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Comparison of different light sources for trapping *Culicoides* biting midges, mosquitoes and other dipterans

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Highlights

- Biting nematocerans and mosquitoes showed a clear affinity to specific wavelenghts, being fluorescent light sources more efficient than incandescent and LED lights.

- Proven vectors of important economically diseases such as *Culicoides obsoletus/C. scoticus* are highly attracted to traps fitted with ultraviolete (UV) and green LEDs.

- The paper highlighted the potential use of traps equipped with light sources emitting in wavelenghts close to 570 nm (green colour).

ABSTRACT

The response of *Culicoides* biting midges, mosquitoes and other dipterans to different wavelengths was evaluated in a farm meadow in northern Spain. A total of 9449 specimens of 23 species of *Culicoides*, 5495 other ceratopogonids (non-biting midges), 602 culicids and 12428 other mixed dipterans were captured. Centers for Disease Control and Prevention (CDC) suction light traps fitted with five light emitting diodes (LEDs) (white, green, red, blue, ultraviolet) were run for 15 consecutive nights. Significantly more *Culicoides* were collected in those traps fitted with green, blue or ultraviolet (UV) lights than in red and white-baited LED traps for the most abundant species captured: *C. punctatus* (37.5%), *C. cataneii* (26.5%) and *C. obsoletus*/*C. scoticus* (20.4%). Similar results were obtained for non-*Culicoides* ceratopogonids, mosquitoes and other mixed dipterans. Wavelengths in green (570 nm) resulted effective for targeting some *Culicoides* species, culicids and other midges. In a second trial, the effectiveness of 4-watt white and UV tubes was compared to traps fitted with UV LED and a standard incandescent light bulb. More specimens of all taxa were collected with fluorescent black light (UV) traps than with the other light sources, except culicids, which were recovered in high numbers from fluorescent white light traps.

Key words: Diptera, Ceratopogonidae, Culicidae, *Culicoides*, fluorescent traps, LED traps.

1. Introduction

Various trapping devices for insects are commercially available, which are used for nuisance reduction, monitoring or surveillance of Diptera (Lühken et al., 2014). Light traps are one of the most commonly used devices, particularly to attract insects with phototaxis. The use of light traps for sampling dipterans with relevance as disease vectors has been studied by many researchers since the mid-twenties (Odetoyinbo, 1969). Among the wide range of light traps developed, CDC-light traps (Centers for Disease Control and Prevention light traps) were introduced originally for arbovirus surveillance and other short-term mosquito investigations. They provide a reliable method for monitoring disease vectors with minimal exposure (Cohntaedt et al., 2008), avoiding unsafe methods such as animal or human bite collection. Since their introduction, several modifications to these traps have been made to improve their effectiveness, being the modern models (CDC miniature trap models 512 and 1212), the most common tools for monitoring *Culicoides* species. These traps have routinely been used in surveillance programs in the USA (Smith and Mullens, 2003), but also in many European countries, including France (Venail et al., 2012), Spain (Pérez et al., 2012; González et al., 2013) or Portugal (Ramilo et al., 2012). Black lights (UV) are superior to white light in terms of specimens and species collected (Venter et al., 2009), depending on the type of trap (design, size and intensity of light source, etc.).

In an effort to develop a highly effective visual target for improved surveillance of different economically important vectors, Burket et al. (1998) used for first time a new generation of lighting technology based on super-bright light-emitting diodes (LEDs). These are energy efficient, often producing a greater total photon flux (TPF) than incandescent globes in the visible spectrum (400-780 nm), making them optimal for battery operation (Bishop et al., 2004). LEDs have become widely available and popular substitutes for incandescent light over the past 18 years. Their advantages include greatly reduced power consumption, high efficiency, accuracy in specific wavelength achievement, cool operating temperatures, durability, less prone to shock damage, compact size, excellent colour saturation, and monochromatic light production in a wide variety of possible wavelengths (Hoel et al., 2007).

Several recent studies have tested LED colours and whether LEDs can serve as effective substitutes for incandescent lamps in standard CDC mosquito traps for mosquito surveillance as well as to determine the most appropriate colour for attracting these vectors (Tchouassi et al., 2012). Blood-feeding Diptera such as mosquitoes, sand flies and biting midges, often are attracted to specific wavelengths of light: UV, blue and green (Wilton and Fay, 1972; Mellor and Hamilton, 2003; Bishop et al., 2004; Burkett and Butler, 2005; Fernández et al., 2015). LED tests on *Culicoides* biting midges have been done in Australia (Bishop et al., 2004, 2006), Africa (Tchouassi et al., 2012), and most recently in South America (Silva et al., 2015) and Europe (Hope et al., 2015). Only a few publications describe the attractiveness of LEDs to different Diptera species.

