The attraction range of the Onderstepoort 220 V light trap for *Culicoides* biting midges as determined under South African field conditions

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Abstract

Despite some limitations suction light traps are the primary tool used for the collection of *Culicoides* species (Diptera: Ceratopogonidae). The range of attraction of the Onderstepoort light trap is not known but an insight into the range of a trap will determine where the trap must be positioned relative to the hosts present, possible breeding sites and environmental structures in the trapping vicinity. It will therefore contribute to a more meaningful interpretation and comparison of results between trapping events. In the present study the number of *Culicoides* midges collected in a single trap was compared to those of traps made with an additional trap respectively 1 m, 4 m and 8.5 m away from the first. Treatments between sites were rotated in three replicates of a 4x4 Latin square design. While interactions were found in traps 4 m apart no statistically significant interactions were found when they were 8.5 m apart. The range of attraction, indicated by the interaction between two traps, will be between 2 m and 4 m. In interpreting light trap results the limitations of this collection method needs to be taken into consideration.

Key words: attraction range, Collection, *Culicoides imicola*, Vectors, orbiviruses.

1. Introduction

Blood feeding midges in the genus *Culicoides* (Diptera: Ceratopogonidae) are associated with the transmission of several pathogens of veterinary importance (Meiswinkel et al., 2004; Borkent, 2005). At least three orbiviruses (*Reoviridae*), African horse sickness- (AHSV), bluetongue- (BTV) and epizootic haemorrhagic disease of deer virus (EHDV), transmitted by certain members in this genus, cause diseases of such international importance that they have been allocated Office International des Epizooties (OIE) list status (Mellor et al., 2000). Outbreaks of bluetongue (BT) in northern Europe have indicated that the virus can effectively be transmitted by several species in this genus (Mellor et al., 2009; Carpenter et al., 2009). A similar multi-vector potential has also been demonstrated for BTV, AHSV and EHDV in South Africa (Paweska et al., 2003; 2005; Venter et al., 2011b).

Risk assessment of vector-borne diseases obtained through entomological surveys will influence decisions on the implementation of effective integrated control measures. Entomological surveys can, however, be time consuming, expensive and can potentially delay control efforts. Information on vector presence and abundance must be obtained in the shortest possible time in order to make appropriate decisions without consuming unnecessary resources.

Since 1928 various models of light traps have been used for the collection and monitoring of night-active insects (Service, 1977). Despite being an artificial system and the great variety of factors that can influence light trap results (Nelson and Bellamy, 1971; Murray, 1987; Bellis and Reid, 1996; Garcia-Saenz et al., 2011) it has become a standard tool for the collection of *Culicoides* midges. In a comparative study in South Africa, the 220 V down-draught Onderstepoort black-light trap was shown to collect significantly more *Culicoides* midges under field conditions than the Rieb, mini-CDC, Pirbright and BG-sentinel light traps (Venter et al., 2009a). Taking into account the more powerful light source and fan of the 220 V Onderstepoort trap, compared to that of the others, this result was not surprising.

Onderstepoort light traps are routinely used to determine the risk of a virus moving into, becoming established and spreading in an area (Goffredo et al., 2004; Patakakis, 2004; Cagienard et al., 2006; Conte et al., 2007; Meiswinkel et al., 2008; Racloz et al., 2008). In the absence of laboratory colonies Onderstepoort light traps are also used to collect live *Culicoides* midges for biological studies requiring live specimens (Paweska et al., 2003; 2005; Veronesi et al., 2009; Venter et al., 2011b). Numerous factors that may contribute to variability in the numbers of specimens collected render the interpretation and comparison of data between different trapping events challenging. It is well established that the presence of livestock near the light trap will increase the numbers of certain species of biting midges (Bellis and Reid, 1996; Garcia-Saenz et al., 2011; Venter et al., 2011a). However, the range of attraction of the Onderstepoort trap is not known. An insight into the potential range and the factors that may contribute to this attraction may help in deciding where a trap needs to be positioned in relation to the hosts present, possible breeding sites and environmental structures in the trapping vicinity. This will contribute to the standardization of a surveillance protocol, the interpretation and the comparison of light trap data between trapping occasions.

