The influence of farm management factors on localized *Culicoides* species on a lowland farm in South-West England

BY

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Declaration

I declare that this dissertation hereby submitted to the University of Pretoria for the degree of Master of Science (Veterinary Tropical Diseases) has not been previously submitted by me for the degree at this or any other University, that it is my own work in design and in execution, and that all material contained therein has been duly acknowledged.

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Abbreviations

BF	Blood fed
BT	Bluetongue
BTV	Bluetongue virus
F	Females
G	Gravid
Hum set	Humidity reading at the time of the trap setting (%)
Hum coll	Humidity reading at the time of the trap collection/removal (%)
N	Nulliparous
Obs	*Culicoides obsoletus*
P	Parous
Prox bedding	Proximity of straw bedding (metres)
Prox cattle	Proximity of cattle (metres)
Prox forage	Proximity of forage store/silage (metres)
Prox manure	Proximity of manure/slurry store (metres)
Prox trees	Proximity of trees (metres)
Prox sheep	Proximity of sheep (metres)
Prox water	Proximity of water source (metres) (includes water trough, stream, ditch
Puli	*Culicoides pulicaris*
T coll	Temperature °C at the time of the trap removal
T max	Maximum temperature °C during the trapping period
T min	Minimum temperature °C during the trapping period
T set	Temperature °C at the time of the trap setting
Tr	Trapping
TSCC (days since contact cattle)	Time, in days, since contact with cattle (within 100 metres of the trap location)

TOCC (days of contact cattle)	Time, in days, of contact with cattle overnight (within 100 metres of the trap location)
TSCS (days since contact sheep)	Time, in days, since contact with sheep (within 100 metres of the trap location)
TOCS (days of contact sheep)	Time, in days, of contact with sheep overnight (within 100 metres of the trap location)
U.K.	United Kingdom
Wind sp. set	Wind speed at the time of the trap setting (m/s)
Wind sp. coll	Wind speed at the time of the trap collection/removal (m/s)

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Abstract

The influence of farm management factors on localized *Culicoides* species on a lowland farm in South-West England

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A survey of the localised distribution of *Culicoides obsoletus* and *Culicoides pulicaris* was performed on a dairy and sheep farm in south-west England. *Culicoides obsoletus* and *C. pulicaris* have both been confirmed as vector species for the transmission of bluetongue virus in Europe. Sampling was done using motorised black-light suction insect traps. Seventeen traps were set around the farmyard and animal housing and five traps were set in varying pasture locations. Sampling was carried out on eight occasions between mid-September and mid-October 2008. The trapped *Culicoides* were counted, speciated, sexed and the reproductive stages of the females were recorded. *Culicoides obsoletus*, *C. chiopterus*, *C. scoticus*, *C. dewulfi* and *C. pulicaris* (group) were identified during the study. The trap sites were selected to examine the *Culicoides* populations associated with a wide range of microclimates. The selected sites included manure stores; forage feed stores; yard areas and sites surrounding as well as inside the animal housing. Comparisons were made between *Culicoides* numbers trapped from different directional sides of the animal buildings and the numbers found inside compared to numbers found outside the buildings. *Culicoides* numbers collected from the animal areas were compared to the non-animal areas and to the manure and forage sites. The field sites consisted of a marsh area; stream; water trough; open field site and a group of trees in a hedge field boundary. *Culicoides* catch sizes from the field trap sites were

compared to each other and to the farm holding sites. The highest number of *Culicoides* trapped were at the farm holding sites, apart from one catch on one occasion from a single field site.

Weather changes, particularly high wind speeds with direction changes appeared to reduce the catch sizes during some of the trapping occasions. A greater number of *C. obsoletus* were collected from both the farm and field sites although a higher relative proportion of *C. pulicaris* was collected from the field sites. Of the *C. obsoletus group*, *C. dewulfi* was only found in farm holding catches, not at any of the field sites. The remaining three sibling species were found in both the farm and field catches. Relatively high numbers of *Culicoides* were found within the animal housing, with external numbers apparently influencing those found within the housing. An increase in numbers of *Culicoides* trapped inside the buildings may have been associated with a small shed size and possibly with straw bedding. A relative shift in the *Culicoides* population into the buildings appeared associated with prolonged high wind speeds.

Widely varying female life stages found at all of the farm trap sites suggested possible dispersal of the *Culicoides* populations between these sites. Populations appeared to remain localised around the farm holding, but possibly dispersed over greater distances from the pasture locations. A wide distribution of breeding sites was suspected around the farm holding. A ranking system was used to identify specific areas associated with increased numbers of female *Culicoides* collected from these sites. Three sites surrounding a straw bedded cow shed were highlighted as higher risk *Culicoides* exposure sites; two sites adjacent to a cubicle shed; inside the calf housing; the manure store area and the silage store area. A field site with trees in a hedge boundary was the only high-risk field site identified.

Multilevel modelling was used to examine for possible factors influencing *Culicoides* numbers. Factors examined included wind, temperature and humidity variables; distance from manure, forage, water and trees and livestock variables such as: time of contact, time since contact and distance from sheep and cattle. The model suggested wind speed at light trap setting and an increased time since contact with cattle both appeared significantly associated with reduced *Culicoides* numbers. *Culicoides obsoletus* numbers also appeared significantly reduced with increasing distance from manure. From an on-farm risk assessment point of view the farm holding area of a dairy farm as a whole should generally be considered a high-risk site for *Culicoides* exposure and specific pasture sites can periodically become high exposure sites.

Chapter 1

Literature review

1.1 Introduction

Bluetongue virus (BTV) is a non-contagious, insect-transmitted disease of domestic and wild ruminants. Bluetongue virus causes disease predominantly in sheep, but also can also infect cattle, goats and camelids (Radostits, Gay, Hinchcliff and Constable, 2007). The disease is characterized by a high fever, catarrhal inflammation of the oral mucous membranes, rhinitis, enteritis, widespread haemorrhages and oedema in affected animals (Radostits *et al*., 2007), resulting in swelling particularly of the lips, tongue and head and lameness due to muscle necrosis and coronitis. Twenty four serotypes of the virus have so far been identified worldwide (Savini, Goffredo, Monaco, Di Gennaro, Cafiero, Baldi, de Santis, Meiswinkel and Caporale, 2005) and often several serotypes will circulate together. The virulence varies widely between serotypes, resulting in varying severity of disease (Radostits *et al.*, 2007). Due to the significant impact of the disease on the global trade of ruminants and their germplasm there are serious socio-economic implications for countries where the infection occurs. Due to the economic impact of the disease, BT is one of sixteen diseases classified as a serious notifiable disease by the Office International des Epizooties (OIE).

When BT is introduced into an area with naive or unvaccinated livestock, the disease incidence can reach 50-75% in sheep, with mortality rates potentially anywhere between 20-50% or even up to 70%, as reported during BT outbreaks in Cyprus and Spain (Radostits *et al.*, 2007). The incidence of clinical disease due to BT is higher in the "improved" sheep breeds commonly farmed in Europe (Darpel, Batten, Veronesi, Shaw, Anthony, Bachanek-Bankowska, Kgosana, bin-Tarif, Carpenter, Müller-Doblies, Takamatsu, Mellor, Mertens and Oura, 2007). Losses due to BT result not only from trade restrictions and livestock deaths, but also from lost production due to embryonic deaths, abortions, malformed foetal calves and lambs and transient infertility of bulls and rams (Osburn, 1994). Other important production losses include livestock body condition loss and prolonged recovery periods, reduced milk yield, as well as muscle necrosis (potentially leading to carcase down-grading) and fleece loss (Radostits *et al.*, 2007) as well as veterinary costs (Wilson and Mellor, 2008). The BTV-8 outbreak in northern Europe resulted in the death of approximately 12.5% of the national sheep flock in Belgium during 2007, and costs to the cattle and sheep industries in Europe have been estimated at £100 million (Gloster, Burgin, Witham, Athanassiadou and Mellor, 2008). Although BTV-8 in northern Europe has resulted in less than 10% of animals on affected holdings showing clinical disease, the mortality rate of affected sheep has reached between 30 and 50% (Darpel *et al.,* 2007). Less than 10% of cattle in BTV-8 affected herds have been reported as clinically ill, however, the case fatality rate of these cases has been up to 10% in some countries (Darpel *et al.,* 2007). A case fatality rate of 41.5% in sheep and of 13.1% in cattle was reported during the 2007 outbreak in Germany. (Conraths, Gethmann, Staubach, Mettenleiter, Beer, Hoffmann and 2009).

1.2 Bluetongue virus distribution-worldwide and in Europe

Bluetongue virus is found primarily in areas of the world situated between the latitudes of 40 ^{o}N and 35 ^{o}S, which includes Africa, Middle East, Asia, Australia, North and South America (Radostits *et al.,* 2007; Sewell and Brocklesby, 1990; Dungu, Gerdes, Smit, 2004). Bluetongue virus has from 1924 regularly caused outbreaks in Cyprus, and prior to the 1998 BT outbreak, had otherwise caused only two outbreaks in Europe and so was considered an exotic disease (Wilson and Mellor, 2008). Bluetongue virus first become established in Europe during the BTV epidemic in the Mediterranean Basin between 1998 and 2005. At least five BTV serotypes, serotypes BTV-1, BTV-2, BTV-4, BTV-9 and BTV-16 were involved (Saegerman, Berkvens and Mellor, 2008). Bluetongue virus gradually spread causing outbreaks in many southern European countries up until 2005, by which time twelve countries had been involved (Purse, Mellor, Rogers, Samuel, Mertens and Baylis, 2005). The epidemic provided a clear illustration of the serious consequences of BT in newly introduced areas, with an estimated 1.5 to 2 million sheep thought to have died (Purse *et al.,* 2005). This epidemic was unprecedented in terms of the extent of disease spread. The introduction of BT into southern Europe was attributed to a change in the northern-most distribution of *Culicoides imicola*, an Afro-Asiatic species of *Culicoides* midge (Purse *et al.,* 2005). The incursion into southern Europe appeared to result from a northerly extension of the distribution range of the major BTV vector *C. imicola,* from northern Africa. As the distribution of *C. imicola* is largely influenced by temperature, the expansion in the range of *C. imicola* was believed to have occurred due to climate change related rising temperatures and in particular warming of winter temperatures (Purse *et al.,* 2005). The Mediterranean Basin epidemic provided the first evidence of *Culicodes* midges, other than *C. imicola,* that could function as effective BTV vectors within Europe. The first indication followed the detection of BT beyond the northern-most extent of the range of *C. imicola* (Mellor and Wittmann, 2002). There was also evidence during this outbreak of an apparent ability for BTV to overwinter in the affected countries (Mellor and Wittmann, 2002).

Bluetongue virus serotype-8 was detected for the first time in Europe following testing of clinically affected livestock on the Begian/Dutch/German border during August 2006, and subsequently the infection became established and widespread across northern Europe (Gloster *et al.,* 2008; Wilson and Mellor, 2008). BT was thought to have been circulating for ten weeks prior to detection in sheep showing typical clinical signs (Darpel *et al.,* 2007). New cases were continually detected in the Netherlands, Belgium and Germany up to December 2006, and then successful overwintering of infection resulted in new cases in June 2007 (Gloster *et al.,* 2008). By November 2007 more than 45000 holdings in Europe were affected, with cases reported in the Netherlands, Belgium, Luxemburg, Germany, northern France, Denmark, Czech Republic, Switzerland and there was the first confirmation of BTV-8 in the United Kingdom (U.K.) during September 2007 (Gloster *et al.*, 2008).

