Received: 8 February 2024 | Accepted: 7 August 2024

DOI: 10.1111/mve.12756

ORIGINAL ARTICLE

Medical and Veterinary Entomology

Winter activity of *Culicoides* (Diptera: Ceratopogonidae) inside and outside stables in Germany

Sarah Groschupp[1] | **Helge Kampen**[2] | **Doreen Werner**[1]

[1]Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany

[2]Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Infectology, Greifswald, Germany

Correspondence
Sarah Groschupp, Leibniz Centre for Agricultural Landscape Research, Eberswalder Straße 84, 15374 Müncheberg, Germany.
Email: sarah.groschupp@zalf.de

Funding information
Federal Office for Agriculture and Food; Federal Ministry of Food and Agriculture, Grant/Award Numbers: 281B101816, 281B101916

Associate Editor: Marion England

Abstract

Culicoides biting midge species (Diptera: Ceratopogonidae) of the Obsoletus Group and the Pulicaris Complex are considered the major vectors of bluetongue and Schmallenberg viruses in Europe. Overwintering strategies of these arboviruses are controversially discussed, with the ongoing activity of vector species and a non-disrupted transmission cycle during winter being a plausible explanation. Although data on *Culicoides* winter activity are relatively scant, a seasonal vector-free period (SVFP), during which adult *Culicoides* are not or hardly active, is questionable. To determine winter activity and define SVFPs according to the EU Commission Regulation No 1266/2007, adult *Culicoides* were trapped weekly with UV-light traps from October to April 2019/2020 and 2020/2021 inside and outside stables on 16 farms throughout Germany. Temperature measurements were taken regularly at each trapping site since the temperature is a known driver of biting midge activity. In 960 indoor and outdoor catches, 32,377 *Culicoides* were trapped, with 90.9% of them belonging to the Obsoletus Group, 6.1% to the Pulicaris Complex and 3.0% to 'other *Culicoides*' according to morphological identification. The majority (61.3%) of *Culicoides* were trapped indoors, with substantial numbers of specimens collected from October to December, in March and in April, and only a few or no specimens in January and February. Obsoletus Group biting midges were active indoors for almost the entire winter. Outdoors, *Culicoides* numbers decreased from October to December, few or no specimens were caught from January to March, and high numbers were captured in April. Of the collected *Culicoides*, 2028 were blood-fed, of which 94.6% were trapped in the stables. The indoor SVFP, although calculated for blood-fed instead of parous females, lasted for almost 4 months (late November until mid-March) in winter 2019/2020 and 2 months (January and February) in winter 2020/2021. The outdoor SVFPs covered almost the entire study period in both winters, with slight differences between the onsets and the ends. The *Culicoides* activity significantly depended on temperature. Specimens of the Obsoletus Group were caught at an average temperature of 7.4°C (minimum 0.3°C) and of the Pulicaris Complex at an average temperature of 10.3°C (minimum 1.2°C). These temperatures were reached inside the stables over more extended periods than outside. The average indoor temperatures were 1.2 K higher than the average outdoor temperatures, although absolute temperature differences of up to 9.0 K were recorded. Based on *Culicoides* activity, the results of the present study

indicate an almost continuous potential for virus transmission in winter within livestock houses.

KEYWORDS
biting midges, bluetongue virus, Obsoletus Group, overwintering, Pulicaris Complex, Schmallenberg virus, vector-free period

INTRODUCTION

In Europe, biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae) are vectors of arboviruses pathogenic to domesticated and wild ruminants, such as bluetongue virus (BTV), Schmallenberg virus (SBV) and the recently emerged epizootic haemorrhagic disease virus (Kampen & Werner, 2023).

BTV (family Sedoreoviridae, genus *Orbivirus*) was first described in South Africa in the 18th century and has since spread worldwide (Gerdes, 2004). In the 1950s, bluetongue disease broke out on the Iberian Peninsula and was later also reported from other European areas bordering the Mediterranean (Wilson & Mellor, 2009). Only since the 1990s, disease cases have been diagnosed in Europe north of 40° N latitude (Wilson & Mellor, 2009). The northward spread became evident again when a large-scale epidemic occurred in central and northern Europe from 2006 to 2009 (Wilson & Mellor, 2009). This outbreak caused considerable harm to animals and had an enormous economic impact on the livestock industry in the affected countries due to the management of infected animals, trade restrictions and vaccination campaigns (Gethmann et al., 2020; Pinior et al., 2015; Velthuis et al., 2010).

In 2011, SBV (family Peribunyaviridae, genus *Orthobunyavirus*) was detected for the first time in Germany and the Netherlands (Beer & Wernike, 2021; Hoffmann et al., 2012). Within 2 years, it spread across numerous European countries (De Regge, 2017). Unlike BTV, SBV did not disappear entirely, and new cases are reported annually (Wernike & Beer, 2020).

During the European bluetongue epidemic from 2006 to 2009, it became obvious that native *Culicoides* species were vector-competent (Kampen & Werner, 2023). Various studies suggested *Culicoides obsoletus* (Meigen, 1818), *Culicoides scoticus* Downes and Kettle, 1952, *Culicoides chiopterus* (Meigen, 1830) and *Culicoides dewulfi* Goetghebuer, 1935, of the Obsoletus Group as well as *Culicoides pulicaris* (Linnaeus, 1758) and *Culicoides punctatus* (Meigen, 1804) of the Pulicaris Complex to be the most abundant livestock-associated species and major potential viral vectors in Europe north of the Mediterranean basin (Caracappa et al., 2003; De Regge et al., 2012, 2015; Hoffmann et al., 2009; Larska et al., 2013; Mehlhorn et al., 2007; Meiswinkel et al., 2007; Rasmussen et al., 2012; Saegerman et al., 2008; Savini et al., 2005).

Following the BTV outbreak in Europe in 2006, the European Union launched a surveillance programme to gain more knowledge about the occurrence and ecology of *Culicoides* species (Commission Regulation (EC) No 1266/2007). In addition, various regulations were introduced to prevent the virus from spreading, including restrictions of movement and trade in ruminants and their products. These strict control measures are subject to reduction or suspension during a seasonal vector-free period (SVFP) (Brugger et al., 2016; Carpenter et al., 2009) when vectors are considered inactive or active at a very low level. According to the Commission Regulation, the SVFP is defined as follows: *Culicoides* imicola Kieffer, 1913, must be completely absent and less than five parous *Culicoides* specimens be captured per trap, although no details are provided on the number and type of traps to be used and about the places where the trap(s) should be operated. This definition applies in the absence of sound evidence for establishing another threshold that can be used to determine the SVFP.

It has been hypothesised that BTV cannot be passed from one vector season to the next in central and northern Europe because winter conditions, such as low temperatures, are unfavourable for the vectors, and virus replication and transmission come to a standstill (Takamatsu et al., 2003; Wilson et al., 2008). Epidemiologic data, however, reveal that virus transmission cycles continue early in a year, without evidence of new introduction, at places where the virus circulated in the previous year (Collins et al., 2016; Hoffmann et al., 2008; Wernike et al., 2013; Wilson et al., 2007). These observations suggest that the virus can persist locally during winter. While studies concluded that transovarial transmission of BTV and other orbiviruses does not occur in *Culicoides* (Mellor et al., 2000; Nunamaker et al., 1990), several possible overwintering mechanisms have been proposed. For example, the virus might latently persist in the host population longer than the maximum duration of detectable viremia or be maintained by an alternative transmission cycle involving one or more unknown host populations or vector species/groups (Takamatsu et al., 2003; Wilson et al., 2008). Furthermore, infected but inactive adult *Culicoides* may survive the winter. While the virus does not replicate in infected biting midges at low temperatures as found in winter, it may continue replication to transmission level as soon as temperatures rise again (Mullens et al., 1995; Paweska et al., 2002; Wellby et al., 1996). The threshold temperature for orbivirus replication in *Culicoides* is about 11–13°C (Carpenter et al., 2011), such as found in spring. It has also been shown that calves and lambs from infected mothers became infected intauterinally and were born viraemic. Transplacental virus transmission in domestic animals is therefore another way for the virus to overwinter (Menzies et al., 2008; Rasmussen et al., 2013; Van der Sluijs et al., 2013). Another possibility could be the continuous virus circulation between active midges and susceptible livestock during winter, facilitated by suitable climatic conditions (Clausen et al., 2009; Hoffmann et al., 2008; Magliano et al., 2018; Takamatsu et al., 2003; Wernike et al., 2013). SBV was

demonstrated in nulliparous *Culicoides* adults suggesting transovarial transmission (Larska et al., 2013), although this finding still awaits confirmation. As with BTV, it is more likely that SBV persists in livestock or in adult *Culicoides* that survive the winter (De Regge, 2017; Doceul et al., 2013). This hypothesis is supported by studies in which BTV and SBV were detected in blood samples from livestock and in active *Culicoides* caught during wintertime (Baldet et al., 2008; Clausen et al., 2009; Hoffmann et al., 2008; Kiel et al., 2009; Losson et al., 2007; Vorsprach et al., 2009; Wernike et al., 2013). Additional PCR-confirmed BTV infections were diagnosed in the winters of 2018/2019, 2019/2020 and 2020/2021 in Central Europe (Belgium, Germany, Switzerland) (WOAH, 2024).

Based on these findings, virus overwintering in *Culicoides* seems possible, and it was pointed out that the concept of an SVFP should be reconsidered as such a period might not exist (Clausen et al., 2009; Hoffmann et al., 2008; Mehlhorn et al., 2009; Meiswinkel, Baldet, et al., 2008). Consequently, more data are necessary to assess whether biting midges are active during winter to maintain the transmission cycle and to investigate whether an SVFP exists. Hence, *Culicoides* collections were carried out indoors and outdoors of livestock stables in Germany during the winters of 2019/2020 and 2020/2021. The study focused on the Obsoletus Group and the Pulicaris Complex, which contain the major putative BTV and SBV vector species. Furthermore, the minimum temperature required for the flight activity of *Culicoides* was determined. Special attention was given to evidence of winter blood-feeding by *Culicoides* and temperature differences inside and outside the stables.

