Flight and swarming behaviour of Culicoides *species (Diptera: Ceratopogonidae) on a livestock farm in Northern Spain*

Mikel A. González[1], Pedro M. Alarcón-Elbal[2], Gert J. Venter[3] and Sergio López[4*]

[1]Division of Biomedical and Life Sciences, Faculty of Health and Medicine, Lancaster University, Bailrigg, Lancashire, LA1 4YG, United Kingdom.
[2]Instituto de Medicina Tropical & Salud Global, Universidad Iberoamericana (UNIBE), Av. Francia 129, Santo Domingo, República Dominicana.
[3]Parasites, Vectors and Vector Borne Diseases, ARC-Onderstepoort Veterinary Institute, Private bag X5, Onderstepoort, 0110, South Africa.
[4] Department of Biological Chemistry and Molecular Modelling, IQAC-CSIC, Jordi Girona 18-26, E08034 Barcelona, Spain.

** Corresponding author at: Department of Biological Chemistry and Molecular Modelling, IQAC-CSIC, Jordi Girona 18-26, E08034, Barcelona, Spain. Tel.: +34 934006108, e-mail: sergio.lopez@iqac.csic.es.*

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Culicoides,
Light trap,
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Swarm,
Sweeping,
Vertical stratification.

Summary
The efficacy of sweep nets and a CDC white light-suction trap for the sampling of *Culicoides* species (Diptera: Ceratopogonidae) were compared on a livestock farm in Northern Spain during the Summer of 2013. A total of 6,082 specimens representing 26 species were collected with sweep nets in 4 areas at different heights (ground level, 1.5 m, and 3 m), and 8,463 specimens representing 28 species with a single white light trap. Eight species - *Culicoides brunnicans, Culicoides punctatus, Culicoides obsoletus/Culicoides scoticus, Culicoides lupicaris, Culcoides picturatus, Culicoides achrayi*, and *Culicoides simulator* - were dominant and accounted for 97.4% and 97.2% of the total specimens collected with both methods, sweep nets, and light traps, respectively. The sex ratios with sweep netting and light trapping were strongly female biased (78.4% and 97.1%, respectively). Nulliparous and parous females were predominantly captured with both methods. A high percentage (17%) of gravid females was, however, captured on manure at ground level while sweeping. Searches for male swarms revealed the presence of several *C. punctatus* swarms consisting of 26 to 196 males and 3 swarms of *C. obsoletus* that ranged from 1 to 12 males in size. This study suggested that both methods are suitable and complementary tools for *Culicoides* sampling.

Presenza e pattern di volo delle specie di Culicoides *(Diptera: Ceratopogonidae) in una fattoria nel Nord della Spagna*

Parole chiave
Culicoides,
Stati fisiologici,
Retino entomologico,
Proporzioni generi maschili e femminili,
Sciame,
Trappola a ventilazione a luce bianca,
Stratificazione verticale.

Riassunto
Durante l'estate del 2013 sono stati comparati in un allevamento in Spagna settentrionale il retino entomologico con la trappola a ventilazione a luce bianca del tipo CDC, testandone l'efficacia nel campionamento delle specie di *Culicoides* (Diptera: Ceratopogonidae). Con il retino entomologico sono stati raccolti in 4 aree a diverse altezze (livello di terra, 1,5 m e 3 m) 6.082 campioni di 26 specie, mentre con una sola trappola a luce bianca sono stati raccolti 8.463 campioni di 28 specie. Otto specie sono risultate dominanti: *Culicoides brunnicans, Culicoides punctatus, Culicoides obsoletus/Culicoides scoticus, Culicoides lupicaris, Culcoides picturatus, Culicoides achrayi*, and *Culicoides simulator*. Queste specie rappresentano il 97,4% e il 97,2% dei campioni complessivamente raccolti, rispettivamente con il retino entomologico e con le trappole a luce bianca. Con entrambi i metodi (rispettivamente il 78,4% e il 97,1%) sono state catturate prevalentemente femmine e nullipare; sullo stallatico a livello del suolo è stata tuttavia catturata una percentuale elevata (17%) di femmine gravide. Le ricerche per gli sciami maschili hanno rivelato la presenza di molti sciami di *C. punctatus* con un numero di maschi da 26 a 196 unità e 3 sciami di *C. obsoletus* che variano da 1 a 12 esemplari maschili. Questo studio ha suggerito che entrambi i metodi sono strumenti appropriati e complementari per il campionamento di *Culicoides*.

Introduction

Culicoides midges (Diptera: Ceratopogonidae) are minute haematophagous flies, some species are responsible for the transmission of pathogens (*e.g.* arboviruses) of medical and veterinary importance for both domestic and wild animals (Purse *et al.* 2015). Among these, bluetongue virus (BTV) has been highlighted as one of the most relevant disease transmitted exclusively by females of certain species of *Culicoides* in West Palearctic region (Mellor and Wittmann 2002). Abundant and widespread *Culicoides* species considered to have played a crucial role in the bluetongue epidemics in Europe include *Culicoides imicola*, the Obsoletus complex (*Culicoides obsoletus* and *Culicoides scoticus*), *Culicoides chiopterus*, *Culicoides dewulfi*, and the Pulicaris complex (*Culicoides pulicaris* and *Culicoides lupicaris*) (Dijkstra *et al.* 2008, Mellor *et al.* 2009, Stephan *et al.* 2009). Although BTV ribonucleic acid was recently detected in *Culicoides montanus*, *Culicoides punctatus*, *Culicoides newsteadi*, and *Culicoides nubeculosus* in Italy (Goffredo *et al.* 2015), the role of these species as potential vectors of BTV remains unproven. Considering that a variety of biologically diverse *Culicoides* species could be involved in the transmission of these viruses, it becomes evident that the accurate detection of all potential vectors in livestock situations would be crucial to clarify the epidemiology of the related diseases.

Following the unexpected outbreak of BTV in 1998 in Europe, exhaustive monitoring and surveillance programmes were initiated using suction light traps. These have subsequently become the standard and most widespread trapping method to detect and quantify the abundance of vectors in the field (Del Río *et al.* 2013, Kirkeby *et al.* 2013, Venter *et al.* 2009a). Although effective in collecting large numbers of night flying insects, light trap results do not necessarily reflect biting rates on the livestock involved, due to the inherent artificial stimulus of the light source (Gerry *et al.* 2009, Viennet *et al.* 2011). Obviously, light traps will not collect day-active species (Meiswinkel and Elbers 2016) and sex distribution may also be biased, as traps apparently tend to capture mainly females (González *et al.* 2013a, González 2014).