The U.K. index cases were clinically affected cattle in South East England. Wind-borne introduction of infected *Culicoides* midges from the mainland of continental Europe was thought to have introduced BT infection during early August 2007 following analysis of wind data (Gloster *et al.,* 2008). There were in total 65 farms in the U.K. that were confirmed with BT infection or exposure to BT infection during the outbreak (Gloster *et al.,* 2008) which rose to 127 farms before the end of the year following pre-movement testing (Burgin, Gloster and Mellor, 2009). There appeared to be no subsequent over-wintering of the infection in the U.K., with no new natural cases detected during 2008 (Burgin *et al.,* 2009). Several BT positive results from animals tested in the U.K. in 2008 were from imported animals only (Burgin *et al.,* 2009).

Two other BTV serotypes, BTV-1 and BTV-6 were identified in northern Europe by the end of the midge season in 2008 (Burgin *et al.,* 2009). Bluetongue virus-1 had spread rapidly through France to reach Brittany on the north coast, the northerly spread possibly following livestock movements and an overlap between *C. imicola* and the northern European *Culicoides* species ranges, allowing transfer of infection to new *Culicoides* vector species via infected ruminants. BTV-1 is of particular concern as it is considered to have a high pathogenic index and a high epidemic potential (Dungu *et al*., 2004). Bluetongue virus-6 was found in the Netherlands and in Germany and BTV-11 was also identified in Belgium during February 2009. The source of BTV-6 and BTV-11 is still uncertain. These newly occurring serotypes pose a significant threat to the U.K., as only BTV-8 vaccination is currently practiced within the U.K.

The risk of re-introduction to the U.K. of BTV-8 or the introduction of other newly emerging BTV serotypes during 2009 and subsequent years should be considered high. Introduction could occur either via imported infected animals or by wind-borne carriage of infected midges across the channel. Imported cows found to have carried congenitally infected BTV positive calves were confirmed in Northern Ireland illustrating a new potential method for disease introduction (Menzies, McCullogh, McKeown, Forster, Jess, Batten, Murchie, Gloster, Fallows, Pelgrim, Mellor and Oura, 2008).

1.3 Bluetongue virus transmission

Although BTV can be transmitted vertically and venereally, these methods of transmission are considered relatively unimportant in comparison to transmission by insect vectors (Maclachlan, 1994). Mechanical transmission by *Ornithodoros* ticks, *Melophagus* sheep keds and *Aedes* and *Anopheles* mosquitoes has been reported but appears relatively unimportant (Radostits *et al.,* 2007). The major insect vectors responsible for BTV transmission are from the Ceratopgonidae family, genus *Culicoides* (Mellor, Boorman and Baylis, 2000). Due to the importance of the *Culicoides* vectors for transmission of the disease, the distribution of BTV coincides with the distribution of competent vector species (Maclachlan, 1994). Various *Culicoides* species have been identified as potential BTV vectors, and will function as biological vectors, that is, the virus replicates within the insect vector after infection, following blood-feeding on infected, viraemic ruminant hosts of the virus (Mellor, 2000). Transmission occurs when an infected female *Culicoides* then blood feeds on a susceptible mammalian host. Only female *Culicoides* will blood feed and function as virus vectors (Kettle, 1990).

Countries with well established BT have recognized effective *Culicoides* BTV vectors, which will often transmit several or numerous different serotypes concurrently. *Culicoides sonorensis* is the major BTV vector in the United States and *C. insignis* is one of the major vectors in the Caribbean and Central/South America (Maclachlan, 1994) and *C. pusillus* is considered the other major vector species. *Culicoides imicola* is considered a major BTV vector in Africa, the Middle East, Asia and now also in southern Europe, although other potentially significant species may still remain to be identified (Mellor *et al.,* 2000). In Australia there are four important BTV vector species, *C. fulvus, C. wadai, C. actoni* and *C. brevitarsis*.

1.4 *Culicoides* vectors of bluetongue virus in Europe

During the Mediterranean Basin BT epidemic it appeared *C. obsoletus* and *C. pulicaris* group midges were functioning as BTV vectors (Mellor and Wittman, 2002). Midge species from the *C. obsoletus* and *C. pulicaris* species groups are found in the U.K. (Tweddle, 2002) and are common and widespread across the whole of central and northern Europe (Baldet and Delecolle, 2007). Several studies have confirmed BTV infection in both of these *Culicoides* species groups, both in the field and in the laboratory (Caracappa, Torina, Guercio, Vitale, Calabro, Purpari, Ferrantelli, Vitale and Mellor, 2003; De Liberato, Lorenzetti, Amaddeo, Cardeti, Scicluna, Ferrari and Autorino, 2005; Savini *et al.*, 2005; Carpenter, Lunt, Arav, Venter and Mellor, 2006). Bluetongue virus has been isolated from field collected *C. obsoletus* and *C. pulicaris* in Italy (Caracappa *et al.,* 2003; De Liberato *et al.,* 2005; Savini *et al.*, 2005) and these species were shown able to be experimentally infected with BTV serotype 9 in a study using *Culicoides* midges taken from the field population in the U.K. (Carpenter *et al.,* 2006). Bluetongue virus-8 positive *C. dewulfi* (midges of the *C. obsoletus* species group) were identified following midge trapping and testing during the BTV outbreak in Gulpen in the Netherlands (Meiswinkel, Van Rijn, Leijs and Goffredo, 2007). *Culicoides obsoletus* infected with BTV-8 were also confirmed in field collected midges in central Europe (Mehlhorn, Walldorf, Klimpel, Birgit, Friedhelm, Eschweiler, Hoffmann and Beer, 2007). Experimentally *C. obsoletus* is able to replicate BTV to high titres following intrathoracic inoculation of the virus (De Liberato, Scavia, Lorenzetti, Scaramozzino, Amaddeo, Cardeti, Scicluna, Ferrari and Autorino, 2005). Studies have also shown that members of the *C. obsoletus* complex may be able to transmit multiple BTV serotypes, but their efficacy at transmitting many of the serotypes is still unknown and may vary between serotypes. For example the competency of *C. obsoletus* complex midges for transmitting BTV serotype-4 in some studies appeared reduced (Savini *et al.*, 2005; De Liberato *et al.*, 2005). It has been suggested, however, that the phylogenetic similarities between *C. obsoletus* and *C. imicola* may result in a genetic predisposition and therefore similarities between these two species for effective BTV replication, and for a comparable function as BTV biological vectors (Savini *et al.*, 2005). Oral susceptibility levels of members of the *C. obsoletus* complex that are equivalent to *C. imicola* have been shown in the laboratory (Carpenter *et al.,* 2006).

An epidemiological study examined the *Culicoides* midge populations found in association with livestock in the 2006 BT affected region in north-eastern France (Baldet and Delecolle, 2007). During the study Onderstepoort-type blacklight traps with a downdraught suction motor were used on fourteen cattle and sheep farms, to determine the range of different *Culicoides* midge species present. A wide variety of *Culicoides* midges were identified, with the species composition of the catches varying marginally between the different trapping sites. The catches were dominated by *C. obsoletus* complex species midges, namely *C. obsoletus* and *C. scoticus* with *C. dewulfi* also found but in lower numbers. *Culicoides pulicaris* was the next most numerous species trapped. During the study *C. nubeculosus* midges were also identified, and were suggested as potential BTV vectors in northern Europe, although no BTV infection was found in this species during 2006. *Culicoides nubeculosus* is often abundant near livestock and has been shown to competently transmit BTV in the laboratory (Baldet and Delecolle, 2007). Due to the close geographical proximity of this region in France to the U.K., the information from this study has many applications to the likely distribution of vectors found in the southern regions of the U.K.

1.5 Influence of *Culicoides* vectors on bluetongue virus transmission

Due to the close correlation between the *Culicoides* vector distribution and the risk of BTV transmission to susceptible livestock, assessments of the risk of livestock exposure to BTV are likely to be largely influenced by the level of contact of livestock with infected BTV vector *Culicoides* species. Factors affecting the vector *Culicoides* species population size, such as breeding potential, midge survival, the potential for and factors affecting midge blood-feeding activity and also factors affecting the midge viral vector competence therefore could be considered when determining the BT disease risk for livestock.

1.6 *Culicoides* population size

1.6.1. *Culicoides* breeding sites and lifecycle

Culicoides lays eggs in batches in suitable breeding sites, the breeding site preferences varying greatly between the different *Culicoides* species (Kettle, 1990). Larvae hatch from the eggs and there are four larval instars before pupation occurs, after which the winged adults emerge. *Culicoides* larvae, of species such as *C. nubeculosus*, have been found to feed on the surface bacterial film, algal and fungal growth suggesting *Culicoides* larvae are able to feed on a range of organisms in their environment during development (Kettle, 1990). *Culicoides* adults are believed not to hibernate or aestivate, and so the largest proportion of the *Culicoides* lifespan is spent in the larval stages (Kettle, 1990). The egg and pupal stages are of a relatively short duration for all *Culicoides* species (Kettle, 1990). The duration of the larval period varies greatly between *Culicoides* species, with the dung breeding species spending, during ideal developmental conditions, approximately two to three weeks in the larval stages (Kettle, 1990). The development of *C. obsoletus* from egg to the mature midge is temperature dependant and takes at least three weeks, with the egg stage lasting between 2-9 days, the larval stage 14-25 days and the pupal stage lasts about 3-10 days (Mehlhorn *et al.*, 2007). The larval stages of temperate bogland species such as *C. impunctatus* can last for as long as a year. Most *Culicoides* females require a blood feed for egg development (Kettle, 1990).

Culicoides typically breed in wet soil or decaying vegetable matter (Kettle, 1990). The larvae rarely swim freely in water (Kettle, 1990) and have been found in the top-layer of permanently non-submerged environments (Zimmer, Haubruge, Francis, Bortels, Simonon, Losson, Mignon, Paternostre, Deken De, Deken De, Deblauwe, Madder, Fassotte, Cors and Defrance, 2008). *Culicoides pulicaris* has been found breeding in vegetated swamps where the water level is above the soil surface level (Kettle, 1990) and seems to prefer breeding in wet soil and marshy areas (Tweddle, 2002). *Cullicoides impunctatus* breed in peaty areas (Kettle, 1990). *Culicoides dewulfi* and *C. chiopterus* breed in dung, particularly cattle dung (Kettle, 1990). The dependence on cattle dung for suitable breeding sites therefore may significantly influence the distribution of these two species. The larvae of both species have been found overwintering in cow dung during a *Culicoides* breeding site study in Belgium (Zimmer *et al.*, 2008). The influence of differing cattle densities, cattle management practices and maybe soil types were suggested by Baldet and Delecolle (2007) as potential factors resulting in relatively low numbers of *C. dewulfi* found on northern France holdings, when compared to a similar study carried out in Gulpen in the Netherlands. *Culicids obsoletus* will breed in a wide range of habitats including marshes, swamps, leaf litter and organically enriched areas of stable habitats (Carpenter, Szmaragd, Barber, Labuschagne, Gubbins and Mellor, 2008) and prefers damp soil, composted organic material and manure heaps as breeding sites (Tweddle, 2002). Abundant numbers of larvae, later identified following adult emergence as *C. scoticus,* were found in maize silage residues during a study on breeding sites in Belgium, along with numerous *C. obsoletus* (Zimmer *et al.*, 2008). Certain fungi have been suggested as potential breeding sites for *C. scoticus* (Kettle, 1990). Potential breeding sites for *Culicoides* midges were examined in a study in Florida U.S.A. Sites such as mud contaminated by milking parlour effluent, waste lagoons, muddy areas in pastures and vegetated pond margins were identified as productive *Culicoides* midge breeding sites (Kline and Greiner, 1985). As the individual *Culicoides* species tend to have specific preferences for breeding sites, the availability of suitable breeding sites where livestock are kept may significantly influence the local midge population and the likelihood of contact between different midge species and livestock.