MATERIALS AND METHODS

Collection sites, trapping and recording of temperature

Field collections of *Culicoides* biting midges were conducted on 14 livestock farms holding ruminants (sheep, goat or cattle) and two horse farms in the German federal states of Baden-Wuerttemberg, Brandenburg, Lower Saxony, North Rhine-Westphalia and Thuringia (Figure 1 and Table 1).

Biting midges were collected during the winters of 2019/2020 and 2020/2021 from October to April using 12 V-ultraviolet(UV)-light traps (Biogents, Regensburg, Germany). Attracted insects were aspirated by the ventilator of the trap and collected in a beaker filled with approx. 200 mL ethanol (75% v/v). After removal from the trap, the samples were stored in the collection medium in a dark environment until morphological identification. On each farm, one UV-light trap was operated indoors and one outdoors. Traps were hung at about 1.5–2-m height (top edge) in the immediate vicinity of the animals (<10 m) inside the stables and close to the stables (<50 m) outside. If trapping was carried out on the same farm in both winters, traps were placed at the very same positions. The two traps on each farm were operated simultaneously once a week for 24 h.

During the trapping periods, livestock was mainly kept in the stables on all farms. Constructional openings (doors and windows) of the livestock buildings were closed in late autumn as soon as outdoor temperatures became chilly and cold wind began to enter the stables. Except for doors being opened several times a day to supply the animals with food, routes of entry into the stables for biting midges were limited to cracks between doors and walls or other crevices.

To record the temperatures on an hourly basis, data loggers (EBI 20-TH1; Xylem Analytics, Ingolstadt, Germany) were installed directly on each UV-light trap during exposure.

Morphological biting midge identification

Collected *Culicoides* were separated from other insects and morphologically identified under a stereo-microscope (M205 C; Leica Microsystems GmbH, Wetzlar, Germany) according to the identification keys of Delécolle (1985) and Mathieu et al. (2012). Due to isomorphism of the females of some species (e.g., *C. obsoletus* and *C. scoticus*), specimens were not identified to species level but were assigned to the following species groups or complexes: Obsoletus Group (including *Culicoides obsoletus* s.s., *Culicoides scoticus*, *Culicoides dewulfi*, and *Culicoides chiopterus*), Pulicaris Complex (including *Culicoides pulicaris* s.s., *Culicoides punctatus*, *Culicoides impunctatus* Goetghebuer 1920, *Culicoides newsteadi* Austen, 1921, and *Culicoides lupicaris* Downes and Kettle, 1952) and 'other *Culicoides*' (all other *Culicoides* species). The determined *Culicoides* species were subdivided into male and female specimens. Female *Culicoides* were further classified as freshly blood-fed (engorged) and non-blood-fed.

Data analyses

To compare the number of *Culicoides* collected indoors and outdoors, the average numbers of specimens per location were calculated as absolute number of specimens per location over the total number of captures per location. In this way, variations in the number of samples taken indoors and outdoors or at different sites were taken into account. In order to determine and compare the monthly numbers of *Culicoides* collected indoors and outdoors, the numbers of specimens were converted into average numbers of specimens per month: absolute numbers of *Culicoides* specimens collected at the respective locations per month in relation to the total number of captures at the respective location per month. Both calculations were additionally done for blood-fed females at each location and every month.

The SVFPs were determined for each trapping location indoors and outdoors for both winters, based on the finding of active culicoids as required by Annex V of the Commission Regulation (EC) No 1266/2007, although blood-fed instead of parous females were considered (c.f. discussion).

A Poisson generalised linear model (GLM) was applied to determine if the abundance of all *Culicoides* and the three distinct *Culicoides* species groups (Obsoletus Group, Pulicaris Complex, 'other *Culicoides*') were temperature-dependent. The response variable in the

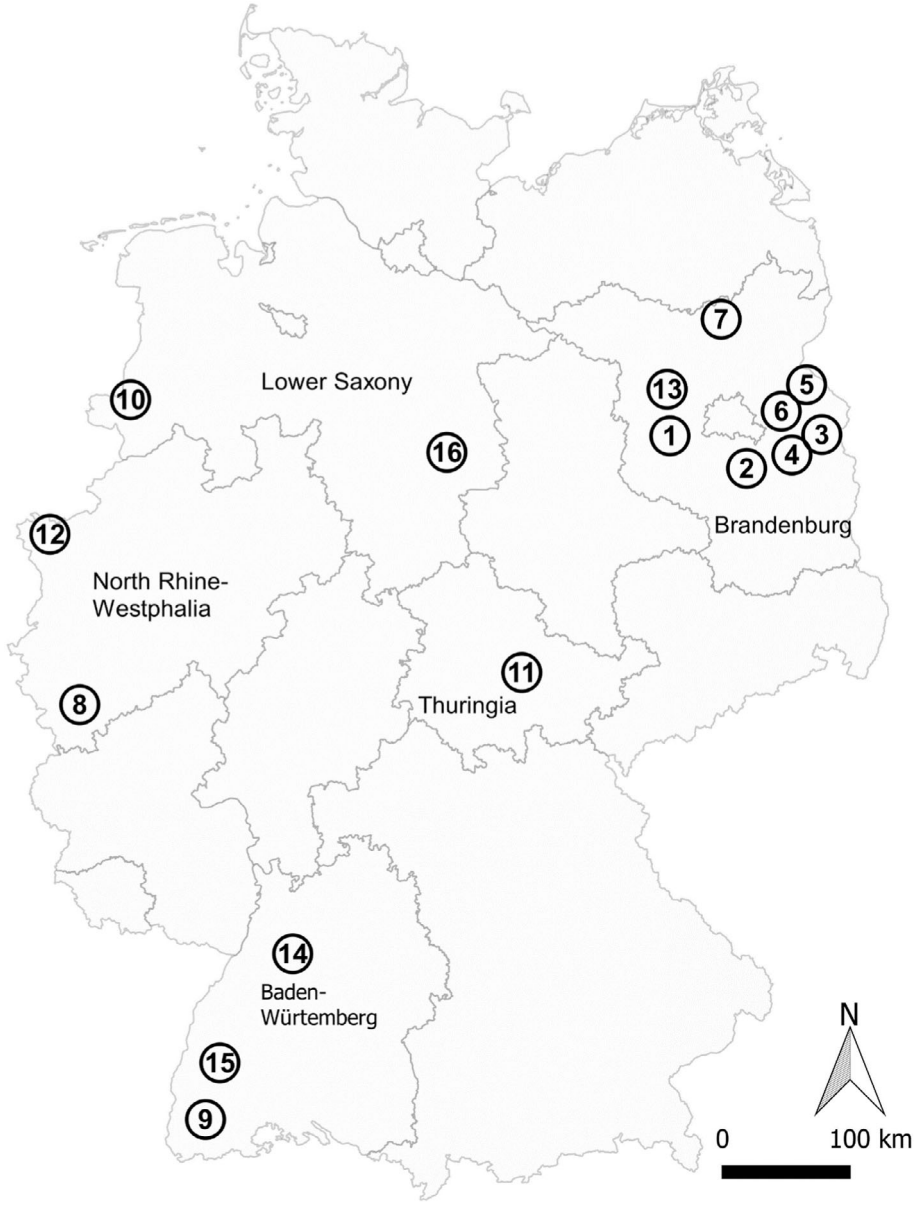

FIGURE 1 Map showing the locations of the study farms in Germany where *Culicoides* biting midges were collected by UV-light traps in the winters 2019/2020 and 2020/2021. The numbers correspond to the farm IDs according to Table 1.

model was the number of counted *Culicoides* (per group), and the predictor variable was the average temperature of the trapping day.

To calculate the lowest temperature at which Obsoletus Group and Pulicaris Complex specimens were active, the average temperature of a 7-day period before each trapping day was determined (t_7). To gain more precise information about the temperature requirement of Obsoletus Group and Pulicaris Complex activity, the same calculations were performed with the lowest temperatures of the first day of capture (t_1).

Measured indoor and outdoor temperatures were checked for significant differences. For this purpose, the average, median, minimum and maximum temperatures per calendar week were calculated separately for indoors and outdoors.

The raw data were managed in Microsoft Excel 2013 (Microsoft, Readmond, WA, USA) and analysed in R version 4.1.3 (R Core Team, 2022) using the packages readxl (Wickham & Bryan, 2019) and tidyverse (Wickham et al., 2019). For all statistical analyses, a *p*-value <0.05 was considered significant.

RESULTS

Within the two study periods, 32,377 *Culicoides* were caught in 960 trap collections (480 both indoors and outdoors, respectively). Biting midges of the Obsoletus Group were most abundant ($n = 29{,}429$; 90.9%), followed by specimens of the Pulicaris Complex

TABLE 1 Characteristics of the study farms where UV-light traps for the collection of *Culicoides* were operated inside and outside stables.