To gain some insight into this attraction range the distance of interaction between two light traps was determined. The number of *Culicoides* midges collected, species composition and age grading results, as determined using a single stationary trap, were compared to those of three other stationary traps each with a second trap 1 m, 4 m or 8.5 m away. The distance at which the second trap influences the numbers collected in the stationary trap could give an indication of the attraction range of the trap. Although not the main purpose, this placement provided an opportunity to compare the results obtained in two Onderstepoort traps which were respectively 1 m, 4 m and 8.5 m apart.

2. Material and Methods

2.1 Collection sites

The study was conducted in early summer from 8 to 28 October 2010 in South Africa. Down-draught 220 V Onderstepoort black-light traps (Venter et al., 2009a) were deployed in four sites at the ARC-Onderstepoort Veterinary Institute and the nearby Onderstepoort Veterinary Academic Hospital, Faculty of Veterinary Science (25°39'S:28°11'E; 1219 m above sea level).

These four sites were at least 200 m to 600 m apart. At the first site traps were placed underneath the eaves of an open-sided stable housing 15 to 20 cattle at night. During the day the cattle were in an open pen (900 m^2) with a concrete floor in front of the stable. The stationary trap was operated at one of the corners of the stable and the second trap, if present, was operated either 1 m, 4 m or 8.5 m, alongside the northern side, facing the open pen. Both traps were therefore in the immediate vicinity of the cattle. More cattle, in similar stables, were present in a radius of 50 m to 100 m from the study area.

The second site where a stationary trap was operated was 200 m to 250 m from the first. These two sites were separated by several office buildings. In this area the traps were placed underneath the eaves of a stable housing two horses. In the front of the stable was an open yard (50 m^2) with a concrete floor where the horses spent most of their time during the day and night. A stationary light trap was operated in the centre of this area and a second, if present, either at 1 m, 4 m or 8.5 m away. Both traps were operated inside the enclosure where the horses could move around freely.

The third site where a stationary trap was operated was 300 m to 400 m from the second area. The traps were placed underneath the eaves of a stable housing 20 to 30 horses at night. The stable was surrounded by open camps with some trees and soil with patches of grass. In addition to the horses inside the stable 10 to 15 horses would usually spend the night in the open camps next to the stable. More horses were present in open camps 100 m to 200 m away.

The fourth study area was at least 600 m away from the third. Here, the traps were operated in a roofed service area between five camps which housed five to ten horses at night. More horses and some cattle were present in open camps and kikuyu pastures 10 m to 50 m away.

The whole study area had relatively many trees and irrigated kikuyu lawns, varying in size, were located throughout the area. Wild birds and small rodents of various species were present at all of the sites.

2.1. Collection procedure

The stationary light trap at each of the four sites was not moved (Fig. 1a-d). On every night of collection a second Onderstepoort light trap (mobile trap) (Fig.1e-g) was operated at three of the four sites where a stationary trap had been installed. The second trap was placed at respectively 1 m, 4 m and 8.5 m away from the stationary trap (Fig. 1e-g). Every night there would have been a trap with no other trap nearby, a site with two traps 1 m apart, another with two traps 4 m apart and one with two traps 8.5 m apart (Fig. 1). At each of the four sites an effort was made to keep comparable animal densities at the two collection points. To ensure that distance treatments were independent of site or occasion the distance at which the second trap was operated from the stationary one was randomised in three repeats of a 4x4 Latin square design (Snedecor and Cochran, 1980).

Traps were hung 1.4 m above ground level and as close to the host animals as possible. Insects were collected into water to which 0.5% 'Savlon' (Johnson & Johnson, South Africa) (contains Clorhexidine gluconate 0.3 g/100 ml and Cetrimide 3.0 g/100 ml) antiseptic had been added as described by Venter et al., 2009a. Traps were run from dusk to dawn and in the morning the insects were transferred to 80% ethanol and stored until analysed. Large collections were sub-sampled (Van Ark and Meiswinkel, 1992). Based on abdominal pigmentation (Dyce, 1969), females of all species were age-graded into nulliparous (unpigmented), parous (pigmented), gravid or freshly blood-fed. Captured males were also counted. On all trial nights when, due to adverse weather conditions or trap failure, no or very few *Culicoides* midges were collected, trapping was repeated the following night.