Therefore, the objective of this study was to determine wavelength preference of adult *Culicoides*, culicids, and selected non-target dipterans, using LEDs technology and standard fluorescent and incandescent light sources.

2. Material and methods

2.1. Study area

Trapping studies were done at Neiker-Tecnalia, Basque Institute for Agricultural Research and Development, Vitoria-Gasteiz, Northern Spain, coordinates: 42º 51´ 43´´ N; 2º 38´ 84´´ W, elevation 517 masl. This area consists primarily of extensive sheep farming, with a flat landscape bearing a variety of trees and bushes. Sheep flocks were enclosed at night until the next morning to avoid interferences in the study. Traps were placed in the middle of a meadow (200 m x 150 m), which was occasionally irrigated, creating temporary pools of water that provided suitable conditions for the development of *Culicoides* species as well as other dipterans.

2.2. Collection methods

The first trial occurred from mid-July to early August 2013 over 15 consecutive nights. Traps were hung 15 m apart in a randomised block design at a height of 1.5 m and separated by 15 m from each other to prevent interference between traps. Five CDC-miniature portable light traps model 512 (John W. Hock Company, Florida, U.S.A.) featured five different LED platform arrays (Bioquip, Rancho Dominguez, U.S.A). Different LED bulbs emitted light which was: white between 425 to 750 nm, red 660 nm, green 570 nm, blue 430 nm and UV 390 nm. Adapters consisted of eight LED units oriented in all directions (360º). Each day, all traps were rotated to new positions to reduce sampling point specific differences.

A second trial was run over 12 nights in mid-August. Traps were hung, rotated and positioned same manner as the first trial. Two CDC standard miniature traps (John W. Hock Company, Florida, U.S.A., model 1212) were used, one equipped with a 4-watt UV light (320-420 nm) and the other with a 4-watt white light (peaks at 450 and 580 nm). The other two CDC-miniature portable traps model 512 were baited with a UV LED array light (390 nm) and with an incandescent bulb. The portable models were connected to the power supply by means of transformers (6 V to 220 V).

All traps fitted with the same model of fan and dimensions were operated overnight from dusk till dawn. Dipterans were collected into 500 ml plastic jars containing water and a drop of detergent and were emptied early in the morning. Trapping was repeated during extra nights in case of strong wind and/or trap failure. Insects collected were stored in 70% ethanol until processing. In total, four Diptera groups we studied: *Culicoides*, other ceratopogonids, culicids referred as mosquitoes and other mixed Diptera. *Culicoides* specimens were identified to species level based on the appropriate keys for northern Spain biting midges (González, 2014). Other common ceratopogonids and culicids were identified at genus level (González and Goldarazena, 2011; Schaffner et al. 2001). For the common members of the subgenus *Avaritia*, *Culicoides obsoletus* and *C. scoticus* (sibling species) were grouped, while *C. chiopterus* and *C. dewulfi* were identified by their characteristic morphological features (Nielsen and Kristensen, 2011). The number of *Culicoides* collected were counted and sexes pooled to simplify the data analysis, as for example *Culicoides* males are relatively rare representing only 0.9% of the total collections.

2.3. Statistical analysis

All data analyses were performed using the program R 3.3.0 (Fox, 2005; R Core Team, 2016) and graphs were prepared with SPSS statistics 23 (IBM corporation, Armonk, U.S.A). Data were analysed with generalized linear models (GLMz) using Poisson response as variables are discrete. Due to data overdispersion, a binomial negative response was applied to compare captures among light traps with the following criteria: if residual deviance was double the degrees of freedom, data were readjusted with negative binomial response (McCullagh and Nelder, 1989). Post-Hoc multiple comparisons of mean trap catches between the different traps were assessed using Tukey´s test.