			Winter 2019/2020		Winter 2020/2021		
Farm ID	Study location	Type of livestock	Oct–Dec	Jan–Apr	Oct–Dec	Jan–Apr	Total number of indoor/outdoor collections
1	Groß Kreutz	Sheep	12/12				12/12
2	Heidesee	Horse	12/12				12/12
3	Platkow	Cattle	7/7				7/7
4	Beerfelde	Cattle	11/11	16/16			27/27
5	Quappendorf	Sheep	13/13	16/16			29/29
6	Dahmsdorf	Horse	8/8	10/10	6/6	10/10	34/34
7	Lychen	Goat	13/13	18/18	12/12	17/17	60/60
8	Zülpich	Cattle	14/14	17/17	12/12	13/13	56/56
9	Oberried	Cattle		16/16			16/16
10	Geeste	Cattle		6/6	10/10		16/16
11	Kiliansroda	Cattle		12/12	13/13	13/13	38/38
12	Kleve	Cattle		11/11	12/12	16/16	39/39
13	Paulinenaue	Sheep		17/17	10/10	14/14	41/41
14	Schmie	Cattle		15/15	13/13	16/16	44/44
15	Mühlenbach	Cattle			13/13	17/17	30/30
16	Braunschweig	Cattle			7/7	12/12	19/19
	Total number of samples		90/90	154/154	108/108	128/128	480/480

Note: Numbers of indoor/outdoor collections are specified per period and farm. Farm IDs correspond to the locations as indicated on the map in Figure 1.

($n = 1962$; 6.1%) and of 'other *Culicoides*' ($n = 986$; 3.0%) (Table 2). More than half, 61.3% ($n = 19{,}831$), of all *Culicoides* were caught inside the stables, whereas 38.7% ($n = 12{,}546$) were collected outdoors (Table 2). The ratio between the number of specimens collected indoors and outdoors was 1.7:1 for the Obsoletus Group, 1:1.1 for the Pulicaris Complex and 1:3.4 for 'other *Culicoides*'. Almost all caught *Culicoides* (98.6%) were female. Of the Obsoletus Group females, 6.7%, and of the Pulicaris Complex females, 0.2% were blood-fed. Of these, 94.8% and 88.5%, respectively, were caught indoors.

The number of *Culicoides* specimens caught per location varied between calendar weeks and winters (Figure 2). Specimens were continuously trapped indoors during all study months and both winters, with relatively high numbers from October to December and in March and April. In January and February, numbers of *Culicoides* collected inside the stables were much lower, but only in a few calendar weeks no *Culicoides* were caught (Figure 2 and Table 3). Numbers of individuals caught outdoors gradually decreased from October to December in both winters, followed by no to low numbers of *Culicoides* at any location in January and February. Relative high numbers of *Culicoides* were trapped outdoors in March and April (Figure 2 and Table 3).

When species groups are considered separately, differences became obvious. Representatives of the Obsoletus Group were trapped inside and outside the stables almost continuously throughout both study periods (Figure 3). By contrast, specimens of the Pulicaris Complex were caught indoors until the beginning of December (49th calendar week) and—after a break of several weeks (except for two individuals in the 1st calendar week of January 2020)—again in April in 2020 and the last week of February in 2021, respectively. Outdoors, specimens of this complex were caught until mid-November (46th calendar week) and again early April in 2020 and early March in 2021 (Figure 3).

Even though more *Culicoides* were caught outdoors, blood-fed specimens were mostly caught indoors (Table S1). While the latter happened only occasionally or not at all in January and February, up to more than 100 blood-fed specimens were collected in all other study months (Table 3 and Figure 4). Outdoors, blood-fed *Culicoides* were mainly captured in October and April, whereas only sporadic or no blood-fed specimens were caught in the other study months of both winters (Table 3 and Figure 4). Only at one farm (Paulinenaue, farm 13), no blood-fed *Culicoides* were caught inside or outside (Table S1).

Significant differences exist between the numbers of blood-fed specimens of the Obsoletus Group and the Pulicaris Complex collected inside and outside the stables regarding the periods in which they were trapped. Inside, no blood-fed specimens of the Obsoletus Group were caught in February only, while blood-fed Pulicaris Complex specimens were not caught for 5 months in either winter (November to March in 2019/2020 and December to April in 2020/2021). Outside the stables, blood-fed specimens of both groups were captured only occasionally (Figure 3).

Based on the indoor catches, an SVFP should have lasted for almost 4 months (late November to mid-March) in the winter 2019/2020 and 2 months (January and February) in the winter 2020/2021 (Figures 3 and 4). Outdoors, the SVFP would have comprised almost the whole study period in both winters, with slight differences between the two winters (Figures 3 and 4).

TABLE 2 Numbers of *Culicoides* and of blood-fed females in 480 indoor and outdoor UV-light trap catches, respectively, carried out in the winters of 2019/2020 and 2020/2021 (percentages relative to total number of collected biting midges).

Culicoides species group	Indoors			Outdoors			Total indoors and outdoors
	Non-blood fed	Blood-fed	Total	Non-blood-fed	Blood-fed	Total	
Obsoletus Group	16,814 (51.9%)	1868 (5.8%)	18,682 (57.7%)	10,644 (32.9%)	103 (0.3%)	10,747 (33.2%)	29,429 (90.9%)
Pulicaris Complex	878 (2.7%)	46 (0.2%)	924 (2.9%)	1033 (3.2%)	6 (<0.05%)	1038 (3.2%)	1962 (6.1%)
Other *Culicoides*	221 (0.7%)	4 (<0.05%)	225 (0.7%)	759 (2.3%)	2 (<0.05%)	761 (2.3%)	986 (3.0%)
Total	17,913 (55.3%)	1918 (6.0%)	19,831 (61.3%)	12,436 (38.4%)	110 (0.3%)	12,546 (38.7%)	32,377 (100%)

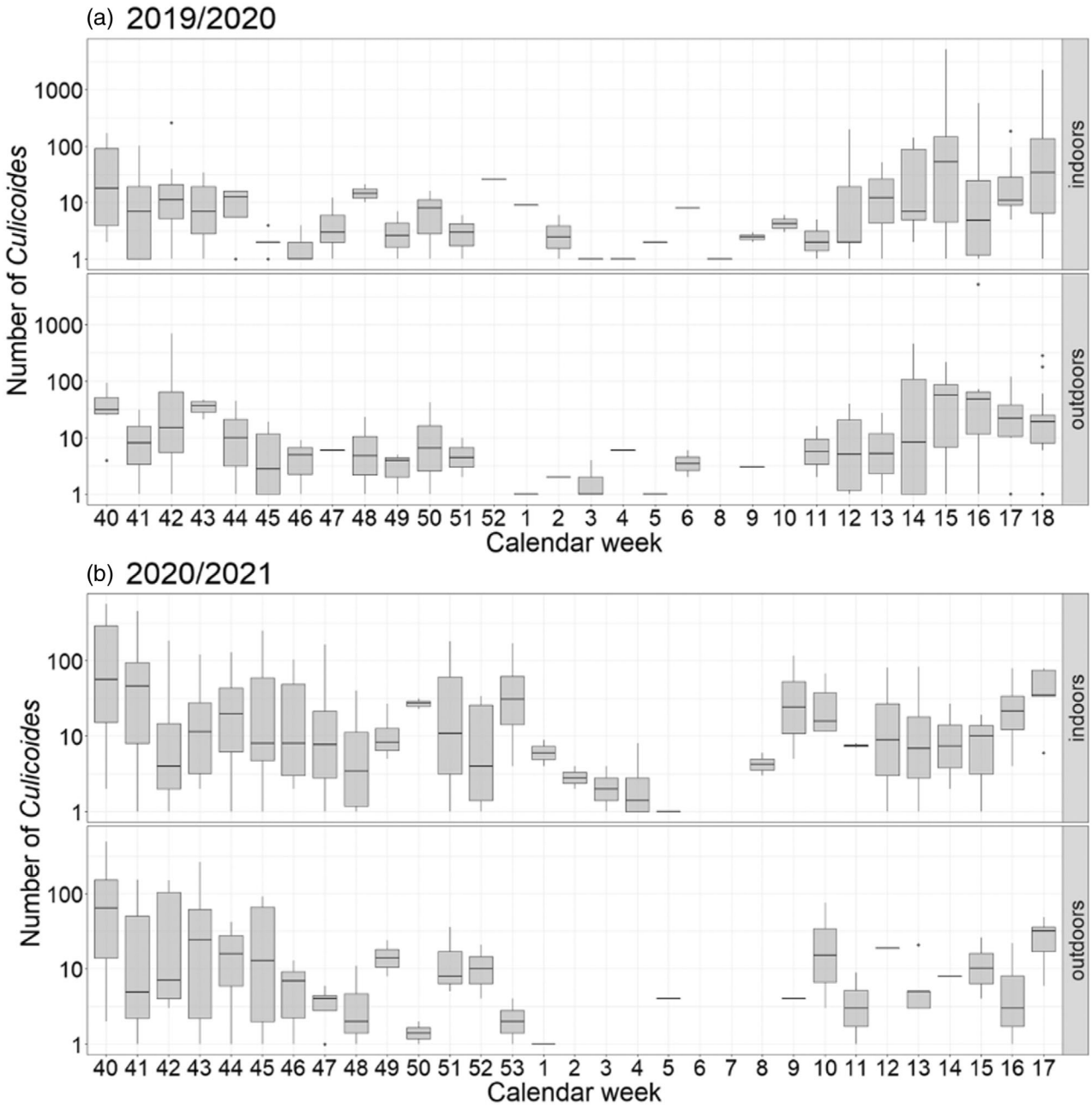

FIGURE 2 Boxplots of the numbers of *Culicoides* caught indoors and outdoors per location by calendar week (*x*-axis) in the winter periods 2019/2020 (a) and 2020/2021 (b). The *y*-axis is log-transformed. The black solid lines in the boxes represent the medians. Boxes show the 25th and 75th percentiles, and error bars reflect the 10th and 90th percentiles. Black dots mark the minimum and maximum data points outside the 10th and 90th percentiles.

TABLE 3 Monthly averages ± standard deviations of total and blood-fed *Culicoides* (in parentheses) from 480 indoor and outdoor UV-light trap catches in the winters of 2019/2020 and 2020/2021.

