: Corine Land Cover 2006 vector; min.: 25 ha/polygon. URL: https://www.eea.europa.eu/data-and-maps/data/clc-2006-vector-4).	NatGras	Percentage of Natural grassland zones in the surrounding 1 km around each trapping site.	X	X	X
	PastGras	Percentage of Pasture grassland zones in the surrounding 1 km around each trapping site.	X	X	X
	AgFor	Percentage of Agro-forestry areas in the surrounding 1 km around each trapping site.	X	X	X
	SchVeg	Percentage of Schlerophyllous vegetation in the surrounding 1 km around each trapping site.	X	X	X
	BrdMix	Broad-leaved forest and mixed forest in the surrounding 1 km around each trapping site.	X	X	X
Photoperiod (source CEH models)	Phmarch	Days in Mar. with more than 9 h of daylight.	X		
	Phapr	Days in April with more than 9 h of daylight.	X		
	Phsep	Days in Sept. with more than 9 h of daylight.		X	X
	Phnov	Days in Nov. with more than 9 h of daylight.		X	X
Females abundance	Sum females	Mean annual average abundance of *Culicoides* females for a site.	X	X	X

we cannot assume that they have a standard statistical distribution. For analyses of the length of overwinter period, however, we used a standard normal distribution. In all analyses, models included normally distributed random effects to account for variation between years (unstructured temporal heterogeneity) and sites (unstructured spatial heterogeneity).

All models were fitted using the WinBUGS program (Lunn et al. 2000, 2009) on the R-Development Core Team 2009 software and fitted following the approach of Searle et al. (2014). Standard diffuse priors, Normal (0, 100000) for slope parameters and Uniform (0,100) for standard deviations, were assumed for all parameters other than the intercept of the WN model. That parameter is assumed to have a prior of the form Normal ($-\pi$, π), because the use of a more diffuse prior may lead to problems with convergence (Searle et al. 2014). Stepwise selection using the Deviance Information Criterion was used to select environmental variables to find the most parsimonious model for each seasonal timing metrics. DIC is a generalization of the Akaike Information Criterion (AIC), and is derived as the mean deviance adjusted for the estimated number of parameters in the model—DIC accounts for both model fit and complexity, and providing a relative measure of out-of-sample predictive performance. DIC comparisons to 'null' models are presented for all best-fitting models, where the null model contained all fixed and random effects but no environmental variables.

More specifically, a preliminary saturated model (with all environmental parameters) was environmental models performed better than a model with no environmental variables ('null' model). Subsequently, all environmental variables were selected in subsets of broad categories and combined, assuming they resulted in an improvement in model fit, assessed using DIC. The order of the broad categories added to the models was: female abundance, temperature, topography, precipitation, interactions between temperature and precipitation, hosts, land cover, and photoperiod. Finally, we identified the model receiving the greatest support in the data for each of the timing metrics by successively adding and removing environmental covariates from the broad categories of environmental covariates. Before model fitting, we first checked the collinearity between the variables in each model. Variance Inflation Factors (VIF) were calculated, and values below 5 were considered as having negligible collinearity and therefore used within the same model (Daoud 2017).

Results

Obsoletus complex were the most prevalent species throughout Spain (*n* = 106, 61 sites over 6 yr) (Table 2) followed by *C. newsteadi* (*n* = 93, 50 sites over 5 yr), *C. imicola* (*n* = 92, 48 sites over 6 yr) and *C. pulicaris* (*n* = 81, 44 sites over 5 yr). The total number of *Culicoides* females captured during the National Surveillance in sites with at least 45 trapping weeks per year and no more than 3 consecutive weeks with no-trapping, was 1,317,103 individuals. Of these individuals, *C. imicola* represented 50.6% of the total captures (666,563 individuals), followed by Obsoletus complex species: 478,819 (36.3%), *C. newsteadi*: 115,783 (8.8%), and *C. pulicaris* 55,938 (4.3%). Applying the species abundance thresholds for the modeling of seasonal activity resulted in a total of 18,377 midges collected (mean 322.4 ± 602.9) for *C. imicola*; 9,757 (mean 160.0 ± 491.5) for the Obsoletus complex; 3,477 (mean 59.9 ± 84.3) for *C. newsteadi* and 1,853 (mean 46.3 ± 56.4) for *C. pulicaris* (Table 4).

The maximum nightly for each taxa catch was 39,000 individuals for *C. imicola* in Cáceres in September 2005; 14,571 individuals of Obsoletus complex in Girona (41° 59′ 00″ N; 2° 49′ 00″ E) in July 2008; 6,400 individuals of *C. newsteadi* Badajoz (38° 53′ 00″ N; 6° 58′ 00″ W) in March 2006 and 1,174 individuals of *C. pulicaris* in Toledo (39° 52′ 00″ N; 4° 02′ 00″ W) in June 2007.