Techniques available for the collection of *Culicoides* midges include, amongst others, vehicle-mounted nets, sweep nets, mouth aspirators, pan traps, Malaise traps, emergence traps, drop traps, non-baited suction traps, and coloured traps (González and Goldarazena 2011). Although less commonly used than light traps, sweep nets can be considered as an unbiased and balanced method for the collection of *Culicoides* midges. Due to their lack of attraction, sweep nets will be reliable in determining the spatial distribution of *Culicoides* midges on farm level. The current tendency is to combine sweeping techniques with light traps in the preparation of faunistic inventories (Culicoides.net 2014).

Sweep nets may also be useful for sampling swarms of *Culicoides*. Swarming is a common phenomenon in dipterans, in which males congregate in discrete 'dancing swarms' as part of their mating behaviour (Blackwell *et al.* 1992). Although swarming has been described for some biting midge species (Blackwell *et al.* 1992, Campbell and Kettle 1979, Downes 1955, Zimmerman *et al.* 1982), there is still a lack of information for many species of medico-veterinary interest.

The present study aimed to compare the efficacy of sweep-netting and the white light suction trap as monitoring tools and also to determine the *Culicoides* midge spatial stratification at farm level. In addition, novel data on male swarms of *Culicoides* in Spain are provided.

Materials and methods

Study site

The study was conducted on a livestock farm in Elguea (42°55′59″N; 02°30′51″E; 754 m above sea level), Basque Country, Spain. The farm consisted of a semi-open barn (30 m x 35 m) and a large meadow (120 m x 180 m), where sheep were grazing on pasture during the day (Figure 1). On the right side of the barn there was an asphalted area with some fresh scattered organic matter and a heap of old-composted manure (15 m long, 4 m wide and 1.5 m high) located 15 m away from the barn (Figure 1). The farm housed approximately 300 head of sheep and 15 horses, which rest overnight inside the barn. The perimeter of the farm was surrounded by a mixed and diverse forest, consisting mainly of *Quercus pyrenaica, Cupressus sempervirens, Populus alba, Alnus glutinosa*, and *Pinus* spp. Large numbers of *Culicoides*, including species implicated in the transmission of BTV (*C. obsoletus*/*C. scoticus*, and *C. lupicaris*), had been collected between 2009 and 2012 on this farm (González *et al.* 2013a, Romón *et al.* 2012). Summer in the area is warm and wet, Winter is cold with intense frost. According to Köppen classification, this is an oceanic climate (Cfb), with an average annual temperature of 11˚C and total annual precipitation of 1,100 mm/m^2.

Sampling period and field sampling

Insects were collected once a week for 18 consecutive weeks from May to mid-September

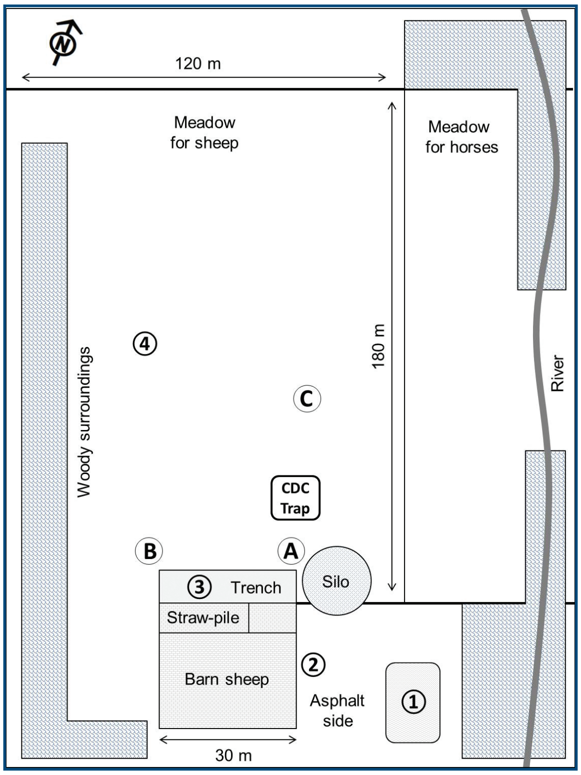

Figure 1. *Map of the farm of Elguea, Basque Country, Spain were the collections have been conducted from May to mid-September 2013.* Alphabetical letters refer to the locations where male swarms where collected (A = silo, B = straw-pile and C = over the meadow). Numbers refer to the four areas selected for sweep net sampling (1 = manure heap, 2 = asphalt, 3 = trench, and 4 = meadow).

2013. Sweep netting was done at a heap of old-composted manure, the asphalt side of the barn, the grassed side below the runoff area that joins the barn with a meadow 'trench', and with the meadow (Figure 1). At each of these 4 sites sweeping was simultaneously performed by sampling midges at ground level, also referred to as 0 m then, 1.5 m, and 3 m. The sweep net consisted of entomological polyester net with a hoop diameter of 38 cm and a 2-part telescopic handle of 75 cm (model F100, Entomopraxis S.C.P., Barcelona, Spain). Collections were made by 2 series of 90 strokes, 45 minutes and 15 minutes before dusk, walking along zig-zag transects, resulting in 180 strokes per area. The collections conducted 45 minutes and 15 minutes before dusk were pooled. The mean time needed to complete one transect (area) was approximately 1 minute, and the order of the sweep netting at the different areas was randomized. Captured specimens were aspirated from the net and transferred to 70% ethanol. Although meteorological data (*i.e.*, temperature, wind speed, and direction) were not recorded, collections from days with unfavourable weather (rain and/or wind) were discarded and repeated the following day.

On the same days of the sweep netting, a CDC white tube light trap (model 1212, J.W. Hock, Gainesville, Florida, USA) was placed on the meadow 1.8 m above the ground and 8 m from the front of the barn (Figure 1). The CDC trap was set at dusk and retrieved at dawn. The trap was switched on immediately after completion of the sweep netting and emptied the next morning. The insects were collected in bottles with water and a drop of odourless soap. The collected flies were transferred to 70% ethanol and stored in the dark until analysed.