	Indoors		Outdoors	
	2019/2020	2020/2021	2019/2020	2020/2021
October	29.16 ± 2.38 (1.38 ± 0.17)	62.04 ± 8.10 (6.65 ± 1.10)	44.56 ± 4.15 (0.03 ± 0.01)	32.21 ± 4.98 (0.58 ± 0.07)
November	2.58 ± 0.34 (0.19 ± 0.18)	30.06 ± 4.83 (16.34 ± 3.18)	2.84 ± 0.32 (0.03 ± 0.01)	7.81 ± 1.80 (0.13 ± 0.02)
December	2.42 ± 0.44 (0.04 ± 0.02)	24.70 ± 4.39 (9.57 ± 2.24)	2.11 ± 0.77 (0 ± 0)	3.82 ± 0.62 (0.03 ± 0.01)
January	0.49 ± 0.10 (0.02 ± 0.01)	1.41 ± 0.21 (0.03 ± 0.02)	0.26 ± 0.06 (0 ± 0)	0.03 ± 0.02 (0 ± 0)
February	0.40 ± 0.10 (0 ± 0)	0.36 ± 0.16 (0 ± 0)	0.35 ± 0.13 (0 ± 0)	0.14 ± 0.07 (0 ± 0)
March	8.04 ± 2.69 (0.82 ± 0.40)	10.43 ± 1.33 (0.37 ± 0.17)	3.18 ± 0.87 (0 ± 0)	3.06 ± 0.49 (0.14 ± 0.05)
April	225.86 ± 44.50 (11.80 ± 1.79)	17.67 ± 2.59 (0.18 ± 0.04)	157.71 ± 35.30 (1.69 ± 0.35)	7.30 ± 1.49 (0 ± 0)

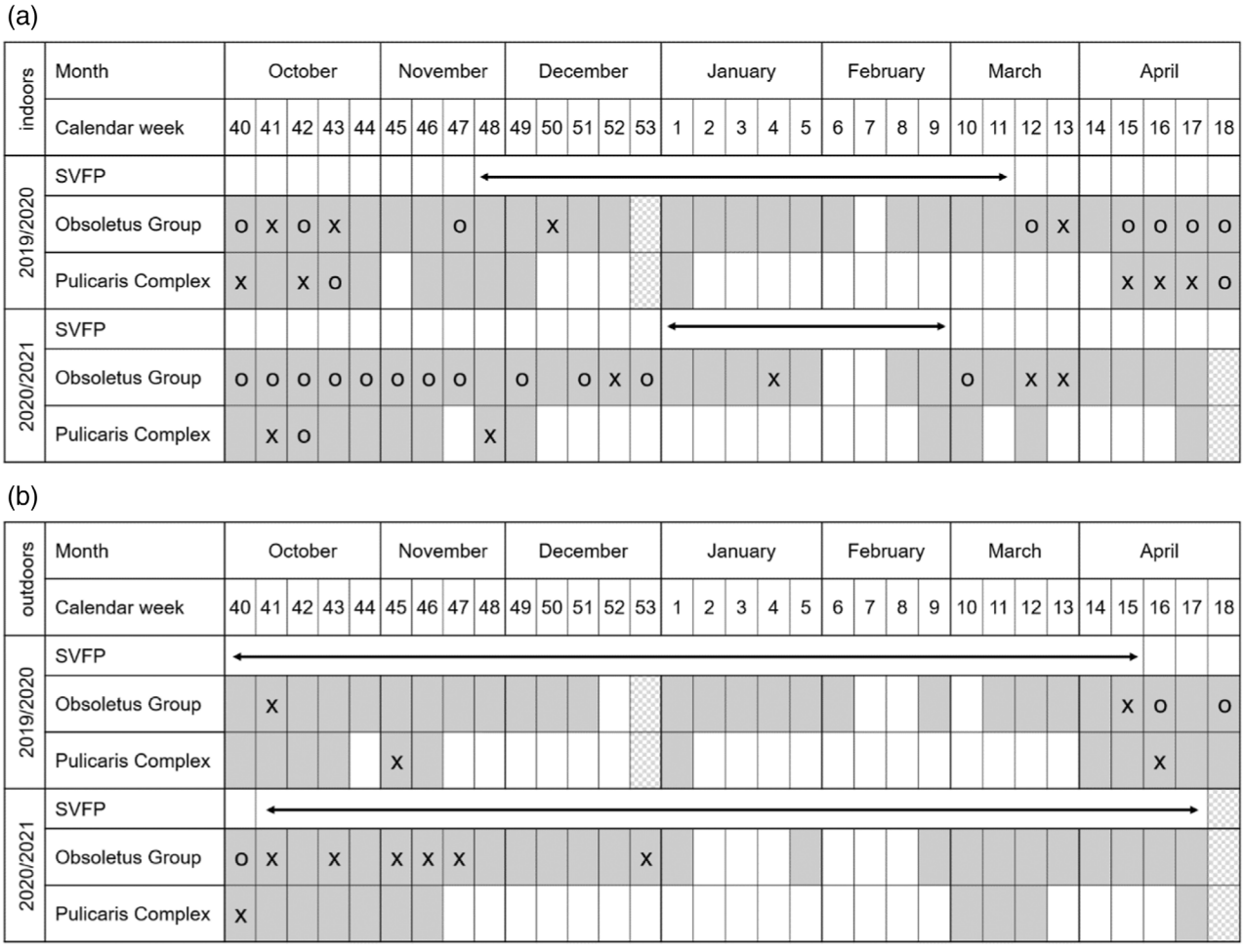

FIGURE 3 Qualitative overview of *Culicoides* occurrence in general and of blood-fed females inside (a) and outside (b) of stables. Grey tiles indicate that at least one *Culicoides* was collected at any one of the trapping locations. White tiles indicate that no *Culicoides* were caught at any trapping location. Hatched tiles mark calendar weeks not existing in the respective month or year. x: up to four freshly blood-fed females were caught at any one trapping location; o: at least five freshly blood-fed females were caught at any one trapping location. The SVFPs ascertained for the respective winters are marked by arrows.

The temperature significantly affected the total number of collected *Culicoides*, as demonstrated by a Poisson GLM (Table S2), which was also true for the three *Culicoides* groups (Obsoletus Group, Pulicaris Complex and 'other *Culicoides*') when analysed individually. Collection numbers of the Pulicaris Complex and 'other *Culicoides*' were significantly more affected than those of the Obsoletus Group (Table S3).

On the first trapping day, the average temperatures at which Obsoletus Group or Pulicaris Complex specimens were trapped were

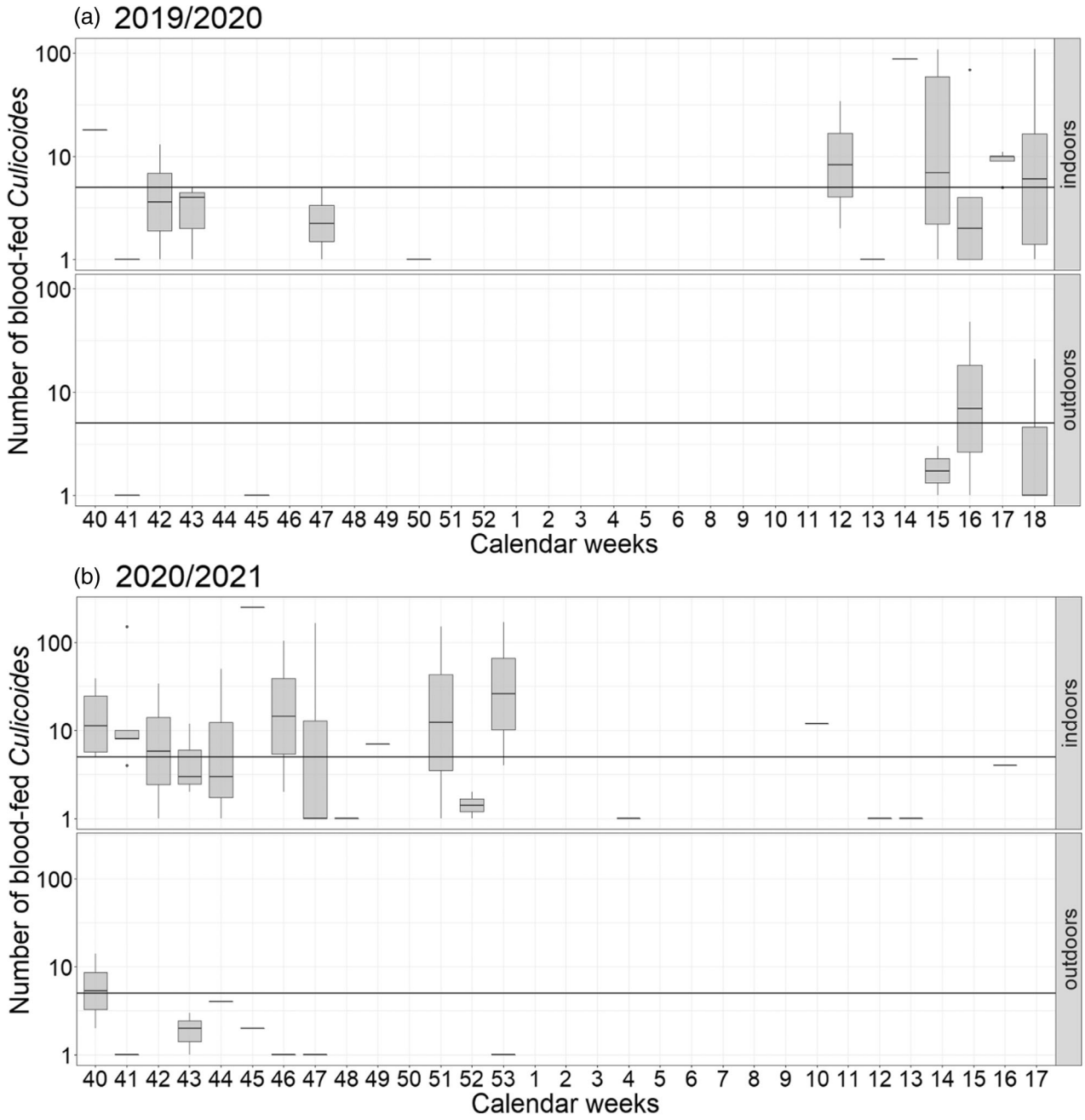

FIGURE 4 Boxplots of the numbers of blood-fed *Culicoides* caught indoors and outdoors per location by calendar week (*x*-axis) in the winter periods 2019/2020 (a) and 2020/2021 (b). The horizontal line represents the proposed treshold of five blood-fed *Culicoides* used for defining an SVFP. The SVFP lasts as long as the values are below the solid line. The *y*-axis is log-transformed. The boxes shows the 25th and 75th percentiles with the black solid lines marking the medians. The error bars on the boxes represent the 10th and 90th percentiles. The black dots mark the minimum and maximum data points outside the 10th and 90th percentiles.