Comparing Phenology Between *Culicoides* Taxa

After applying the data thresholds to the raw trapping data, the median length of the adult season was quite similar between the *Culicoides* taxa studied, with adult *C. imicola* absent during the winter for 19.25 (first Quartile [Qu.]) to 30.5 (third Qu.) weeks (M_e = 25.5 d) (Fig. 2C), *C. newsteadi* absent between 16 (first Qu.) ad 30 (third Qu.) weeks (M_e = 23), the Obsoletus complex absent 23.5 (first Qu.) to 35 wk (third Qu.) (M_e = 29) and *C. pulicaris* absent 21.5 (first Qu.) to 35.5 wk (third Qu.) (M_e = 29). Despite this, there were substantial differences in the timing of the adult season between taxa. Obsoletus complex and *C. newsteadi* appeared earlier, followed by *C. pulicaris*, then *C. imicola* (Fig. 2A). The timing of the end of the season was more variable across the sample sites and years than the start of the season. In autumn, *C. pulicaris* disappeared first, followed closely by the Obsoletus complex and *C. newsteadi*, with *C. imicola* ending approximately 4 wk later in October (Fig. 2B).
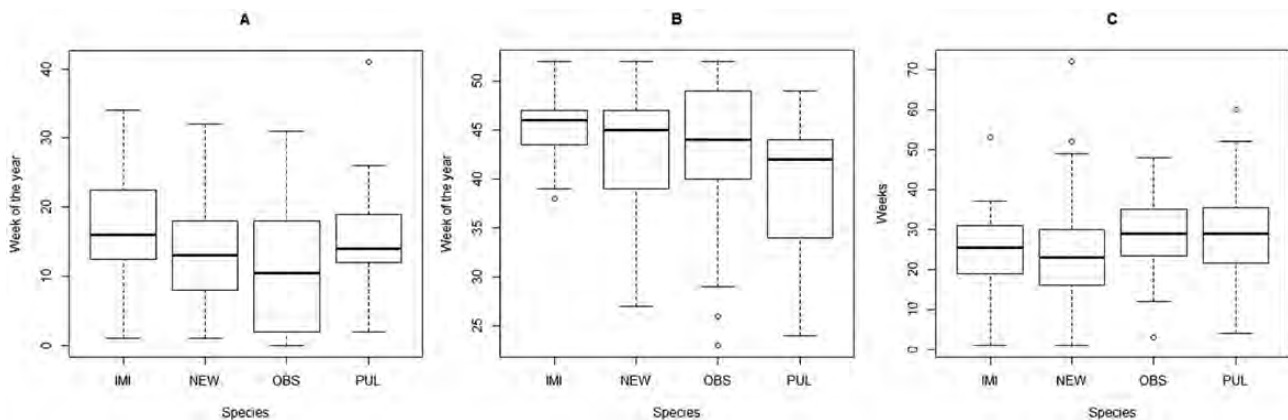
Fig. 2. Differences between species in the timing of the start (A) and end (B) of seasonal activity (weeks of the year), and length of overwinter period (C; weeks) derived from Spanish National Surveillance Program data during 2005 to 2010, after species abundance thresholds had been applied. Box plots show the median (central line), box denotes first Qu. and third Qu., error bars represent maximum and minimum, and dots are outliers. Data are shown for *C. imicola* (IMI, *n* = 57), *C. newsteadi* (NEW, *n* = 58), Obsoletus complex species (OBS, *n* = 61), and *C. pulicaris* (PUL, *n* = 40).

Table 4. Total and mean number of *Culicoides* caught by site and year used in analysis with thresholds applied for Obsoletus complex, *C. newsteadi*, and *C. pulicaris*: >5 females / trap catch and for *C. imicola* >20 females/trap catch

| Species | N | Sites | Years | Total (mean ± SD) | Mean by year | | | | | |
					2005	2006	2007	2008	2009	2010
Obsoletus complex	61	42	6	9,757 (160.0 ± 491.5)	76.1	93.2	165.3	246.5	61.7	57.8
C. imicola	57	37	6	18,377 (322.4 ± 602.9)	1,332.7	223.1	309.3	245.1	63.2	126.4
C. newsteadi	58	38	5a	3,477 (59.9 ± 84.3)	-	19.9	96.1	53.9	33.0	29.2
C. pulicaris	40	25	5a	1,853 (46.3 ± 56.4)	-	26.8	86.4	31.6	8.1	19.6

N, number of samples.
[a]No catches in 2005.

According to the median obtained across all sites, the *Culicoides* spp. appeared on average between May and July (Fig. 2A). Obsoletus complex showed the widest variability in the start of the season across all the sites, with records varying between week 2 (first Qu.) and 19 (third Qu.) (M_e = 13). This species was also the earliest to appear, followed by *C. newsteadi* (weeks 8.25–17.75, M_e = 13), *C. pulicaris* (weeks 12–19.75, M_e = 14.5) and *C. imicola* (weeks 13–23, M_e = 17). In fact, the first median appearance of the Obsoletus complex species occurred in early April, with the first records occurring in early January in several years in the Balearic Islands (39° 30′ 00″ N; 3° 00′ 00″ E), Girona, Navarra (42° 49′ 00″ N; 1° 39′ 00″ W), and Pontevedra (42° 26′ 01″ N; 8°38′51″W). Otherwise, the site recording the latest start of the Obsoletus complex season was recorded in Arcos de la Frontera (Cádiz, 36° 44′ 54″ N; 5° 48′ 23″ W) in early August of 2005. *Culicoides imicola* appeared the latest with an average median date of appearance in early July, but first records were also observed in early January (M_e = 17) in the Balearic Islands (2008), Badajoz (2007) and Málaga (36° 43′ 00″ N; 4° 25′ 00″ W) in 2005 and 2006. The latest first appearance records for this species were in late August in Málaga in 2008.

