Comparison of the efficiency of the Onderstepoort- and CDC ultraviolet light traps for the collection of livestock associated *Culicoides* species in South Africa

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Abstract

Comparative monitoring of the abundance and distribution of *Culicoides* biting midges (Diptera: Ceratopogonidae), the biological vectors of the causative agents of several diseases of global veterinary importance, will be crucial in determining the risk of disease outbreak and spread. Ultraviolet (UV) suction traps have become the most frequent method used for the monitoring of *Culicoides* diversity and abundance. The current study compared the trapping efficiency of the two most used UV suction light traps, i.e., the Onderstepoort (OP)- and the CDC trap, for the collection of livestock associated *Culicoides* species in South Africa. The study confirmed the superiority of the OP trap and indicated a correlation in species composition and age grading results as determine with the two trap types. Substantial variations in the comparative trap efficiency, as found between areas and sites within an area, suggest that a universal conversion factor between the two trap types may not be advisable as it is unclear to what extent species composition and environmental factors may influence the conversion factor. Light traps, independent of trap model, can be considered acceptable for determining the serial comparison of population numbers for seasonal fluctuation and species abundance in distribution surveys.

Key words. *Culicoides imicola, Culicoides* species diversity, Trap type, Conversion factor.

INTRODUCTION

Since the discovery that blood feeding midges in the genus *Culicoides* (Diptera: Ceratopogonidae) are the biological vectors of bluetongue virus (du Toit, 1944) an excess of 75 arboviruses have been isolated from a variety of *Culicoides* species worldwide (Meiswinkel *et al*., 2004). At least six of these viruses, African horse sickness-, Akabane-, bluetongue-, epizootic hemorrhagic disease- and Schmallenberg virus, are of global veterinary importance (Purse *et al*., 2015).

In most cases *Culicoides*-borne arbovirus episystems potentially involve two or more viruses/pathogens, serotypes of the virus and various species of the vector and hosts within a geographical region. An excess of 1357 described *Culicoides* species (Borkent, 2017), coupled to wide-spread susceptibility to orbivirus infection in the genus (Carpenter *et al.,* 2008; Del Rio López *et al*., 2012; Ruder *et al*., 2012; Venter, 2016), emphasise the need to collect and identify all *Culicoides* species that may feed on susceptible hosts to pinpoint the vectors in an area. Seasonal outbreaks of these viral diseases in livestock are associated with high *Culicoides* abundance as reflected in light trap collections (Venter *et al.,* 1996ab).

Field collection of *Culicoides* will be crucial in identifying the geographical distribution of vector species, the factors that regulate this and the seasonal abundance and age structure of populations to indicate periods of vector activity and risk of transmission, as well as for the field evaluation of repellents (Braverman *et al*., 1999; EFSA, 2017; Medlock *et al.,* 2018). Field monitoring is used to define seasonal vector free periods that will influence vaccine regimes and the movement of animals and animal products between areas. Due to the logistical and funding constraints involved in conducting field surveys over large areas, models that predict the distribution and abundance of *Culicoides* may help to focus these surveys and make them more cost effective. The development, and subsequent validation, of these models will depend on reliable collection methods (Leta *et al*., 2017).

Due to relative ease of use and minimal influence from human error various models of light traps, albeit with variable levels of efficiency, have become the most extensive tools used to monitor *Culicoides* abundance (Venter *et al*., 2009; Del Río *et al*., 2013; Probst *et al*., 2015; Wilson *et al*., 2021). Currently, the two most commonly traps used are the Onderstepoort- (OP) and the Centres for Disease Control (CDC) downdraft traps with UV light (McDermott & Lysyk, 2020). Additional collection methods, amongst others, include direct collections from host animals, drop traps, vechile mounted traps and unbaited suction traps (Medlock *et al.,* 2018).

The OP trap, as manufactured and distributed by the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR, Pretoria, South Africa), is a modification of a discontinued commercially available trap imported from Europe in the early 1970s (Venter *et al*., 2009). Variations of this trap have been used since the 1970s for the collection of *Culicoides* midges for virus isolations (Nevill *et al*., 1992) and to determine species distribution and abundance in southern Africa (Venter *et al*., 1996a). Since 1996 this trap has been used in several countries in Europe, e.g. Italy (Goffredo *et al*., 2004), Greece (Patakakis, 2004), Switzerland (Cagienard *et al*., 2006) and France (Balenghien *et al*., 2008). Although the 220-V OP trap can be adapted, via a generator or an inverter system, to run on 12-V ts dependence on 220-V power supply limits the application thereof in rural areas and field situations (de Beer *et al.,* 2021).

A commercially available 12-V CDC trap (J.W. Hock, Gainesville, USA), often baited with CO_2, is the preferred trap in North America (Mullens & Schmidtmann, 1982). This trap was used in some countries in Europe, e.g. Spain (Miranda *et al*., 2004), Portugal (Capela *et al*., 1993) and Belgium (De Deken *et al*., 2008).

Discrepancies in the efficiency of the OP- and CDC trap have been described in several studies and the lack of an universal conversion factor between the two trap types makes it difficult to compare data between trapping events (Venter *et al.,* 2009; 2013; Porbst *et al.,* 2015; Del Río López, 2012; Del Río *et al.,* 2013). These discrepancies in trapping efficiency render it problematic to compare abundance results obtained with different trap models. Although trapping efficiency of the various trap models may differ between *Culicoides* species (Bishop *et al*., 2004) several factors contribute to this discrepancy. The efficient monitoring of vectors may be of greater importance in situations with low disease occurrence and envisaged low *Culicoides* abundance. In the current study the trapping efficiency of the OP- and CDC traps for the collection of livestock associated *Culicoides* were compared in two geographical areas.

MATERIAL & METHODS

Study area and collection period

Trap comparisons were conducted independently in two geographical areas, i.e., the Agricultural Research Council – Onderstepoort Veterinary Research (ARC-OVR) (-25.651081, 28.184421; 1219 m above sea level) in Pretoria, Gauteng Province, and the Paradys Experimental Farm (-29.219516, 26.2136765; 1400 m above sea level) of the University of the Free State in Bloemfontein, Free State Province, in South Africa. The mean annual rainfall at the ARC-OVR ranges between 430 mm and 1017 mm with a peak in summer between November and March (Venter *et al*., 1996b). Although the Free State is also a summer rainfall area the mean annual rainfall, ranging between 400 mm and 500 mm, is lower than at the ARC-OVR (Liebenberg, 2012). At the ARC-OVR the annual mean daily maximum and minimum temperatures are 26.3 °C and 9.3 °C, respectively (Venter *et al*., 1996b). In the higher-lying, Free State the mean maximum summer and winter temperatures are 26 °C and 16 °C, respectively, with night-time temperatures falling to a monthly mean of -2 °C (Liebenberg, 2012).

Comparative collections at the ARC-OVR were conducted two nights a week over 24 weeks, between 15 April and 25 September 2019, and over 12 weeks, between 8 April and 9 July 2019, at Paradys Farm. Due to project constrains collections could not be made during August and September at Paradys Farm. Since the two trap types were compared individually at the two sites, with no comparisons between sites, the influence of the shorter collection period at Paradys Farm will be neglectable. The OP- and CDC traps were randomly alternated on two successive nights between two comparable sites in each area, resulting in four collections per week in each geographical area. In each area the sites were at least 50 m apart and out of direct sight from each other. At the ARC-OVR the traps were operated under the eaves of stables housing 20 to 30 cattle nightly. During the day the cattle were in open pens (900 m^2) with concrete flooring in front of the stables where some of the cattle will spend the night. Trees and kikuyu lawns surrounded the stables. Vervet monkeys (*Chlorocebus pygerythrus*) were abundant in the area. At Paradys Farm, the traps were operated at an open camp housing between 5 and 10 cattle each. Wild birds and small rodents were abundant in both areas.