During the same period, Mondays to Fridays, the farm was visited daily at 1 hour, 30 min, and 15 min before dusk following a designated itinerary to search for male swarms of *Culicoides* species. All flies corresponding to swarms of males were completely netted. The date, time, location, height, and number of individuals collected were recorded.

Identification and storage

All collected specimens were separated by sex and then morphologically identified under a stereomicroscope to species level with the aid of appropriate identification keys (Delécolle 1985, Glukhova 2005, González and Goldarazena 2011). *Culicoides* midges were sorted according to wing pattern and palpi. If morphological identification with a stereomicroscope was not achievable, specimens were dissected and slide mounted in a medium of Hoyer for microscopic identification. Physiological stages of females were categorized according to the pigmentation of the abdomen as nulliparous, parous, freshly blood-engorged or gravid (Dyce 1969). Sibling species of the subgenus Avaritia were identified to species level except for the members of the Obsoletus complex where only the males were differentiated based on genital characters. Voucher specimens of each species collected were deposited in Neiker-Tecnalia, Basque Institute for Agricultural Research and Development, Vitoria, Spain.

Data analysis

The numbers of midges collected were subjected to Analysis of variance (ANOVA) at a significance level of $\alpha = 0.05$, followed by Tukey's post hoc test for separation of means (SPSS 2004). Prior to the analysis, data were transformed ($\log X + 1$) to correct heteroscedasticity and non-linearity. Proportions of insect counts between treatments were compared using chi-squared tests. Linear regression analysis was carried out in Excel to compare the correlation in numbers collected with sweep netting and the light trap.

Table I. *Total number of* Culicoides *species collected with a white light trap and sweep netting from May to mid-September 2013 at the farm of Elguea, Basque Country, Spain.*

No of collections made	White-light		Sweep-net		Total (%)	% of females	
	18		720 strokes per night over 18 nights				
Species	**Total (%)**	**(X ± SE)**	**Total (%)**	**(X ± SE)**		**W**	**S**
C. brunnicans	3816 (45.1)	**(212.0 ± 95.3) a**	2968 (48.8)	**(164.9 ± 74.8) a**	**6784 (46.6)**	**93.0**	**72.2**
C. punctatus	1302 (15.4)	**(72.3 ± 32.8) a**	812 (13.4)	**(45.1 ± 12.8) a**	**2114 (14.5)**	**98.0**	**62.8**
C. obsoletus/C. scoticus	972 (11.5)	**(54.0 ± 24.8) a**	888 (14.6)	**(49.3 ± 9.0) a**	**1860 (12.8)**	**97.2**	**96.3**
C. lupicaris	984 (11.6)	**(54.7 ± 18.5) a**	536 (8.8)	**(29.8 ± 11.1) a**	**1520 (10.5)**	**99.9**	**95.2**
C. achrayi	422 (5.0)	**(23.4 ± 10.7) a**	338 (5.6)	**(18.8 ± 10.1) a**	**760 (5.2)**	**97.8**	**61.9**
C. picturatus	363 (4.3)	**(20.2 ± 12.2) a**	366 (6.0)	**(20.3 ± 11.8) a**	**729 (5.0)**	**99.1**	**98.1**
C. simulator	361 (4.3)	**(20.1 ± 9.7) a**	11 (0.2)	**(0.6 ± 0.2) b**	**372 (2.6)**	**99.9**	**99.4**
C. tauricus	68 (0.8)	3.7	5 (<0.1)	0.27	**73 (0.5)**	-	-
C. vexans	9 (<0.1)	0.5	46 (0.8)	2.55	**55 (0.4)**	-	-
C. poperinghensis	41 (0.5)	2.27	11 (0.2)	0.61	**52 (0.4)**	-	-
C. kibunensis	3 (<0.1)	0.16	29 (0.5)	1.61	**32 (0.2)**	-	-
C. furcillatus	10 (<0.1)	0.5	13 (0.2)	0.72	**23 (0.2)**	-	-
C. pulicaris	18 (0.2)	1	4 (<0.1)	0.22	**22 (0.2)**	-	-
C. santonicus	15 (0.2)	0.83	5 (<0.1)	0.26	**20 (0.1)**	-	-
C. festivipennis	20 (0.2)	1.1	0 (0)	0.0	**20 (0.1)**	-	-
C. dewulfi	2 (<0.1)	0.11	16 (0.3)	0.88	**18 (0.1)**	-	-
C. pallidicornis	9 (<0.1)	0.5	7 (<0.1)	0.38	**16 (0.1)**	-	-
C. pictipennis	15 (0.2)	0.83	1 (<0.1)	0.05	**16 (0.1)**	-	-
C. fascipennis	12 (<0.1)	0.6	1 (<0.1)	0.05	**13 (0.1)**	-	-
C. fagineus	9 (<0.1)	0.5	2 (<0.1)	0.11	**11 (0.1)**	-	-
C. chiopterus	1 (<0.1)	0.05	7 (<0.1)	0.38	**8 (0.1)**	-	-
C. circumscriptus	3 (<0.1)	0.16	4 (<0.1)	0.22	**7 (<0.1)**	-	-
C. heliophilus	0 (0)	0.0	5 (<0.1)	0.27	**5 (<0.1)**	-	-
C. kurensis cf.	3 (<0.1)	0.16	2 (<0.1)	0.11	**5 (<0.1)**	-	-
C. parroti	0 (0)	0.0	3 (<0.1)	0.16	**3 (<0.1)**	-	-
C. minutissimus	0 (0)	0.0	2 (<0.1)	0.11	**2 (<0.1)**	-	-
C. paradisionensis	2 (<0.1)	0.11	0 (0)	0.0	**2 (<0.1)**	-	-
C. alazanicus	1 (<0.1)	0.05	0 (0)	0.0	**1 (<0.1)**	-	-
C. longipennis	1 (<0.1)	0.05	0 (0)	0.0	**1 (<0.1)**	-	-
C. shaklawensis	1 (<0.1)	0.05	0 (0)	0.0	**1 (<0.1)**	-	-
Total	**8463**	**(470.2 ± 110.8) a**	**6082**	**(337.9 ± 79.6) a**	**14545**	**97.1**	**78.4**
Species richness	**28**		**26**		**31**		

Different letters in bold denote statistical differences at 5% level for the most abundant *Culicoides* species according to Tukey´s test. W = White-light; S = Sweep-net.