The median values obtained across all sites and years for the last *Culicoides* captures were mainly recorded between October and November (Fig. 2B). The widest variability at the end of season adult activity was shown by *C. pulicaris*, with the season ending between the week 34 (first Qu.) and 44 (third Qu.) (M_e = 42) in most sites across Spain. Activity of *C. imicola* ended later, between weeks 43 and 47 (M_e = 46). Obsoletus complex species and *C. newsteadi* had similar end of season periods ranging between week 39 (first Qu.) and 49 (third Qu.) (M_e = 43) and 39.5 (first Qu.) and 47 (third Qu.) (M_e = 45), respectively. The earliest final captures were for individuals of *C. pulicaris* recorded in Medina-Sidonia (Cádiz, 36° 27′ 28″ N; 5° 55′ 38″ W) in early June 2008, whereas *C. imicola* species were active until December in Málaga, Cádiz, Badajoz, Mallorca (39° 37′ 00″ N; 2° 59′ 00″ E) and Menorca (39° 58′ 00″ N; 4° 05′ 00″ E) in 2008.

The overwinter period of *C. newsteadi* and *C. pulicaris* were the longest, reaching 72 and 60 wk, respectively in Lugo (43° 00′ 42″ N; 7° 33′ 26″ W) in 2007 for *C. newsteadi* and Guadalajara (40° 38′ 00″ N; 3° 10′ 00″ W) on 2008 for *C. pulicaris*.

Factors Affecting the Start of Season

The best-fitting model for the start of the season for Obsoletus complex (DIC = 95.94, Supp. Table S1 [online only]) showed a significant negative effect of the number of accumulated degree days over 10°C and precipitation in spring, and the abundance of *Culicoides* females, in addition to positive significant effects of elevation above sea level and the length of the photoperiod in March (Table 5). Thus, the

Table 5. Estimated posterior mean values and credible intervals when more than 95% of posterior values are above or below zero for the coefficients for environmental fixed variables of the best Obsoletus complex model within the three different seasonal metrics

Obsoletus seasonal metric	Variable	Estimate (95% credible interval)	r^2	Appearance (%)
Start of season	DDspr	–0.37* (–0.57, –0.16)	0.93	100
	Pspr	–0.32* (–0.57, –0.06)		100
	Elev	0.27* (0.04, 0.50)		100
	Sheep	–0.18 (–0.39, 0.02)		80
	Phmarch	0.33* (0.08, 0.57)		10
	Sum females	–.29* (–0.43, –0.14)		100
End of season	DDaut	0.06 (–0.17, 0.28)	0.69	100
	(DDaut*Paut)	–0.29* (–0.56, –0.02)		100
	Elev	–0.12 (–0.38, 0.14)		90
	Cattle	0.30* (0.04, 0.57)		80
	Sum females	0.22* (0.02, 0.43)		100
Length of overwintering period	DDaut	–2.27 (–6.51, 1.59)	0.99	100
	(DDaut*Paut)	4.59 (–1.77, 10.22)		90
	Slope	1.51 (–4.40, 7.46)		70
	Phnov	5.10 (–0.16, 10.39)		10
	Sum females	–3.95 (–9.56, 1.98)		100

*Significance for environmental variables. Appearance: percentage of the best performing species models in which the significant variables appear, according to DIC.

season started earlier in sites with higher temperatures and precipitation in spring, and with higher abundance of *Culicoides* females. Moreover, the season started later when the elevation was higher and when more days in March exceeded 9 h of daylight. A second model received similar support in the data as the best model (ΔDIC < 2 Supp Table S1 [online only]), which also included landcover

of pastures and grasslands but with no significant effects. The null model with no environmental covariates received essentially no support in the data in comparison to the best model (ΔDIC 34.83, Supp Table S1 [online only]). Including random effects, the best model explained 93% of the variation in the observed data (Table 5).

Adding environmental variables to the null model for the start of season model for *C. imicola* did not result in improved support in the data (null model DIC = 128.62, Supp Table S2 [online only]); however, two models that did include environmental variables showed a significant negative effect of the number of accumulated degree days over 10°C in spring, the length of the photoperiod in April and the abundance of *Culicoides* females. These models were within 2 DIC units from the model with no covariates, suggesting some influence of these variables on this seasonal metric (Supp Table S2 [online only]). Including random effects, the best model explained 87% of the variation in the observed data (Table 6).