7.4°C (minimum 0.3°C) and 10.3°C (minimum 1.2°C), respectively. The average temperature for the 7 day-period before capture was 7.8°C (minimum 1.1°C) for Obsoletus Group specimens and 10.1°C (minimum 3.2°C) for the Pulicaris Complex (Table 4).

The difference between the weekly average indoor and outdoor temperatures over both study periods was, on average, 1.2°C in all stables (median: 1.0°C, minimum: −4.3°C, maximum: 9.0°C). The differences between indoor and outdoor weekly average temperatures were most significant from December to February (Figure 5), in which average indoor temperatures were higher than average outdoor temperatures. In some weeks in October and November 2019/2020 and in March 2021, however, the average indoor temperature was lower than the average outdoor temperature (Figure 5). Generally, the highest average indoor and outdoor temperatures were recorded in April and October (up to 15.0°C). The lowest temperatures were recorded from December to February, with the weekly average indoor and

TABLE 4 Lowest temperatures at which specimens of the Obsoletus Group and the Pulicaris Complex were caught.

	First trapping day (t_1)		Seven-day period before trapping (t_7)	
	Obsoletus Group	Pulicaris Complex	Obsoletus Group	Pulicaris Complex
Average	7.4°C	10.3°C	7.8°C	10.1°C
Median	7.5°C	10.1°C	7.6°C	11.1°C
Minimum	0.3°C	1.2°C	1.1°C	3.2°C
Maximum	15.7°C	17.1°C	14.5°C	15.0°C

Note: Average temperatures of the first trapping day (t_1) and of a 7-day period before the trapping day (t_7) are displayed, including medians, minimum and maximum.

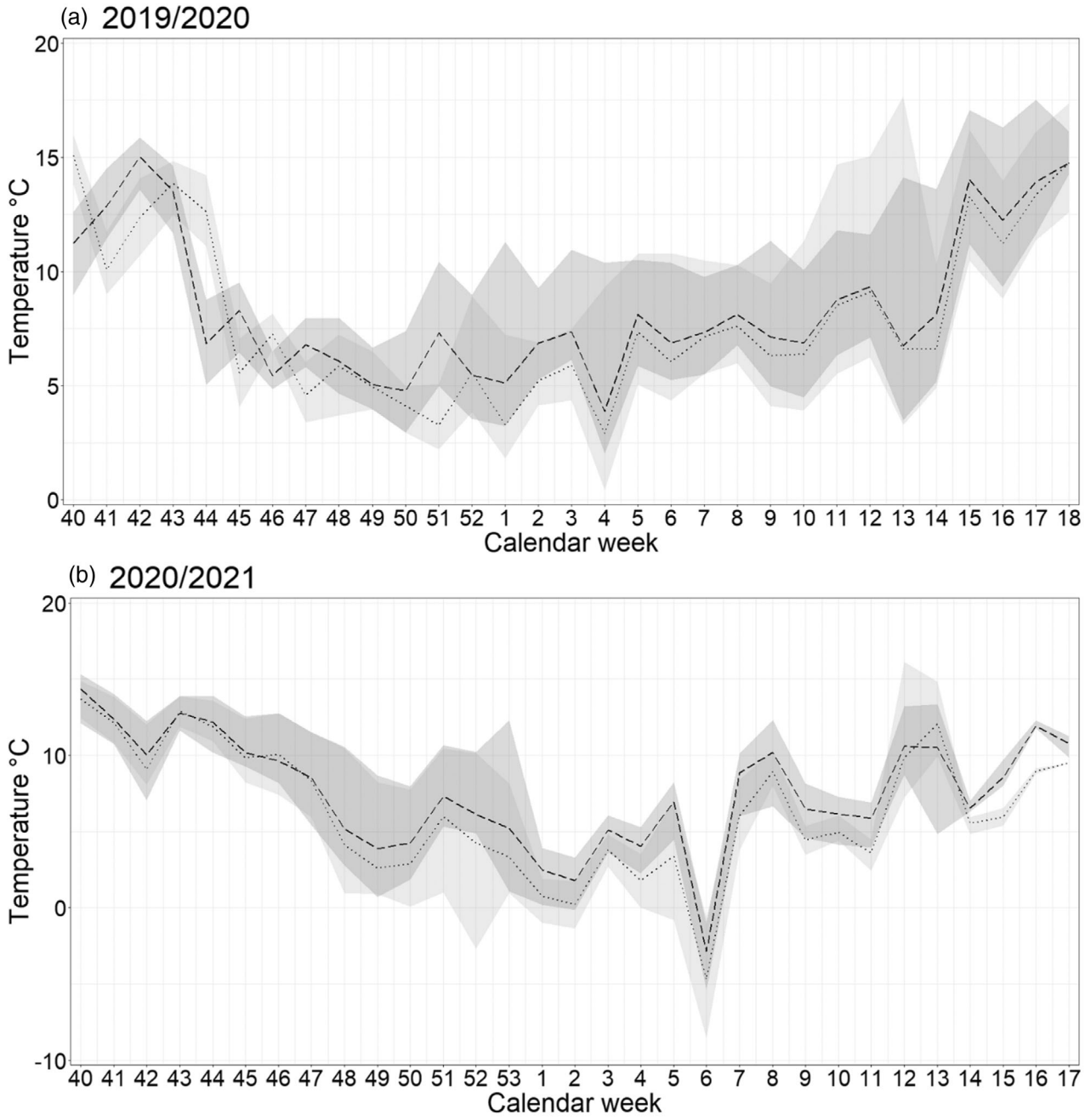

FIGURE 5 Average indoor (dashed line) and outdoor temperatures (dotted line) of the trapping sites according to calendar week during the winters 2019/2020 (a) and 2020/2021 (b). The shaded areas show the respective minimum and maximum temperatures, with dark grey representing indoor temperatures and light grey outdoor temperatures.

outdoor temperatures dropping below freezing point only in calendar week 6 in February 2021 (Figure 5). In the other weeks during these 3 months, the outside temperatures only occasionally fell below 0°C, but never inside the stables.

DISCUSSION

This study investigated the flying activity of biting midges of the genus *Culicoides* during wintertime in Germany. *Culicoides* adults were trapped in considerable numbers inside and outside of stables harbouring ruminants or horses, including blood-fed females that were mainly caught indoors.

The majority (more than 90%) of the captured *Culicoides* belonged to the Obsoletus Group. This was expected, as members of this group live and breed in various kinds of environments, are widely distributed and are usually predominant in the European biting midge fauna (Cuéllar et al., 2018). By contrast, the Pulicaris Complex seems to exhibit a more stenoecious and specialised adaptation to eco-climatic conditions (Werner et al., 2020). This might be one of the reasons why members of the Pulicaris Complex are usually caught in lower numbers than those of the Obsoletus Group.

Due to the adverse weather conditions (such as wind, precipitation or low temperatures), typical for the winter season in temperate regions, *Culicoides* flight activity is more suppressed outdoors than indoors (Baylis et al., 2010). On the other hand, in protected indoor areas, where there is little wind and higher temperatures, light traps could be more efficient, resulting in higher numbers of *Culicoides* collected (Baylis et al., 2010). Therefore, a much higher proportion of *Culicoides* specimens was trapped inside the stables than outside, in agreement with other European studies conducted during the cold season in temperate regions (Baldet et al., 2008; Brugger et al., 2016; Clausen et al., 2009; Kameke et al., 2017). This proportion is mainly attributable to the high number of Obsoletus Group specimens caught inside the stables, although the proportion of Pulicaris Complex specimens was higher than that in other studies conducted indoors (Baldet et al., 2008; Meiswinkel, Goffredo, et al., 2008).

Blood-fed *Culicoides* were also captured much more frequently and for extended periods inside the stables than outside, even if a higher total number of *Culicoides* specimens was trapped outdoors (e.g., on farms 3 or 9). Other studies (Baldet et al., 2008; Magliano et al., 2018; Sarvašová et al., 2016) also registered more blood-fed specimens indoors, for which Magliano et al. (2018) propose two explanations. First, females having fed on blood outdoors enter stables immediately after the blood meal. On farms included in this study, the livestock was kept inside closed stables for extended periods, while no domesticated animals were outside. Therefore, it can be assumed that the blood meals were taken indoors. This idea is supported by studies that show that keeping livestock indoors entails a higher number of *Culicoides* indoors, but also that the number of livestock in the vicinity of a trap might influence the number of *Culicoides* caught (Sanders et al., 2011; Zimmer et al., 2008). In particular, a significantly higher number of blood-fed *C. obsoletus* was detected indoors when livestock was present (Baylis et al., 2010), as probably reflected by higher numbers of Obsoletus Group specimens in this study. The second explanation by Magliano et al. (2018) is that nulliparous females try to avoid the lower temperatures outside and enter the stables to take a blood meal. This behaviour seems to be unlikely in this study, as the stables were relatively tightly closed and openings to enter them were limited, except for times when the livestock was provided with food.

