Host location and selection by British *Culicoides* species associated with farms.

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Andrew Hope.

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Culicoides biting midges (Diptera: Ceratopogonidae) are biological vectors of economically important arboviruses of livestock. Two such arboviruses, bluetongue virus (BTV) and Schmallenberg virus (SBV) have recently emerged in northern Europe inflicting unprecedented outbreaks of disease in this region. The aim of the current investigation was to explore both host seeking behaviour and surveillance methods for livestock-associated *Culicoides* species in the UK.

To achieve this aim, a series of field-based, manipulative experiments were conducted using three farm sites in southern England. These studies demonstrated that host preference had a significant impact upon several parameters important in determining arbovirus transmission. *Culicoides* were found to be differentially attracted to different breeds of sheep ($p<0.05$) and blood feeding efficiency was shown to be determined in part by whether the sheep had been sheared ($p<0.05$). In addition the presence of an alternative host (a cow and its calf) was demonstrated to lead to an increased *Culicoides* biting rate on sheep held in close proximity ($p<0.05$), increasing the risk of arbovirus transmission.

Preliminary studies of volatile chemicals produced by hosts illustrated that while these attracted livestock-associated *Culicoides* at rates higher than those recorded in un-baited traps ($p<0.05$), collections only represented a small proportion of those collected on hosts themselves. These studies, however, provided a platform for future investigations of this area.

Finally, the use of light-emitting diode (LED) baited suction traps was trialled as a means of improving detection sensitivity in surveillance of *Culicoides* populations. This study found that certain *Culicoides* species demonstrated increased sensitivity to specific wavelengths ($p<0.05$) and integration of these commercially available traps could improve our understanding of the abundance, geographic distribution and behaviour of these species.

Contents

List of Figures

List of Tables

Chapter 1: Introduction

Forty-eight species of *Culicoides* (Diptera: Ceratopogonidae) have been identified in the UK (Boorman 1986) and, until recently, their primary economic significance lay in nuisance biting of humans and equids. *Culicoides impunctatus* Goetghebuer, is a notorious biting nuisance that impacts on the tourism and forestry industries of northern England, Wales and Scotland. Attacks of this species result in up to 20% of summer working days being lost in the forestry industry in Argyll (Hendry and Godwin 1988). *Culicoides* also inflict an allergic dermatitis on equids in the UK, colloquially termed 'sweet-itch' which is prevalent across the country, although economic impact has not been quantified (Mellor and McCaig 1974, Carpenter *et al.* 2008b). While these areas remain important, the primary focus of attention on UK *Culicoides* species has shifted in recent years, following the unprecedented emergence and spread of bluetongue and Schmallenberg viruses. These events have highlighted the importance of ruminant livestock-associated *Culicoides* in the UK and led to renewed interest in their biology and ecology. This thesis therefore examines the behaviour of such species in a series of primarily field-based studies, as a means to better understand the interactions between vector species of *Culicoides* and their hosts.

1.1. *Culicoides* Biology and Ecology

Culicoides are holometabolous, passing through four physiological stages of egg, larva, pupa and adult (Figure 1.1). The life cycle duration is dependent on both environmental temperature and species and generally proceeds more rapidly in tropical regions where there may be continuous presence of all four life stages

(Kettle 1962, Mellor *et al.* 2000). In northern Europe, the vast majority of species are thought to be either bi- or trivoltine and generally overwinter at breedingsites as fourth instar larvae (Boorman 1986, Holmes and Boorman 1987, Blackwell *et al.* 1992b, Sanders *et al.* 2011).

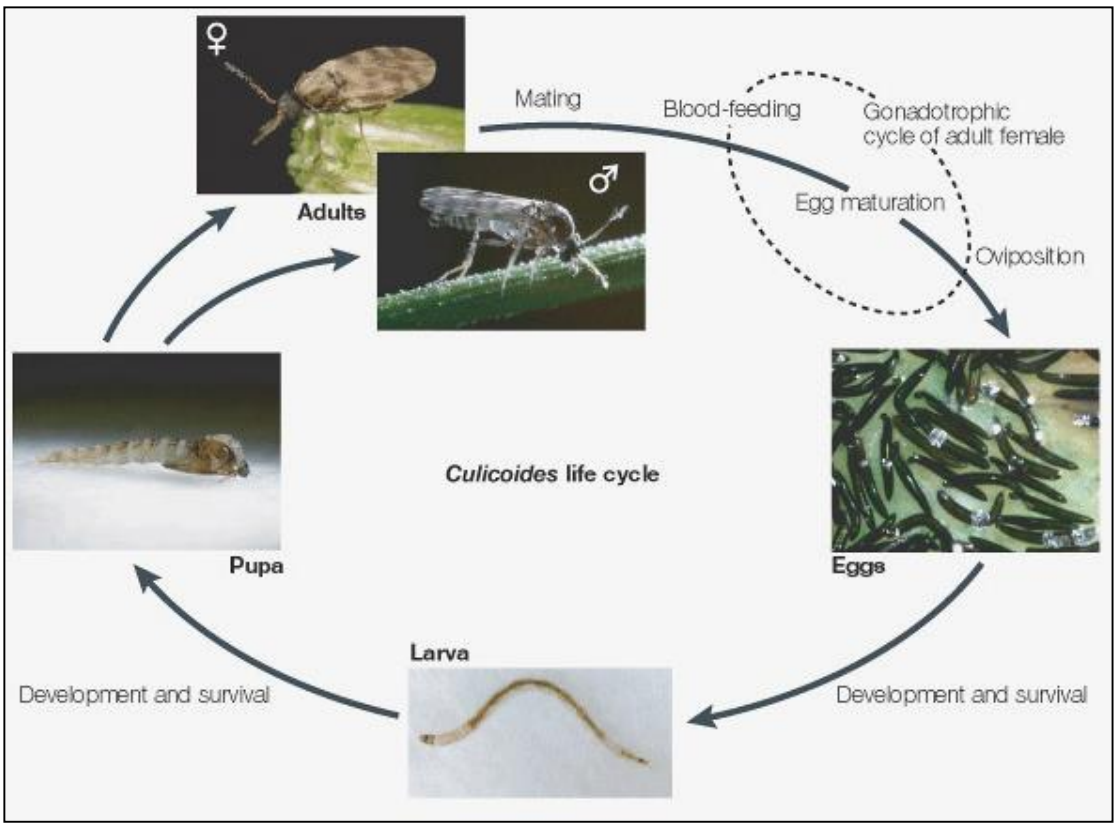

Figure 1.1. Life cycle of *Culicoides nubeculosus* (reproduced with permission from the author from Purse *et al.*, 2005)

Culicoides eggs are cigar-shaped and translucent when laid but darken to an opaque brown within half an hour. The size of eggs varies between species with *Culicoides obsoletus* Meigen eggs reported to be on average 366µm in length by 49µm in width (Jamnback 1961) while *C. impunctatus* eggs are 490µm by 80µm (Hill 1947). Fecundity in *Culicoides* ranges from 30-450 follicles according to both species and a wide range of host and environment related parameters (Service 1968, Kettle 1984). An important factor determining egg production appears to be the

source of blood-meal, with bird-feeding species generally producing a far larger number of follicles than those that feed on mammals (Kettle 1977). As an example, the ornithophilic species *C. circumscriptus* Kieffer has been shown to develop batches of up to 450 follicles while *C. impunctatus*, a primarily mammalophilic species, produces an average of approximately 50 eggs in its first batch (Service 1968, Kettle 1984, Carpenter *et al.* 2006b). Similar differences in egg batch size as a result of blood meal source being mammalian or avian are observed with mosquitoes (Shroyer and Siverly 1972), although underlying biological reasons have not been clearly identified. Other determinants of egg batch size include full completion of the blood-meal (Kettle 1962) and intra-specific variation in female body size (Akey *et al.* 1978). The duration of oogenesis varies according to both *Culicoides* species and climate and has repeatedly been found to be temperature dependent under laboratory conditions (Linley 1966, Carpenter *et al.* 2006b, Veronesi *et al.* 2009). In the Republic of South Africa, *C. imicola* Kieffer eggs were found to hatch after 1 day at 25ºC and 28ºC in the laboratory, but at 20ºC hatching took three days (Veronesi *et al.* 2009). Similar experiments with *C. subimmaculatus* Lee and Reye in Australia found egg hatching occurred in 3.9 days at 28ºC and 10.7 days at 18ºC (Edwards 1982).

Emerging *Culicoides* larvae are vermiform, semi-aquatic and free swimming (Kettle 1977). While largely generalist feeders, *Culicoides* larvae can be roughly divided into species with heavy, sclerotised pharyngeal apparatus and those possessing lighter structures (Kettle 1977, Mullen and Hribar 1988). This has been hypothesised to reflect diet, with the heavier mouthparts inferred to allow algal feeding, whereas *Culicoides* larvae with light mouthparts have a more predatory lifestyle (Mullen and Hribar 1988). It is usually difficult to pinpoint preferred food sources, however, due to difficulties in identifying diet selection in complex habitats

(Aussel and Linley 1994). Larvae pass through four instars during development and this part of the lifecycle often constitutes the longest part of the *Culicoides* lifespan (Mullens and Rutz 1983). In the afrotropic region development may be brief, for example 8-10 days in the Southern African species *C. bolitinos* Meiswinkel, where the immature stages develop in animal dung (Meiswinkel 1989). In the Nearctic and Palaearctic, however, development can last for over six months as a result of 4th stage larval instars entering diapause as a means of overwintering (Kettle 1984).

Culicoides pupae are either light or dark brown in colour and in the UK fauna can measure up to 4.5mm in length, although most species do not exceed 3mm (Kettle and Lawson 1952). The pupae do not feed and are largely inactive, often being visible on the surface of larval habitat (Kettle 1977). Pupation usually occurs over one to two days, but at low temperatures may be extended to several weeks (Edwards 1982, Mellor *et al.* 2000). In species identification, pupae are useful in possessing diagnostic characters, and a preliminary key has been published for the UK fauna (Kettle and Lawson 1952).

Adult *Culicoides* are amongst the smallest haematophagous insects and many UK species possess wing lengths of one millimetre or less (Campbell and Pelham-Clinton 1960). They are thought to be short lived, with the majority of emerging *Culicoides* surviving for fewer than ten days, although a small number of individuals are thought to be able to persist for longer periods of up to 90 days (Mellor *et al.* 2000). The uncertainty surrounding this fundamental area is caused by the difficulty in maintaining *Culicoides* in a laboratory setting, the inability to effectively apply capture-mark-recapture methodologies and the lack of straightforward age grading methods for the group. The latter has been partially addressed by dividing females according to the appearance of a burgundy pigment in the abdomen that is thought to

be associated with the accumulation of waste products following oogenesis (Dyce 1969). While widely adopted to distinguish females into nulliparous (those that have not matured an egg batch) and parous (those that have matured at least one egg batch) individuals, the method does not allow the worker to determine the number of egg batches that have been matured which would give a more accurate reflection of age (Dyce 1969). In addition, recent studies have shown that newly emerged females can also have pigmented abdomens and so results using this method should be viewed with some caution (Braverman and Mumcuoglu 2009, Harrup *et al.* 2013).

Adult diel periodicity in UK *Culicoides* species is primarily crepuscular, with peak appetitial activity in both males and females recorded at dusk and dawn (Hill, 1947; Blackwell, 1997; Sanders *et al*., 2012). The primary advantages of crepuscular activity are that *Culicoides* avoid meteorological conditions that lead to desiccation and conduct blood feeding at a time of low host animal activity. True diurnally active species have been described in the UK including *Culicoides heliophilus* Edwards (Boorman and Goddard 1970b) and *Culicoides riethi* Kieffer (Hendry 2011), while other species, such as *C. impunctatus*, have been shown to exhibit diurnal behaviour when disturbed by a host outside of their primary periods of activity (Blackwell *et al.* 1992b). Activity is also modulated by a range of other factors, most notably meteorological conditions (Blackwell 1997, Carpenter *et al.* 2008c, Sanders *et al.* 2012), season and moon and tidal phases (Kettle *et al.* 1998, Bishop *et al.* 2000).

Mating in *Culicoides* can be stenogamous, (involving the use of markers or hosts), or eurygamous and facultative (Downes 1955, Glukhova and Dubrovskaya 1974, Blackwell *et al.* 1992c). The mating behaviour of the UK *Culicoides* fauna is among the best described worldwide from observational studies and stenogomy has been observed for *C. obsoletus*, *C. pulicaris* Linnaeus and *C. punctatus* Meigen.

Culicoides nubeculosus Meigen has also been observed to mate whilst the female is blood-feeding on a host (Downes 1954). The Scottish biting midge, *C. impunctatus*, displays eurygamous behaviour using landmarks for the formation of swarms (Blackwell *et al.* 1992c). Females of the majority of *Culicoides* species worldwide are haematophagous, although autogeny has been documented in some 38 species which are capable of developing a first egg batch without a blood meal (Boorman and Goddard 1970a, Linley 1983). Of the major UK species, autogeny has been reported in *C. impunctatus*, but is not thought to occur in primary livestock associated species (Boorman and Goddard 1970a). The number of gonotrophic cycles successfully completed by a female *Culicoides* is dependent upon survival and the availability of hosts and oviposition sites during periods of oogenesis (Kettle 1962).

Adult *Culicoides* are poor fliers and active movement is greatly limited by meteorological conditions (Mellor *et al.* 2000). Dispersal is usually limited to within several kilometres from the emergence site (Kettle 1951, Lillie *et al.* 1981), although individuals may be carried over far greater distances through wind dispersal, largely inferred from the spread of *Culicoides*-borne disease (Sellers *et al.* 1977, Sellers *et al.* 1979, Gloster *et al.* 2008, Burgin *et al.* 2013). This semi-passive flight is one of the reasons why *Culicoides*-borne arboviruses are capable of rapid spread, particularly across large water bodies where airflows are thought to be more uniform (Burgin *et al.* 2013).

1.2 *Culicoides* as Arbovirus Vectors

Worldwide, *Culicoides* is by far the most important genus within the family Ceratopogonidae in their impact on animal and human health (Kettle 1977, Mellor *et al.* 2000). The genus contains species responsible for the transmission of a range of internationally important pathogens including viruses, bacteria, protozoa and nematodes of both animals (Linley 1985, Tabachnick 1996, Mellor *et al.* 2000) and humans (Linley *et al.* 1983, Carpenter *et al.* 2013). The most important of these pathogens are arboviruses, of which over fifty have been isolated from *Culicoides* species to date (Mellor *et al.* 2000). Currently the most important of these arboviruses in Europe are bluetongue virus (BTV); African horse sickness virus (AHSV) and the newly emerged Schmallenberg virus (SBV).

1.2.1 Bluetongue virus

Bluetongue virus is an *Orbivirus* belonging to the Reoviridae family which occurs in 26 serotypes (Mann *et al*., 2011). Only limited cross-protection from infection occurs across these serotypes, resulting in the co-circulation of diverse strains of different serotypes in endemic regions (Maclachlan and Mayo 2013). Bluetongue virus is the aetiological agent of bluetongue (BT), a haemorrhagic disease that occurs primarily in sheep (MacLachlan 1994), but which can also affect cattle (Darpel *et al.* 2007, Dal Pozzo *et al.* 2009) and deer (Vosding *et al.* 1968). Due to the impact of BT and the potential of BTV for rapid spread, it is classified as a notifiable disease by the World Organisation for Animal Health (OIE). A strong regulatory framework has been developed that is designed to control outbreaks, including the imposition of ruminant movement restrictions upon discovery of cases (Purse *et al.* 2005).

Bluetongue was first described in the Republic of South Africa following the importation of exotic merino sheep breeds that were highly susceptible to the disease (Hutcheon 1902). In Europe, BTV was historically confined to the southern fringes of the Mediterranean basin (Mellor *et al*., 2009). This distribution was interpreted as representing the northern limit of the only implicated vector of BTV in the region, *C. imicola* (Mellor *et al.*, 1985). From 1998, however, BTV expanded northwards into areas where *C. imicola* was known to be either spatially or temporally absent during outbreaks including Italy (Torina *et al.* 2004, De Liberato *et al.* 2005), the Balkans (Mellor 2004) and Bulgaria (Purse *et al.* 2006). This raised concerns that outbreaks might spread to more northerly latitudes through a so-called 'baton effect' of initial incursions of BTV driven by *C. imicola* populations allowing movement into new areas dominated by Palaearctic species.

Farm species that are commonly encountered and abundant in the Southern Mediterranean and Palaearctic regions include the *C. obsoletus* group and the *C. pulicaris* group (Mellor and Wittmann 2002). In Europe, the *C. obsoletus* group comprises *Culicoides obsoletus* Meigen; *Culicoides scoticus* Downes and Kettle; *Culicoides dewulfi* Goetghebuer; *Culicoides chiopterus* Meigen and *Culicoides montanus* Shakirzjanova (Boorman 1986, Gomulski *et al.* 2005). These species are easily separable in male specimens through genital morphology, but to a variable degree cryptic in the case of female specimens. Intact and well preserved specimens of *C. dewulfi* and *C. chiopterus* females can usually be separated relatively straightforwardly from other species via morphology of the wing and spermathecae (Delecolle 1985). Specimens of the other three species, which are grouped as the *C. obsoletus* complex are generally inseparable without detailed morphometric studies or the use of DNA analysis (Gomulski *et al.* 2005, Mathieu *et al.* 2007, Nolan *et al.*

2007, Schwenkenbecher *et al.* 2009). The *C. pulicaris* group similarly includes *C. pulicaris*, *C. punctatus* and an unknown number of other species that remain poorly described (Gomulski *et al.* 2006, Pages *et al.* 2009). In this case, *C. pulicaris* and *C. punctatus* can usually be separated by wing pattern, but there is some overlap in these characters (Lane 1981).

In Italy BTV serotype 2 and BTV-9 were initially isolated from field collected members of the *C. obsoletus* group and BTV-2 from members of the *C. pulicaris* group (Caracappa *et al.* 2003, De Liberato *et al.* 2005, Savini *et al.* 2005), recalling a previous study that had isolated BTV from pools of the *C. obsoletus* complex in Cyprus (Mellor and Pitzolis 1979). All of the members of the *C. obsoletus* group were known to be highly abundant on farms in the UK and northern Europe, with the exception of *C. montanus* which, although potentially under-reported due to difficulties in separation by morphology, appeared to be highly restricted in distribution in continental Europe, Turkey and Russia (Gomulski *et al.* 2005). The *C. pulicaris* group had also been recorded on farms across the UK although the taxonomic status and presence of cryptic species had not been assessed (Campbell and Pelham-Clinton 1960, Boorman 1986).

Vector competence studies in the laboratory had demonstrated that the *C. obsoletus* and *C. pulicaris* groups possessed a very low oral susceptibility to BTV infection leading to initial doubts that these species could act as primary vectors (Jennings and Mellor 1988). Field populations of both groups from multiple locations, however, were subsequently sampled in the UK and fed on infected blood using a pledglet feeding technique known to underestimate competence when compared to membrane methods (Venter *et al.* 2005, Carpenter *et al.* 2006a). Oral susceptibility rates of infection of 13% for *C. pulicaris* group and 7.4% for *C.*

obsoletus group were recorded indicating that this parameter was previously underestimated (Carpenter *et al.* 2006a). Later laboratory studies paired with species diagnostic PCR additionally demonstrated specifically that *C. scoticus* was capable of replicating BTV to high viral loads (Carpenter *et al.* 2008a).

In a second major change in its epidemiology, BTV was discovered in northern Europe for the first time in recorded history near Maastricht, the Netherlands in 2006 (Anonymous 2006). From molecular phylogenetic analyses, the new serotype 8 strain responsible was subsequently traced to sub-Saharan Africa (Maan *et al.* 2008), but the specific route of entry into Europe remains undefined (Mintiens *et al.* 2008, Carpenter *et al.* 2009b, Carpenter *et al.* 2013). Following emergence, BTV-8 expanded into Belgium, Germany, Luxembourg and France, before activity ceased during the winter (Mellor *et al.* 2009). The virus successfully overwintered at multiple loci, although a specific mechanism(s) for this phenomenon has not been defined (Wilson *et al.* 2008). In 2007, BTV-8 expanded its range to much of France, Germany and the Low Countries, placing the UK at high risk of incursion (Gloster *et al.* 2008). The index clinical case of BTV was recorded in the UK at Baylham Farm, Suffolk in September 2007 and was traced to infected wind-borne *Culicoides* from Belgium (Anonymous 2007, Gloster *et al.* 2008). A total of 125 affected holdings were identified from the outbreak (Szmaragd *et al.* 2010). Entomological surveillance carried out using light traps in the 2007-2008 autumn/winter period allowed the declaration of a "vector-free period", defined as when less than five parous (pigmented) *Culicoides* are found per trap for two successive trapping nights, which allowed livestock movement restrictions to be partially lifted (Carpenter *et al.* 2009a).

Following the cessation of BTV-8 transmission in the UK during the winter of 2007, a major voluntary vaccination campaign was initiated to eradicate the virus. This was driven by the expectation that re-emergence of BTV-8 would occur during spring 2008 with disastrous economic consequences for farmers across the country. A commercially-produced, inactivated vaccine was offered in a voluntary vaccination programme with substantial rates of uptake achieved in the south east of England, the region at greatest risk of emergence (Carpenter *et al.* 2009a). The success of this approach was demonstrated during 2008 when no new BTV-8 circulation in Britain was detected from clinical report cases. In subsequent years from 2009-10, BTV-8 was systematically eradicated from northern Europe (Figure 1.2), largely through compulsory vaccination and immunity of previously infected livestock.

The economic impact of BTV-8 in Europe was substantial; studies in the Netherlands estimated that costs during the 2007 outbreak were in the region of €163-€175 million (Velthuis *et al.* 2010). As BTV-8 outbreaks in the Netherlands represented a small fraction of the total area affected in Europe, the overall costs are likely to have run into the €1,000 millions, resulting in this being the most damaging single strain outbreak in history (Carpenter *et al.* 2009b).

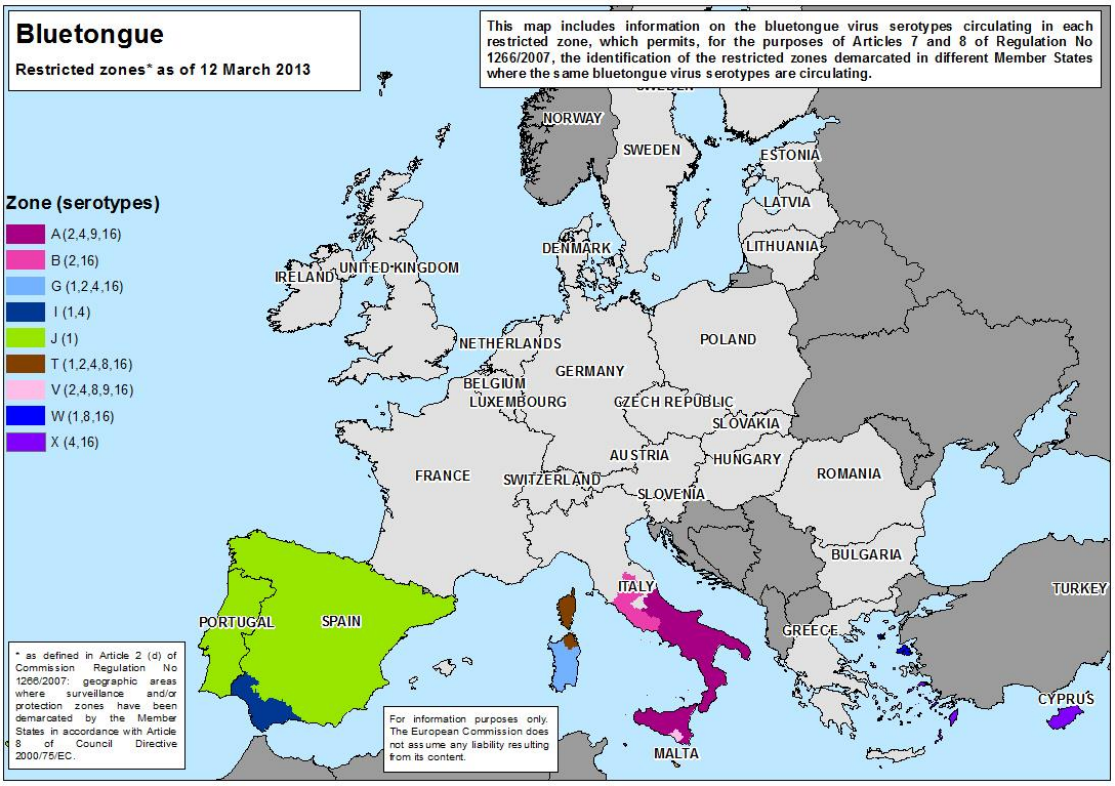

Figure 1.2. Current status of bluetongue virus in Europe in 2013
(http://ec.europa.eu/food/animal/diseases/controlmeasures/bt_restrictedzones-map_2012.jpg)

1.2.2 African Horse Sickness Virus

Like BTV, African horse sickness virus (AHSV) is also placed in the genus *Orbivirus*. African horse sickness exists in 9 serotypes and is the most lethal virus of horses known, inflicting mortality rates that can exceed 90% in susceptible populations (Mellor and Hamblin 2004). Mules, donkeys and zebras can also be infected by AHSV, although zebras do not exhibit clinical signs (Wilson *et al.* 2009). The virus is endemic in parts of sub-Saharan Africa, but has previously spread as far east as Pakistan and India in a devastating AHSV emergence which caused the death of over 300,000 equids in 1959-61 (Mellor and Hamblin 2004). A persistent outbreak

of AHSV additionally occurred in Spain and Portugal during the late 1980s and early 1990s, triggered by the importation of a viraemic zebra (Mellor 1993).

A series of experiments carried out at the Onderstepoort Veterinary Institute (OVI) in the Republic of South Africa implicated *Culicoides* species in the transmission of both AHSV and BTV (Du Toit 1944). *Culicoides* collected using a light-suction trap in the field were allowed to feed on a horse infected with AHSV, then re-fed 12 days later on a susceptible horse. This horse then demonstrated clinical signs of AHSV after a further 12 days. During the 1988 epizootic in Spain, AHSV was isolated from pools of field collected *Culicoides* (Mellor *et al.* 1990). Pools of *C. imicola*, a vector of AHSV in sub-Saharan in Africa, were found to contain infectious virus, but two pools of mixed species including *C. obsoletus* and *C. pulicaris* were also detected as positive for AHSV. This finding of AHSV isolations from Palaearctic species could have important implications for AHSV epidemiology and potential spread further north in Europe as it echoes previous experience with BTV. A key factor influencing spread in this region may be the lower population density of susceptible hosts compared to BTV (Lo Iacono *et al.* 2013).

1.2.3 Schmallenberg Virus

In autumn of 2011 a novel Orthobunyavirus affecting cattle was detected in Germany. Serum samples were obtained from dairy cows displaying clinical signs (reduced milk yield, fever and diarrhoea) and these were screened using metagenomic analyses (Hoffmann *et al.* 2012). A novel virus was identified belonging to the Simbu serogroup and was provisionally named Schmallenberg Virus (SBV), after the city near to which it was initially found. The major clinical

impact of SBV lies in the development of congenital defects in the foetus of ruminants infected during pregnancy (Elbers *et al.* 2013). Field collected *Culicoides* from surveillance in the Netherlands and Belgium were shown to contain SBV in their heads implying SBV dissemination and the potential for transmission (De Regge *et al.* 2012, Elbers *et al.* 2013). In an improvement to the studies with BTV, species were specifically implicated using a DNA barcode and identified as *C. obsoletus, C. scoticus* and *C. chiopterus*. Vector competence work in the laboratory also confirmed that the model species *C. sonorensis* which originates in the USA is capable of replicating the SBV to transmissible levels (Veronesi *et al.* 2013).

Schmallenberg was first detected in the UK during 2012 and has now spread rapidly throughout England, Wales, Scotland and Northern Ireland (Defra, Dardni) and continues to persist (see, http://www.defra.gov.uk/ahvla-en/files/20130114sbv-statistics.pdf; http://www.dardni.gov.uk/index/animal-health/animal-diseases/schmallenberg-virus.htm). The virus has also spread across a vast area of Europe from Italy in the south to Scandinavia in the north and from Spain to Latvia and Estonia (Figure 1.3). Cases of SBV infection reported to date are a substantial under-estimation of prevalence as clinical disease is only manifested in a small proportion of cases and is not an OIE notifiable disease in all EU states. This spread has been substantially more rapid than that recorded for the BTV-8 outbreak and while this may in part be due to a lack of movement restrictions imposed on livestock, although it has been hypothesised that a contributing factor could also be enhanced vector competence for SBV (Elbers *et al.* 2013). A vaccine against SBV is due to be available to UK farmers in summer 2013, but it is not clear what level of uptake there will be due to the uncertainty regarding the persistence of SBV in northern Europe and the economic impact of clinical disease.

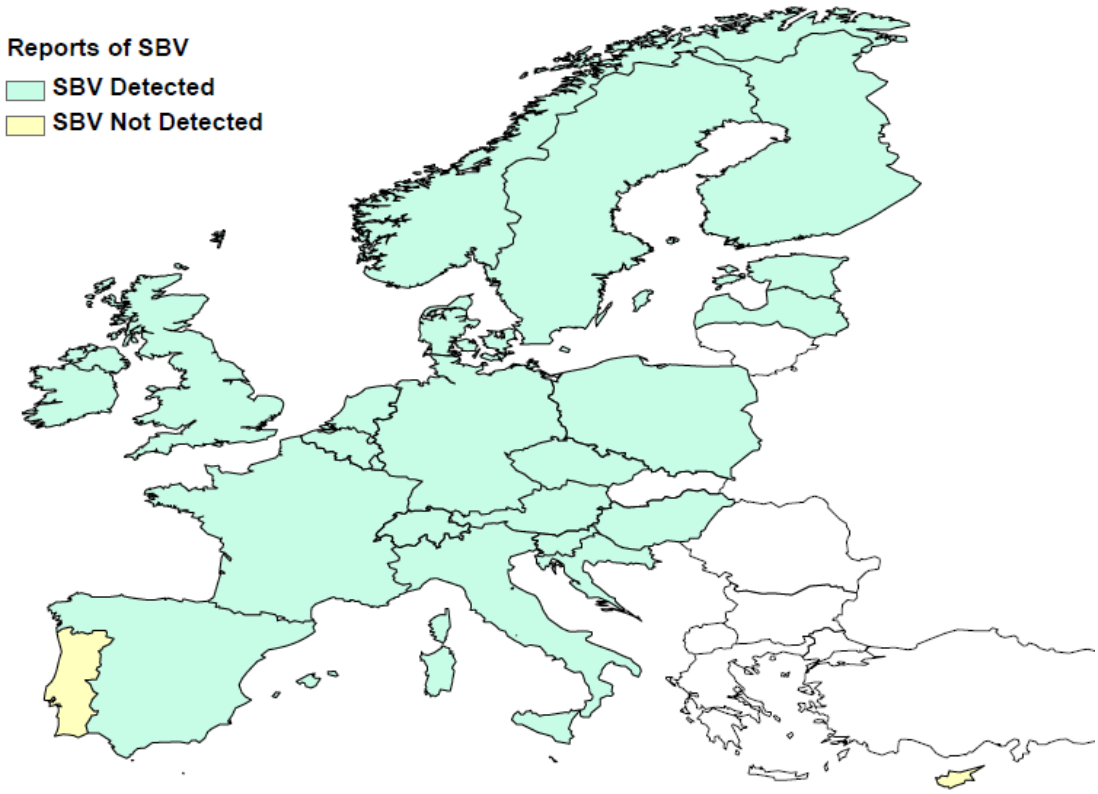

Figure 1.3. Current Status of Schmallenberg virus in Europe in 2013
http://www.efsa.europa.eu/en/search/doc/429e.pdf

1.3 Surveillance and Control of *Culicoides*

Culicoides surveillance has a role both in understanding the epidemiology of arboviruses and in ameliorating their impact. The standard method for surveillance for *Culicoides* in Europe is the use of UV light-suction traps of which several models are commercially available. Among these designs, what is commonly called the Onderstepoort Veterinary Institute (OVI) trap is considered to be the "gold-standard" and is the most commonly used trap in the region (Figure 1.4) (Mellor *et al.* 2004). This trap is usually used at permanent trapping sites requiring robust, low maintenance use, particularly in Italy (Goffredo and Meiswinkel 2004), France (Venail *et al.* 2012) and the UK (Carpenter *et al.* 2009a). In smaller scale studies and

particularly in those requiring setting up of traps with no mains power supply, the OVI trap is usually replaced by lightweight battery powered units including the CDC UV light-suction trap (Figure 1.4) (Gerry *et al.* 2009). This division is blurred in Spain, however, where these traps are also used for permanent surveillance sites (Calvete *et al.* 2006). Following the introduction of BTV to Germany, the surveillance programme that was initiated employed the BG-Sentinel trap (BioGents, Germany), which is another design variation on the UV light-suction trap (Mehlhorn *et al.* 2009). Attempts have been made to compare the performance of the OVI and CDC traps in addition to several historically utilised designs in South Africa (Venter *et al.* 2009) and studies are also underway in Europe. While the OVI trap was found to collect almost twice the number of *C. imicola* in total during the experiment as the second most successful trap in South Africa (Venter *et al.* 2009), statistical differences in collections were only observed in the age of the *C. imicola* collected when assessed by pigmentation of the abdomen. On the basis of the size and power of both bait UV light and suction fan used, it is highly likely that the OVI trap will outperform the other designs in both abundance and diversity of species collected.

(a) (b)

Figure 1.4. (a) OVI and (b) CDC light-suction traps commonly used for *Culicoides* collection

In addition to the limitations imposed by a lack of standardisation of surveillance trap models across Europe, light-suction trapping in itself has well known limitations in monitoring vector populations (Service 1993). As in other vector groups, the mechanism by which *Culicoides* are attracted to light is not fully understood, although it may be caused by disorientation as the light disrupts normal navigation cues. It has been demonstrated that *Culicoides* surveillance using light does not accurately reflect the abundance of biting individuals found on host animals and that abundance at light is influenced by a wide range of parameters beyond population density such as moonlight (Linhares and Anderson 1990, Bishop *et al.* 2000), meteorological conditions (Edwards *et al.* 1987, Linhares and Anderson 1990, Blackwell *et al.* 1992b) and host animals abundance and proximity to traps (Garcia-Saenz *et al.* 2011).

In Europe, recent studies have compared UV light-suction trap collections of *Culicoides* to collections made directly on host animals (Carpenter *et al.* 2008c, Gerry *et al.* 2009, Viennet *et al.* 2011, Viennet *et al.* 2012, Viennet *et al.* 2013). In a UK study, species composition in an OVI light trap was found to underestimate *C. chiopterus* and *C. dewulfi* compared with host seeking females found on sheep in a drop trap, with the former species being considered relatively rare in light traps (Carpenter *et al.* 2008c). The sheep-baited drop traps also did not yield any *C. pulicaris* despite the group making up 5.2% of the catch in light traps. Light-suction trap collections were carried out overnight after the drop trapping had finished, however, hence collections were not directly comparable. The low abundance of *C. dewulfi* and *C. chiopterus* in the light trap may also be due to the timing of activity of these species where activity ceases earlier than *C. obsoletus* and *C. scoticus* and the efficacy of the light-suction trap against ambient light is reduced (Sanders *et al.* 2012).

Similar results to those found in the UK have been reported in France where drop trap collections on sheep were compared to OVI light-suction traps (Viennet *et al.* 2011). An over-estimation of the abundance of *C. obsoletus* in the light trap and an under-estimation of *C. dewulfi* was recorded and very few *C. chiopterus* were collected (Viennet *et al.* 2011). The study also included collections using direct aspiration from penned sheep and the use of a sticky trap on the host animal, with both techniques catching fewer *Culicoides* than drop trapping. In a second study conducted at the same site, drop trap collections were replaced by collections using sticky traps on host animals, again drawing comparisons with catches in an OVI light-suction trap (Viennet *et al.* 2013). The authors compared *Culicoides* response to horse, sheep, cow, goat and hen; of the host animals the collections were greatest on

the horse (625 females) and the sheep collected very low numbers (5 females). *Culicoides obsoletus* was found to be the most abundant species in the UV light-suction trap, yet was only the third most abundant on host animals after *C. scoticus* and *C. dewulfi*. In contrast to the study in the UK, *C. chiopterus* was collected in greater numbers in the light trap collections compared to the on animal collections (Carpenter *et al.* 2008c, Viennet *et al.* 2013). Comparisons between catches on sheep, CO_2 traps and UV CDC traps in Spain found significant differences between the abundance of species collected (Gerry *et al.* 2009). In the case of *C. obsoletus* 313 individuals were collected on sheep but only 2 and 16 in CO_2 traps and UV traps respectively and no *C. dewulfi* or *C. chiopterus* were collected in stark contrast to the UK and French studies which found higher numbers of *C. obsoletus* in the light trap compared to on the sheep. These studies highlight the need for improved surveillance techniques that are more representative of the biting pressure to which hosts are exposed and the different methodologies used in each also highlights the importance of standardised study designs so that comparisons can be made.

Culicoides control techniques have generally been applied to nuisance biting species, rather than vectors of arboviruses, where vaccination tends to form the primary means of reducing transmission (Carpenter *et al.* 2008b). Methods most commonly used include the use of larvicides, adulticides, larval habitat modification or destruction, stabling of livestock, the application of repellent compounds and employing attractant traps (Kettle 1962, Carpenter *et al.* 2008b). While these techniques have been used with transient success against isolated populations of *Culicoides*, in major larval development areas their use is often impracticable. A key example in the UK was attempts by the Department of Health in Scotland to devise control programs for *C. impunctatus* between 1945 and 1958 (Kettle 1996). Despite a

systematic approach, these attempts proved unsuccessful due primarily to the vast larval habitats utilised by *C. impunctatus* and their inaccessibility (Kettle 1962).

Following the outbreak of BTV-8, greater attention was paid to the use of insecticides applied directly to cattle and sheep as a protection against adult *Culicoides* (Carpenter *et al.* 2008b, Venail *et al.* 2011). These products were already in use against a wide range of ectoparasites and are typically pour-on formulations, applied along the back of ruminants. In laboratory bioassays encouraging results have been shown for the effect of deltamethrin insecticides on hair samples from sheep and cattle (Schmahl *et al.* 2009) and using WHO insecticide assays (Venail *et al.* 2011). These results were obtained from laboratory trials rather than from feeding on hosts in the field, however, where results have been equivocal at best (Venail *et al.* 2011). It was found that when *Culicoides* were allowed to feed on sheep treated with a commercially available deltamethrin pour on the mortality rate peaked at just 45%. This echoes studies in the USA that demonstrated a permethrin treatment had no significant effect on seroconversion to BTV in cattle (Mullens *et al.* 2001). The latter was in spite of the fact that a previous study demonstrated a reduction of *C. sonorensis* by 80% following permethrin treatment up to 7 days post-treatment (Mullens *et al.* 2000).

Housing animals at the greatest times of *Culicoides* biting was also recommended during the BTV outbreaks in northern Europe, however this would require that farmers have enough sheds for all their animals and that they are sufficiently midge-proofed as *C. obsoletus* and *C. imicola* have been demonstrated to enter animal housing (Baldet *et al.* 2008, Baylis *et al.* 2010, Calvete *et al.* 2010, Romon *et al.* 2012). Key concerns with studies that examined entry of *Culicoides* into buildings were a lack of standardisation in the degree of enclosure and midge-

proofing used and the increased efficacy of light-suction trapping in collections indoors. An exception is a study conducted in France where indoor and outdoor collections were carried out with both drop traps and suction traps (Viennet *et al.* 2012). In this study *C. obsoletus* was collected inside the stable but was ten times more abundant in the outdoor collections, showing that while hosts are still at some risk from biting indoors it is to a far lesser extent than outdoors (Viennet *et al.* 2012). This relationship is also thought to vary with time of year as demonstrated in a study in England (Baylis *et al.* 2010).

Studies of *Culicoides* to date demonstrate that there is a need to further our understanding of their host location behaviour to develop improved tools for surveillance and control. Current surveillance techniques in particular have significant limitations and their improvement is required given the clear on-going threat of *Culicoides*-borne arboviruses to the livestock industry. The *Culicoides* surveillance program in the UK was crucial during the 2007 BTV outbreak as these data provided evidence of a vector-free period when animal movement restrictions could be lifted (Carpenter *et al.* 2009a). The subsequent demonstration of the differences between collections on hosts and the collections in light based surveillance traps highlights the need for improved techniques that better reflect the biting rate that occurs on hosts. A clearer understanding of host location can be achieved by examining the behaviour of vector species in relation to hosts and by attempting to identify what cues, particularly olfactory, are driving this attraction. Finally, host-seeking behaviour and host preference are in part responsible for driving the epidemiology of arbovirus outbreaks, constituting a major part of attempts to describe transmission using mathematical models (Gubbins *et al.* 2008).