The best model for the start of the season for *C. newsteadi* (DIC = 122.39, Supp Table S3 [online only]) indicated a significant negative effect of the number of sheep and the abundance of *Culicoides* females (Table 7). Therefore, the season for this species started earlier in places with higher numbers of sheep and higher average annual abundance of *Culicoides* in the farm. However, four other models with similar environmental variables had

approximately similar support in the data (ΔDIC < 2; Supp Table S3 [online only]). The null model with no environmental covariates essentially received no support in the data in comparison to the best model (ΔDIC 11.62, Supp Table S3). Including random effects, the best model explained 84% of the variation in the observed data (Table 7).

The best model for *C. pulicaris* species (DIC = 88.48, Supp Table S4 [online only]) showed a significant influence of the accumulated degree days greater than 10°C during winter (Table 8), which means that for this species the season started earlier in places with higher accumulated temperatures during winter. There was also a negative interaction between accumulated degree days in winter and precipitation in winter, meaning that the negative effect of temperature leading to earlier seasonal activity was further strengthened in sites with higher precipitation. Therefore, sites with warmer and wetter winters tended to have earlier starts to seasonal activity for this species. Accumulated degree days in winter were also present and significant in five other top models that had a ΔDIC < 2 (Supp Table S4 [online only]), indicating an important influence of winter temperature on the start of the season. The null model with no environmental covariates also received some support in the data in comparison to the best model (ΔDIC 2.64, Supp Table S4 [online only]). Including random

Table 6. Estimated posterior mean values and credible intervals when more than 95% of posterior values are above or below zero for the coefficients for environmental fixed variables of the best *C. imicola* model within the three different seasonal metrics

C. imicola seasonal metric	Variable	Estimate (95% credible interval)	r^2	Appearance (%)
Start of season	No significant effects detected		0.87	-
End of season	DDsum	−0.16* (−0.30, −0.02)	0.79	90
	Paut	0.04 (−0.21, 0.30)		80
	Elev	−0.30* (−0.45, −0.15)		100
	Cattle	−0.04 (−0.19, 0.11)		50
	Sum females	0.10 (−0.03, 0.22)		100
Length of overwintering period	DDwin	4.77 (−5.35, 14.82)	0.98	100
	(DDwin*Pwin)	6.89* (2.83, 10.60)		100
	Elev	3.10 (−4.94, 10.81)		90
	Sheep	0.07 (−5.95, 5.72)		80
	BrdMix	−6.96* (−12.76, −1.21)		30
	Sum females	−6.66* (−12.27, −1.80)		100

*Significance for environmental variables. Appearance: percentage of the best performing species models in which the significant variables appear, according to DIC.

Table 7. Estimated posterior mean values and credible intervals when more than 95% of posterior values are above or below zero for the coefficients for environmental fixed variables of the best *C. newsteadi* model within the three different seasonal metrics

C. newsteadi seasonal metric	Variable	Estimate (95% credible interval)	r^2	Appearance (%)
Start of season	DDspr	−0.03 (−0.31, 0.24)	0.84	100
	(DDspr*Pspr)	−0.12 (−0.34, 0.10)		100
	Slope	0.04 (−0.24, 0.34)		90
	Sheep	−0.32* (−0.60, −0.04)		80
	NatGras	−0.16 (−0.38, 0.07)		30
	Sum Females	−0.37* (−0.60, −0.13)		100
End of season	DDaut	0.15 (−0.05, 0.35)	0.40	90
	(DDaut*Paut)	0.15 (−0.01, 0.31)		90
	Cattle	−0.15 (−0.34, 0.05)		90
	Sum females	0.08 (−0.12, 0.27)		100
Length of overwintering period	DDaut	−10.83* (−18.26, −2.25)	0.82	100
	Elev	−3.95 (−10.72, 3.58)		80
	SchVeg	−2.00 (−4.64, 0.60)		30
	Sum females	−5.12* (−7.99, −2.16)		100

*Significance for environmental variables. Appearance: percentage of the best performing species models in which the significant variables appear, according to DIC.

effects, the best model explained 41% of the variation in the observed data (Table 8).

Factors Affecting the End of Season

The best model for the end of the season for the Obsoletus complex (DIC = 136.74, Supp Table S5 [online only]) included a nonsignificant positive effect of accumulated degree days greater than 10°C in autumn coupled with a significant negative interaction between accumulated degree days greater than 10°C in autumn and precipitation in autumn, as well as significant positive effects of cattle livestock and *Culicoides* females abundance (Table 5). This suggests that the season for this species tended to end later in sites with warmer autumns, but that this effect was attenuated by interaction with precipitation in autumn, with warmer sites receiving more precipitation tending to have an earlier end to seasonal activity than warmer sites with less precipitation. The season also tended to end later in sites with a higher abundance of cattle and *Culicoides* females. Seven other models received similar support in the data, being within 2 DIC units of the top model (Supp Table S5 [online only]). The null model received essentially no support in the data in comparison to the best model (ΔDIC 14.11, Supp Table S5 [online only]). Including random effects, the best model explained 69% of the variation in the observed data (Table 5).