Collection method

The OP trap is a downdraft suction trap equipped with a 30 cm 8 W UV light tube (Fig. 1). This relatively heavy (4 kg) and robust powder coated metal trap can be left in situ for several months (Venter *et al*., 2009). The CDC trap, also a down-draught trap, is equiped with a 15 cm 4 W UV tube. This lightweight (0.8 kg), mostly plastic, trap has a rechargeable battery witch enables it to be operated in areas witout an electricity supply (Fig. 1).

The 12-V CDC traps were operated on 220-V via a transformer. The OP traps were operated on 220-V. Bigger insects were excluded by polyester netting (mesh size 2 mm) covering the entrance portals of the traps. Traps were hung 1.5 m above ground level and operated between dusk and dawn. Insects were collected into distilled water to which 0.5% ‘Savlon’ (Johnson & Johnson, South Africa) (contains

Chlorhexidine gluconate 0.3 g/100 ml and Cetrimide 3.0 g/100 ml) antiseptic was added as a wetting and bactericidal agent (Goffredo & Meiswinkel, 2004).

Fig. 1. The CDC- (left) and Onderstepoort 220-V (right) down draught light trap (Photocredit: C. J. de Beer).

After retrieval in the morning, the collected insects were filtered through fine gauze netting, transferred to 70% ethanol, and stored in the dark at 4 °C until analysed. Large collections were sub-sampled (Van Ark & Meiswinkel, 1992). At least a thousand individuals were identified from each collection made. The collected *Culicoides* were counted and identified to species level using appropriate keys and wing photographs (Labuschagne, 2016). Females were, based on abdominal pigmentation (Dyce, 1969), age-graded as either nulliparous, parous, gravid, or freshly blood-fed. Males captured were also counted. On nights where no or very few insects were collected, due to adverse weather conditions or trap failure, collections were repeated the following night.

Statistical analyses

Shannon diversity indexes were calculated based on the *Culicoides* species composition as determined with each trap type (Virtue-s.eu., 2020). The numbers collected in each trap type were subjected to an analysis of variance (ANOVA). As the data was not normally distributed nonparametric Wilcoxon matched-pairs signed-ranks tesing was employed as a post hoc test to establish significant differences between treatment means. The mean weekly collection size of the four most frequently collected species in each area were correlated between trap types. Variation in the weekly conversion factor between the trap types was graphically expressed as a deviation from 1, with "1" indicating that equal mean numbers were collected. Depending on sample size either Chi-square or Fisher's exact testing was conducted to determine significant proportional differences between treatments. Statistical testing was conducted at a 5% significance using the GenStat (VSN International, 2012).

RESULTS

ARC-OVR

At the ARC-OVR 24 collections were made with each trap type at each of two sites and 192 374 *Culicoides* were collected in 96 collections made between 15 April and 25 September 2019 (Table 1). The overall higher mean weekly numbers, 2295.7 ±2384.9, collected with the two OP traps were significantly ($P < 0.001$) different from that of 1712.1 ±2399.2 in the two CDC traps.

Of 100 760 midges collected at the first site (A), 55 963 (55.5%) were collected in the OP- and 44 797 (44.5%) in the CDC trap (Table 1). Although 1.2 times more midges were collected in the OP traps, the higher mean number, 2331.8 ±2853.5, collected did not differ significantly ($P = 0.207$) from that of 1866.5 ±3023.3 collected in the CDC traps. Of 91 614 *Culicoides* collect at the second site (B), 54 231 (59.2%) were collected with the OP- and 37 383 with the CDC traps (Table 1). Overall, 1.5 times more midges were collected with the OP traps and the higher mean numbers, 2259.6 ±2245.6, collected, was significantly different ($P = 0.003$) from that of 1557.6 ±2826.2 in the CDC traps.

Table 1. Compararive *Culicoides* species composition as determined with OP- and CDC down draught light traps at two sites at the ARC-OVR, Pretoria, South Africa, between 15 April and 25 September 2019. 24 colections were made with each trap at each site.

	Site A		Site B		Total (Site A + B)			Frequency of collection
	OP (%)	CDC (%)	OP (%)	CDC (%)	OP (%)	CDC (%)	Total (%)	
Species richness	20	17	17	18	20	20	22	
Shannon-Wiener Index	0.32	0.31	0.29	0.33	0.31	0.32	0.32	
C. imicola	52 718 (94.2)	42 237 (94.3)	51 573 (95.1)	35 247 (94.3)	104 291 (94.6)	77 484 (94.3)	181 775 (94.5)	96
C. enderleini	698 (1.2)	746 (1.7)	506 (0.9)	720 (1.9)	1204 (1.1)	1466 (1.8)	2670 (1.4)	81
C. zuluensis	196 (0.4)	183 (0.4)	194 (0.4)	123 (0.3)	390 (0.4)	306 (0.4)	696 (0.4)	81
C. magnus	1036 (1.9)	734 (1.6)	686 (1.3)	598 (1.6)	1722 (1.6)	1332 (1.6)	3054 (1.6)	71
C. brucei	881 (1.6)	690 (1.5)	702 (1.3)	297 (0.8)	1583 (1.4)	987 (1.2)	2570 (1.3)	65
C. nivosus	56 (0.1)	26 (0.1)	106 (0.2)	94 (0.3)	162 (0.1)	120 (0.1)	282 (0.1)	53
C. leucostictus	99 (0.2)	25 (0.1)	44 (0.1)	37 (0.1)	143 (0.1)	62 (0.1)	205 (0.1)	48
C. subschultzei	67 (0.1)	40 (0.1)	96 (0.2)	105 (0.3)	163 (0.1)	145 (0.2)	308 (0.2)	38
C. bolitinos	17 (<0.1)	19 (<0.1)	55 (0.1)	27 (0.1)	72 (0.1)	46 (0.1)	118 (0.1)	38
C. pycnostictus	30 (0.1)	19 (<0.1)	40 (0.1)	24 (0.1)	70 (0.1)	43 (0.1)	113 (0.1)	38
C. bedfordi	89 (0.2)	34 (0.1)	142 (0.3)	44 (0.1)	231 (0.2)	78 (0.1)	309 (0.2)	31
C. nevilli	13 (<0.1)	19 (<0.1)	30 (0.1)	13 (<0.1)	43 (<0.1)	32 (<0.1)	75 (<0.1)	28
C. trifasciellus	45 (0.1)	16 (<0.1)	36 (0.1)	31 (0.1)	81 (0.1)	47 (0.1)	128 (0.1)	20
C. schultzei	2 (<0.1)	5 (<0.1)	13 (<0.1)	17 (<0.1)	15 (<0.1)	22 (<0.1)	37 (<0.1)	18
C. similis	1 (<0.1)	2 (<0.1)	2 (<0.1)	2 (<0.1)	3 (<0.1)	4 (<0.1)	7 (<0.1)	6
C. exspectator	3 (<0.1)		4 (<0.1)	1 (<0.1)	7 (<0.1)	1 (<0.1)	8 (<0.1)	5
C. ravus	3 (<0.1)		2 (<0.1)	2 (<0.1)	5 (<0.1)	2 (<0.1)	7 (<0.1)	5
C. coarctatus	5 (<0.1)			1 (<0.1)	5 (<0.1)	1 (<0.1)	6 (<0.1)	3
C. nigripennis grp.	3 (<0.1)				3 (<0.1)		3 (<0.1)	1
C. angolensis grp	1 (<0.1)				1 (<0.1)		1 (<0.1)	1
C. gulbenkiani		1 (<0.1)				1 (<0.1)	1 (<0.1)	1
C. neavei		1 (<0.1)				1 (<0.1)	1 (<0.1)	1
Total	55 963	44 797	54 231	37 383	110 194	82 180	192 374	

Both trap types indicated a species richness of 20 species (Table 1). Representatives of the C. nigripennis group (3 specimens) and C. angolensis group (1 specimen) were only collected in the OP traps. On the other hand, one specimen each of *Culicoides gulbenkiani* Caeiro and *Culicoides neavei* Austen were only collected with the CDC traps. Despite these differences there was a strong correlation (r^2 = 1.00; P < 0.001) in the species composition as determine with the trap types.