Results

Comparison of white light trap and sweep net sampling

A total of 14,545 *Culicoides* specimens belonging to at least 31 species were collected over 18 consecutive weeks from May to mid-September 2013 (Table I). Of these, 6,082 specimens representing 26 species were collected with sweep netting, and 8,463 specimens representing 28 species with the white light trap (Table I). The lower mean number of 470.2 ± 110.8 midges collected in 18 nights of sweep netting (180 swipes at 4 sites per night) was not significantly different

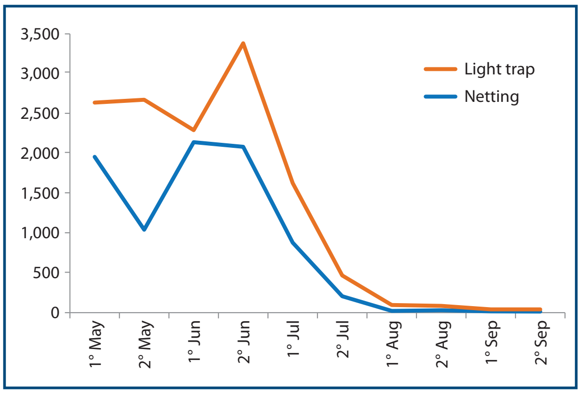

Figure 2. Culicoides *abundance (grouped in fortnights) from May to mid-September 2013 in the farm of Elguea (Basque Country, Spain) as determined with 2 collection methods.*

from the mean number of 337.9 ± 79.6 collected in the 18 light trap collections. The mean number of *Culicoides* for the 8 most abundant species was not statistically different when comparing both collection methods, with the exception of *C. simulator* species (Table I). With both methods, higher numbers were captured during the first part of Summer, between May and July, with a peak in June (Figure 2). The numbers collected gradually decreased from the first week in July during Summer, with just a few specimens captured from August to September (Figure 2).

Linear regression indicated a correlation coefficient of $r^2 = 0.98$ in the species composition as determined with light trapping and sweep netting. Both methods indicated *Culicoides brunnicans*, representing 45.1% and 48.8% in the white light and sweep netting collections respectively, to be the most abundant (Table I). Only single specimens of *Culicoides festivipennis*, *Culicoides longipennis*, *Culicoides paradisionensis*, and *Culicoides shaklawensis* were collected with the light traps. Similarly, low numbers of *Culicoides heliophilus*, *Culicoides minutissimus*, and *Culicoides parroti* were collected with sweep nets (Table I). Based on male genitalia, 36% of the collected specimens of *C. obsoletus*/*C. scoticus* with sweep netting were *C. obsoletus* and 64% *C. scoticus*; whereas light trap collections accounted for 68% *C. obsoletus* and 32% *C. scoticus*.

Most of the species in light trap collections showed a strong female-biased sex ratio (97.1%) (Table I). Sweep netting results were also biased toward females, but to a lesser extent (78.4%), *e.g.* *C. obsoletus*/*C. scoticus*, *C. lupicaris*; *C. simulator* female proportion values were above 95%, in contrast to *C. brunnicans* (72.2%), *C. achrayi* (61.9%), and *C. punctatus* (62.8%) (Table I). Comparison of the physiological stages of the females collected with sweep netting revealed that 48% were nulliparous, 33% parous, 17% gravid, and 2% blood-engorged, while light trap showed 52% of nulliparous, 41% parous, 4% gravid, and 3% blood-engorged.

Culicoides abundance and distribution as determined with sweep netting

Comparison of sweep netting results for the 4 areas indicated that *Culicoides* were not equally abundant throughout the farm ($\chi = 2481.2$, df = 3, $P < 0.001$) (Figure 3). Of 6,082 *Culicoides* collected, the majority – 2,611 (42.9%) – was found in the trench area (Figure 1). Relatively high numbers, 2,077 (34.2%), were also collected in the asphalt area. The manure (897) and meadow (497) areas accounted for only 14.7% and 8.2% of the collected midges, respectively. While a number of different species were found to be about equally abundant in the meadow area, the other 3 areas were dominated by single species (Figure 3). *Culicoides brunnicans*, accounting for 61% and 67% of the collected samples, was the most common midge in the trench and asphalt, respectively. Whereas *C. obsoletus*/*C. scoticus* dominated in manure environments, comprising 77% of the midges collected (Figure 3). *Culicoides achrayi* species was well-represented in the meadow (34%), but it was less abundant in the other areas (< 6%) (Figure 3).

Vertical stratification

With sweep netting the majority of the midges were collected at 1.5 m (2,550 specimens, 42%) and 3 m (2,674 specimens, 44%). Only 858 specimens (14%) were collected at ground level (0 m). The proportional representation at the various heights was statistically significant ($\chi^2 = 1523.2$, df = 2, $P < 0.001$). The higher mean numbers collected at 1.5 m (141.6 ± 44.9) and 3 m (148.5 ± 48.2) were statistically different ($P < 0.05$) from that collected at 0 m (47.6 ± 16.0) (Table II).

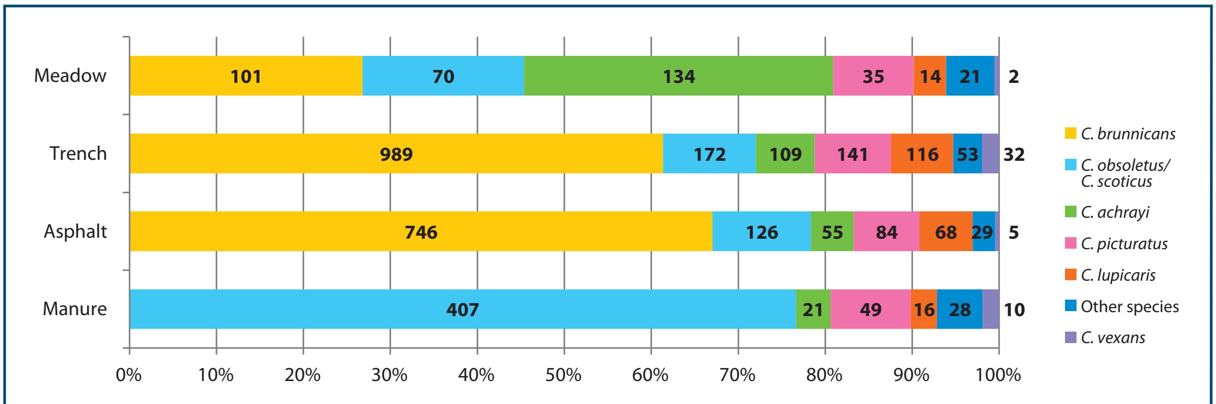

Figure 3. *Distribution of the abundant* Culicoides *species in the 4 sampled areas (different heights gathered) from May to mid-September 2013 in the farm of Elguea (Basque Country, Spain).* "Other species" refer to the remaining 21 *Culicoides* species collected.