3. Results

A total of 27974 specimens of Diptera was collected over the 27 consecutive nights divided into two independent experiments (15 nights and 12 nights), giving a total of 123 collections. The majority (53.4%) were ceratopogonids. *Culicoides* midges comprised 33.7% of the total (*n* = 9449), while other ceratopogonids represented 19.6% (*n* = 5495). A total of 22 species/6485 specimens of *Culicoides* biting midges were collected in the first trial and 11 species/2964 specimens in the second trial.

The most abundant species were *Culicoides punctatus* (67.7%; *n* = 6400), *C. cataneii* (13.5%; *n* = 1282) and *C. obsoletus/C. scoticus* (9.6%; *n* = 906). Other species collected in declining order of abundance were: *Culicoides alazanicus* (3.1%; *n* = 291), *C. festivipennis* (1.6%; *n* = 148) and *C.*

kibunensis (1.2%; *n* = 117). The remaining 16 species comprised less than 3.2% of the total collections. Non-biting midges within Ceratopogonidae were represented by *Forcipomyia* (92.6%; *n* = 5090), *Dasyhelea* (4.4%; *n* = 242), *Atrichopogon* (2.8%; *n* = 154) and *Stilobezzia* (0.2%; *n* = 9). Culicids comprised a total of 602 specimens (2.2% of the total collections) representing four genera: *Culex, Culiseta*, *Anopheles* and *Aedes*. Other mixed Diptera (Nematocera suborder), specially Chironomidae, Sciaridae and Cecidomiidae, were also recorded in a single group which accounted for 12428 individuals (44.4% of the total collections).

In the first trial (Table 1, Fig. 1A), there were significant differences in the total mean numbers of *Culicoides* collected between traps ($X^2 = 194.91$, d.f. = 4, $P < 0.001$). Comparison of the efficacy of different LEDs in *Culicoides* collections indicated that UV-LED traps ($X \pm SD = 159.8 \pm 121.9$) and green-baited LED traps (118.8 ± 74.3) collected significantly higher numbers of *Culicoides* than traps using white (34.7 ± 25.2) and red (21.8 ± 12.5) LEDs. Blue LED-baited traps (96.2 ± 61.3) were not significantly different from each other ($P > 0.05$). Similar patterns were observed for the most common *Culicoides* species: *C. punctatus*, *C. cataneii* and *C. obsoletus*/*C. scoticus,* but with subtle differences. Green LED light traps showed the highest numbers of captures for the 18 remaining species (10.7 ± 9.3) ahead of UV LED light (7.9 ± 9.3), and significantly different from blue LED (4.1 ± 3.6), red LED (1.3 ± 1.4) and white LED lights (3.7 ± 4.9) ($P < 0.001$). In non-biting ceratopogonids, mean numbers collected with green LED-baited traps (103.5 ± 211.6) was significantly higher than that with white (30.3 ± 56.0) and red (13.8 ± 29.8) LED light traps ($X^2 = 53.25$, d.f. = 4, $P < 0.001$). Similar responses were observed for culicids ($X^2 = 13.05$, d.f. = 4, $P = 0.001$). However, in other Diptera, a major response to blue and green wavelengths was observed ($X^2 = 210.64$, d.f. = 4, $P < 0.001$). Regarding *Culicoides* species richness, only 9 species where trapped using red LED traps, while the other wavelengths attracted between 15 and 19.

In the second trial (Table 2, Fig. 1B), proportional representation of *Culicoides* differed significantly between treatments ($X^2 = 294.91$, d.f. = 3, $P < 0.001$). Comparison of the efficacy between UV (tube and LED) and white (tube and incandescent bulb) lights for *Culicoides* collection, showed that the mean number of midges collected with the UV tube traps (136.6 ± 129.8), was nearly double than numbers collected with the white tube trap (73.9 ± 77.2). The mean numbers collected in traps equipped with UV LED lights (21.1 ± 27.5) and white incandescent bulb lights (15.2 ± 16.7) were significantly lower than fluorescent baited traps. Similar tendency was observed for other ceratopogonids, where the mean collections with the UV tube traps were significantly higher than the others (X^2 =191.13, d.f. = 3, $P < 0.001$). However, culicids showed better responses to the fluorescent white light (6.3 ± 5.0) than to the other three light sources (X^2 = 28.33, d.f. = 3, $P < 0.001$). In other mixed dipterans, traps equipped with fluorescent wavelengths collected significantly higher numbers than LED traps (X^2 = 180.44, d.f. = 3, $P < 0.001$). In relation to the number of *Culicoides* species, the white-baited trap trapped the smallest number of species (6), followed by UV LED (8), white tube (9) and UV tube (11), therefore the latter light trap captured not only a higher mean number of individuals but also higher species diversity.