The OP- and CDC traps indicate a species richness of 20 and 17 species respectively at Site A (Table 1). At Site B the OP trap indicates the presence of 17 species while 18 species were collected in the CDC trap. Although overall higher numbers were collected for most species in the OP trap the weekly conversion factor differs between species and sites. Of a total of 22 species collected overall only 18 were present in both trap types. *Culicoides enderleini* Cornet and Brunhes, *Culicoides schultzei* (Enderlein) and *Culicoides similis* Carter, Ingram and Macfie were collected in lower numbers in the OP- than in the CDC traps (Table 1). The Shannon-Wiener Index, ranging from 0.31 to 0.33, indicate that *Culicoides* species diversity did not vary between the two trap types or sites (Table 1).

The four most frequently collected species in both trap types were *C. imicola, C. enderleini, Culicoides zuluensis* de Meillon and *Culicoides magnus* Colaco (Table 1).

Culicoides imicola

Both trap types indicate *C. imicola* to be the dominant species, with no significant difference in the proportional representation, i.e., 94.6% and 94.3% in the OP- and CDC traps, respectively. It was the only species to be present in all 96 collections made (Table 1). Although the higher weekly mean numbers collected with the two OP traps (2172.7 ±2326.6) differ significantly ($P < 0.001$) from that of the two CDC traps (1614.3 ±2322.8) there was a strong linear correlation ($r^2 = 0.92$; $P < 0.001$) in the weekly mean numbers collected (Fig. 2). The linear trendline indicated that while midges may be absent in the CDC- up to 625.8 may still be collected with the OP traps (Fig. 2).

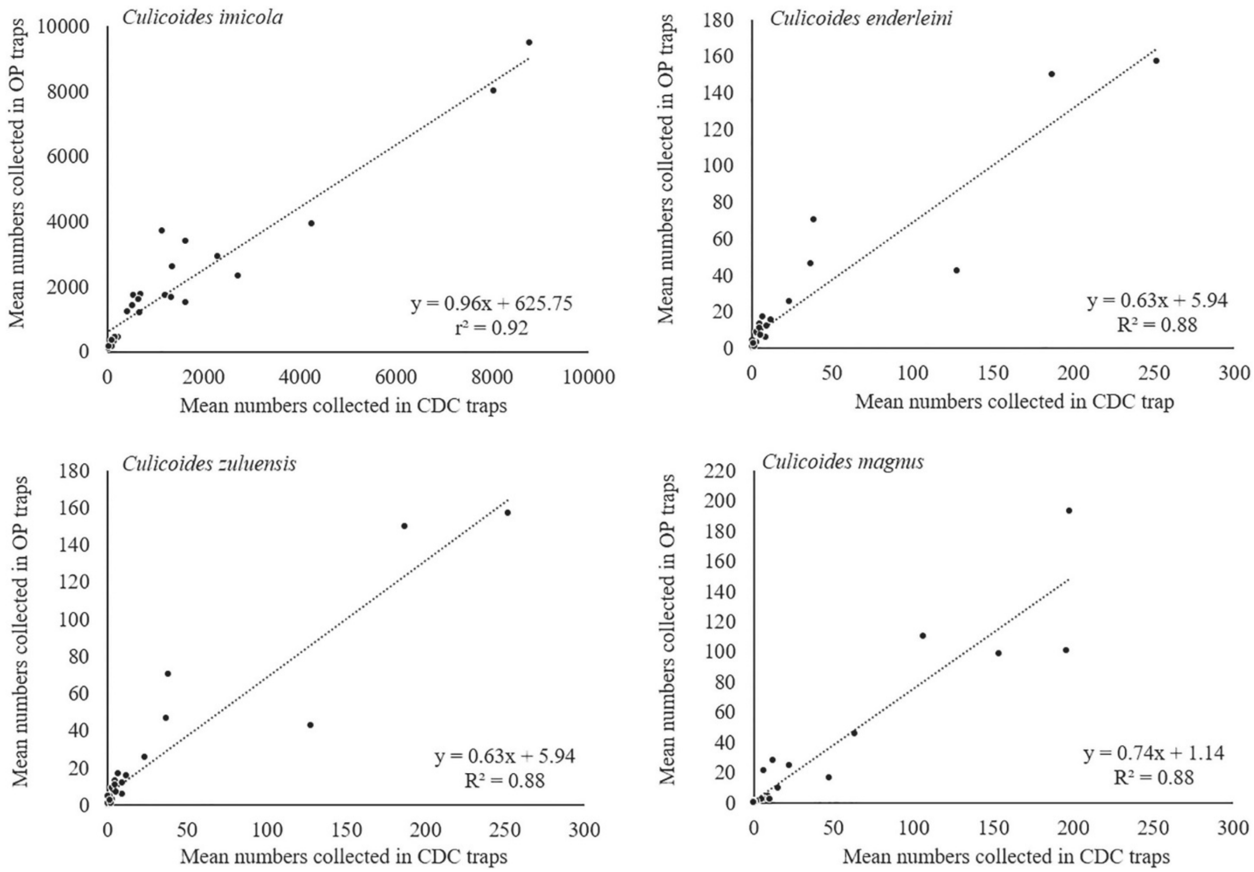

Fig. 2. Linear regression in weekly mean numbers as collected with OP- and CDC traps at two sites at the ARC-Onderstepoort Veterinary Research between 15 April and 25 September 2019.

On average the OP traps collected 1.3 times more *C. imicola* than the CDC trap and on occasion it collected up to four times more individuals than the CDC trap (Fig. 3). In four weekly trapping events higher mean numbers were collected in the two CDC traps with up to 0.9 times fewer midges in the two OP traps (Fig. 3).