Table II. *Vertical stratification of* Culicoides *(at 0, 1.5 and 3 m) for the 4 sampled areas in the farm of Elguea (Basque Country, Spain) in the period ranging from May to mid-September 2013.*

Zone	Total	0 m (X ± SE)	1.5 m (X ± SE)	3 m (X ± SE)
Manure	897	29.4 ± 7.6 (48) **a**	21.5 ± 5.5) (31) **a**	12.6 ± 3.3 (21) **b**
Asphalt	2077	7.7 ± 2.0 (6) **b**	40.0 ± 10.3 (36) **a**	75.5 ± 19.5 (59) **a**
Trench	2611	17.8 ± 4.3 (9) **a**	93.7 ± 24.2 (51) **b**	69.9 ± 10.1 (40) **ab**
Meadow	497	5.0 ±1.3 (17) **a**	12.8 ± 3.3 (39) **ab**	22.3 ± 5.7 (44) **b**
Total	**6082**	**47.6 ± 16.0 b**	**141.6 ± 44.9 a**	**148.5 ± 48.2 a**

Different letters in bold denote statistically differences at the 5% level (Tukey´s test).

In the manure area, significantly (P < 0.05) more midges were collected at 0 m (29.4 ± 7.6) and 1.5 m (21.5 ± 5.5) than at 3 m (12.6 ± 3.3) (Table II). In the asphalt area, significantly fewer midges were collected at 0 m (7.7 ± 2.0) than at 1.5 m (40.0 ± 10.3) and 3 m (75.5 ± 19.5) (Table II). In the trench and meadow areas, the lower mean number collected at ground level was not significantly different than the one for the collections at 3 m and 1.5 m (Table II).

A relative high proportion of *C. obsoletus*/*C. scoticus* (46%) was captured at ground level, whereas the remaining species comprised less than 17% at this level. The number of *C. achrayi* captured at 1.5 m was lower than at 3 m, while in *C. punctatus*, *C. brunnicans*, and *C. obsoletus*/*C. scoticus* the proportions were quite similar, although more specimens of *C. picturatus* and *C. achrayi* were collected at 1.5 m than at 3 m.

Overall the main differences among areas were observed at ground level, while the proportions of the different gonotrophic stages at 1.5 and 3 m were similar (Figure 4). At ground level, the percentage of freshly-blood fed specimens was 31% and 18% in trench and meadow respectively, whereas in asphalt and manure comprised less than 0.5%. Similarly, the manure and the meadow contained 73% and 56% of gravid females (Figure 4).

Swarms

A total of 15 male swarms were observed and sampled between mid-June to mid-July (Table III). All these swarms were collected in the vicinity of the barn (Figure 1). Most of them consisted of *C. punctatus*, with 919 males ($\chi \pm SD = 76.5 \pm 50.3$) captured at 2 specific sites (Table III). The number of males in these swarms varied from 26 to 196 (Table III). Swarms of *C. obsoletus* were only observed over the meadow and swarm sizes were smaller than that of *C. punctatus* (Table III). A single mating couple of *C. obsoletus* was captured 'in fraganti' copulating in the air over the meadow (Figure 5). All swarms were found in June and July, between 1.8 m and 2.5 m above ground level and within the interval of 1 hour before sunset (Table III).

Discussion

The relatively high correlation in species composition as determined with light traps and sweep nets indicated that both methods are suitable for monitoring *Culicoides* species. Nearly the same number of species (26 with sweep nets versus 28 with light trap) were captured in the same proportions with the two methods. Species collected included the most common vectors for arboviruses in Europe (*e.g.*, *C. obsoletus*, *C. scoticus* and *C. lupicaris*).

In the present study, sweep netting was conducted before sunset and indicated that most of *Culicoides* species were active before sunset. As suggested by Meiswinkel and Elbers, the large numbers collected in a relative short period with sweep netting may indicate that the light trap underestimates the abundance of *Culicoides* in an area (Meiswinkel and Elbers 2016). This may be particularly relevant for potential vectors, such as *C. chiopterus* and *C. dewulfi*, which are usually less abundant in light trap collections. Sweep netting may also be useful for trapping species with diurnal preferences,

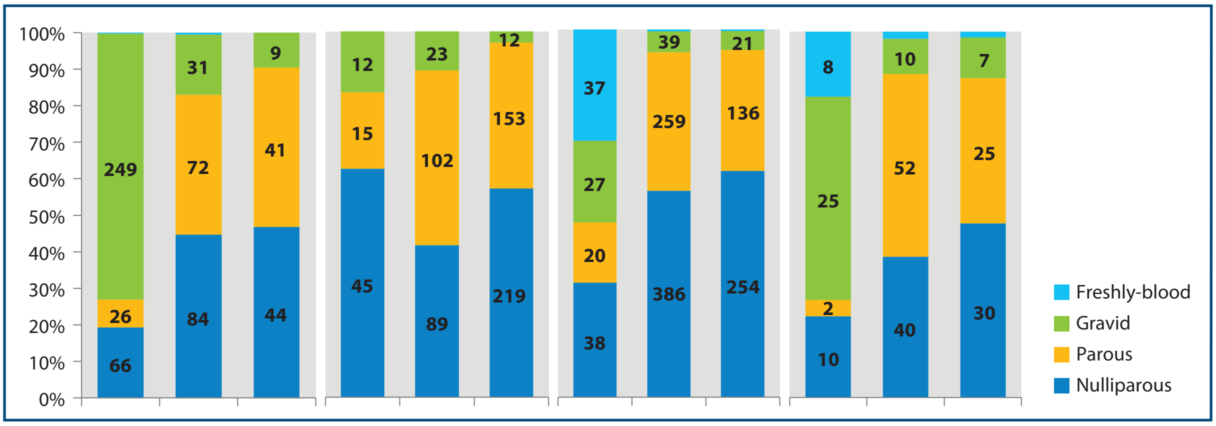

Figure 4. *Stratification of the different gonotrophic stages of* Culicoides *females trapped with aerial sweeping at different heights (0, 1.5 and 3 m) in 4 sampling areas (manure, asphalt, trench and meadow) from May to mid-September 2013 in the farm of Elguea (Basque Country, Spain).*