The *C. imicola* best model (DIC = 45.64, Supp Table S6 [online only]) for the end of the season included a significant negative effect of elevation and accumulated degree days greater than 10°C during summer (Table 6). Therefore, the season for this species tended to end earlier in sites with higher elevation and higher accumulated temperatures during summer. Several other models received similar support in the data, including a range of environmental covariates (ΔDIC < 2; Supp Table S6 [online only]). The null model received essentially no support in the data in comparison to the best model (ΔDIC 17.88, Supp Table S6 [online only]). Including random effects, the best model explained 79% of the variation in the observed data (Table 6).

The environmental variables included in the best end of the season model for *C. newsteadi* (DIC = 124.63, Supp Table S7 [online only]) were temperature in autumn, the interaction between temperature and precipitation in autumn, cattle livestock and abundance of *Culicoides* females; however, none of these effects were significant (Table 7). Six other models showed similar support in the data, being within 2 DIC units of the top model, and all included effects of autumn temperature combined with interaction with autumn precipitation, as well as the abundance of females (Supp Table S7 [online only]). The null model received very little support in the data in relation to the best model (ΔDIC 5.82, Supp Table S7 [online only]). Including random effects, the best model explained 40% of the variation in the observed data (Table 7).

The best end of the season model for *C. pulicaris* (DIC = 80.10, Supp Table S8 [online only]) included a significant negative effect of natural grassland areas (Table 8), which means that the seasons for *C. pulicaris* ended earlier in sites with higher presence of natural grassland areas. The best model received similar support in the data compared with a second model, which contained the same environmental variables as the best model excluding photoperiod in November (ΔDIC < 2; Supp Table S8 [online only]). The null model received essentially no support in the data in comparison to the best model (ΔDIC 10.4, Supp Table S8 [online only]). Including random effects, the best model explained 92% of the variation in the observed data (Table 8).

Table 8. Estimated posterior mean values and credible intervals when more than 95% of posterior values are above or below zero for the coefficients for environmental fixed variables of the best *C. pulicaris* model within the three different seasonal metrics

C. pulicaris seasonal metric	Variable	Estimate (95% credible interval)	r^2	Appearance (%)
Start of season	DDwin	−0.40* (−0.83, −0.02)	0.41	100
	(DDwin*Pwin)	−0.38 (−1.15, 0.15)		30
	Sum females	0.05 (−0.21, 0.33)		100
End of season	DDsum	−0.92 (−1.85, 0.06)	0.92	100
	DDaut	0.19 (−0.72, 1.09)		100
	Slope	−0.01 (−0.57, 0.57)		70
	Cattle	−0.42 (−0.97, 0.15)		50
	NatGras	−0.90* (−1.33, −0.43)		20
	Phnov	0.21 (−0.19, 0.60)		10
	Sum females	0.24 (−0.16, 0.59)		100
Length of overwintering period	DDaut	4.46 (−11.35, 18.26)	0.99	100
	(DDaut*Paut)	−4.56 (−10.79, 2.12)		100
	Elev	7.91 (−7.36, 22.75)		100
	Cattle	4.35 (−6.83, 15.72)		80
	AgFor	3.14 (−5.92, 12.66)		30
	Sum females	−1.13 (−6.75, 4.38)		100

*Significance for environmental variables. Appearance: percentage of the best performing species models in which the significant variables appear, according to DIC.

Factors Affecting the Length of Overwintering Period

The environmental variables included in the best length of overwinter model for the Obsoletus complex (DIC = 117.60, Supp Table S9 [online only]) were temperature in autumn, and interaction between temperature and precipitation in autumn, slope, photoperiod in November and abundance of *Culicoides* females; however, none of these effects were significant (Table 5). The null model received essentially no support in the data in comparison to the best model (ΔDIC 53.0, Supp Table S9 [online only]). Including random effects, the best model explained 99% of the variation in the observed data (Table 5).

The best length of overwinter model for *C. imicola* (DIC = 168.73, Supp Table S10 [online only]) showed a nonsignificant positive effect for accumulated degree days in winter, coupled with a significant positive effect for interaction between accumulated degree days over 10°C and precipitation in winter, and significant negative

effects of the presence of broad-leaved and mixed forest areas and *Culicoides* females abundance (Table 6). Thus, sites with higher temperatures in winter that also received more precipitation had a longer overwintering period. Conversely, sites with higher presence of Broad-leaved and mixed forest, in addition to higher abundance of *Culicoides* females, had shorter overwintering periods. The best model had similar support in the data to two other models that also included either photoperiod in November or in September (ΔDIC < 2, Supp Table S10 [online only]). The null model received essentially no support in the data in comparison to the best model (Δ DIC 54.59, Supp Table S10 [online only]). Including random effects, the best model explained 98% of the variation in the observed data (Table 6).