4. Discussion

A total of 9449 *Culicoides* midges belonging to 23 species was collected in this study. This represents 44.9% of the *Culicoides* species recorded for the Basque Country region (González, 2014) and 26.8% of the 82 species reported in Spain (Sánchez Murillo et al., 2015). Interestingly, the two most abundant species in the current study, *C. punctatus* and *C. cataneii*, have not been recorded in similar numbers in adjacent regions. At livestock farms, González et al., (2013) recorded mainly *C. obsoletus*/*C. scoticus* followed by *C. lupicaris.* Variation in species composition could be attributed to differences in sampling dates and/or habitat and location, as the farm is located next to an urban zone. In fact, *C. punctatus* is the most common species in urban parks in the Basque Country, according to collections done using a battery powered-aspirator (González et al., 2015). This species is of potential interest as it could play a role in the epidemiology of Schmallenberg virus (SBV) and bluetongue virus (BTV) in Europe (Larska et al., 2013).

Culicoides species exhibited a similar response to green, blue and UV LEDs, but seemed to respond poorly to red (Bishop et al., 2004; Hope et al., 2015). Only 9 species were collected using red lights while the other wavelengths attracted more than 15 species. White light also was less attractive to *Culicoides* midges, although it could collect a large number of species.

It is interesting to point out that the most powerful LED light for *C. obsoletus/C. scoticus,* the main relevant BTV vectors across Europe (Purse et al., 2005), was green, which agrees with Hope et al. (2015) observations, where *C. obsoletus/C. scoticus* and *C. brunnicans* were collected in higher numbers using the same colour LED traps. Notwithstanding the lower numbers collected, *C. brunnicans*, *C. kibunensis*, *C. alazanicus* and *C. festivipennis* seemed to be highly attracted to green LED traps. This is in line with studies from Australia, where *Culicoides* midges are routinely collected with green light sources (Bishop et al., 2006; Eagles et al., 2014; Melville et al., 2015). Silva et al. (2015) reported that traps emitting green light captured the most Brazilian *Culicoides* (53.9%), followed by incandescent (34.9%) and blue light (11.2%), but they did not include any UV light source in their comparisons. These results suggest a spectral sensitivity for *Culicoides* females around 570 nm with decreasing sensibility in wavelengths toward the red spectrum. Regarding fluorescent lights, previous studies using Onderstepoort traps fitted with bright black light (8W) have highlighted its superiority for collecting *Culicoides* compared to other trap designs and light sources (del Río et al., 2013; Venter et al., 2009; Probst et al., 2015). Our results with CDC traps showed the superiority of the UV tube baited traps in comparison to UV LEDs, incandescent bulb and white tube lights for the collection of *Culicoides* midges. This can be clearly observed when all light sources are deployed simultaneously, as traps equipped with UV tubes collected six times more *Culicoides* midges than did UV LEDs. Similarly, white tube light traps were five times more efficient than the incandescent bulb. Fluorescent UV light was up to two times more efficient than white light in collecting *Culicoides*, while this difference was three times with South African traps (Venter and Hermanides, 2006).

Predaceous, nectar and haemolymph-feeding ceratopogonids are usually sampled by means of a wide variety of trapping methods including light traps (Tóthóva et al., 2005), but often, are accidentally collected in UV light traps used for monitoring *Culicoides* biting midges (González et al., 2014). Nonetheless, there is little wavelength-specific information, as many members of this family are not studied due to their limited sanitary interest. The specimens mostly taken using green, blue and UV LED traps for the four genera studied, except *Dasyhelea,* which was trapped well with white sources as well. Some species of *Forcipomyia* of medical interest such as *F. taiwana*, are monitored with blue light, 405 nm (Liu et al., 2009). In general, fluorescent lights were more attractive than LED lights within this genus.