Understanding how *Culicoides* biting rates vary on hosts directly contributes to improving such modelling exercises.

1.4 Behavioural Studies of Host Location by Haematophagous Diptera

Haematophagous behaviour is prevalent within the Diptera, having been recorded in the Culicidae, Psychodidae, Simuliidae, Glossinidae, Tabanidae, Muscidae and Ceratopogonidae families, among others. In the majority of these families a blood meal is required for the development of egg batches and hence blood-feeding is found only in females. Exceptions to this include Glossinidae, Tabanidae and Muscidae, where both sexes blood-feed. Host location by Diptera is a complex process involving both endogenous and exogenous factors (Takken and Knols 1999, Pickett *et al.* 2010, Takken and Verhulst 2013). These may include: the circadian and seasonal rhythm of ectoparasites and their nutritional and physiological status in addition to meteorological variables that influence host and ectoparasites activity, seasonal fluctuations in populations, light intensity and olfactory and visual cues from the host (Torr 1989, Gibson and Torr 1999). The response to host cues in haematophagous Diptera is largely modulated through stimuli such as kairomones, body heat and visual cues (including movement, size, shape and contrast) (Sutcliffe 1986, Colvin and Gibson 1992, Gibson and Torr 1999, Takken and Knols 1999, Takken and Verhulst 2013).

The process of locating a host on which to feed can be represented as a continuum of behaviours encompassing three inter-related processes from inactivity to finding a suitable host on which to land (Sutcliffe 1986, Dodd and Burgess 1995, Gibson and Torr 1999, Day 2005). These steps can be summarised as:

1. Activation: governed by circadian rhythms resulting in ranging flight to seek host stimuli;
2. Long range orientation: usually via olfactory responses but in some species may also include response to visual cues;
3. Close range orientation and landing: via olfactory, visual and thermal cues.

While undoubtedly an over-simplification of a continuum of processes this basic categorization provides a useful general framework to compare host location between vector groups. Contrasting patterns of activity across blood-feeding Diptera can be clearly discerned and related largely to adaptive advantages for the groups concerned. In general, large, strong flying species that are resistant to desiccation such as those found within the families Glossinidae and Tabanidae tend to be diurnally active with a highly developed visual capacity for detecting movement of hosts (Allan *et al.* 1987). In contrast, the Ceratopogonidae and Psychodidae are relatively weak fliers and susceptible to desiccation, utilise olfaction to a greater degree in host location and are either crepuscular or nocturnal (Mellor *et al.* 2000, Ready 2013). The Culicidae lie in an intermediate position, being smaller and somewhat more fragile than the Glossinidae and Tabanidae, yet within the family there are species which are predominantly diurnal, while others are crepuscular or nocturnal (Barrozo *et al.* 2004).

To illustrate host location behaviour, this review will focus on the two families of haematophagous Diptera that are most extensively studied; the Glossinidae and the Culicidae. Within the Glossinidae, *Glossina morsitans morsitans* Westwood and *Glossina pallidipes* Austen of the *Morsitans* group, are of interest due to their importance in the transmission of trypanosomiasis. The low reproductive rate and obligate requirement of both sexes in this family to feed on blood also offers a

real opportunity for effective control. Similarly, our knowledge of host location in mosquitoes largely rests on major vectors of malaria, dengue and yellow fever viruses and lymphatic filariasis. Necessarily, the review is limited to areas of relevance to the current study as vastly more detailed and complete reviews are available (Sutcliffe 1986, Torr 1989, Gibson and Torr 1999, Logan and Birkett 2007, Pickett *et al.* 2010, Takken and Knols 2010, Takken and Verhulst 2013).

1.4.1 Host location in the Glossinidae

Step One: Activation and Ranging Flight

Both *G. m. morsitans* and *G. pallidipes* are diurnally active, with peaks of biting activity in the field observed in the morning and late afternoon and seasonal changes in daily flight duration (Brady and Crump 1978, Bursell and Taylor 1980). In addition to circadian factors, activation is also linked to nutritional status with flight activity increasing with starvation (Brady 1972) meteorological conditions (Brady and Crump 1978) and the presence of host odours and visual stimuli (Brady 1972, Warnes 1992). In the absence of host odour plumes, field experiments using video recording of ranging tsetse have shown that they orientate downwind and then revert to upwind flight when host odour is introduced (Gibson *et al.* 1991). Downwind orientation while ranging would be advantageous as it would require less energy expenditure and also means that the ranging insect is more likely to come into contact with an odour plume closer to its source (Sabelis and Schippers 1984).

Significant inter-specific differences in response have been recorded in the laboratory to whole host odour according to species of tsetse (Warnes 1992). The sole study to investigate activation of naturally resting field tsetse flies in the field measured emergence, (i.e. activation), from a resting refuge in response to olfactory

and moving visual stimuli (Torr 1988). Limited activation was observed in response to olfactory stimuli, with just 18.7% of flies responding to 240 L/min ox odour and 28.5% responding to 0.2 L/min carbon dioxide. The moving visual target, however, resulted in the highest rate of activation (34.8%) and with no significant differences between the two species identified in the study (Torr 1988).

Step Two: Long-range Orientation

Long range responses to hosts in the Glossinidae are hypothesised to be largely mediated by host odour, rather than visual or thermal cues. This was demonstrated by early studies that investigated the detection of hosts at long-range by placing them into underground pits and then collecting tsetse that were attracted to vented airstreams in the absence of visual, motion or thermal cues from the host (Vale 1974). Tsetse were shown to respond to whole host odours up to 90 metres downwind of the host following activation (Vale 1977). This experimental design also demonstrated attraction of male and female *G. morsitans* and *G. pallidipes* to ox odour and an inhibitory effect on attraction when human odour was added (Vale 1974). Similar methodologies later demonstrated that attraction increased with mass of the preferred host animals placed in the pit (Hargrove and Vale 1978).

Attempts have also been made to identify the individual components of host-odour that elicit the greatest response in tsetse, although these studies have been plagued by a lack of agreement between laboratory and field findings. Carbon dioxide has been shown to activate tsetse and induce upwind flight in both the laboratory (Turner 1971, Bursell 1984a, Colvin *et al.* 1989) and in the field, using electric nets to intercept flight towards the odour source (Torr 1990, Torr and Mangwiro 1996). As a single compound, however, CO_2 is only mildly attractive to

tsetse in the field, collecting approximately 25-30% of the catch attracted to whole host odour (Vale 1979, Vale 1980). A wide range of other volatile chemicals have been shown to elicit increase responses from tsetse in the laboratory when combined with CO_2, most prominently acetone (Bursell 1984b, Hall *et al.* 1984) and 1-octen-3-ol (hereafter referred to as octenol) (Hall *et al.* 1984), both of which are significant components of oxen odour. In the field, this led to synergistic effects in certain studies (Torr *et al.* 1995), although in most cases acetone and octenol release rates were far higher than that naturally released from hosts (Torr *et al.* 1995).

Attraction of tsetse flies has also been recorded to traps baited with the urine of host animals (Owaga 1985, Hassanali *et al.* 1986, Vale *et al.* 1986). Fractions of urine and individual phenolic compounds have been found to induce responses in tsetse through electrophysiological and behavioural testing (Hassanali *et al.* 1986, Bursell *et al.* 1988). Field trials carried out using visually attractive traps demonstrated that one of the urine fractions resulted in significantly higher collections of *G. pallidipes* than a control trap with no olfactory stimulus (Hassanali *et al.* 1986). Individual phenolic compounds were then trialled in high doses in combination with acetone and octenol and three were found to significantly increase collections relative to traps baited with acetone and octenol alone (Bursell *et al.* 1988). Despite these advances in understanding components of host odour, a range of different synthetic ox odours comprising CO_2, acetone, octenol, butanone and phenols at natural doses have been trialled and remain inferior in attracting tsetse with collections around half the size when compared to natural odour (Hargrove *et al.* 1995, Torr *et al.* 1995). These data suggest that there must be other, as yet unidentified, components in ox odour that are important for host location in *Glossina*.

Step Three: Close-range Orientation and Landing

At close-range, host location appears to represent a transition from primarily olfactory to visual cues, although the former still appear to play a minor role (Torr and Solano 2010). It has been demonstrated that tsetse following an odour plume have difficulty locating the exact source, unless it is marked with a visual cue, and fly beyond the source before then turning and flying back downwind to re-join the plume (Vale 1974, Bursell 1984b, Torr 1989). This phenomenon is not just applicable to synthetic host cues; in the ventilated pit tests with oxen, a visual cue had to be employed in order to concentrate tsetse at the killing net (Vale 1974). Tsetse are also known to respond to mobile baits, collections using these targets were not enhanced by the addition of odour suggesting that the response is largely mediated by vision (Vale 1974).

Using electric nets in a field experiment it was demonstrated that tsetse could be diverted by a visual cue from an odour plume to another odour plume at six metres distance (Torr 1990). Increased collections of tsetse were made in plumes of acetone and octenol with a visual cue implying that olfaction plays a role in mediating the response of tsetse in combination with vision. Four electric nets surrounded the visual target but collections were not different between nets with no significant upwind bias in the odour plume after encountering the visual target (Torr 1990). In a separate study responses of tsetse were recorded as they approached and left a square black target positioned downwind from an odour source (Brady and Griffiths 1993). Flies were shown to turn upwind towards the odour source in plumes of acetone or a combination of octenol and two phenols (Brady and Griffiths 1993). At five metres distance from the source of an acetone plume, there was no significant

increase in arrival rates, however, the octenol/phenol combination odour release increased arrivals. The flight direction of tsetse leaving the field of vision was significantly upwind for both treatments, however, in contrast to the previous study on acetone and octenol (Torr 1990). Recordings at 10 metres did not show the same results for acetone as arrival and departure were not significantly different to those observed in the no odour control, but for the octenol/phenol combination there was still significant upwind arrival and departure, albeit less than at 5 metres suggesting close range attraction.

The results from these two studies may have been influenced by the visual target used. It has been demonstrated that using a similar methodology with electric nets, tsetse are generally collected upwind and that adding a black target to the net where the odour is dispensed results in tsetse concentrating at that particular net (Torr 1989). When the target is moved to a side net, the collection is again concentrated at the visual cue rather than at the odour source. This could explain the finding by Torr (1990) that the tsetse flies diverted by the visual cue from the original odour plume did not show upwind bias in the second odour plume, if the black target in the centre had been electric then this would perhaps have had the highest collection of tsetse (Torr 1990). In a second trial in this study it was found that when a target was placed on the upwind electric net this led to a significant increase in catch (Torr 1990). Similarly in the second experiment it is not known what happens to the tsetse that leave the field of vision in an upwind orientation as there is no collection at the actual odour source (Brady and Griffiths 1993).

At close range it appears that lactic acid inhibits tsetse fly landing and this is possibly why humans are less attractive for these species as they emit approximately 15 times more lactic acid than bovines (Vale 1974, Hargrove 1976, Dekker *et al.*

2002). The fact that *G. morsitans* are still attracted towards oxen in the presence of humans would suggest that this repellent effect is a close range cue, although it is clear that semiochemical output from cattle has the potential to screen that of humans given size differences (Vale 1974). Feeding of *G. morsitans* and *G. pallidipes* was observed on oxen that had been sprayed with lactic acid in comparison to un-sprayed controls. Untreated oxen attracted about twice as many *G. moristans* females and male and female *G. pallidipes* and the number of fed individuals was far higher on the untreated animals, although these results were not analysed statistically (Vale 1979).

The significant body of work on the Glossinidae has also highlighted the attraction of tsetse to different colours. Spectral sensitivity using electro-retinograms found that *G. morsitans* was sensitive to ultraviolet and most of the spectral range visible to man and this provided the basis for what colours to test for attraction in the field (Green and Cosens 1983). A variety of coloured traps were trialled in the field and efficacy was dependent on their reflectivity in different wavelength bands, with blue proving to be the most effective and black being important to induce landing (Green 1986). In addition to colour of targets, size and shape have also been shown to be important (Hargrove 1980, Torr *et al.* 1989, Torr *et al.* 2011). While response to colour is not necessarily a host-seeking response, it could be related to the search for a resting site, mating location or an larviposition site, nonetheless, the discovery of this behaviour in tsetse has been hugely beneficial and aided the development of control techniques which are now widely used and employ both olfactory and visual cues (Torr and Vale 2011). It is clear from the extensive body of work conducted on tsetse flies that host location is a complex process governed by a number of different olfactory and visual cues which are influential both individually and in combination.

1.4.2 Host Location in the Culicidae

The Culicidae comprises 37 genera with over 3,000 species described, exhibiting a very broad range of host location behaviours (Service 2000). In the species examined to date, olfaction is the principal means by which Culicidae locate a host (Takken and Knols 1999, Takken and Verhulst 2013) and is additionally a source of inter- and intra-specific differential attraction between hosts (Lindsay *et al.* 1993, Knols *et al.* 1995, Brady *et al.* 1997, Dekker and Takken 1998, Mboera and Takken 1999). Differential attraction can be induced by the presence or absence of certain kairomones as well as by their relative quantities and understanding what drives this attraction could provide useful tools for the development of baits or repellents (Logan *et al.* 2008). The vast majority of studies of host location in the Culicidae have centred upon three major vector species: *Aedes aegypti* Linnaeus, *Anopheles gambiae* Giles and *Culex quinquefasciatus* Say and this comparative review is restricted to these. Reviews of the vast number of studies associated with understanding host-seeking behaviour are provided elsewhere (Clements 1999, Gibson and Torr 1999, Takken and Knols 2010, Takken and Verhulst 2013).

Step One: Activation and Ranging Flight

Circadian activity within the Culicidae is diverse, ranging from primarily diurnal/crepuscular host-seeking in *Ae. aegypti* to primarily nocturnal activity in *An. gambiae* and *Cx. quinquefasciatus*. Similar to studies of the Glossinidae, physiological status has also been thoroughly investigated in relation to host-seeking and also shown to affect flight activity in both *Anopheline* and *Culex* females. These factors include mosquito age, nutritional and hydration status, the presence or

absence of eggs, mating status and the number of gonotrophic cycles already completed (Klowden 1996, Clements 1999, Gibson and Torr 1999).

Activation by CO_2 has been demonstrated for all three representative species in the laboratory: *Ae. aegypti* and *An. gambiae* have been shown to be activated by minor changes in CO_2 concentration relative to background levels (0.01-0.15%) and *Cx. quinquefasciatus* is activated by releases at the equivalent of human CO_2 emission (Eiras and Jepson 1991, De Jong and Knols 1995a, Healy and Copland 1995, Takken *et al.* 1997, Geier *et al.* 1999a, Bosch *et al.* 2000, Dekker *et al.* 2005, Dekker and Carde 2011, Lacey and Carde 2011, Lacey and Carde 2012). It is notable, however, that the relative impact of CO_2 as an activating agent varies substantially, even within closely related species complexes and populations and its complicated role in activation has been reviewed (Gillies 1980, Grant and O'Connell 2010). To a far greater degree than the Glossinidae, both electrophysiological and molecular studies of this process have been made and are beginning to be integrated into wider studies of genomics (Justice *et al.* 2003, Manoharan *et al.* 2013). Interestingly, unlike the Glossinidae, where refuges for resting flies could be used for monitoring activation, very few studies have directly examined initial activation of Culicidae in the field. An exception is a study that demonstrated evening mass movement of *An. gambiae* in relation to an apparent circadian host-seeking response in Africa (Gillies 1961). Ranging flight additionally remains an area of some confusion in the optimal flight pattern to detect host odour plumes (Carde and Willis 2008).

Step Two: Long-range Orientation

Defining host-seeking behaviours in the Culicidae at long range has proved challenging, not least due to difficulties in defining standardised techniques (Grant and O'Connell 2010). In wind tunnels, where attraction is measured by upwind flight towards a source of CO_2, attraction has been found to vary from 50-98% in *Ae. aegypti* (Eiras and Jepson 1991, Dekker *et al.* 2005, Dekker and Carde 2011). Bioassays using dual port olfactometers, however, gave a reduced response of between 10-19% *Ae. aegypti* entering the port with the kairomone source, despite apparent activation in the region of 90% of individuals tested (Bernier *et al.* 2007). These contrasting results make it difficult to determine the extent to which CO_2 acts as an activator, long range attractant and/or short range attractant for *Ae. aegypti*.

A degree of caution must be employed when interpreting the results of trials examining behavioural responses in laboratory bioassays. By necessity, these trials are conducted in extremely controlled environments that bear little relation to what is experienced by the insects in the field setting. Test insects are usually supplied from colony strains which may have been established for decades, where normal active host seeking behaviour is unnecessary and behaviour has developed that is different to that which is observed in the field. With clean air controls *An. gambiae* and *Cx. quinquefasciatus* have been shown to take to flight in the laboratory and in some cases they are shown to land in the trapping ports of olfactometers which would be classed as attraction if it were with a test stimulus, this is less pronounced in *Ae. aegypti* (Knols *et al.* 1994, Mboera *et al.* 1998, Geier *et al.* 1999b, Dekker *et al.* 2005, Lacey and Carde 2011, Spitzen *et al.* 2013). This could be interpreted as ranging flight, but in reality it highlights the need for field testing of any kairomones

that are found to be behaviourally active in a laboratory setting to fully assess the role that they may play in the host location process.

In the field, CO_2 has long been used as a bait for trapping mosquitoes and dose response to this kairomone by field populations was first established in the early 1950s (Reeves 1953). Early studies tended to use traps that had light sources making it difficult to truly determine the effect of CO_2 alone, however subsequent studies demonstrated the attraction (Reeves 1953, Newhouse *et al.* 1966, Gillies and Wilkes 1969, Gillies and Wilkes 1970). The effect was also shown by comparing attack rates on humans when CO_2 was removed from breath which resulted in up to 80% less attraction for some species (Snow 1970). Interestingly, of the mosquitoes that did still locate the host, the percentage attempting to feed was not different to what was observed when CO_2 was present, suggesting that CO_2 is a long range attractant, but at close range other factors are important. For *An. gambiae*, a highly anthropophilic species, the use of human baits resulted in consistently higher numbers than collections made using only CO_2 (Costantini *et al.* 1996, Mboera *et al.* 1997). A five-fold increase in the rate of CO_2 flow did not give a corresponding increase in collections and numbers were still significantly lower than those attracted to the human.

Step Three: Close Range Orientation and Landing

To a greater degree than in the Glossinidae, close-range orientation and landing cues in mosquitoes are extremely difficult to separate from long-range cues and may overlap. A key technical issue in this area is the use of disrupted plumes (simulating long-distance encounters with host-odour in the field) and homogenous plumes or still air (simulating close-range release of semiochemicals) (Carde and

Gibson 2010). Due to this, and the fact that the vast majority of studies have been conducted in the laboratory over relatively short distances, the following volatile chemicals are considered to be primarily short range cues.

As in the Glossinidae, octenol has also been shown to be an important stimulant for mosquitoes in the laboratory and use in host location has been demonstrated in the response of *Anopheles* species to a racemic formulation, the effect was synergistically enhanced when delivered with CO_2 (Takken *et al.* 1997). *Aedes aegypti* and *Cx. quinquefasciatus* also demonstrated this response where activation was evaluated with racemic octenol, S-1-octen-3-ol and R-1-octen-3-ol, activation was generally greater for *Ae. aegypti* (Cook *et al.* 2011). In the field, results are more equivocal with *Cx. quinquefasciatus* failing to show responses to octenol and CO_2 when combined as a bait (Mboera *et al.* 2000). Octenol has not been isolated from birds and with many *Culex* species being primarily ornithophilic this kairomone may not be behaviourally important for this species.

Unlike the Glossinidae, lactic acid has been demonstrated to be an attractant to *Ae. aegypti* in wind tunnel bioassays when delivered in combination with CO_2 (Acree *et al.* 1968) and *Cx. quinquefasciatus* is also activated in laboratory assays (Allan *et al.* 2010). This effect has also been recorded for *An. gambiae* in a dual port olfactometer (Dekker *et al.* 2002). In the field, attraction to lactic acid in combination with CO_2 has been demonstrated for *Anopheles* (Murphy *et al.* 2001). Lactic acid is also one of the important components of host odour for the mediation of host selection, particularly in anthropophilic species and highlights the fact that single chemicals can have a significant impact upon host location (Steib *et al.* 2001).

In addition to these volatile chemicals, a vast range of other compounds have been identified as being behaviourally active in the laboratory, primarily through the use of extraction techniques, electrophysiological screening and then secondary testing of individual chemical in the laboratory (Bernier *et al.* 2000, Meijerink *et al.* 2000, Verhulst *et al.* 2010, Verhulst *et al.* 2011b, Smallegange *et al.* 2012). A key advance in this respect arose from an increased understanding of the importance of the emission from microbial fauna on hosts (Braks *et al.* 1999, Meijerink *et al.* 2000, Smallegange *et al.* 2011). Human feet have been shown to be a preferential landing site for *An. gambiae* (De Jong and Knols 1995b); leading to the investigation of feet odours as attractants and the use of Limburger cheese as a substitute for foot odours. Significantly higher response to Limburger cheese than to clean air control has been recorded for two strains of *An. gambiae* originating from East and West Africa in a wind tunnel (De Jong and Knols 1995a). *Cx. quinquefasciatus* also responds to foot odour and significantly higher rates of response are observed compared to controls and compared to CO_2 alone (Mboera *et al.* 1998, Lacey and Carde 2011, Lacey and Carde 2012). In *Ae. aegypti*, responses towards odour from a sock were significantly lower than towards a human hand but significantly greater than for a clean air control (Kline, 1998).

These studies have culminated in highly complex odour combinations that demonstrate considerable promise in improving monitoring tools in the field for *An. gambiae*, while attempts to generate similar data for *Ae. aegypti* and *Cx. quinquefasciatus* have not been provided. A recent example of this process has been provided in Africa with *An. gambiae*, (Verhulst *et al.* 2011a), where it has been suggested that the improvement in efficacy of the bait will increase both monitoring accuracy and additionally could be used in strategies to reduce mosquito numbers

(Logan and Birkett 2007). An issue for investigating the host seeking orientation of *Ae. aegypti* in the field is the fact that this species is typically found in habitats within or in close proximity to human dwellings thus rendering field trials challenging and hence most information pertaining to this species is derived from laboratory based assays.

The role of both heat and increased humidity in close proximity of the host remains poorly understood although convection currents are thought to guide selection of biting areas in *An. gambiae* (De Jong and Knols 1995b) and act as an additive effect with human odour (Spitzen *et al.* 2013). Visual cues for landing are extremely poorly understood, but must necessarily represent a switching from optomotor amenotaxis to distance led landing on a rapidly expanding object (Carde and Gibson 2010). While heat has commonly been integrated into traps used for control of mosquitoes (Hougaard and Dickson 1999), despite only a basic fundamental understanding of influence on behaviour, movement has generally been largely ignored in field-based mosquito trapping.

1.5 Host Location by *Culicoides*

In comparison with the substantial literature examining host location in the Glossinidae and Culicidae, there is a paucity of knowledge regarding this behavioural process in *Culicoides*. This in itself is not surprising, taking into account the comparatively limited socioeconomic impact of the group, but importantly also reflects the substantial technical difficulties of study in species that are significantly smaller than model species in the Glossinidae and Culicidae. Studies of *Culicoides* olfaction have been reviewed in relation to host location (Logan *et al.* 2010). A key limitation in the study of olfactory responses in *Culicoides* has been the restriction of

detailed studies of host location in the laboratory to just one species, *C. nubeculosus*. This species was originally colonised in the UK (Boorman 1974) and is now maintained at laboratories in the UK, France, Switzerland and the Netherlands. While a relatively common farm-associated species, *C. nubeculosus* is not thought to play a significant role in arbovirus transmission in northern Europe and hence is not an ideal subject for investigation. This has led to a far greater reliance on field-based studies than laboratory-based, in direct contrast to the Culicidae in particular.

A second bias in the exploration of host-seeking behaviour in *Culicoides* is the fact that a majority of studies have been conducted on nuisance biting species of humans in preference to livestock arbovirus vectors. At present, the most detailed studies have been conducted on *C. impunctatus* in the Scotland, where detailed investigations of host-seeking behaviour have been conducted almost continuously for over twenty years (Bhasin 1996, Carpenter 2001, Logan *et al.* 2010). In addition, field investigations have also been carried out on salt marsh nuisance biting species of *Culicoides*, most commonly in the USA, but also in Australia. Despite the paucity of laboratory data, and technical limitations imposed by their biology, the primary stages of host location in *Culicoides* appear to share clear parallels with those in the Glossinidae and Culicidae.

Our understanding of ranging flight in *Culicoides* is virtually non-existent and suitable electric nets to assist in monitoring this activity have yet to be devised. Activation in *Culicoides* has largely been inferred from suction and truck-trap catches and collections from human hosts. In northern Europe, the majority of species follow a crepuscular endogenous circadian cycle modulated by factors including temperature, humidity and physiological status (Hill 1947, Parker 1949, Service 1971, Blackwell 1997, Sanders *et al.* 2012). Activation and upwind flight in

response to CO_2 has been inferred by studies conducted using electrophysiology, a field-located wind tunnel and laboratory based y-tube assays for *C. impunctatus* (Bhasin 1996, Bhasin *et al.* 2000a). Field-based collections using CO_2 as a bait has also been demonstrated for a wide range of species including *C. furens*, *C. hollensis* and *C. melleus* (Kline *et al.* 1994) and *C. sonorensis* (Gerry and Mullens 1998). Interestingly, despite close association with livestock and similarities to the Glossinidae, responses to CO_2 in the *C. obsoletus* group appear poor in the few studies conducted to date (Gerry *et al.* 2009, Harrup *et al.* 2012).

Responses to additional olfactory host location cues have also been recorded from laboratory and field studies for octenol (Kline *et al.* 1994, Ritchie *et al.* 1994, Blackwell *et al.* 1996, Bhasin *et al.* 2001, Harrup *et al.* 2012) and lactic acid was attractive to the generalist feeder *C. impunctatus* (Bhasin *et al.* 2000a). In addition, more recent studies of *C. impunctatus* utilising air entrainment extracts from humans have identified a wide range of physiological active compounds (Logan *et al.* 2008). This study demonstrated differential attraction to humans in *C. impunctatus* and the existence of apparent repellent compounds to this species (6-methyl-5-hepten-2-one and geranylacetone) (Logan *et al.* 2008).

The primary aim of the current study is to investigate the relationship between livestock-associated species of *Culicoides* and their hosts in the UK through a series of field-based studies. As discussed, our understanding of this relationship is extremely poor, both in comparison to nuisance biting *Culicoides* species of humans and, more obviously, other Dipteran vector groups.

Aims of the Present Study

The aim of the present study is to further the knowledge and understanding of the behaviour of host seeking *Culicoides* through a range of field and laboratory experiments. The primary hypotheses tested in the chapters are listed below.

Chapter 3: Host preference in *Culicoides* is investigated through field trials collecting specimens directly from the same host species and different species.

Hypotheses tested during studies:

I. *Culicoides* exhibit a differential response in host location between two breeds of sheep.
II. *Culicoides* exhibit differential host location and blood-feeding on sheared and unsheared sheep.
III. Sheep are protected from the bites of *Culicoides* by preferential feeding on an alternative host (cattle).
IV. *Culicoides* exhibit a differential response in host location to individual sheep within a flock.

Chapter 4: The response of *Culicoides* to host odour cues is investigated through field trials.

Hypotheses tested during studies:

I. The response of *Culicoides* to CO_2 is dose dependent.
II. *Culicoides* show a differential attraction to the whole host odour of two different breeds of sheep.
III. *Culicoides* show a differential attraction to volatiles isolated from host odour when delivered with CO_2.

Chapter 5: The role of light wavelength in surveillance tools is investigated through field trials.

Hypothesis tested during study:

I. *Culicoides* species exhibit a differential attraction to different wavelengths of light in standard surveillance light-suction traps.

Chapter 2: Materials and Methods

The materials and methods described within this chapter are common to studies in more than one of the data chapters within the thesis. Within each data chapter additional materials and methods are presented which are unique to the studies of that chapter.

2.1 Study Sites

Investigations reported in this thesis were carried out at three different study sites in the south east of England (see Figure 2.1). Field site 1 was located in Compton, Berkshire, 51º30'21.25"N, 1º16'19.06"W, on a mixed cattle and sheep farm and was used in Chapter 4 to investigate responses to CO_2. This location has been used for previous studies of *Culicoides* and had a well described fauna (Carpenter *et al.* 2008c, Harrup *et al.* 2012, Sanders *et al.* 2012). The habitat type of the 1 km^2 cell into which the field site fell was pre-dominantly "arable and horticulture" with adjacent cells of "arable and horticulture" and "improved grassland", as defined by the Joint Nature Conservation Committee Broad Habitat classification scheme (Morton *et al.* 2011). Field site 1 was only used during one field season due to a declining *Culicoides* population which was likely related to changing grazing pattern at the farm.

Field site 2 was located near Bradfield, Berkshire, 51º27'09.40"N, 1º09'41.82"W, on a mixed cattle and sheep farm and was used in Chapters 3 and 4 where *Culicoides* collections were made from host animals and their responses to semiochemical baits were assessed. Field site 2 was selected following a general decline in the *Culicoides* population at Field site 1, and was thought to be

representative of a typical organic farm with a well-established *Culicoides* population assessed through preliminary surveys with UV light-suction traps. The specific field location used fell into a 1 km^2 cell with dominant habitat type of "broadleaved, mixed and Yew woodland" and adjacent cells dominated by "broadleaved, mixed and Yew woodland", "improved grassland" and "arable and horticulture" (Morton *et al.* 2011).

Field site 3 was located near to Horsell Common, Woking, Surrey, 51º20'09.60"N, 0º33'55.87"W. It was a smallholding with horses and two pigs and was used in Chapter 5. The site provides a sheltered location for trapping with an established *Culicoides* population identified through preliminary UV light-suction trap surveys and anecdotal reports of sweet itch on the horses. The site fell within a 1 km^2 cell that was dominated by "broadleaved, mixed and Yew woodland" with adjacent cells of "improved grassland", "coniferous woodland" and "suburban" habitat classifications (Morton *et al.* 2011).

Figure 2.1. Location of field sites in south east England

2.2 Collection of *Culicoides* in UV Light-suction Traps

All studies were completed with the use of a UV light-suction trap as a positive control and the model selected was the downdraught miniature blacklight (UV) Centers for Disease Control (CDC) model 912 (John W Hock, USA) (Figure 2.2). These traps operate with a 4W UV tube emitting in the near UV range, 320-420 nm, and were powered using a 12V lead acid sealed battery (Yuasa, Japan). Light-suction traps were suspended at a height of approximately 1.5 metres and at a distance of at least 50 metres from any other traps to avoid interference. Insects attracted to the light-suction traps were blown into a plastic killing jar containing 200 ml of water with a drop of detergent to break surface tension. At the end of the sampling period the contents of the kill jar were poured through a fine mesh sieve with an aperture less than 0.25 mm to retain insects and then transferred to 70% ethanol for storage.

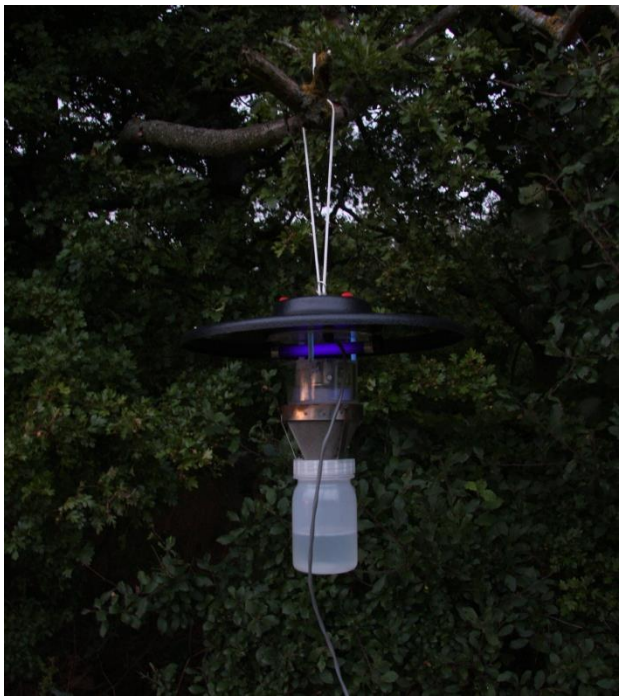

Figure 2.2. CDC miniature UV light-suction trap

2.3 Morphological Identification of *Culicoides*

All collections were initially identified morphologically using a stereo-microscope with non-*Culicoides* removed. In general, it is possible to identify *Culicoides* to species level based on characteristic wing patterns (Figure 2.3), and with the aid of an identification key (Campbell and Pelham-Clinton 1960). Females were identified to physiological state: un-pigmented; pigmented; gravid or blood fed by examination of the abdomen (Dyce 1969). For the *C. obsoletus* group identification of females can only be made morphologically for *C. dewulfi* and *C. chiopterus*. In *C. dewulfi* the spermathecae are of unequal size while, *C. chiopterus* is characterised by very pale wing markings and generally smaller in size than other *C. obsoletus* group species (Campbell and Pelham-Clinton 1960). For *C. obsoletus* and *C. scoticus* molecular techniques must be used to differentiate the species. This technique can also be used for *C. dewulfi* and is more convenient for very large collections. By contrast the males of the *C. obsoletus* group can be identified to species based on their genitalia with the shape of the ninth sternite being of particular diagnostic importance (Figure 2.4).

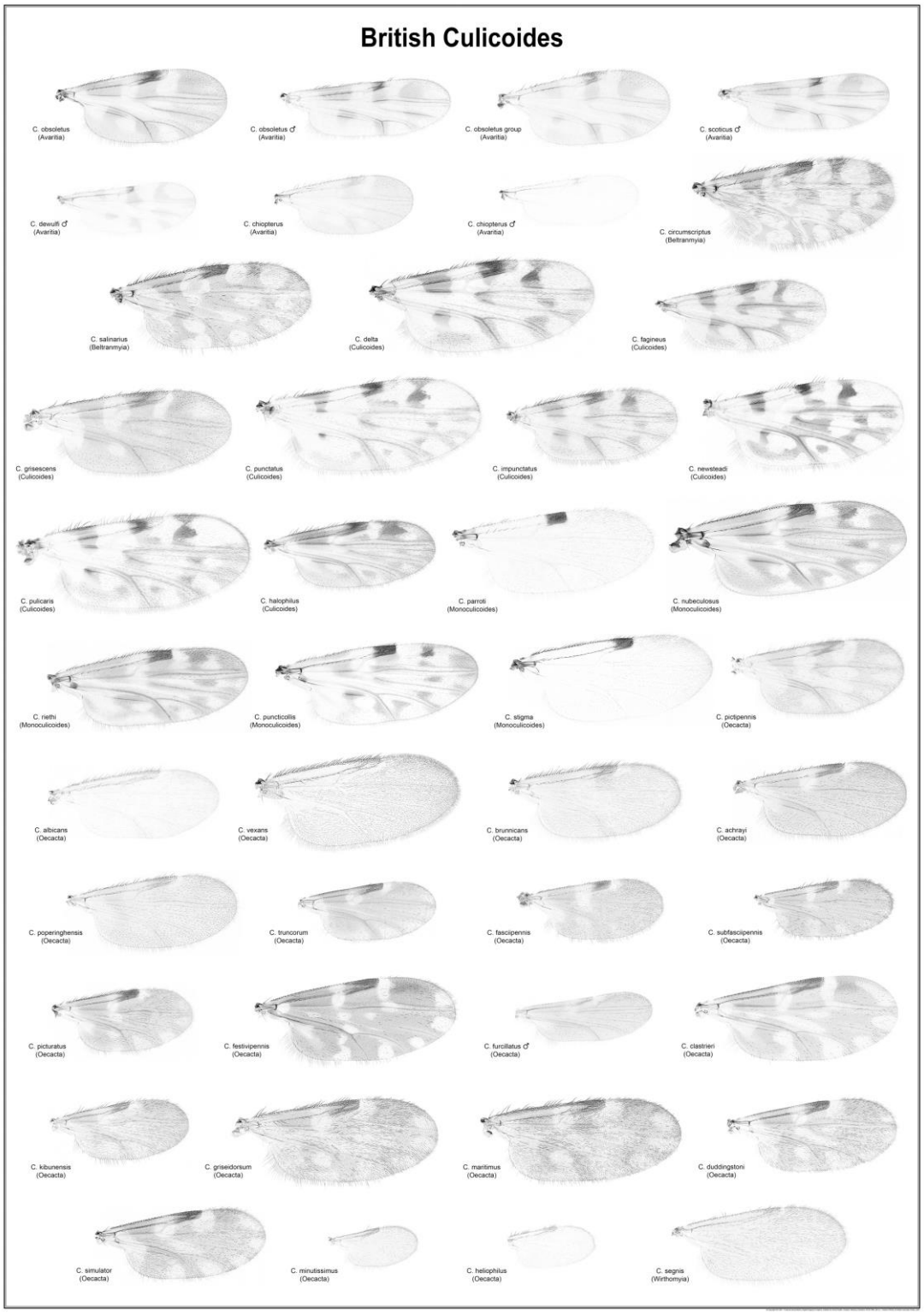

Figure 2.3. Wing patterns of UK *Culicoides* species (Copyright: The Pirbright Institute)

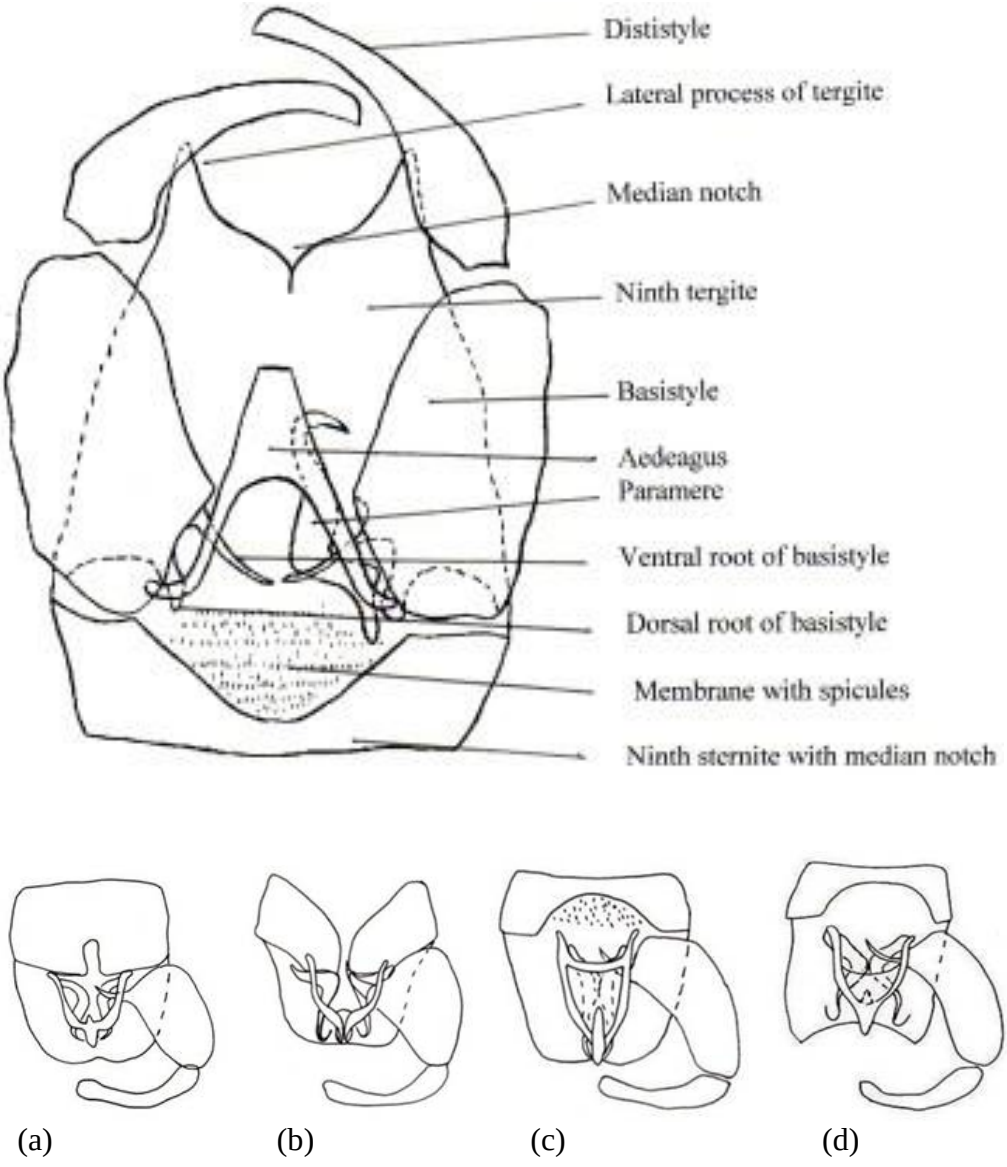

Figure 2.4. Structure of male genitalia of *Culicoides* and specific structure of members of the *C. obsoletus* group: a) *C. obsoletus,* b) *C. scoticus,* c) *C. dewulfi* and d) *C. chiopterus*

From Campbell and Pelham-Clinton, 1960

2.4 Molecular Identification of *Culicoides*

Molecular identification of *C. obsoletus* and *C. scoticus* females was carried out using a multiplex PCR method targeting the COI gene region (Schwenkenbecher *et al.* 2009). In cases of very large data sets *C. dewulfi* was also processed molecularly to save time, rather than examining the size of the spermathecae of every individual. As a result of the large numbers of females of the *C. obsoletus* group being collected it was not possible to identify all individuals using molecular techniques due to costs and time constraints and under such circumstances sub-samples of the total collection were taken and subjected to molecular analysis; this is described in each data chapter where it applies.