The best-supported length of overwinter model for *C. newsteadi* (DIC = 184.57, Supp Table S11 [online only]) showed that accumulated degree days greater than 10°C in autumn and the abundance of *Culicoides* females had significant negative effects on this seasonal metric (Table 7). Hence, sites with more accumulated days over 10°C in autumn and high abundance of *Culicoides* females showed shorter periods of inactive overwintering of *C. newsteadi* females. Three other models received similar support in the data, being within 2 DIC units of the best model (Supp Table S11 [online only]); however, the null model received essentially no support in the data compared to the best model (ΔDIC 13.49, Supp Table S11 [online only]). Including random effects, the best model explained 82% of the variation in the observed data (Table 6).

The predictors for *C. pulicaris* included in the best length of overwintering period model (DIC = 183.96, Supp Table S12 [online only]) were temperature in autumn, and interaction between autumn temperature and precipitation in autumn, elevation, cattle livestock, agro-forestry areas and abundance of *Culicoides* females; however, none of these effects were significant (Table 8). Five other models showed similar support in the data, being within 2 DIC units of the top model, all of which included effects of temperature in autumn, an interaction between autumn temperature and precipitation, elevation, abundance of cattle and abundance of *Culicoides* females (Supp Table S12 [online only]). The null model received essentially no support in the data compared to the best model (ΔDIC 20.04, Supp Table S12 [online only]). Including random effects, the best model explained 99% of the variation in the observed data (Table 8).

In summary (Table 9), Obsoletus complex and *C. imicola* were the species for which the most significant environmental effects on seasonality were detected. The abundance of *Culicoides* females had the greatest number of significant effects across the species (41.7 %). This variable lengthened the activity period of Obsoletus complex, *C. imicola*, and *C. newsteadi*. The number of accumulated degree days over 10°C in winter, the accumulated degree days over 10°C in autumn, and the elevation (16.7%) also played an important role across the species in shaping the seasonality of females.

All the species showed seasonal effects for one or more climate variables. Conversely, topography was linked to seasonal effects in the Obsoletus complex and *C. imicola*, whereas landcover variables influenced seasonal effects on *C. imicola* and *C. pulicaris*. Host densities only affected the seasonality of the Obsoletus complex and *C. newsteadi*, while photoperiod influenced the seasonality of the Obsoletus complex species.

Discussion

This is the first time that *Culicoides* adult seasonality has been quantified for these four-vector species over such a large surveillance system in Spain (6 yr) and related to such a comprehensive range of environmental drivers encompassing climate, topography, hosts, and landscape. The results obtained are useful for improving our knowledge of seasonal activity and the environmental requirements of these four species in Spain. These findings can help us to understand suitable zones and the environmental circumstances that are favorable for the activity of these vector species, which should be taken into account to mitigate against possible Bluetongue virus outbreaks.

Despite its southerly distribution in Europe, *C. imicola*, one of the major Bluetongue virus vector species in Europe, was the most abundant and frequent species collected during the Spanish National Surveillance Program, which agrees with results

Table 9. Summary of favorable and unfavorable environmental conditions for the activity of each species and seasonal metric color-coded

Species	Seasonal metric	DDwin	DDspr	DDsum	DDaut	Pwin	Pspr	Paut	Elev	Cattle	Sheep	BrdMix	NatGras	Phmarch	Sum fem
Obsoletus complex	Start		■				■		▨		░			▨	■
	End				▨				▨	■					■
	OW			░	░										░
C. imicola	Start														
	End			▨					▨						■
	OW	▨			░	▨						■			■
C. newsteadi	Start			░			░			░	░	■	■	░	■
	End				░				░						
	OW				■					░					■
C. pulicaris	Start	■													
	End			░	░					░			▨		
	OW														
	App.	16.7 %	8.3 %	8.3 %	16.7 %	8.3 %	8.3 %	8.3 %	16.7 %	8.3 %	8.3 %	8.3 %	8.3 %	8.3 %	41.7 %

Full black: significant favorable effect, black lines: significant unfavorable effect, grey: variable retained in the model but with no significant effect. Appearance (App.), Percentage of seasonal metrics in which the variable was significant across all the species. Elev, Elevation; DDwin/spr/sum/aut, Accumulated degree days over 10°C in winter, spring, autumn, and summer; Pwin/spr/aut, Precipitation in winter, spring, and autumn; BrdMix, Broad-leaved forest and mixed forest; NatGras, Natural grassland zones; Phmarch, Photoperiod in March; Sum fem, *Culicoides* female abundance.

recorded by Calvete et al. (2008, 2009a,b), Talavera et al. (2015), and Cuéllar et al. (2018b) using the same data. The maximum number of individuals collected of *C. imicola* was in Cáceres, for *C. newsteadi* in Badajoz, for the Obsoletus complex in Girona, and for *C. pulicaris* in Toledo—demonstrating that the Obsoletus complex were dominant in northern sites, while *C. newsteadi* and *C. imicola* seemed to be more abundant in central and southern parts of mainland Spain.