2.2. Statistical analyses

The experimental layout was a 4x4 Latin square repeated three times with Row = Day, Columns = Site and Treatment = Trap Distance (0 m, 1 m, 4 m and 8.5 m). The daily stationary trap counts at each site were $Log_{10}(x+1)$ transformed before subjected to the appropriate Analysis of Variance (ANOVA). The residuals were tested for deviation from normality and homogeneity of treatment variances. Treatment means were separated using Fisher's protected t-LSD (Least Significant Difference) at the 5% level of significance (Snedecor and Cochran, 1980). Proportions of insect counts between the single and the stationary plus a second distance trap were compared using Chi-squared (χ^2) tests. Data was analysed using the statistical program GenStat version 13.1 (Payne et al., 2010).

Two tailed paired t-tests were used to compare the numbers of *Culicoides* midges collected in the stationary and non-stationary traps using GraphPad InStat Version 3. Species diversity at each site was calculated with the Shannon Weiner index, which describes the evenness in distribution of species abundances taking sample size into account. Evenness in distribution of species abundance as determined at the different sites was compared using linear regression GraphPad InStat Version 3.

3. Results

There was not enough evidence against normality and homogeneity of variances therefore the data results can be considered reliable.

3.1 Stationary traps

As prescribed by the minimum requirements of the Latin square design 48 collections were made with the four stationary traps (Fig. 1a-e) on 12 nights between 8 and 28 October 2010. A total of 288512 *Culicoides* midges was collected. The highest mean number (7574, Standard Deviation (SD) = 9620) was collected with the stationary trap with no additional light trap nearby (Table 1). Taking into account the substantial day to day variation in the numbers collected the mean number was not significantly different from that collected in the stationary trap with a second trap at respectively 1 m (5186, SD = 6795) or 8.5 m (6740, SD = 8725) away (Table 1). Significantly (P = 0.024) fewer midges (4542, SD = 7149) were, however, collected in the stationary trap when the second one was 4 m away (Table 1).

Midges belonging to 25 *Culicoides* species were collected in the four stationary traps (Table 1). *Culicoides imicola* was the most abundant species collected in all four treatments (Table 1). Its abundance ranged from 92.6% (in the stationary trap with a second 8.5 m away) to 86.9% (in the stationary trap with a second trap 1 m away). Similar to the total number the larger mean number (6937, STD = 9380) of *C. imicola* collected in the trap with no additional trap nearby was also not significantly different from that with a trap 1 m (4508, SD = 6693) or 8.5 m (6240, SD = 8413) away (Table 1). The stationary trap with a second one 4 m away did, however, collect significantly fewer (4004, SD = 6987) *C. imicola* than the stationary trap with no second trap (Table 1). For the second (*Culicoides pycnostictus* Ingram & Macfie) ($P = 0.369$) and third (*Culicoides bedfordi* Ingram & Macfie) ($P = 0.089$) most abundant *Culicoides* species no significant differences were found in the mean numbers collected in the four stationary traps.

Linear regression showed that the proportion of the different species collected with a stationary trap alone was nearly identical to those with a second trap 1 m (R^2 = 93%), 4 m (R^2 = 89%) and 8.5 m away (R^2 = 92%). Differences in species richness between treatments were the result of single specimens of some species which were collected on only a few trapping occasions (Table 1). Species diversity as represented by the Shannon Weiner Index in the trap alone ($H' = 0.46$) was nearly identical to that of a trap with one 8.5 m away ($H' = 0.42$) (Table 1). Species diversity and evenness was higher in the other two treatments (Table 1).

Typical for light trap collections females were the most abundant gender to be collected in all four treatments (Table 2). A comparison of the age-grading results for *C. imicola* females indicates that there were relatively small but statistically significant differences in the proportions of nulliparous, parous, freshly bloodfed and gravid females collected in the stationary trap alone and the stationary with a second trap either 1 m, 4 m or 8.5 m away (Table 2). There were, however, no significant differences in the portion of parous (χ^2 = 1.330, *df* = 1, *P* = 0.249) as determined in the stationary trap with a second one 4 m away and the stationary trap without a second nearby (Table 2).