Culicoides breeding may be influenced by both photoperiod and temperature effects, which appear to significantly effect midge development (Baldet and Delecolle, 2007). Both factors potentially determine the seasonality of midge emergence and the timing of seasonal peaks in midge populations. The duration of the midge ovarian cycle is influenced by temperature, and can be determined due to red pigment colouration of the abdomen which appears during the first ovarian cycle (Kettle, 1990. The duration of the ovarian cycle is four days for members of the *C. obsoletus* group during the summer in southern England (Birley and Boorman, 1982; Kettle, 1990).

In temperate areas many *Culicoides* species have just one generation a year and the the majority of adults emerge during the summer (Kettle, 1990). *Culicoides obsoletus* was found to have two generations in one study in the north of England (Kettle, 1990) and in southern England there can be three generations with a generation time of seven weeks (Birley and Boorman, 1982). This differs from the population dynamics of *Culicoides* species found in BT endemic non-European countries, where the *Culicoides* vector species present can have numerous generations annually (Kettle, 1990). *Culicoides variipennis* can have numerous generations a year and in some cases will be present all year round, (Barnard and Jones, 1980). Environmental conditions that increase numbers of *Culicoides* midges are likely to optimise the transmission rate of BTV (Maclachlan, 2004).

In general *Culicoides* disperse only short distances from the breeding site, usually not more than 500 metres and potentially remain within 70 metres of it (Kettle, 1990) unless wind transport them over longer distances. The availability of mammalian hosts may influence the population density at the breeding site, and a tendency for midges to disperse may occur due to a lack of vertebrate hosts for blood-feeding (Kettle, 1990).

1.6.2. *Culicoides* survival

The efficacy of BTV transfer is directly influenced by the frequency of blood meals and therefore potentially by the daily survival rate of the midge vector. *Culicoides obsoletus* was found in a study by Birley and Boorman (1982) to have a daily survival rate of 0.74 and to have a four day interval between blood meals. This provided a figure of 30% survival from meal to meal.

The lifespan of adult *Culicoides* has not been precisely measured and appears to vary according to ambient temperature (Kettle, 1990; Baldet and Delecolle, 2007). A lifespan of between 1-3 months is quoted for some *Culicoides species* (Mellor *et al.*, 2000) and *Culicoides* appear to survive longer in cool temperatures (Mullens, Tabachnick, Holbrook and Thompson, 1995). The lifespan is likely to be less than 10-20 days in most cases but in rare cases can be up to 44-90 days (Mellor *et al.,* 2000) and during this time females can take multiple blood feeds. In colder climate areas many *Culicoides species* overwinter as a diapause larval stage (Mellor *et al.,* 2000).

1.6.3. *Culicoides* blood-feeding activity

It is only the female *Culicoides* that feeds upon blood, to fuel egg development. A female may blood-feed every 3-4 days if a mammalian host is available (Birley and Boorman, 1982). Female *Culicoides* feeds on nectar but this energy source does not enable egg development. The peak times for feeding activity of *Culicoides* is typically around sunset and sunrise, behaviour possibly influenced by light intensity (Kettle, 1990) and biting generally continues through the night. Daytime biting activity occurs with some species (Savini *et al.,* 2005), particularly if there are overcast conditions or in focal microclimates. Preliminary results from a study in France suggest there could be diurnal activity of *C. obsoletus/scoticus*, *C. dewulfi* and *C. chiopterus* and therefore there may be significant daytime biting activity on mild autumn or winter days (Balenghien, Cêtre-Sossah, Grillet, Delécolle, Mathieu and Baldet, 2008). Environmental factors also may potentially alter behaviour, for example *C. impunctatus* can become active at anytime if a host arrives in the vicinity of unfed resting females (Kettle, 1990).

1.6.4. Vector competence

Once infected with BTV in the *Culicoides* vector persists for the entire life-span of the midge (MacLachlan, 2004). Higher ambient temperatures will enhance and accelerate virus replication and development in the midge vector, and will stimulate insect activity resulting in increased feeding rates (Mullens *et al.* 1995). Lower ambient temperatures may inversely influence the BTV transmission efficacy of temperate region *Culicoides* vectors. On-ward transmission from infected vector insects to susceptible ruminants can only happen following an incubation period in the *Culicoides* host of 10-14 days, when the virus is disseminated from the insect intestines to the salivary glands (MacLachlan, 2004). This incubation period is applicable at about 25 °C, at which point sufficiently large numbers of transmittable virus particles will have been produced inside the female midge (Mellor, 1990). The incubation period is shortened at higher ambient temperatures. Laboratory trials suggest temperatures of between 27-30 °C are optimal for BTV replication within the midge vector and temperatures of less than 9-15 °C can inhibit replication of BTV in the midge, depending on the strain of BTV involved (Mellor, 2000; Wittmann, Mellor and Baylis, 2002; Paweska, Venter and Mellor, 2004; Purse *et al*., 2005).

Following light trapping of *C. obsoletus* complex midges in BTV outbreak regions in Italy, relatively low BTV infection rates were detected in the trapped midges (Savini *et al.,* 2005; De Liberato *et al.*, 2005). Savini *et al.* (2005) found midge infection rates of at least 0.02% for BTV-2, and De Liberato *et al.* (2005) found an infection rate of at least 0.22%. Infection rates of 0.51% were recorded by Caracappa *et al.* (2003) in *C. pulicaris* midges trapped during a BTV outbreak in Italy. Although the infection rates for both *C. obsoletus* and *C. pulicaris* were relatively low in these studies, a high population density was thought to compensate, resulting in significant BTV transmission and disease rates in animals in the outbreak regions (De Liberato *et al.*, 2005). The highest sheep mortality was found associated with the highest BTV infected *C. obsoletus* complex midge prevalence (Savini *et al.*, 2005).

1.7 Effects of climate and season on adult *Culicoides* population numbers and midge activity and bluetongue epidemiology

The epidemiology of BT appears closely linked to climate and weather, with influences on both the seasonal and spatial distribution of disease, which in turn appears influenced by the seasonal fluctuations in *Culicoides* midge numbers (Radostits *et al.,* 2007). The adult midge population in the U.K. peaks in May or June, and then peaks again in September, with midge numbers falling from mid to late October (Tweddle, 2002). By December adult midges are either not detectable or are only present in low numbers until the following April or May (Tweddle, 2002). This specific period of seasonal midge activity appeared to influence the temporal incidence of BT in the U.K. during 2006, and may prove significant for disease control if BT becomes established. A study in central Europe indicated *C. obsoletus* activity can persist, with engorged females detected, until late December in comparison to the previously quoted midge season from Mach until October (Mehlhorn *et al.*, 2007).

In the Mediterranean Basin BT cases are usually first identified during the late summer and autumn, when the midge vector population is large and the population consists predominantly of old females (Baldet and Delecolle, 2007). Older female *Culicoides* are thought to be the most effective BTV hosts for both virus replication and transmission (Baldet and Delecolle, 2007). Bluetongue virus-8 appears to have had a similar seasonal occurrence in northern Europe, with most clinical cases occurring in late summer and autumn. In Germany during 2006 cases were initially detected during late August, with a peak incidence during mid-October, and a slow decline in cases until the end of the year (Conraths *et al.*, 2009). The first clinical cases during the following year were then detected during June 2007 (Burgin, Gloster and Mellor, 2009).

Populations of *Cuicoides* midges can rapidly change depending on weather conditions. In the Baldet and Delecolle epidemiological study (2007) in France the midge population rapidly increased during early September 2006. Baldet and Delecolle (2007) found that midge populations increased three fold when midge numbers trapped during September were compared to midge numbers at the end of August 2006. During August lower temperatures and heavy rains may have reduced larval production and heavy rain in particular was thought to have reduced the numbers of adults, due to flowing water disrupting breeding sites, reduced egg hatching and slowed larval development. Heavy rain may also have reduced adult activity and survival. In the northern European outbreaks, although the peak BTV incidence occurred at the same time as the peak midge populations, some new cases have been detected after December. This may have resulted from residual infected midges remaining active in focal locations with conditions favourable for midge survival and activity (Baldet and Delecolle, 2007).

1.7.1 Temperature

Ambient temperatures will strongly influence the geographical distribution of some *Culicoides* species, such as *C. imicola* (Wittman *et al*., 2001). Ambient temperatures can also influence the localized midge populations and midge activity of other *Culicoides* midge species. Although *C obsoletus* is most active at temperatures between 7 and 16 °C, it can still remain active at temperatures as low as 3.5 °C (Savini *et al.,* 2005), Baldet and Delecolle (2007) found that when the minimum ambient temperature was below 5 °C and the maximum temperature was below 10 °C no midges were caught in outdoor midge traps. It is thought low ambient temperatures may have two affects, one of reducing the flight activity and survival of adult *Culicoides* midges and secondly low ground temperatures may prevent *Culicoides* larval development (Wittmann and Baylis, 2000). Low ambient temperatures also reduce the vector competence of some *Culicoides* species, by reducing viral replicators within the midge vector, as discussed previously (Wittmann *et al.,* 2002; Paweska *et al*., 2004). As midge activity is affected by ambient temperatures, temperature can influence the timing of disease risk periods and the potential for disease persistence (overwintering) in newly introduced areas. If ambient temperatures during the winter period prevent adult survival, persistence of BTV may be prevented, as studies suggest vertical transmission of the BTV does not occur in *Culicoides* midges (Nunamaker, Sieburth, Dean, Wigington, Nunamaker and Mecham, 1990). BTV persistence is therefore dependent on adult midge carriage or mammalian host infection. The relationship between BTV infection distribution and *Culicoides* midge distribution using the thermal requirements for BTV transmission during winter has been studied (Sellers and Mellor, 1993).

Baldet and Delecolle (2007) found on study farms in northern France, when ambient temperatures reached a minimum temperature of less than 10 °C and a maximum temperature of less than 15 °C, there were four times more *C. obsoletus* and *C. scoticus* midges found inside the animal buildings than outside. Meiswinkel, Baylis and Labuschagne (2000) also found *Culicoides* species midge numbers inside animal buildings will increase when outside temperatures fall. Housing therefore may in some circumstances potentially increase the contact rate between livestock and BTV midge vectors. Baldet and Delecolle (2007) suggested, however, there were unlikely to be productive breeding sites within livestock buildings, as the indoor trapping rate appeared to fluctuate relative to ambient temperature. The presence of animals inside housing also may influence midge activity within buildings (Meiswinkel *et al.,* 2000). The potential significance of *Culicoides* activity within housing needs consideration as a risk for the winter transmission of BTV in temperate areas (Wilson, Carpenter, Gloster and

Mellor, 2007). The indoor biting activity may require all year round monitoring, along with factors such as temperature, rainfall as well as shed typology (Baldet and Delecolle, 2007). During a typical winter in the U.K. there are likely to be days when temperatures are warm enough for midge activity and possibly even for virogenesis to occur (Gloster *et al.,* 2008) potentially allowing continued BTV transmission. Baldet and Delecolle (2007) also suggested adult midges with residual BTV infection could overwinter in housing, perhaps leading to overwintering of BTV via infected midges, rather than from continuing BTV transmission through midge biting activity. Another possible means of BTV overwintering in Europe includes the potential for extended virus carriage by some ruminant hosts from the previous season, although this has not been reported in the field (Gloster *et al.,* 2008).