Regarding culicids, trapping is usually conducted with a combination of light traps and chemical attractants, e.g., BG-Sentinel traps or CDC traps baited with CO_2 and/or 1-octen-3-ol, (Roiz et al., 2012; Lühken et al., 2014). Mosquitoes wavelength preferences seems to be in the blue-green range (400-600 nm), with a decrease in attraction as wavelengths increase (> 600 nm) (Burkett et al., 1998). Using LED technology, green and UV light traps collected the highest numbers of culicids, followed by blue colour, which agrees with Silva et al. (2014). Similarly, Bentley et al. (2009) suggested that, in the absence of host stimuli, wavelengths in the lower green (502 nm) spectral range would be optimal for targeting a broad range of mosquitoes. However, when fluorescent lights are run with LEDs, the responses in culicids were greater with a white fluorescent tube according to other similar studies (Hoel, 2005). This suggests that incandescent bulbs are less attractive to culicids than fluorescent tubes. However, giving the accuracy in trapping considerable host-seeking mosquitoes, LEDs can be used as an alternative trapping system thereby eliminating the cost of heavy tanks, volatile chemicals or where access or equipment is limited (Bentley et al., 2009).

Finally, regarding other mixed Diptera, our results showed a preference to blue light, followed by green and UV light, and to a lesser extent white and red. It is documented that UV and blue light are usually most attractive to arthropods, although the degree of attraction varies among families (Longcore et al., 2015). Small collections in red spectrum agree with (Li et al., 2015), as members of the Diptera order, including mosquitoes, tend not to be attracted to light wavelengths > 580 nm.

5. Conclusion

The use of green LED lights is quite promising in collecting *Culicoides* midges and other dipterans. We suggest further studies be done using green fluorescent lights (4 to 8 watt tubes) instead of LEDs technology and this should be compared with standard UV lamps for *Culicoides*. The investigation of green light as a new light source in surveillance and monitoring trapping programs might have important implications, particularly for *C. obsoletus/C. scoticus* species, as these species are the most abundant across Europe. Fluorescent lamps are powerful lures for the collection of huge numbers and species richness. However, LED array technology could be used as an alternative suitable for monitoring blood-sucking dipterans in areas where external power supply is not available or where the weight of batteries is prohibitive. The expected results will have potential for use by ecologists and epidemiologists for improving collection efficiency of certain species of biting midges, obtaining greater sample sizes, higher species biodiversity and saving time and money.

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Conflict of interest

The authors disclose no conflicts of interest in this work.

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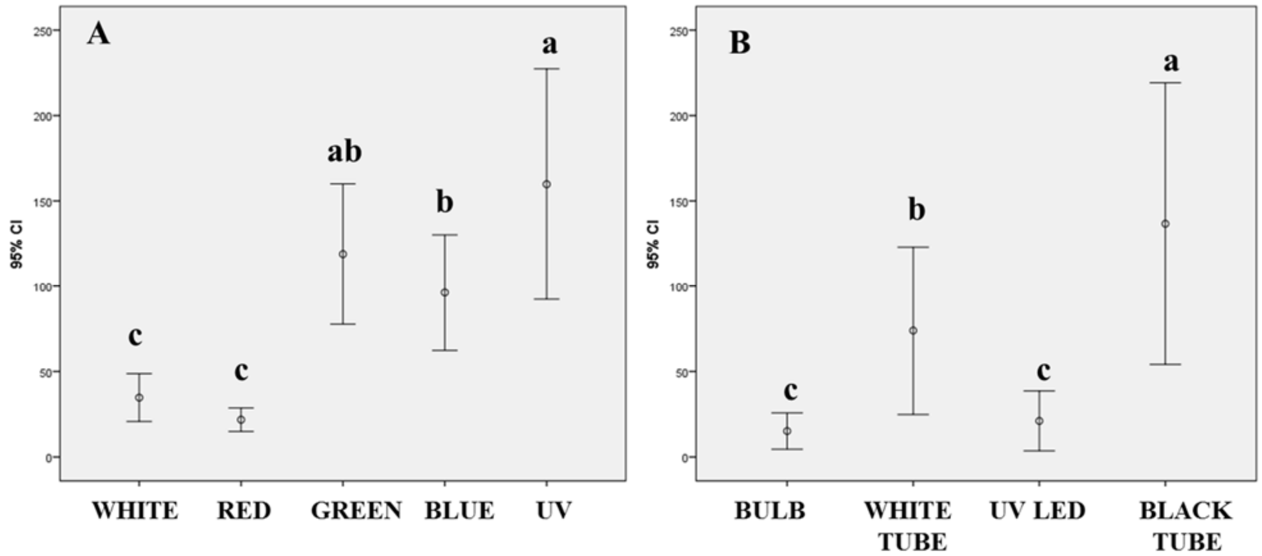
Figure 1. Numbers of *Culicoides* midges collected with suction light traps at a sheep meadow in the Basque Country, Spain: (A) LEDs trial (white, red, green, blue and UV lights) and (B) mixed light sources trial (incandescent bulb, white tube, UV LED and UV tube).