To extract DNA, *Culicoides* were removed from 70% ethanol storage and allowed to dry for 10 minutes before being placed individually into 2 ml micro-collection tubes (Qiagen, UK). Each tube then had 10 µl of 2% proteinase-k (Bioline, UK) made in solution with tris calcium acetate added along with 200 µl of 5% chelex (Bio-Rad, UK). Samples were then homogenised in two cycles of two minutes at 25 Hz in a Tissuelyser (Qiagen, UK). Homogenised samples were incubated overnight at 37 ºC. Following incubation 4 µl of each sample was removed and added to a PCR plate (Abgene, UK). These samples were then subjected to an eight minute cycle at 99 ºC to de-activate the proteinase-k. PCR mastermix for each PCR plate consisted: 25 µl of 10 µM forward primer specific to each species; 25 µl DNAse free water; 100 µl of 10 µM universal reverse primer (see Table 2.1 for primer sequences); 7 µl MgCl solution; 400 µl Biomix Red solution (Bioline, UK), 6 µl of mastermix was added to each 4 µl sample of extracted DNA. In addition to the test samples, each plate also contained 3 positive controls, using DNA extracted from males of each species using spin column methods with the protocol supplied by the manufacturer

(Qiagen, UK), and 3 negative controls, consisted of 4 µl of DNAse free water. Samples were subjected to PCR with the following profile: initial denaturing step at 94 ºC for 4 minutes; 32 cycles of 94 ºC for 30 seconds, 60 ºC for 30 seconds, 72 ºC for 1 minute; followed by a final extension step at 72 ºC for 5 minutes. PCR products were examined by electrophoresis using 2% agarose e-gels (Invitrogen, UK) and identified as species according to band position against positive controls.

Primer	Primer Sequence	Reference
C. obsoletus forward	TGCAGGAGCTTCTGTAGATTTG	(Nolan *et al.* 2007)
C. scoticus forward	ACCGGCATAACTTTTGATCG	(Nolan *et al.* 2007)
C. dewulfi forward	ATACTAGGAGCGCCCGACAT	(Nolan *et al.* 2007)
Reverse	CAGGTAAAATTAAAATATAAACTTCTGG	(Schwenkenbecher *et al.* 2009)

Table 2.1. Primer sequences used during multiplex *Culicoides* PCR.

2.5 Collection of Meteorological Data

As *Culicoides* activity is heavily influenced by meteorological conditions, it was important to be able to include these data in analysis of studies. Each field site where studies were conducted had an automatic weather station (CR800 data logger, Campbell Scientific, UK) that recorded conditions every 15 minutes throughout sampling periods (Figure 2.5). Data collected were: air temperature (ºC); relative humidity (%); solar intensity (Wm^{-2}); wind speed (ms^{-1}) and wind direction (º). The values given at each 15 minute data point correspond to mean values for the 15 minute period rather than values for that fixed point in time.

For data analysis, wind direction was transformed using the ArcTangent2 function in Excel (Microsoft Corporation 2010) as it is a circular variable and therefore wind direction at 0º and 360º represent the same direction.

Figure 2.5. Automatic weather station *in situ* at field site 3

2.6 Data Analysis

All experiments in this thesis generated insect count data that was typically over dispersed with non-normal error distributions, in order to deal with this kind of data analyses were conducted using negative binomial generalised linear models (GLM) with a log-link function. Statistical analyses were carried out using R version 2.15.2 (R Core Team 2013) with "MASS" (Venables and Ripley, 2002) and "multcomp" (Hothorn, Bretz and Westfall, 2008) packages. The construction of the GLMs specifically used the "glm.nb" function from the MASS package. For each trial, GLMs were constructed to include trap collection data in addition to

meteorological, location and temporal variables. Where data were sufficient, analyses were carried out for total females of each species and separately for physiological states. Initial GLMs included all meteorological variables: air temperature (°C); relative humidity (%); solar radiation (Wm^{-2}); wind speed (ms^{-1}); transformed wind direction and, where appropriate, variation in wind direction (°). In addition a linear and quadratic temporal trend was included to model the effect of seasonality on collections. In data sets where collections were made using traps that rotated through different locations, the position effect was also included in analysis. The construction of final models proceeded by stepwise deletion of non-significant ($p>0.05$) variables, with the final model corresponding to the one where all terms are significant. The final model explains the collections of *Culicoides* by the different traps analysed accounting for variation caused by meteorological, temporal and location variables.

The effects of individual factors (e.g. trap type) in the final models were examined using Tukey's honest significant differences to identify significant differences ($p<0.05$) between factors, this was done using the "glht" command in the multcomp package. This analysis allows for the comparison the means of multiple factors at the same time, examining the difference between the parameter coefficients that have been estimated in the GLM. This term explains the direction in which collections vary between factors and the size of the differences when all other significant variables are accounted for.

Chapter 3: The Differential Responses of *Culicoides* to Hosts

3.1 Introduction

The relationship between haematophagous arthropods and their hosts has a direct influence on vectorial capacity, defined as the daily rate of new infections arising from each infective case per day. In addition, the number of *Culicoides* successfully feeding on livestock (determined in part by the preference that a species has for one host over another) has been highlighted as a key parameter that is currently poorly understood for this genus (Carpenter *et al.* 2008c, Gubbins *et al.* 2008, Lo Iacono *et al.* 2013). Previous studies of *Culicoides* on hosts have generally taken one of two forms. Indirect methods infer host use from immunological or molecular analysis of blood meals in engorged female *Culicoides* collected in the field. In contrast, direct methods attempt to quantify biting rate and/or host preference from catches on, or close to, hosts either through observation or active collection of host-seeking *Culicoides*.

Prior to the 2006 incursion of BTV-8, indirect studies of *Culicoides* host preference in Europe relied upon enzyme-linked immunosorbant assay (ELISA) for identification of blood meals and were focused on *C. impunctatus* (Blackwell *et al.* 1994, Blackwell *et al.* 1995), although a very limited study demonstrated the *C. obsoletus* group feeding on sheep and rabbits (Service *et al.* 1986). The studies of *C. impunctatus* demonstrated that this species fed on a wide variety of mammalian hosts including cattle, sheep, deer, rabbit, mice, dog and cat. Preliminary attempts were also made to calculate ‘forage ratios’ to estimate the utilisation of hosts in relation to

availability, with apparent preference for cattle and deer over sheep (Blackwell *et al.* 1995).

Following the 2006 BTV-8 incursion and the interim development of PCR-based analyses, indirect tracing the origin of blood meals in *Culicoides* has been a popular area of work as it is highly compatible with general trapping surveys (Table 3.1). One difficulty in conducting these studies, however, is that UV light-suction traps do not collect large numbers of blood fed individuals, restricting most work to small data sets. In general, studies tend to analyse less than 350 blood fed *Culicoides*, although one conducted in Sweden reported collecting 2,164 blood fed individuals of which only a small proportion were processed (Lassen *et al.* 2012). Similarly, passive suction traps also collect few blood fed individuals and in one study of 23,637 *Culicoides* trapped only 64 were engorged (Pettersson *et al.* 2013).

A second major challenge in interpreting indirect studies is that collections of *Culicoides* tend to be determined to a great degree by the placement of traps, with blood fed individuals typically being found to have fed on whatever host animals are grazed in proximity to the traps. This explains the preponderance of species containing blood meals from livestock in Table 3.1. It is noticeable that since the initial attempt to calculate forage ratios for *C. impunctatus* (Blackwell *et al.* 1995), no subsequent author has presented quantitative information regarding potential host position or accurately assessed the presence of wildlife in the vicinity of traps. Hence, due to low numbers of *Culicoides* processed and the biased nature of collections, blood meal analysis is probably best regarded as a presence/absence of feeding on a specific host rather than as a tool to discern host-preference.

Species	Host									
	Cattle	Sheep	Horse	Human	Pig	Deer	Goat	Rodent	Rabbit	Bird
C. obsoletus	3,4,6,8,9	2,3,4,7,9	3,4,1,9	3,4,8		3	3,7	3		6
C. scoticus	2,4,6,9	2,4,9	3,8,9	3,8	4	3,6	2,3		4	6
C. dewulfi	2,3,4	2	4,9	3,8	4				4	6
C. chiopterus	2,3,6	2	9	8		3	2			
C. obsoletus grp	1,5	5,9	1	5	1				5	5
C. pulicaris	3,5,6	2,5	3	5,8		3	3		4	5
C. punctatus	2,3,4,6		4,6,9	5,8		3	3	5	4	5,6
C. pulicaris grp	1				1					
C. impunctatus		9	9							
C. festivipennis		5		5,8						9
C. brunnicans	4	2								
C. parroti	5									
C. newsteadi		2								
C. pictipennis		2		8						8,9
C. lupicaris	3,4,9	2	4		4				4	
C. circumscriptus				3						9
C. furcillatus	3,4								4	
C. kibunensis	3			8						
C. pallidicornis	3,4			8			3		4	
C. poperinghensis	3,4			8						
C. riethi	3									
C. vexans	3			3		3				
C. achrayi	4,9									
C. picturatus	4									
C. deltus	6			8						
C. clastieri				8						
C. semiaculatus				8						
C. grisescens	9									
C. salinarius										9

Table 3.1. Origin of blood meals in European *Culicoides* following analysis by PCR from 2009-2013. Collated from: 1: (Bartsch *et al.* 2009), 2: (Garros *et al.* 2011), 3: (Lassen *et al.* 2012), 4: (Ninio *et al.* 2011), 5: (Calvo *et al.* 2012), 6: (Lassen *et al.* 2011), 7: (Martinez-de la Puente *et al.* 2012), 8: (Santiago-Alarcon *et al.* 2012), 9: (Pettersson *et al.* 2013)

The numbers of direct studies of biting rate and host preference on *Culicoides* in Europe have similarly increased since the 2006 BTV-8 outbreak, but not to the same degree as these require far greater logistical effort to perform. Prior to this event, landing and engorgement sites on cattle and horses had been investigated in

order to relate the location of *Culicoides* bites with the presence of mastitis and sweet-itch (Nielsen 1971, Mellor and McCaig 1974, Townley *et al.* 1984).

Subsequent studies dedicated to the investigation of biting rates by Palaearctic species on livestock hosts have primarily concentrated on sheep as these hosts suffer the most severe clinical signs of BTV and are also more straightforward to contain during manipulative experiments. Collections of *Culicoides* in Europe have most commonly been carried out using drop-traps, which are used for studying a wide variety of other haematophagous arthropods worldwide and whose design has been reviewed (Silver 2008). In these studies a putative host is penned prior to the dropping of a net with a suitable mesh size over the holding corral, allowing collection of any arthropods either present on the host or in the immediate vicinity. The use of a drop trap, when correctly deployed, reduces the potentially biasing impact of the collector's presence next to the host during the attraction and feeding behaviour of arthropods. An alternative method is to attach adhesive panels or tape to hosts which intercepts arthropods in the process of host-seeking and allows collections to be made overnight, if required, which can be difficult to safely achieve using drop-trapping. Sticky trapping also has biases, the traps need to be placed on preferential engorgement sites on the host animal which may not always be practical and there is also the risk that the adhesive material may be repellent to the host seeking insect.

Since 2006, direct studies of *Culicoides* biting behaviour have been carried out in the UK (Carpenter *et al.* 2008c), Spain (Gerry *et al.* 2009) and France (Viennet *et al.* 2011, Viennet *et al.* 2012, Viennet *et al.* 2013). These studies were in part designed to examine the potential differences in *Culicoides* diversity and abundance

collected in UV light-suction trapping networks with those feeding on hosts susceptible to BTV in the field. Comparisons were drawn against the OVI UV light-suction trap, concurrently with on-host sampling in France (Viennet *et al.* 2011), or following host-based sampling in the UK (Carpenter *et al.* 2008c), while in Spain a CO_2 baited CDC suction trap and CDC UV light-suction trap were compared with collections on the host (Gerry *et al.* 2009). This lack of standardisation was also reflected in the means of capture, with *Culicoides* collected by drop-trap in the UK study (Carpenter *et al.* 2008c), by direct aspiration in Spain (Gerry *et al.* 2009), and using drop trapping, sticky trapping and direct collection in the other (Viennet *et al.* 2011). The studies in France and Spain additionally used a single sheep for collection in isolation from the rest of the flock, while the UK study kept the flock in close proximity to the drop trap resulting in a more natural host behaviour.

In all the studies, the diversity and abundance of *Culicoides* collected in the animal-baited traps did not reflect that found in the surveillance trapping methods. The most striking observation was a substantial underestimation of the numbers of *C. chiopterus* in the UV light-suction OVI trap samples in the UK (Carpenter *et al.* 2008c) confirming previous catches of this species on horses (Townley *et al.* 1984) and cattle (Nielsen 1971). This result was not confirmed at the site used in France which did not appear to support large numbers of *C. chiopterus* (Viennet *et al.* 2011), or in Spain where this species appears to be absent. In addition, *C. brunnicans* was also underestimated in abundance by the light-suction OVI trap in the study in France when compared to the sheep host (Viennet *et al.* 2011), although again this species was absent from the other two sites. While the underlying biological reasons for these differences remain unknown, the studies clearly demonstrated the difficulties in relying on a UV light-suction trap as the primary means of *Culicoides*

surveillance. A key observation from all three studies, however, was the fact that the sites used, while logistically convenient did not appear to be representative of the wider surveillance networks that are in place as they collected relatively few *Culicoides* at light.

Following on from the collections of *Culicoides* from sheep in France using sticky traps (Viennet *et al.* 2011), this methodology was later used in collections from a sheep, calf, pony, goat and hens in the only systematic host-preference study conducted in Europe to date (Viennet *et al.* 2012). In this, a high proportion of *Culicoides* (>95%) were collected on the pony host using sticky trapping when compared to other hosts (0.8% on the sheep, 2.1% on the calf, 1.2% on the goat and 0.9% on the hens). When data were corrected to account for differences in body weight and surface area, abundance of *C. obsoletus*, *C. scoticus* and *C. dewulfi* remained significantly higher on the horse than on all other hosts (Viennet *et al.* 2012). The low abundance of *Culicoides* collected in this study is a major concern, which when combined with the fact that meteorological data was not included in the analysis and few replicates were completed led to difficulties in interpreting the dataset produced.

In addition to underpinning modelling of transmission of arboviruses, understanding biting rate and host-preference has also been suggested anecdotally to be a potential means of reducing transmission. A potential host-preference related factor is the hypothesis that sheep may vary in their attraction to *Culicoides* according to breed. Given the apparent intra-breed variation in host preference for cattle observed in other vector groups (Birkett *et al.* 2004, Jensen *et al.* 2004), it is highly likely that different breeds of sheep have varying attraction to *Culicoides*,

although many confounding factors that could influence this process have been identified (Torr *et al.* 2006). If differences in *Culicoides* attraction to sheep breeds are significant, the underlying reasons for this variation are likely to be highly complex and could involve visual, thermal or semiochemical-related cues. These in turn could be underpinned by a diverse range of factors including age and physiological condition that could be more significant than breed in determining fly load. Interestingly, BT is known to have greater impact on specific breeds, although this may primarily be a differential immunological response to infection.

A second method for reducing biting rates of *Culicoides* on sheep lies in the use of shearing at certain times of the year, which has again been employed to reduce biting rates of *C. imicola* on sheep in the Republic of South Africa with only anecdotal evidence of success (Erasmus 1975, Coetzee *et al.* 2012). Here, the fleece is thought to act as a more substantial mechanical barrier to *Culicoides* feeding at times of high biting rates, if shearing is timed correctly. Again, however, no quantitative trials of these observations have been conducted.

By far the most commonly stated example of these hypotheses is the suggestion that an apparent higher degree of host preference for cattle rather than sheep in *C. imicola* could be exploited as a means of zooprophylaxis for the protection of susceptible sheep against BT in South Africa (Du Toit 1962, Nevill 1978). In an initial study, five susceptible sheep were grazed in close proximity to cattle from which BTV had been isolated in order to determine whether the virus would be transmitted from the cattle reservoir to the sheep (Du Toit 1962). Over a five month period the sheep were tested for BTV infection on three occasions and again at the end of the trial, but were found to be negative despite the close proximity

to the infected cattle and high *Culicoides* abundance as measured using UV light-suction traps (Du Toit 1962). In the second study a sheep and dairy farm with historical cases of BT among sheep adopted an approach of grazing cattle near sheep (Nevill 1978). In addition to maintaining cattle close to the sheep, vaccination was carried out on an annual basis and from 1970-1975 no serious cases of BT were observed in the sheep. In the summer of 1975-76 it was not possible to keep the cattle near the sheep and all of the older rams were found to be infected with BTV at the next test (Nevill 1978).

The primary aim of this chapter is to examine all three of these potential means of mitigating transmission using a site that is relatively representative of standard trapping network sites in Europe. In the first trial the responses of *Culicoides* to two different breeds of sheep grazed together on the farm were investigated to see whether differences in attack rates could be determined. In the second trial, completed a year later, the breed with the highest attack rates in trial one were further investigated to determine differences in collections on sheared and unsheared sheep. The third trial was designed to determine whether cattle could protect sheep from *Culicoides* bites as described in South Africa as a method of preventing BT transmission to sheep. During the bluetongue outbreak the farming community had questioned whether changing shearing and grazing practices could provide some protection to their animals. In all three cases, this is the first time that these assessments have been made for Palearctic *Culicoides* and provides direct practical information of relevance to famers with regard to grazing practices. A final preliminary investigation of diurnal host seeking activity was also conducted through direct collections on sheep made in the drop trap. Such activity has been reported

elsewhere and could have practical implications for the use of light-suction traps for surveillance (Balenghien *et al.* 2008, Rijt *et al.* 2008, Viennet *et al.* 2012).

3.2 Materials and Methods

3.2.1 Collection Methods

All studies were carried out at the same site, a mixed cattle and sheep farm in Berkshire, see description of Site 2 in Chapter 2. A drop trap (Figure 3.1) was developed based on designs used previously (McCreadie *et al.* 1984, Carpenter *et al.* 2008c) and was used during each of the trials. The drop trap had a rectangular metal base frame measuring 3 m length by 2.4 m width and three arches were attached to the base frame giving a maximum height of 2.1 m. The drop trap was further supported by a wooden frame on the outside of the structure. White netting with mesh size of less than 0.25 mm^2 was attached to the metal frame and could be raised and lowered as required. In order to retain sheep inside the drop trap a rectangular enclosure was created using open sided fencing panels.

Collections were made using two commercially grazed sheep breeds: pure Hartline breed and Hartline/Suffolk cross breed (see Figure 3.2). These breeds were chosen due to their similarity in size and weight in order to control for bias in this respect. The only apparent difference between the two breeds is that the cross breed had black legs and faces.

Prior to starting *Culicoides* collections, sheep were herded into a corral positioned next to the drop trap where they could be held throughout the sampling period. For each sample collection, between 1 and 3 sheep were herded from the corral into the drop trap where the netting was raised (see Figure 3.1). The investigator then moved to a distance of at least 100 metres from the drop trap for a 10 minute exposure period. Following the 10 minute exposure the investigator

returned to the drop trap and the netting was dropped trapping all *Culicoides* within the net. The investigator then proceeded to collect all the *Culicoides* within the drop trap using a manual aspirator during a ten minute period. *Culicoides* were transferred to pillboxes (Watkins & Doncaster, UK), which were then placed in sealed plastic containers with chloroform to kill samples before transfer to 70% ethanol. On completion of the ten minute collection period the netting was raised and the sheep were returned to the rest of the flock within the adjacent corral, a further 1-3 sheep were then herded into the drop trap for the next sample collection. These twenty minute exposure and collection periods for each sample were repeated throughout the trapping evening. For each trial a UV light-suction trap was operated as a positive control (Model 912, John W Hock Inc., USA), this was positioned at a distance of at least 50 metres from the drop trap. Two light trap positions were used with the trap switching position each night.

Figure 3.1. Drop trap apparatus used for on-animal collections of *Culicoides* showing netting up and down

3.2.2 Trial 1 – Collection of *Culicoides* from two breeds of sheep

This trial was conducted from late June to late July 2011. Both the Hartline and Hartline/Suffolk cross breeds of sheep were used (Figure 3.2.). Ten females of

each breed, weighing between 70-80 kg were used. The sheep also each had a lamb, although collections were only carried out on adults. The field site was set-up as shown in Figure 3.3.; a herd of suckler cows with calves was also grazed in the field but were predominantly found in the upper part of the field, several hundred metres from where the drop trap was located. For each drop trap collection three individuals of the same breed were used with collections alternated between breeds, drop trap collections were carried out as described above. In addition to the drop trap collections a separate study carrying out air entrainments on the sheep was conducted in parallel (see Chapter Four). The use of three sheep in the air entrainment each night excluded them from the study resulting in a total of 17 sheep being used for drop trap collections thus, throughout the evening the drop trap contained 3 sheep with 14 remaining in the adjacent corral. Collections were carried out from 3 hours before sunset to one hour after in order to coincide with air entrainments and collections made from an odour baited trap attached to the entrainment unit (see Chapter 4). A UV light-suction trap was operated at the same time as drop trap collections with one collection made for each night of sampling.

(a) **(b)**

Figure 3.2. Pure Hartline (a) and Hartline/Suffolk Cross (b) sheep used to determine host breed preferences for *Culicoides*

Figure 3.3. Map of field site where trial 1, the investigation of *Culicoides* attraction to different breeds of sheep, was conducted

3.2.3 Trial 2 – Collection of *Culicoides* from sheared and unsheared sheep

This trial was conducted in May/June 2012 in the same field as trial one but the location of the drop trap was moved (Figure 3.4.), a temporary fence was erected crossing the field between the light-suction traps to prevent mixture of sheep with the remaining flock in the field. No cattle were present during the trial period. Twelve Hartline/Suffolk ewes weighing between 70-80 kg were used in two groups, six sheared and six unsheared (Figure 3.5). Sheep were sheared in the week prior to the start of the trial. Drop trap collections were made on groups of three sheep for consistency with the first trial following the method described above. Trapping was conducted from three hours before sunset to one hour after sunset. The UV light-suction trap was also operated during the trial but it was examined after each drop trap collection in order to be directly comparable.

Figure 3.4. Map of field site where drop-trap trials 2, 3 and 4 were conducted in 2012

Figure 3.5. Sheared and unsheared Hartline/Suffolk cross ewes

3.2.4 Trial 3 – Collection of *Culicoides* from sheep in the presence of cattle

Trial three followed directly from trial two and was completed in July 2012. Of the six sheared ewes that had been used in trial two, five were retained for this study. The primary focus of the investigation was to examine the effect of the presence of cattle on biting rate of *Culicoides* on sheep. This study also investigated whether differential attraction existed between the individual sheep, so, unlike trials 1 and 2, drop trap collections were carried out on individuals rather than groups of three. Collections of *Culicoides* from a cow were made on an Angus/Stabiliser cross of approximately 500 kg, the cow had a calf but collections were made from the adult only. A corral was constructed next to the drop trap where the cow and calf could be held (Figure 3.4). *Culicoides* were collected from the cow through the use of a sweep net with 35 cm diameter with mesh size less than 0.255 mm^2 (Watkins & Doncaster, UK).

The sweep net collections on the cow were made by a second investigator during the same 10 minute collection periods on the sheep (when drop trap netting is down) in order to be directly comparable. Sweep netting was carried out by sweeping in figures of eight around the adult cow for a ten minute period. On completion of the collection the sweep net was transferred to a sealed plastic container with chloroform to kill the *Culicoides* before transfer to 70% ethanol. The UV light-suction trap was operated at the same time and was also checked after each on-animal collection. The field site set up is shown in Figure 3.4. The cow was present every second night so that drop trapping on the sheep could be done in the presence and absence of the cow. On nights where sampling was not carried out on the cow, it was held in a barn at the main farm. In order to demonstrate that collections from the cow were due

directly to the response of *Culicoides* to the host rather than due to the position of the corral or the collector, sweep net collections were also made in the corral on sheep only nights during every third sheep exposure period. On nights when only sheep were present, only one investigator was present for the study therefore sweep net collections in the cow corral were conducted during the ten minute period when the sheep was in the drop trap with the netting raised. On these nights the actual third collection on the sheep with the drop trap netting down was carried out immediately after the 10 minutes of sweep netting in the empty cow corral.

3.2.5 Trial 4 - Diurnal Collection of *Culicoides*

On completion of the third trial a final pilot study was conducted to assess diurnal activity of *Culicoides*. The same sheep that were used in trial three, Hartline/Suffolk cross, were used for this trial but unlike trial three, where collections were made on individual sheep, for diurnal collections three sheep were used for each exposure period. Collections were made once per hour from one hour after sunrise to one hour after sunset and a UV light-suction trap was operated at the same time. The drop trapping followed the same procedure described earlier however, in order that the sheep could graze and take water they were released back into the field for an hour after every third collection before being re-herded into the corral.

3.2.6 *Culicoides* Identification

Collections were identified initially based on morphological characteristics as described in Chapter 2. Females identified morphologically as being *C. obsoletus*/*C. scoticus*/*C. dewulfi* were identified molecularly through multiplex PCR (see Chapter 2). Due to the high numbers of individuals identified as belonging to these three

species a sub-sample approach was used for PCR identification. For trials one and two, five nights of collections were randomly selected and all *C. obsoletus*/*C. scoticus*/*C. dewulfi* females were subjected to molecular identification. For trial three, six nights of collection were analysed molecularly, 3 nights when the cow was absent and 3 nights when it was present. The proportions of species and physiological states identified through the PCR for each trap (i.e. Cross breed, sheared, sweep net) were then applied to the remaining nights where identification was solely based on morphology. Final abundance estimates are presented with any individuals that failed to be amplified through the PCR being excluded.

3.2.7 Meteorology

Meteorological data were collected throughout each study period using a weather station positioned in the same field as described in Chapter 2. Data were recorded every 15 minutes allowing the matching up of meteorological conditions to each drop trap collection.

3.2.8 Analysis

Data were analysed as described in Chapter 2 using negative binomial generalised models (GLM) with a log link function in R version 2.15.2 (R Core Team 2013). The effects of individual factors in the final model were examined using Tukey's honest significant differences to identify significant differences ($p<0.05$) between factor levels. Final models showing the model script with parameter estimates and 95% confidence intervals are presented in Appendix 1.

3.3 Results

3.3.1 Trial 1 – Response of *Culicoides* to two breeds of sheep

A total of 224 collections were made from the sheep, 112 from each breed, over 22 nights of trapping, with 22 corresponding UV light-suction trap collections. A total of 16,170 *Culicoides* were collected, 8,381 on the cross breed, 6,483 on the pure breed and 1,306 in the light trap. The average *Culicoides* collection calculated per 10 minute exposure period was 74.8 for the cross bred sheep and 57.9 for the pure breed this equates to 24.9 and 19.3 for individual sheep per 10 minute exposure. The greatest single ten minute collection on the cross breed was 495 *Culicoides* and for the pure breed 472, equating to a mean rate on individuals of 165 and 157.3, respectively. In the light trap the highest collection was 510, but this was recorded for the entire four hour trapping period. Total and mean collections are shown in Table 3.2.

Trap	Species					
	Total *Culicoides*	***C. obsoletus* Females**	***C. obsoletus* Males**	***C. scoticus* Males**	***C. dewulfi* Males**	***C. chiopterus* Males**
Cross Breed n=112	8,381 (74.8±7.8)	8,247 (73.6±7.7)	20 (0.18±0.04)	7 (0.1±0.0)	1	1
Pure Breed n=112	6,483 (57.9±6.9)	6,364 (56.8±6.8)	34 (0.3±0.06)	10 (0.1±0.0)	1	1
Light Trap n=22	1,306 (59.4±24.2)	1,122 (51±21.8)	7 (0.32±0.19)	19 (0.9±0.4)	4 (0.2±0.1)	1
Total	**16,170**	**15,733**	**61**	**36**	**6**	**3**

Table 3.2 *Culicoides obsoletus* group collected using drop trap sampling on two breeds of sheep and from light-suction trap controls

Of the total *Culicoides* collected, 97.3% were females of the *C. obsoletus* group (Table 3.2). Additional species constituted 2.7% of catches and included *C. achrayi* (1.28%), *C. punctatus* (0.5%) and the remaining 0.92% comprised rarer species such as *C. pulicaris*, *C. brunnicans* and *C. impunctatus*. A total of 2,572 individuals identified morphologically as *C. obsoletus*, *C. scoticus* or *C. dewulfi* were subjected to molecular identification through multiplex PCR, 2,458 (95.5%) of samples were successfully amplified with the remaining 76 (3%) failing as a result of poor DNA extraction. Of the successfully amplified samples 1,268 (51.6%) were *C. obsoletus*, 936 (38.1%) *C. scoticus* and 254 (10.3%) *C. dewulfi*. Estimated total numbers for these species were calculated on these sub-sample proportions and are presented in Table 3.3.

Species	Physiological Status	Cross Breed	Pure Breed	Light Trap	Total
C. obsoletus	Un-pigmented	1672 (40.4%)	1612 (40.1%)	333 (58.5%)	3,617
	Pigmented	1714 (41.4%)	1482 (36.8%)	159 (27.9%)	3,355
	Blood-fed	700 (16.9%)	862 (21.4%)	7 (1.2%)	1,569
	Gravid	30 (0.7%)	12 (0.3%)	63 (11.1%)	105
	Male	20 (0.5%)	34 (0.8%)	7 (1.2%)	61
	Total	**4,136**	**4,022**	**569**	**8,707**
C. scoticus	Un-pigmented	870 (28.6%)	710 (41%)	295 (61.2%)	1,875
	Pigmented	377 (12.4%)	407 (23.5%)	141 (29.2%)	925
	Blood-fed	1778 (58.4%)	570 (32.9%)	7 (1.4%)	2,355
	Gravid	15 (0.5%)	35 (2%)	21 (4.3%)	71
	Male	7 (0.2%)	10 (0.6%)	19 (3.9%)	36
	Total	**3,047**	**1,732**	**483**	**5,262**
C. dewulfi	Un-pigmented	368 (39.9%)	217 (40.6%)	22 (42.3%)	607
	Pigmented	446 (48.4%)	205 (38.3%)	17 (32.7%)	668
	Blood-fed	102 (11.1%)	112 (20.9%)	0	214
	Gravid	5 (0.5%)	0	9 (17.3%)	14
	Male	1 (0.1%)	1 (0.1%)	4 (7.7%)	6
	Total	**922**	**535**	**52**	**1,509**
***C. chiopterus*[1]**	Un-pigmented	2 (1.4%)	1 (0.8%)	3 (11.5%)	6
	Pigmented	73 (52.1%)	67 (55.4%)	19 (73.1%)	159
	Blood-fed	63 (45%)	52 (43.0%)	0	115
	Gravid	1 (0.7%)	0	3 (11.5%)	4
	Male	1 (0.7%)	1 (0.8%)	1 (3.8%)	3
	Total	**140**	**121**	**26**	**287**
Total *Culicoides* collected		**8,245**	**6,390**	**1,130**	**15,765**

Table 3.3. Final estimated abundance of *C. obsoletus* group species calculated from sub-samples of collections

[1] *C. chiopterus* results are actual results based on morphological identification

Statistical analyses of collections on the two breeds of sheep were restricted to investigating differences between the breeds without the inclusion of data from the light-suction trap. This was due to the fact that the light trap operated continually throughout the sampling period while drop trap collections were made in ten minute blocks. As a result of the analyses being concerned with only two factors, pure breed and cross breed, Tukey's testing was not necessary as the differences between the two factors are revealed in the models where the pure breed is compared to the cross. Four models were generated to describe the collections of *C. obsoletus*, *C. scoticus* and *C. dewulfi* females from the two breeds of sheep: total females (includes all physiological states); un-pigmented females, pigmented females and blood fed females (Tables 3.4-3.7). Due to low numbers of un-pigmented *C. chiopterus* females collected only three models were generate for this species: total *C. chiopterus* females; pigmented females and blood fed females (Table 3.8).

Parameter	***C. obsoletus* Total Females**	***C. obsoletus* Un-pigmented**	***C. obsoletus* Pigmented**	***C. obsoletus* Blood Fed**
Intercept	3.971***	3.192***	0.977*	0.899
Temporal Trend				
Linear	0.174***	0.180***	0.179***	0.126***
Quadratic	-0.005***	-0.006***	-0.005***	-0.004***
Trap				
Cross Breed	Baseline	Baseline	Baseline	Baseline
Pure Breed	-0.058	-0.075	-0.119	0.188
Temperature	NS	NS	NS	0.096**
Humidity	NS	NS	0.024***	NS
Solar Radiation	-0.004***	-0.004***	NS	-0.007***
Wind Speed	-0.659***	-0.706***	-0.661***	-0.574***

Table 3.4. Regression coefficients for the final negative binomial GLMs for *C. obsoletus* females collected on two breeds of sheep (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Parameter	**Total *C. scoticus* Females**	***C. scoticus* Un-pigmented**	***C. scoticus* Pigmented**	***C. scoticus* Blood Fed**
Intercept	4.089***	2.675***	-0.575	3.793***
Temporal Trend				
Linear	0.115***	0.162***	0.168***	0.066*
Quadratic	-0.003***	-0.005***	-0.005***	-0.002*
Trap				
Cross Breed	Baseline	Baseline	Baseline	Baseline
Pure Breed	-0.569***	-0.215	0.089	-1.118***
Humidity	NS	NS	0.026***	NS
Solar Radiation	-0.005***	-0.004***	NS	-0.007***
Wind Speed	-0.703***	-0.729***	-0.698***	-0.667***

Table 3.5. Regression coefficients for the final negative binomial GLMs for *C. scoticus* females collected on two breeds of sheep (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Parameter	**Total *C. dewulfi* Females**	***C. dewulfi* Un-pigmented**	***C. dewulfi* Pigmented**	***C. dewulfi* Blood Fed**
Intercept	2.218***	1.504***	-0.477	-4.915
Temporal Trend				
Linear	0.215***	0.196***	0.203***	0.349***
Quadratic	-0.007***	-0.006***	-0.006***	-0.011***
Trap				
Cross Breed	Baseline	Baseline	Baseline	Baseline
Pure Breed	-0.497***	-0.529***	-0.809***	0.106
Temperature	NS	NS	NS	0.243***
Humidity	NS	NS	0.025***	NS
Solar Radiation	-0.004***	-0.004***	NS	-0.010***
Wind Speed	-0.705***	-0.709***	-0.774***	-0.488***

Table 3.6 Regression coefficients for the final negative binomial GLMs for *C. dewulfi* females collected on two breeds of sheep (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Parameter	**Total *C. chiopterus* Females**	***C. chiopterus* Pigmented**	***C. chiopterus* Blood Fed**
Intercept	4.348***	4.262***	3.755***
Temporal Trend			
Linear	NS	-0.071***	NS
Quadratic	-0.001***	NS	-0.001**
Trap			
Cross Breed	Baseline	Baseline	Baseline
Pure Breed	-0.031	0.135	-0.203
Humidity	-0.042***	-0.044***	-0.048***
Wind Speed	-0.782***	-0.847***	-0.665***

Table 3.7 Regression coefficients for the final negative binomial GLMs for *C. chiopterus* females collected on two breeds of sheep (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Across all four models generated to describe collections of *C. obsoletus* and the three models for *C. chiopterus*, the analyses revealed that catches on the pure and cross breeds did not differ significantly (p>0.05). Analysis of *C. scoticus* data revealed that collections differed significantly between the two breeds when considering total females and blood fed females (p<0.001), but that no significant differences were found for un-pigmented or pigmented individuals. Collections of *C. dewulfi* differed significantly between breeds (p<0.001) with greater catches made on the cross breed with the exception of blood fed females where no difference was found (p>0.05).

Temporal trends were significant across all four species (p<0.05) and of the meteorological conditions included, wind-speed was significant in all models of all species and physiological states as a very highly significant variable (p<0.001). Among other meteorological conditions the response to solar radiation was more equivocal with less response in pigmented individuals when compared to all other physiological states in *C. obsoletus*, *C. scoticus* and *C. dewulfi*. Similarly,

temperature and humidity had limited effects except in the case of *C. chiopterus* which demonstrated a stronger relationship with humidity than in other species with the exception of pigmented individuals.

In summary, the data reveal that collections on the cross breed are higher than those on the pure breed and in terms of species abundance *C. obsoletus* is collected in the greatest number followed by *C. scoticus, C. dewulfi* and *C. chiopterus*. The models generated for these species demonstrate that there are only significant differences in collections of *C. scoticus* and *C. dewulfi*. For *C. scoticus* there are significantly less total females and blood fed females collected on the pure breed. For *C. dewulfi* there are also significantly less total females on the pure breed, in addition there were significantly fewer un-pigmented and pigmented females on that breed.

3.3.2 Trial 2 – Response of *Culicoides* to Sheared and Unsheared Sheep

A total of 362 collections were made during the trial, 181 in the UV light-suction trap, 90 on the unsheared sheep and 91 on the sheared sheep over 17 nights of trapping. The total collection of *Culicoides* was 15,163, including 14,613 *C. obsoletus* group females representing 96.4% of the total (Table 3.8). Other species collected included: *C. brunnicans* (1.92%); *C. achrayi* (0.5%) and *C. pulicaris* (0.4%) while *C. punctatus* and *C. impunctatus* were collected in smaller numbers.

Trap	Species					
	Total *Culicoides*	***C. obsoletus* group females**	***C. obsoletus* Males**	***C. scoticus* Males**	***C. dewulfi* Males**	***C. chiopterus* Males**
Sheared n=91	7,571 (83.2±11.5)	7,239 (79.5±11.3)	24 (0.3±0.1)	4	2	15 (0.2±0.05)
Un-sheared n= 90	6,755 (75.1±11.3)	6,565 (72.9±11.2)	26 (0.3±0.1)	3	8	13 (0.1±0.04)
Light-suction trap n= 181	837 (4.6±3.3)	809 (4.5±3.2)	2 (0.01±0.1)	0	0	0
Total	**15,163**	**14,613**	**52**	**7**	**10**	**28**

Table 3.8. *Culicoides* collected on sheared and unsheared sheep and with a UV light-suction trap

The largest single drop trap collection on the sheared sheep was 523, while the drop-trap with the unsheared sheep collected a maximum of 505 individuals. The largest UV light-suction trap collection was 586 *Culicoides*. Assuming all these *Culicoides* fed successfully, this equated to 17.43 bites/minute on unsheared individuals and 16.83 for sheared. Over the course of the trial there were only two collections on sheep where zero *Culicoides* were collected, one for each type of sheep treatment, compared to 160 zero samples when using the light-suction trap.

Of the 14,613 females identified as *C. obsoletus* group, 1,080 were classified morphologically as *C. chiopterus*. Of the remaining *C. obsoletus*, *C. scoticus* and *C. dewulfi*, 5,050 individuals were subjected to molecular identification as a subsample (Table 3.8). A total of 4,915 individuals, representing 97.3%, were successfully identified through PCR, the remaining 135 (2.67%) failing as a result of poor DNA extraction. The results of the PCR revealed that the sub-sample comprised 1,824 (37.1%) *C. obsoletus*, 2,903 (59.67%) *C. scoticus* and 188 (3.36%) *C. dewulfi*. For each treatment the proportion of physiological state per species calculated from the sub-sample was then applied to the remaining samples to provide estimates of total numbers and physiological states for each species, the results are shown in Table 3.9.