1.7.2 Wind speed

Wind speed is thought to affect the abundance of *Culicoides* midges, as high wind speeds have been associated with reduced adult midge activity and increased midge mortality (Baylis and Rawlings, 1998). The number of *Culicoides* reduces and there is usually little midge activity at wind speeds above 2.5 m/s, although activity can still be seen at higher wind speeds with some species (Kettle, 1990). In studies where light traps are used this could be due to reduced efficacy of the light traps during windy conditions.

1.7.3 Humidity and precipitation

Precipitation/rain appears to significantly reduce midge activity although this effect has not been directly quantified (Gloster *et al.*, 2008; Mellor *et al.*, 2000). A mid-range relative humidity, of between 75-85% is thought ideal for midge survival and activity (Wittmann *et al.*, 2002) as at lower humidity levels there is a risk midges may desiccate, and at higher levels midge saturation may result. Humid, calm and warm conditions are most conducive for adult midge activity (Carpenter *et al*, 2008).

1.8 Microclimates and their influence on adult *Culicoides* activity for bluetongue epidemiological assessments

Climate has been used to predict the activity of *Culicoides* midge vectors in several different countries, to model the spread of BTV infection to susceptible livestock (Wittmann *et al.*, 2001; Calistri Goffredo, Caporale and Meiswinkel, 2003; Baylis Tatem, Mellor, Purse, Rogers, Braverman, Van Ham and Chizov-Ginzburg, 2004). Models for predicting BTV distribution that incorporate temperature and moisture variables are successful on a country and regional level, but are only moderately effective at a farm level, as demonstrated in Sicily (Baylis, Mellor, Wittman and Rogers, 2001; Tatem, Baylis, Mellor, Purse, Capela, Pena and Rogers, 2003). Soil type and local vegetation types have been implicated as important factors affecting the distribution and population of some of the major BTV vector *Culicoides* species, including *C. imicola* and *C. pulicaris* (Purse *et al*., 2004).

The effect of insect microhabitats on *Culicoides* distribution has been considered in relation to vegetation biomass, chlorophyll abundance and light absorption as well as vegetation coverage and productivity, which have been correlated with soil moisture and rainfall (Purse, Tatem, Caracappa, Rogers, Mellor, Baylis and Torina, 2004). Surface temperature and the structure of vegetation canopies have also been considered. These factors possibly influence midge distribution due to the availability of *Culicoides* midge breeding sites (Purse *et al.*, 2004). The size and quality of available *Culicoides* breeding sites may influence midge population size, and may depend not only on soil type and the terrain of the area but also on farm-related factors such the rate of dung removal from animal holdings and the availability of water sources such as channels and leaking water-taps (Purse *et al.,* 2004).

Culicoides larvae will desiccate rapidly in soil with a low moisture content and the pupae of some *Culicoides* species can drown if the midge breeding sites flood (Baylis, O'Connell and Purse, 2004). Adult midges are susceptible to desiccation at low humidities (Murray, 1991). Moist microhabitats are therefore important for the survival of adult *Culicoides* midges as well as for providing suitable *Culicoides* midge breeding sites. Generally *Culicoides* midges inhabit a wide range of moist habitats and can reach high numbers under suitable climatic conditions (Mellor *et al*, 2000). These populations can then become passively dispersed on the wind, rapidly spreading *Culicodes*-borne diseases such as BT. The significance of these local environmental factors should therefore be considered when predicting BTV vector distribution (Pili, Ciuccè, Culurgioni, Figus, Pinna and Marchi, 2006).

Due to the variability of climatic influences on the different *Culicoides* midge species, the distribution of different species and their involvement in BTV spread should be considered individually. *Culicoides obsoletus* distribution can to some degree be predicted by ambient temperature, as they prefer warmer conditions without temperature variation (Purse *et al.*, 2004). *Culicoides pulicaris* however is influenced more by vegetation type, rainfall and soil moisture content, preferring high stable environmental moisture levels (Purse *et al.*, 2004). The availability of vertebrate sources for blood meals can also determine the distribution and local population of *Culicoides* midge, both by numbers of animals available and the type of animal present (Meiswinkel *et al.,* 2000). Models to predict BTV distribution, which ignore farm-level factors, potentially may produce inaccurate results (Purse *et al.,* 2004).

As climatic factors can alter *Culicoides* midge abundance and distribution, including the species distribution, several catches made during the peak midge periods are needed at each site, to reduce the effects of variances in midge numbers due to climatic changes (Baylis and Rawlings, 1998). Previous studies on BTV vector distribution have suggested localized microclimates may be an important consideration when examining BTV transmission risk during BT outbreaks. This study aims to examine in more depth the typical management factors found on farms in the U.K. that could provide microclimates associated with increased localized *Culicoides* midge populations. An increased localized *Culicoides* midge population may result in an increased risk of BT exposure, in the event of further BT outbreaks in the U.K.. As control strategies designed to reduce BTV transmission will include methods to reduce livestock exposure to BTV vectors, the identification of management factors that may increase livestock exposure to *Culicoides* midges and alteration of these factors could therefore form a significant part of integrated disease control strategies in the event of BT outbreaks. Identifying risk factors for livestock exposure to BTV vectors could aid the compilation of a disease risk assessment, for advising on disease limiting actions for use on individual farm holdings. This information is of particular significance due to the risk of aditional BTV serotypes occurring in countries where vaccination is currently limited to specific BTV serotypes, as is currently the case in Europe.

The main objectives/aims of the study were to:

- Determine possible microclimates present on a typical dairy and sheep farm in the U.K. that may be associated with increased localized *Culicoides* midge populations.
- Identify possible factors, including farm management practices, that may influence the localized *Culicoides* midge population.
- Aid the compilation of on-farm BTV risk assessments and farm disease control plans using identified risk factors, to help prevent and control the spread of BTV in the event of future outbreaks in the U.K..

Chapter 2

Materials and Methods

2.1 Initial preparation and sample farm information

The criteria determined for a suitable study farm was a lowland, non-coastal farm in the south-west of England, with an average sized dairy cow herd and sheep flock and which employed typical management practices. This included summer grazing of livestock, the use of winter housing and the use of cubicle housing for the dairy cows and straw-bedded loose housing for calving cows, calves and youngstock. A twice daily milking routine is typical in the U.K.

The farm of 267 acres (106 hectare) is at an elevation of between 6-20 m above sea level, with an average annual rainfall of 36 inchs (910 mm) and a soil- type consisting of clay, alluvial, loam soil with some sand. The pasture was generally well drained, but with some focal poorly drained areas. The farm was approximately 10 miles (16 km) from the coast.

The farm at the time of sampling had a total of 107 Holstein-Friesian dairy cows, 16 in-calf heifers, 43 growing heifers, 5 post-weaned calves and from 8 increasing up to 13 pre-weaned calves. The group of in-calf heifers and 13 dry cows were at pasture close to the farm holding, within a 300 m range. A group of growing heifers were at pasture at least 300 m away. There were a total of 180 ewes, 5 rams, 40 ewe lambs and 380, six to seven month old lambs on the farm. The lambs would be routinely weaned in mid-July and are sold for meat from that time onwards through the year. At the start of the study the sheep were at pasture approximately 500 m or more from the farm holding. Only the group of 180 ewes came into close contact with any of the study sites.

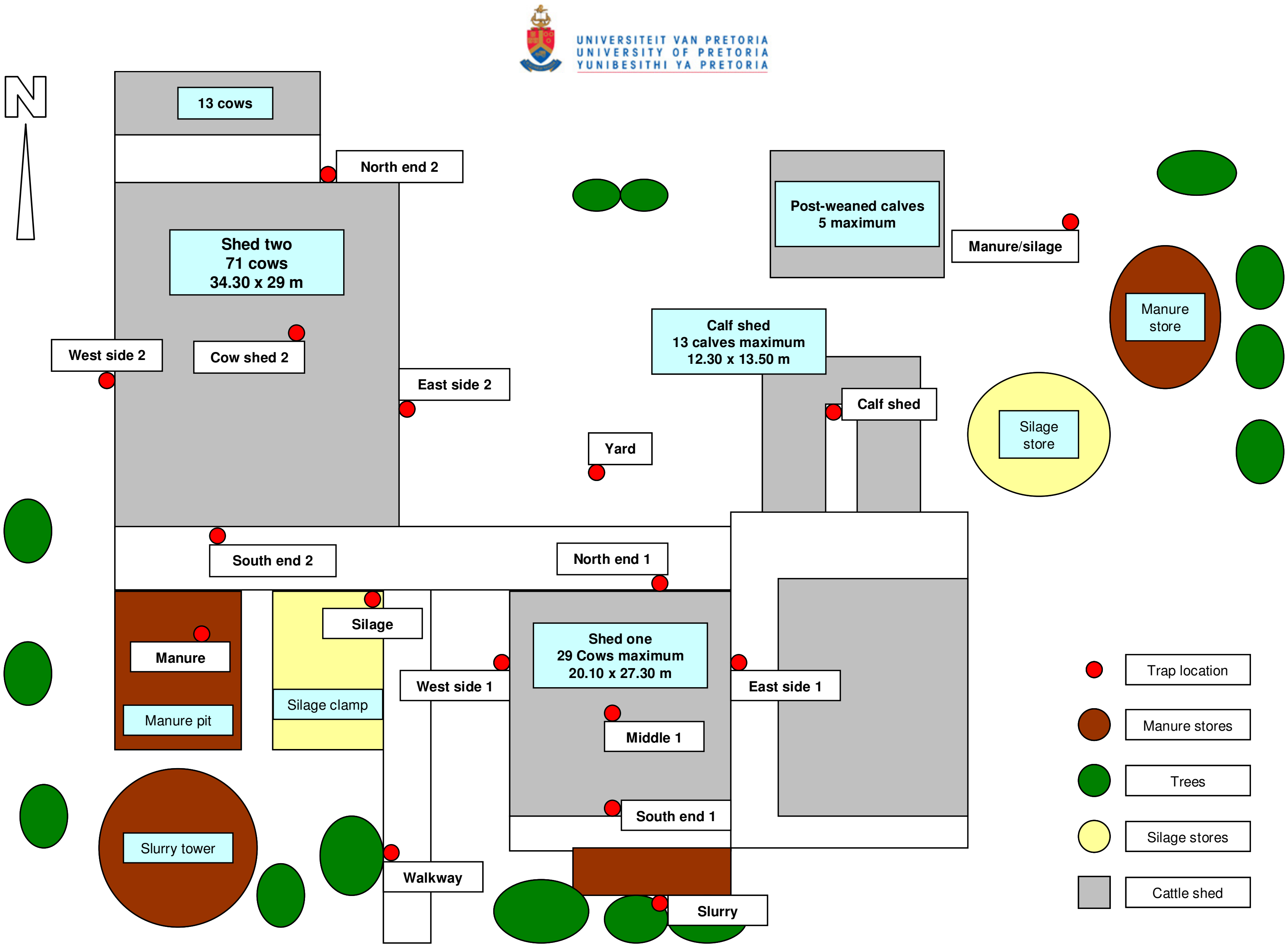

Figure 1 Study farm: Plan of farm holding (farm trap sites)