The length of the adult activity periods differed across the four species depending on location. Temperature, which is a key factor for insects development and activity (Alekseev et al. 2007), played an important role in shaping seasonal activity in all four vector species and should be taken into account for the SVPF because temperature also impacts vector behavior (EFSA 2017). In fact, temperature, measured as accumulated degree days during winter and autumn, was an important explanatory variable appearing in 16.7% of all the best performing models, demonstrating the importance of temperature during lower activity seasons, affecting the development, survival and later seasonal appearance of these insects (Sanders et al. 2011, Burgin et al. 2013, White et al. 2017).

The longest *Culicoides* adult activity periods were in southern sites of the Iberian Peninsula and the Mediterranean, due to these regions' high temperatures throughout the year. For example, previous studies have shown *C. imicola* had longer activity periods in sites with high humidity (Rawlings and Mellor 1994) such as coastal zones (Málaga, Cádiz, Balearic Islands) supporting the study of Conte et al. (2007), Talavera et al. (2015), Cuéllar et al. (2018b), and Barceló et al. (2020). Therefore, the transmission season will be longer in these sites because the seasonal activity of this species overlaps with that of Palearctic species (e.g., Obsoletus complex), resulting in multi-vector assemblages in this region, thereby extending vector seasonal activity over longer periods of the year.

Sites in the North (Pontevedra and Navarra) had longer activity periods for the Obsoletus complex species, while *C. newsteadi* and *C. pulicaris* recorded shorter activity periods in northern sites such as Lugo and Guadalajara. These results should have a bearing on evaluating the SVFP in these regions, allowing a more nuanced assessment of transmission risk.

Northern Species

Obsoletus complex activity started earlier in sites with greater accumulated degree days over 10°C in spring and higher spring precipitation and ended later in areas where the weak effect of autumn temperature on elongating the end of seasonal activity was amplified by lower autumn precipitation. These findings are consistent with previous studies that demonstrated the Obsoletus complex species had a low optimum temperature for the adult activity of 14.2°C in the field appearing earlier in the year with seasonal peaks between May and July in Spain and Italy (Miranda et al. 2004, Conte et al. 2007). However, in contrast to our findings, other studies have demonstrated low adult recordings of this species during autumn (Miranda et al. 2004, Conte et al. 2007, Talavera et al. 2015, Barceló et al. 2020). This phenomenon could be linked with the precipitation during this season. Our results demonstrated that precipitation combined with precipitation in autumn could extend the activity period of the Obsoletus complex species. Furthermore, despite Obsoletus complex previously being found to be more abundant in arid sites (Conte et al. 2007); our results demonstrated that precipitation in spring increased the activity period of this species, probably related to the increase of availability of breeding sites. *Culicoides* females abundance also had an important positive effect on the abundance

of Obsoletus complex as well as the photoperiod. Effects of the latter were nonsignificant in other studies of the Obsoletus complex species in laboratory conditions (Lühken et al. 2015) or in-field data in the United Kingdom (Searle et al. 2014), but were not supported in the present study. These disparities could be related to the lower latitudinal range used in Searle et al. (2014) that spanned less variability in photoperiod than the present study. The presence of multiple species within the Obsoletus complex group with different ecologies and phenologies must be considered when interpreting the results. In fact, studies in Italy found that *C. scoticus* abundance was higher between winter and spring, while *C. obsoletus* was more abundant in summer (Pili et al. 2010).

The topography, namely high elevation, had significant negative effects on adult seasonal activity for many of the species included in this study. In the case of the Obsoletus complex, elevation played an important role for this species showing longer activity periods in lower elevation sites in contrast to other findings in Italy where Obsoletus complex species were favored by high slope and elevation (Capela et al. 2003, Torina et al. 2004, Conte et al. 2007). These results could be related to the host distribution in Spain because cattle density was another influential factor that increased the length of the activity season. Obsoletus complex species are generally associated with livestock farms and breed upon old and fresh mature (Takken et al. 2008, González et al. 2013b, Talavera et al. 2015); thus this species might be more abundant in sites with cattle livestock, which tend to be at lower elevation.

The species with the longest overwintering period in Spain was *C. pulicaris*. This result differed from the long activity periods recorded by Sanders et al. (2011) for this species in the United Kingdom. It is a widespread species in Spain with a similar distribution to the Obsoletus complex; therefore, this species could adapt to a wide range of eco-climatic circumstances (Ortega et al. 1998, Ducheyne et al. 2013). Our results only demonstrated significant effects for two environmental variables on the seasonal activity of this species, and, in agreement with the results obtained by Purse et al. (2012), the environmental variables did not explain much variance in population parameters for *C. pulicaris*. The adult activity of this species was closely related to temperature (Purse et al. 2004, Sanders et al. 2011, Searle et al. 2012), accumulated degree days over 10°C during winter showed a significant increase in the length of adult activity of *C. pulicaris*. Natural grassland zones showed an unfavorable effect on the seasonality of this species, probably related to its breeding sites and distribution. Cattle usually graze on grassland areas; however, it was aforementioned that *C. pulicaris* is not related to livestock farms (Harrup et al. 2013, 2016). Other unmeasured environmental factors such as remotely sensed climatic variables (e.g., land surface temperature (LST) or enhanced vegetation index (EVI)) (Purse et al. 2004a,b, 2012) should be considered in further studies as drivers of the phenology and abundance of this species.