Species	Physiological Status	Sheared	Unsheared	Light Trap	Total
C. obsoletus	Un-pigmented	1,224 (50.1%)	1,456 (67.3%)	383 (63.4%)	3,063
	Pigmented	356 (14.6%)	501 (23.1%)	169 (28.0%)	1,026
	Blood-fed	817 (33.4%)	173 (7.9%)	3 (0.5%)	993
	Gravid	24 (9.8%)	7 (0.3%)	47 (7.8%)	78
	Male	24 (9.8%)	26 (1.2%)	2 (0.3%)	52
	Total	2,445	2,163	604	**5,212**
C. scoticus	Un-pigmented	2,667 (66.9%)	2,503 (69.8%)	114 (74.0%)	5,284
	Pigmented	788 (19.8%)	597 (16.7%)	22 (14.3%)	1,407
	Blood-fed	526 (13.2%)	482 (13.4%)	0	1,008
	Gravid	0	0	18 (11.7%)	18
	Male	4 (0.1%)	3 (0.1%)	0	7
	Total	3,985	3,585	154	**7,724**
C. dewulfi	Un-pigmented	146 (64.6%)	184 (70.5%)	27 (56.3%)	357
	Pigmented	51 (22.6%)	35 (13.4%)	15 (31.3%)	101
	Blood-fed	18 (8.0%)	21 (8.0%)	0	39
	Gravid	7 (3.1%)	13 (5.0%)	6 (12.5%)	26
	Male	4 (1.7%)	8 (3.1%)	0	12
	Total	226	261	48	**535**
C. chiopterus[1]	Un-pigmented	14 (2.5%)	14 (2.6%)	0	28
	Pigmented	312 (54.9%)	304 (56.3%)	0	616
	Blood-fed	226 (39.8%)	207 (38.3%)	0	433
	Gravid	1 (0.2%)	2 (0.4%)	0	3
	Male	15 (2.6%)	13 (2.4%)	0	28
	Total	568	540	0	**1,108**
Total *Culicoides* collected		**7,224**	**6,549**	**806**	**14,579**

Table 3.9. Final estimated abundance and of *C. obsoletus* group species collected in drop trap trial 2

[1] Numbers of *C. chiopterus* are actual numbers identified from morphological identification

For *C. obsoletus* and *C. scoticus* four models were generated to describe collections made on sheared and unsheared sheep: total females (including all physiological stages); un-pigmented females; pigmented females and blood fed females. Significant parameters for each model are summarised in Table 3.10 for *C. obsoletus* and Table 3.12 for *C. scoticus*.

Parameter	***C. obsoletus* females**	***C. obsoletus* Un-pigmented**	***C. obsoletus* Pigmented**	***C. obsoletus* Blood Fed**
Intercept	-1.091*	1.641***	-0.532	-2.309**
Temporal Trend				
Linear	NS	NS	-0.078***	NS
Quadratic	-0.008***	-0.009***	NS	-0.007***
Trap				
Light Trap 1	Baseline	Baseline	Baseline	Baseline
Light Trap 2	0.623*	0.945**	1.046**	-0.378
Sheared	3.409***	2.984***	2.803***	6.231***
Unsheared	3.295***	3.123***	3.209***	4.728***
Temperature	0.058*	NS	0.084**	NS
Solar Radiation	-0.008***	-0.007***	-0.009***	-0.005***
Wind Speed	-0.387**	-0.361**	-0.493***	-0.333**
Wind Direction	-0.002*	-0.002*	NS	NS

Table 3.10 Regression coefficients for the final negative binomial GLMs for collections of *C. obsoletus* females collected on sheared and unsheared sheep(* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Analysis of collections across the four models revealed that collections of *C. obsoletus* females depended significantly on trap ($p<0.05$), and for the light-suction traps, location ($p<0.05$). There was a significant temporal trend ($p<0.001$) and slight differences in significant meteorological variables were observed between models. Temperature was only significant for the total female *C. obsoletus* and pigmented female models having a positive effect on collections. Solar radiation and wind speed were significant across all models and wind direction was significant for total females and un-pigmented females, all of these variables having a negative effect on

collections. Further analysis revealed that the light-suction traps collected significantly less than the drop trap collections on the sheep for all models ($p<0.001$) (Table 3.10). Analysis also revealed that when the light-suction trap was at position one it collected significantly fewer un-pigmented and pigmented females than at position two ($p<0.05$). Between sheared and unsheared sheep no significant differences were observed in the total female model, un-pigmented model or pigmented model but for blood fed *C. obsoletus* the sheared sheep collected significantly higher numbers than the unsheared ($p<0.001$).

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-0.62	-	
Sheared	-3.41***	-2.78***	-
Unsheared	-3.29***	-2.67***	0.11

(a) *C. obsoletus* total females

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-0.95*	-	
Sheared	-2.98***	-2.04***	-
Unsheared	-3.12***	-2.18***	-0.14

(b) *C. obsoletus* un-pigmented

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-1.05*	-	
Sheared	-2.80***	-1.76***	-
Unsheared	-3.21***	-2.16***	-0.40

(c) *C. obsoletus* pigmented

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	0.38	-	
Sheared	-6.23***	-6.61***	-
Unsheared	-4.73***	-5.11***	1.50***

(d) *C. obsoletus* blood fed

Table 3.11. Differences in collections between sheared and unsheared sheep and UV light-suction trap controls for *C. obsoletus* for total females (a), un-pigmented females (b), pigmented females (c) and blood fed females (d). Estimates are given for factors on the top row relative to factors in the left hand column (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Consistent models were obtained to describe collections of total females, un-pigmented females and pigmented females for *C. scoticus*. Collections were significantly dependent on trap ($p<0.001$), other significant variables in all models were: quadratic temporal trend; temperature; solar radiation; wind speed ($p<0.05$) and for the light-suction traps, positioning was also significant ($p<0.01$). Analysis of blood fed *C. scoticus* females excluded the light-suction traps as these caught no individuals. The collections of blood fed females on sheep was found to be

significantly dependent on meteorological variables ($p<0.01$): temperature; solar radiation; wind speed and temporal trend but no differences were observed between sheared and unsheared sheep. Models are summarised in Table 3.12.

Parameter	***C. scoticus* Females**	***C. scoticus* Un-pigmented**	***C. scoticus* Pigmented**	***C. scoticus* Blood Fed**
Intercept	-1.505*	-1.718***	-2.585***	2.421***
Temporal Trend				
Quadratic	-0.011***	-0.014***	-0.004***	-0.009***
Trap				
Light Trap 1	Baseline	Baseline	Baseline	Excluded
Light Trap 2	1.590***	2.065***	1.918**	Excluded
Sheared	5.611***	5.729***	5.702***	0.028
Unsheared	5.677***	5.867***	5.516***	Baseline
Temperature	0.111***	0.098***	0.043*	0.057**
Solar Radiation	-0.008***	-0.007***	-0.007***	-0.006***
Wind Speed	-0.424***	-0.491***	-0.469***	-0.236**

Table 3.12. Regression coefficients for the final negative binomial GLMs for collections of *C. scoticus* females from sheared and unsheared sheep (* $p<0.05$, ** $p<0.001$, *** $p<0.001$, NS $p>0.05$)

Further analysis of *C. scoticus* collections demonstrated that the light-suction traps collected significantly fewer females, un-pigmented females and pigmented females than the sheep ($p<0.001$) (Table 3.13). Between the light-suction traps the collections at position one were consistently and significantly less than at position two ($p<0.05$). No significant differences were found in the collections that were made on the two sheep treatments.

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-1.590***	-	
Sheared	-5.611***	-4.021***	-
Unsheared	-5.677***	-4.087***	-0.066

(a) *C. scoticus* females

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-2.065***	-	
Sheared	-5.729***	-3.664***	-
Unsheared	-5.867***	-3.802***	0.864

(b) *C. scoticus* un-pigmented

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-1.918*	-	
Sheared	-5.702***	-3.784***	-
Unsheared	-5.516***	-3.598***	0.608

(c) *C. scoticus* pigmented

Table 3.13. Differences in collections between sheared and unsheared sheep and UV light-suction trap controls for *C. scoticus* females (a), un-pigmented females (b) and pigmented females (c). Estimates are given for factors on the top row relative to factors in the left hand column (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Analyses of *C. dewulfi* were restricted to total females, un-pigmented and pigmented females due to low numbers of blood fed individuals. Collections depended significantly on trap type ($p<0.05$) and temporal trend ($p<0.05$). Meteorological variables were consistent across models except that wind variables were not significant for pigmented females (Table 3.14).

Parameter	***C. dewulfi* Females**	***C. dewulfi* Un-pigmented**	***C. dewulfi* Pigmented**
Intercept	-1.815***	-2.358***	-4.668***
Temporal Trend			
Quadratic	-0.009***	-0.016***	-0.003*
Trap			
Light Trap 1	Baseline	Baseline	Baseline
Light Trap 2	1.236**	1.374*	1.626*
Sheared	3.286***	3.518***	3.251***
Unsheared	3.499***	3.675***	2.789***
Temperature	0.069**	0.088**	0.121***
Solar Radiation	-0.006***	-0.006***	-0.013***
Wind Speed	-0.303*	-0.331*	NS
Wind Direction	-0.002*	-0.002*	NS

Table 3.14. Regression coefficients for the final negative binomial GLMs for collections of *C. dewulfi* females from sheared and unsheared sheep (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Further examination of the data revealed differences between the different trap types (Table 3.15) for *C. dewulfi* collections. The sheared and unsheared sheep consistently collected significantly higher numbers of total females, un-pigmented females and pigmented females than the light traps ($p<0.05$), although no differences were found between the two sheep treatments. Light-suction traps catches differed significantly between locations only in the total females model with location one collecting significantly less than location two ($p<0.05$).

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-1.236*	-	
Sheared	-3.286***	-2.050***	-
Unsheared	-3.499***	-2.263***	-0.213

(a) *C. dewulfi* females

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-1.374	-	
Sheared	-3.518***	-2.144***	-
Unsheared	-3.675***	-2.301***	-0.157

(b) *C. dewulfi* un-pigmented

Trap	Light Trap 1	Light Trap 2	Sheared
Light Trap 2	-1.626	-	
Sheared	-2.789**	-1.624***	-
Unsheared	-3.251***	-1.162*	0.462

(c) *C. dewulfi* pigmented

Table 3.15. Differences in catch collections between sheared and unsheared sheep and UV light-suction trap controls for *C. dewulfi* females (a), un-pigmented females (b) and pigmented females (c). Estimates are given for factors on the top row relative to factors in the left hand column (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

As with *C. scoticus*, three models were generated to describe collections of *C. chiopterus*: total females, pigmented females and blood-fed females. Too few un-pigmented individuals were collected for analysis. Models are summarised in Table 3.16., light-suction traps were excluded from the analysis as these failed to collect any *C. chiopterus*.

Parameter	***C. chiopterus* Females**	***C. chiopterus* Pigmented**	***C. chiopterus* Blood Fed**
Intercept	2.408***	3.331***	0.558
Temporal Trend			
Linear	-0.156***	-0.121***	NS
Quadratic	NS	NS	-0.010***
Trap			
Sheared	-0.007	-0.029	0.181
Unsheared	Baseline	Baseline	Baseline
Temperature	0.112***	NS	0.136***
Solar Radiation	-0.003**	NS	-0.003**
Wind Speed	-0.711***	-0.813***	-0.684***
Wind Direction	0.002*	NS	0.003*

Table 3.16. Regression coefficients for the final negative binomial GLMs for collections of *C. chiopterus* females on sheared and unsheared sheep (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Collections of *C. chiopterus* were not found to be significantly dependent upon trap type. Temporal trends were significant ($p<0.001$) for all models as were meteorological variables ($p<0.05$). These were consistent between the female model and the blood-fed female model, but the model for pigmented females only included wind speed. No significant differences were found between collections on sheared and unsheared sheep.

In summary, the data for the sheared and unsheared sheep reveal that overall the sheared sheep collected the largest number of *Culicoides*. Amongst the *C. obsoletus* group it is *C. scoticus* that is most abundant followed by *C. obsoletus*, *C. chiopterus* and *C. dewulfi*. For statistical analyses, where data were sufficient models were generated to describe differences in total females of each of the species and then by physiological state. The only significant difference found was that the numbers of blood fed *C. obsoletus* females were significantly lower on the unsheared sheep compared to the sheared.

3.3.3 Trial 3 – Collection of *Culicoides* from sheep in the presence of cattle

Over 14 nights of sampling 419 collections were completed through drop trapping, sweep netting and collections in UV light-suction traps, these yielded a total of 16,130 *Culicoides* (Table 3.17). Catches were heavily dominated by the *C. obsoletus* group, representing 96.4% of the total trap catch. Unlike trial 2, however, *C. chiopterus* was less abundant than *C. dewulfi* in estimated numbers following subsampling (Table 3.18).

Of the 15,013 females identified as *C. obsoletus* group, 14,594 were classified morphologically as *C. obsoletus*, *C. scoticus* and *C. dewulfi*, 3,505 were subjected to molecular identification as a sub-sample. A total of 3,471 (99%) were successfully amplified with only 34 (1%) failing. The results of the multiplex PCR revealed that 1,434 (41.3%) were *C. obsoletus*, 1,553 were *C. scoticus* (44.7%) and 484 (14%) were *C. dewulfi*. For each treatment the proportion of physiological state per species calculated from the sub-sample was then applied to the remaining samples to provide estimates of total numbers and physiological states for each species, the results are shown in Table 3.18.

Host Treatment	**Trap**	**Total *Culicoides***	***C. obsoletus* females**	***C. obsoletus* Males**	***C. scoticus* Males**	***C. dewulfi* Males**	***C. chiopterus* Males**	**Other**
Cow Absent	Sheep n=84	2,718 (32.4±4.9)	2,533 (30.2±4.7)	58 (0.69±0.14)	48 (0.6±0.2)	0	0	79
	Sweep n=26	9 (0.4±0.2)	6 (0.2±0.1)	0	3 (0.1±0.1)	0	0	0
	Light n=84	92 (1.1±0.5)	54 (0.6±0.3)	1	6 (0.07±0.04)	0	0	31
	Total	2,819	2,593	59	57	0	0	110
Cow Present	Sheep n=75	6,381 (85.1±11.2)	5,903 (78.7±10.8)	139 (1.85±0.33)	216 (2.9±0.7)	0	0	123
	Sweep n=75	6,902 (92±12.7)	6,497 (86.6±12.2)	32 (0.43±0.11)	21 (0.01±0.01)	1	0	351
	Light n=75	28 (0.4±0.2)	20 (0.3±0.2)	4 (0.05±0.03)	0	1	0	3
	Total	13,311	12,420	175	237	2	0	477
Total		16,130	15,013	234	294	2	0	587

Table 3.17. Collections of *Culicoides* made through direct collections on sheep, sweep netting in a cow corral and in UV light-suction trap in the presence and absence of a cow.

Species and Physiological Status		Sheep		Sweep Net		Light Trap		Total
		Cow Absent	**Cow Present**	**Cow Absent**	**Cow Present**	**Cow Absent**	**Cow Present**	
C. obsoletus	Un-pigmented	578 (55.4%)	1,826 (65.9%)	6 (66.7%)	1,528 (61.4%)	7 (50.0%)	2 (25.0%)	3,947
	Pigmented	197 (18.9%)	535 (19.3%)	0	461 (18.5%)	4 (28.6%)	2 (25.0%)	1,199
	Blood-fed	211 (20.2%)	271 (10.0%)	0	456 (18.3%)	0	0	938
	Gravid	0	0	0	12 (0.5%)	2 (14.3%)	0	14
	Male	58 (5.6%)	139 (5.0%)	3 (33.3%)	32 (1.3%)	1 (7.1%)	4 (50.0%)	237
	Total	**1,044**	**2,771**	**9**	**2,489**	**14**	**8**	**6,335**
C. scoticus	Un-pigmented	856 (63.5%)	1,589 (57.0%)	0	1,532 (63.6%)	27 (64.3%)	5 (45.5%)	4,009
	Pigmented	259 (19.2%)	628 (22.5%)	0	585 (24.3%)	7 (16.7%)	3 (27.3%)	1,482
	Blood-fed	183 (13.6%)	300 (10.8%)	0	258 (10.7%)	0	0	741
	Gravid	2 (0.1%)	54 (1.9%)	0	12 (0.5%)	2 (4.8%)	3 (27.3%)	73
	Male	48 (3.6%)	216 (7.8%)	0	21 (0.8%)	6 (14.3%)	0	291
	Total	**1,348**	**2,787**	**0**	**2,408**	**42**	**11**	**6,596**
C. dewulfi	Un-pigmented	106 (64.2%)	362 (74.8%)	0	1,111 (73.8%)	3 (100%)	1 (50.0%)	1,583
	Pigmented	47 (28.5%)	60 (12.4%)	0	247 (16.4%)	0	0	354
	Blood-fed	12 (7.2%)	62 (12.8%)	0	146 (9.7%)	0	0	220
	Gravid	0	0	0	0	0	0	0
	Male	0	0	0	1 (0.06%)	0	1 (50.0%)	2
	Total	**165**	**484**	**0**	**1,505**	**3**	**2**	**2,159**
C. chiopterus[1]	Un-pigmented	5 (7.4%)	5 (2.3%)	0	25 (17.7%)	0	0	35
	Pigmented	35 (51.5%)	160 (76.6%)	0	96 (68.1%)	1 (100%)	0	292
	Blood-fed	28 (41.2%)	1 (0.5%)	0	20 (14.2%)	0	0	49
	Gravid	0	43 (20.6%)	0	0	0	0	43
	Male	0	0	0	0	0	0	0
	Total	**68**	**209**	**0**	**141**	**1**	**0**	**419**
Total *Culicoides*		**2,625**	**6,251**	**9**	**6,543**	**60**	**21**	**15,509**

Table 3.18. Final estimated abundance and physiological status of *C. obsoletus* group species collected on sheep and cattle and in a UV light-suction trap [1] The numbers for *C. chiopterus* are actual totals rather than estimates, based on morphological identification

The number of *C. obsoletus* females collected on sheep when in close proximity to a cow and its calf was 2.4 times greater than when collections were made without these additional hosts being present. This relationship was constant across the species examined following estimation of numbers from subsampling (*C. obsoletus*: 2.7; *C. scoticus*: 2; *C. dewulfi*: 3.1; *C. chiopterus*: 3.1). The *C. chiopterus* population also appeared noticeably older than the other species with few un-pigmented individuals identified when compared to other species in the group (Table 3.18). Sweep-netting in the absence of the cattle hosts led to the collection of only 9 *C. obsoletus* group individuals during the study, while in the presence of cattle a total of 6543 individuals were collected (Table 3.18). This represented a reduction in catches of *C. obsoletus* group females of 99.9% and very low levels of attraction to the collector. While numbers of female *Culicoides* were extremely low in light trap collections on evenings with only sheep present, these were further reduced by the presence of the cattle hosts. Catches of *Culicoides* made across the five sheep used in the study were consistent in both the presence and absence of the cattle hosts (Table 3.19). Mean rates of total *Culicoides* catches varied from 24.5-38.2 per 10 minute exposure in the absence of cattle hosts to 58.1-91.7 per 10 minute exposure in the presence of cattle.

Species and Physiological Status		Sheep 1		Sheep 2		Sheep 3		Sheep 4		Sheep 5		Total
		Cow Absent (n=17)	Cow Present (n=15)	Cow Absent (n=18)	Cow Present (n=14)	Cow Absent (n=15)	Cow Present (n=17)	Cow Absent (n=18)	Cow Present (n=13)	Cow Absent (n=16)	Cow Present (n=16)	
C. obsoletus	**Un-pigmented**	91 (57.2%)	402 (68.8%)	128 (52%)	325 (58.2%)	96 (58.5%)	388 (60.3%)	153 (62.4%)	432 (80.1%)	110 (47.6%)	278 (62.5%)	2,403
	Pigmented	33 (20.8%	110 (18.8%)	45 (18.3%)	122 (21.9%)	28 (17.1%)	140 (21.8%)	57 (23.3%)	75 (13.9%)	34 (14.7%)	87 (19.5%)	731
	Blood-fed	25 (15.7%)	51 (8.7%)	61 (24.8%)	72 (12.9%)	29 (17.7%)	74 (11.5%)	0	21 (3.9%)	68 (29.5%)	53 (11.9%)	454
	Gravid	0	0	0	0	0	0	29 (11.8%)	0	0	0	29
	Male	10 (6.3%)	21 (3.6%)	12 (4.9%)	39 (7%)	11 (6.7%)	41 (6.4%)	6 (2.5%)	11 (2.1%)	19 (8.2%)	27 (6.1%)	197
	Total	**159**	**584**	**246**	**558**	**164**	**643**	**245**	**539**	**231**	**445**	**3,814**
C. scoticus	**Un-pigmented**	142 (67.6%)	356 (58.7%)	150 (53.9%)	297 (50.2%)	155 (65.9%)	333 (50.3%)	242 (69.5%)	360 (71.6%)	168 (60.4%)	243 (57.2%)	2,446
	Pigmented	32 (15.2%)	131 (21.6%)	73 (26.3%)	143 (24.9%)	34 (14.5%)	171 (25.8%)	73 (21%)	83 (16.5%)	47 (16.9%)	101 (23.7%)	888
	Blood-fed	23 (11%)	58 (9.6%)	43 (15.5%)	69 (11.7%)	35 (14.9%)	105 (15.9%)	25 (7.2%)	22 (4.4%)	57 (20.5%)	46 (10.8%)	483
	Gravid	0	1 (0.2%)	0	24 (4.1%)	0	27 (4.1%)	1 (0.3%)	0	1 (0.4%)	2 (0.5%)	56
	Male	13 (6.2%)	60 (9.9%)	12 (4.3%)	59 (9.9%)	11 (4.7%)	26 (3.9%)	7 (2%)	38 (7.5%)	5 (1.8%)	33 (7.8%)	264
	Total	**210**	**606**	**278**	**592**	**235**	**662**	**348**	**503**	**278**	**425**	**4,137**
C. dewulfi	**Un-pigmented**	14 (50%)	82 (73.9%)	13 (56.5%)	62 (68.1%)	20 (71.4%)	75 (71.4%)	36 (69.2%)	82 (84.5%)	23 (69.7%)	61 (75.3%)	468
	Pigmented	12 (42.9%)	15 (13.5%)	7 (30.4%)	14 (15.4%)	6 (21.4%)	13 (12.4%)	15 (28.9%)	10 (10.3%)	7 (21.2%)	9 (11.1%)	108
	Blood-fed	2 (7.1%)	14 (12.6%)	3 (13.1%)	15 (16.5%)	2 (7.2%)	17 (16.2%)	1 (1.9%)	5 (5.2%)	3 (9.1%)	11 (13.6%)	73
	Gravid	0	0	0	0	0	0	0	0	0	0	0
	Male	0	0	0	0	0	0	0	0	0	0	0
	Total	**28**	**111**	**23**	**91**	**28**	**105**	**52**	**97**	**33**	**81**	**649**

Species and Physiological Status		Sheep 1		Sheep 2		Sheep 3		Sheep 4		Sheep 5		Total
		Cow Absent (n=17)	Cow Present (n=15)	Cow Absent (n=18)	Cow Present (n=14)	Cow Absent (n=15)	Cow Present (n=17)	Cow Absent (n=18)	Cow Present (n=13)	Cow Absent (n=16)	Cow Present (n=16)	
C. chiopterus	**Un-pigmented**	1 (33.3%)	2 (4.9%)	3 (17.6%)	2 (4.4%)	0	1 (1.5%)	1 (7.7%)	0	0	0	10
	Pigmented	0	22 (53.7%)	7 (41.2%)	36 (80%)	5 (55.6%)	52 (78.8%)	7 (53.8%)	18 (94.7%)	16 (61.5%)	32 (84.2%)	195
	Blood-fed	2 (66.7%)	17 (41.4%)	7 (41.2%)	7 (15.6%)	4 (44.4%)	12 (18.2%)	5 (38.5%)	1 (5.3%)	0	6 (15.8%)	61
	Gravid	0	0	0	0	0	1 (1.5%)	0	0	10 (38.5%)	0	11
	Male	0	0	0	0	0	0	0	0	0	0	0
	Total	**3**	**41**	**17**	**45**	**9**	**66**	**13**	**19**	**26**	**38**	**277**
Total *Culicoides*		**400**	**1,342**	**564**	**1,286**	**436**	**1,476**	**658**	**1,158**	**568**	**989**	**8,877**

Table 3.19. Final estimated abundance and physiological status of *C. obsoletus* group species collected on individual sheep in the presence and absence of cattle [1] Numbers of *C. chiopterus* are actual numbers identified from morphological identification

Analysis of data initially examined the effect of the presence of cattle on total *Culicoides* collections made on sheep; using the sweep net; and in UV light-suction traps. During the construction of the model an interaction term between the trap type and presence of the cow was included. The final model to describe total *Culicoides* collections is summarised in Table 3.20. Collections were found to be significantly dependent on trap type ($p<0.05$) in the presence and absence of cattle, temporal trends ($p<0.001$) and a number of meteorological variables ($p<0.05$).

Parameter	**Total *Culicoides***
Intercept	5.591***
Temporal Trend	
Linear	0.404***
Quadratic	-0.019***
Trap	
Light Trap 1 – Cow Present	-3.054***
Light Trap 1 – Cow Absent	Baseline
Light Trap 2 – Cow Present	-0.960*
Light Trap 2 – Cow Absent	0.027
Sheep – Cow Present	4.359***
Sheep – Cow Absent	3.421***
Sweep – Cow Present	4.369***
Sweep – Cow Absent	-1.712**
Temperature	-0.128*
Humidity	-0.034**
Solar Radiation	-0.006***
Wind Speed	-0.997***

Table 3.20. Regression co-efficients for final GLM to describe total collections of *Culicoides* using different traps in the presence and absence of cattle (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Further analysis revealed significant differences in collections between different trap types in the presence and absence of cattle. Collections on sheep when the cattle was present were significantly higher than when the cattle was absent

(estimated difference 0.938; p<0.001) and collections in the sweep net in the presence of cattle were not significantly different to collections on sheep on those nights (estimated difference 0.009; p>0.05). Data were subsequently analysed focusing on the collections made on sheep in the drop trap to model how these collections differed between nights with the cow present and nights where it was absent. Models were generated to describe collections of *C. obsoletus, C. scoticus, C. dewulfi* and *C. chiopterus*. Four models were generated to describe abundance of *C. obsoletus* on sheep: total females collected (including all physiological states); un-pigmented females; pigmented females and blood fed females. Significant parameters included in final models are summarised in Table 3.21.

Parameter	***C. obsoletus* Females**	***C. obsoletus* Un-pigmented**	***C. obsoletus* Pigmented**	***C. obsoletus* Blood fed**
Intercept	3.671***	2.503***	6.389***	2.257***
Temporal Trend				
Linear	0.229**	0.351***	-0.065**	NS
Quadratic	-0.129***	-0.017***	NS	NS
Trap Type				
Sheep – Cow Present	1.012***	1.127***	0.707**	0.215
Sheep – Cow Absent	Baseline	Baseline	Baseline	Baseline
Humidity	NS	NS	-0.035*	NS
Solar Radiation	-0.003***	-0.003***	-0.005***	-0.002*
Wind Speed	-1.081***	-1.105***	-0.863***	-0.714***

Table 3.21. Regression co-efficients for final GLMs to describe collections of C. obsoletus females from sheep in the presence and absence of cattle (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

All models except for blood fed *C. obsoletus* were significantly dependent on trap type (p<0.01), with collections made on nights when the cow was present being significantly higher than those made on nights where it was absent. Other significant variables included temporal trend (except in the blood fed model) and meteorological

variables ($p<0.05$), particularly solar radiation and wind speed, humidity was also significant for the pigmented model.

A further four models were generated for *C. scoticus* to describe collections of females; these are summarised in Table 3.22.

Parameter	***C. scoticus* Females**	***C. scoticus* Un-pigmented**	***C. scoticus* Pigmented**	***C. scoticus* Blood fed**
Intercept	4.316***	2.933***	7.417***	2.426***
Temporal Trend				
Linear	0.184*	0.335***	-0.075***	NS
Quadratic	-0.010*	-0.016***	NS	NS
Trap Type				
Sheep – Cow Present	0.567**	0.513*	0.597*	0.423
Sheep – Cow Absent	Baseline	Baseline	Baseline	Baseline
Humidity	NS	NS	-0.040*	NS
Solar Radiation	-0.003***	-0.003***	-0.003*	-0.002*
Wind Speed	-1.206***	-1.144***	-1.088***	-0.952***

Table 3.22. Regression co-efficients for final GLMs to describe collections of *C. scoticus* females from sheep in the presence and absence of cattle (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Analysis of data on *C. scoticus* females revealed similar patterns to those observed for *C. obsoletus*. With the exception of blood fed individuals all other collections were significantly dependent on trap type ($p<0.05$) with greater collections made on nights when cattle were present. Temporal and meteorological variables were also significant ($p<0.05$).

Due to limited numbers of blood fed *C. dewulfi* collected analyses for this species were restricted to three models as summarised in Table 3.23.

Parameter	***C. dewulfi* Females**	***C. dewulfi* Un-pigmented**	***C. dewulfi* Pigmented**
Intercept	3.381*	3.078*	5.054**
Temporal Trend			
Linear	0.504***	0.584***	NS
Quadratic	-0.023***	-0.026***	NS
Trap Type			
Sheep – Cow Present	1.197***	1.307***	0.044
Sheep – Cow Absent	Baseline	Baseline	Baseline
Humidity	-0.038**	-0.043**	-0.051**
Solar Radiation	-0.005***	-0.005***	-0.006***
Wind Speed	-0.848***	-0.894***	-0.479**

Table 3.23. Regression co-efficients for final GLMs to describe collections of *C. dewulfi* females from sheep in the presence and absence of cattle (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

Collections of total female *C. dewulfi* and un-pigmented females were significantly dependent on trap type ($p<0.001$) with greater collections made when the cow was present; other significant variables included temporal trends, humidity, solar radiation and wind speed. ($p<0.01$) This was not the case for pigmented individuals, where trap type was found to be non-significant and only humidity, solar radiation and wind speed were significant in the model ($p<0.01$).

Due to low numbers of un-pigmented and blood fed *C. chiopterus* collected through the sampling period analyses were limited to looking at total females and pigmented individuals, the models are summarised in Table 3.24.

Parameter	***C. chiopterus* Females**	***C. chiopterus* Pigmented**
Intercept	-3.703*	-2.102
Temporal Trend		
Linear	-0.086**	-0.136***
Trap Type		
Sheep – Cow Present	0.710*	0.987**
Sheep – Cow Absent	Baseline	Baseline
Temperature	0.347***	0.240***
Solar Radiation	-0.004*	NS
Wind Speed	-0.633***	-0.888***

Table 3.24. Regression co-efficients for final GLMs to describe collections of *C. chiopterus* females from sheep in the presence and absence of cattle (* p<0.05, ** p<0.001, *** p<0.001, NS p>0.05)

The analysis of *C. chiopterus* data revealed that collections were dependent on trap type ($p<0.05$) showing that the presence of cattle led to greater collections. Collections were also dependent on temporal trend and meteorological variables including temperature and wind speed in both models and solar radiation in the total *C. chiopterus* model ($p<0.05$).

Further analysis was carried out to investigate differences in *Culicoides* catch on individual sheep taking into account the presence or absence of the cow. GLMs were constructed as per the previous analysis on the sheep with models constructed for total *Culicoides* and then for the different species and physiological states However, significant differences were only found in two of the models. For the collections of *C. obsoletus* blood fed females, a significant difference was found between sheep 4 and 2, with significantly fewer being caught on sheep 4 (estimated difference -0.979; $p<0.05$). Collections of *C. obsoletus* blood fed females were dependent on trap, solar radiation and wind speed. For pigmented *C. chiopterus*, significantly more individuals were caught on sheep 5 than on sheep 1 (estimated difference 1.390; $p<0.05$). The collection of pigmented *C. chiopterus* was

significantly dependent on trap, temporal trend, the presence of the cow, temperature and wind speed. Final models with parameter estimates and 95% confidence intervals and showing model scripts are presented in Appendix 1.

In summary when collections on sheep were made in the presence of cattle numbers of *Culicoides* were significantly higher than on nights without cattle. Collections on sheep and cattle did not differ significantly. Analysis of collections of *C. obsoletus* group species compared collections on sheep on nights with cattle present and nights when cattle were absent. *For C. obsoletus* the analysis showed that collections of total females of this species were significantly higher on nights when cattle were present, this result was also demonstrated when analysing collections of un-pigmented and pigmented females. The same results were shown for *C. scoticus* with significantly more total females found on nights when cattle were present and significantly more un-pigmented and pigmented females. For *C. dewulfi* significantly more total females and un-pigmented females were collected on nights with cattle present. For *C. chiopterus* low numbers meant that analysis was restricted to looking at total females and pigmented females, in both models collections were significantly greater on nights when cattle were present.

Investigation of attraction to individual sheep revealed that for the most part there were no differences between the sheep. There were two exceptions: significantly fewer blood fed *C. obsoletus* were collected from sheep 4 than sheep 2; significantly fewer pigmented *C. chiopterus* were collected on sheep 1 than sheep 5.

3.3.4 Trial 4 – Diurnal Collection of *Culicoides*

Diurnal host seeking *Culicoides* were collected on two consecutive days from sheep in the drop trap with a total of 32 collections made on hosts and 32 corresponding UV light-suction trap samples. A total of 1,282 *Culicoides* were collected from sheep and one single specimen was taken at light as summarised in Table 3.25.

Time	**Day 1**				**Day 2**			
	***C. obsoletus* group Females**		**ºC**	**Solar Intensity Wm^{-2}**	***C. obsoletus* group Females**		**ºC**	**Solar Intensity Wm^{-2}**
	Sheep	Light			Sheep	Light		
0600-0700	11	0	11.74	23.1	37	0	13.83	20.9
0700-0800	30	0	16.01	139.7	24	0	16.0	147.6
0800-0900	30	0	18.62	279.6	51	0	17.0	240.1
0900-1000	20	0	21.19	422.2	15	0	18.73	390.0
1000-1100	102	0	23.94	549.4	9	0	20.72	518.3
1100-1200	39	0	25.3	692.3	27	0	22.21	662.6
1200-1300	21	0	26.26	736.9	5	0	23.44	640.1
1300-1400	7	0	26.88	774.7	10	0	24.41	714.7
1400-1500	18	0	27.02	690.9	1	0	25.92	732.3
1500-1600	23	0	27.72	749.7	3	0	26.27	680.2
1600-1700	20	0	27.91	528.4	1	0	26.75	611.5
1700-1800	11	0	26.61	260.2	1	0	26.88	489.2
1800-1900	32	0	27.87	381.6	5	0	26.75	378.1
1900-2000	55	0	27.19	214.5	35	0	26.03	225.9
2000-2100	55	0	25.97	94.6	143	0	23.68	84.0
2100-2200	142	1	23.45	7.7	159	0	22.15	3.5
Total	**616**	**1**			**526**	**0**		

Table 3.25. Diurnal collections of *C. obsoletus* group females from sheep and UV light-suction traps showing temperature and solar radiation at time of collection

These results were not subjected to statistical analysis as this was a pilot study with limited sampling. Temperature and solar intensity are also presented,

highlighting that collections were carried out on days of high temperature and bright sunshine with little cloud cover. The results demonstrate that there is some host-seeking activity outside normal crepuscular activity periods.

3.4 Discussion

The studies presented in this chapter provide a comprehensive investigation of the differential responses of *Culicoides* to hosts within a typical farm setting in the UK. *Culicoides* were demonstrated to exhibit a preference for sheep breed and the presence of cattle in close proximity was shown to increase biting rates on these hosts. In addition, shearing of sheep increased the efficiency of feeding in *Culicoides* when compared to unsheared hosts. The studies were additionally notable for being conducted at a site supporting large populations of livestock-associated *Culicoides*, resulting in biting rates that far exceeded those recorded previously in the UK (Carpenter *et al.* 2008c), France (Viennet *et al.* 2011, Viennet *et al.* 2013) or Spain (Gerry *et al.* 2009) and significant levels of biting were recorded in preliminary trials during diurnal conditions. This imposed significant demands in identification of *Culicoides* to species level by PCR, but was also crucial in achieving sufficiently large datasets for detailed analysis. The collection of these datasets will also significantly improve future modelling of arbovirus transmission by providing a more realistic range of potential biting rates under varied meteorological conditions.

This study represents the first investigation to demonstrate breed preference towards any host for *Culicoides*. While previous studies have demonstrated the attraction of *Culicoides* to sheep, they have invariably focused on a single breed (Schmidtmann *et al.* 1980, Carpenter *et al.* 2008c, Gerry *et al.* 2009, Viennet *et al.* 2011, Viennet *et al.* 2012). Inter-breed differences in attraction to Diptera have to date generally been investigated for large biting flies that can be identified and recorded visually as feeding on cattle e.g. *Haematobia irritans* (Ernst and Krafsur 1984, Guglielmone *et al.* 2000). In the current study, the two breeds selected for the

trial were purposefully closely related and of very similar size, one being pure Hartline breed and the other being Hartline/Suffolk cross, in an attempt to reduce the diversity of cues used for differentiation. The sheep could, however, still be separated as the cross breed had black faces and legs while the pure breed was all white. A second key consideration in the experimental design was the use of three sheep during each exposure. In addition to more accurately reflecting sheep flocking behaviour in the field, previous work has shown that responses of *Culicoides* to a group of three sheep are greater than to a single sheep (Garcia-Saenz *et al.* 2011). This experimental design also minimised the impact of individual variation in attraction due to physiological status as described in studies of other vector groups (Birkett *et al.* 2004, Torr *et al.* 2006).

The species collected at light and on the hosts were representative of farms in northern Europe, being dominated by *C. obsoletus*, *C. scoticus*, *C. dewulfi* and *C. chiopterus* (Boorman 1986, Cagienard *et al.* 2006, Meiswinkel *et al.* 2008, Venail *et al.* 2012). Statistical analyses detected no significant differences in responses to either breed at a total female *C. obsoletus* level, or when analyses were performed on different physiological states. By contrast, both total and blood fed female *C. scoticus* populations and total female *C. dewulfi* exhibited a preference for the cross breed sheep over the pure breed. As a whole, collections of *Culicoides* were greater on the cross bred sheep with an average collection rate of 24.9 *Culicoides* per 10 minute exposure and 19.3 per 10 minute exposure for the pure breed, although the maximum recorded rates were 165 and 157.3 respectively.

The biological reasons underlying this species-specific host selection require further investigation, as the only obvious physical difference between the breeds was the different colour of the face and legs. There is little information available

concerning the response of *Culicoides* species to visual cues. A previous study on *C. sanguisuga* in the USA found that collections on darker coloured hosts were higher than those on lighter hosts, though these differences were not statistically significant (Humphreys and Turner Jr. 1973). An early study of *Culicoides* circadian activity in the UK also used black cloth as a target on which to collect specimens and was found to be effective for the collection not only of *C. impunctatus* but also *C. obsoletus* and *C. chiopterus* (Hill 1947). *Culicoides impunctatus* has also been shown to discriminate between vertical and horizontal black stripes on a target (Bhasin 1996). Studies conducted as part of a related PhD have also illustrated that of twelve compounds isolated from the odour profile of the breeds of sheep that were found to be electrophysiologically active, four were found to occur in significantly different quantities between the two breeds (J. Cook, personal communication).

The implication of breeds not being equally attractive to *Culicoides* may not provide a practical solution to farmers to prevent the flocks being infected by arboviruses, but it is an important observation in understanding transmission. It is relatively common for multiple sheep breeds to be kept at specific locations and this may represent a driver of variation in infection prevalence on farms (and the common anecdotal observation that certain breeds are affected less by BT in endemic countries such as India and Africa). At present, epidemiological modelling also largely relies on having an accurate assessment of biting rates on host animals, although these are largely inferred from light-suction trap catches used with a wide margin of error (Gubbins *et al.* 2008). A practical follow up work would be to assess biting rate on more common commercially grazed sheep breeds.

The effect of being freshly sheared on the biting and successful feeding rate of *Culicoides* on sheep had also not been previously quantitatively investigated. Prior

to shearing, the sheep possessed an extremely thick fleece which covered the majority of the body surface and potentially acted as a barrier to *Culicoides* bites in certain areas of the body (particularly the belly). In this study it was hypothesised that the presence of the fleece would decrease biting rates due to this mechanical barrier, but that there could also be a secondary effect on attraction of *Culicoides* to the host. This could be mediated by the emission of greater quantities of semiochemicals from the fleece (which could have a positive or negative effect on host location) or as a by-product of increased respiration due to thermal stress. It was also clear that the silhouettes of the sheared and unsheared sheep were substantially different which could also potentially influence host location (Bishop *et al.* 2008).

Collections on both sheared and unsheared sheep were again dominated by *C. obsoletus* and *C. scoticus* but, unlike the previous trial examining breed preference, it was the latter species that was most abundant. *Culicoides chiopterus* was more abundant than in the previous trial and numbers of *C. dewulfi* were much reduced when compared to 2011, perhaps as a result of the field not being used for cattle grazing in 2012, but a parallel reduction in *C. chiopterus* would also be expected if this were the case. In *C. obsoletus* the abundance of blood fed females was significantly greater in collections from sheared sheep when compared to unsheared. There was no significant variation, however, in host location prior to feeding, indicating that other factors had a negligible impact on preference. Interestingly in *C. dewulfi*, *C. chiopterus* and *C. scoticus*, no significant differences were found between the sheared and unsheared sheep. This division between species was driven by reductions in feeding efficiency on unsheared sheep in *C. obsoletus*. Feeding efficiency was generally poor in this species, an observation that remains unexplained.