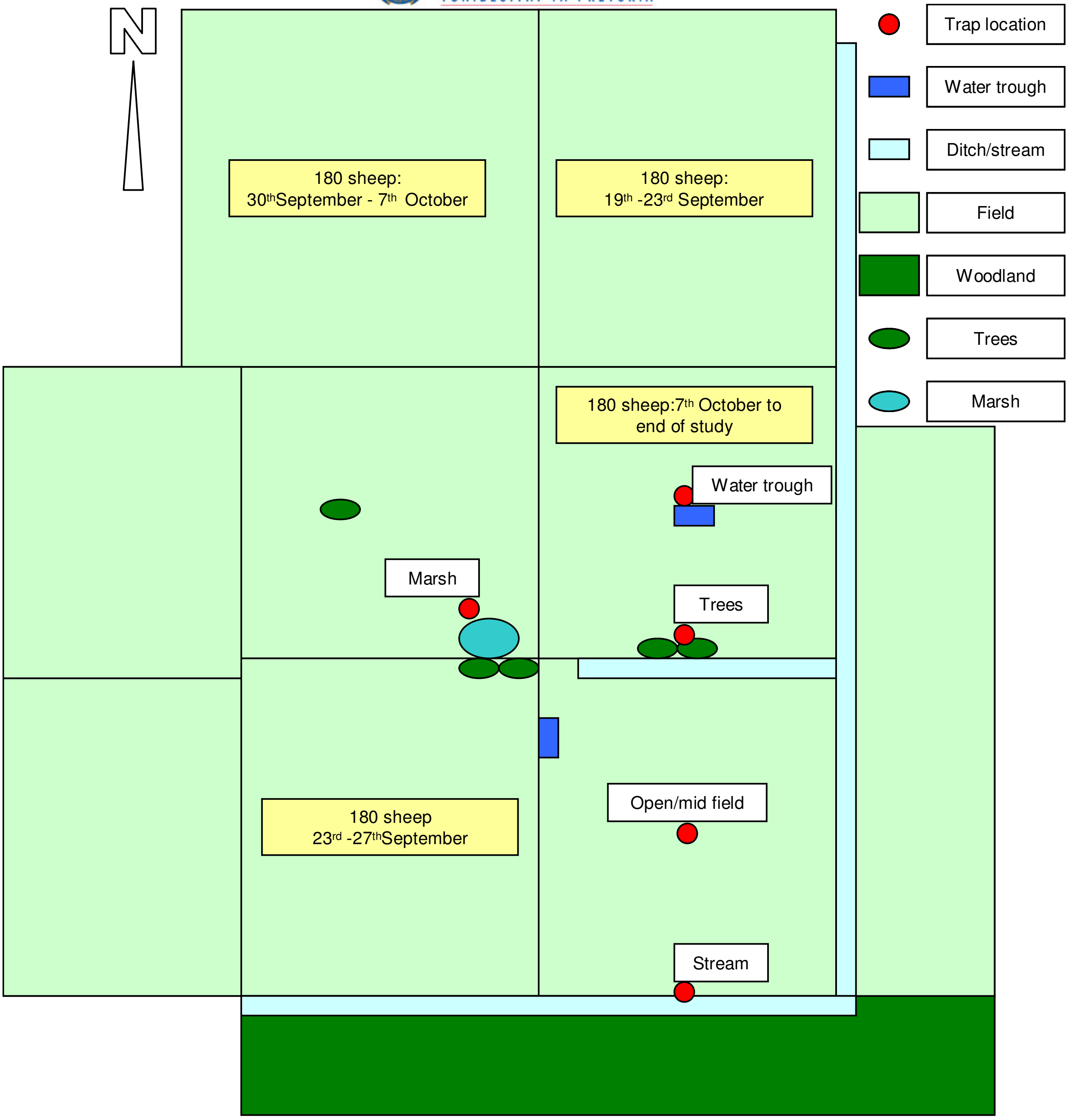

Figure 2 Study farm: Field plan (field trap sites)

2.2 Management information for the sample farm

The farm management details including animal movements and management changes, such as manure removal, the milking routines and the times of animal turnout, housing or pasture changes were recorded during the study.

2.2.1 Cattle management

Milking of the lactating cows was done daily between 5:15 and 8:00 in the morning and in the evening between 15:00 and 18:00, and adult cows returned to the farm holding, including from the pastures, every day during these times. The animal housing at the farm holding was used solely for the cattle. The majority of the mature animals had been out at pasture since the beginning of April, and since the beginning of June for the youngstock. Some cows and calves had been periodically housed in Cow shed 1 and the Calf shed during the summer (Figure 1). The high yielding cows could have night-time access to the cubicle shed during the summer for access to supplementary feed. During 2008 the mid- to late-lactation milking cows had been housed ten days prior to the first trapping, on the 2nd September, due to wet weather. The pastures used for the field traps had been used for dry cow and heifer summer gazing during 2008 prior to sampling, with the cattle rotating on roughly a weekly basis around the group of fields. The field trap pastures were used for sheep grazing at the times described below, during the sampling period.

Fresh silage was fed daily to the housed cows, sourced at the time of sampling from the silage store in the eastern yard. A new cut of silage was added to the silage store located in the yard south of shed 2 on the 23rd of September.

2.2.2 Sheep management

The sheep were winter housed at the beginning of 2008 in buildings approximately 1 km away from the farm holding, and were turned-out to pasture at lambing during February and March. The sheep were at pasture during the study period and a group of 180 ewes were moved to a field a minimum of 100 m to the north of the trapping fields on the 19th September, the day before trapping 3. This group of sheep was then moved to a field to the west of the Open/mid field, Stream and Marsh field trap sites on the 23rd of September, two days before trapping 4, to a minimum distance of 10 m from the Marsh site and 55 m from the Open/mid field site. The sheep remained there until 27th of September. The same group of sheep then returned to fields at least 180 m north of the Marsh site on the 30th of September for the following seven days. The sheep were finally moved to the Water trough and Trees field site on the 9th of October, at the time of trapping 8 (Figure 2).

2.3 Sampling technique and data collection design

Farm plans were drawn-up, detailing the farm holding and pasture layout, and the locations for the midge traps were predetermined to examine a wide range of locations around the farm holding and of five pasture sites. The sites used for midge trapping were marked and recorded, allowing for repeated sampling in the same locations. Figures 1 and 2 outline the plans of the farm holding and field plan (trap sites marked).

2.3.1 Description of the trap site locations

Unless otherwise specified the traps were set at a height of 1.5 m above the ground. The traps were hung on structures already present on farm buildings or on the manure and forage stores. Free-standing traps were otherwise used, consisting of a 180 cm to 200 cm plastic pole fixed in a weighted plastic base.

- **Shed 1**

Shed 1, was a straw-bedded cattle shed measuring 20.1 m by 27.3 m. The shed had a concrete central passage, approximately 5 m wide, with a feed barrier for the animal pens along both the east and west sides of the shed. Fresh silage was fed daily on the floor of the central passage. There were two water troughs, one on either side of the central passage located in the centre of the building. There was also a water trough at the north end on both the left and right sides of the central passage. The passage was open at the north and south ends of the shed. The east and west sides of the shed had solid walls 2 m in height with slatted Yorkshire boarding above. The shed was a single span with a central roof ridge. In the cattle pens there was a manure channel along the front of the bedding, which was cleaned-out daily to the small slurry lagoon south of shed 1 (Figure 1).

Initially 10 cows were housed in shed 1, consisting of lame and freshly calved cows and up to 3 calves. Numbers would vary as newly calved cows or newly lame cows were added or removed to the lactating cow group. Numbers reduced at one stage to 7 cows and 1 calf. Transition cows were allowed access to the shed from the 23rd of September with a potential maximum of 29 cows overnight, but with access to grazing during the day.

Shed 1 trap locations

North end 1: The trap was located on the north side of the shed, on the outside of the building adjacent to the east side pen. The closest water was a water trough, 3.2 m away.

West side 1: The trap was located on the western outside wall of the shed adjacent to the side ventilation opening through the Yorkshire boarding, 4.5 m from the north end of the shed. The trap site was surrounded by an undisturbed grassy area.

East side 1: The trap was located on the eastern outside wall of the shed adjacent to the side ventilation opening through the Yorkshire boarding, 4.5 m from the north end of the shed. The trap was located alongside a muddy track with shallow standing water and grass covered edges, running between shed 1 and an empty second shed parallel to shed 1.

South end 1: The trap was located at the south end outside wall of the shed, adjacent to the central passage opening.

Middle 1: The trap was situated in the central passage of the shed, adjacent to the west-side central-shed water trough. The trap was 14 m from the north end and 13.3 m from the south end of the shed (Figure 1).

- **Shed 2**

Shed 2 was a single span cubicle cow shed measuring 34.3 m by 29 m. The milking parlour and collecting yard was located within the northern end of this shed. The collecting yard measured 15.9 m by 15.7 m, the parlour 13.3 m by 16.2 m and the remaining shed area consisted of cow cubicles. The shed was in two halves. There were 3 rows of cubicles and an outside feed barrier where silage was fed, in the east half of the shed. Thirty three low milk yield cows were housed in this half. The west half of the shed housed 38 high yielding cows in two rows of cubicles, with a central feed barrier where silage was fed. There were 3 water troughs within the shed. Sawdust was used in the cubicles. The walkways between the cubicles were concrete based and were where the cow dung/slurry accumulated. The walkways were cleaned out twice a day into a grilled channel that was located in the yard south of the shed. The slurry was then pumped into a cylindrical slurry tower.

North end 2: The trap was located beside the outside wall of the north end of the shed, at the edge of the yard to the north, 13.3 m from the east wall of the shed. To the north of this yard was a shed where lame and sick cows were housed. At the time of sampling 13 cows were housed here. On mild days this group of cows would have access to the yard. Overnight this trap was at least 11 m away from livestock and 15 m from the nearest water trough located in shed 2.

East side 2: The trap was located on the outside of the east side of the shed, adjacent to the open fronted feed barrier where fresh silage was fed to the low milk yield cows daily. The trap was hung at a height of 1.8 m to avoid cow interference. The east side of the shed adjoined the open yard, which was a non-livestock area.

West side 2: The trap was located on the outside of the west wall of the shed adjacent to a slot ventilation opening between the wall and roof. The trap was located 13.5 m from the south end of the building at a height of 1.9 m from the ground, to a level consistent with the ventilation inlet. The adjoining area to the west wall was a small grassy paddock with a water trough 14.5 m away and trees at a similar distance. The water trough pipe was leaking, resulting in water logging of a wide area of the paddock, from a period prior to the first trapping on the 12th of September until the pipe was repaired on the 2nd of October.

South end 2: The trap was located on the outside of the south end of the shed adjacent to the central door opening. The trap was set at a height of 2.7 m to avoid cow interference. The trap site was adjacent to a cow yard with cow access during the day, the manure pit was at a distance of 8.6 m and the silage store was at a distance of 8.6 m. The closest water trough was at a distance of 8.1 m, within shed 2.

Cow shed: The trap was situated within cow handling pens inside the cubicle shed, adjacent to the low milk yield cow group, 11.1 m from the east side and 18.1 m from the south end of the shed. The trap was 3.1 m from the closest water trough, which was located within the shed (Figure 1).

- **Calf shed**

Calf shed trap: The calf shed consisted of two covered halves with an open-roofed central concrete passage and measured 12.3 m by 13.5 m. The west half of the shed consisted of 4 straw pens each containing up to 4 pre-weaned calves. A maximum of 13 calves were housed here during the study period. The east half consisted of 3 large straw pens, which were used for housing a cow and calf from the 2nd of October to the 6th of October. One pen was used as a manure store from the 27th of September and there was manure stored in a trailer in the central passage. The water source in the calf pens consisted of small corner water bowls. The trap was located at the north end of the shed adjacent to the west side calf pens.

- **Manure/slurry and forage stores**

The manure and slurry from the farm was stored in several locations:

Manure trap: The manure pit was 16.8 m from the southern end of shed 2, adjacent to the southern cow yard. The manure pit measured 20.7 m by 11 m. Manure containing straw bedding, soiled sawdust from the cubicles and discarded forage were regularly added to the north end of the pit. The north end of the pit consisted of solid manure and the southern end of slurry. The trap was located 5.8 m from the north end of the manure pit on a concrete platform, 1.5 m above the surface of the manure. The site was relatively exposed, with the closest trees 14 m away.