In addition to climatic conditions in autumn and winter, which cause a reduction of *Culicoides* activity outdoors, studies have shown that UV-light trap collections do not reflect the actual abundance of blood-fed *Culicoides* females and, thus, the biting rate (Baylis et al., 2010; Viennet et al., 2011). Therefore, the relatively low number of blood-fed *Culicoides* caught can also be attributed to the low sensitivity of the trap type (Gerry et al., 2009). For comprehensive epidemiological monitoring and the assessment of virus transmission risk, biting rates should be determined by direct aspiration of *Culicoides* from livestock (Gerry et al., 2009; Viennet et al., 2011).

Several studies have demonstrated that temperature has a decisive impact on *Culicoides* activity and, thus, on the risk of biting (Gubbins et al., 2008; Sarvašová et al., 2016; Silbermayr et al., 2011; Vorsprach et al., 2009; Wittman et al., 2002). The significant influence of temperature on *Culicoides* activity was confirmed in this study. During the coldest study months (December to February), a temperature difference of up to 9.0 K was measured between indoor and outdoor areas, with higher temperatures indoors. Compared to outdoors, milder temperatures appear to lead to a constant or an extended flight activity of *Culicoides* inside the stables, particularly of the putative vector species of the Obsoletus Group. The lower number of *Culicoides* trapped outdoors, on the other hand, is likely to be caused by the colder outdoor temperatures, which may result in a higher mortality rate (Magliano et al., 2018). This observation clearly shows that the selection of trap positions (indoors or outdoors) considerably influences the results of monitoring programmes, which should be the basis for the determination of an SVFP. As demonstrated by this study, outdoor catches would clearly define longer SVFPs than indoor catches. This is supported by results from an Austrian study, in which indoor and outdoor catches were compared over some years (Brugger et al., 2016). It is, therefore, crucial which trap results (inside or outside) are used as the basis for regulatory restrictions. *Culicoides* activity and blood-feeding in winter will probably only affect the situation in a stable itself, since few biting midges are active outside. Virus spread between farms, however, is driven by *Culicoides* outdoor activity which in turn depends on outside temperatures. Moreover, when outdoor temperatures rise again, farmers will open the stables and potentially infected *Culicoides* can leave and fly to other livestock facilities.

In this study, the temperature was measured directly at the traps and, even indoors, was often lower than 11–13°C, the temperature required for virus replication in the biting midge (Carpenter et al., 2011). However, temperatures at other places in the stables may be higher and allow virus replication, the development and oviposition of eggs, and a second blood meal in winter. Therefore, studies

on microclimatic conditions inside stables should be conducted to investigate whether there are places with temperatures sufficient for virus replication (e.g., in direct proximity to livestock).

A continuous trapping rhythm with short time intervals (e.g., weekly) is essential for the meaningful interpretation of collection data during wintertime, since *Culicoides* activity may change quickly as has been demonstrated in this study. Brugger et al. (2016) report that the vector season begins when a mean daily temperature of 10°C is reached for seven consecutive days. The average daily temperature triggering the activity of the Obsoletus Group, as measured for seven consecutive days before trapping, was found to be even lower in this study (7.4°C), while the average daily activity temperature for the Pulicaris Complex (10.1°C) corresponded to that suggested by Brugger et al. (2016). Due to the almost continuously higher average daily temperature in the stables, the temperature necessary for *Culicoides* activity was achieved or exceeded more frequently indoors than outdoors. At the same time, individuals of the Obsoletus Group and the Pulicaris Complex were trapped at much lower temperatures (0.3 and 3.2°C, respectively). Furthermore, the number of blood-fed *Culicoides* caught each week in this study shows short interruptions of the SVFP because the threshold was temporarily exceeded (calendar week 12 in 2020, indoors) and undershot (e.g., calendar weeks 13 and 14 in 2020, indoors). This could entail the misleading assumption that either the SVFP has come to an end or that an SVFP is declared, although not existing.

To determine the SVFPs in this study, blood-fed *Culicoides* specimens were analysed instead of parous *Culicoides*, as required by the Commission Regulation (EC), 1266/2007. Parous and nulliparous *Culicoides* are commonly classified according to the presence or absence of burgundy-red pigmentation in the abdominal wall. This pigmentation is generated during the first gonotrophic cycle in females, intensifies on subsequent feedings, and remains lifelong (Dyce, 1969). This method of determining previous blood-feeding of a female and the possibility of being infected with a virus has some limitations and uncertainties, though, since freshly hatched *Culicoides*, for example, may also have a pigmented abdomen (Braverman & Mumcuoglu, 2009; Harrup et al., 2013). In addition, Goffredo et al. (2021) observed that abdominal pigmentation in *Culicoides* was absent after artificial blood-feeding. To be on the safe side, only females visibly blood-fed were therefore taken into account in this study. It is, however, possible that some specimens considered non-blood-fed had in fact taken a blood meal and were parous. Consequently, the SVFPs deduced from this study could actually have been shorter than determined.

Location factors such as the surrounding landscape of farms, vegetation cover, land cover, and soil characteristics such as type, moisture, or organic matter can influence the occurrence of *Culicoides* (Conte, Goffredo, et al., 2007; Conte, Ippoliti, et al., 2007; De Regge et al., 2015; Kameke et al., 2021; Lühken et al., 2015; Purse et al., 2012; Zimmer et al., 2010). However, studies were mainly conducted outdoors and in summer, and their results may only partly be transferable to winter conditions. Therefore, conditions found indoors may play a crucial role in the development and occurrence of *Culicoides*, although requirements of biting midges to indoor habitats are inadequately known. Zimmer et al. (2010) described dried dung adhering to the walls inside stables as breeding substrate for *Culicoides* species of the Obsoletus Group, and Steinke et al. (2016) noted dung heaps as productive breeding sites. Fresh dung heaps may be found indoors in winter when livestock is kept there. In addition, it is conceivable that manure management and the building construction influence the occurrence of *Culicoides*. So far, no evident influence by manure management has been demonstrated (Groschupp et al., 2023), and studies on the effect of different types of building construction, including air ventilation systems, on culicoids have yet to be carried out.

CONCLUSION

The present study shows that species of the Obsoletus Group are active in stables during wintertime, coexisting with, and feeding on, livestock. By all means, Obsoletus Group species developing and residing indoors could therefore play a role in virus persistence in the cold months of the year. This hypothesis is supported by the demonstration of blood-fed specimens, which were trapped in significantly higher numbers indoors than outdoors. Should infected *Culicoides* survive the winter, virus replication may proceed with increasing spring temperatures and the virus be transmitted during the next blood meal. Staying indoors could increase survival rates of biting midges, and the vector capacity is higher indoors than outdoors. Further investigations on winter activity should be conducted to find out how climate change affects the occurrence and activity of *Culicoides* during the cold season and whether the concept of an SVFP should be modified to a regionally adaptable vector-poor period with respective restrictions. When defining an SVFP, it should be taken into account that the location of traps used for collecting blood-fed or gravid-biting midge females and the trapping regime will impact the results substantially.

AUTHOR CONTRIBUTIONS

Sarah Groschupp: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing. **Helge Kampen:** Conceptualization; funding acquisition; writing – review and editing; project administration; supervision; validation; visualization; methodology; investigation. **Doreen Werner:** Conceptualization; investigation; funding acquisition; project administration; supervision; writing – review and editing; methodology.

ACKNOWLEDGEMENTS

The authors thank the farmers who allowed to operate the traps on their premises. They would also like to thank Christiane Eska, Christin Henneberg, Evelin Leich, Nathalie Richter, Sebastian Schuran and Maria Will for excellent technical assistance. The study was funded by the Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE), grant numbers 281B101816 and 281B101916. Open Access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data supporting the conclusions of this article are included within the article and its additional files.

ORCID

Sarah Groschupp https://orcid.org/0009-0006-0686-8806

REFERENCES

Baldet, T., Delécolle, J.C., Cêtre-Sossah, C., Mathieu, B., Meiswinkel, R. & Gerbier, G. (2008) Indoor activity of *Culicoides* associated with livestock in the bluetongue virus (BTV) affected region of northern France during autumn 2006. *Preventive Veterinary Medicine*, 87(1–2), 84–97.

Baylis, M., Parkin, H., Kreppel, K., Carpenter, S., Mellor P.S. & McIntyre, K.M. (2010) Evaluation of housing as a means to protect cattle from *Culicoides* biting midges, the vectors of bluetongue virus. *Medical and Veterinary Entomology*, 24(1), 38–45.

Beer, M. & Wernike, K. (2021) Akabane virus and Schmallenberg virus (Peribunyaviridae). In: Bamford, D.H. & Zuckerman, M. (Eds.) *Encyclopedia of virology*, Vol. 2, 4th edition. Oxford: Academic Press, pp. 34–39.

Braverman, Y. & Mumcuoglu, K. (2009) Newly emerged nulliparous *Culicoides imicola* Kieffer (Diptera: Ceratopogonidae) with pigmented abdomen. *Veterinary Parasitology*, 160(3–4), 356–358.

Brugger, K., Köfer, J. & Rubel, F. (2016) Outdoor and indoor monitoring of livestock-associated *Culicoides* spp. to assess vector-free periods and disease risks. *BMC Veterinary Research*, 12, 88.