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Table III. *Characteristics of* Culicoides *swarms in the farm of Elguea (Basque Country, Spain) from May to mid-September 2013.*

Species	Date	Average height (m)	Sampling hour (pm)[a]	No of males	Farm position[b]
C. punctatus	June 18	2.5	09:30	90	A
C. punctatus	June 18	2.2	09:55	44	A
C. punctatus	June 25	2	09:10	61	A
C. punctatus	June 25	2	09:30	52	A
C. punctatus	June 25	2	09:50	70	B
C. punctatus	June 26	2	09:40	196	A
C. obsoletus	June 27	2	09:40	1 + 1*	C
C. punctatus	July 2	2.2	09:10	26	A
C. obsoletus	July 2	2	09:25	12	C
C. obsoletus	July 8	1.8	09:35	8	C
C. punctatus	July 16	1.8	09:00	55	A
C. punctatus	July 16	2	09:15	120	B
C. punctatus	July 16	2	09:25	147	B
C. punctatus	July 17	2.5	09:50	30	B
C. punctatus	July 17	2	09:00	32	B

[a] As reference, sunset 5th of June occurred at 9:50 p.m.; [b] Capital letters indicate the farm position; A = Near silo (edge of the barn corner); B = Near straw-bale; C = Over the meadow (about 20-25 m away from the front of the barn).
* A single female.

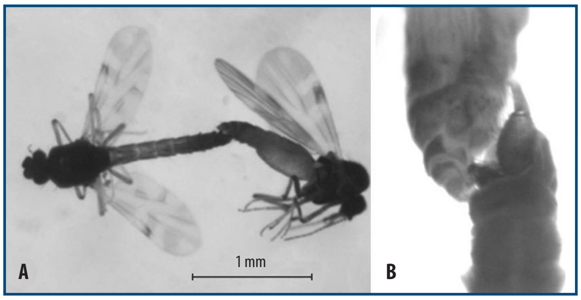

Figure 5. *Mating couple of* Culicoides obsoletus *captured with aerial sweeping in the meadow at 2 m above ground level (**A**) and detail of the genitalia of both sexes while mating (**B**) from May to mid-September 2013 in the farm of Elguea (Basque Country, Spain).*

such as *C. heliophilus* (Kettle 1962), which was only collected with sweep netting in this study.

The proportional representation of *C. obsoletus*/*C. scoticus*, based on males, contrasted with previous studies in this area, in which ultraviolet-traps collected higher proportions of *C. obsoletus* (González *et al.* 2013b). Similarly, sweep netting probably overrated the numbers of *C. scoticus*. This species was, thus, either less attracted to white light or the weather conditions that year may have been more favourable for its breeding, *i.e.* leaf litter in the forest near the farm. Nonetheless, the shared habitat preferences, along with apparent similarities in host preferences (Ninio *et al.* 2011) may explain why *C. obsoletus* and *C. scoticus* are often found equally distributed in light-suction trap surveys in some European countries (Gomulski *et al.* 2005).

The sex ratios in biting midge populations are assumed to be close to 1:1. Although trapping techniques, such as emergence traps, show balanced and realistic sex ratios, skewed sex ratios have been reported in biting midges (Braverman 1978, Kettle and Lawson 1952, Root and Gerhardt 1991). Light trap catches are prone to be female biased with males usually representing less than 2% to 5% of the collected midges (González *et al.* 2013a, Schulz 2012, Venter *et al.* 2009 a, b). This trend was also observed in the present study. Different hypotheses have been proposed to explain this unbalanced gender distribution, such as:

i. light sources predominantly attract females (Venter and Hermanides 2006);
ii. males disperse over shorter distances than females (Mullen 2009);
iii. females have a longer life span than males (Hunt and Schmidtmann 2005);
iv. larval developmental sites and swarming occur far away from traps (Mehlhorn *et al.* 2007, Vorsprach *et al.* 2009).

The latter hypothesis can, however, be dismissed in view of the evidence presented in present and previous studies on this farm (González *et al.* 2013b). Although it was envisaged that sweep netting would display a more balanced sex ratio in the present study, the gender ratio was still found to be female biased. This can partly be ascribed to the fact that the swarms sampled around the barn housing the sheep at night were mostly female.

Although light-trap results are influenced by a number of critical factors, such as trap type and light source, the height and position of the trap, the animal species, number of hosts, and the vicinity of the trap, they lure and capture mainly nulliparous and parous females actively flying around in search of a blood meal (Del Río 2012, Venter *et al.* 2009 a, b). Taking into account the seasonal variation in emergence, the proportions of parous females may be higher in Winter (Lysyk 2007). In the present study, conducted in the Summer, proportionally more nulliparous than parous females were collected by both methods. Sweep netting, however, captured a substantial number of gravid females on the vegetation (17%), particularly at ground level in the meadow and on manure habitats. Interestingly, during a drought in July and August, a remarkable number of gravid females of *C. obsoletus*/*C. scoticus* and *C. brunnicans* were found resting on the manure and grassed runoff areas, and only a few specimens were captured while flying. Ground level would allow females to explore the suitability of potential oviposition sites. Furthermore, a considerable number of blood-engorged females were found resting at ground level. Thus, it may be probable that after feeding on a preferred host, females tend to move rapidly to their breeding sites and do not spend much time flying. Blood engorged and gravid females were common in grassed habitats (meadow and trench), and this may partly explain the low numbers of blood-engorged females collected with the light trap. These observations, however, need to be verified with large-scale surveys.