Bars represent means confidence interval ± SD and different letters between boxes denote statistically significant differences ($p < 0.05$).

Table 1. Summary of dipterans collected with five different LED lights between mid-July to the beginning of August 2013 over 15 consecutive nights at a meadow in the Basque Country, Spain.

Species	**LIGHT EMITTING DIODES (LEDS)**						
	WHITE	**RED**	**GREEN**	**BLUE**	**UV**	**TOTAL**	**% species**
Culicoides a							
C. punctatus	417 (27.8 ± 19.1)**b**	257 (17.1 ± 10.4)**b**	1411 (94.1 ± 60.5)**a**	1254 (83.6 ± 54.9)**a**	1927 (128.4 ± 90.6)**a**	**5266**	**81.1**
C. cataneii	20 (1.3 ± 2.3)**c**	32 (2.1 ± 1.6)**c**	113 (7.5 ± 5.6)**b**	63 (4.2 ± 2.8)**b**	262 (17.4 ± 28.7)**a**	**490**	**7.5**
C. obsoletus/scoticus	28 (1.9 ± 2.0)**bc**	19 (1.3 ± 1.7)**c**	98 (6.5 ± 8.5)**a**	65 (4.3 ± 5.2)**ab**	89 (5.9 ± 6.0)**a**	**299**	**4.6**
C. kibunensis	6	5	31	16	30	**88**	**1.4**
C. alazanicus	9	5	28	13	24	**79**	**1.2**
C. circumscriptus	19	6	13	7	28	**73**	**1.1**
C. brunnicans	3	0	34	3	12	**52**	**0.8**
C. festivipennis	2	1	17	1	7	**28**	**0.4**
C. lupicaris	3	0	8	3	10	**24**	**0.4**
C. achrayi	1	0	9	4	5	**19**	**0.3**
C. fascipennis	2	0	7	5	1	**15**	**0.2**
C. duddingstoni	3	2	0	0	9	**14**	**0.2**
C. picturatus	3	0	6	4	0	**13**	**0.2**
C. poperinghensis	1	0	5	0	1	**7**	**0.1**
C. simulator	0	0	1	2	3	**6**	**0.1**
C. dewulfi	0	0	4	0	0	**4**	**0.1**
C. tauricus	1	0	1	0	0	**2**	**0.0**
C. vexans	1	0	0	1	0	**2**	**0.0**
C. parroti	0	0	2	0	0	**2**	**0.0**
C. furcillatus	1	0	0	0	0	**1**	**0.0**

C. pulicaris	1	0	0	0	0	**1**	**0.0**
Total (other species) b	**56 (3.7 ± 4.9)c**	**19 (1.3 ± 1.4)d**	**166 (10.7 ± 9.3)a**	**59 (4.1 ± 3.6)bc**	**130 (7.9 ± 9.3)ab**	**430**	
Total *Culicoides*	**521**	**327**	**1788**	**1441**	**2408**	**6485**	**100**
Species collected	19	9	18	15	15	**22**	
Mean collected	**34.7 ± 25.2 b**	**21.8 ± 12.5 b**	**118.8 ± 74.3 a**	**96.2± 61.3 ab**	**159.8± 121.9 a**		
Other Ceratopogonidae							
Forcipomyia	403	207	1457	1347	1355	**4769**	**95.0**
Dasyhelea	44	9	47	30	44	**174**	**3.4**
Atrichopogon	8	1	47	10	7	**73**	**1.4**
Stilobezzia	0	0	2	4	0	**6**	**0.1**
Total " other Ceratopogonidae "	**455**	**217**	**1553**	**1391**	**1406**	**5022**	**100**
Mean collected	**30.3 ± 56.0 cd**	**13.8 ± 29.8 d**	**103.5 ± 211.6 a**	**92.7 ± 228.9 bc**	**93.7 ± 189.0 ab**		
Culicidae							
Culex	54	42	109	67	59	**331**	**78.3**
Culiseta	19	9	13	11	21	**73**	**17.3**
Anopheles	1	2	2	7	3	**15**	**3.5**
Aedes	1	1	1	1	0	**4**	**0.9**
Total Culicidae	**75**	**54**	**125**	**86**	**83**	**423**	**100**
Mean collected	**5.0 ± 3.8 ab**	**3.6 ± 2.6 b**	**8.3 ± 8.0 a**	**5.7 ± 5.4 ab**	**5.5 ± 7.0 ab**		
Other Diptera							
Total " other Diptera "	**714**	**504**	**2810**	**3227**	**1473**	**8728**	**100**
Mean collected	**47.6 ± 32.2 c**	**33.6 ± 27.5 c**	**187.3 ± 107.4 a**	**215.1 ± 129.8 a**	**98.2 ± 84.4 b**		