Caracappa, S., Torina, A., Guercio, A., Vitale, F., Calabrò, A., Purpari, G. et al. (2003) Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. *The Veterinary Record*, 153(3), 71–74.

Carpenter, S., Wilson, A., Barber, J., Veronesi, E., Mellor, P., Venter, G. et al. (2011) Temperature dependence of the extrinsic incubation period of orbiviruses in *Culicoides* biting midges. *PLoS One*, *6*(11), e27987.

Carpenter, S., Wilson, A. & Mellor, P.S. (2009) *Culicoides* and the emergence of bluetongue virus in northern Europe. *Trends in Microbiology*, 17(4), 172–178.

Clausen, P.H., Stephan, A., Bartsch, S., Jandowsky, A., Hoffmann-Köhler, P., Schein, E. et al. (2009) Seasonal dynamics of biting midges (Diptera: Ceratopogonidae, *Culicoides* spp.) on dairy farms of Central Germany during the 2007/2008 epidemic of bluetongue. *Parasitology Research*, 105(2), 381–386.

Collins, Á.B., Barrett, D., Doherty, M.L., Larska, M. & Mee, J.F. (2016) Post-epidemic Schmallenberg virus circulation: parallel bovine serological and *Culicoides* virological surveillance studies in Ireland. *BMC Veterinary Research*, 12(1), 234.

Commission Regulation (EC) 1266/2007 of 26 October 2007 on implementing rules for Council Directive 2000/75/EC as regards the control, monitoring, surveillance and restrictions on movements of certain animals of susceptible species in relation to bluetongue. *Official Journal of the European Union*, 2007(L283), 37–52.

Conte, A., Goffredo, M., Ippoliti, C. & Meiswinkel, R. (2007) Influence of biotic and abiotic factors on the distribution and abundance of *Culicoides imicola* and the Obsoletus complex in Italy. *Veterinary Parasitology*, 150(4), 333–344.

Conte, A., Ippoliti, C., Savini, L., Goffredo, M. & Meiswinkel, R. (2007) Novel environmental factors influencing the distribution and abundance of *Culicoides imicola* and the Obsoletus Complex in Italy. *Veterinaria Italiana*, 43(3), 571–580.

Cuéllar, A.C., Kjær, L.J., Kirkeby, C., Skovgard, H., Nielsen, S.A., Stockmarr, A. et al. (2018) Spatial and temporal variation in the abundance of *Culicoides* biting midges (Diptera: Ceratopogonidae) in nine European countries. *Parasites & Vectors*, 11(1), 112.

De Regge, N. (2017) Akabane, Aino and Schmallenberg virus—where do we stand and what do we know about the role of domestic ruminant hosts and *Culicoides* vectors in virus transmission and overwintering? *Current Opinion in Virology*, 27, 15–30.

De Regge, N., De Deken, R., Fassotte, C., Losson, B., Deblauwe, I., Madder, M. et al. (2015) *Culicoides* monitoring in Belgium in 2011: analysis of spatiotemporal abundance, species diversity and Schmallenberg virus detection. *Medical and Veterinary Entomology*, 29(3), 263–275.

De Regge, N., Deblauwe, I., De Deken, R., Vantieghem, P., Madder, M., Geysen, D. et al. (2012) Detection of Schmallenberg virus in different *Culicoides* spp. by real-time RT-PCR. *Transboundary and Emerging Diseases*, 59(6), 471–475.

Delécolle, J.-C. (1985) Nouvelle Contribution à l'Étude Systématique et Iconographique des Espèces du Genre *Culicoides* (Diptera: Ceratopogonidae) du Nord-Est de la France. PhD thesis, Universität Strasbourg, France.

Doceul, V., Lara, E., Sailleau, C., Belbis, G., Richardson, J., Bréard, E. et al. (2013) Epidemiology, molecular virology and diagnostics of Schmallenberg virus, an emerging orthobunyavirus in Europe. *Veterinary Research*, 44(1), 31.

Dyce, A.L. (1969) The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Journal of the Australian Entomological Society*, 8(1), 11–15.

Gerdes, G.H. (2004) A South African overview of the virus, vectors, surveillance and unique features of bluetongue. *Veterinaria Italiana*, 40(3), 39–42.

Gerry, A.C., Sarto i Monteys, V., Moreno Vidal, J.O., Francino, O. & Mullens, B.A. (2009) Biting rates of *Culicoides* midges (Diptera: Ceratopogonidae) on sheep in northeastern Spain in relation to midge capture using UV light and carbon dioxide-baited traps. *Journal of Medical Entomology*, 46(3), 615–624.

Gethmann, J., Probst, C. & Conraths, F.J. (2020) Economic impact of a bluetongue serotype 8 epidemic in Germany. *Frontiers in Veterinary Science*, 7, 65.

Goffredo, M., Quaglia, M., De Ascentis, M., d'Alessio, S.G., Federici, V., Conte, A. (2021) The absence of abdominal pigmentation in livestock associated *Culicoides* following artificial blood feeding and the epidemiological implication for arbovirus surveillance. *Pathogens*, 10(12), 1571.

Groschupp, S., Kampen, H. & Werner, D. (2023) Occurrence of putative *Culicoides* biting midge vectors (Diptera: Ceratopogonidae) inside and outside barns in Germany and factors influencing their activity. *Parasites & Vectors*, 16, 307.

Gubbins, S., Carpenter, S., Baylis, M., Wood, J.L.N. & Mellor, P.S. (2008) Assessing the risk of bluetongue to UK livestock: uncertainty and sensitivity analyses of a temperature-dependent model for the basic reproduction number. *Journal of the Royal Society Interface*, 5(20), 363–371.

Harrup, L.E., Purse, B.V., Golding, N., Mellor, P.S. & Carpenter, S. (2013) Larval development and emergence sites of farm-associated *Culicoides* in the United Kingdom. *Medical and Veterinary Entomology*, 27(4), 441–449.

Hoffmann, B., Bauer, B., Bauer, C., Bätza, H.J., Beer, M., Clausen, P.H. et al. (2009) Monitoring of putative vectors of bluetongue virus serotype 8, Germany. *Emerging Infectious Diseases*, 15(9), 1481–1484.

Hoffmann, B., Sasserath, M., Thalheim, S., Bunzenthal, C., Strebelow, G. & Beer, M. (2008) Bluetongue virus serotype 8 reemergence in Germany, 2007 and 2008. *Emerging Infectious Diseases*, 14(9), 1421–1423.

Hoffmann, B., Scheuch, M., Höper, D., Jungblut, R., Holsteg, M., Schirrmeier, H. et al. (2012) Novel orthobunyavirus in cattle, Europe, 2011. *Emerging Infectious Diseases*, 18(3), 469–472.

Kameke, D., Kampen, H., Wacker, A. & Werner, D. (2021) Field studies on breeding sites of *Culicoides* Latreille (Diptera: Ceratopogonidae) in agriculturally used and natural habitats. *Scientific Reports*, 11(1), 10007.

Kameke, D., Kampen, H. & Walther, D. (2017) Activity of *Culicoides* spp. (Diptera: Ceratopogonidae) inside and outside of livestock stables in late winter and spring. *Parasitology Research*, 116(3), 881–889.

Kampen, H. & Werner, D. (2023) Biting midges (Diptera: Ceratopogonidae) as vectors of viruses. *Microorganisms*, 11(11), 2706.

Kiel, E., Liebisch, G., Focke, R., Liebisch, A. & Werner, D. (2009) Monitoring of *Culicoides* at 20 locations in northwest Germany. *Parasitology Research*, 105(2), 351–357.

Larska, M., Lechowski, L., Grochowska, M. & Żmudziński, J.F. (2013) Detection of the Schmallenberg virus in nulliparous *Culicoides obsoletus/scoticus* complex and *C. punctatus*—the possibility of transovarial virus transmission in the midge population and of a new vector. *Veterinary Microbiology*, 166(3–4), 467–473.

Losson, B., Mignon, B., Paternostre, J., Madder, M., de Deken, R., de Deken, G. et al. (2007) Biting midges overwintering in Belgium. *The Veterinary Record*, 160(13), 451–452.

Lühken, R., Kiel, E., Steinke, S. & Fladung, R. (2015) Topsoil conditions correlate with the emergence rates of *Culicoides chiopterus* and *Culicoides dewulfi* (Diptera: Ceratopogonidae) from cowpats. *Parasitology Research*, 114(3), 1113–1117.

Magliano, A., Scaramozzino, P., Ravagnan, S., Montarsi, F., Darold, G., Cincinelli, G. et al. (2018) Indoor and outdoor winter activity of *Culicoides* biting midges, vectors of bluetongue virus in Italy. *Medical and Veterinary Entomology*, 32(1), 70–77.

Mathieu, B., Cêtre-Sossah, C., Garros, C., Chavernac, D., Balenghien, T., Carpenter, S. et al. (2012) Development and validation of IIKC: an interactive identification key for *Culicoides* (Diptera: Ceratopogonidae) females from the Western Palaearctic region. *Parasites & Vectors*, 5, 137.

Mehlhorn, H., Walldorf, V., Klimpel, S., Jahn, B., Jaeger, F., Eschweiler, J. et al. (2007) First occurrence of *Culicoides obsoletus*-transmitted bluetongue virus epidemic in Central Europe. *Parasitology Research*, 101(1), 219–228.

Mehlhorn, H., Walldorf, V., Klimpel, S., Schaub, G., Kiel, E., Focke, R. et al. (2009) Bluetongue disease in Germany (2007-2008): monitoring of entomological aspects. *Parasitology Research*, 105(2), 313–319.

Meiswinkel, R., Baldet, T., de Deken, R., Takken, W., Delécolle, J.C. & Mellor, P.S. (2008) The 2006 outbreak of bluetongue in northern Europe—the entomological perspective. *Preventive Veterinary Medicine*, 87(1–2), 55–63.