3.2 Mobile traps

The placement of traps provided the opportunity to compare the results obtained in two traps which were respectively 1 m, 4 m and 8.5 m apart operated on the same night. Results were obtained for three replicates of each distance at four sites. A comparison of the numbers of *Culicoides* midges collected in these stationary traps compared to the numbers in a second trap 1 m away indicated considerable day to day variation (Table 3). For example the total number of *Culicoides* per night in the stationary trap at 1 m varied from 275 to 20068 (Table 3). The mean number of midges (5185, SD = 6795) collected in the stationary traps in 12 collections made at four sites was nearly double that (2641, SD = 2711) collected in the 12 collections made with the 1 m trap (Table 3). Taking into account the substantial day to day variation the number collected per night per trap was not significantly different (*P* = 0.089, r = 0.85). Similarly the numbers collected in traps 4 m (*P* = 0.228, r = 0.92) and 8.5 m (*P* = 0.280, r = 0.89) apart were not statistically significant.

There were also no significant differences in the mean number of the dominant species, *C. imicola*, collected in traps 1 m (*P* = 0.088, r = 0.91), 4 m (*P* = 0.284, r = 0.92) and 8.5 m (*P* = 0.294, r = 0.90) apart. A relative good linear correlation was found in the proportion of different species collected at trap 1 m (R^2 = 92%), 4 m (R^2 = 88%) and 8.5 m (R^2 = 92%). Variations in species richness and species diversity between treatments were the result of single specimens of some species which were collected on only a few trapping occasions (Table 3).

4. Discussion

The statistically significant higher mean number of *Culicoides* specimens as well as *C. imicola* collected with a single Onderstepoort light trap compared to one with a second trap 4 m away indicates an interaction between those two traps 4 m apart (Table 1). In the light trap with a second 8.5 m away no statistically significant difference was found in the mean numbers and species composition compared to that collected in a solitary trap (Table 1).

The lack of a statistically significant differences found in the mean numbers in a trap with no other nearby compared to that of a trap with a second 1 m away may indicate that this distance was too small for these traps to function as two separate entities. The two traps may have acted as a single unit and midges may have been collected indiscriminately by either of the two.

The range of attraction of the Onderstepoort light trap, as indicated by the interaction between two traps 4 m apart and lack of interaction between two traps 8 m apart, will be between 2 m and 4 m. The area covered by a single Onderstepoort light trap will therefore not exceed 50 m^2. The influence of climatic and environmental factors still needs to be determined. The proportion of midges in this range actually captured by the trap will partly depends on the suction strength of the fan and is not known. This relatively short range is supported by previous findings showing that significantly lower numbers of *C. imicola*, a species known to feed on livestock, are collected in traps only 5 m away from the host compared to that in one immediately next to the animals (Venter et al., 2009b). This indicates the light source of a trap to be much less efficient than the odour plume and other stimuli generated by hosts in attracting *Culicoides* midges. Odour plumes can be distributed more widely by air streams and will therefore have the potential to attract midges over a much larger distance than a fixed light source. Olfactory cues, on their own or as an additional attractant to light, may increase the trapping efficiency (Kline et al., 1994; Ritchie et al., 1994; Braverman et al., 2000; Harrup et al., 2012) but this may also differ between species (Cilek and Kline 2002).

The present results were largely dictated by the dominance of *C. imicola* in the collections made near livestock. The fact that no statistical differences were found for the second (*C. pycnostictus*) and third (*C. bedfordi*) most abundant species may indicate differential attraction of the traps for these species. The preferred hosts of these two species are considered to be birds (Meiswinkel et al., 2004) and midge presence in the traps can be ascribed to wild birds at all the sites. Host preference may influence the average height at which species will be active and the dispersal capacity of the species. This will have direct influence on the efficacy of the traps, placed at livestock, to collect these and other species not attracted by livestock.