Slurry trap: Slurry (liquid manure) was cleaned out regularly, usually once a day, from the manure channels in shed 1. The slurry store was a shallow, concrete lined 7 m by 10 m pit, 9.5 m south of shed 1, containing mostly liquid and some straw containing manure. The trap was located on the southern edge of the slurry lagoon, within the shelter of several trees, 18 m from the southern end of shed 1. The area was adjacent to a 2.2 hectare field to which cattle had access during the study period. The closest water sources were the water troughs in shed 1 (distance of 18 m) and a water trough in the adjacent field (distance of 40 m).

Manure/silage trap: The trap was located in a yard at the eastern end of the farm holding. At the eastern edge of this yard was a manure stack consisting of mature manure taken from the animal housing during early summer (May/June). There was a pool of stagnant water alongside the manure stack, and trees bordered the eastern side of the yard. In the yard area there was also a silage store in the westerly section of the yard. The trap was located on a grass area in the northern part of the yard, at a distance of 12.1 m from the manure stack, 15.9 m from the silage store, 21.9 m from the stagnant pool of water and 30 m from the trees on the eastern edge. The trap was 19 m east of the post-weaned calf shed. The yard was an open area with a stone-base, which accumulated some mud and minor standing water after rain.

Silage trap: The trap was located adjacent to the silage store located 8.7 m from the southern end of shed 2, adjacent and to the east of the manure pit, and adjoining the southern cow yard. The silage store had concrete walls on three sides and gates across the front to prevent livestock access. The cow walkway was along the eastern side of the silage store. The silage store had been refilled with a new cut of silage on the 25th of September, but prior to this there had been some well fermented silage from the previous season. The trap was located at the north-east corner of the silage clamp, adjacent to an area with some pooled liquid run-off from the silage and grass at the edge of the cow yard.

- **Other**

Yard trap: The trap was located in the main open yard, which had no livestock access and was a stony, well drained yard area with some grass. The north side of the yard consisted of the office and entrance road, the east side of the calf sheds, the southern boundary was the yard north of shed 1 and the west boundary consisted of the feed barrier of shed 2. The trap was located 10 m from the north end of shed 1.

Walkway trap: The trap was located below a tree, along a track leading from the fields to the farm holding, running between the silage store and a grass border west of shed 1. The trap was 41.1 m from the southern end of shed 2 and was 11.7 m from the south end of the silage clamp. The closest livestock to the trap were those housed in shed 1, at a distance of 17 m and the closest water was a water trough in shed 1, at a distance of 22 m.

- **Field sites**

The field sites were at least 500 m south of the farm holding (from the Water trough trap, the most northerly field trap site). The relevant sheep movements to these sites are detailed in the sheep management section and on the field plan (Figure 2).

Marsh: The Marsh trap site was a poorly drained area of approximately 10 m diameter, 20 m from the entrance gate of a 2.4 hectare field, the most westerly field site sampled. The area consisted of some shallow standing water, waterlogged soil and reeds. There were trees 7.7 m south of the trap in a small group, within a hedge boundary. The remaining field consisted of open, sloping, grassland with a single tree in the centre and with hedge boundaries.

Water trough: The trap site was adjacent to a large mains-water filled trough located in the centre of a 2 hectare field, the most northerly field trap site. The trap was 77 m east from the Marsh trap and 49 m north of the Tree trap site. The field consisted of flat open grassland with a ditch along the eastern boundary. All of the field boundaries consisted of hedges.

Trees: The trap site consisted of a row of mature trees within the mid-section of the southern boundary hedge of a 2 hectare field, the same field as the Water trough trap. The hedge boundary was the northern boundary of the Open/mid field and Stream trap field. On the southern side of the hedge there was a deep drainage ditch 4 m from the trap with vegetation covered banks. The trap site was 47 m east from the field entrance gate and 92 m from the eastern boundary. The site was 49 m directly south of the Water trough and 52 m north of the Open/mid field trap sites.

Open/mid field: The trap site was located in the centre of the most southern field sampled. The trap was 63 m directly south of the Trees trap site and 63 m north of the Stream site. The trap site was 52 m from the western field boundary and 92 m from the eastern boundary. The field was a 2.2 hectare flat grassland field with hedge boundaries on the north, east and west sides. The north and east borders had a drainage ditch and the southern boundary consisted of a shallow running stream. There was a water trough 10 m from the field entrance gate.

Stream: The trap site was midway along the southern boundary of the southern field and consisted of a shallow, slow running fresh-water stream with deciduous woodland on the southern side. The wooded area was 4 m south of the Stream trap site. The Stream trap was 63 m directly south of the Open/mid field trap site.

2.3.2 *Culicoides* collection

A total of 22 insect traps were set. The traps used were 912 blacklight downdraft, 12 volt DC, ultraviolet-light insect traps, each using a 12 volt-10AmpHr sealed gelled-electrolyte battery, (John Hock Company, Florida, U.S.A) (Figure 3).

Eight trappings were carried out on the dates listed in Table 1, with 22 trap site catches attempted during each trapping. The times of sunset and sunrise are recorded along with the setting and collection dates and the moonphase for the trapping night.

Table 1 Trapping dates and sunset and sunrise times during 2008

Trapping number	Trap setting date	Sunset time	Trap removal date	Sunrise time	Moonphase
Trapping 1	12th September	19.22	13th September	06.32	Three quarter moon
Trapping 2	18th September	19.08	19th September	06.43	Just passed full moon
Trapping 3	20th September	19.03	21st September	06.46	Half moon
Trapping 4	25th September	18.48	26th September	06.53	Crescent moon
Trapping 5	27th September	18.43	28th September	06.57	Crescent moon
Trapping 6	2nd October	18.31	3rd October	07.05	New moon
Trapping 7	4th October	18.27	5th October	07.08	Crescent moon
Trapping 8	8th October	18.22	9th October	07.14	Half moon

Figure 3 A 912 blacklight downdraft insect trap in location

The midge traps were set up to 1 hour before sunset and remained running overnight until at least one hour after sunrise, to allow trapping during the periods of maximum *Culicoides* activity. The ambient temperature, relative humidity (%) and wind speed (m/s) was measured and recorded for each of the trapping sites, at trap level, at the time of setting and collection using a handheld Kestrel 3000 weather meter ("Weather Shop" Internet Company). Digital thermometers were set at a single field trap site, a farm holding location and within the cubicle shed to monitor the maximum and minimum temperature during each trapping period (Gardman dual display maximum/minimum thermometer). A Pro X (IR-PROX) weather station ("Weather Shop" Internet Company) was used to give comparative temperature, humidity, wind speed, wind direction and rainfall readings for the trapping period. The blacklight traps were located at a height of 1.5 m above the ground except in specific locations (described in section 2.3.1) where trap height was altered to prevent livestock interference or to address key features of the trapping location.

The insect collecting pot supplied with the light trap had a mesh base, therefore a 500 ml sealable collecting pot was added to the mesh based pot. Water and approximately 0.5 ml of washing-up detergent was added to the sealable collecting pot to a depth of 30% of the pot volume, after the traps had been set in position. Detergent was added to the collecting pot water to reduce the water surface tension, to aid midge submersion. At the time of trap removal the sealable collecting pot was removed, sealed with a lid for transport and labelled with the site of collection using a code number with the trapping day number (prefix T), and a number for the trap site. A record was kept of the trap site and the corresponding sample pot number. The collected contents were transferred to the laboratory, where the pot contents were drained through a sieve lined with filter paper. The sieved trap contents were then placed into 70% ethyl alcohol solution in 60 ml polypropylene storage pots and the pots were labelled with the location of collection. The pots were then stored in a cool dark location until the catches were examined.

2.4 Midge identification

The trap contents were examined under a trap code number, allowing examination without knowledge of the sample origin. The trap contents were filtered from the ethyl alcohol preservative through a fine plastic mesh filter, and were placed in a Petri dish in a small quantity of ethyl alcohol to prevent desiccation. The trap contents were then examined under a Kyowa Optical model SDZ-PL dissecting Parasitology microscope (HWF10x eye-piece) on setting 1.0 (range 0.7x to 4.5x objective lens with 5 diopters and a 6.5:1 zoom ratio) and the *Culicoides* were separated from the mixed non-*Culicoides* insects present. Counting and preliminary speciation of the *Culicoides* midges were then performed under setting 1.0 under the dissecting microscope. More detailed speciation and staging of the females present was performed under setting 1.5, under the dissecting microscope. The *C. obsoletus* group males were speciated to species level after mounting on a microscope slide and examination under a Leitz DIALUX 20 EB light microscope, under low power magnification x10/0.25 (160/- Nikon lens x10 eye piece). Speciation to the *C. obsoletus* species level involved examination of the male genitalia as described below.

A day of training on visual *Culicoides* identification and speciation was provided at the Institute of Animal Health, Pirbright on the 13th of January 2009. Training was provided using sample species and diagrams. Training was also given in male *Culicoides* identification as well as the identification of the varying female stages of development, namely nulliparous, parous, gravid and blood fed stages.

The following characteristics were used in the identification of *Culicoides* species: Briefly, many *Culicoides* have a pale wing with darker markings, the patterning produced by pigmentation of the wing membranes. On the medial edge of the wing the costa extends more than half-way and to less than two thirds the length of the wing. The radius forms two usually equally sized radial cells which vary in definition between some species (Kettle, 1990). The second radial cell is pale. The genus has legs of even lengths. The females have a fatter body and in some species more defined wing markings than the males. The females have broader wings and antennae consisting of 15 segments with only short setae. The males of some species have paler wing markings than the female, have a narrower body and "feathered" antennae (Institute of Animal Health website; notes provided during training at the Institute of Animal Health, Pirbright). Female *C. obsoletus* and *C. pulicaris* were identified only to a species group level. Females of *C. obsoletus* (Meigen) are relatively small with a pale second radial cell on the wings. The species group was identified to sibling species level using only the males. Identification of the male *C. obsoletus* group species was predominantly carried out using the species-specific variations in the shape of the ninth sternite, of the male genitalia. Male *C. obsoletus* were identified by the presence of a pale second radial cell on the wings as well as a deeply clefted, but not divided, ninth sternite. *Culicoides chiopterus* (Meigen) males were identified by clear wings and a deeply emarginate ninth sternite with a bare membrane. *Culicoides chiopterus* males are also relatively small when compared to the other sibling species males. *Culicoides dewulfi* (Goetghebuer) males have pale wing markings and also a deeply emarginate ninth sternite with a spiculate membrane. *Culicoides scoticus* (Downes and Kettle) males are larger more definitely marked males with a completely divided ninth sternite.

Culicoides pulicaris (Linnaeus) and *C. punctatus* (Meigen) are both medium to large sized species, have pale wings with blackish spots and a blackish spot in the cubital cell of the wings. The two species are differentiated using subtle wing pattern differences. *Culicoides pulicaris* and *C. punctatus* are very similar in appearance and behaviour and were not differentiated during this study, due to the difficulties associated with reliably identifying the distinguishing features between the two species. These two species were therefore only identified to the species group level for both the males and females, and have been referred to as *C. pulicaris* group during this study.

After speciation and examination the *Culicoides* collected were placed into either 2 ml or 8 ml polypropylene bijoux containers and 70% ethyl alcohol was added to the collected midges for preservation and storage.