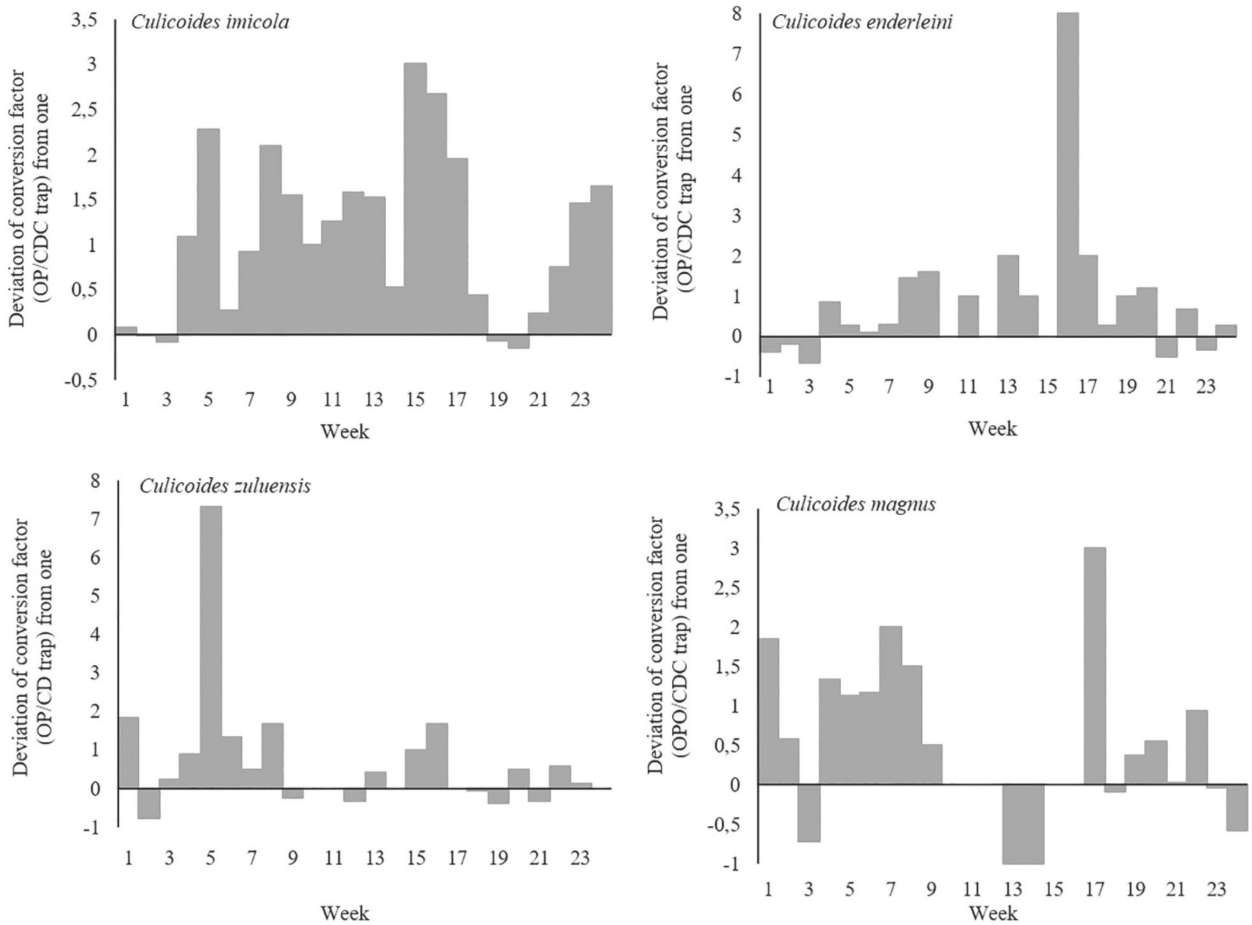

Fig. 3. Variation in the weekly conversion factor (OP trap/CDC trap) for the four most frequently *Culicoides* species collected between 15 April and 25 September 2019 at the ARC-Onderstepoort Veterinary Research expressed as a deviation from 1 (1 = no difference in the mean numbers collected with the OP- and CDC trap).

With proportional representations of 64.1% and 65.9% in the OP- and CDC traps respectively both trap types indicated nulliparous females to be the dominant age grouping (Table 2) and that it differs significantly ($P < 0.001$) between trap types. Similarly, the proportion of parous females differ significantly ($P < 0.001$) between the OP- (31.9%) and CDC trap (32.6%) (Table 2). The proportions of gravid females (0.3% and 0.2%), as well as the proportion of males (3.2% and 0.8%), in the OP- and CDC traps respectively, were low but not identical. Both trap types collected comparable low proportions of blood-engorged (0.5%) females (Table 2).

Table 2. Comparive age grading results for the four most frequently *Culicoides* collected with the OP- and CDC down draught traps between 15 April and 25 September 2019 at the ARC-OVR.

	OP					CDC				
	Female				Male	Female				Male
Culicoides species	Nulli Parous	Parous	Blood-engorged	Gravid		Nulli Parous	Parous	Blood-engorged	Gravid	
C. imicola										
Total collected	66 833	33 265	548	284	3361	51 043	25 237	425	134	645
(%)	(64.1)	(31.9)	(0.5)	(0.3)	(3.2)	(65.9)	(32.6)	(0.5)	(0.2)	(0.8)
Mean	1392.4	693.0	11.4	5.9	70.0	1063.4	525.8	8.9	2.9	13.4
(STD)	(1543.0)	(832.7)	(14.1)	(10.1)	(232.0)	(1846.5)	(935.4)	(22.3)	(5.9)	(29.5)
C. enderleini										
Total collected	704	321	24	6	149	865	450	4	4	143
(%)	(58.5)	(26.7)	(2.0)	(0.5)	(12.4)	(59.0)	(30.7)	(0.3)	(0.3)	(9.8)
Mean	14.7	6.7	0.5	0.1	3.1	18.0	9.4	0.1	0.1	3.0
(STD)	(28.4)	(14.0)	(1.9)	(0.7)	(7.1)	(52.1)	(24.5)	(0.5)	(0.3)	(8.2)
C. zuluensis										
Total collected	298	89	1	1	1	227	68	1	3	7
(%)	(76.4)	(22.8)	(0.3)	(0.3)	(0.3)	(74.2)	(22.2)	(0.3)	(1.0)	(2.3)
Mean	6.2	1.9	<0.1	<0.1	<0.1	4.8	1.4	<0.1	0.1	0.1
(STD)	(8.1)	(2.8)	(0.1)	(0.1)	(0.1)	(6.8)	(3.4)	(0.1)	(0.3)	(0.7)
C. magnus										
Total collected	1352	355	7	0	8	1066	252	11	1	2
(%)	(78.5)	(20.6)	(0.4)		(0.5)	(80.0)	(18.9)	(0.8)	(0.1)	(0.2)
Mean	28.2	7.4	0.1	0	0.2	22.2	5.4	0.2	<0.1	<0.1
(STD)	(55.8)	(14.0)	(0.9)		(0.5)	(42.0)	(9.3)	(1.0)	(0.1)	(0.2)

Culicoides enderleini

Together with *C. zuluensis, C. enderleini* was the second most frequently collected species. Both species were present in 81 of the 96 collections made (Table 1). *Culicoides enderleini* was encountered in 40 and 41 of the 48 collections made with OP- and CDC traps, respectively. With proportional representations of 1.8 % and 1.1 % respectively it was the second and fourth most abundant species collected with the CDC- and OP traps (Table 1).

The apparent lower weekly mean numbers collected with the OP traps, 25.1 ±43.2, did not differ significantly (P = 0.157) from that of 30.5 ±64.7 in the CDC traps. As for *C. imicola* there was a strong correlation (r^2 = 0.88; P < 0.001) in the mean weekly numbers collected with the two trap types (Fig. 2). The trendline indicated that while midges may be absent in the CDC traps up to 5.9 may still be collected in the OP traps (Fig. 2).

In one trapping event the two OP traps collected nine times more individuals than the two CDC traps (Fig. 3). In two weekly trapping events, equal numbers were collected in the two trap types and in five events the mean numbers collected in the OP traps were lower than that in the CDC traps (Fig. 3).

Both trap types indicate nulliparous females to be the dominant age grouping (Table 2). The proportional representation of nulliparous females, 58.5%, as determined with the OP traps did not differ significantly (P = 0.812) from that of 59.0% in the CDC traps. The proportion of parous females differ significantly (P = 0.025) between the OP- (26.7%) and CDC trap (30.7%) (Table 2). Both trap types indicated low proportions of blood-engorged (2.0%) and gravid females (0.5%). The relatively high proportions of males collected, in both the OP- (12.4%) and CDC (9.8%) traps, differ significantly (P = 0.036).

Culicoides zuluensis

Similar to *C. enderleini, C. zuluensis* was present in 81 of the 96 collections made and was encountered in 42 and 39 of the 48 collections made with OP- and CDC traps, respectively (Table 1). The relatively low weekly mean numbers collected with the OP trap, 8.1 ±8.4, did not differ significantly (P = 0.050) from that of 6.4 ±7.4 collected with the CDC traps. In contrast to *C. imicola, C. enderleini* and *C. magnus* there was a relatively weak correlation (r^2 = 0.60; P < 0.001) in the mean weekly numbers collected with the two trap types (Fig. 2).

The mean weekly numbers collected in the OP traps were up to 8.3 times higher compared to that in the CDC traps. In six of the trapping events, lower mean numbers were collected in OP traps (Fig. 3).

The proportional representation of nulliparous females representing 76.4% and 74.2% in the OP- and CDC traps respectively did not differ significantly (P = 0.535) (Table 2). Similarly, the proportional representation of parous females collected in the OP- (22.8%) and CDC trap (22.2%) did not differ significantly (P = 0.927). Low numbers of blood-fed (<0.05%) and gravid females (0.1%) and males (0.1%) were collected in both trap types (Table 2).