In cattle, *C. chiopterus* has been found primarily to feed on the belly and legs (Nielsen 1971) and has also been observed to approach the host at a very low altitude (S. Carpenter, personal communication). In contrast, *C. obsoletus* did not exhibit preferential feeding sites on this host (Nielsen 1971), although this could have been hidden by the lack of separation by the author of *C. obsoletus* and *C. scoticus*. More detailed studies of these differences in behaviour (particularly in *C. scoticus* and *C. dewulfi*) could explain this apparent differential response to shearing and allow prediction of impact on other species. As mitigation against infection with arboviruses, the significant reduction in feeding of *C. obsoletus* is of interest given its consistent implication as a vector (Carpenter *et al.* 2008a, Elbers *et al.* 2013). Sheep shearing is usually conducted in June-July in the UK, and this timing coincides with seasonal peaks in *Culicoides* numbers (Sanders *et al.* 2011). The use of earlier or later shearing, informed by both awareness of local transmission of arboviruses and the presence of reservoir hosts, could therefore impact upon infection. This action, however, would need to be balanced with the cost of delaying or bringing forward shearing and the likely reduction in efficacy of insecticidal treatments on unsheared sheep (Venail *et al.* 2011).

The suggestion that grazing cattle with sheep might reduce *Culicoides* biting rates also warranted investigation, as this factor is not only cited as a method of mitigation for BT, but also forms an important component of modelling exercises where attraction to these hosts is treated as a competing process. Unlike the first two trials where collections were made on groups of three sheep, this trial used single sheep and, hence, total numbers of *Culicoides* on nights when the cow are absent are lower, as would be expected (Garcia-Saenz *et al.* 2011), although still higher than in previous studies. Contrary to expectation, the presence of a heifer and calf increased

the numbers of *Culicoides* feeding on sheep substantially, presumably by increasing the range of attraction, followed by spill over of populations attracted by the cattle onto the secondary host. The fact that the presence of cattle increased *Culicoides* abundance on sheep by 2.4 times has significant repercussions for modelling transmission of arboviruses as cattle and sheep are commonly grazed together or in close proximity. The study additionally systematically demonstrated for the first time the highly zoophilic nature of livestock associated *Culicoides* in the UK, with very few individuals caught by sweep-netting when only a human host was present.

A key area of interest now exists in understanding to what degree this effect of cattle presence can be extrapolated to different ecosystems and livestock-associated *Culicoides* species. While the current study was limited to one site only, light trapping carried out across the trials gave results that were representative of the northern European farm fauna as defined by light-suction trapping (Boorman 1986, Meiswinkel *et al.* 2008, Venail *et al.* 2012). In addition, while subtle differences in the impact of this effect according to breed and individual have been recorded in the present study, the difference in size between sheep and cow hosts (and therefore semiochemical output) was generally representative of scenarios likely to occur elsewhere, as an adult heifer was used rather than a calf alone as in other studies (Mullens and Gerry 1998, Viennet *et al.* 2013). An assessment should be made, however, of the impact of distance of cattle from sheep on biting rate as this is likely to provide direct and detailed information regarding the likely range of visual, semiochemical and thermal cues.

An important *Culicoides* species absent from the study region is *C. impunctatus*, which reaches vast populations on farms in Scotland and northern England (Purse *et al.* 2012). This species has a wide host range and is the primary

nuisance biting species on humans, which implies that responses may differ from the highly zoophilic *C. obsoletus* group. While the status of this species as a vector of arboviruses is doubtful due to this wider host range, the presence of autogeny in populations and a more restricted seasonal abundance peak, similar studies would be useful in exploring the potential for this species to act as vectors of arboviruses of both livestock and humans (Carpenter *et al.* 2013). An additional and more surprising feature of all the studies carried out over the two years is the very low numbers of *C. pulicaris* and *C. punctatus* collected from the sheep and from the sweep net collections around the cattle. These results are broadly similar to other studies that have been carried out in the UK and Europe (Carpenter *et al.* 2008c, Gerry *et al.* 2009, Viennet *et al.* 2011, Harrup *et al.* 2012). While represented in light trap catches the numbers of these species were low in comparison to other recorded sites and investigations of the biting habits of these groups could be investigated as an additional area of interest.

While *Culicoides* are primarily crepuscular in their activity there have been reports of diurnal activity, including on sheep (Balenghien *et al.* 2008, Rijt *et al.* 2008, Viennet *et al.* 2012). These findings are supported by the pilot study of diurnal collections made in trial four where successful collections were made throughout the day. With the exception of one collection on day one that was made between 10-11 am the diurnal collections are lower than those made at times around sunset. The surprising feature of these collections is that they were made on two days with high temperatures, peaking at 29 ºC on the first day, and high levels of solar radiation with very little cloud cover. It may be the case that the *Culicoides* collected in daylight were responding opportunistically to immediate host availability, over-riding an innate temperature-mediated response to light intensity and it would be interesting to

assess the level of diurnal activity when meteorological conditions would be more suitable for *Culicoides*.

Chapter 4: The Responses of *Culicoides* to Olfactory Stimuli

4.1 Introduction

Host-derived chemicals play a primary role in host location of haematophagous Diptera, eliciting both activation and directional flight (see Chapter 1). The process of understanding host location is complicated by the substantial range of chemicals that are released by hosts; as an example, human beings have been demonstrated to emit between 300-400 volatiles from their hands alone (Bernier *et al.* 2000). Kairomones are a type of semiochemical produced and emitted by a host that provide a chemical cue to a host-seeking insect. A considerable body of work has already investigated responses of *Culicoides* to specific kairomones either used individually or in blends (Logan *et al.* 2010). The majority of these studies, however, have been conducted on nuisance-biting species in the USA and the UK. To date investigations of how livestock associated species find their hosts have been extremely limited.

Carbon dioxide, which is emitted as a by-product of respiration by all animals and plants, is an important cue in the host location of a majority of haematophagous Diptera. In *Culicoides*, CO_2 was first shown to be effective as an attractant in Buttonwillow, California, USA in studies from 1963-4 (Nelson 1965). In this study up to 3,755 individuals of the *C. variipennis* complex were collected in a single night using modified mosquito trap baited with 1.4-2.3 kg of dry ice. The vast majority of these were not blood fed or gravid leading to a conclusion that CO_2 played an important role in host location (although small numbers of male *C. variipennis* complex were also caught). Since these initial findings, many other studies have

investigated the role of CO_2 across a range of *Culicoides* species. These studies can roughly be divided into those that have used field based experimental techniques to compare baits of CO_2 with un-baited traps of a standardised design and those that examine the response of *Culicoides* to this semiochemical in the laboratory. The latter studies can include examination of responses through behavioural analyses, inference of antenna or maxillary palp function via their morphology and electroantennagram (EAG) experimentation (Table 4.1).

Species	Laboratory/Field	Reference
*C. sonorensis**	Field	(Nelson 1965, Holbrook 1985, Anderson and Linhares 1989, Mullens 1995, Gerry and Mullens 1998, Mullens and Gerry 1998, Mullens *et al.* 2005, Gerry *et al.* 2008)
C. furens	Field	(Kline *et al.* 1990, Kline *et al.* 1994, Kline and Lemire 1995)
	Laboratory	(Grant and Kline 2003)
C. mississippiensis	Field	(Cilek and Kline 2002)
	Laboratory	(Grant and Kline 2003)
C. melleus	Field	(Kline *et al.* 1994, Cilek and Kline 2002)
C. hollensis	Field	(Kline *et al.* 1994)
	Laboratory	(Grant and Kline 2003)
C. barbosai	Field	(Cilek and Kline 2002)
C. impunctatus	Field	(Bhasin *et al.* 2000b, Bhasin *et al.* 2001)
	Laboratory	(Blackwell *et al.* 1992a, Bhasin *et al.* 2000b, Bhasin *et al.* 2000a)
C. histrio	Field	(Ritchie *et al.* 1994)
*C. subimmaculatus**	Field	(Ritchie *et al.* 1994)
C. molestus	Field	(Ritchie *et al.* 1994)
C. marmoratus	Field	(Ritchie *et al.* 1994)
C. brevitarsis	Field	(Bishop *et al.* 2008)
*C. obsoletus**	Field	(Mullens *et al.* 2005, Carpenter *et al.* 2008c, Gerry *et al.* 2009, Harrup *et al.* 2012)
C. parroti	Field	(Gerry *et al.* 2009)
*C. pulicaris**	Field	(Harrup *et al.* 2012)
C. nubeculosus	Field	(Harrup *et al.* 2012)
	Laboratory	(Blackwell *et al.* 1992a)

Table 4.1. *Culicoides* species demonstrating responses to CO_2 in the field or laboratory. (* = species level taxonomy of subject uncertain).

The response of *Culicoides* species to CO_2 varies significantly from species that can be collected in large numbers (>1000/trap night), as is the case in *C. sonorensis* in the Nearctic (Mullens 1995, Mullens and Gerry 1998) to those that are rarely caught in CO_2 baited traps such as *C. obsoletus* in the northern Palaearctic (Gerry *et al.* 2009, Harrup *et al.* 2012). Even in those species of *Culicoides*

exhibiting the strongest responses, however, the CO_2 collections always vastly underestimate the true biting rate on natural hosts. In the best characterised example of this phenomenon, numbers of *C. sonorensis* collected using dry ice baited suction traps underestimated those collected on a calf by 7.2 times, illustrating the limitations of pure CO_2 as an artificial attractant (Mullens and Gerry 1998). In species less attracted to CO_2, including the *C. obsoletus* complex, this can lead to only very small numbers of individuals being recovered in baited traps despite relatively high biting rates on hosts held in proximity (Mullens *et al.* 2005, Gerry *et al.* 2009). As an example, in Spain 313 *C. obsoletus* and 4 *C. scoticus* were collected directly from a sheep host, but only 2 *C. obsoletus* were collected at CO_2 (Gerry *et al.* 2009). This finding suggests that additional host odours in combination with CO_2 may be required to elicit the olfactory response in *C. obsoletus*.

A key area in examining *Culicoides* responses to CO_2 has been the degree to which the rate and method of release determines the degree of response and/or range of collections. A positive relationship between increased release rate from baits and the numbers of *Culicoides* collected has been demonstrated in the field for *C. furens*, *C. melleus* and *C. hollensis* (Kline *et al.* 1994); *C. sonorensis* (Mullens 1995) and *C. impunctatus* (Bhasin *et al.* 2001). Preliminary studies with only two release rates of CO_2 did not demonstrate such a relationship in *C. obsoletus* (Harrup *et al.* 2012). The range chosen for studies of attraction to CO_2 is generally between 200 ml/min (representing release from a calf, sheep or human), to 2500 ml/min (representing output from a large ruminant). A laboratory study of EAG responses to different CO_2 release rates in *C. furens* confirmed field studies in terms of response intensity (Grant and Kline 2003), but remains the only detailed investigation using this method to date.

After CO_2, racemic octenol is the most widely studied kairomone for *Culicoides* and has been investigated in detail for other vector groups (see Chapter 1). Responses to the release of octenol in isolation in the field range from weak in *C. furens* (Kline *et al.* 1994) to insignificant in *C. impunctatus* (Bhasin *et al.* 2000b, Bhasin *et al.* 2001), *C. hollensis* and *C. melleus* (Kline *et al.* 1994), *C. molestus*, *C. ornatus* group, *C. subimmaculatus* group and *C. marmoratus* (Ritchie *et al.* 1994) and *C. brevitarsis* (Bishop *et al.* 2008). Interestingly, laboratory studies of *C. impunctatus* demonstrate electrophysiological and behavioural responses to octenol in the laboratory (Blackwell *et al.* 1996, Bhasin *et al.* 2000a) and the reason for this lack of agreement between laboratory and field studies has not been investigated.

Despite this lack of response, octenol can act synergistically with CO_2 to dramatically increase trap catches, although this effect is known to be highly species-specific. A combination of CO_2 and octenol has been shown to increase *C. impunctatus* collections by 23 fold compared to CO_2 alone (Bhasin *et al.* 2001) and by 35.8 fold in *C. furens* (Kline et al, 1994). In the latter study, however, two additional species, *C. melleus* and *C. hollensis*, demonstrated no such increase. Similarly, the addition of racemic octenol to CO_2 baited traps in the UK did not significantly improve catches of *C. obsoletus* or *C. nubeculosus* (Harrup *et al.* 2012).

In Australia contrasting results were shown during two trials with the addition of CO_2. During the first trial no differences were observed between CO_2 and octenol and CO_2 alone, while in the second trial *C. molestus*, *C. ornatus* group, *C. subimmaculatus* group and *C. marmoratus* were collected in significantly higher numbers in the combined bait (Ritchie *et al.* 1994). These differences in results may have reflected changes in experimental design between the two trials as during the second trial three release rates were tested for octenol (0.099, 5.66 and 28.5 mg/h)

compared to 6.05 mg/h in the first trial all of which are considerably higher than natural release rates, ie 0.01 mg/h from oxen (Torr *et al.* 1995). Significant differences in collections relative to CO_2 alone were only observed with the medium and high release rates and there were no significant differences between these in numbers of *Culicoides* collected. In addition, the second trial used CO_2 at 412 ml/min in contrast to 200 ml/min during the first trial. In another trial conducted in Australia, collections of the major arbovirus vector *C. brevitarsis* in CO_2 baited traps were enhanced 6 fold with the addition of octenol although release rates were not measured (Bishop *et al.* 2008).

In their entirety, these results demonstrate that racemic octenol has a mixed effect with regards *Culicoides*, being effective in improving capture rates for some species but not inducing any enhanced response for others. While the reasons underlying this response remain poorly characterised, it has been suggested that this variation in response may be partly driven by host preference, with mammalian, or less specific feeders being more strongly attracted (Kline *et al.* 1994). A major point of contention is that the racemic octenol used in studies is typically a 1:1 ratio of the two enantiomeric components, R-octenol and S-octenol. The natural composition of host-derived octenol has been found to vary between 80:20 and 92:8 R:S, hence studies with racemic octenol are not a true representation of emissions from hosts (Hall *et al.* 1984).

To date, only one study has investigated the effects of different enantiomeric composition of octenol in field collections of *Culicoides* (Harrup *et al.* 2012). The trials were conducted in two areas: one with high abundance of *C. impunctatus* and one in an area with livestock associated species. Collections of *C. impunctatus* were made using increasing proportions of R:S enantiomers compared to CO_2 at 500

ml/min. Using an R:S ratio of 4:96, no difference was observed between the treatment trap and the CO_2 control. As the ratio of R to S increased, however, the collections became significantly greater in numbers compared to CO_2 alone, although the different proportions of R:S were not directly compared against each other to see whether the collections differed significantly. Substantial numbers of *C. impunctatus* were collected with all treatments and it was found that increasing the proportion of the R enantiomer in baits yielded significantly higher collections compared to the CO_2 control. For the comparison in the livestock farm, R-octenol and S-octenol were combined with 500 ml/min CO_2 and compared to racemic octenol with CO_2 and CO_2 alone. Both enantiomers collected significantly greater catches of *C. obsoletus* than the CO_2 control and R-octenol was also significantly more attractive than racemic octenol. In addition, large collections of *C. nubeculosus* were made using R-octenol that substantially exceeded previous light-trap based surveys of this species, highlighting the variation in surveillance results according to trapping method used (Harrup *et al.* 2012).

A range of other compounds have been tested for behavioural activity in *Culicoides.* Mixtures of acetone at cattle release rate equivalents elicited an enhanced response in *C. impunctatus* using a wind tunnel (Bhasin *et al.* 2000b) and increased field catches of *C. achrayi* as a supplement bait in light traps (Romon *et al.* 2012). There is also preliminary evidence that lactic-acid may act as an attractant for *C. furens* (Kline *et al.* 1990). More commonly, however, these have been combined as constituents of host odour blends, summarised in Table 4.2.

Species	Blend	Effect	Reference
C. impunctatus	Acetone and CO_2	5.8x increase relative to CO_2	(Bhasin *et al.* 2001)
	Octenol, acetone and CO_2	3.2x* increase relative to CO_2	
	Butanone and CO_2	2x increase relative to CO_2	
	Phenols and CO_2	3x increase relative to CO_2	
	Cow urine and CO_2;	10x* increase relative to CO_2	
	Cow urine, acetone and CO_2;	11x* increase relative to CO_2	
	Cow urine, octenol and CO_2	7.3x* increase relative to CO_2	
	Goat hair extract, octenol and CO_2	1.2x increase relative to octenol and CO_2	(Mands *et al.* 2004)
	Water buffalo hair extract, octenol and CO_2	2.6x* increase relative to octenol and CO_2	
	Red deer hair extract, octenol and CO_2	0.25x decrease relative to octenol and CO_2	
	Sheep fleece extract, octenol and CO_2	0.5x decrease relative to octenol and CO_2	
	Pony hair extract, octenol and CO_2	1.4x increase relative to octenol and CO_2	
C. furens	Lactic acid and CO_2	64x increase relative to CO_2	(Kline *et al.* 1990)
	Lactic acid, octenol and CO_2	123x increase relative to CO_2	
	Butanone, 1-octen-3-ol and CO_2	31x increase relative to CO_2	
	Acetone, 1-hexen-3-ol, octenol	17.6x* increase relative to CO_2	(Kline *et al.* 2012)
	Acetone, lactic acid, glycolic acid	No increase relative to CO_2	
	Acetone, Lactic acid, dimethyl disulphide	7x increase relative to CO_2	
C. floridensis	Acetone, 1-hexen-3-ol, octenol	No increase relative to CO_2	
	Acetone, lactic acid, glycolic acid	No increase relative to CO_2	
	Acetone, Lactic acid, dimethyl disulphide	No increase relative to CO_2	

Species	Blend	Effect	Reference
C. mississippiensis	4:1:8 octenol phenol mix with CO_2	2x* increase relative to CO_2	(Cilek and Kline 2002)
C. barbosai	4:1:8 octenol phenol mix with CO_2	2-3x* increase relative to CO_2	
C. melleus	4:1:8 octenol phenol mix with CO_2	No increase relative to CO_2	

Table 4.2. The response of *Culicoides* to blends of semiochemicals under field conditions (* = statistically significant increase in trap catches vs control).

The aim of this chapter is to investigate three separate, but interrelated aspects of host location of livestock associated species in the UK. Initially, the variation in response to CO_2 is investigated as a more thorough follow up to preliminary studies conducted previously (Gerry *et al.* 2009, Harrup *et al.* 2012). A major observation of both these studies was that the response of *C. obsoletus* to CO_2 was poor, however, both studies utilised extremely limited numbers of replicates and the CO_2 rate was undefined (from dry ice) in one study while the second only used two release rates. Hence, a range of release rates are investigated in the current study to act as a baseline for future experimentation.

In a second trial, the response of livestock associated species to the odour of two breeds of sheep with the removal of visual and thermal cues is examined in order to assess the likely contribution of kairomones to host location. This is the first time

that this experimental design has been used for *Culicoides* and provides an overall assessment of the likelihood that semiochemicals can be used to assess biting rates in the field.

A third trial then examines the response of field populations of *C. obsoletus* to specific host derived semiochemicals that have been shown to induce electrophysiological and behavioural responses in the laboratory. All studies included detailed recording of meteorological conditions throughout each sampling period and this was used during analysis of results with generalised linear models to explain trap catch abundance.

4.2 Materials and Methods

In all three trials miniature CDC light-suction traps (Model 512, J.W. Hock Company, USA) with bulbs removed were used to measure the responses of *Culicoides* to semiochemicals and UV CDC light-suction traps were used to monitor background population changes (Model, 912, J.W. Hock Company, USA). Semiochemical traps were powered by D-cell battery adapters (J.W. Hock Company, USA) and the light trap was powered by a lead acid sealed 12v battery (Yuasa, Japan). Carbon dioxide was supplied from 14.5 kg compressed cylinders (Aire Liquide, UK) fitted with 4 bar two stage regulators (C.S. Milne, UK) and CO_2 was passed through tygon tubing (Type R3603, Saint Gobain Performance Plastics, USA) to an adjustable flow metre (Platon model, Roxspur, UK). The regulated flow rate was passed through tygon tubing with a final release point on the underside of the rain shield of the trap. Semiochemical-baited traps and light traps were operated over the same period of time in each trial, *Culicoides* responding to the traps were sucked in through the fan into kill jars containing 200ml water and a drop of detergent.

4.2.1 Trial 1 – The response of *Culicoides* to increasing release rates of CO_2

The study was conducted at a mixed arable and livestock farm, site 1 described in Chapter 2, from late July to mid-September 2010. The field selected for the study, 150 metres by 75 metres, contained a herd of adult Holstein-Friesian cows. Two sides of the field were surrounded by deciduous woodland, with grazing fields beyond, one side bordered a crop field and a farm road ran along the fourth side with an arable field on the opposite side of the road.

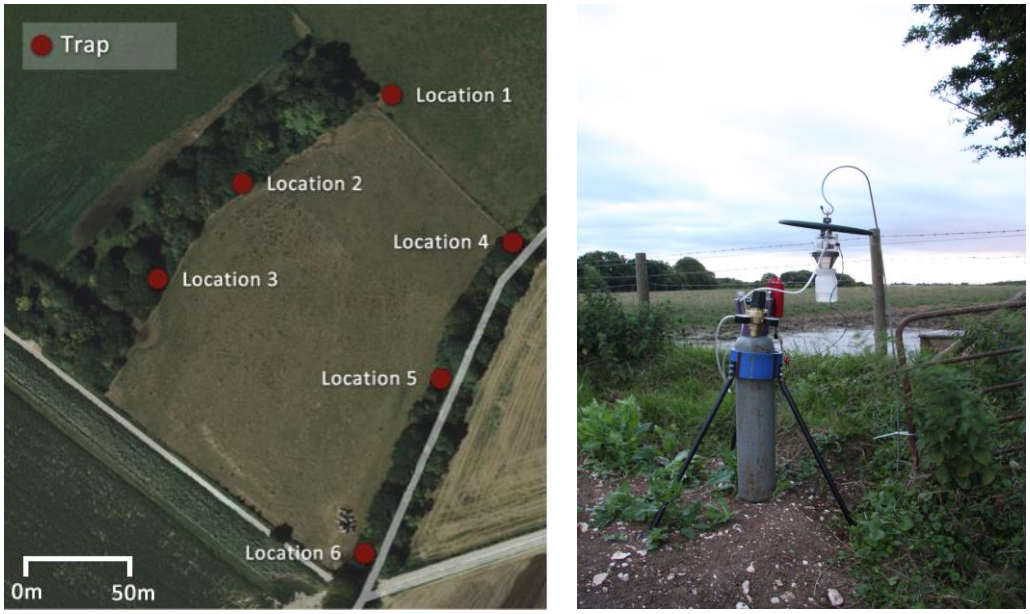

Figure 4.1. Field site for CO_2 trial showing trap locations and the trap site at location 1

Carbon dioxide was released at five flow rates: 500 ml/min; 1,000 ml/min; 1,500 ml/min; 2,000 ml/min and 2,500 ml/min and a UV light-suction trap was run as a control. Traps were operated from two hours before sunset to one hour afterwards to coincide with peak activity of *Culicoides.* To prevent interference from cattle, traps were positioned outside the field around the edge of the fence with an estimated inter-trap distance of 50 metres (Figure 4.1). On the first night of the trap rotation treatments were randomly assigned to each trap position, on subsequent nights treatments rotated clockwise until the end of the rotation so that each trap would occupy each location. Treatments were then re-randomised at the start of the second rotation. Meteorological data were recorded in an adjacent field, approximately 500 metres from the furthest treatment location.

4.2.2 Trial 2 – The response of *Culicoides* to sheep odour

The study was conducted at a livestock farm, site 2 as described in Chapter 2. The trial was carried out in a large grazing field, 1,000 metres by 600 metres, with mixed sheep and suckler cattle, the experiment was conducted in the lower part of the field which measured 200 metres by 70 metres (Figure 4.2). The field was surrounded on three sides by deciduous woodland and the fourth side was grazing pasture.

Figure 4.2. Map showing study site for sheep odour trial

As part of a separate PhD study, by James Cook from Rothamsted Research/LSHTM, air entrainments were being carried out on the two breeds of sheep, Hartline and Hartline/Suffolk cross, described in Chapter 3, hereafter termed pure and cross. This required three sheep to be contained in a specially constructed apparatus where volatile odours could be collected onto Porapak polymers. The apparatus comprised a sealed metal box manufactured from steel with aluminium

sheeting lining the inside walls and ceiling, the entrainment box measured 1.82 m in length by 1.06 m in width and 1.64 m in height. While inside the entrainment unit the sheep were supplied with air pumped through a "push" fan (ebm-papst UK Ltd., UK) which was passed through a charcoal filter (Vokes Air Group, UK) to remove environmental impurities. The air inside the box therefore only contained the odour of the sheep that were being entrained, the volatiles released from the sheep were collected onto porapak polymers that were suspended from the ceiling of the entrainment box and fitted with a pump to draw air from inside the box across the polymer (Rothamsted Research, UK). Air was exhausted out of the box through a second fan (RS Components, UK). The set-up of the entrainment box is illustrated in Figure 4.3. Exhausted air containing pure sheep odour, was delivered via ventilation tubing (102 mm internal diameter) (Part number 340-01444, RS Components, UK) to a miniature CDC trap at two metres distance from the entrainment box. The ventilation tube was attached to the underside of the rain shield of the trap and sheep odour alone was used without combining it with CO_2 from compressed cylinders, (Figure 4.3). A second miniature CDC trap was also used with no bait attached and traps were placed 3 metres apart with positions switched each night (these two traps are represented by the Odour Traps in Figure 4.2). A UV light-suction trap was positioned 50 metres away at one of two sites each night to monitor *Culicoides* populations in the field. Air entrainments of sheep ran for 4 hours each day from 3 hours before sunset to 1 hour after and therefore odour baited trapping was carried out over the same period. The sheep breed used in the entrainment apparatus was alternated each night.

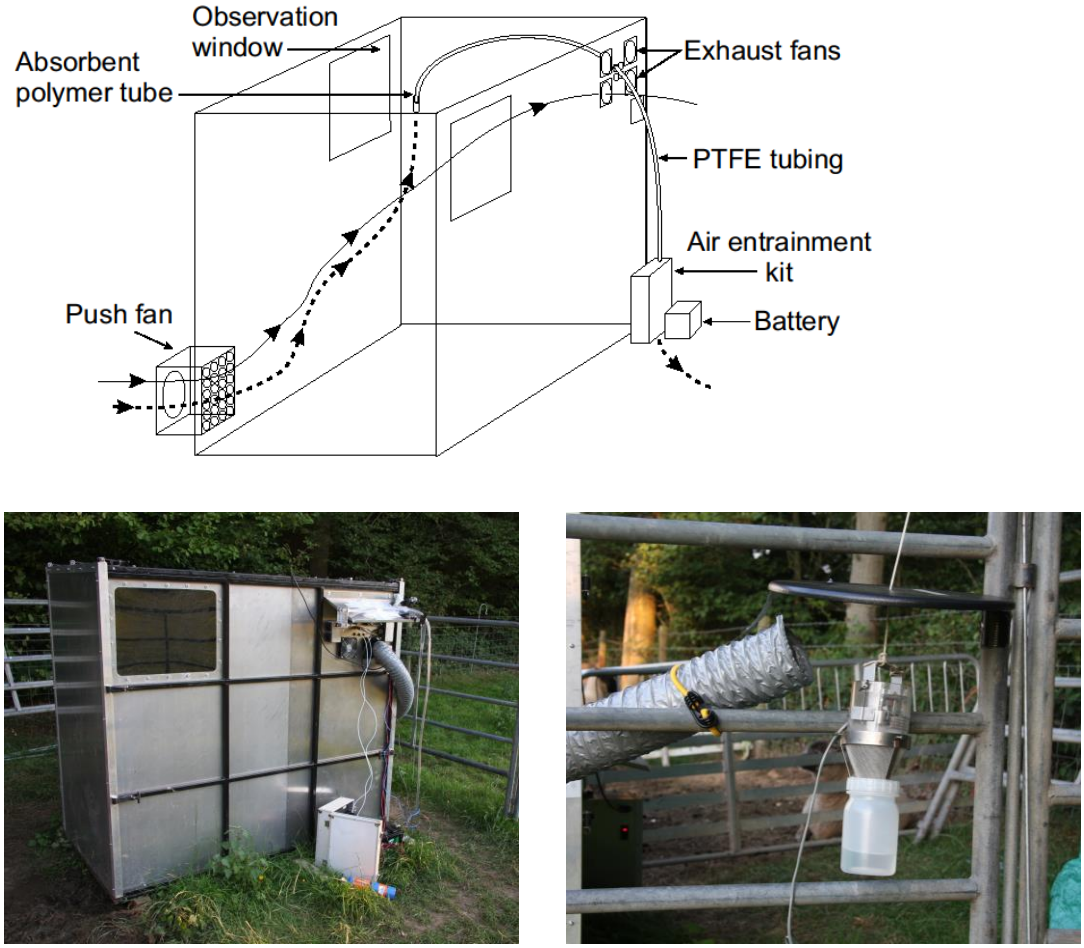

Figure 4.3. Schematic diagram of air flow though air entrainment unit (courtesy James Cook, Rothamsted Research) and the air entrainment unit *in situ* with ventilation duct attached to an exhaust fan and the delivery of odours to the unlit suction-trap

4.2.3 Trial 3 – The response of *Culicoides* to host derived semiochemicals

This trial was conducted in September 2012 at the same site that was used in trial two. Three chemicals collected through air entrainments on sheep, described above, and found to be behaviourally active in a laboratory setting through electrophysiological testing (EAG) and behavioural assays (y-tube) were tested. Further details regarding the laboratory work and with specific regard to the

chemicals identified can be obtained from James Cook (james.cook@lshtm.ac.uk) or James Logan (james.logan@lshtm.ac.uk). The chemicals identified through the laboratory work are the subject of intellectual property and will therefore be referred to as chemical A, B and C. Test chemicals were supplied by Rothamsted Research: chemicals A and B were impregnated into cellulose sponges (500 µl) and heat sealed in 500 (chemical A) and 1,500 (chemical B) gauge bagging; Chemical C was supplied in 3 polyvials, each containing 400 µl of the chemical. Lures were attached to the underside of the rain shield of the suction trap using wire. R-octenol was also trialled in the field study as a positive control, it was released from a 0.8 ml amber borosilicate vial (Chromacol, UK). A four centimetre pipe cleaner wick was fitted through a 1 mm hole in the vial cap with 2 cm of wick inside the vial and 2 cm exposed outside the vial this was then attached to the fan mounting on the trap. Release rates for chemicals are shown in Table 4.3, these were obtained for chemicals A, B and C through wind tunnel experiments by James Cook, for R-octenol the release rate was measured by weighing the vial before and after each trapping period.

Semiochemical	**Mean Release Rate**
CO_2 (Aire Liquide, UK)	500 ml/min
Chemical A (99%, Sigma-Aldrich, UK	0.16 (±0.02) mg/day
Chemical B (97%, Sigma-Aldrich, UK)	2.97 (±0.60) mg/day
Chemical C (95%, Sigma-Aldrich, UK)	1.28 (±0.02) mg/day
R-octenol (99%, Bedoukian Reseach Inc., USA)	4.21 (±0.26) mg/hour

Table 4.3. Mean release rates (±S.E.M.) of semiochemical treatments, chemical purity and supplier information

The chemicals tested in the field trial were combined with 500 ml/min of CO_2, in addition to the four traps baited with chemicals A, B, C and R-octenol a fifth trap contained a blend of all 4 chemicals plus CO_2, a sixth trap was supplied with

CO_2 alone, a seventh trap had no bait, negative control, and the final eighth trap was a UV light-suction trap operated as a positive control. For all semiochemical baited traps the CO_2 release point was fixed approximately 2-3 cm from the test chemical, see Figure 4.4.

Figure 4.4. Semiochemical-baited trap *in situ* at field location site and close up of trap baited with R-octenol showing CO_2 release point position relative to semiochemical

Eight trap positions were chosen around the outside of the field to avoid interference from cattle and there was an inter-trap distance of at least 50 metres (Figure 4.5). On the first night of the trial, each treatment was randomly assigned to a trap position and on subsequent nights treatments were moved in a clockwise direction. Following the completion of each 8 night trap rotation, treatments were re-randomised to positions. Trapping was carried out from one hour before sunset to three hours after sunset.

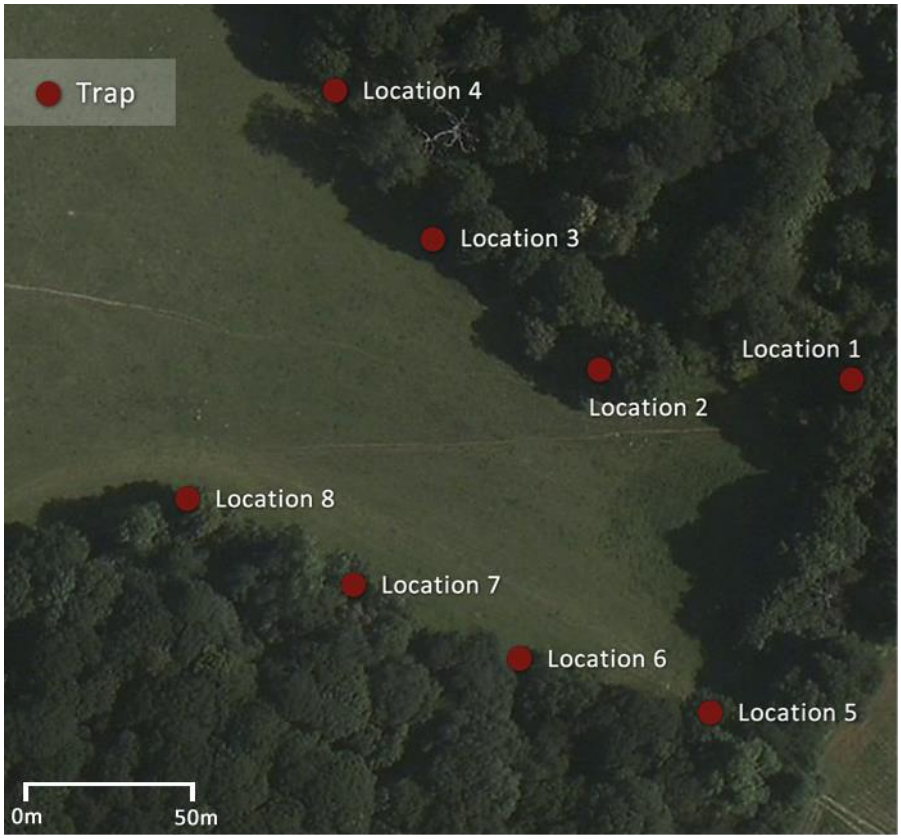

Figure 4.5. Map of field site showing trap positions in semiochemical trial

4.2.4 Sample Identification

Culicoides collected in test bait traps were identified by morphological characteristics as described in Chapter 2, and are presented as *C. obsoletus/scoticus*, *C. dewulfi* and *C. chiopterus*. The only exception is the collections made in the light-suction trap during trial two; these were identified molecularly as part of the drop trap experiment in Chapter 3 but for comparison to other data in that trial are presented here as *C. obsoletus/scoticus*. Other light trap collections are only identified to *C. obsoletus* group level. Females were further sorted according to physiological state.

4.2.5 Statistical Analysis

Due to low numbers of *C. obsoletus* group females collected, analyses were restricted to group level rather than species level for all trials. Analyses of collections were made through the construction of negative binomial GLMs with a log link function in R version 2.15.2 (R Core Team 2013) as described in Chapter 2. The effects of individual factors in final models were examined using Tukey's significant differences. Final models with parameter estimates and 95% confidence intervals as well as model scripts are presented in Appendix 2.

4.3 Results

4.3.1 Trial 1 - The response of *Culicoides* to increasing release rates of CO_2

Over twelve nights of trapping, constituting two complete rotations of traps, seventy two trap collections were made collecting a total of 4,422 *Culicoides*. The majority of *Culicoides* collected were *C. nubeculosus* constituting 4,342 (98%) of total *Culicoides* collected and only 73 *C. obsoletus* group individuals were collected. Of the female *C. obsoletus* group collected, all were found to be *C. obsoletus/scoticus* complex with the exception of 2 *C. dewulfi* collected in the 1,500 ml/min trap. Other species collected in small numbers included *C. festivipennis* (3), *C. circumscriptus* (3) and *C. punctatus* (1). Carbon-dioxide baited traps collected 98.2% of the total *Culicoides* catch with 1.8% collected in the UV light-suction trap. Collections for each trap are shown in Table 4.5. The highest single collection was 1,201 *C. nubeculosus* in the 2,500 ml/min trap at trap location 1 and the highest collection for each treatment was made at this location.

Amongst the CO_2 baited traps the collections of female *C. nubeculosus* comprised 88.7% un-pigmented, 11.28% pigmented and 0.02% blood fed individuals while in the UV trap the proportions were 48.4% un-pigmented, 46.8% pigmented and 4.8% gravid. The composition of *C. obsoletus* group females from CO_2 baited traps was 45.1% un-pigmented, 31.4% pigmented and 23.5% gravid. Of the *C. obsoletus* group males collected these comprised 17 *C. obsoletus* in the 1,500 ml/min trap, 1 *C. scoticus* in the light trap and 3 *C. dewulfi*, 2 from the light trap and 1 from the 1,500 ml/min trap.

Trap bait (CO_2 release rate)	**Total (mean ± s.e.m.)**					
	***C. nubeculosus* Females**	***C. nubeculosus* Males**	***C. obsoletus* group Females**	***C. obsoletus* group Males**	**Other *Culicoides***	**Total *Culicoides***
500 ml/min	270 (22.5±20.8)	0	0	0	0	270
1,000 ml/min	1,423 (118.6±79.7)	59 (4.9 ±4.8)	0	0	0	1,482
1,500 ml/min	577 (48.1±30.2)	10 (0.8 ±0.7)	44 (3.7±3.7)	18 (1.5±1.5)	2	651
2,000 ml/min	30 (2.5 ±2.1)	3 (0.3 ±0.2)	0	0	1	34
2,500 ml/min	1,859 (155.0 ±102.0)	38 (3.2 ±2.)	7 (0.6±0.6)	0	0	1,904
Light	62 (5.2 ±3.6)	11 (0.9 ±0.7)	1	3 (0.3±0.2)	4	81

Table 4.4. Collections of *C. nubeculosus* and *C. obsoletus* group by CO_2 baited traps showing totals, means and standard error of mean

Due to a large number of zero catches analysis was restricted to the development of two models to explain total *Culicoides* and total female *C. nubeculosus*. For both analyses initial models could not be run with all variables included, therefore a forward step approach was taken. In this case, variables are added to the model one at a time with the variable producing the most significant ($p<0.05$) reduction in deviance being selected at each step. The final model for total *Culicoides* included trap type ($p<0.05$) and location, temporal trend and wind speed, see Table 4.5. Analysis revealed that the 1,500 ml/min trap collected significantly higher numbers of total *Culicoides* than the 1,000 ml/min, 500 ml/min and the UV light-suction trap ($p<0.05$), but no other significant differences were observed between traps (Table 4.6). Analysis of the effect of trap location shows that collections at location 1 were significantly higher than at all other locations ($p<0.001$) and no other differences were found between locations (Table 4.7). This can be explained by a leaking water trough close to location 1 which would provide a suitable development site for *C. nubeculosus*.

Parameter	**Total *Culicoides***	***C. nubeculosus* Females**
Intercept	2.114*	1.810*
Temporal Trend		
Quadratic	-0.001***	-0.001***
Trap		
500	-0.593	-0.270
1,000	Baseline	Baseline
1,500	2.926***	2.572**
2,000	0.901	0.870
2,500	1.787*	1.313
Light Trap	-0.320	-0.607
Location		
Location 1	5.079***	5.343***
Location 2	0.586	1.107
Location 3	Baseline	Baseline
Location 4	-2.134*	-1.987*
Location 5	-1.773	-3.268**
Location 6	-1.732	-2.324*
Wind Speed	-1.242***	-1.140***

Table 4.5 Regression co-efficients for final models to describe total *Culicoides* and total *C. nubeculosus* females collected (*=p<0.05, **=p<0.01, ***=p<0.001)

Treatment	**1,000**	**1,500**	**2,000**	**2,500**	**Light**
500	0.593	3.519**	1.495	2.380	0.272
1,000	-	2.926*	0.902	1.787	-0.321
1,500		-	-2.024	-1.139	-3.247**
2,000			-	0.885	-1.223
2,500				-	-2.108

Table 4.6. Differences between traps for Total *Culicoides* estimates are treatments on the top line relative to treatments on the left (*=p<0.05, **=p<0.01, ***=p<0.001)

Location	**2**	**3**	**4**	**5**	**6**
1	-4.492***	-5.079***	-7.214***	-6.853***	-6.812***
2	-	-0.587	-2.721	-2.360	-2.319
3		-	-2.134	-1.773	-1.732
4			-	0.360	0.402
5				-	0.041

Table 4.7. Analysis of differences between traps locations for Total *Culicoides* model, estimates are treatments on the top line relative to treatments on the left (*=p<0.05, **=p<0.01, ***=p<0.001)

Collections of female *C. nubeculosus* depended significantly on trap type ($p<0.01$), temporal trend, wind speed and location, see Table 4.5. Further analysis showed that the 1,500 ml/min trap collected significantly higher numbers than the 1,000 ml/min, 500 ml/min and UV light-suction trap ($p<0.05$), no other significant differences were observed between traps (see Table 4.8). Analysis of trap locations showed that location 1 collected significantly higher numbers than all other locations, amongst the other locations, location 2 collected higher numbers than locations 4, 5 and 6 and location 3 collected higher numbers than location 5, no other significant differences were found (see Table 4.9).