Swarming behaviour for *C. punctatus* and *C. obsoletus* are not well documented (Downes 1955). To our knowledge, this is the first evidence of the mating behaviour of *C. obsoletus* species in Spain. Finding a mating pair of *C. obsoletus* in the air, suggesting that mating occur in flight, has only been recorded once before (Downes 1955). Male swarms have been recorded in species of the same subgenus, *e.g. C. imicola* (Morag *et al.* 2013). Despite the low number of swarms sampled, the present study showed *C. obsoletus* swarms to be smaller than *C. punctatus*. Male swarms of *C. punctatus* were common near their emergence sites, where the males apparently used features such as a straw-bale and silo, which may have provided protection against wind, as specific markers. Interesting is the confirmation that the *C. punctatus* swarms consisted almost entirely of males located close to putative larval development sites (moist patches of grass and mud below the roof). In contrast, the ecological markers for *C. obsoletus* were less specific and swarms were collected over mud patches in the meadow, slightly farther away from their main emergence sites (composted-old heaps of manure) (González *et al.* 2013b). A similar lack of specificity has also been recorded for *C. impunctatus* (Blackwell *et al.* 1992). Although swarms are almost entirely formed by males, virgin females may enter these swarms to mate before initiating host-seeking behaviour (Anderson 1974). In contrast to our results, *C. punctatus* females may be present in low numbers in these swarms.

The female/male ratio within swarms varies, *e.g.* in *Culicoides impunctatus* it ranged from 1 to 9.5 (Blackwell *et al.* 1992), in *Culicoides brevitarsis* from 1 to 77 (Campbell and Kettle 1979), and from 1 to 167 in *Culicoides variipennis* (Gerry and Mullens 1998, Zimmerman *et al.* 1982). Although females appear also to fly in aggregates, there is little information on the significance of the spectacular clouds of females observed around the barn during May and June. Downes described immense groups of *Culicoides* females found in dense clouds along both sides of a byre divided into an upper, shaded region, and a lower, brightly illuminated layer (Downes 1955). Although these female swarms may be misidentified as male swarms, midges were unequivocally identified based on specific formations (huge horizontal clouds) and the fact that females display irregular flight patterns different to the regular dance of male swarming (Blackwell *et al.* 1992, Downes 1955).

Taking into account the similarity in species composition and abundance as determined with the 2 techniques, sweep netting can be used as a complementary method to light traps for the collection of *Culicoides* midges. Sweep netting will minimize the female-biased sex ratio in some species. While light traps will give an accurate representation of the midges present in an area, sweep netting more accurately determines the spatial distribution of the species and the various gonotrophic stages throughout an area. This information will be essential for inferring the age-grading of *Culicoides* populations and estimation of the survival of the vectors. Potential shortcomings of light-suction traps, (*e.g.*, estimating diurnal midge activity, host preference, and biting rates) (Elbers and Meiswinkel 2014), could be offset by complementary sampling with other collection methods, such as sweep-netting and direct collection on host animals. The new and interesting observations on swarming behaviour contributes to a better understanding of their biology and might serve to improve current techniques for midge surveillance and control.

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References

Anderson J.R. 1974. Symposium on reproduction of arthropods of medical and veterinary importance. II. Meeting of the sexes. *J Med Entomol*, **11**, 7-19.

Blackwell A., Mordue (Luntz) A.J., Young M.R. & Mordue W. 1992. The swarming behaviour of the Scottish biting midge, *Culicoides impunctatus* (Diptera: Ceratopogonidae). *Ecol Entomol*, **17**, 319-325.

Braverman Y. 1978. Characteristics of *Culicoides* (Diptera, Ceratopogonidae) breeding places near Salisbury, Rhodesia. *Ecol Entomol*, **3**, 163-170.

Campbell M.M. & Kettle D.S. 1979. Swarming of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) with reference to markers, swarm size, proximity of cattle, and weather. *Aust J Zool*, **27**, 17-30.

Culicoides.net. 2014. Taxonomy. Developed and hosted by the Institute for Animal Health. Powered by (http://www.culicoides.net/ accessed on 18 February 2014).

Del Río R. 2012. Estrategias de control y competencia vectorial de las especies de *Culicoides* vectores del virus de la lengua azul en Península Ibérica y Baleares. Thesis, Universitat de les Illes Balears, Spain, 240 pp.

Del Río R., Monerris M., Miquel M., Borràs D., Calvete C., Estrada R., Lucientes J. & Miranda M.A. 2013. Collection of *Culicoides* spp. with four light trap models during different seasons in the Balearic Islands. *Vet Parasitol*, **195**, 150-156.

Delécolle J.C. 1985. Nouvelle contribution à l'étude systematique et iconographique des espèces du genre *Culicoides* (Diptera: Ceratopogonidae) du nord-est de la France. Thesis, Université Louis Pasteur de Strasbourg, UER Sciences, Vie et Terre, 238 pp.

Dijkstra E., Van der Ven I.J., Meiswinkel R., Holzel D.R. & Van Rijn P.A. 2008. *Culicoides chiopterus* as a potential vector of bluetongue virus in Europe. *Vet Rec*, **162**, 422.

Downes J.A. 1955. Observations on the swarming flight and mating of *Culicoides* (Diptera: Ceratopogonidae). *Trans Entomol Soc Lond*, **106**, 439-453.

Dyce A.L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *J Aust Entomol Soc*, **8**, 11-15.

Elbers A.R.W. & Meiswinkel R. 2014. *Culicoides* (Diptera: Ceratopogonidae) host preferences and biting rates in the Netherlands: comparing cattle, sheep and the black-light suction trap. *Vet Parasitol*, **205**, 330-337.

Gerry A. & Mullens B.A. 1998. Response of male *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) to carbon dioxide and observations of mating behavior on and near cattle. *J Med Entomol*, **35**, 239-244.

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. *J Med Entomol*, **46**, 615-624.

Glukhova V.M. 2005. *Culicoides* (Diptera, Ceratopogonidae) of Russia and adjacent lands. *Int J Dipterol Res*, **16**, 3-75.

Goffredo M., Catalani M., Federici V., Portanti O., Marini V., Mancini G., Quaglia M., Santilli A., Teodori L. & Savini G. 2015. Vector species of *Culicoides* midges implicated in the 2012-2014 Bluetongue epidemics in Italy. *Vet Ital*, **51**, 131-138.

Gomulski L.M., Meiswinkel R., Delecolle J.C., Goffredo M. & Gasperi G. 2005. Phylogenetic relationships of the subgenus *Avaritia* Fox, 1955 including *Culicoides obsoletus* (Diptera, Ceratopogonidae) in Italy based on internal transcribed spacer 2 ribosomal DNA sequences. *Syst Entomol*, **30**, 619-631.

González M. 2014. The genus *Culicoides* (Diptera: Ceratopogonidae) in the Basque Country. Thesis, University of the Basque Country, Spain. 327 pp. (in Spanish).