Culicoides magnus

The fourth most frequently collected species, *C. magnus,* was present in 71 of the 96 collections made (Table 1). It was encountered in 35 and 36 of the 48 collections made with the OP- and CDC traps, respectively. In both trap types, it represented 1.6% of the species composition (Table 1). The higher mean numbers, 35.9 ±62.1, collected in the OP traps did not differ significantly (P = 0.038) from that of 27.8 ±49.0 in the CDC traps. There was a correlation (r^2 = 0.88; P < 0.001) in the mean weekly numbers collected with the two trap types and the trendline indicated that while midges may be absent in the CDC trap up to 2.8 may still be collected in the OP trap (Fig. 2).

In one trapping event the OP traps collected up to four times more individuals than the CDC traps (Fig. 3) and on average it collected 1.3 times more individuals than the CDC traps. In six trapping events the CDC- outperform the OP traps.

Both trap types indicate high proportions of nulliparous females with no significant difference (P = 0.327) in the proportional representation between the OP- (78.5%) and CDC trap (80.0%) (Table 2). Likewise, the proportion of parous females also did not differ significantly (P = 0.263) between the OP- (20.6%) and CDC traps (18.9%). As for most other species low numbers of freshly blood-engorged and gravid females and males were collected in both trap types (Table 2).

Paradys Experimental Farm

At Paradys Farm, 2788 *Culicoides* were collected in 48 collections made between 8 April and 9 July 2019 (Table 3). As at the ARC-OVR, most, 1893 (67.9%), of these were collected with the two OP traps and the higher mean number, 78.9 ±82.40, collected in the 24 collections made with the OP traps differ significantly (P = 0.004) from that of 43.0 ±50.02 in the 24 collections made with the CDC traps.

Table 3. Compararive *Culicoides* species composition as determined with OP- and CDC downdraught light traps at two sites at the Paradys Experimental Farm, between 8 April and 9 July 2019. 12 colections were made with each trap at each site.

	Site A		Site B		Total (site A + B)			Frequency of collection
	OP (%)	CDC (%)	OP (%)	CDC (%)	OP (%)	CDC (%)	TOTAL (%)	
Species richness	12	11	12	13	14	13	15	
Shannon-Wiener Index	1.42	1.47	1.41	1.44	1.45	1.46	1.46	
C. imicola	454 (53.8)	138 (34.2)	374 (35.7)	252 (40.1)	828 (43.7)	390 (37.8)	1218 (41.6)	44
C. nivosus	161 (19.1)	158 (39.1)	450 (42.9)	245 (39.0)	611 (32.3)	403 (39.0)	1014 (34.7)	33
C. bolitinos	90 (10.7)	31 (7.7)	75 (7.1)	34 (5.4)	165 (8.7)	65 (6.3)	230 (7.9)	30
C. leucostictus	47 (5.6)	44 (10.9)	34 (3.2)	38 (6.0)	81 (4.3)	82 (7.9)	163 (5.6)	29
C. pycnostictus	61 (7.2)	23 (5.7)	77 (7.3)	29 (4.6)	138 (7.3)	52 (5.0)	190 (6.5)	24
C. magnus	10 (1.2)	1 (0.2)	8 (0.8)	10 (1.6)	18 (1.0)	11 (1.2)	29 (1.0)	16
C. subschultzei	4 (0.5)	4 (1.0)	9 (0.9)	6 (1.0)	13 (0.7)	10 (1.0)	23 (0.8)	12
C. zuluensis	12 (1.4)	2 (0.5)	11 (1.0)	4 (0.6)	23 (1.2)	6 (0.6)	29 (1.0)	12
C. enderleini	2 (0.2)	1 (0.2)	2 (0.2)	3 (0.5)	4 (0.2)	4 (0.4)	8 (0.3)	7
C. cornutus		1 (0.2)	4 (0.4)	1 (0.2)	4 (0.2)	2 (0.2)	6 (0.2)	6
C. similis	1 (0.1)	1 (0.2)	3 (0.3)	3 (0.5)	4 (0.2)	4 (0.4)	8 (0.3)	5
C. brucei	1 (0.1)			3 (0.5)	1 (<0.1)	3 (0.3)	4 (0.1)	3
C. bedfordi			2 (0.2)		2 (0.1)		2 (<0.1)	2
C. glabripennis				1 (0.2)		1 (<0.1)	1 (<0.1)	1
C. nigripennis grp	1 (0.1)				1 (<0.1)		1 (<0.1)	1
Total	844	404	1 049	629	1 893	1 033	2 788	

At the first site (A) the OP traps collected 2.1 times more midges than the CDC traps and the higher mean number collected, 70.3 ±74.1, was significantly (P = 0.027) different from that of 33.7 ±36.1 collected with the CDC traps. At the second site (B) the OP traps collected 1.7 times more midges than the CDC trap and the higher mean numbers, 87.4 ±92.4, collected with OP traps were significantly (P = 0.027) different than that of 52.4 ±61.1 collected with the CDC traps.

Overall, 15 species of *Culicoides* were collected in the 48 collections made, with 14 and 13 species collected in the OP- and CDC traps respectively (Table 3). The OP- and CDC traps indicate a species richness of 12 and 11 species respectively at the first site. At the second site the OP traps indicate the presence of 12 species while 13 species were collected in the CDC traps (Table 3). Overall, one specimen of the C. nigripennis group and two specimens of *Culicoides bedfordi* Ingram and Macfie were only collected in the OP trap. On the other hand, *Culicoides glabripennis* Goetghebuer (1 specimen) was only collected in the CDC trap (Table 3). As at the ARC-OVR there was a strong correlation ($r^2 = 0.96$; $P < 0.001$) between the *Culicoides* species composition as determine with the two trap types.

Low, but equal numbers of *C. enderleini* and *C. similis,* were collected in both trap types. The Shannon-Wiener Index, ranging from 1.41 to 1.47, indicates that *Culicoides* species diversity did not differ between trap type or site.

The four most frequently collected species in both trap types were *C. imicola, Culicoides nivosus* de Meillon, *Culicoides bolitinos* Meiswinkel and *C. leucostictus* (Table 3).

Culicoides imicola

As at the ARC-OVR *C. imicola* was the most frequently collected species. It was present in 44 of the 48 collections made and represented 41.6% of all midges collected (Table 3). While the OP traps indicate it to be the dominant species, representing 43.7% of the species collected, the CDC trap indicate it to be the second most abundant (Table 3). The marginally higher numbers of *C. nivosus* collected in the CDC traps, 16.8 ±22.4, however, did not differ significantly ($P = 0.886$) from that of the mean number, 16.3 ±16.3, of *C. imicola* collected.

The higher mean number, 34.5 ±33.2, of *C. imicola* collected in the OP traps differ significantly ($P = 0.008$) from that of 16.3 ±16.3 in the CDC traps and there was a strong linear correlation ($r^2 = 0.82$, $P < 0.001$) in the weekly mean numbers collected in the two trap types. The trendline indicated that while midges maybe absent in the CDC trap, low numbers, up to 4.6, may be collected in the OP trap (Fig. 4).