Treatment	**1,000**	**1,500**	**2,000**	**2,500**	**Light**
500	0.270	2.842*	1.140	1.583	-0.337
1,000	-	2.572*	0.870	1.313	-0.607
1,500		-	-1.702	-1.258	-3.179*
2,000			-	0.443	-1.477
2,500				-	-1.921

Table 4.8. Analysis of differences between traps for *C. nubeculosus* Females, estimates are treatments on the top line relative to treatments on the left (*=p<0.05, **=p<0.01, ***=p<0.001)

Location	**2**	**3**	**4**	**5**	**6**
1	-4.236***	-5.343***	-7.331***	-8.611***	-7.668
2	-	-1.107	-3.095*	-4.375**	-3.432**
3		-	-1.987	-3.268*	-2.324
4			-	-1.280	-0.337
5				-	0.943

Table 4.9. Analysis of differences between trap locations, for *C. nubeculosus* Females, estimates are treatments on the top line relative to treatments on the left. (*=p<0.05, **=p<0.01, ***=p<0.001)

In total the collections of *Culicoides* were dominated by *C. nubeculosus* with very few *C. obsoletus* group collected in comparison. Statistical analysis was restricted to looking at the total *Culicoides* collection and the total female *C.*

nubeculosus collection with too few individuals of other species collected to allow for analysis. The models for total *Culicoides* and total female *C. nubeculosus* both demonstrated that CO_2 at a release rate of 1,500 ml/min collected significantly more individuals than the 500 and 1,000 ml/min rates or the UV light-suction trap, beyond 1,500 ml/min no further significant increases in collection were observed.

4.2.2 Trial 2 – The response of *Culicoides* to sheep odour

Collections of *Culicoides* were made over 17 nights using the whole sheep odour from the air entrainment unit. On seven nights the trap was baited with the odour of the Hartline/Suffolk cross breed (two nights were abandoned for this breed due to heavy rain). On ten nights the trap was baited with the odour of the pure Hartline breed. A total of 51 collections were made: 17 in odour baited traps; 17 in un-baited traps and 17 in UV light-suction traps; the results are summarised in Table 4.10. A total of 1,389 *Culicoides* were collected in the traps of which 1,202 (86.5%) were *C. obsoletus* group females. The UV light-suction trap collected 1,253 (90.2%) of the total *Culicoides* with 108 (7.8%) collected using the pure sheep breed baits (7.8%) and 19 (1.4%) with the cross breed sheep bait. A total of 9 *Culicoides* were collected in un-baited traps. The greatest collection of *Culicoides* in a single evening was made using the UV light-suction trap (509); the greatest in the pure breed odour being 42 and for the cross breed 4. By comparison, direct collections from an animal bait conducted over the same period collected a total of 12,509 *Culicoides* (see Chapter 3). The female *C. obsoletus* group were further identified to *C. obsoletus/scoticus* complex, *C. dewulfi* and *C. chiopterus* from the odour and un-baited traps based on morphological characteristics, see Table 4.10. Females from UV light-suction traps were identified molecularly as part of the direct collection

study in Chapter 3 but for comparability are reported here as *C. obsoletus/scoticus* complex. In addition to female *C. obsoletus* group, 39 males were also collected comprising: 16 *C. obsoletus*, 18 *C. scoticus*, 4 *C. dewulfi* and 1 *C. chiopterus*.

Species and Physiological Status		**Total *Culicoides* Collected**				
		Pure Breed	**Cross Breed**	**Un-baited**	**Light Trap**	**Total**
C. obsoletus/scoticus	Un-pigmented	45 (60.8%)	7 (50%)	3 (42.8%)	620 (59.6%)	675
	Pigmented	19 (25.7%)	3 (21.4%)	1 (14.3%)	306 (29.4%)	329
	Blood-fed	5 (6.8%)	0	1 (14.3%)	14 (1.3%)	20
	Gravid	1 (1.3%)	0	0	76 (7.3%)	77
	Damaged	1 (1.3%)	0	0	0	1
	C. obsoletus Male	3 (4%)	4 (28.6%)	2 (28.6%)	7 (0.7%)	16
	C. scoticus Male	0	0	0	18 (1.7%)	18
	Total	**74**	**14**	**7**	**1,041**	**1,136**
C. dewulfi	Un-pigmented	0	0	0	22 (42.3%)	22
	Pigmented	1 (50%)	0	0	17 (32.7%)	18
	Blood-fed	0	0	0	0	0
	Gravid	1 (50%)	0	0	9 (17.3%)	10
	Male	0	0	0	4 (7.7%)	4
	Total	**2**	**0**	**0**	**52**	**54**
C. chiopterus	Un-pigmented	0	0	0	3 (11.5%)	3
	Pigmented	21 (100%)	2 (66.7%)	1 (100%)	19 (73.1%)	43
	Blood-fed	0	0	0	0	0
	Gravid	0	1 (33.3%)	0	3 (11.5%)	4
	Male	0	0	0	1 (3.8%)	1
	Total	**21**	**3**	**1**	**26**	**51**
Total *Culicoides*		**97**	**17**	**8**	**1,119**	**1,241**

Table 4.10. Collections of *Culicoides* from sheep odour traps showing totals and life stage per species

Two models were generated to explain the results of the effect of whole host odour on *Culicoides* collections: one examining total *Culicoides* caught and a second for *C. obsoletus* group females. Both models were found to be significantly dependent on trap ($p<0.05$) with no other variables having a significant impact (Table 4.11).

Parameter	**Total *Culicoides***	***C. obsoletus* group Females**
Intercept	0.998	0.619
Trap		
Light Trap 1	3.88***	4.118***
Light Trap 2	2.107**	2.354***
Pure Breed	1.381*	1.621*
Cross Breed	Baseline	Baseline
Un-baited Trap	-1.634*	-1.66*

Table 4.11 Regression co-efficients included in final models to describe collections of Total *Culicoides* and *C. obsoletus* group females (*=p<0.05, **=p<0.01, ***=p<0.001)

Tukey's analysis revealed significant differences ($p<0.005$) between the traps for both models (Table 4.12)). In the Total *Culicoides* model, when the light trap was at location 1 it collected significantly greater numbers compared to collections made at location 2, the sheep odour traps and the un-baited trap. When the light trap was at position 2 it also collected significantly more *Culicoides* than the cross breed and the un-baited trap, but was not significantly different to the pure breed. Between the two sheep breeds there is no significant difference but the pure breed collected significantly greater numbers than the un-baited trap ($p<0.001$) in contrast to the cross breed which did not.

Treatment	Light Trap 2	Pure Breed	Cross Breed	Un-baited
Light Trap 1	-1.773*	-2.499***	-3.880***	-5.515***
Light Trap 2	-	-0.726	-2.107*	-3.742***
Pure		-	-1.381	-3.015***
Cross			-	-1.634

Table 4.12. Analysis of differences between traps for Total *Culicoides* model, estimates are treatments on the top line relative to treatments on the left. (*=p<0.05, **=p<0.01, ***=p<0.001)

In the *C. obsoletus* group females model a similar pattern was observed (Table 4.12). When the light trap was at location 1 it collected more than all other traps ($p<0.05$) while when the light trap was at location 2 it still collected more than the cross breed and the un-baited trap but was not significantly different to the pure breed. No significant difference was observed between the two breeds but the pure breed collected significantly more than the un-baited trap ($p<0.001$) while the cross breed and un-baited trap did not differ significantly (Table 4.13).

Treatment	Light Trap 2	Pure Breed	Cross Breed	Un-baited
Light Trap 1	-1.764*	-2.496***	-4.118***	-5.579***
Light Trap 2	-	-0.732	-2.354	-4.014***
Pure		-	-1.622	-3.282***
Cross			-	-1.660

Table 4.13. Differences between traps for Total *C. obsoletus* group females, estimates are treatments on the top line relative to treatments on the left. (*=p<0.05, **=p<0.01, ***=p<0.001)

The collections of *Culicoides* in the sheep odour traps and UV light-suction traps were dominated by females of the *C. obsoletus* group. The majority of *Culicoides* were collected in the UV light-suction traps and analysis looking at total *Culicoides* and total *C. obsoletus* group females showed that light trap 1 collected

significantly more than all of the other traps. No differences were found in the responses of *Culicoides* to the odours of the two sheep breeds.

4.2.3 Trial 3 – The response of *Culicoides* to host derived semiochemicals

Three rotations were completed for the trial resulting in 24 nights of trapping and 192 collections. A total of 5,704 *Culicoides* were collected of which 98% were collected in the UV light-suction trap. Of the semiochemical baited traps, R-octenol collected the greatest number of *Culicoides* (63), followed by the blended semiochemicals (29) while chemical A failed to catch any *Culicoides* and CO_2, chemical B and chemical C each collected a single specimen. The results are summarised in Table 4.14 (an additional 8 individuals belonging to 2 species, *C. achrayi* and *C. festivipennis*, were collected in the UV light-suction trap that are not shown in the table).

Treatment	Total *Culicoides* caught (Mean ±SEM)						Total
	***C. obsoletus* group Females**	***C. obsoletus* group Males**	***C. pulicaris* Females**	***C. pulicaris* Males**	***C. punctatus* Females**	***C. punctatus* Males**	
Light trap	4,957 (207±146)	60 (2.5±1.99)	428 (17.83±8.92)	13 (0.54±0.25)	132 (5.5±2.5)	11 (0.46±0.16)	5,601
Un-baited	0	0	0	0	0	0	0
Chemical A	0	0	0	0	0	0	0
Chemical B	1	0	0	0	0	0	1
Chemical C	1	0	0	0	0	0	1
R-octenol	63 (2.63±2.24)	0	0	0	0	0	63
CO_2	1	0	0	0	0	0	1
Blend	29 (1.2±0.5)	0	0	0	0	0	29
Total	**5,052**	**60**	**428**	**13**	**132**	**11**	**5,696**

Table 4.14. *Culicoides* collected in miniature CDC suction traps baited with a range of putative semiochemicals

The semiochemical-baited traps collected only *C. obsoletus* group species while the UV light-suction trap collected a greater diversity of species that included *C. pulicaris* and *C. punctatus* along with a small number of *C. festivipennis* and *C. achrayi*. The largest collection was made using the UV light-suction trap (3,787 *Culicoides*), while the largest semiochemical trap collection was 54 in the R-octenol baited trap. Both of these trap collections were made on the same evening. Semiochemical baited traps predominantly collected un-pigmented and pigmented host–seeking *Culicoides*, with the exception of 1 gravid female, *C. obsoletus/scoticus* complex in the R-octenol trap, whereas the UV light-suction trap collected 35 gravid and 23 blood fed individuals. The low numbers of *C. obsoletus* group females meant that analysis would not be possible at species level. For the purpose of illustrating what species were responding to the semiochemical-baited traps females from these collections were identified to *C. obsoletus/scoticus* complex and *C. chiopterus* based on morphological characteristics (Table 4.15), no *C. dewulfi* were identified. No species identification of females beyond *C. obsoletus* group level was made on collections from UV light-suction traps. *C. obsoletus* group females were separated by physiological state and found to comprise 71.2% un-pigmented, 27.6% pigmented, 0.7% gravid and 0.5% blood fed. Males were only collected in the UV light-suction trap and included *C. obsoletus* (3); *C. scoticus* (54)*;* and *C. dewulfi* (3).

		Total *Culicoides* Collected					
Species	**Life Stage**	**Chemical B**	**Chemical C**	**R-octenol**	**CO_2**	**Blend**	**Total**
C. obsoletus/scoticus	Un-pigmented	0	1 (100%)	34 (54%)	1 (100%)	16 (57.1%)	52
	Pigmented	0	0	28 (44.4%)	0	12 (42.9%)	40
	Blood-fed	0	0	0	0	0	0
	Gravid	0	0	1 (1.6%)	0	0	1
	Male	0	0	0	0	0	0
	Total	**0**	**1**	**63**	**1**	**28**	**93**
C. chiopterus	Un-pigmented	0	0	0	0	0	0
	Pigmented	1 (100%)	0	0	0	1 (100%)	2
	Blood-fed	0	0	0	0	0	0
	Gravid	0	0	0	0	0	0
	Male	0	0	0	0	0	0
	Total	**1**	**0**	**0**	**0**	**1**	**2**
Total *Culicoides* collected		**1**	**1**	**63**	**1**	**29**	**95**

Table 4.15. *C. obsoletus* group females collected using semiochemical-baited traps

For the analysis the two traps which recorded zero *Culicoides* (un-baited and chemical A) were excluded. Two models were generated to explain trap collections of total *Culicoides* and total *C. obsoletus* group females.

Parameter	**Total *Culicoides***	***C. obsoletus* group Females**
Intercept	-13.777***	-13.924***
Temporal Variables		
Linear	0.64***	0.664***
Quadratic	-0.016***	-0.017***
Trap		
Light trap	5.114***	4.965***
Chemical B	-3.286**	-3.28**
Chemical C	-3.338**	-3.339**
R-octenol	-0.171	-0.179
CO_2	-3.732**	-3.747**
Blend	Baseline	Baseline
Temperature	0.648***	0.645**

Table 4.16. Regression coefficients for final models to describe total *Culicoides* and total *C. obsoletus* group Females collected in semiochemical baited traps (*=p<0.05, **=p<0.01, ***=p<0.001)

In both models collections were shown to depend significantly on traps ($p<0.01$), temporal variables and air temperature; in both cases trap location was not significant and so was excluded from the models (Table 4.16). Significant differences between treatments were also found in both models (Table 4.17 and Table 4.18). The UV light-suction trap collected significantly greater numbers of *Culicoides* and female *C. obsoletus* group than all other traps ($p<0.001$). Among semiochemical-baited traps the blend collected significantly higher numbers than CO_2, chemical B and chemical C in both models ($p<0.05$); no other significant differences were observed.

Treatment	Chemical B	Chemical C	R-octenol	CO_2	Blend
Light Trap	-8.400***	-8.543***	-5.286***	-8.847***	-5.114***
Chemical B	-	0.052	3.114	-0.446	3.286*
Chemical C		-	3.166	-0.394	3.338*
R-octenol			-	-3.560	0.181
CO_2				-	3.732*

Table 4.17. Analysis of differences between traps, estimates for Total *Culicoides*, estimates are treatments on the top line relative to treatments on the left. (*=p<0.05, **=p<0.01, ***=p<0.001)

Treatment	Chemical B	Chemical C	R-octenol	CO_2	Blend
Light Trap	-8.246***	-8.304***	-5.144***	-8.713***	-4.965***
Chemical B	-	-0.058	3.568	-0.467	3.280*
Chemical C		-	3.159	-0.408	3.280*
R-octenol			-	-3.568	0.179
CO_2				-	3.747*

Table 4.18. Analysis of differences between traps, estimates for total *C. obsoletus* females, estimates are treatments on the top line relative to treatments on the left. (*=p<0.05, **=p<0.01, ***=p<0.001)

The vast majority of *Culicoides* were collected in the UV light-suction trap, of the novel chemicals under investigation chemical A failed to collect any *Culicoides* and chemicals B and C each collected one individual. For the analysis two models were generated, one to explain total *Culicoides* and one to explain total *C. obsoletus* goup females, both demonstrated that the UV light-suction trap collected significantly more than the semiochemical baited traps. Of the semiochemicals the blend was found to collect significantly more than chemicals B and C but there was no significant difference to the R-octenol collections.

4.3 Discussion

The results of this chapter contribute to a clearer understanding of the host location of livestock associated *Culicoides* in the UK and in a wider sense represent an attempt to isolate and test novel chemicals used in host location for this group. Initially, responses to a range of CO_2 release rates were characterised at a livestock farm that had been used in previous studies of *Culicoides* host location (Harrup *et al.* 2012), biting rate (Carpenter *et al.* 2008c) and diel periodicity (Sanders *et al.* 2012). This study demonstrated a significant preference for CO_2 release rates of 1,500 ml/min for *C. nubeculosus*, a common and widespread farm species in northern Europe (Boorman 1986). While the abundance of the *C. obsoletus* group as a whole was low at the site, collection of *C. obsoletus* males at CO_2 baited traps also indicates the possibility of host-associated mating, with males responding to host kairomones in order to encounter females, to date this has only been reported anecdotally (Downes 1954).

At a second site, the impact of semiochemicals on host location when other visual or thermal cues were excluded was attempted through the use of an air entrainment unit. Against a relatively high background *Culicoides* population density it was demonstrated that while individuals of several species could be collected through the use of a vented air stream from the sheep hosts, these catches represented a very small fraction of the total available population. In addition, for the first time in arthropod vectors, semiochemical emissions from two separate breeds of sheep were compared in their attraction to *Culicoides* using this method and found not to differ significantly. Finally, a series of novel compounds isolated from sheep were tested as possible attractants for livestock associated *Culicoides*. Of these compounds, more

Culicoides were attracted to R-octenol and a blend of kairomones than to any of the novel chemicals tested individually.

Through modelling of trap collections, *C. nubeculosus* was demonstrated to exhibit a dose dependent response to CO_2. The optimal release rate for *C. nubeculosus* collections was 1,500 ml/min, resulting in significantly higher collections than 500 ml/min or 1,000 ml/min and the UV light-suction trap ($p<0.05$), increasing the release rate beyond 1,500 ml/min did not yield significantly different results suggesting that there is a plateau in response at higher concentrations. A key advance in the analysis of the data was the assessment of site specific and meteorological parameters in contrast to previous studies that had only compared total collections using analysis of trap collection variance. It is clear that if this type of analysis had been applied to the dataset, very different results would have been generated and this may have previously led to the generation of potentially erroneous optimal release rates for the collection of other *Culicoides* species (the results of an ANOVA analysis where no significant differences were found between treatments are shown in Appendix 2, table APP2.2).

Previously, two studies have been carried out that systematically examined attraction of *Culicoides* to CO_2 baited traps using a series of release rates (Kline *et al.* 1994, Mullens 1995). In both *C. furens* and *C. sonorensis* it was found that the number of individuals collected was positively correlated with release rate to maximum exposures of 2,000 ml/min in *C. furens* and 3,000 ml/min in *C. sonorensis*. This was considered to represent a simple effect of trapping range whereby increasing CO_2 release extended the range of the trap through increased dispersal of the kairomone (Mullens 1995). The finding in the current study that release rates above 1,500 ml/min do not significantly increase catches may imply that

the primary emergence and resting sites of *C. nubeculosus* were already within range of the bait, or that there may be inhibition in flight towards very high concentrations of CO_2.

A notable feature of the study was the dominance of *C. nubeculosus* in the field collections, which had been recorded during a previous study at the same farm holding in 2008 (Harrup *et al.* 2012). While demonstrating the same high proportion of *C. nubeculosus* in trap catches, the previous study used a fixed site for the light-suction trap to monitor background populations with the result that the small number of other livestock associated *Culicoides* could have been overlooked due to local scale variation in incidence. In the current trial, however, the light trap was included in the rotation of semiochemical-baited traps and broadly reflected the abundance of *C. nubeculosus* in the CO_2 baited traps implying a true low abundance of other common livestock-associated species at the sites used. Despite large-scale surveys of *Culicoides* populations being conducted across northern Europe (see Chapter 1), *C. nubeculosus* has to date not been found to dominate any trapping site to the degree found in this study. This may in part reflect intra-farm differences in *Culicoides* abundance that are not captured by standardised trapping measures, as postulated in other studies (Kirkeby *et al.* 2013a, Kirkeby *et al.* 2013b), the abundance of *C. nubeculosus* only within a short range of discrete breeding habitats, or, alternatively, a general underestimation of *C. nubeculosus* populations on farms due to a poor response for the UV light bait.

The collection of male *Culicoides* in CO_2 baited traps has been suggested to be indicative of either host-associated mating or the use of traps as flight markers in studies of *C. sonorensis* in the USA (Mullens 1995). In the current study these factors are also difficult to separate due to the much smaller numbers of male

Culicoides collected. In *C. nubeculosus*, mating on the host has already been recorded anecdotally in the field (Downes 1954) and although this behaviour has not been confirmed directly in *C. obsoletus* this study provides preliminary evidence that attraction to the host may enable effective mate location in this species.

The second and third sections of this chapter were carried out at a separate field site that was more representative of UK livestock holdings than the first in background populations of *Culicoides*, being dominated by the *C. obsoletus* group (Boorman 1986). The second trial examined the response of *Culicoides* species to natural whole host odour that was largely isolated from thermal and visual cues. Somewhat surprisingly, this experimental design had not previously been used for *Culicoides* despite a similar design forming the basis of successful studies of tsetse fly host location in Zimbabwe (Vale 1974). The collections of *Culicoides* in the odour-baited suction traps were significantly different to the numbers of *Culicoides* intercepted by a passive suction trap. The number of *Culicoides* collected, however, appeared very limited in comparison to collections made using drop traps (discussed in Chapter 3 and conducted within 10m of the collection site) and a UV-baited light-suction trap, one possibility is that the flock of sheep held for the drop trap experiment out-competed the odour baited trap due to their relatively close proximity.

The lack of difference in collections between the two different breeds is perhaps not surprising given that they are closely related, one being pure Hartline while the other was Hartline/Suffolk cross, although significant differences were found during the direct collections from the breeds. Slight differences were found in the odour profiles of the two breeds from the entrainments (J. Cook, personal communication) with differences in concentrations of four chemicals released.

Whether such differences would be detectable to *Culicoides* in the current set-up is unclear and this was not assessed during the trial.

There are several potential inter-related explanations for the limited numbers of *Culicoides* collected in the odour-baited traps. Firstly, it is unlikely that the semiochemical profile released the trap attached to the air entrainment apparatus is accurately representative of that emitted from the three sheep. While the content of the odour stream from the entrainment box was broadly representative of that produced from the sheep themselves, the release rate from the entrainment box, containing the three sheep, was not controlled and CO_2 was not added to the bait (to supplement that already present in the emissions). This is likely to have resulted in significant changes in the relative proportions of semiochemical constituents during the venting process. While the ultimate impact of this process was not monitored (due primarily to the fact that semiochemical monitoring was limited to entrainments of several hours), it is likely to have led to a concentration of odours of high volatility at the single point of release. Given that inhibitory effects on host location have been recorded using super-normal concentrations of semiochemicals, such as octenol, in laboratory-based behavioural studies (Bhasin *et al.* 2000a), it is possible that these may have inhibited trap catches in the field. One other possibility for the low levels of *Culicoides* collected is that the odour is released relatively close to the trap fan which is designed to suck insects downward and this suction may have a negative impact on the dispersal of odour from the trap. In addition, the low abundance of *Culicoides* collected at the odour baits may also be due to the lack of close range landing cues such as visual and thermal stimuli which would normally be part of the host location process. Little is known about close range cues for *Culicoides* but heat is likely to be important (Kline and Lemire 1995). It has been

demonstrated that the addition of heat to CO_2 and octenol can give a significant increase in *C. furens* collections (Kline and Lemire 1995), although even in the absence of heat this species was collected in high numbers in a CO_2 baited trap.

While the response to the whole sheep odour was limited, the attempt to produce a novel system for isolating host odours from visual and heat cues showed promise. With modifications to the experimental set-up including more accurate recording of chemicals released and the potential for adding supplementary CO_2 there is potential to improve the collections of *Culicoides* and create a truly representative bait that can then be screened for further semiochemicals involved in host location. This potential was demonstrated by the final study of this chapter which involved the use of three chemicals identified during screening of entrainments from the equipment. Interestingly, while the blend of the three novel putative attractants with R-octenol led to increased catches of *Culicoides*, the individual components did not elicit a significant response. The study did not confirm statistically the relative attraction of the *C. obsoletus* group to R-octenol, which had previously been demonstrated to collect significantly higher numbers of *C. obsoletus* group females than CO_2 baits alone (Harrup *et al.* 2012). A current deficiency of the entrainment system used is a lack of control in both assessing the comparability of semiochemicals at the point of the release, those released in the box itself and those emitted under natural conditions. These will require substantial standardisation before attaining the accuracy required for screening chemicals systematically, but the system does have the advantage of both being flexible with regard to host used and in conforming to UK Home Office guidelines for animal use.

A surprising finding from the analyses of the three trials is that most meteorological variables were not found to significantly influence trap collections

despite the importance demonstrated in other studies (Carpenter *et al.* 2008c, Baylis *et al.* 2010, Sanders *et al.* 2011, Harrup *et al.* 2012). The CO_2 dose-response study models included wind speed and the trial of novel semiochemicals was significantly influenced by temperature but no effect was shown in the sheep odour study. Wind in particular would have been expected to be important as this would have a key impact on the dispersal of semiochemicals (Murlis *et al.* 1992).

The work presented in this chapter is a significant advance in our knowledge of the response of farm associated *Culicoides* species to semiochemical cues. It has been demonstrated that *C. nubeculosus* exhibits a dose-response to CO_2 and is collected in significantly higher numbers with this kairomone than in UV light-suction traps. This discovery and the possibility that the abundance of other *Culicoides* species may be similarly misinterpreted from UV light-suction trapping has important implications for surveillance that is based purely on this method. The data presented for *C. obsoletus* group females shows that this group responds significantly to the odour of their hosts, while numbers were low this was the first time that such a study had been done for *Culicoides* and provides a basis for development in future work. The responses of *C. obsoletus* group females to individual semiochemicals was also low but was shown to be significantly higher when chemicals were presented as a blend providing encouraging results for future investigations.

Chapter 5: The Response of Livestock-Associated *Culicoides* to Wavelengths of Light-Emitting Diode Baited Light-Suction Traps

5.1 Introduction

Light traps have a long history of use in surveillance of crepuscular or nocturnally active phototactic populations of insects (Southwood and Henderson 2000). This popularity of use stems from the commercial availability of standardised traps that can be deployed with minimal logistical considerations under a wide range of environments and across wide geographic areas (Silver 2008). The first widely used standardised trap for vector populations of Diptera was the New Jersey trap which was developed in the late 1920s and used an incandescent light bait (Mulhern 1985). This model was then in part superseded by the less cumbersome miniature Centre for Disease Control (CDC) light-suction trap, which has been used since the 1960's with both incandescent and ultraviolet (UV) light baits (Sudia and Chamberlain 1962).

While the CDC light-suction trap is used in Spain to routinely monitor *Culicoides* populations (Calvete *et al.* 2006), the Onderstepoort Veterinary Institute (OVI) light-suction trap is the most commonly used method of sampling populations in the Palaearctic region. This light-suction trap, which was originally produced from a Russian design (R. Meiswinkel, personal communication.), uses an 8w UV tube as bait (significantly more powerful than the 4w tube used in the UV CDC trap) and is more suited to permanent site operation where mains electricity is available. Major

surveillance schemes using this trap have been maintained for many years in Italy (De Liberato *et al.* 2003, Goffredo and Meiswinkel 2004) and more recently in France (Venail *et al.* 2012). 'Snapshot' countrywide surveys have also been completed through use of the OVI light-suction trap in the Netherlands (Meiswinkel *et al.* 2008), Belgium (De Deken *et al.* 2008), Switzerland (Cagienard *et al.* 2006), Bulgaria (Purse *et al.* 2006), Greece (Patakakis *et al.* 2009) and many other European countries.

It has long been known that insects vary in their response to light according to the specific spectrum of wavelengths emitted (Silver 2008). Most simply, this has been observed in an increasing use in vector surveillance of UV baited traps (operating in the 320-420 nm range) over incandescent baits (operating over a wider variable spectrum) although sensitivity appears to vary widely according to species. The majority of insects are trichromats, possessing compound eyes with colour receptors that are sensitive to UV, blue and green wavelengths (Briscoe and Chittka 2001). Spectral sensitivity of these eyes to specific wavelengths has been investigated in the laboratory using the electroretinogram technique that relies on extracellular recording of a neural signal in response to exposure to colours of light. Families of Dipteran vectors investigated to date include the Culicidae, Glossinidae and Psychodidae, all of which have shown peaks in sensitivity in the ultraviolet (UV) and blue/green range as determined from electroretinograms (Green and Cosens 1983, Muir *et al.* 1992, Mellor *et al.* 1996). While these studies demonstrate that insects are able to distinguish between different wavelengths, with the exception of *Lutzomyia longipalpis*, the other species investigated are diurnally active making it difficult to interpret the results as they would typically not respond to light traps in the field.

While evaluation of different colours of light in their attraction has historically been the focus for a number of studies, considerable difficulties have been experienced in standardisation with the use of widely varying intensities, wavelengths and types of light source (Bargren and Nibley 1956, Breyev 1963, Gjullin *et al.* 1973, Ali *et al.* 1984). The recent commercial development of super-bright light emitting diodes (LEDs) as a source of light has partially addressed this issue by providing greater specificity in the wavelength and intensity of light used as bait (Cohnstaedt *et al.* 2008). A major advantage over traditional light baits also exist in the reduced power consumption of LEDs when compared with standard incandescent and UV light sources, a key logistical factor in trapping where mains electricity for charging batteries is limited in supply (Bishop *et al.* 2004b).

Miniature CDC light-suction traps baited with coloured LEDs were initially trialled for collection of Culicidae in Florida, using a Latin square design (Burkett *et al.* 1998). Catches of mosquitoes were compared to standard unlit and incandescent miniature CDC light-suction traps with or without supplementary CO_2. This experimental design allowed the assessment of whether single LED baits could be used to replace the more logistically challenging use of CO_2. Across trials of red (613 ±50nm), orange (605 ±50nm), yellow (587 ±50nm), green (567 ±50nm), blue (450 ±50nm) and infra-red (IR) (940 ±50nm) LED baits, results were inconsistent due in part to the limited number of nights used for trapping (6-8 days for each of the trials). The study did demonstrate, however, species specific differences in collections, the most convincing being the greater attraction of *Anopheles crucians* for white light over all three trials when compared with more specific LED wavelengths (Burkett *et al.* 1998). The addition of CO_2 led to trap collections that were in general more than ten times larger and had a greater diversity of species (18 vs 13 in the first two trials).

Light emitting diodes have also been assessed as bait in surveillance systems for adult Psychodidae in two separate studies (Hoel *et al.* 2007, Mann *et al.* 2009). In Egypt, a study was carried out using modified CDC light-suction traps and blue (470 ±30nm), green (502 ±25nm), and red (660 ±30nm) LED baits (Hoel *et al.* 2007). Unlike the previous study on mosquitoes, four LEDs were attached to each trap, taking account of the directional nature of the light produced (in contrast to standard incandescent light). One kilogram of dry ice was placed in each trap to generate CO_2 as additional bait, but release rate was not assessed in the trial. A control CDC trap with incandescent bait was also included in the trial and a total of twelve nights of sampling were conducted over three months. Samples were dominated by *Phlebotomus papatasi* comprising >94% of the trap catch and this species appeared to be significantly attracted to red light with >55% collected in this treatment.

While surprising in light of the paradigm that sensitivity for vectors was within the blue-green-UV range, these results were then partially confirmed with other sandfly species in Florida (Mann *et al.* 2009). The study used the same LED wavelengths as in Egypt, but in this case three LEDs were fitted to commercial Mosquito Magnet X-MM-X traps. In addition, a blue-green-red combination with nine LEDs was also used as an additional treatment. Carbon dioxide was released from each trap via a cylinder at 500 ml/min and collections were made for twenty four hours rather than between dusk and dawn as in the study in Egypt. In 108 nights of trapping, 2613 sandflies were collected of two species, *Lutzomyia shannoni* (77%) and *Lu. vexator* (23%). While no statistically significant differences were detected across treatments, the trap baited with the red light collected the highest number of *Lu. shannoni*, while the blue-green-red baited trap collected the most *Lu. vexator*. Subsequently, the authors tested a series of combinations of the red LED baits with

semiochemicals and concluded that inclusion of ‘red mixture’ (a combination of octenol and 1-hexen-3-ol), with the CO_2 led to an additive effect (Mann *et al.* 2009).

A key issue in assessment of these studies was the increasing understanding that to achieve full 360° of light around cylindrical trap entry required an octagonal arrangement of eight units with the standard 45° visibility of LEDs (Cohnstaedt *et al.* 2008). This arrangement was subsequently patented and commercially developed by Bioquip Inc. (USA), using a design based on the original CDC light-suction trap but with a far lower overall weight and taking advantage of lower power consumption. These traps were initially compared to a standard incandescent baited CDC light-suction trap in Kenya (Tchouassi *et al.* 2012). Light emitting diode baits of UV (390 nm), blue (430 nm), green (570 nm) and red (660 nm) were used, in addition to a combination bait with three green, three blue and two red LEDs. A total of forty-two trap nights were carried out in 2010 and 2011 under both low and high abundance periods of mosquito activity, although the randomisation procedure for trap placement during the trial was unclear, which could have resulted in bias in analysis (Tchouassi *et al.* 2012). Throughout both low and high abundance periods of the trial, the standard incandescent CDC light-suction trap collected consistently higher numbers of mosquitoes, although significant differences were not apparent in the vast majority of comparisons due to the relatively small number of days trapped.

The use of LEDs as bait in light-suction traps for *Culicoides* was implemented at an early stage of their development in Australia during 2002-3. Spatial and temporal changes in the *Culicoides* fauna of arbovirus epidemic areas of Australia have been monitored since 1975, initially using incandescent light-suction traps as part of the National Arbovirus Monitoring Program (NAMP) (Kirkland *et al.* 1996). In certain areas, however, Akabane virus had been detected in sentinel cattle

in the absence of *C. brevitarsis* Kieffer the principle vector in this region (Bishop *et al.* 2004a). This led to a hypothesis that *C. brevitarsis* was under-represented by incandescent light-suction trap collections and that different wavelengths from light emitting diodes might collect different abundances of this species (Bishop *et al.* 2004b). The study used LEDs of red (640 nm), yellow (595 nm), green (520 nm), blue (475 nm) and white (460/570nm) in comparison to a standard incandescent light-suction trap. Uniquely for these studies, the intensity of the incandescent and LED baits were assessed in a chamber using a quantum sensor and light diffusers were additionally integrated into the traps to diffuse the light produced. Three LEDs were mounted on each trap and no additional semiochemical baits were used during the trial. At the two locations used during the trial, the green LED collected significantly higher numbers of *C. brevitarsis* than the standard incandescent control. A number of other species were also collected in significantly higher numbers using the green LED and *C. austropalpalis* Lee & Reye, *C. bunrooiensis* Lee & Reye, *C dycei* Lee & Reye and *C. marksi* Lee & Reye were significantly more abundant in blue LED traps. Yellow and red traps either did not differ significantly from the incandescent collections or collected significantly fewer individuals, depending on species (Bishop *et al.* 2004b).

In a follow-up study at the same two sites in 2004, the red and white LEDs were replaced with a UV LED in the experimental design (Bishop *et al.* 2006). Those *Culicoides* species that had shown the greatest response to blue light in the previous experiment now demonstrated a significant preferential response to the UV LED (namely *C. marksi*, *C. austropalpalis*, *C. bunrooensis* and *C. dycei*). Specific comparisons were also made between green LEDs (520 nm) and standard incandescent traps at sites in New South Wales, Northern Territory and East Timor

and it was found that with the exception of two species, caught in low numbers, the green LEDs consistently collected higher numbers of *Culicoides* including for those that had shown preference for UV light. The green LED also collected five rarer species that were not found in incandescent collections, albeit in small numbers. As a result of these findings, and the fact that LED-based traps consume less power making trapping more logistically straightforward, the Australian National Arbovirus Monitoring Program employs green LED traps for monitoring *C. brevitarsis* in low density areas (Bishop *et al.* 2006).

The attraction of northern Palaearctic *Culicoides* species to different wavelengths of light has not been assessed. Following the commercial development of standardised LED-based traps, an assessment of their utility in a study of the species present in this region is required, as current surveillance is entirely based on UV-baited light-suction traps. This reliance on a highly specific wavelength of light has the potential to significantly distort both the abundance and diversity of species in areas inferred as containing *Culicoides* vectors of arboviruses. In addition, the logistical flexibility of lightweight LED-baited traps has the potential to make studies of *Culicoides* in the field far more straightforward to perform, in particular in defining intra-farm assessments of population density. In this chapter a comparison is therefore made between LED wavelengths in attraction for *Culicoides* using standardised traps.

5.2 Materials and Methods

5.2.1 Study Site

The trial was conducted from May to September 2011 at a small farm holding in Surrey (see description of Field Site 3 in Chapter 2). The site comprised a large field (140m x 120m) subdivided into smaller grazing enclosures that in total accommodated four horses and two pigs. Two sides of the site were surrounded by deciduous woodland and two sides bordered further grazing land used for horses.

5.2.2 Trap Treatments

The response of *Culicoides* species to different wavelengths of light was assessed using commercially available light-suction traps (Model 2770, Bioquip Inc., USA) fitted with LED platforms consisting of 8 individual LEDs emitting at different wavelengths (Cohnstaedt *et al.* 2008). Six different colours of LED were used (Figure 5.1): ultra-violet (390 nm); Blue (430 nm); Green (570 nm); Yellow (590 nm); Red (660 nm) and White (425 nm – 750 nm with peaks at 450 nm and 580 nm), an additional standard CDC light trap (320-420 nm) (Model 912, J. W. Hock, USA) fitted with a 4w UV tube was used as a positive control. Traps were hung at a height of 1.5m *Culicoides* attracted to the light traps were collected into beakers containing 200ml of water and transferred to 70% ethanol for storage following collection.

Figure 5.1. LED light sources used during investigation of differential attraction to wavelengths of light (UV, Blue, Green, Yellow, Red and White)

Collections were made overnight with traps operating from late afternoon until the following morning in order to encompass the sunset and sunrise peaks in UK *Culicoides* activity (Hill 1947, Carpenter *et al.* 2008c, Sanders *et al.* 2012). On night one the trap treatments were randomly assigned to locations and on subsequent nights the treatments were rotated to the next location in a clockwise direction. Trap

locations were at least 50 metres apart to eliminate the risk of interference between treatments (Figure 5.2). After seven nights of trapping the treatments were again re-randomised to trap locations for the start of the next rotation, a total of seven rotations were completed giving 49 nights of data collection. Meteorological data were collected throughout the sampling period using a weather station as described in Chapter 2.

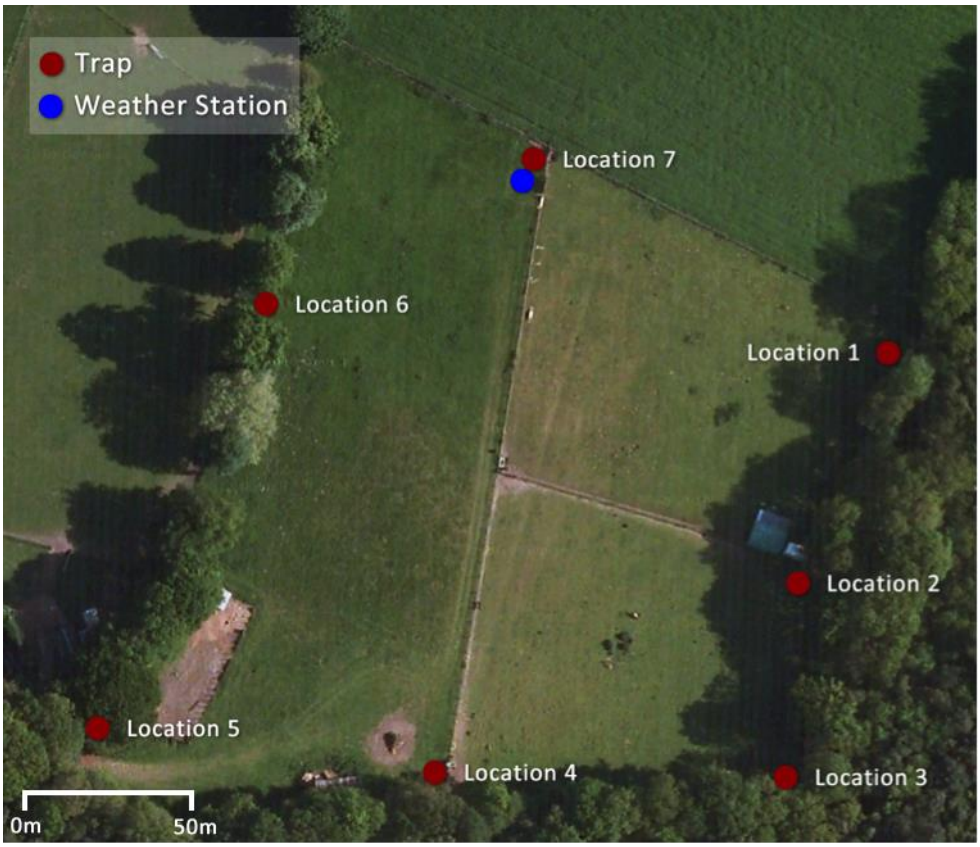

Figure 5.2. Map of field site for trial to investigate differential attraction to wavelengths of light

5.2.3 Sample Identification

Culicoides collected were initially identified by morphological characteristics (see Chapter 2). While *C. chiopterus* was identified by morphology (pale wings and small size), the other members of the *C. obsoletus* group females were identified as

sub-samples by multiplex PCR (see Chapter 2). For each trap treatment, two nights were randomly selected from each seven night rotation and all *C. obsoletus*/*C. scoticus*/*C.dewulfi* females within the trap catch were identified by PCR to species level. The PCR results were then combined and for each seven night rotation the proportions of each species and physiological states within each species were applied to the collections from the remaining five nights of the rotation. If collections failed to amplify then another night was randomly selected for analysis and failed samples were excluded from final estimates.