A light trap will only reflect midge flight activity within a few metres of its immediate vicinity. Flight activity is influenced, however, by a variety of climatic conditions such as ambient temperature, relative humidity, wind (Murray, 1991) and the physiological status and host-seeking activity of the females (Bellis and Reed, 1996). As was found for mosquitoes (Service, 1977) light traps may collect significantly more midges when placed near the flight paths of the active blood-seeking females and near aggregation sites of suitable hosts. The relative low number of males collected in the different treatments (Table 2) may indeed indicate a low representation of males near livestock. The quantity and quality of the midges collected will not only depend on the trap per se, weather conditions and other variables but also on trap location (Service, 1977). The many environmental factors that may influence the dispersal capacity of midges linked to those that influence the efficacy of light trap will make it very problematic to compare trap results between trapping occasions. This phenomenon is highlighted by the considerable variation in the *Culicoides* numbers collected on a daily basis in the present study (Table 3).

Culicoides midges are mobile and the composition and size of a population measured at a specific site may vary dramatically within relatively short periods of time. The similarity in species composition at the four sites and also between traps in the present study can be ascribed to the presence of livestock at all four sites. This shows that despite a variety of factors that can influence the numbers collected with light traps, as indicated by considerable variation in the age grading (Table 2) and numbers collected from day to day (Table 3), they are still a practical and reliable way to determine presence and abundance in a given area. In interpreting light trap results the limitations of this collection method and the factors that can influence the results need to be taken into consideration. In determining the risk of virus transmission light trap results need to be linked to what is known about the biology, capacity and competence of the *Culicoides* species involved.

Conflict of interest

The authors declare no conflict of interest.

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Table 1

Summary of mean numbers of *Culicoides* midges collected in four stationary 220 V down-draught Onderstepoort light traps at which an additional trap was operated 1 m, 4 m and 8.5 m away at three of the sites at the ARC-Onderstepoort Veterinary Institute, South Africa (8 to 28 October 2010)

Distance (m) of second trap from a stationary trap	No secondary trap		1		4		8.5	
Culicoides species	#	Total collected	#	Total collected	#	Total collected	#	Total collected
C. imicola	12	6937.1 (229-30190)**a**	12	4507.5 (164-19528)**ab**	12	4004.0 (125-25192)**b**	12	6239.6 (226-26598)**ab**
C. pycnostictus	12	142.3 (6-870)	12	224.7 (5-1596)	12	213.3 (4-1674)	12	127.3 (2-690)
C. bedfordi	12	140.7 (15-672)	12	175.7 (16-820)	12	110.2 (5-545)	12	129.4 (5-816)
C. leucostictus	11	84.3 (0-290)	12	100.7 (6-366)	12	61.7 (8-335)	12	68.7 (5-354)
C. nivosus	12	133.7 (4-270)	12	52.7 (8-234)	12	49.4 (2-162)	11	40.6 (0-210)
C. nevilli	12	83.8 (1-305)	11	40.1 (0-267)	10	39.4 (0-164)	12	48.5 (1-138)
C. magnus	9	21.8 (3-50)	10	26.5 (0-123)	10	15.2 (0-60)	10	17.6 (0-64)
C. neavei	11	20.3 (0-75)	12	15.1 (1-48)	11	10 (0-30)	9	13.9 (0-72)
C. enderleini	6	9.1 (0-48)	6	5 (0-42)	6	11.2 (0-76)	8	14.5 (0-88)
C. expectator	11	7.5 (0-20)	11	5.7 (0-22)	10	8 (0-40)	9	12.8 (0-84)
C. similis	9	10.4 (0-54)	7	9.7 (0-60)	9	6.5 (0-24)	10	8.7 (0-54)
C. brucei	10	9.9 (0-51)	9	9.6 (0-78)	8	4.8 (0-24)	5	5.8 (0-45)
C. zuluensis	9	9.7 (0-50)	7	5.4 (0-27)	6	5.3 (0-28)	6	4.7 (0-16)
C. coarctatus	10	4.1 (0-14)	7	3.1 (0-15)	8	2.4 (0-8)	10	6.0 (0-20)
C. glabripennis	3	0.7 (0-4)	3	1.4 (0-12)	3	1 (0-5)		
C. subschultzei	3	1.3 (0-12)	3	0.4 (0-2)	3	0.8 (0-4)	2	0.8 (0-6)
C. tropicalis	4	0.7 (0-3)	3	0.6 (0-3)			2	0.3 (0-3)
C. schultzei	2	0.6 (0-5)	1	0.2 (0-2)			2	0.8 (0-6)
C. trifasciellus	1	0.4 (0-5)			2	0.3 (0-4)	2	0.3 (0-2)
C. bolitinos	1	0.2 (0-2)	1	0. 1 (0-1)	3	0.3 (0-1)		
C. nigripennis grp			1	0.1 (0-1)			1	0.1 (0-1)
C. cornutus	2	0.5 (0-5)			1	0.3 (0-3)		
C. sp#54df*	1	0.3 (0-3)	1	0.2 (0-2)	1	0.1 (0-1)		
C. gulbenkiani	1	0.2 (0-2)						
C. sp#107			1	0.1 (0-1)				
Total		7574.1 (374-30825)**a**		5185.8 (275-20068)**ab**		4541.8 (166-25736)**b**		6740.5 (313-26880)**ab**
Shannon Weiner index		0.46		0.63		0.59		0.42
Species evenness		0.15		0.2		0.2		0.14