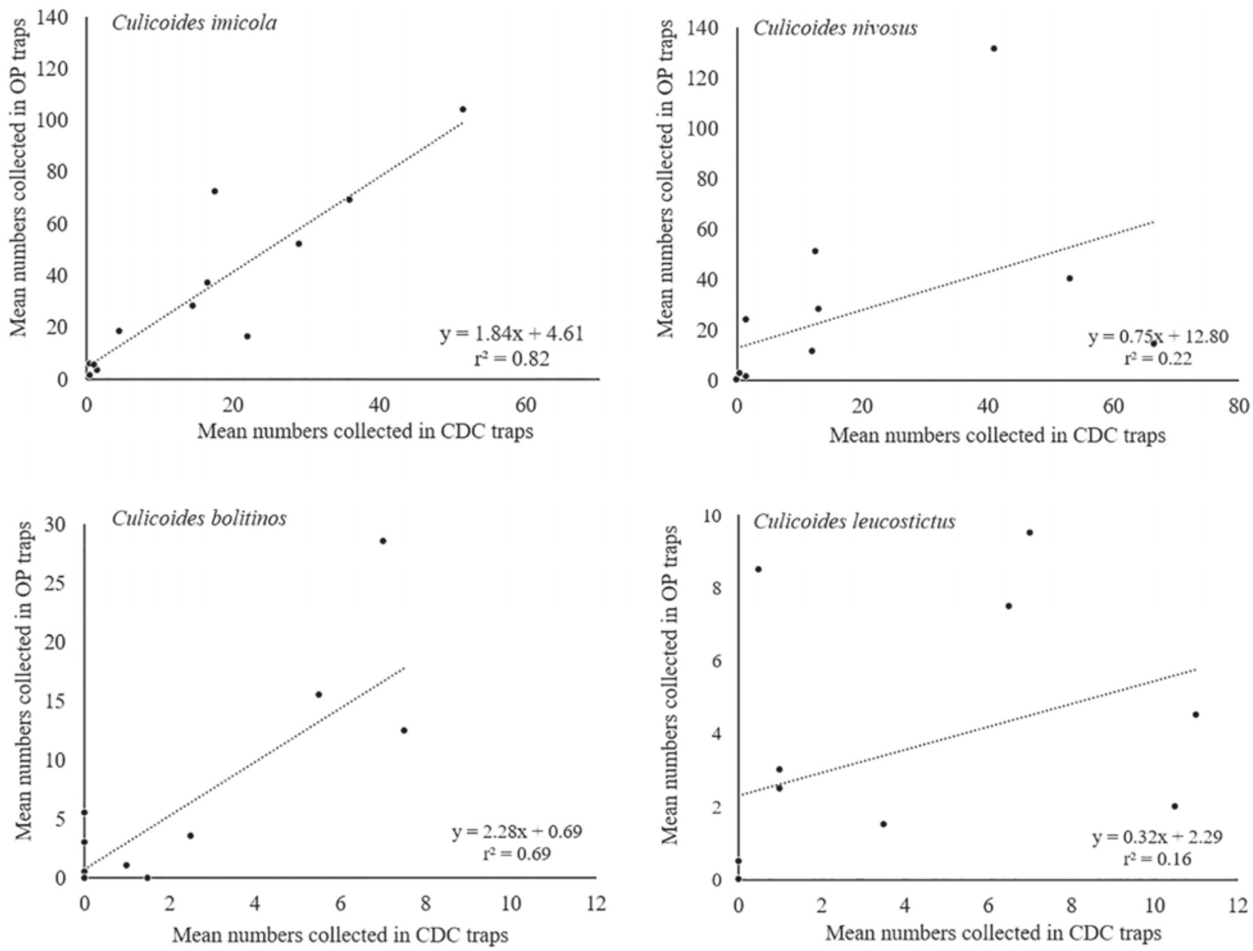

Fig. 4. Linear regression in weekly mean numbers as collected with an OP- and CDC traps at two sites at Paradys Experimental Farm between 8 April and 9 July 2019.

On average the OP traps collected 2.1 times more *C. imicola* specimens than the CDC traps. In one trapping event the mean numbers collected in the two OP traps were 12 times higher than that in the CDC traps and in another the mean numbers in the two CDC traps were 0.75 times higher than that in the OP traps (Fig. 5).

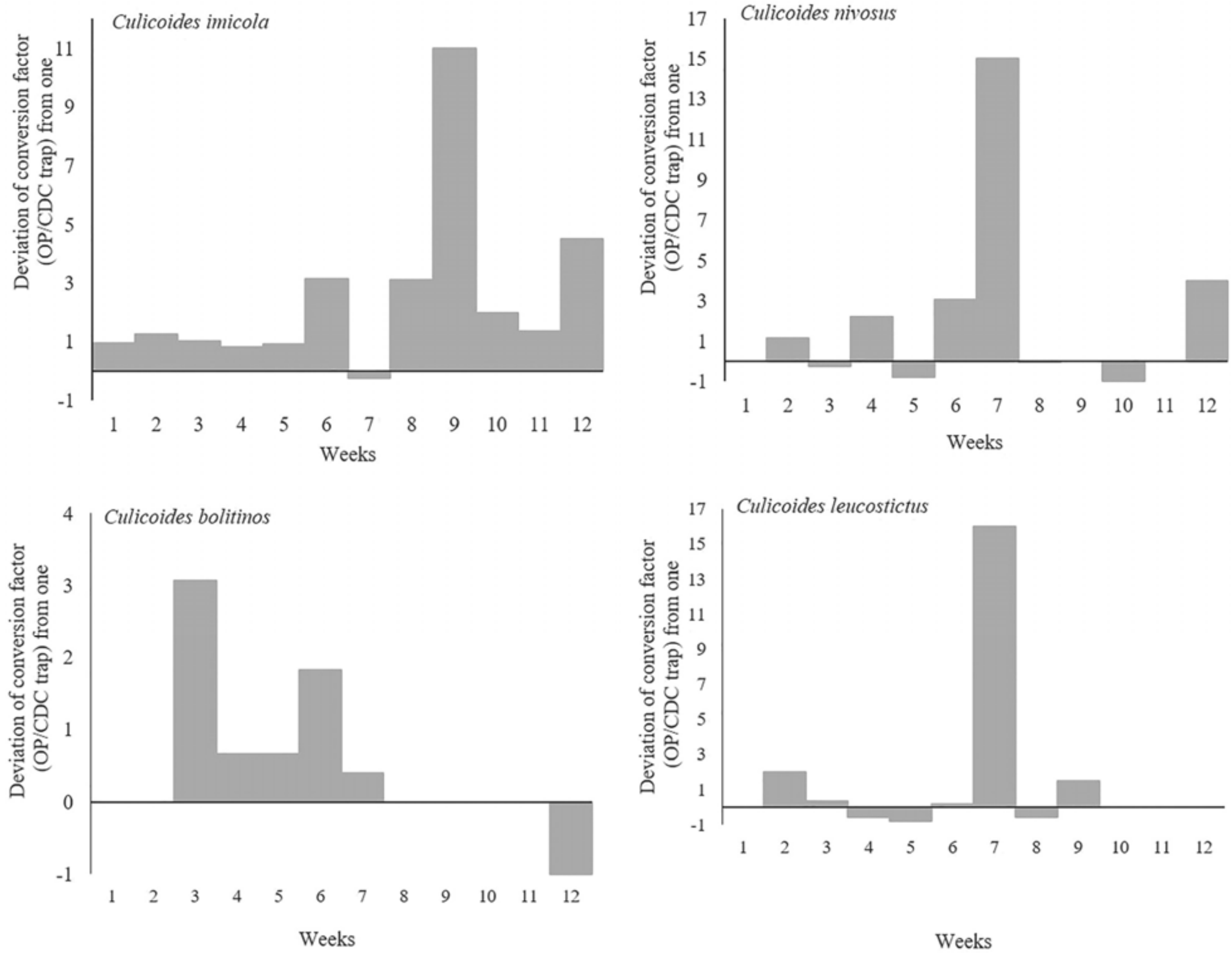

Fig. 5. Variation in the weekly conversion factor (OP trap/CDC trap) for the four most frequently *Culicoides* species collected between 8 April and 9 July 2019 at Paradys Experimental Farm expressed as a deviation from 1 (1 = no difference in the mean numbers collected with the OP- and CDC trap).

A representation of 56.3% in the OP- and 60.0% in the CDC traps respectively indicated nulliparous females to be the dominant age grouping and that the proportional representation did not differ significantly (P = 0.238) between the trap types. Similarly, the proportional representation of parous females, 31.0% and 28.5% in the OP- and CDC trap, respectively did not differ significantly (P = 0.385) (Table 4). Both trap types collected low numbers of freshly blood-engorged and gravid females. The relatively high proportions, 8.1% and 7.2% of males collected in the OP- and CDC traps respectively, did not differ significantly (P = 0.647).

Table 4. Comparive age grading results as determined for the four most frequently *Culicoides* collected with the OP- and CDC down draught traps between 8 April and 9 July 2019 at the Paradys Experimental Farm.