[a] In brackets, mean ± SD for the most abundant *Culicoides* species. [b] Includes the remaining species. Different letters in bold denote statistically differences at the 5% level according to Tukey´s comparison test. *Aedes* spp. and *Ochlerotatus* spp. were pooled as *Aedes* spp. according to the criteria of Wilkerson et al. (2015).

Table 2. Summary of dipterans collected with different light sources in mid-August over 12 nights at a meadow in the Basque Country, Spain.

Species	BULB	WHITE-TUBE	UV-LED	UV-TUBE	TOTAL	% species
Culicoides a						
C. punctatus	56 (4.7 ± 4.9)**c**	362 (30.1 ± 32.3)**b**	81 (6.7 ± 12.4)**c**	635 (52.9 ± 52.7)**a**	**1134**	**38.3**
C. cataneii	97 (8.1 ± 9.9)**c**	221 (18.4 ± 18.2)**b**	71 (5.9 ± 6.3)**c**	403 (33.6 ± 33.1)**a**	**792**	**26.8**
C. obsoletus/scoticus	6 (0.5 ± 1.2)**c**	174 (14.5 ± 17.1)**b**	19 (1.6 ± 2.1)**c**	408 (34.0 ± 31.5)**a**	**607**	**20.5**
C. alazanicus	10	50	41	111	**212**	**7.1**
C. festivipennis	13	36	32	39	**120**	**4.1**
C. kibunensis	0	13	4	12	**29**	**0.9**
C. kibunensis	0	20	5	19	**44**	**1.5**
C. circumscriptus	0	14	0	6	**20**	**0.7**
C. duddingstoni	0	0	0	5	**5**	**0.2**
C. nubeculosus	0	0	0	1	**1**	**0.0**
Total (other species) b	**23 (1.9 ± 2.6) d**	**133 (10.8 ± 12.4) b**	**82 (6.8 ± 8.7) c**	**193 (16.2 ± 16.8) a**	**431**	**14.5**
Total *Culicoides*	**182**	**890**	**253**	**1639**	**2964**	**100**
Species collected	6	9	8	11	**11**	
Mean collected	**15.2 ± 16.7 c**	**73.9 ± 77.2 b**	**21.1 ± 27.5 c**	**136.6 ± 129.8 a**		
Other Ceratopogonidae						
Forcipomyia	37	122	34	129	**321**	**68.0**
Atrichopogon	2	11	8	61	**81**	**17.1**
Dasyhelea	5	12	15	36	**68**	**14.3**
Stilobezzia	0	0	0	3	**3**	**0.6**
Total " other Ceratopogonidae "	**44**	**145**	**57**	**229**	**473**	**100**

Mean collected	**3.6 ± 4.2 c**	**12.1 ± 16.3 b**	**4.7 ± 5.2 c**	**19.1 ± 24.0 a**		
Culicidae						
Total Culicidae	**28**	**76**	**34**	**41**	**179**	**100**
Mean collected	**2.3 ± 2.2 b**	**6.3 ± 5.0 a**	**2.8 ± 2.0 b**	**3.4 ± 3.2 b**		
Other Diptera						
Total "other Diptera"	**510**	**1319**	**445**	**1426**	**3700**	**100**
Mean collected	**42.5 ± 28.4 b**	**109.9 ± 81.4 a**	**37.1 ± 22.8 b**	**118.8 ± 75.5 a**		

[a] In brackets, mean ± SD for the most abundant *Culicoides* species. [b] Includes the remaining species. Different letters in bold denote statistically differences at the 5% level according to Tukey´s comparison test. Culicidae was pooled due to the low numbers collected.