Total counts in a row followed by a different letter were significantly different at the 5% level
These letters are based on the t-LSD calculated for the mean $\log_{10}$(Count+1)

Number of collections in which this species was present

*The numbering system is that of R. Meiswinkel and refers to yet undescribed*Culicoides* species

Table 2

Age grading results of *C. imicola* given as total (% in brackets) collected in four stationary 220 V down-draught Onderstepoort light traps at which an additional trap was operated 1 m, 4 m and 8.5 m away at three of the sites, at the ARC-Onderstepoort Veterinary Institute, South Africa (8 to 28 October 2010)

Distance (m) of second trap from a stationary trap	No secondary trap	1	4	8.5
Nulliparous	35897 (43.1)	19065 (35.3)	19040 (39.6)	33455 (44.7)
P value		<0.001	<0.001	<0.001
Parous	39907 (47.9)	28109 (52.0)	22874 (47.6)	34937 (46.7)
P value		<0.001	(0.249)	<0.001
Blood fed	1488 (1.8)	933 (1.7)	1100 (2.3)	1436 (1.9)
P value		<0.001	<0.001	<0.001
Gravid	4248 (5.1)	4964 (9.2)	4124 (8.6)	3640 (4.9)
P value		<0.001	<0.001	0.028
Total Female	81540 (98.0)	53071 (98.1)	47138 (98.1)	74875(98.1)
Total Males	1705 (2.1)	1019 (1.9)	909 (1.9)	1407 (1.9)
P value		0.035	0.053	0.017
Total *C. imicola*	83245	54090	48047	74875

P values < 0.05 indicate a statistical significant difference from that collected in a trap with no additional trap from a Chi-square test for comparison of proportions in two independent samples (2x2 table).

Table 3

Mean number of midges collected in each of two 220 V down-draught Onderstepoort traps which were respectively 1 m, 4 m and 8.5 m apart at the ARC-Onderstepoort Veterinary Institute, South Africa. The range in numbers collected is given in brackets. (8 to 28 October 2010)