Meiswinkel, R., Goffredo, M., Dijkstra, E.G., van der Ven, I.J.K., Baldet, T. & Elbers, A. (2008) Endophily in *Culicoides* associated with BTV-infected cattle in the province of Limburg, south-eastern Netherlands, 2006. *Preventive Veterinary Medicine*, 87(1–2), 182–195.

Meiswinkel, R., van Rijn, P., Leijs, P. & Goffredo, M. (2007) Potential new *Culicoides* vector of bluetongue virus in northern Europe. *The Veterinary Record*, 161(16), 564–565.

Mellor, P.S., Boorman, J. & Baylis, M. (2000) *Culicoides* biting midges: their role as arbovirus vectors. *Annual Review of Entomology*, 45, 307–340.

Menzies, F.D., McCullough, S.J., McKeown, I.M., Forster, J.L., Jess, S., Batten, C. et al. (2008) Evidence for transplacental and contact transmission of bluetongue virus in cattle. *The Veterinary Record*, 163(7), 203–209.

Mullens, B.A., Tabachnick, W.J., Holbrook, F.R. & Thompson, L.H. (1995) Effects of temperature on virogenesis of bluetongue virus serotype 11 in *Culicoides variipennis sonorensis*. *Medical and Veterinary Entomology*, 9(1), 71–76.

Nunamaker, R.A., Sieburth, P.J., Dean, V.C., Wigington, J.G., Nunamaker, C.E. & Mecham, J.O. (1990) Absence of transovarial transmission of bluetongue virus in *Culicoides variipennis*: immunogold labelling of bluetongue virus antigen in developing oocytes from *Culicoides variipennis* (Coquillett). *Comparative Biochemistry and Physiology*, 96A(1), 19–31.

Paweska, J.T., Venter, G.J. & Mellor, P.S. (2002) Vector competence of South African *Culicoides* species for bluetongue virus serotype 1 (BTV-1) with special reference to the effect of temperature on the rate of virus replication in *C. imicola* and *C. bolitinos*. *Medical and Veterinary Entomology*, 16(1), 10–21.

Pinior, B., Lebl, K., Firth, C., Rubel, F., Fuchs, R., Stockreiter, S. et al. (2015) Cost analysis of bluetongue virus serotype 8 surveillance and vaccination programmes in Austria from 2005 to 2013. *Veterinary Journal*, 206(2), 154–160.

Purse, B.V., Falconer, D., Sullivan, M.J., Carpenter, S., Mellor, P.S., Piertney, S.B. et al. (2012) Impacts of climate, host and landscape factors on *Culicoides* species in Scotland. *Medical and Veterinary Entomology*, 26(2), 168–177.

R Core Team. (2022) R: a language and environment for statistical computing. Available from: https://www.Rproject.org/ [Accessed 8th February 2024].

Rasmussen, L.D., Kristensen, B., Kirkeby, C., Rasmussen, T.B., Belsham, G.J., Bødker, R. et al. (2012) Culicoids as vectors of Schmallenberg virus. *Emerging Infectious Diseases*, 18(7), 1204–1206.

Rasmussen, L.D., Savini, G., Lorusso, A., Bellacicco, A., Palmarini, M., Caporale, M. et al. (2013) Transplacental transmission of field and rescued strains of BTV-2 and BTV-8 in experimentally infected sheep. *Veterinary Research*, 44(1), 75.

Saegerman, C., Berkvens, D. & Mellor, P.S. (2008) Bluetongue epidemiology in the European Union. *Emerging Infectious Diseases*, 14(4), 539–544.

Sanders, C.J., Shortall, C.R., Gubbins, S., Burgin, L., Gloster, J., Harrington, R. et al. (2011) Influence of season and meteorological parameters on flight activity if *Culicoides* biting midges. *Journal of Applied Ecology*, 48(6), 1355–1364.

Sarvašová, A., Kočišová, A., Liptáková, E., Hiavatá, H. & Mathieu, B. (2016) First insights into indoor and outdoor *Culicoides* activity related to the risk period for bluetongue virus transmission in eastern Slovakia. *Acta Parasitologica*, 61(4), 743–755.

Savini, G., Goffredo, M., Monaco, F., di Gennaro, A., Cafiero, M.A., Baldi, L. et al. (2005) Bluetongue virus isolations from midges belonging to the Obsoletus Complex (*Culicoides*, Diptera: Ceratopogonidae) in Italy. *The Veterinary Record*, 157(5), 133–139.

Silbermayr, K., Hackländer, K., Doscher, C., Koefer, J. & Fuchs, K. (2011) A spatial assessment of *Culicoides* spp. distribution and bluetongue disease risk areas in Austria. *Berliner und Münchener Tierärztliche Wochenschrift*, 124(5–6), 228–235.

Steinke, S., Lühken, R., Balczun, C. & Kiel, E. (2016) Emergence of *Culicoides obsoletus* group species from farm-associated habitats in Germany. *Medical and Veterinary Entomology*, 30(2), 174–184.

Takamatsu, H., Mellor, P.S., Mertens, P.P.C., Kirkham, P.A., Burroughs, J.N. & Parkhouse, R.M.E. (2003) A possible overwintering mechanism for bluetongue virus in the absence of the insect vector. *The Journal of General Virology*, 84(1), 227–235.

Van der Sluijs, M.T., Schroer-Joosten, D.P., Fid-Fourkour, A., Smit, M., Vrijenhoek, M.P., Moulin, V. et al. (2013) Transplacental transmission of BTV-8 in sheep: BTV viraemia, antibody responses and vaccine efficacy in lambs infected in utero. *Vaccine*, 31(36), 3726–3731.

Velthuis, A.G., Saatkamp, H.W., Mourits, M.C., de Koeijer, A.A. & Elbers, A.R.W. (2010) Financial consequences of the Dutch bluetongue serotype 8 epidemics of 2006 and 2007. *Preventive Veterinary Medicine*, 93(4), 294–304.

Viennet, E., Garros, C., Lancelot, R., Allène, X., Gardès, L., Rakotoarivony, I. et al. (2011) Assessment of vector/host contact: comparison of animal-baited traps and UV-light/suction trap for collecting *Culicoides* biting midges (Diptera: Ceratopogonidae), vectors of orbiviruses. *Parasites & Vectors*, 4, 119.

Vorsprach, B., Meiser, C.K., Werner, D., Balczun, C. & Schaub, G.A. (2009) Monitoring of Ceratopogonidae in southwest Germany. *Parasitology Research*, 105(2), 337–344.

Wellby, M.P., Baylis, M., Rawlings, P. & Mellor, P.S. (1996) Effect of temperature on survival and rate of virogenesis of African horse sickness virus in *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) and its significance in relation to the epidemiology of the disease. *Bulletin of Entomological Research*, 86(6), 715–720.

Werner, D., Groschupp, S., Bauer, C. & Kampen, H. (2020) Breeding habitat preferences of major *Culicoides* species (Diptera: Ceratopogonidae) in Germany. *International Journal of Environmental Research and Public Health*, 17(14), 5000.

Wernike, K. & Beer, M. (2020) Re-circulation of Schmallenberg virus, Germany, 2019. *Transboundary and Emerging Diseases*, 67(6), 2290–2295.

Wernike, K., Kohn, M., Conraths, F.J., Werner, D., Kameke, D., Hechinger, S. et al. (2013) Transmission of Schmallenberg virus during winter, Germany. *Emerging Infectious Diseases*, 19(10), 1701–1703.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L.D., François, R. et al. (2019) Welcome to the Tidyverse. *Journal of Open Source Software*, 4, 1686.

Wickham, H. & Bryan, J. (2019) readxl: read Excel files. Available from: https://cran.r-project.org/package=readxl [Accessed 8th February 2024].

Wilson, A., Carpenter, S., Gloster, J. & Mellor, P. (2007) Re-emergence of bluetongue in northern Europe in 2007. *The Veterinary Record*, 161(14), 487–489.

Wilson, A., Darpel, K. & Mellor, P.S. (2008) Where does bluetongue virus sleep in the winter? *PLoS Biology*, 6(8), e210.

Wilson, A.J. & Mellor, P.S. (2009) Bluetongue in Europe: past, present and future. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 364(1530), 2669–2681.

Wittman, E., Mellor, P. & Baylis, M. (2002) Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. *Medical and Veterinary Entomology*, 16(2), 147–156.

WOAH. (2024) World organisation for animal health—WAHIS—world animal health information system. Available from: https://wahis.woah.org/#/event-management [Accessed 17th June 2024].

Zimmer, J.Y., Haubruge, E., Francis, F., Bortels, J., Joie, E., Simonon, G. et al. (2008) Distribution of potential bluetongue vectors on Belgium farms. *The Veterinary Record*, 162(21), 700.

Zimmer, J.Y., Saegerman, C., Losson, B. & Haubruge, E. (2010) Breeding sites of bluetongue virus vectors, Belgium. *Emerging Infectious Diseases*, 16(3), 575–576.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. Average numbers of total and of blood-fed *Culicoides* per catch and study location.

Table S2. Coefficients and statistical output of the variable 'temperature' as calculated by the Poisson GLM. The response variable is the number of collected *Culicoides* biting midges.

Table S3. Coefficients and statistically significant output of the variable 'temperature' as calculated by the Poisson GLM. The response variables are the number of the collected *Culicoides* biting midges.

How to cite this article: Groschupp, S., Kampen, H. & Werner, D. (2024) Winter activity of *Culicoides* (Diptera: Ceratopogonidae) inside and outside stables in Germany. *Medical and Veterinary Entomology*, 38(4), 552–565. Available from: https://doi.org/10.1111/mve.12756