2.5 Data analysis

The following data were collected and analyzed: The *Culicoides* species trapped; the numbers of males and females of each species or species group; the reproductive stage of the female *Culicoides* as well as weather data were recorded during sampling.

The following data were compared:

- The species of *Culicoides* trapped and the relative distribution and numbers of the different species found.
- The numbers of *Culicoides* trapped from different locations around the farm buildings and yards, comparing catches from inside and outside the animal housing and also examining the *Culicoides* numbers relative to animal contact, straw bedding, the manure stores, the forage stores and water sources. The variation in the *Culicoides* distribution between the northern, southern, eastern and western locations around the holding was also examined.
- The *Culicoides* populations at various pasture microenvironment locations were examined, and results were also compared to the farm holding *Culicoides* population sizes and distributions.
- The variation of *Culicoides* numbers between the different sampling dates.
- Examination of the *Culicoides* population data along with ambient air temperature, wind speed, wind direction, humidity and precipitation measurements.
- The influence of recorded farm management practices on *Culicoides* numbers.

Visual examination of the data was used along with a scoring system and Multi-level modelling. Multilevel modelling was used to aid examination of the data for determining the likely significance of varying co-existing factors on the *Culicoides* populations found in the trap locations.

2.5.1 Ranking of results based on a female *Culicoides* scoring system

A ranking system was used for six determinants, for assessing risk of exposure to *C. obsoletus* and *C. pulicaris* females, the potential vectors for BTV transmission. The five determinants used to assess each of the trapping sites are described below:

- The mean female count, calculated from the total female catch from that trap site during all trappings.
- The mean percentage females, calculated from the percentages from all of the catches (the percentage value was the number of females caught at that trap site, as a percentage of the total female catch from all of the trap sites during that trapping).
- The peak percentage of females for that trap site, after examining the percentage results from all of the catches from that site.
- The peak female catch from the trap site.
- The total number of females collected at the trap site (sum of all of the catches for the trap site).

A score was then allocated to each of these measurements according to the rank of the trap site when listed on a descending scale. There were 22 trap sites therefore the highest score was 22. For example the trap site with the highest mean female count, was allocated a score of 22. Scores were then allocated to the other trap sites in order of descending values, to the lowest value in the list which was allocated a score of 1. The score for each of these measurements, for each trap site, were then added together to give an overall score.

The score was then adjusted to incorporate the regularity of catches with an above average number of females, to represent sites considered to have a more persistent or increased regularity of exposure risk to female *Culicoides*, the potential BTV vectors. The number of catches with an above mean percentage of females was recorded for each site. These were allocated a score per result above the mean, as described below:

<u>Number of trap sites= 22 (to allow a maximum potential score of 22)</u>

Number of catches of which an above mean value could be considered

(for most catches this value was eight and the value was adjusted for trap sites with reduced numbers of catches)

Score used for sites with all eight catches = 22/8 = 2.75

Score used for the Manure/silage, Manure, Open/mid field and East side 2 sites with seven catches = 22/7 = 3.14

Score used for the Trees, Marsh and Stream sites with six catches = 22/6 = 3.67

Score used for the Water trough site with five catches = 22/5 = 4.4

2.5.2 Multilevel modelling

(The multilevel modelling was performed by Dr Nick Bell and Professor William (Bill) Brown. The interpretation was edited by Dr Nick Bell).

Multilevel modelling is a system of data analysis designed to aid the examination of data with hierarchical or clustered structures. Variation was analysed at the different levels within the dataset using multilevel logistic regression within MLwiN (V.2.12). Clustering due to time within location was accounted for in this model to generate parameter estimates for a range of predictor variables.

Each predictor variable was separately entered into, and removed from the models for each of the *Culicoides* species, and assessed for significance. Overall there was a decline in *Culicoides* numbers over time. This was accounted for within the model by the addition of a term for time within season. To account for variation over time the first time point was used as a reference point (zero) and the remaining trappings were included as time points. The data was normalized using a log+1 transformation. A final model was created using a step-wise inclusion of the predictor variables that were significant in the single variable analysis to create a succinct model. The final model only retained variables that maintained significance when all of the variables were offered.

Chapter 3

Results

3.1 *Culicoides* midges trapped at different trapping sites (*Culicoides obsoletus* group and *Culicoides pulicaris*)

In this section the populations of *Culicoides* found at 22 trapping sites are reported. Twenty two black light insect traps were set at the locations detailed in the Materials and Methods section, on eight trapping occasions between the 12th September and 9th October 2008 (trapping dates are listed in Table 1 in Materials and Methods). To determine the prevalence of *Culicoides* at these sites, data were collected in the form of numbers of *Culicoides* collected at each of the insect traps at the various locations. Identification of the trapped *Culicoides* midges during the project found only midges from the *C. obsoletus* and *C. pulicaris* groups (the species identification method used is described in section 2.4 in Materials and Methods).

Trap sites with missing data:

Trappings were successfully carried out at all of the trap sites apart from the Water trough site during trapping 1, 7 and 8; the Marsh and Stream sites during trappings 6 and 7; the Trees site during trappings 7 and 8; the Open/mid field site during trapping 7; Manure/silage and Manure sites during trapping 7 and the east side of shed 2 during trapping 8. Missing data are indicated in all tables by a shaded box. The missing data occurred due to trap failure or adverse weather conditions and resulted in these sites having a reduced number of catches assessed.

3.1.1 Prevalence of female *Culicoides obsoletus* and *Culicoides pulicaris*

The total number of female *C. obsoletus* and *C. pulicaris* was determined at the different trapping locations on each of the trapping ocassions, as recorded in Table 2.

Table 2 Prevalence of female *C. obsoletus* and *C. pulicaris* at the different trapping locations

Trap location	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 6	Tr 7	Tr 8	Total
Farm trap sites									
Manure /silage	865	40	36	266	6	6		0	1219
Slurry	245	86	456	1517	259	16	42	7	2628
Manure	3113	414	333	2091	169	2		6	6128
Silage	982	131	332	3304	281	4	36	17	5087
Yard	1004	255	103	450	41	0	5	0	1858
Walkway	1015	121	246	1971	78	13	182	12	3638
East side 1	3189	194	615	1116	106	5	235	40	5500
North end 1	2998	31	728	2435	299	7	88	30	6616
South end 1	3332	558	496	1577	94	10	27	27	6121
West side 1	725	23	28	70	15	0	13	11	885
South end 2	1249	614	495	2688	157	2	3	4	5212
West side 2	436	73	57	184	12	0	6	2	770
East side 2	3013	468	490	1426	225	14	34		5670
North end 2	1381	70	123	455	54	3	18	17	2121
Cow shed	705	44	36	97	86	2	21	10	1001
Middle 1	716	110	299	371	58	58	111	98	1821
Calf shed	1493	80	839	864	175	68	254	45	3818
Field trap sites									
Marsh	116	25	3	55	10			1	210
Stream	21	4	1	7	18			3	54
Trees	8175	8	29	705	76	2			8995
Water trough		0	5	114	2	2			123
Open/mid field	665	21	10	396	12	1		0	1105
Total	35438	3370	5760	22159	2233	215	1075	330	70580

Tr = Trapping

The total numbers of *C. obsoletus* and *C. pulicaris* trapped during the study at each farm trap site ranged between 770 (West side 2) and 6616 (North end 1) (Table 2). The percentage of these identified as *C. obsoletus* from the farm trap sites ranged between 94.67% (Manure/silage) and 98.56% (Manure) with a mean percentage of 96.29%. The percentage of *C. pulicaris* ranged between 1.54% (Manure) and 5.33% (Manure/silage) with a mean of 3.71%. The higher percentages of *C. pulicaris* (more than 1% above the mean) were at the Manure/silage site and also the west side of shed 2, the east side of shed 2 and the middle of shed 1 (4.94%). The next highest percentages were at the Walkway site (4.84%) and the Slurry site (4.79%). The lowest percentages (more than 1% below the mean) were the Manure site (1.54%) and the west side of shed 1 (2.03%).

From the field trap sites the percentages of *C. pulicaris* trapped were higher than the percentages found at the farm trap sites, with a range of 9.98% to 17.65%. The field site *C. pulicaris* percentages were Trees (9.98%), Marsh (14.29%), Water trough (14.63%), Stream (14.81%) and Open/mid field (17.65%). The range of *C. obsoletus* percentages at the field sites therefore ranged between 82.35% (Open/mid field) and 90.02% (Trees).

A graph of the total numbers of female *C. obsoletus* and *C. pulicaris* collected at each of the trap sites is shown in Figure 4.

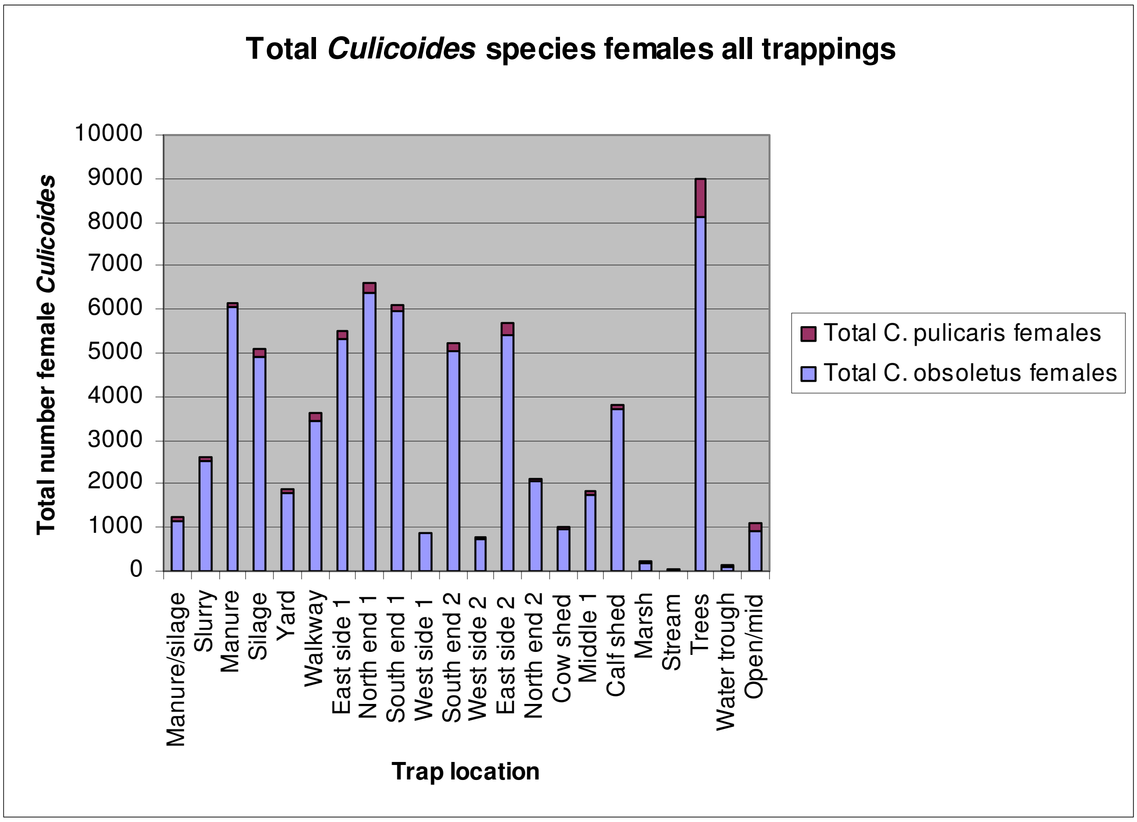

Figure 4 Total female *C. obsoletus* and *C. pulicaris* collected from all trappings for each trap site

3.1.2 Total catches of *Culicoides obsoletus* and *Culicoides pulicaris* per trapping

The total catches from each trapping of *C. obsoletus* and *C. pulicaris* are detailed in Table 3, and the percentage of the *Culicoides* collected during each trapping has been calculated for both species groups, taken as a percentage of the total numbers of that species collected during the study.