Distance (m) between traps	1		4		8.5	
	Stationary	Non stationary	Stationary	Non stationary	Stationary	Non stationary
Species richness	22	20	20	21	19	22
Culicoides species						
C. imicola	4507.5 (164-19528)	2122.8 (247-9732)	4004.0 (125-25192)	2600.9 (173-13312)	6239.6 (226-26598)	5065.1 (367-24444)
C. pycnostictus	224.7 (5-1596)	217.3 (0-1818)	213.3 (4-1674)	91.7 (6-554)	127.3 (2-690)	86.0 (3-192)
C. bedfordi	175.7 (16-820)	110.9 (6-512)	110.2 (5-545)	74.6 (8-308)	129.4 (5-816)	94.8 (4-420)
C. leucostictus	100.7 (6-366)	88.1 (2-417)	61.7 (8-335)	61.0 (3-264)	68.7 (5-354)	58.0 (1-237)
C. nivosus	52.7 (8-234)	42.7 (0-102)	49.4 (2-162)	28.8 (2-104)	40.6 (0-210)	32.2 (2-108)
C. nevilli	40.1 (0-267)	12.8 (0-30)	39.4 (0-164)	20.6 (0-82)	48.5 (1-138)	42.1 (0-208)
C. magnus	26.5 (0-123)	6.9 (0-32)	15.2 (0-60)	12.2 (0-39)	17.6 (0-64)	15.8 (0-72)
C. neavei	15.1 (1-48)	18.7 (0-160)	10 (0-30)	6.7 (0-17)	13.9 (0-72)	12.4 (1-54)
C. enderleini	5 (0-42)	2.8 (0-20)	11.2 (0-76)	5.7 (0-42)	14.5 (0-88)	7.5 (0-36)
C. expectator	5.7 (0-22)	5.5 (0-18)	8 (0-40)	5.6 (0-44)	12.8 (0-84)	5.7 (0-27)
C.similis	9.7 (0-60)	3.8 (0-16)	6.5 (0-24)	4.1 (0-24)	8.7 (0-54)	6.1 (0-27)
C. brucei	9.6 (0-78)	5.8 (0-34)	4.8 (0-24	4.1 (0-15)	5.8 (0-45)	8.8 (0-60)
C. zuluensis	5.4 (0-27)	3 (0-12)	5.3 (0-28)	2.6 (0-10)	4.7 (0-16)	5.8 (0-24)
C. coarctatus	3.1 (0-15)	1.2 (0-4)	2.4 (0-8)	2.2 (0-8)	6.0 (0-20)	3.8 (0-24)
C. glabripennis	1.4 (0-12)	0.3 (0-3)	1 (0-5)	1.2 (0-4)		0.2 (0-2)
C. subschultzei	0.4 (0-2)	0.2 (0-2)	0.8 (0-4)	0.6 (0-4)	0.8 (0-6)	1.0 (0-3)
C. tropicalis	0.6 (0-3)	0.7 (0-3)		0.2 (0-1)	0.3 (0-3)	0.5 (0-3)
C. schultzei	0.2 (0-2)			0.1 (0-1)	0.8 (0-6)	0.5 (0-4)
C. trifasciellus		0.1 (0-1)	0.3 (0-4)		0.3 (0-2)	0.3 (0-2)
C. bolitinos	0.1 (0-1)	0.3 (0-2)	0.3 (0-1)	0.1 (0-1)		
C. nigripennisgrp	0.1 (0-1)				0.5 (0-6)	
C. cornutus			0.3 (0-3)			0.1 (0-1)
C. gulbenkiani				0.3 (0-4)		
C. sp#54df*	0.2 (0-2)		0.1 (0-1)			
C. engubandei						0.2 (0-2)
C. onderstepoortensis						0.2 (0-2)
C. sp#50				0.2 (0-2)		
C. dekeyseri		0.1 (0-1)				
C. sp#107	0.1 (0-1)					
Total	5185.8 (275-20068)	2640.8 (247-9732)	4541.8 (166-25736)	2923.2 (251-10854)	6740.5 (313-26880)	5428.9 (367-24444)
Shannon Weiner index	0.63	0.83	0.59	0.57	0.42	0.39
Species evenness	0.2	0.28	0.2	0.19	0.14	0.13

*The numbering system is that of R. Meiswinkel and refers to yet undescribed *Culicoides* species.

Fig.1

A stationary Onderstepoort light trap (a-d) was operated at four sites. On every night of collection a second light trap (e-g) was operated at respectively 1 m, 4 m and 8.5 m away from the stationary trap at three of four sites where a stationary trap had been installed. To ensure that distance treatments were independent of any effect due to site or occasion the distance at which the second trap was operated was randomised in three repeats of a 4 x 4 Latin square design.

Figure