5.2.4 Analysis

Where *Culicoides* numbers for each species or physiological group were sufficient for analyses, data were analysed using negative binomial generalised models (GLM) in R version 2.15.2 (R Core Team 2013) as described in Chapter 2. The effects of individual factors in the final model were examined using Tukey's honest significant differences to identify significant differences ($p<0.05$) between factor levels. Final models with parameter estimates and 95% confidence intervals and model scripts are presented in Appendix 3.

5.3 Results

Sampling was conducted over 49 nights to give a total of 329 successful collections after the exclusion of 14 trap failures due to mechanical breakdown of trap fans, failure of LEDs and, on one occasion, battery failure. A total of 42,696 *Culicoides* were collected, the majority of which were females of *C. obsoletus*, *C. scoticus* and *C. dewulfi*, accounting for 37,367 (87.5%) of the trap collections (Table 5.1). Other species collected, in order of abundance, were *C. brunnicans* (4.9%); *C. pulicaris* (2.1%); *C. punctatus* (1.0%); and *C. impunctatus* (0.8%) (Table 5.1). The remaining 3.7% of individuals constituted rarer species including *C. achrayi*, *C. festivipennis, C. pictipennis, C. nubeculosus* and *C. chiopterus*.

A total of 9,918 female individuals identified morphologically as *C. obsoletus, C. scoticus* or *C. dewulfi* were subjected to molecular identification by multiplex PCR, of which 88.9% were successfully identified and 11.1% failed due to poor DNA extraction. Of the 8,853 individuals successfully identified, 5,862 (66.2%) were *C. obsoletus*, 2,789 (31.5%) were *C. scoticus* and 202 (2.3%) were *C. dewulfi*. The majority of *C. obsoletus* processed were un-pigmented (3,643; 62.1%), with fewer pigmented (1,880; 32.1%), gravid (239; 4.1%) and blood-fed (101; 1.7%) individuals. In *C. scoticus*, an almost equal number of un-pigmented (1,365; 48.9%) and pigmented (1,391; 49.9%) individuals were processed, with few gravid (25; 0.9%) and blood-fed (9; 0.3%) females. Of the relatively small numbers of *C. dewulfi* identified, numbers of un-pigmented individuals (85; 42.1%) were less than pigmented (98; 48.5%) with few gravid (14; 6.9%) and blood-fed (5; 2.5%) females. Estimated total numbers were calculated following subsampling of populations

(Table 5.2). Collections of female *C. pulicaris* and *C. brunnicans* were also identified to physiological status (Table 5.3).

Culicoides **Species**	**Total *Culicoides* collected (Mean ±SEM)**							
	CDC (n=48)	**UV (n=47)**	**Blue (n=46)**	**Green (n=49)**	**Yellow (n=48)**	**Red (n=45)**	**White (n=46)**	**Total (n=329)**
C. obsoletus; C. scoticus; C. dewulfi	20,569 (429 ±110)	3,077 (65.5 ±18.6)	3,515 (76.4 ±24.1)	3,965 (80.9 ±17.3)	2,810 (58.5 ±25.0)	122 (2.7 ±0.6)	3,379 (73.5 ±23.0)	**37,437**
C. pulicaris	389 (8.1 ±2.8)	49 (1.0 ±0.3)	119 (2.6 ±0.9)	157 (3.2 ±1.0)	69 (1.4 ±0.5)	1 (0.02 ±0.0)	100 (2.2 ±0.7)	**884**
C. punctatus	210 (4.4 ±1.7)	20 (0.4 ±0.2)	55 (1.2 ±0.9)	77 (1.6 ±0.5)	31 (0.6 ±0.3)	0	13 (0.3±0.1)	**406**
C. impunctatus	93 (1.9 ±0.8)	54 (1.1 ±0.6)	91 (2.0 ±1.2)	72 (1.5 ±0.4)	16 (0.3 ±0.1)	2 (0.04 ±0.0)	16 (0.3 ±0.1)	**344**
C. brunnicans	264 (12.6 ±4.9)	103 (4.9 ±1.9)	542 (25.8 ±22.4)	744 (35.3 ±27.3)	213 (10.1 ±5.3)	19 (1.0 ±0.4)	186 (10.3 ±5.5)	**2,071**
Other Species	527	154	319	357	164	3	100	**1,624**
Total	**22,052**	**3,457**	**4,641**	**5,372**	**3,303**	**147**	**3,794**	**42,766**

Table 5.1. *Culicoides* collected using light emitting diode (LED) baited suction traps in the UK

Species	**Life Stage**	**Estimated total *Culicoides* collected**							
		CDC	**UV**	**Blue**	**Green**	**Yellow**	**Red**	**White**	**Total**
C. obsoletus	Un-pigmented	7,336 (69.4%)	1,259 (63.7%)	1,351 (56.4%)	1,628 (55.8%)	979 (57.1%)	24 (44.4%)	1,458 (66.1%)	14,035
	Pigmented	2,545 (24.1%)	604 (30.6%)	752 (31.4%)	814 (27.9%)	529 (30.9%)	21 (38.9%)	537 (24.3%)	5,802
	Blood-fed	92 (0.8%)	9 (0.5%)	44 (1.8%)	72 (2.5%)	46 (2.7%)	0	96 (4.4%)	359
	Gravid	219 (2.1%)	42 (2.1%)	153 (6.4%)	189 (6.5%)	123 (7.2%)	1 (1.9%)	27 (1.2%)	754
	Male	384 (3.6%)	62 (3.1%)	97 (4%)	209 (7.2%)	37 (2.1%)	8 (14.8%)	88 (4%)	885
	Total	**10,576**	**1,976**	**2,397**	**2,912**	**1,714**	**54**	**2,206**	**21,835**
C. scoticus	Un-pigmented	4,826 (50.3%)	489 (51.7%)	301 (44%)	316 (37.9%)	552 (60.5%)	28 (52.8%)	381 (54.5%)	6,893
	Pigmented	4,405 (45.9%)	425 (45%)	307 (45%)	441 (52.9%)	310 (34%)	21 (39.6%)	252 (36.1%)	6,161
	Blood-fed	3 (0.03%)	1 (0.1%)	7 (1%)	17 (2%)	4 (0.4%)	0	12 (1.7%)	44
	Gravid	96 (1%)	6 (0.6%)	28 (4.1%)	3 (0.4%)	9 (1%)	1 (1.9%)	30 (4.3%)	173
	Male	264 (2.8%)	24 (2.5%)	41 (5.9%)	56 (6.7%)	37 (4.1%)	3 (5.7%)	24 (3.4%)	449
	Total	**9,594**	**945**	**684**	**833**	**912**	**53**	**699**	**13,720**

Species	**Life Stage**	**Estimated total *Culicoides* collected**							
		CDC	**UV**	**Blue**	**Green**	**Yellow**	**Red**	**White**	**Total**
C. dewulfi	Un-pigmented	147 (42.7%)	14 (30.4%)	45 (26.9%)	19 (17.9%)	33 (55.9%)	0	40 (47.6%)	298
	Pigmented	92 (26.7%)	7 (15.2%)	70 (41.9%)	36 (34%)	22 (37.3%)	2 (50%)	37 (44%)	266
	Blood-fed	2 (0.6%)	0	37 (22.2%)	3 (2.8%)	0	0	0	42
	Gravid	86 (25%)	19 (41.3%)	13 (7.8%)	38 (35.8%)	1 (1.7%)	0	1 (1.2%)	158
	Male	17 (5%)	6 (13%)	2 (1.2%)	10 (9.4%)	3 (5.1%)	2 (50%)	6 (7.2%)	46
	Total	**344**	**46**	**167**	**106**	**59**	**4**	**84**	**810**
Total *Culicoides* collected		**20,514**	**2,967**	**3,248**	**3,851**	**2,685**	**111**	**2,989**	**36,365**

Table 5.2. Final estimated abundance and physiological status of *C. obsoletus, C. scoticus and C. dewulfi* calculated from subsamples of collections

Species	Physiological status	Total *Culicoides* collected							
		CDC	**UV**	**Blue**	**Green**	**Yellow**	**Red**	**White**	**Total**
C. pulicaris	Un-pigmented	189 (48.6%)	20 (40.8%)	71 (59.7%)	49 (31.2%)	30 (43.5%)	1 (100%)	46 (46%)	406
	Pigmented	170 (43.7%)	24 (49%)	31 (26.1%)	68 (43.3%)	32 (46.4%)	0	48 (48%)	373
	Blood-fed	0	0	1 (0.8%)	0	1 (1.4%)	0	0	2
	Gravid	29 (7.5%)	5 (10.2%)	16 (13.4%)	37 (23.6%)	4 (5.8%)	0	5 (5%)	96
	Male	1 (0.2%)	0	0	3 (1.9%)	2 (2.9%)	0	1 (1%)	7
	Total	**389**	**49**	**119**	**157**	**69**	**1**	**100**	**884**
C. brunnicans	Un-pigmented	59 (22.3%)	33 (32%)	40 (7.4%)	104 (14%)	39 (18.3%)	6 (31.6%)	27 (14.5%)	308
	Pigmented	161 (61%)	54 (52.4%)	410 (75.6%)	355 (47.8%)	84 (39.4%)	10 (52.6%)	140 (75.3%)	1,214
	Blood-fed	11 (4.2%)	3 (2.9%)	22 (4.1%)	13 (1.7%)	6 (2.8%)	0	6 (3.2%)	61
	Gravid	30 (11.4%)	11 (10.7%)	53 (9.8%)	265 (35.6%)	83 (39%)	2 (10.5%)	11 (5.9%)	455
	Male	3 (1.1%)	2 (1.9%)	17 (3.1%)	7 (0.9%)	1 (0.5%)	1 (5.3%)	2 (1.1%)	33
	Total	**264**	**103**	**542**	**744**	**213**	**19**	**186**	**2,071**

Table 5.3. Abundance and physiological status of *C. pulicaris and C. brunnicans* collected in light-suction traps

The results show that the CDC light-suction trap consistently collects higher numbers of *C. obsoletus* group females and the red LED-suction trap always collected the least. Looking at the species level response of the group to the different LED-suction traps, *C. obsoletus* was collected most at the green trap, *C. scoticus* at UV and *C. dewulfi* at blue. *C. pulicaris* is also similar to *C. obsoletus* group having been collected in highest numbers with the CDC light-suction and lowest numbers at the red trap, amongst the LED–suction traps the highest numbers were found at green light. Unlike the aforementioned species, the CDC light-suction trap did not collect the highest numbers of *C. brunnicans*, for this species the largest collections were made with the green LED and the lowest, again, with red.

Three models were generated to describe *C. obsoletus* abundance in traps: total females (including all physiological stages); un-pigmented females and pigmented females. Significant parameters included in each model are summarised in Table 5.4.

Parameter	***C. obsoletus* Females**	***C. obsoletus* Un-pigmented**	***C. obsoletus* Pigmented**
Intercept	-5.804***	-6.764***	-7.365***
Temporal Trend			
Linear	-0.032***	NS	-0.046***
Quadratic	0.0002***	NS	0.0002***
Trap			
CDC	1.657***	1.669***	1.571***
UV	0.203	0.126	0.262
Blue	Baseline	Baseline	Baseline
Green	0.612	0.613	0.634
Yellow	-0.172	-0.202	0.029
Red	-3.272***	-3.641***	-2.941***
White	0.555	0.122	0.087
Trap Location			
Position 1	Baseline	Baseline	Baseline
Position 2	-0.365***	-0.280	-0.550
Position 3	-1.172***	-0.915**	-1.400***
Position 4	-2.288	-2.674***	-2.374***
Position 5	-0.522	-0.563	-0.731*
Position 6	-0.590	-0.587	-0.879**
Position 7	-2.685***	-2.687***	-2.819***
Temperature	0.327***	0.264***	0.371***
Humidity	0.063***	0.060***	0.068***
Solar Radiation	NS	-11.922**	NS
Wind Speed	-0.481***	-0.344*	-0.720***
Variation in Wind Direction	0.015***	0.017**	0.0179***

Table 5.4. Regression coefficients in final negative binomial GLMs for *C. obsoletus* females attracted to wavelengths of light (*=p<0.05, **=p<0.01, ***=p<0.001, NS*p>0.05)

Analysis of trap collections for all classifications of female *C. obsoletus* revealed that catches were significantly dependent on trap (p<0.001). Final models for all analyses included trap location, where all locations were found to collect lower numbers than location 1, although not all were significant. Meteorological variables were broadly consistent across all models with the exception than solar radiation was only significant for un-pigmented female collections. Temporal trends were also significant for total females and pigmented females models but not for un-pigmented. Analysis of differences between traps found that the CDC trap collected

significantly greater numbers of *C. obsoletus* females ($p<0.001$) irrespective of physiological status than any other trap with the exception of pigmented individuals where no difference was seen compared to the green LED-baited trap. In addition, the red LED baited trap collected significantly fewer *C. obsoletus* females than all other traps ($p<0.001$) (Table 5.5).

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	1.454***	-				
Blue	1.656***	0.202	-			
Green	1.045**	0.409	-0.612	-		
Yellow	1.828***	0.374	0.172	0.784	-	
Red	4.929***	3.475***	3.272***	3.884***	3.100***	-
White	1.601***	0.147	-0.055	0.556	-0.227	-3.327***

(a)

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	1.543***	-				
Blue	1.669***	0.126	-			
Green	1.055*	-0.487	-0.613	-		
Yellow	1.872***	0.329	0.202	0.816	-	
Red	5.310***	3.767***	3.641***	4.254***	3.438***	-
White	1.547***	0.004	-0.122	0.491	-0.324	-3.763***

(b)

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	1.307**	-				
Blue	1.570***	0.262	-			
Green	0.936	-0.371	-0.633	-		
Yellow	1.541***	0.233	-0.029	0.604	-	
Red	4.511***	3.203***	2.941***	3.574***	2.970***	-
White	1.483***	0.175	-0.087	0.546	-0.058	-3.028***

(c)

Table 5.5. Analysis of differences between traps for a) total *C. obsoletus* females; b) un-pigmented *C. obsoletus* females; c) pigmented *C. obsoletus* females, estimates are for treatments on the top row relative to treatments on the left hand column (*=p<0.05, **=p<0.01, ***=p<0.001)

Data for *C. scoticus* females analysed in a similar manner to those for *C. obsoletus* females. The significant variables included in each model are summarised below in Table 5.6.

Parameter	***C. scoticus* Females**	***C. scoticus* Un-pigmented**	***C. scoticus* Pigmented**
Intercept	-5.683***	-2.904*	-7.556***
Temporal Trend			
Linear	-0.032***	NS	-0.053***
Quadratic	0.0002***	0.00004*	0.0003***
Trap			
CDC	2.988***	3.035***	2.993***
UV	0.928**	0.813*	0.837*
Blue	Baseline	Baseline	Baseline
Green	0.789*	0.688	0.821*
Yellow	0.314	0.343	0.168
Red	-2.079***	-2.205***	-2.224***
White	0.512	0.634	0.339
Trap Location			
Position 1	Baseline	Baseline	Baseline
Position 2	-0.448	-0.148	-0.609
Position 3	-1.162***	-0.993**	-1.270***
Position 4	-2.150***	-0.240***	-2.171***
Position 5	-0.579	-0.475	-0.548
Position 6	-0.868**	-0.704*	-0.907**
Position 7	-2.943***	-2.892***	-2.807***
Temperature	0.212***	NS	0.300***
Humidity	0.063***	0.046***	0.067***
Solar Radiation	-9.020*	-16.38***	NS
Wind Speed	-0.462**	NS	-0.617***
Variation in Wind Direction	0.018***	-0.013**	0.021***

Table 5.6. Regression coefficients included in final negative binomial models for *C. scoticus* females attracted to wavelengths of light (*=p<0.05, **=p<0.01, ***=p<0.001, NS>0.05)

The final models generated for collections of *C. scoticus* females were significantly dependent on trap ($p<0.05$). Final models also included temporal trends, trap location, with similar results to the *C. obsoletus* models, and meteorological variables with relative humidity and variation in wind direction included in all three. Again, collections between traps in all three models varied significantly with the CDC trap collecting more individuals than all other traps ($p<0.001$) (Table 5.7). In an identical fashion to *C. obsoletus*, significantly fewer *C. scoticus* of all physiological

classifications were also collected in the red LED baited trap than any other ($p<0.001$) (Table 5.7).

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	2.059***	-				
Blue	2.988***	0.928	-			
Green	2.199***	0.140	-0.788	-		
Yellow	2.673***	0.614	-0.314	0.474	-	
Red	5.067***	3.008***	2.079***	2.868***	2.393***	-
White	2.476***	0.417	-0.511	0.277	-0.197	-2.590***

(a)

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	2.222***	-				
Blue	3.035***	0.813	-			
Green	2.346***	0.124	-0.688	-		
Yellow	2.691***	0.469	-0.343	0.345	-	
Red	5.240***	3.018***	2.204***	2.893***	2.548***	-
White	2.401***	0.179	-0.633	0.054	-0.290	-2.838***

(b)

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	2.156***	-				
Blue	3.035***	0.837	-			
Green	2.171***	0.154	-0.821	-		
Yellow	2.825***	0.669	-0.168	0.653	-	
Red	5.217***	3.061***	2.224***	3.045***	2.392***	-
White	2.654***	0.498	-0.339	0.482	-0.170	-2.563***

(c)

Table 5.7. Analysis of differences between traps for a) total *C. scoticus* females; b) un-pigmented *C. scoticus* females and c) pigmented *C. scoticus* females, estimates are for treatments on the top row relative to treatments on the left hand column (*=p<0.05, **=p<0.01, ***=p<0.001)

Total female collections of *C. dewulfi* were significantly dependent on trap ($p<0.05$) and influenced by all meteorological conditions recorded with the exception of solar intensity. Trap position was also influential in determining abundance with all locations except for position 5 collecting significantly fewer than position 1.

Parameter	***C. dewulfi* Females**
Intercept	-11.132***
Temporal Trend	
Linear	-0.083***
Quadratic	0.0005***
Trap	
CDC	1.033**
UV	-1.423**
Blue	Baseline
Green	0.415
Yellow	-1.434**
Red	-4.549***
White	-0.157
Trap Location	
Position 1	Baseline
Position 2	-1.019*
Position 3	-2.033***
Position 4	-2.958***
Position 5	-0.574
Position 6	-1.268**
Position 7	-2.980***
Temperature	0.442***
Humidity	0.085***
Wind Speed	-0.627**
Variation in Wind Direction	0.020**

Table 5.8. Regression coefficients included in final negative binomial model for total female *C. dewulfi* attracted to wavelengths of light (*=p<0.05, **=p<0.01, ***=p<0.001)

C. dewulfi females exhibited greater levels of preference between LED baits than *C. obsoletus* and *C. scoticus* (Table 5.8). Analysis of differences between traps demonstrated that the red LED collected significantly lower numbers of *C. dewulfi* females than all other traps ($p<0.05$) (Table 5.9). The CDC collected significantly more than UV, yellow, red and white ($p<0.05$) and the blue LED collected significantly greater numbers than the UV, yellow and red LED baits ($p<0.05$) and is not significantly different to the CDC. The green LED collects significantly greater numbers than UV, yellow and red ($p<0.01$) and was not significantly different to the CDC or blue LED.

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	2.456***	-				
Blue	1.033	-1.423*	-			
Green	0.618	-1.838**	-0.415	-		
Yellow	2.467***	0.010	1.433*	1.848***	-	
Red	5.582***	3.125*	4.549***	4.964***	3.115*	-
White	1.191*	-1.265	0.157	0.572	-1.276	-4.391***

Table 5.9. Analysis of differences between traps for total female *C. dewulfi*, estimates are for treatments on the top row relative to treatments on the left hand column (*=p<0.05, **=p<0.01, ***=p<0.001)

For *C. pulicaris* and *C. brunnicans*, models describing total female catches are summarised in Table 5.10. Collections of *C. pulicaris* were dependent on trap ($p<0.05$), temporal trend and all meteorological variables except for wind speed, trap location was also significant and all positions collected significantly fewer than position 1. *C. brunnicans* collections were also significantly dependent on trap ($p<0.05$), temporal trend and location although not all locations differed significantly from position 1. Unlike in the other models the differences between positions compared to position 1 were not always negative with position 3 collecting significantly higher numbers than position 1. The influence of meteorological variables was also significant with all variables except solar radiation included in the final model.

Parameter	***C. pulicaris* Females**	***C. brunnicans* Females**
Intercept	-2.617	-14.799***
Temporal Trend		
Linear	0.008**	NS
Quadratic	NS	-0.003**
Trap		
CDC	0.957**	1.345***
UV	-0.788*	-0.324
Blue	Baseline	Baseline
Green	0.506	1.000*
Yellow	-0.793*	-0.087
Red	-4.743***	-1.843**
White	-0.051	0.291
Trap Location		
Position 1	Baseline	Baseline
Position 2	-0.867*	0.914
Position 3	-1.088**	1.017*
Position 4	-2.958***	-1.358*
Position 5	-1.045**	0.067
Position 6	-1.375***	-0.101
Position 7	-3.940***	-1.892***
Temperature	0.111*	1.159***
Humidity	0.029*	0.047*
Solar Radiation	-12.651*	NS
Wind Speed	NS	-1.453***
Wind Direction	0.002*	-0.003*

Table 5.10. Regression coefficients included in final negative binomial GLMs for total female *C. pulicaris* and *C. brunnicans* attracted to wavelength of light (*=p<0.05, **=p<0.01, ***=p<0.001)

In *C. pulicaris*, the CDC light-suction trap collected significantly higher numbers of females than the UV, yellow, red and white LED baited traps ($p<0.05$) but was not significantly different to blue and green LED (Table 5.11a). The green LED collected greater numbers than the UV, yellow and red LEDs ($p<0.01$) but was not significantly different from the blue LED. The red LED baited trap caught significantly less *C. pulicaris* than any other trap. In *C. brunnicans*, the CDC light-suction trap collected significantly higher numbers than UV, yellow and red LEDs ($p<0.05$) but was not significantly different to blue, green and white LEDs. The red

LED collected significantly lower numbers than the other traps with the exception of the UV LED baited trap where no significant difference was observed.

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	1.746***	-				
Blue	0.957	-0.788	-			
Green	0.451	-1.295**	-0.506	-		
Yellow	1.751***	0.005	0.793	1.300**	-	
Red	5.701***	3.955**	4.743***	5.250***	3.950**	-
White	1.009*	-0.736	0.051	0.558	-0.741	-4.691***

(a)

Trap	CDC	UV	Blue	Green	Yellow	Red
UV	1.669*	-				
Blue	1.345	-0.324	-			
Green	0.344	-1.325	-1.000	-		
Yellow	1.432*	-0.237	0.087	1.087	-	
Red	3.188***	1.519	1.843*	2.844***	1.756*	-
White	1.054	-0.615	-0.291	0.709	-0.378	-2.134**

(b)

Table 5.11. Analysis of differences between traps for a) total female *C. pulicaris* and b) total female *C. brunnicans*, estimates are for treatments on the top row relative to treatments on the left hand column

In summary, the results show that for *C. obsoletus* and *C. scoticus* there is a significantly greater response to the CDC trap and a significantly lower response to the red LED. Of the remaining wavelengths tested no significant differences were found for these species. For *C. dewulfi* the CDC also collected significantly greater numbers than the UV, yellow, red and white LEDs but there was no difference to green and blue, green and blue also collected significantly more than UV and yellow while red collected significantly less than all other traps. For C. pulicaris the CDC collected significantly more than the UV, yellow, white and red LEDs but was not significantly different to blue or green, the red LED collected significantly less than all others. The green LED collected significantly more than the UV and yellow LEDs. Finally, *C. brunnicans* responded in significantly higher numbers to the CDC

than to the UV, yellow and red LEDs but there was no significant difference between the CDC and the blue or green LEDs.

5.4 Discussion

The demonstration that *C. brevitarsis* was more sensitive to green LEDs in Australia than to UV light-suction baits had a direct impact on surveillance schemes in that country (Bishop *et al.* 2006, Bishop *et al.* 2008). Despite this observation, the current study is the first to assess differential attraction of *Culicoides* to different wavelengths of light in Europe, where the impact of *Culicoides*-borne arboviruses is substantially greater and surveillance schemes larger and based entirely on UV baited trapping (Mellor *et al.* 2004). Key objectives of this study in comparison to previously published work in this area were to collect sufficient data to apply appropriate statistical modelling of parameters determining *Culicoides* abundance and diversity in collections and the integration of meteorological data into the study (which had not previously been attempted). In addition, processing of all *Culicoides* to species level had rarely been attempted in studies of this scale, allowing accurate demarcation in response between *C. obsoletus* and *C. scoticus* in particular. Finally, the study also utilised commercially available traps for testing. While this had the disadvantage of not allowing specific design of a dedicated trap for northern European *Culicoides* populations, it did have the advantage of allowing traps to be rapidly replaced if the studies highlighted significant differences in species-specific responses.

The study site chosen for the trial contained large populations of most of the common livestock-associated species of *Culicoides* in the UK, confirmed through the use of the control CDC light-suction trap (Boorman 1986). These collections were dominated by *C. obsoletus* and *C. scoticus*, which are ubiquitous across Europe, with a lesser abundance of *C. dewulfi, C. brunnicans, C. pulicaris, C. punctatus* and *C.*

impunctatus, all of which species have been recorded in previous trials carried out locally to this area (Boorman and Goddard 1970b, Birley and Boorman 1982). It was notable, however, that cattle-dung breeding species (namely *C. dewulfi* and *C. chiopterus*) were under-represented as a proportion of total catch when compared to farm studies conducted elsewhere in northern Europe (De Deken *et al.* 2008, Meiswinkel *et al.* 2008). This may have been due to the close relationship between these species and cattle (Kettle and Lawson 1952), which were not directly present at the site during the trial (although they were grazed in adjacent fields to the study area).

Throughout the study, the UV baited CDC light-suction trap consistently outperformed the Bioquip® LED traps in the abundance of *Culicoides* collected, with the exception of *C. brunnicans*. This observation was also recorded to a lesser degree for mosquito collections in Kenya with an incandescent CDC (Tchouassi *et al.* 2012), where the authors suggested that this difference was due to the increased scatter of incandescent light. While these differences may partly be a consequence of trap design (including the use of different rain shields that may have influenced catches), it is clear that the greater power of the 4W tube could have been a key component in increasing *Culicoides* catch size. Hence the fact that the UV baited CDC light-suction trap did not catch significantly more *C. brunnicans* than blue, green or white LED baited Bioquip® traps is indicative of true differences in the spectral sensitivity of this species rather than just a response to increased brightness.

In France, *C. brunnicans* has been found during live host collections in greater abundance than *C. obsoletus* (Viennet *et al.* 2011). It is not clear, however, whether the species plays a role in arbovirus transmission in Europe as it is predominantly an early season species which does not correlate with outbreaks of

BTV and SBV occurring primarily in the autumn. Previous studies have been carried out to test the vector competence of *C. brunnicans* for BTV, though only small numbers were tested (Jennings and Mellor 1988). A clearer characterisation of the ecology and vector competence of *C. brunnicans* would therefore be useful and these studies could be aided by the use of green LED baited traps.

When the response to different LED wavelengths was assessed across the Bioquip® traps, the only highly consistent pattern in response was a poor attraction to the red (660 nm) LED baited trap in comparison to all others. This was in contrast to studies with the Psychodidae that used light of an identical wavelength and had demonstrated at least a degree of attraction in comparison to other wavelengths (Hoel *et al.* 2007, Mann *et al.* 2009). The authors of those studies hypothesised that this attraction in sandflies is indicative of host plant location for sugar feeding, although given that this sugar feeding behaviour has been recorded in mosquitoes and *Culicoides*, these apparent differences require further elucidation. It has been demonstrated through behavioural studies that *An. gambiae* mosquitoes may be able to see red and infra-red light at certain intensities (Gibson 1995). For *Culicoides* it may be that the lack of response to the red is due to this wavelength being beyond their visual range, this could be investigated through electroretinnograms in the laboratory. Excluding the red LED baited trap, the major putative arbovirus vectors *C. obsoletus* and *C. scoticus* appeared to exhibit an indiscriminate response to the LED-baited traps. This would indicate that, irrespective of differences in design, the UV baited CDC light-suction trap would be unlikely to be substantially improved in sensitivity for collection of these species through the use of different wavelengths of light.

In contrast, while *C. dewulfi* was collected in fewer numbers than *C. obsoletus* and *C. scoticus*, the CDC collections were not significantly different to the collections made in the blue and green LED baited Bioquip® traps and when just looking at the LED traps, the blue and green traps have significantly higher catches than the UV. *C. dewulfi* does show differential attraction to wavelengths of light and UV runs the risk of under-estimating the population of this species. Very similar results were also found in attraction of *C. pulicaris* and both species demonstrate clear similarities to *C. brevitarsis* which responds significantly to green light (Bishop *et al.* 2004b, Bishop *et al.* 2006). Further study to define attraction wavelengths more accurately in these species would be useful to define these differences.

A key concern in the study was the use of variable light intensities across the treatments. As a broad estimate, the UV CDC light trap produces approximately 4 watts output, while the LEDs produce 1-2 watts and the UV LED only about 0.8 W (Tchouassi *et al.* 2012). Light intensity was shown to be a significant factor in increasing collections of *C. brevitarsis* with green LEDs in Australia with a 42% rise in intensity giving an almost 3 fold increase in catch size (Bishop *et al.* 2004b). Increasing intensity will give an increase in the range of attraction of a light source and so will result in a larger proportion of the local population being sampled but has the trade-off of increasing power consumption. Behavioural responses of *Lu. longipalpis* to wavelengths in a choice chamber delivered at low, equivalent and high intensity in relation to a 400 nm control illustrate that the intensity of delivery may influence attraction to specific wavelengths (Mellor and Hamilton 2003). At low light intensities peak responses for female *Lu. longipalpis* were found in the blue-green region, while for males this occurred in the green-yellow region, and a second peak for both sexes was found at UV while at the higher intensities the response peak

was greatest to UV. This agrees with the *Culicoides* work in Australia (Bishop *et al.* 2004b).

Flight behaviour of *Culicoides* is heavily influenced by meteorological variables making it essential to include these data in any analysis of field collections of these species (Carpenter *et al.* 2008c, Baylis *et al.* 2010, Sanders *et al.* 2011). The models generated are broadly in agreement with previous *Culicoides* studies in showing that temperature and humidity have a positive impact on trap collections whereas wind speed has a negative impact.

The present study demonstrates that for *C. obsoletus* and *C. scoticus* all wavelengths tested, with the exception of red, are equally effective for collecting these species. By contrast green light was found to collect significantly higher numbers of females of *C. dewulfi* and *C. pulicaris* compared to UV this suggests that for the most sensitive surveillance of vector species, green light might be the most appropriate to use for Palearctic *Culicoides*. Further investigation using LEDs with a uniform light intensity would reveal beyond doubt whether or not the green LED is superior to UV or whether this effect for *C. dewulfi* and *C. pulicaris* is due to intensity as seen with sand flies (Mellor and Hamilton 2003). A separate laboratory study to investigate spectral sensitivity through electroretinograms would also yield very useful information on the response of *Culicoides* to different wavelengths. To truly assess the appropriateness of any wavelength for surveillance it would be necessary to evaluate light colours along with direct collections on hosts to tests whether light trap collections give an accurate measure of host seeking activity.

Chapter 6: General Discussion

The introduction and transmission of BTV and SBV in northern Europe by *Culicoides* species has highlighted the requirement for a clearer understanding of the relationship between vector species and their hosts. Host location is an essential part of *Culicoides* biology as females of the majority of species require a blood meal in order to mature egg batches. This interaction is crucial in driving the transmission of arboviruses between susceptible hosts. Previous investigations of *Culicoides* host location in northern Europe have primarily focused on the nuisance biting species *C. impunctatus*. With the implication of *C. obsoletus* and *C. pulicaris* group species in the transmission of BTV and SBV (Carpenter *et al.* 2006a, De Regge *et al.* 2012), it is clear that there is an urgent need to fill the gaps in our knowledge concerning the host location behaviour of these species. A clearer understanding of these behaviours would not only assist in understanding the transmission of these pathogens, but could also provide the opportunity for the development of novel tools for surveillance and control. In addition, novel, convenient surveillance methods based on LED-baited light-suction traps had not previously been tested for *Culicoides* in the Palaearctic region. These were seen as potentially providing an interim means of more accurately sampling the genus in northern Europe. The work presented in this thesis therefore provides a systematic investigation of responses of *Culicoides* species to host animals; response to specific host-derived olfactory stimuli; and differential response to visual cues.

The substantial data sets that were generated as part of this thesis were important in providing a more realistic representation of *Culicoides* activity on

livestock holdings than previous work. Samples collected were identified to species level through the use of multiplex PCR with 21,045 individuals subjected to molecular analysis and an overall amplification success rate of 93.6%. The importance of identifying to species level is shown by the significant differences observed at this level throughout the studies conducted. Detailed analyses were also carried out to include meteorological data collected throughout sampling periods as this is known to be a key factor in determining *Culicoides* flight activity (Sanders *et al.* 2012).

Investigation of differential responses of *C. obsoletus* group females to hosts was conducted through a series of studies involving the direct collection of *Culicoides* from hosts. Previous work has been carried out to investigate responses of *Culicoides* to hosts, but the aims of these studies were primarily to establish biting-rates on hosts rather than host preference (Carpenter *et al.* 2008c, Gerry *et al.* 2009, Viennet *et al.* 2011). One study has attempted to investigate host preferences by collections on a range of host species but the number of *Culicoides* collected was very low (Viennet *et al.* 2013). In the present study, three separate investigations were conducted to assess the differential responses of *Culicoides* to different breeds of sheep, sheared and unsheared sheep, and the effect of cattle on biting rates on sheep.

No previous investigation has examined differential responses of *Culicoides* to breeds of sheep despite BT being documented as affecting some breeds more severely than others. In the work carried out in this thesis 16,170 *Culicoides* were collected in this trial and it was demonstrated that *C. dewulfi and C scoticus* exhibit differential attraction to breeds, even when the breeds are closely related. For both of these species significantly fewer females were collected on the pure sheep breed

compared to the cross. Of the *C. dewulfi* collections numbers of un-pigmented and pigmented females were significantly lower on the pure breed while in the *C. scoticus* collections there was a significant reduction in the number of blood fed individuals on the pure breed. It is not clear what drives these differences, one explanation for the differences in attraction of the breeds could be due to different odour profiles of the breeds, differences were found in the concentrations of some chemicals released from the breeds (J. Cook, personal communication). In a separate study, however, using the odour of the sheep breeds in isolation from other host cues, no differences were observed in attraction and the numbers collected in that work were far lower than the collections made on the host. This difference could be due to the different physical attributes of the two breeds, although little is known regarding the response to visual cues in *Culicoides*. The phenomena of intra-breed variation in host preference is not unique to *Culicoides* and has been described in other vector groups (Birkett *et al.* 2004, Jensen *et al.* 2004). Following the discovery of breed preference a practical continuation of this work would be to investigate the differential attractiveness of other, more commercially important, sheep breeds.

The investigation into differential attraction and feeding on sheared and unsheared sheep collected significantly greater numbers of blood fed *C. obsoletus* on sheared sheep compared to unsheared with 4.7 times as many collected in sheared sheep. No significant differences were noted in any other species including *C. scoticus* which was the most abundant during this trial. In addition, no significant difference was observed for total numbers of *Culicoides* females of any species on the hosts which was surprising as it was hypothesised that unsheared sheep would be likely to have a different odour and thermal profile and increased respiration rate that would lead to increased attractiveness to host seeking individuals. This indicated that

shearing of sheep did not influence the number of *Culicoides* initially attracted to the host. The difference in feeding success of *C. obsoletus* is of significant interest due to the ubiquitous nature of this species on livestock holdings across northern Europe and its status as a putative vector of BTV and SBV (Carpenter *et al.* 2006a).

The reasons why not shearing failed to impact upon the other members of the *C. obsoletus* group species remains unknown and could be related to differences in feeding efficiency and feeding site selection. This area would be of significant interest to pursue in detail during future studies. The findings of the current study demonstrate that choosing not to shear could have a mitigating effect in the event of an arbovirus outbreak where there are large populations of *C. obsoletus*, however the likely trade-off with reducing the efficacy of insecticides on unsheared sheep (Venail *et al.* 2011) would need to be assessed as this could lead to an overall heightened risk of transmission. Another consideration for such action would be the fact that *C. scoticus* is also found in high abundance on farms and has been shown to replicate BTV to high levels in the laboratory (Carpenter *et al.* 2008a). While sheep may be protected to some degree from BTV transmission from *C. obsoletus* bites if left unsheared the risk remains for transmission from *C. scoticus* which demonstrated no significant reduction in blood feeding between sheared and unsheared sheep.

Grazing cattle in close proximity to sheep has been reported as a means of protecting sheep from *Culicoides* bites in South Africa (Du Toit 1962, Nevill 1978). No entomological investigation of the effect of this husbandry had been carried out prior to the current study. Surprisingly, considering the South Africa trial, the results convincingly demonstrate that in the case of Palaearctic *Culicoides* species, grazing cattle with sheep would provide no protection. The impact of cattle being held in close proximity lead to a doubling of *Culicoides* collections on sheep. One limitation

of the study design was that the cow corral was in very close proximity to the drop trap and in a natural grazing situation cattle and sheep would be likely to have more distance between them. Follow up work with the cow either in the same field but at a greater distance from the sheep or in a neighbouring field would provide useful information as to how this alters the responses of *Culicoides*. An interesting observation of this trial was the numbers of *C. dewulfi*, in the sheared and unsheared trial that was conducted immediately prior to this one where there was a low abundance of *C. dewulfi* but this changed completely once the cow trial started, emphasising the close association of this species with cattle.

Olfaction is known to be an important component of host location for haematophagous Diptera including *Culicoides* species (Gibson and Torr 1999). Three studies were conducted to investigate the olfactory response of *Culicoides* on farms. CO_2 is known to be an attractant for many haematophagous species and collections of the north American BTV vector, *C sonorensis*, are typically in suction traps supplemented with this kairomone (Mullens 1995). The dose response work presented here demonstrates that *C. nubeculosus* displays a significantly greater response to 1,500 ml/min CO_2 than to 500 or 1,000 ml/min and that beyond 1,500 there is no significant increase in collections. The findings are in accordance with a previous study at the same site which reported no significant difference in response to 500 and 1,000 ml/min CO_2 (Harrup *et al.* 2012). This species is not typically collected in large numbers in light-suction trap surveillance and the current work indicates that this could be a result of low response to the standard surveillance tool rather than to a low abundance of the species. Similar behaviour is seen in *C. sonorensis* and both species are members of the Monoculicoides sub-genus and this response to olfactory cues over visual cues may be a common trait for this group.

The results highlight the potential that other species could be under-estimated in light traps for these same reasons. In contrast Avaritia group species, including *C. obsoletus* are found to be more responsive to light-baited traps than to CO_2 (Gerry *et al.* 2009).