González M. & Goldarazena A. 2011. El género *Culicoides* en el País Vasco: guía práctica para su identificación y control. Servicio Central de Publicaciones del Gobierno Vasco, Vitoria, España. 247 pp.

González M., López S., Mullens B.A., Baldet T. & Goldarazena A. 2013b. A survey of *Culicoides* developmental sites on a farm in Northern Spain with a brief of immature habitats of European species. *Vet Parasitol,* **191**, 81-93.

González M., López S., Romón P., Baldet T., Delécolle J.C. & Goldarazena A. 2013a. Monitoring of *Culicoides* Latreille (Diptera: Ceratopogonidae) after BTV outbreaks in sheep farms and in natural habitats from the Basque Country (N Spain). *Proc Entomol Soc Wash*, **115**, 48-69.

Hunt G.J. & Schmidtmann E.T. 2005. Care, maintenance and experimental infection of biting midges. *In* Biology of Disease Vectors, 2nd Ed. (W.C. Marquardt, ed). Academic Press, London, 816 pp.

Kettle D.S. 1962. The bionomics and control of *Culicoides* and *Leptoconops*. (Diptera, Ceratopogonidae = Heleidae). *Ann Rev Entomol*, **7**, 401-408.

Kettle D.S. & Lawson J.W.H. 1952. The early stages of British midges *Culicoides* Latreille (Diptera: Ceratopogonidae) and allied genera. *Bull Entomol Res*, **43**, 421-467.

Kirkeby C., Græsbøll K., Stockmarr A., Christiansen L.E. & Bødker R. 2013. The range of attraction for light traps catching *Culicoides* midges (Diptera: Ceratopogonidae). *Parasit Vectors*, **6**, 67.

Lysyk T.J. 2007. Seasonal abundance, parity, and survival of adult *Culicoides sonorensis* (Diptera: Ceratopogonidae) in Southern Alberta, Canada. *J Med Entomol*, **44**, 959-969.

Mehlhorn H., Walldorf V., Klimpel S., Jahn B., Jaeger F., Eschweiler J., Hoffmann B. & Beer M. 2007. First occurrence of *Culicoides obsoletus*-transmitted bluetongue virus epidemic in Central Europe. *Parasitol Res*, **101**, 219-228.

Meiswinkel R. & Elbers A.R. 2016. The dying of the light: crepuscular activity in *Culicoides* and impact on light trap efficacy at temperate latitudes. *Med Vet Entomol*, **30**, 53-63.

Mellor P.S., Carpenter S. & White D.M. 2009. Bluetongue virus in the insect host. *In* Bluetongue (P.S. Mellor, ed). Academic Press, Amsterdam.

Mellor P.S. & Wittmann E. 2002. Bluetongue virus in the Mediterranean basin 1998 to 2001. *Vet J*, **164**, 20-37.

Morag N., Mullens B.A. & Gottlieb Y. 2013. Assessment of survival and body size variation in *Culicoides imicola* (Diptera: Ceratopogonidae) as a function of *Cardinium* (Bacteriodetes) infection status. *Appl Environ Microbiol*, **79**, 6260-6263.

Mullen G.R. 2009. Biting Midges (Ceratopogonidae). *In* Medical and Veterinary Entomology, 2nd Ed. (G.R. Mullen & L.A. Durden, eds). Academic Press, London, 169-188.

Ninio C., Augot D., Delécolle J.C., Dufour B. & Depaquit J. 2011. Contribution to the knowledge of *Culicoides* (Diptera: Ceratopogonidae) host preferences in France. *Parasitol Res*, **108**, 657-673.

Purse B.V., Carpenter S., Venter G.J., Bellis G. & Mullens B.A. 2015. Bionomics of temperate and tropical *Culicoides* midges: knowledge gaps and consequences for transmission of *Culicoides*-borne viruses. *Ann Rev Entomol*, **60**, 373-392.

Romón P., Higuera M., Delécolle J.C., Baldet T., Aduriz G. & Goldarazena A. 2012. Phenology and attraction of potential *Culicoides* vectors of bluetongue virus in Basque Country (northern Spain). *Vet Parasitol*, **186**, 415-424.

Root D.S. & Gerhardt R.R. 1991. Seasonal emergence patterns of *Culicoides* (Diptera: Ceratopogonidae) in eastern Tennessee. *J Agri Entomol,* **8**, 127-135.

Schulz C. 2012. *Culicoides* fauna and bluetongue virus serotype 8 infection in South American camelid herds in Germany. Thesis, Justus-Liebig-Universität Gießen, Germany, 168 pp.

SPSS. 2004. SYSTAT Statistical Package, Version 13.0. SPSS, Chicago, IL.

Stephan A., Clausen P.H., Bauer B. & Steuber S. 2009. PCR identification of *Culicoides dewulfi* midges (Diptera: Ceratopogonidae), potential vectors of bluetongue in Germany. *Parasitol Res*, **10**, 367-371.

Venter G.J. & Hermanides K.G. 2006. Comparison of black and white light for collecting *Culicoides imicola* and other livestock-associated *Culicoides* species in South Africa. *Vet Parasitol*, **142**, 383-385.

Venter G.J., Hermanides K.G., Boikanyo S.N., Majatladi D.M. & Morey L. 2009b. The effect of light trap height on the numbers of *Culicoides* midges collected under field conditions in South Africa. *Vet Parasitol*, **166**, 343-345.

Venter G.J., Labuschagne K., Hermanides K.G., Boikanyo S.N.B., Majatladi D.M. & Morey L. 2009a. Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of *Culicoides* species. *Vet Parasitol*, **166**, 299-307.

Viennet E., Garros C., Lancelot R., Allène X., Gardès L., Rakotoarivony I., Crochet D., Delécolle J.C., Moulia C., Baldet T. & Balenghein T. 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. *Parasit Vectors*, **4**, 119.

Vorsprach B., Meiser C.K., Werner D., Balczun C. & Schaub G.A. 2009. Monitoring of Ceratopogonidae in southwest Germany. *Parasitol Res*, **105**, 337-344.

Zimmerman R.H., Barker S.J. & Turner Jr E.C. 1982. Swarming and mating behaviour of a natural population of *Culicoides variipennis* (Diptera. Ceratopogonidae). *J Med Entomol*, **19**, 151-156.