South-Western Species

Topography and climate were important variables shaping the abundance and seasonality of *C. imicola*, which agrees with the results obtained by Acevedo et al. (2010) that showed that *C. imicola* was favored by hot days in lower altitude sites and extensive plains (also shown by that of Conte et al. (2003, 2007) and Torina et al. (2004) in Italy). Otherwise, the overwinter period of *C. imicola* was longer in sites with higher temperatures and rainfall during winter, probably related to the fact that immature stages prefer to breed in areas that are not waterlogged (Conte et al. 2007, Purse et al. 2007, Foxi and Delrio 2010, Ramilo et al. 2017). *Culicoides imicola* was

the species that appeared as adult latest during the year, with seasonal peaks during its optimum temperature between August and October when the land surface temperature remains high (Ortega et al. 1999, Miranda et al. 2004, Purse et al. 2004, Talavera et al. 2015, Grimaud et al. 2018). Conversely, this species showed longer activity periods in sites with lower temperatures in summer, probably linked to higher insect mortalities in zones included in the distribution area of this species, with temperatures reaching the 40°C in summer (AEMET 2019).

While *C. newsteadi* has previously demonstrated similar distribution patterns to *C. imicola* (Ducheyne et al. 2013, Ramilo et al. 2017), the present study has shown that these species were affected by four environmental factors. As well as the Obsoletus complex, the temperature during autumn also had a significant impact on this species showing high activity in sites with high minimum temperatures (Ortega et al. 1999, Purse et al. 2004).

Culicoides female abundance and hosts were also important factors for the activity of *C. newsteadi*. This result is supported by prior studies documenting that this species prefers sheep as a host (Garros et al. 2011, Calvo et al. 2012, Martínez-de la Puente et al. 2015, Slama et al. 2015). In fact, early stages of this species have been recorded in sheep livestock holdings (Foxi and Delrio 2010, González et al. 2013b).

This study has demonstrated important eco-climatic and seasonal differences for these four major Bluetongue virus vector species present in Spain, as well as a contribution to understand the seasonal activity and the biology of these species. Temperature, topography, and *Culicoides* abundance were the elements that most strongly affected the seasonal adult activity of these vector insects and will, in turn, determine periods of risk of transmission of Bluetongue virus as well as the SVFP of these species. These parameters are of capital importance to understand the possible geographical scenarios of Bluetongue virus risk of transmission in Spain, and furthermore, to establish a data baseline to detect changes due to current climate change. Further empirical fieldwork is needed to refine these adult seasonality-environment relationships, and to understand how these interface with species vector competence (Barber et al. 2018) and other seasonal aspects of vectorial capacity (Barceló et al. 2020), before specific recommendations can be made about whether the seasonal risk period for transmission will be longer in some years and some places than others.

The gonotrophic stage of *Culicoides* females must be considered as a variable in further studies because it is an important key for Bluetongue virus transmission. *Culicoides* parous females are the fraction of the population that may transmit the virus; in fact, surveillance programs often identify the individuals within gonotrophic stages (Barceló et al. 2020).

In addition to such life-history parameters, other variables that influence the availability of midge habitat may improve our understanding of how seasonality and abundance are modulated by climate and habitat factors (Hartemink et al. 2015). Other variables like the seasonal water availability metrics that have recently been of value for mosquitoes (Sanz-Aguilar et al. 2018), should be considered for further studies on *Culicoides* activity. Prior studies have also found remotely sensed variables to be valuable in this respect such as the aforementioned LST, EVI (Purse et al. 2012) or also other remotely sensed imagery data, e.g., the normalized difference vegetation index (NDVI); the middle infra-red reflectance of the land cover (MIR) and the air temperature a few meters above ground (TAIR) (Purse et al. 2004a,b). Therefore, areas with suitable environmental circumstances for each particular species should be contemplated in future surveillance of *Culicoides* species in Spain to detect and prevent possible infections of *Culicoides*-borne viruses.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

Table S1. Top 10 models for Obsoletus complex start of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S2. Top 10 models for *C. imicola* start of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 20 individuals per trap.

Table S3. Top 10 models for *C. newsteadi* start of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S4. Top 10 models for *C. pulicaris* start of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S5. Top 10 models for Obsoletus complex end of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S6. Top 10 models for *C. imicola* end of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 20 individuals per trap.

Table S7. Top 10 models for *C. newsteadi* end of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S8. Top 10 models for *C. pulicaris* end of the season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S9. Top 10 models for Obsoletus complex overwintering (OW) season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S10. Top 10 models for *C. imicola* overwintering (OW) season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 20 individuals per trap.

Table S11. Top 10 models for *C. newsteadi* overwintering (OW) season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

Table S12. Top 10 models for *C. pulicaris* overwintering (OW) season (best model in gray) including the null model. a= intercept. pD: Effective number of parameters in each model. DIC: Deviance information criterion. Δ DIC: Difference with the best model. n= Number of observations with > 5 individuals per trap.

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