Culicoides species	OP					CDC				
	Female				Male	Female				Male
	Nulli parous	Parous	Blood-engorged	Gravid		Nulli parous	Parous	Blood-engorged	Gravid	
C. imicola										
Total collected	466	257	11	27	67	234	111	1	16	28
(%)	(56.3)	(31.0)	(1.3)	(3.3)	(8.1)	(60.0)	(28.5)	(0.3)	(4.1)	(7.2)
Mean	19.4	10.7	0.5	1.1	2.9	9.8	4.6	<0.1	0.7	1.2
(STD)	(22.0)	(11.2)	(0.7)	(1.8)	(3.3)	(11.6)	(5.5)	(0.2)	(1.0)	(2.6)
C. nivosus										
Total collected	52	60	2	331	166	49	40	1	195	118
(%)	(8.5)	(9.8)	(0.3)	(54.2)	(27.2)	(12.2)	(9.9)	(0.2)	(48.4)	(29.3)
Mean	2.2	2.5	0.1	13.8	6.9	2.0	1.7	<0.1	8.5	4.9
(STD)	(3.2)	(5.9)	(0.3)	(42.1)	(8.5)	(4.2)	(4.0)	(0.2)	(22.3)	(9.6)
C. bolitinos										
Total collected	72	52	1	27	13	25	17	0	19	4
(%)	(43.6)	(31.5)	(0.6)	(16.4)	(7.9)	(38.5)	(26.2)		(29.2)	(6.2)
Mean	3.0	2.2	<0.1	1.1	0.6	1.2	0.8	0	0.9	0.2
(STD)	(4.7)	(3.3)	(0.2)	(2.1)	(1.5)	(2.4)	(1.2)	(0)	(1.4)	(0.5)
C. leucostictus										
Total collected	31	12	1	9	28	28	18	2	9	25
(%)	(38.3)	(14.8)	(1.2)	(11.1)	(34.6)	(34.1)	(22.0)	(2.4)	(11.0)	(30.5)
Mean	1.3	0.5	<0.1	0.4	1.2	1.2	0.8	0.1	0.4	1.0
(STD)	(1.9)	(0.9)	(0.2)	(1.2)	(1.9)	(1.7)	(1.5)	(0.3)	(0.9)	(2.4)

Culicoides nivosus

Culicoides nivosus was present in 33 of the 48 collections made and was found in 18 and 15 of the 24 collections made with the OP- and CDC traps, respectively. While the CDC trap indicated *C. nivosus* to be the most abundant species it was the second most abundant in the OP trap (Table 3). As indicated for *C. imicola* the mean numbers of *C. nivosus* and *C. imicola* collected in the CDC traps did not differ significantly. Overall, the higher mean numbers, 25.5 ±35.9, of *C. nivosus* in the 24 collections made with the OP traps did not differ significantly ($P = 0.886$) from that of 16.8 ±22.4 in the CDC traps. No correlation ($r^2 = 0.22$; $P = 0.123$) was found in the weekly mean numbers collected in the two trap types (Fig. 4).

On average the OP traps collected 1.5 times more individuals than the CDC trap. In one trapping event 16 times more individuals were collected in the OP- compared to that in the CDC traps and with three events the CDC traps outperform the OP traps (Fig. 5).

Age grading results indicated gravid females to be the dominant age grouping (Table 4). Gravid females represented 54.2% and 48.4% in the OP- and in the CDC trap respectively and the proportional representation did not differ significantly (P = 0.441). The relatively high proportional representation of males, 27.2% in the OP- and 29.3% in the CDC traps, did not differ significantly (P = 0.475). The proportional representation of parous females, 9.8% and 9.9% in the OP- and CDC trap respectively, was nearly identical (P = 1.000). Low proportions of freshly blood-engorged females, 0.3% in the OP- and 0.2% in the CDC traps.

Culicoides bolitinos

The third most frequently collected species, *C. bolitinos* was present in 30 of the 48 collections made (Table 3). It was present in 16 and 14 of the 24 collections made with the OP- and CDC traps, respectively. With a representation of 8.7% and 6.3% respectively it was the third and fourth most abundant species in the OP- and the CDC traps respectively (Table 3). The mean numbers, 2.7 ±3.2, collected, however, did not differ significantly (P = 0.212) from that of *C. leucostictus* (3.4 ±4.2), the fourth most abundant species in the CDC traps (Table 3). The higher mean numbers of *C. bolitinos* collected in the OP traps, 6.9 ±8.8, differ significantly (P = 0.045) from that of 2.7 ±3.2 in the CDC traps. There was a correlation (r^2 = 0.69, P < 0.001) in the mean weekly numbers collected in the two trap types and the trendline indicated that while no midges may be collected in the CDC trap low numbers, 0.7, may still be present in the OP trap (Fig. 4).

On average 2.5 more individuals were collected in the OP- than that in the CDC traps. In one trapping event four times more individuals were collected in the OP trap and in another, the CDC- outperform the OP trap (Fig. 5). In five events both trap types indicated *C. bolitinos* to be absent.

With a representation of 43.6% in the OP- and 38.5% in the CDC trap nulliparous females were the dominant grouping and the proportional representation did not differ significantly (P = 0.554) (Table 4). Similarly, the proportional representation of parous females, 31.5% and 26.2% in the OP- and CDC traps respectively, did not differ significantly (P = 0.523). The relatively high proportions of gravid females, 16.4% in the OP- and 29.2% in the CDC trap differ significantly (P = 0.043). Low proportions of freshly blood-engorged females and males were collected in both trap types (Table 4).

Culicoides leucostictus

Culicoides leucostictus was present in 29 of the 48 collections and it was the fifth most abundant species collected (Table 3). Near equal (P = 0.973) mean numbers, 3.4 ±3.4 in the OP- and 3.4 ±4.2 in the CDC traps, were collected and no correlation (r^2 = 0.16, P = 0.199) could be established in the mean weekly numbers collected (Fig. 3). On occasion, the OP traps collected 17 times more individuals than the CDC traps. In three trapping events the CDC- outperforms the OP trap (Fig. 5).

Both trap types indicated nulliparous females to be the dominant age grouping (Table 3). The proportional representation of 38.3% in the OP traps did not differ significantly (P = 0.627) from that of 34.1% in the CDC traps. Similarly, the proportional representation of parous females, 14.8% in the OP- and 22.0% in the CDC traps did not differ significantly (P = 0.313). The relatively high proportions of males, 34.6% and 30.5% in the OP- and CDC traps respectively, did not differ significantly (P = 0.618).

DISCUSSION

To enable more reliable comparisons between the results of various trapping events the efficiency of the two most common traps used currently, OP- and the CDC downdraft traps (McDermott & Lysyk, 2020), was compared. Considering the potential wide-spread susceptibility to orbivirus infection in the genus *Culicoides* and the possible involvement in avian pathogens the results were, independent of vector status, focused on the four most abundant species collected at each of the two sites. In line with previous studies (Venter *et al.*, 2009; Del Río López, 2012; Probst *et al.*, 2015) the current study confirmed that the OP trap will, on average, collect higher numbers of *Culicoides*, especially of the confirmed orbivirus vector *C. imicola,* than the CDC trap. Considering the more powerful light source and fan of the OP trap the observed superiority is not surprising.

In previous comparisons done in South Africa the OP trap collected 2.0 to 3.5 times more *Culicoides* than the CDC trap (Venter *et al*., 2009). In a subsequent study, it collected 7.1 times more (Venter *et al*., 2013). In a comparison in Germany, 41.5 times more midges belonging to the Obsoletus complex were collected with an OP trap compare to that in a CDC trap (Probst *et al*., 2015). In Majorca, Balearic Islands, Spain, the OP trap collected 3.2 to 3.9 times more midges than the CDC trap, although, occasionally the CDC trap collected up to 1.6 times more midges than the OP trap with no overall difference in the efficiency of the two trap types (Del Río López, 2012; Del Río *et al.,* 2013). As in these studies extensive variation in the comparative efficiency of the OP- and CDC traps was observed in the present study. Similar variations in discrepancy were observed in comparisons of the OP trap with a commercially available Triple Trap (The Kendal Group, South Africa) (Venter *et al.,* 2013). While 7.4 times more midges were collected in the OP trap in summer only 1.2 times more were collected in winter (Venter *et al*., 2013).