A key area of this study was the use of a statistical analysis where GLMs were constructed integrating meteorological variables and position effects to model the responses of *Culicoides* to traps. If the analysis had been carried out using ANOVA then the results would have looked very different with no significant differences found between the treatments, this would have led to different conclusions about the optimum release rate for this species. The CO_2 trial was conducted at a site with low *C. obsoletus* abundance, subsequent semiochemical trials were conducted a site with a large population including all four members of the *C. obsoletus* group. Where studies have used one or two semiochemicals as bait for *Culicoides* the response of *C. obsoletus* has been limited in terms of numbers collected (Mullens *et al.* 2005, Gerry *et al.* 2009, Harrup *et al.* 2012). As a preliminary trial, collections of *C. obsoletus* group were made using the whole odour profile of hosts as this was expected to be most likely to yield a positive response. This was the first time that such a technique has been used for *Culicoides* species but it has previously been demonstrated to be effective for tsetse flies in Zimbabwe (Vale 1974). The study demonstrated promising preliminary results with significantly higher numbers of *Culicoides* collected in odour baited traps than in un-baited traps. Overall, the numbers collected in the odour traps were low but there are many factors that could have contributed to this (e.g. no standardisation of odour release rate; close proximity of other hosts). With field equipment now developed and tested, this trial can form the basis for on-going development of host odour-baited traps.

The follow up study assessing CO_2 combined with chemicals from host odour that induced electrophysiological and behavioural responses in the laboratory produced similar results in terms of numbers of *Culicoides* responding to traps. A blend of three novel chemicals, R-octenol and CO_2 was found to collect significantly higher numbers than when the three novel chemicals were tested individually. R-octenol was not found to induce a significant response despite being shown elsewhere to collect significantly more *C. obsoletus* than CO_2 alone (Harrup *et al.* 2012). The release method of R-octenol in the previous trial was different to that used here in that the former trial mixed the semiochemicals prior to release. In the current study these semiochemicals were released in close proximity but not directly mixed. This may in part have led to the lower numbers of *Culicoides* responding. It is also possible that the entire catch in the blend of chemicals was a response to the R-octenol as the other chemicals when trialled alone with CO_2 had only collected single *Culicoides* in the trial while R-octenol had the highest collection. In terms of numbers of *Culicoides* collected in the semiochemical baited traps, these were of a similar level to those observed in the collections using whole host odour. For future work in this area a number of options could be investigated. The low numbers collected in semiochemical baited traps could be a result of *Culicoides* being attracted to the vicinity but not being efficiently captured due to the lack of landing cues. This could be investigated through examining more efficient ways of eliciting landing behaviour or through making collections from areas contiguous to the trap using a sweep net or drop trap. It would also be useful to consider adding a thermal cue as this has been shown to be very effective as a supplement in collections in the USA (Kline and Lemire 1995).

The final area of work investigated the use of different wavelengths of light for the collection of Palaearctic *Culicoides* species, an area that had been entirely overlooked to date. Work on Australian *Culicoides* has demonstrated the effectiveness of green wavelengths of light for the collection of the arbovirus vector *C. brevitarsis* (Bishop *et al.* 2004b). The standard surveillance trap used in Europe is the OVI UV light-suction trap, but this has been demonstrated to under-estimate abundance of species found on host animals (Carpenter *et al.* 2008c). These findings and the commercial availability of novel LED-baited light-suction traps with precise wavelengths of light provided a timely opportunity to investigate differential attraction of Palearctic species to wavelengths of light to determine whether wavelengths other than UV might provide a more sensitive tool for surveillance in terms of species diversity.

Responses to six wavelengths of light from LED-baited light traps were assessed in comparison to a standard CDC UV light-suction trap with a total collection of 42,696 *Culicoides*. Results demonstrated that the CDC trap collected significantly higher numbers of *C. obsoletus* and *C. scoticus* but between the LED traps there were no differences except that the red trap collected significantly lower numbers which may indicate that they are unable to see red light. These results demonstrate that these species have a broad response to light and that changing from standard UV light to a different wavelength would be unlikely to significantly alter collections. In contrast, *C. dewulfi* does show significant differential attraction to wavelengths of light with blue and green LEDs, collections were more than twice the numbers collected in UV LED baited trap and these differences were significant. More than three times as many *C. pulicaris* were collected in the green LED compared to the UV LED, again the collections were significantly different. These

results indicate that current surveillance risks under-estimating these species and that more sensitive monitoring could be achieved through the use of green wavelengths of light in particular. No significant differences were found in collections of *C. brunnicans* in the CDC trap compared to blue and green LEDs, although in terms of numbers the green trap collected considerably more, almost three times as many as the UV CDC trap. This species is under-reported in UK light trap collections, but was found in greater abundance than *C. obsoletus* on sheep in France (Viennet *et al.* 2011). Future investigations to compare collections with green light to collections on host animals would provide confirmation of whether this wavelength could provide a more sensitive measure of on-host activity than current UV-baited surveillance.

The work presented in this thesis provides quantitative analysis of host location by Palaearctic *Culicoides* species. Studies were carried out at field sites with large populations of *Culicoides* which provide the first investigations of host location behaviour that are representative of the typical activity on farms providing important information for understanding BTV epidemiology.

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Appendix 1. Supplementary Material For Data Chapter 3

Generalised Linear Models with Parameter Estimates and 95% Confidence Intervals

	C. obsoletus **Total Females**		***C. obsoletus*** **Un-pigmented**		***C. obsoletus*** **Pigmented**		***C. obsoletus*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	3.971***	3.571; 4.393	3.192***	2.784; 3.622	0.977*	0.199; 1.774	0.899	-0.349; 2.149
Temporal Trend								
Linear	0.174***	0.121; 0.227	0.180***	0.125; 0.234	0.179***	0.116; 0.241	0.126***	0.066; 0.187
Quadratic	-0.005***	-0.007; -0.004	-0.006***	-0.007; -0.004	-0.005***	-0.007; -0.003	-0.004***	-0.006; -0.002
Trap								
Cross Breed	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Pure Breed	-0.058	-0.301; 0.184	-0.075	-0.325; 0.174	-0.119	-0.390; 0.152	0.188	-0.065; 0.442
Temperature	NS	-	NS	-	NS	-	0.096**	0.029; 0.164
Humidity	NS	-	NS	-	0.024***	0.014; 0.034	NS	-
Solar Radiation	-0.004***	-0.005; -0.002	-0.004***	-0.005; -0.002	NS	-	-0.007***	-0.009; 0.005
Wind Speed	-0.659***	-0.864; -0.456	-0.706***	-0.914; -0.500	-0.661***	-0.868; -0.456	-0.574***	-0.785; -0.366

APP1.1. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. obsoletus*; non-pigmented *C. obsoletus*, pigmented *C. obsoletus* and blood fed *C. obsoletus* made on two breeds of sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. obsoletus Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

C. obsoletus Un-pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

C. obsoletus Pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Humidity + Wind Speed

C. obsoletus Blood Fed ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed

	C. scoticus **Total Females**		***C. scoticus*** **Un-pigmented**		***C. scoticus*** **Pigmented**		***C. scoticus*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	4.089***	3.690; 4.515	2.675***	2.256; 3.112	-0.575	-1.364; 0.213	3.793	3.348; 4.271
Temporal Trend								
Linear	0.115***	0.058; 0.170	0.162***	0.107; 0.216	0.168***	0.108; 0.229	0.066	0.001; 0.132
Quadratic	-0.003***	-0.005; -0.002	-0.005***	-0.007; -0.003	-0.005***	-0.007; -0.003	-0.002	-0.004; -0.0001
Trap								
Cross Breed	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Pure Breed	-0.569***	-0.826; -0.312	-0.215	-0.471; 0.040	0.089	-0.179; 0.358	-1.118	-1.415; -0.821
Humidity	NS	-	NS	-	0.026	0.016; 0.035	NS	-
Solar Radiation	-0.005***	-0.006; -0.003	-0.004	-0.006; -0.003	NS	-	-0.007	-0.008; 0.004
Wind Speed	-0.703***	-0.920; -0.488	-0.729	-0.940; -0.522	-0.698	-0.897; -0.504	-0.667	-0.919; -0.419

APP1.2. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. scoticus*; non-pigmented *C. scoticus*, pigmented *C.scoticus* and blood fed *C. scoticus* made on two breeds of sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. scoticus Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

C. scoticus Un-pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

C. scoticus Pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Humidity + Wind Speed

C. scoticus Blood Fed ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

	C. dewulfi **Total Females**		***C. dewulfi*** **Un-pigmented**		***C. dewulfi*** **Pigmented**		***C. dewulfi*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	2.218***	1.830; 2.618	1.504***	1.052; 1.964	-0.477	-1.250; 0.292	-4.915***	-6.872; -3.032
Temporal Trend								
Linear	0.215***	0.163; 0.266	0.196***	0.135; 0.257	0.203***	0.143; 0.264	0.349***	0.254; 0.449
Quadratic	-0.007***	-0.008; -0.005	-0.006***	-0.008; -0.004	-0.006***	-0.008; -0.004	-0.011***	-0.141; -0.008
Trap								
Cross Breed	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Pure Breed	-0.497***	-0.735; -0.259	-0.529***	-0.809; -0.251	-0.809***	-1.081; -0.539	0.106	-0.284; 0.498
Temperature	NS	-	NS	-	NS	-	0.243***	0.142; 0.346
Humidity	NS	-	NS	-	0.025***	0.016; 0.355	NS	-
Solar Radiation	-0.004***	-0.005; -0.002	-0.004***	-0.006; -0.002	NS	-	-0.010***	-0.144; -0.006
Wind Speed	-0.705***	-0.889; -0.522	-0.709***	-0.926; -0.497	-0.774***	-0.968; -0.586	-0.488***	-0.773; -0.210

APP1.3. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. dewulfi*; non-pigmented *C. dewulfi*, pigmented *C.dewulfi* and blood fed *C. dewulfi* made on two breeds of sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. dewulfi Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

C. dewulfi Un-pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

C. dewulfi Pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Humidity + Wind Speed

C. dewulfi Blood Fed ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

	C. chiopterus **Total Females**		***C. chiopterus*** **Pigmented**		***C. chiopterus*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	4.348***	3.014; 5.751	4.262***	2.637; 5.993	3.755***	2.219; 5.381
Temporal Trend						
Linear	NS	-	-0.071***	-0.098; -0.044	NS	-
Quadratic	-0.001***	-0.002; -0.0009	NS	-	-0.001**	-0.002; -0.0004
Trap						
Cross Breed	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Pure Breed	-0.031	-0.484; 0.422	0.135	-0.417; 0.696	-0.203	-0.726; 0.315
Humidity	-0.042***	-0.061; -0.234	-0.044***	-0.067; -0.021	-0.048***	-0.071; -0.026
Wind Speed	*0.782***	-1.094; -0.483	-0.847***	-1.251; -0.473	-0.665***	-1.027; -0.325

APP1.4. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. chiopterus*; pigmented *C.chiopterus* and blood fed *C. chiopterus* made on two breeds of sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. chiopterus Total Females ~ Quadratic Temporal Trend + Trap + Humidity + Wind Speed

C. chiopterus Pigmented ~ Linear Temporal Trend + Trap + Humidity + Wind Speed

C. chiopterus Blood Fed ~ Quadratic Temporal Trend + Trap + Humidity + Wind Speed

	C. obsoletus **Total Females**		***C. obsoletus*** **Un-pigmented**		***C. obsoletus*** **Pigmented**		***C. obsoletus*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	1.091*	0.309; 2.141	1.641***	0.918; 2.391	-0.532	-1.651; 0.579	-2.309**	-4.132; -1.113
Temporal Trend								
Linear	NS	-	NS	-	-0.078***	-0.127; -0.028	NS	-
Quadratic	-0.008***	-0.010; -0.005	-0.009***	-0.012; -0.006	NS	NS	-0.007***	-0.009; -0.005
Trap								
Light Trap 1	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Light Trap 2	0.623*	-0.144; 1.369	0.945**	0.267; 1.622	1.046**	0.304; 1.795	-0.378	-3.468; 2.002
Sheared	3.409***	2.747; 4.073	2.984***	2.300; 3.680	2.803***	2.143; 3.484	6.231***	5.025; 8.060
Unsheared	3.295***	2.635; 3.963	3.123***	2.432; 3.680	3.209***	2.547; 3.895	4.728***	3.512; 6.561
Temperature	0.058*	0.001; 0.119	NS	-	0.084**	0.020; 0.150	NS	-
Solar Radiation	-0.008***	-0.010; -0.006	-0.007**	-0.009; -0.005	-0.009***	-0.011; -0.007	-0.005***	-0.007; -0.004
Wind Speed	-0.387**	-0.642; -0.213	-0.361**	-0.622; -0.091	-0.493***	-0.778; -0.206	-0.333**	-0.539; -0.125
Wind Direction	-0.002*	-0.004; -0.003	-0.002*	-0.004; -0.0002	NS	-	NS	-

APP1.5. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. obsoletus*; non-pigmented *C. obsoletus*, pigmented *C. obsoletus* and blood fed *C. obsoletus* made on sheared and unsheared of sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. obsoletus Total Females ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed + Wind Direction

C. obsoletus Un-pigmented ~ Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed + Wind Direction

C. obsoletus Pigmented ~ Linear Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed

C. obsoletus Blood Fed ~ Quadratic Temporal Trend + Trap + Solar Radiation + Wind Speed

	C. scoticus **Total Females**		***C. scoticus*** **Un-pigmented**		***C. scoticus*** **Pigmented**		***C. scoticus*** **Blood Fed**	
Intercept	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	1.505***	-2.474; -0.566	-1.718***	-2.787; -0.700	-2.585***	-4.119; -1.381	2.421***	1.705; 3.151
Temporal Trend								
Quadratic	-0.011***	-0.013; -0.009	-0.014***	-0.016; -0.012	-0.004***	-0.006; -0.002	-0.009***	-0.011; -0.007
Trap								
Light Trap 1	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Excluded	Excluded
Light Trap 2	1.590***	0.926; 2.285	2.065***	1.297; 2.898	1.918**	0.764; 3.409	Excluded	Excluded
Sheared	5.611***	4.995; 6.268	5.729***	5.013; 6.521	5.702***	4.679; 7.129	0.028	0.267; 0.323
Unsheared	5.677***	5.050; 6.346	5.867***	5.138; 6.672	5.516***	4.489; 6.945	Baseline	Baseline
Temperature	0.111***	0.067; 0.156	0.098***	0.052; 0.145	0.043*	0.003; 0.085	0.057**	0.018; 0.097
Solar Radiation	-0.008***	-0.009; -0.006	-0.007***	-0.008; -0.005	-0.007***	-0.008; -0.005	-0.006***	-0.008; -0.005
Wind Speed	-0.424***	-0.624; -0.222	-0.491***	-0.708; -0.272	-0.469**	-0.658; -0.278	-0.236**	-0.413; -0.057

APP1.6. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. scoticus*; non-pigmented *C. scoticus*, pigmented *C.scoticus* and blood fed *C. scoticus* made on sheared and unsheared sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. scoticus Total Females ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed

C. scoticus Un-pigmented ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed

C. scoticus Pigmented ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed

C. scoticus Blood Fed ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed

	C. dewulfi **Total Females**		***C. dewulfi*** **Un-pigmented**		***C. dewulfi*** **Pigmented**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	-1.815***	-2.936; -0.759	-2.358***	-3.806; -1.038	-4.668***	-6.725; -3.073
Temporal Trend						
Quadratic	-0.009***	-0.011; -0.006	-0.016***	-0.021; -0.122	-0.003*	-0.006; -0.004
Trap						
Light Trap 1	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Light Trap 2	1.236**	0.403; 2.148	1.374*	0.274; 2.620	1.626*	0.266; 3.517
Sheared	3.286***	2.537; 4.137	3.518***	2.537; 4.688	3.251***	2.022; 5.088
Unsheared	3.499***	2.747; 4.354	3.675***	2.695; 4.843	2.789***	1.535; 4.636
Temperature	0.069**	0.021; 0.117	0.088**	0.028; 1.504	0.121***	0.057; 0.188
Solar Radiation	-0.006***	-0.008; -0.004	-0.006**	-0.009; -0.004	-0.013***	-0.017; -0.009
Wind Speed	-0.303*	-0.546; -0.059	-0.331*	-0.638; -0.248	NS	-
Wind Direction	-0.002	-0.003; -0.0002	-0.002*	-0.004; -0.0003	NS	-

APP1.7. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. dewulfi*; non-pigmented *C. dewulfi*, pigmented *C.dewulfi* and blood fed *C. dewulfi* made on sheared and unsheared sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. dewulfi Total Females ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed + Wind Direction

C. dewulfi Un-pigmented ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed + Wind Direction

C. dewulfi Pigmented ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation

	C. chiopterus **Total Females**		***C. chiopterus*** **Pigmented**		***C. chiopterus*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	2.408***	1.224; 3.618	3.331***	2.668; 4.045	0.558	-0.768; 1.927
Temporal Trend						
Linear	-0.156***	-0.209; -0.103	-0.121***	-0.171; -0.070	NS	-
Quadratic	NS	-	NS	-	-0.010***	-0.013; -0.006
Trap						
Sheared	-0.007	-0.453; 0.439	-0.029	-0.508; 0.448	0.181	-0.343; 0.710
Unsheared	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Temperature	0.112***	0.041; 0.186	NS	-	0.136***	0.061; 0.214
Solar Radiation	-0.003**	-0.005; -0.001	NS	-	-0.003**	-0.006; -0.0007
Wind Speed	-0.711***	-1.001; -0.415	-0.813***	-1.118; -0.511	-0.684***	-1.041; -0.326
Wind Direction	0.002*	0.0004; 0.004	NS	-	0.003*	0.0004; 0.005

APP1.8. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. chiopterus*; pigmented *C. chiopterus,* and blood fed *C. chiopterus* made on sheared and unsheared sheep (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. chiopterus Total Females ~ Linear Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed + Wind Direction

C. chiopterus Pigmented ~ Linear Temporal Trend + Trap + Wind Speed

C. chiopterus Blood Fed ~ Quadratic Temporal Trend + Trap + Temperature + Solar Radiation + Wind Speed + Wind Direction

	Total *Culicoides*	
Parameter	Estimate	95% Confidence Interval
Intercept	5.591***	4.046; 7.118
Temporal Trend		
Linear	0.404***	-0.308; 1.116
Quadratic	-0.019***	-0.022; -0.016
Trap		
Light Trap 1 – Cow Present	-3.054***	-3.803; -2.305
Light Trap 1 – Cow Absent	Baseline	Baseline
Light Trap 2 – Cow Present	-0.960*	-1.394; -0.526
Light Trap 2 – Cow Absent	0.027	-0.377; 0.431
Sheep – Cow Present	4.359***	4.028; 4.69
Sheep – Cow Absent	3.421***	3.095; 3.747
Sweep – Cow Present	4.369***	4.038; 4.7
Sweep – Cow Absent	-1.712**	-2.31; -1.114
Temperature	-0.128*	-0.181; -0.075
Humidity	-0.034**	-0.045; -0.023
Solar Radiation	-0.006***	-0.007; -0.005
Wind Speed	-0.997***	-1.109; -0.885

APP1.9. Regression coefficients and confidence intervals for final model to describe collection of total *Culicoides* using different traps in the presence and absence of cattle (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Script:

Total *Culicoides* ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap Cow Interaction + Temperature + Humidity + Solar Radiation + Wind Speed

	C. obsoletus **Total Females**		***C. obsoletus*** **Un-pigmented**		***C. obsoletus*** **Pigmented**		***C. obsoletus*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	3.671***	2.753; 4.647	2.503***	1.555; 3.509	6.389***	3.908; 8.993	2.257***	1.735; 2.808
Temporal Trend								
Linear	0.229**	0.053; 0.396	0.351***	0.164; 0.531	-0.065**	-0.108; -0.024	NS	-
Quadratic	-0.12***	-0.020; -0.004	-0.017***	-0.026; -0.008	NS	-	NS	-
Trap								
Sheep – Cow Present	1.012***	0.615; 1.414	1.127***	0.724; 1.535	0.707**	0.256; 1.156	0.245	-0.192; 0.622
Sheep – Cow Absent	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Humidity	NS	-	NS	-	-0.035*	-0.064; -0.007	NS	-
Solar Radiation	-0.003***	-0.004; -0.001	-0.003***	-0.004; -0.001	-0.005***	-0.008; -0.002	-0.002*	-0.004; -0.006
Wind Speed	-1.081***	-1.345; -0.821	-1.105***	-1.385; -0.831	-0.83***	-1.179; -0.553	-0.714***	-1.006; -0.427

APP1.10. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. obsoletus*; un-pigmented *C. obsoletus*, pigmented *C. obsoletus* and blood fed *C. obsoletus* made on sheep in presence and absence of cattle (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. obsoletus Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Cow + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. obsoletus Un-pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Cow + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. obsoletus Pigmented ~ Linear Temporal Trend + Cow + Humidity + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. obsoletus Blood Fed ~ Cow + Solar Radiation + Wind Speed, subset (Trap = Sheep)

	C. scoticus **Total Females**		***C. scoticus*** **Un-pigmented**		***C. scoticus*** **Pigmented**		***C. scoticus*** **Blood Fed**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	4.316***	3.232; 5.489	2.933***	1.778; 4.171	7.414***	4.414; 10.419	2.426***	1.833; 3.058
Temporal Trend								
Linear	0.184*	-0.027; 0.377	0.335***	0.120; 0.539	-0.075***	-0.114; -0.035	NS	-
Quadratic	-0.010*	-0.019; -0.0004	-0.016***	-0.025; -0.005	NS		NS	-
Trap								
Sheep – Cow Present	0.567**	0.089; 1.050	0.513*	0.027; 1.005	0.597*	0.136; 1.057	0.423	-0.012; 0.860
Sheep – Cow Absent	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Humidity	NS	-	NS	-	-0.040*	-0.036; 0.285	NS	-
Solar Radiation	-0.03***	-0.004; -0.001	-0.003***	-0.004; -0.001	-0.003*	-0.004; -0.001	-0.002*	-0.004; -0.003
Wind Speed	-1.206***	-1.547; -0.875	-1.155***	-1.491; -0.809	-1.088***	-1.395; -0.782	-0.952***	-1.301; -0.616

APP1.11. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. scoticus*; non-pigmented *C. scoticus*, pigmented *C. scoticus* and blood fed *C. scoticus* made on sheep in presence and absence of cattle (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. scoticus Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Cow + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. scoticus Un-pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Cow + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. scoticus Pigmented ~ Linear Temporal Trend + Cow + Humidity + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. scoticus Blood Fed ~ Cow + Solar Radiation + Wind Speed, subset (Trap = Sheep)

	C. dewulfi **Total Females**		***C. dewulfi*** **Un-pigmented**		***C. dewulfi*** **Pigmented**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	3.381*	0.859; 5.986	3.078*	0.203; 6.070	5.054**	2.172; 7.981
Temporal Trend						
Linear	0.504***	0.271; 0.750	0.584***	0.312; 0.880	NS	-
Quadratic	-0.023***	-0.034; -0.012	-0.026***	-0.040; -0.142	NS	-
Trap						
Sheep – Cow Present	1.197***	0.744; 1.656	1.307***	0.803; 1.820	0.044	-0.458; 0.546
Sheep – Cow Absent	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Humidity	-0.038**	-0.066; -0.010	-0.043**	-0.076; -0.011	-0.051**	-0.082; -0. 020
Solar Radiation	-0.005***	-0.007; -0.002	-0.005***	-0.008; -0.002	-0.006***	-0.010; -0.003
Wind Speed	-0.848***	-1.173; -0.536	-0.894***	-1.265; -0.541	-0.479**	-0.824; -0.149

APP1.12. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. dewulfi*; non-pigmented *C. dewulfi*, pigmented *C. dewulfi* made on sheep in presence and absence of cattle (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. dewulfi Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Cow + Humidity + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. dewulfi Un-pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Cow + Humidity + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. dewulfi Pigmented ~ Cow + Humidity + Solar Radiation + Wind Speed, subset (Trap = Sheep)

	C. chiopterus **Total Females**		***C. chiopterus*** **Pigmented**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	-3.703*	-6.655; -0.703	-2.102	-4.696; 0.537
Temporal Trend				
Linear	-0.086**	-0.138; -0.035	-0.136***	-0.194; -0.081
Quadratic	NS	-	NS	-
Trap				
Sheep – Cow Present	0.710*	0.153; 1.263	0.987**	0.361; 1.611
Sheep – Cow Absent	Baseline	Baseline	Baseline	Baseline
Temperature	0.347***	0.183; 0.510	0.240***	0.102; 0.380
Solar Radiation	-0.004*	-0.007; -0.001	NS	-
Wind Speed	-0.633***	-1.063; -0.213	-0.888***	-1.364; -0.442

APP1.13. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. chiopterus*; *C. chiopterus*, pigmented made on sheep in presence and absence of cattle (*=p<0.05, **=p<0.01, ***=p<0.001, NS=p>0.05)

Model Scripts:

C. chiopterus Total females ~ Linear Temporal Trend + Cow + Temperature + Solar Radiation + Wind Speed, subset (Trap = Sheep)

C. chiopterus Pigmented ~ Linear Temporal Trend + Cow + Temperature + Wind Speed, subset (Trap = Sheep)

Parameter	***C. obsoletus* Blood Fed**		***C. chiopterus* Pigmented**	
	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	2.204***	1.633; 2.809	-3.475**	-6.245; -0.729
Temporal Trend				
Linear	NS	-	-0.143***	-0.201; -0.088
Trap Type				
Sheep 1	Baseline	Baseline	Baseline	Baseline
Sheep 2	0.649*	0.061; 1.240	0.815	-0.122; 1.768
Sheep 3	0.069	-0.534; 0.671	1.241*	0.319; 2.185
Sheep 4	-0.329	-0.968; 0.306	0.286	-0.756; 1.338
Sheep 5	0.351	-0.243; 0.946	1.390**	0.449; 2.363
Cow Present	NS	-	1.001**	0.362; 1.641
Temperature	NS	-	0.273***	0.136; 0.413
Solar Radiation	-0.002*	-0.004; -0.0006	NS	-
Wind Speed	-0.742***	-1.015	-0.899***	-1.379; -0.453

APP1.14.Regression co-efficients for final GLMs to describe collections of blood fed *C. obsoletus* and pigmented *C. chiopterus* females from individual sheep during investigation of influence of cattle presence on biting rate on sheep (*=p<0.05, **=p<0.01, ***=p<0.001)

Model Scripts:

C. obsoletus Blood Fed ~ Trap + Solar Radiation + Wind Speed

C. chiopterus Pigmented ~ Linear Temporal Trend + Trap + Cow + Temperature + Wind Speed

Appendix 2. Supplementary Material For Data Chapter 4

Generalised Linear Models with Parameter Estimates and 95% Confidence Intervals

	Total *Culicoides*		***C. nubeculosus*** **Total Females**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	2.114*	0.882; 4.359	1.810*	0.572; 3.992
Temporal Trend				
Quadratic	-0.001***	-0.002; -0.0005	-0.001***	-0.002; -0.0005
Trap				
500	-0.593	-2.752; 1.636	-0.270	-2.444; 1.971
1,000	Baseline	Baseline	Baseline	Baseline
1,500	2.926***	0.709; 5.244	2.572**	0.385; 4.870
2,000	0.901	-1.419; 2.996	0.870	-1.472; 3.038
2,500	1.787	-0.095; 3.689	1.313	-0.481; 3.100
Light	-0.320	-2.644; 1.952	-0.607	-3.145; 2.715
Location				
Location 1	5.079***	3.122; 7.154	5.343***	3.365; 7.449
Location 2	0.586	-1.455; 2.562	1.107	-0.917; 3.065
Location 3	Baseline	Baseline	Baseline	Baseline
Location 4	-2.134*	-4.508; 0.123	-1.987*	-4.299; 0.232
Location 5	-1.773	-4.097; 0.433	-3.268**	-5.881; -0.889
Location 6	-1.732	-3.925; 0.439	-2.324*	-4.993; 0.150
Wind Speed	-1.242***	-2.028; -0.575	-1.140***	-1.953; -0.430

APP2.1. Regression coefficients and confidence intervals for final models to describe collections of: total *Culicoides* and total females of *C. nubeculosus* in CO_2 baited traps (*=p<0.05, **=p<0.01, ***=p<0.001)

Model Scripts:

Total *Culicoides* ~ Quadratic Temporal Trend + Trap + Location + Wind Speed

C. nubeculosus Total Females ~ Quadratic Temporal Trend + Trap + Location + Wind Speed

	***C. nubeculosus* Total Females**	
Parameter	Estimate	95% Confidence Interval
Intercept	0.193	-0.344; 0.73
Temporal Trend		
Quadratic	-0.0005***	-0.0006; -0.0004
Location		
Location 1	3.570***	3.046; 4.094
Location 2	0.668	0.144; 1.192
Location 3	Baseline	Baseline
Location 4	-0.404	-0.928; 0.12
Location 5	-0.443	-0.967; 0.081
Location 6	-0.543	-1.067; -0.019
Solar Radiation	0.073*	0.041; 0.105

APP2.2. Regression coefficients and confidence intervals for final ANOVA to describe collection of *C. nubeculosus* Females in CO_2 baited traps (*=p<0.05, **=p<0.01, ***=p<0.001)

ANOVA Script:

C. nubeculosus Total Females ~ Quadratic Temporal Trend + Location + Solar Radiation

	Total *Culicoides*		***C. obsoletus* Group** **Total Females**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	0.998	0.068; 2.139	0.619	-0.371; 1.786
Trap				
Light Trap 1	3.880***	2.513; 5.222	4.118***	2.727; 5.492
Light Trap 2	2.107**	0.756; 3.410	2.354***	0.978; 3.692
Pure Breed	1.381*	0.045; 2.654	1.621*	0.256; 2.933
Cross Breed	Baseline	Baseline	Baseline	Baseline
Un-baited Trap	-1.634*	-3.055; -0.347	-1.66*	-3.192; 0.270

APP2.3. Regression coefficients and confidence intervals for final models to describe collections of: total *Culicoides* and total females of *C. obsoletus* group in semiochemical baited traps (*=p<0.05, **=p<0.01, ***=p<0.001)

Model Scripts:

Total *Culicoides* ~ Trap

C. obsoletus Group Total Females ~ Trap

	Total *Culicoides*		***C. obsoletus* Group** **Total Females**	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	-13.777***	-20.521; -6.899	-13.924***	-20.929; -6.807
Temporal Trend				
Linear	0.64***	0.343; 0.942	0.664***	0.354; 0.980
Quadratic	-0.016***	-0.026; -0.008	-0.017***	-0.027; -0.008
Trap				
Light Trap	5.114***	3.744; 6.523	4.965***	3.545; 6.422
Chemical B	-3.286**	-6.425; -1.110	-3.28**	-6.438; -1.066
Chemical C	-3.338**	-6.466; -1.192	-3.339**	-6.484; -1.158
R-octenol	-0.171	-1.594; 1.210	-0.179	-1.653; 1.250
CO_2	-3.732**	-6.880; -1.544	-3.747**	-6.914; -1.521
Blend	Baseline	Baseline	Baseline	Baseline
Temperature	0.648***	0.273; 1.016	0.645**	0.258; 1.025

APP2.4. Regression coefficients and confidence intervals for final models to describe collections of: total *Culicoides* and total females of *C. obsoletus* group in semiochemical baited traps (*=p<0.05, **=p<0.01, ***=p<0.001)

Model Scripts:

Total *Culicoides* ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Temperature

C. obsoletus Group Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Temperature

Appendix 3. Supplementary Material For Data Chapter 5

Generalised Linear Models with Parameter Estimates and 95% Confidence Intervals

	C. obsoletus		*C. obsoletus*		*C. obsoletus*	
Parameter	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval	Estimate	95% Confidence Interval
Intercept	-5.804***	-8.483; -3.145	-6.764***	-9.558; -4.034	-7.365***	-10.280; -4.465
Temporal Trend						
Linear	-0.032***	-0.048; -0.015	NS	-	-0.046***	-0.065; -0.027
Quadratic	0.0002***	0.00009; 0.0003	NS	-	0.0002***	0.0001; 0.003
Trap						
CDC	1.657***	1.031; 2.279	1.669***	1.012; 2.323	1.571***	0.869; 2.243
UV	0.203	-0.444; 0.850	0.126	-0.549; 0.802	0.262	-0.433; 0.961
Blue	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Green	0.612	-0.028; 1.245	0.613	-0.070; 1.297	0.634	-0.054; 1.323
Yellow	-0.172	-0.804; 0.459	-0.202	-0.892; 0.486	0.029	-0.660; 0.719
Red	-3.272***	-4.000; -2.545	-3.641***	-4.481; -2.811	-2.941***	-3.804; -2.090
White	0.555	-0.581; 0.691	0.122	-0.565; 0.809	0.087	-0.600; 0.776
Trap Location						
Position 1	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Position 2	-0.365***	-0.995; 0.264	-0.280	-0.953; 0.393	-0.550	-1.232; 0.131
Position 3	-1.172***	-1.816; -0.528	-0.915**	-1.582; -0.247	-1.400***	-2.102; 0.700
Position 4	-2.288	-2.968; -1.606	-2.674***	-3.402; -1.939	-2.374***	-3.132; -1.619
Position 5	-0.522	-1.170; -0.121	-0.563	-1.246; 0.116	-0.731*	-1.442; -0.028
Position 6	-0.590	-1.236; 0.054	-0.587	-1.266; 0.089	-0.879**	-1.596; -0.166
Position 7	-2.685***	-3.349; -2.017	-2.687***	-3.404; -1.964	-2.819***	-3.564; -2.074
Temperature	0.327***	0.227; 0.428	0.264***	0.170-0.363	0.371***	0.257; 0.489
Humidity	0.063***	0.041; 0.086	0.060***	0.045; 0.091	0.068***	0.045; 0.092
Solar Radiation	NS	-	-11.922**	-19.479; -3.756	NS	-
Wind Speed	-0.481***	-0.803; -0.156	-0.344*	-0.672; -0.012	-0.720***	-1.087; -0.351
Variation Wind Direction	0.015***	0.003; 0.026	0.017**	0.005; 0.028	0.0179***	0.005; 0.029

APP3.1. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. obsoletus*; non-pigmented *C. obsoletus* and pigmented *C. obsoletus* in LED suction traps and CDC trap (*=p<0.05, **=p<0.01, ***=p<0.001)

Model Scripts:

C. obsoletus Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Trap Location + Temperature + Humidity + Wind Speed

C. obsoletus Un-pigmented ~ Trap + Trap Location + Temperature + Humidity + Solar Radiation + Wind Speed + Variation Wind Direction

C. obsoletus Pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Trap Location + Temperature + Humidity + Wind Speed

	C. scoticus		***C. scoticus***		***C. scoticus***	
Parameter	Estimate	95% Confidence	Estimate	95% Confidence	Estimate	95% Confidence
Intercept	-5.683***	-8.661; -2.721	-2.904*	-5.165; -6.040	-7.556***	-10.816; -4.340
Temporal Trend						
Linear	-0.032***	-0.051; -0.012	NS	NS	-0.053***	-0.073; -0.033
Quadratic	0.0002***	0.00008; 0.0003	0.00004*	0.000005; 0.00007	0.0003***	0.0001; 0.0004
Trap						
CDC	2.988***	2.314; 3.662	3.035***	2.328; 3.739	2.993***	2.288; 3.695
UV	0.928**	0.2403; 1.619	0.813*	0.088; 1.538	0.837*	0.113; 1.565
Blue	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Green	0.789*	0.089; 1.486	0.688	-0.046; 1.422	0.821*	0.095; 1.546
Yellow	0.314	0.378; 1.007	0.343	-0.395; 1.080	0.168	-0.564; 0.901
Red	-2.079***	-2.855; -1.305	-2.205***	-3.078; -1.338	-2.224***	-3.112; -1.349
White	0.512	-0.192; 1.216	0.634	-0.114; 1.381	0.339	-0.400; 1.080
Trap Location						
Position 1	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Position 2	-0.448	-1.150; 0.255	-0.148	-0.873; 0.579	-0.609	-1.331; 0.113
Position 3	-1.162***	-1.879; -0.451	-0.993**	-1.707; -0.279	-1.270***	-2.016; -0.529
Position 4	-2.150***	-2.903; -1.401	-0.240***	-3.209; -1.593	-2.171***	-2.962; -1.384
Position 5	-0.579	-1.281; 0.116	-0.475	-1.198; 0.246	-0.548	-1.268; 0.164
Position 6	-0.868**	-1.557; -0.180	-0.704*	-1.413; 0.006	-0.907**	-1.626; -0.191
Position 7	-2.943***	-3.659; -2.226	-2.892***	-3.663; -2.116	-2.807***	-3.573; -2.041
Temperature	0.212**	0.101; 0.324	NS	-	0.300***	0.183; 0.419
Humidity	0.063***	0.039; 0.087	0.046***	0.023; 0.068	0.067***	0.042; 0.093
Solar Radiation	-9.020*	-17.156; -3.035	-16.38***	-24.910; -7.228	NS	-
Wind Speed	-0.462**	-0.805; -0.115	NS	-	-0.617***	-0.993; -0.240
Variation Wind Direction	0.018***	0.0069; 0.0265	-0.013**	-0.002; 0.023	0.021***	0.009; 0.033

APP3.2. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. scoticus*; non-pigmented *C. scoticus* and pigmented *C. scoticus* in LED suction traps and CDC trap (*=p<0.05, **=p<0.01, ***=p<0.001)

Model Scripts:

C. scoticus Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Trap Location + Temperature + Humidity + Solar Radiation + Wind Speed + Variation Wind Direction

C. scoticus Un-pigmented ~ Quadratic Temporal Trend + Trap + Trap Location + Humidity + Solar Radiation + Variation Wind Direction

C. scoticus Pigmented ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Trap Location + Temperature + Humidity + Wind Speed + Variation Wind Direction

	C. dewulfi		*C. pulicaris*		*C. brunnicans*	
Parameter	Estimate	95% Confidence	Estimate	95% Confidence	Estimate	95% Confidence
Intercept	-11.132***	-15.570; -7.277	-2.617	-5.719; 0.477	-14.799***	-20.174; -9.591
Temporal Trend						
Linear	-0.083***	-0.112; -0.056	0.008**	0.002; 0.015	NS	-
Quadratic	0.0005***	0.0003; 0.0007	NS	-	-0.003**	-0.004; -0.002
Trap						
CDC	1.033**	0.220; 1.842	0.957**	0.255; 1.659	1.345***	0.297; 2.403
UV	-1.423**	-2.397; -0.498	-0.788*	-1.572; -0.005	-0.324	-1.368; 0.738
Blue	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Green	0.415	-0.396; 1.222	0.506	-0.236; 1.260	1.000*	-0.031; 2.033
Yellow	-1.434**	-2.392; -0.498	-0.793*	-1.565; -0.028	-0.087	-1.096; 0.919
Red	-4.549***	-7.003; -2.817	-4.743***	-7.697; -3.002	-1.843**	-3.062; -0.614
White	-0.157	-1.019; 0.701	-0.051	-0.791; 0.689	0.291	-0.723; 1.310
Trap Location						
Position 1	Baseline	Baseline	Baseline	Baseline	Baseline	Baseline
Position 2	-1.019*	-1.849; -0.192	-0.867*	-1.566; -0.164	0.914	-0.021; 1.856
Position 3	-2.033***	-2.932; -1.142	-1.088**	-1.812; -0.363	1.017*	0.032; 2.011
Position 4	-2.958***	-4.033; -1.911	-2.958***	-3.886; -2.063	-1.358*	-2.431; -0.278
Position 5	-0.574	-1.381; 0.226	-1.045**	-1.732; -0.361	0.067	-0.948; 1.104
Position 6	-1.268**	-2.133; -0.411	-1.375***	-2.085; -0.665	-0.101	-1.147; 0.949
Position 7	-2.980***	-4.037; -1.955	-3.940***	-4.989; -2.961	-1.892***	-3.134; -0.644
Temperature	0.442***	0.289; 0.602	0.111*	0.005; 0.215	1.159***	0.834; 1.515
Humidity	0.085***	0.056; 0.122	0.029*	0.002; 0.057	0.047*	0.006; 0.087
Solar Radiation	NS	-	-12.651*	-21.910; -3.101	NS	-
Wind Speed	-0.627**	-1.112; -0.145	NS	-	-1.453***	-2.330; -0.636
Variation Wind Direction	0.020**	-0.003; 0.037	0.002*	-0.0002; 0.004	-0.003*	-0.006; -0.00003

APP3.3. Regression coefficients and confidence intervals for final models to describe collections of: total female *C. dewulfi*; total female *C. pulicaris* total *C. brunnicans* in LED suction traps and CDC trap (*=p<0.05, **=p<0.01, ***=p<0.001)

Model Scripts:

C. dewulfi Total Females ~ Linear Temporal Trend + Quadratic Temporal Trend + Trap + Trap Location + Temperature + Humidity + Wind Speed + Variation Wind Direction

C. pulicaris Total Females ~ Linear Temporal Trend + Trap + Trap Location + Temperature + Humidity + Solar Radiation + Variation Wind Direction

C. brunnicans Total Females ~ Quadratic Temporal Trend + Trap + Trap Location + Temperature + Humidity + Wind Speed + Variation Wind Direction