The current study highlighted the relatively big variation in the comparative efficiency of the two trap types. The variation in comparative weekly trapping events accentuated that several environmental factors can influence *Culicoides* flight behaviour and consequently light trap efficiency. As defined for mosquitoes (Barr *et al*., 1963; Bidlingmayer, 1967), and in common with most trapping techniques, the quantity and quality of a collection not only depends on the type of trap used but also on weather conditions, several environmental variables, and especially trap location. In the current study, the importance of trap location was illustrated by the differences in the comparative efficiency of the trap types at the same site. E.g., at the ARC-OVR significant differences in the efficiency of the two trap types were only observed at one of the two collection sites.

In the present study, conducted at the end of summer and during winter to include potential low vector periods at both sites, no correlation could be established between low abundance and trap efficiency. Although seasonal abundance at the sites was not analysed, the weekly conversion factor (OP- versus CDC trap) for the four most frequently collected *Culicoides* species collected at each site seems to vary, independently of relative abundance, over the whole collection period (Fig. 3 & 5).

In evaluating the results, it must be considered that *Culicoides* populations are not homogeneously distributed in an area (González *et al*., 2017) and that, as was found for mosquitoes (Barr *et al*., 1963; Bidlingmayer, 1967), traps may function more efficiently when intercepting the flight paths of blood-seeking females, larval developmental sites or near aggregations on appropriate hosts. These flight paths as partly dictated by environmental factors may vary significantly on a nightly basis. It can be envisaged that the random movement of animals and local air currents in combination with yet unidentified factors may significantly influence the numbers of *Culicoides* collected nightly and the apparent efficiency of a trap.

In combination with the random movement of the hosts and vectors, the limited attraction range of the traps may contribute to the variation in trapping efficiency of the traps. Available results suggest that the range of attraction of the OP trap may be less than 4 m if operated near livestock (Venter *et al*., 2012; Elbers & Meiswinkel, 2015) as in the current study. In the absence of livestock the attraction range of OP- and CDC traps maybe extend up to 29.6 m and 15.3 m, respectively (Rigot & Gilbert 2012; Kirkeby *et al*. 2013). To minimise the potential influence of the two traps on the trapping efficiency the two traps were placed out of direct site from each other and 50 m apart in the current study conducted near livestock. A potential interference between the traps could, however, still have played a role. These observations suggest that background olfactory cues may reduce the attraction ranges and efficiency of the traps (Wilson *et al*., 2021). Light traps with shorter ranges of attractions, e.g., the CDC trap, may be appropriate for the evaluation of the effectiveness of control methods implemented to prevent *Culicoides* from entering stables, as it will lower the possibility of midges being artificially attracted to the trap from outside the stable. The stronger light source of the OP- compared to that of the CDC trap, may expand the range of attraction of the trap resulting that a larger proportion of the field population being attracted and sampled. As such it may reduce the number of trapping events needed to effectively sample a given area.

In both geographical areas, a strong correlation was found in the species composition as determined with the two trap types. For three, *C. imicola, C. enderleini* and *C. magnus,* of the four most frequently collected species at the ARC-OVR a strong correlation was found in the mean weekly numbers collected with the two trap types. At Paradys Farm, with an overall lower *Culicoides* abundance, this correlation was less pronounced and was restricted to two, *C. imicola* and *C. bolitinos*, of the four most frequently collected species. The weekly conversion factor between the two traps varies considerably between trapping events for these species.

The overall lower numbers collected in the CDC traps suggest that this trap may miss low abundant species. Although the trendlines generated support this observation, both trap types indicated a similar species richness. Despite the apparent higher efficacy of the OP trap both trap types were able to detect low abundant species and relative low numbers of blood-fed and gravid females and males. This indicate that both traps, despite the apparent lower efficiency of the CDC trap, will be effective in determining vector free or vector low periods.

Although less efficient the independence of the CDC trap on 220-V allow for greater flexibility and implies that the trap can be used in wildlife areas. Collections in wildlife areas will be of importance considering that wild bovine- and equine species can act as cycling hosts for orbiviruses such as bluetongue- and African horse sickness virus. CDC traps may as such be used effectively to determine and compare the species composition near wild live with that at livestock and assist with the identification of species that may act as a bridge mechanism for the transmission of orbiviruses between these two systems.

The age grading results as established by the two trap types were comparable. Significant differences, found in some instances, highlighted that *Culicoides* populations are not homogenously distributed in the area. Parity rates are of importance in determining the relative risk for potential virus transmission of a *Culicoides* population considering the apparent absence of transovarial transmission of orbiviruses in *Culicoides* (Osborne *et al.,* 2015). In evaluating parity results, as obtained by light trapping, it must be considered that orbivirus infections may render infected females adverse to light and that light traps may underestimate the parous rate in field populations (McDermott & Mullens 2017). Larger catches may, however, increase the chances of finding infected females (Bishop *et al*., 2004; Wilson *et al.,* 2021).

Typical to light traps operated near potential hosts both traps collected low numbers of freshly blood-engorged and gravid females as well as males of most livestock associated species. This accentuates that light traps, placed near livestock, mainly collect females flying around in search of a bloodmeal. A noticeable exception may be the apparent ornithophilic species *C. nivosus.* The dominance of gravid females, as indicated in both geographical areas and by both trap types, maybe indicative of autogenesis in this ornithophilic species.

Both trap types indicated relatively high but comparable abundances of the males of certain species, e.g., *C. nivosus* and *C. leucostictus.* This may be indicative of the presence of larval development sites of these species in the vicinity of the traps. While most *Culicoides* species usually mate soon after emergence while still at the larval developmental site some species mate near or on the livestock host (Shults *et al.,* 2021). High proportions of males of certain species in traps operated near the host may indicate the latter.

Since both traps use UV light as an attractant the lack of significant differences in species composition and age grading results is not unexpected. Although larger numbers will be collected with the OP trap it is clear both trap types will give comparable indications of species presence and relative abundance as well as age-grading results of *Culicoides* composition in an area. The variation in the comparative efficiency as found between the ACR-OVR and Paradys Farm, as well as for the sites at the ARC-OVR, emphasised the importance of site selection. Trap location can influence the number of midges captured, even more so than the choice of attractant (McDermott *et al*., 2016). The variation in the relative efficiency of the two traps, as observed in the present and previous studies, however, make it difficult to determine a reliable universal conversion factor between traps and that the conversion factor may be influenced by the species involved. The extent of the differential impact of environmental factors on the trapping may differ between light trap models.

Although a single trap per farm may not produce a reliable estimate of abundance for that farm due to spatial heterogeneity it will provide an indication of species diversity or the presence of a species at the site (McDermott & Lysyk, 2020). Despite several short comings, light traps, independent of the trap model, can be considered acceptable to give indices of absolute population numbers that were serially comparable at any one site for the seasonal fluctuation studies and they would catch most night active species present in the distribution surveys.

Data availability statement

All available data is presented in the manuscript.

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Author contributions

Study conception and design: CJdB (Joint FAO/IAEADivision of Nuclear Techniques in Food and Agriculture, Insect Pest Control Laboratory; Agricultural Research Council–Onderstepoort Veterinary Research), GJV (Agricultural Research Council–Onderstepoort Research; University of Pretoria), SSS (University of the Free State; Clinvet International (Pty) Ltd) and VRS (University of the Free State). Acquisition of data: CJdB, SSS and SNBB (Agricultural Research Council–Onderstepoort Research). Analysis, interpretation of data and manuscript writing: CJdB, GJV, SSS and VRS. All authors read and approved the final version of the manuscript.

Conflicts of interest statement

The authors declare no conflicts of interest.

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