Table 3 Total catches of *C. obsoletus* and *C. pulicaris* per trapping

Trapping time	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 6	Tr 7	Tr 8	Total
Total *C. obsoletus* females	34021	3196	5462	21287	2085	154	877	255	67339
% of *C. obsoletus* total number	50.52	4.75	8.11	31.61	3.10	0.23	1.30	0.38	
Total *C. pulicaris* females	1417	175	298	871	147	61	197	75	3241
% of *C. pulicaris* total number	43.72	5.40	9.19	26.87	4.54	1.9	6.08	2.31	

Tr = trapping

The total between trapping catch variations of *C. obsoletus* and *C. pulicaris* were similar during trappings 1 to 5 (Table 3). During trappings 6, 7 and 8 the *C. obsoletus* catch size reduction was dramatic. The *C. pulicaris* catch size reduced by a lesser degree during trappings 6 and 8 but had a catch size equivalent to catches 2 and 5 during trapping 7 (Table 3).

3.1.3 Mean number of female *Culicoides obsoletus* and *Culicoides pulicaris*

The mean number of females was calculated for *C. obsoletus* and *C. pulicaris* for each trap site, using the total numbers of each species collected during all of the trapping occasions, as illustrated in Table 4.

Table 4 Total and mean numbers of *C. obsoletus* and *C. pulicaris* collected from each trap location

Trap location	Total *C. obsoletus* females	Mean *C. obsoletus* females	Total *C. pulicaris* females	Mean *C. pulicaris* females
Farm trap sites				
Manure/silage	1154	164.86	65	9.29
Slurry	2502	312.75	126	15.75
Manure	6034	862.0	94	13.43
Silage	4898	612.25	189	23.63
Yard	1787	223.38	71	8.88
Walkway	3462	432.75	176	22.00
East side 1	5326	665.75	174	21.75
North end 1	6382	797.75	234	29.25
South end 1	5943	742.88	178	22.25
West side 1	867	108.38	18	2.25
South end 2	5031	628.88	181	22.63
West side 2	732	91.50	38	4.75
East side 2	5425	775.00	245	35.00
North end 2	2054	256.75	67	8.38
Cow shed	963	120.38	38	4.75
Middle 1	1731	216.38	90	11.25
Calf shed	3710	463.75	108	13.5
Field trap sites				
Marsh	180	30.0	30	5.00
Stream	46	7.67	8	1.33
Trees	8097	1349.50	898	149.67
Water trough	105	21.00	18	3.60
Open/mid field	910	130.00	195	27.86
Total	**67339**	**409.7**	**3241**	**721.12**

3.1.3.1 Mean catch values of *C. obsoletus* females

The mean female *C. obsoletus* numbers trapped at each of the trap sites was determined. The manure storage sites included the Manure/silage trap site which had a mean *C. obsoletus* count of 165, the Slurry store with a mean of 313 and the Manure site had a mean of 862. The forage storage sites included the Manure/silage location with a mean of 165 and the Silage trap mean with a mean of 612. The non-livestock areas consisted of the Yard site with a mean of 223 and the Walkway with a mean of 433. Shed 1 had mean values ranging from 108 to 798, with the north, east and south sides having mean counts of between 666 and 798. Shed 2 had mean values of 91.5 to 775. Two of the shed 2 sites, had means of 629 and 775 (the south and east sides) and the remaining two sides (north and west sides) had mean counts of 258 and 91.5. The traps located inside the cattle housing were located within the Cow shed 2, the middle of shed 1 and the Calf shed. The Cow shed 2 had a mean of 120, the middle of shed 1 a mean of 216 and the Calf shed a mean of 464. (The highest mean out of the farm sites was the Manure site with a mean female *C. obsoletus* count of 862) (Table 4).

The lowest mean values from all of the sites were those from both of the west sides of sheds 1 and 2. The Manure site; Silage site; north, east and south sides of shed 1 and the east and south sides of shed 2 all had mean female counts between 612 and 862. The trap at the south end of shed 2 was located close to the Manure site (16.8 m) and the Silage site (11.5 m). The Slurry site, with a moderate mean of 313, was 18 m from the south end of shed 1 but had approximately half the mean count of that found at the south end of shed 1. The Yard mean (223) was comparable to the mean catches of the north end of shed 2 (258) and the Manure/Silage site (165). The internal shed site means varied widely from 120 (Cow shed 2) to 216 (middle of shed 1) and up to 464 (Calf shed). Factors potentially affecting the distribution of the *C. obsoletus* population are examined in the discussion section.

The field trap mean counts varied widely from 7.67 (Stream site) to 1350.00 (Trees site). The remaining traps had low mean values of 21.00 (Water trough), 30.00 (Marsh) and 130.00 (Open/mid field).

3.1.3.2 Mean catch values of *C. pulicaris* females

The *C. pulicaris* catch sizes were significantly lower than the *C. obsoletus* catches, as previously discussed. The mean *C. pulicaris* female catches at the manure storage sites included the Slurry site with a mean count of 16, the Manure site with a mean of 13 and the Manure/silage site with a mean count of 9. The forage storage sites consisted of the Silage site with a mean of 24 and the Manure/silage with a mean of 9. The non-livestock areas were the Yard and Walkway trap sites with catch mean values of 9 and 22 respectively. The north, east and south sides of shed 1 all had similar mean counts of 29, 22 and 22. The west side of shed one had a mean of just 2. Shed two had two sites with higher mean counts of 23 (south end of shed 2) and 35 (east side of shed 2) and two lower means of 8 (north end of shed 2) and 5 (west side of shed 2). Of the sites within the animal housing the Cow shed 2 had the lowest mean of 5, the middle of shed 1 had the next highest mean of 11 and the Calf shed had a mean count of 14. Out of the farm sites the highest mean count was from the east side of shed 2, with a mean of 35. The mean catch values at the field sites indicated the Trees trap site mean was the highest at 150 (influenced by an unusually high catch at this site during trapping 2). Otherwise the mean results were 28 (Open/mid field), 5 (Marsh), 4 (Water trough) and 1 (Stream), respectively (Table 4).

The relative distributions of the mean counts were similar for *C. obsoletus* and *C. pulicaris* with the exceptions of the Manure site, which had a higher relative mean of *C. obsoletus,* and the east side of shed 2, which had a higher relative mean of *C. pulicaris*.

3.1.4 *Culicoides obsoletus* and *Culicoides pulicaris* female distribution

The female *C. obsoletus* and *C. pulicaris* catch sizes from each of the trap sites were examined for each trapping occasion. The data were compared to identify variations in catch size and distribution for each species between individual trappings. The data is illustrated in graphs for each trapping as a percentage of the total number of each species collected during that trapping, for each catch site (Figure 5 to 12).

3.1.4.1 Trapping 1

Trapping 1 was associated with the highest numbers of female *C. obsoletus* and *C. pulicaris* caught during the study (Figure 13). For *C. obsoletus* only one trap site had a catch of less than 100 (Stream = 18) and conversely the peak catch was at the Trees site (7412). The *C. obsoletus* catch from the Trees site was particularly high, with the next highest catch only 3298 (Silage). The farm holding sites had catches of between 244 and 3298, with eight catches of greater than 1000, consisting of shed 1, shed 2, the Manure and Calf shed sites.

The *C. pulicaris* catch during trapping 1 was also relatively high at the Trees site, with a catch of 763. The next highest catch was only 144, from the Open/mid field site. The farm catches of *C. pulicaris* ranged from 1 to 93, with the highest farm site result from the east side of shed 2. The field site catches ranged from 3 to 763. The percentage distribution at each trap site are given in Figure 5.

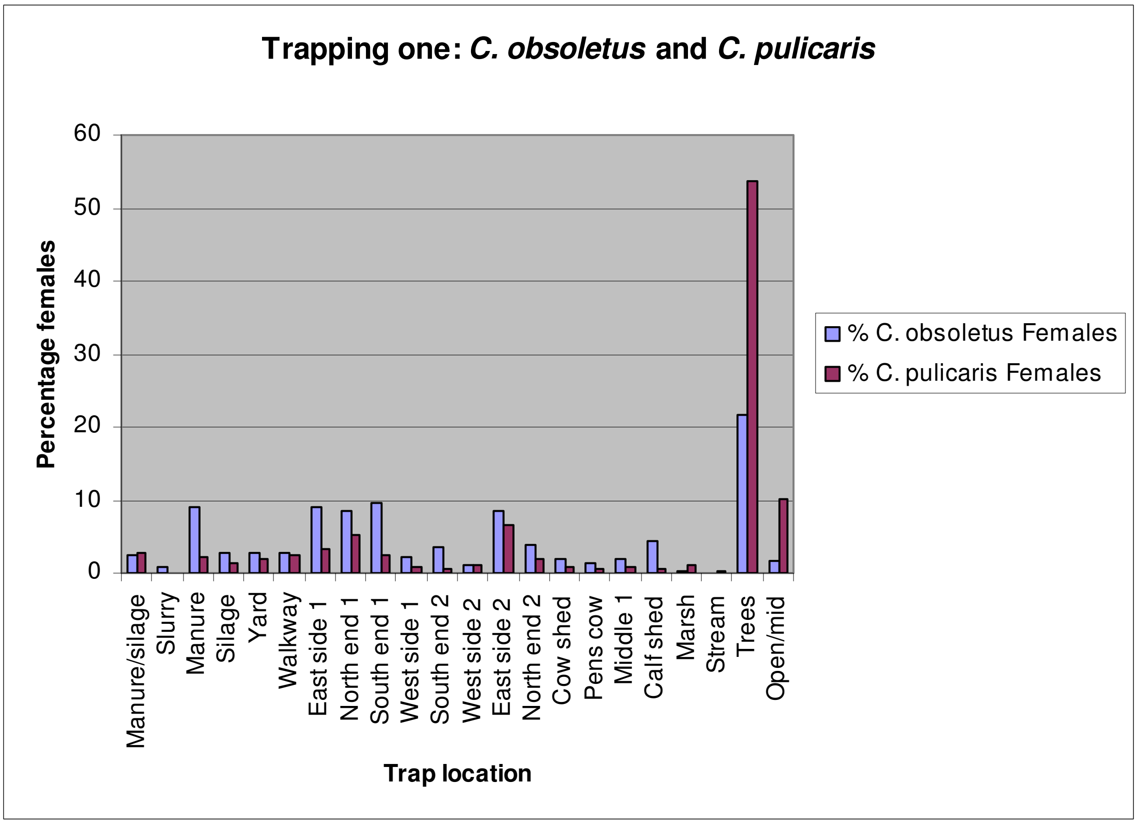

Figure 5 ***Culicoides obsoletus*** **and** ***Culicoides pulicaris*** **catches from each of the trap sites during trapping 1**

3.1.4.2 Trapping 2

All of the *C. obsoletus* catch sizes had reduced during trapping 2 from the catch sizes found during trapping 1 (Figure 13). The numbers dropped by between 54% (South end 2) and 99% (North end 1) from the farm holding traps, and by between 78% (Stream) and 99.5% (Trees) from the field traps. Catches obtained from the farm ranged from 23 (West side 1) to 577 (South end 2) (Figure 6). The field site catches ranged between 0 (Water trough) and 21 (Open/mid field) (Figure 6). The percentage distribution at each trap site are given in Figure 6.

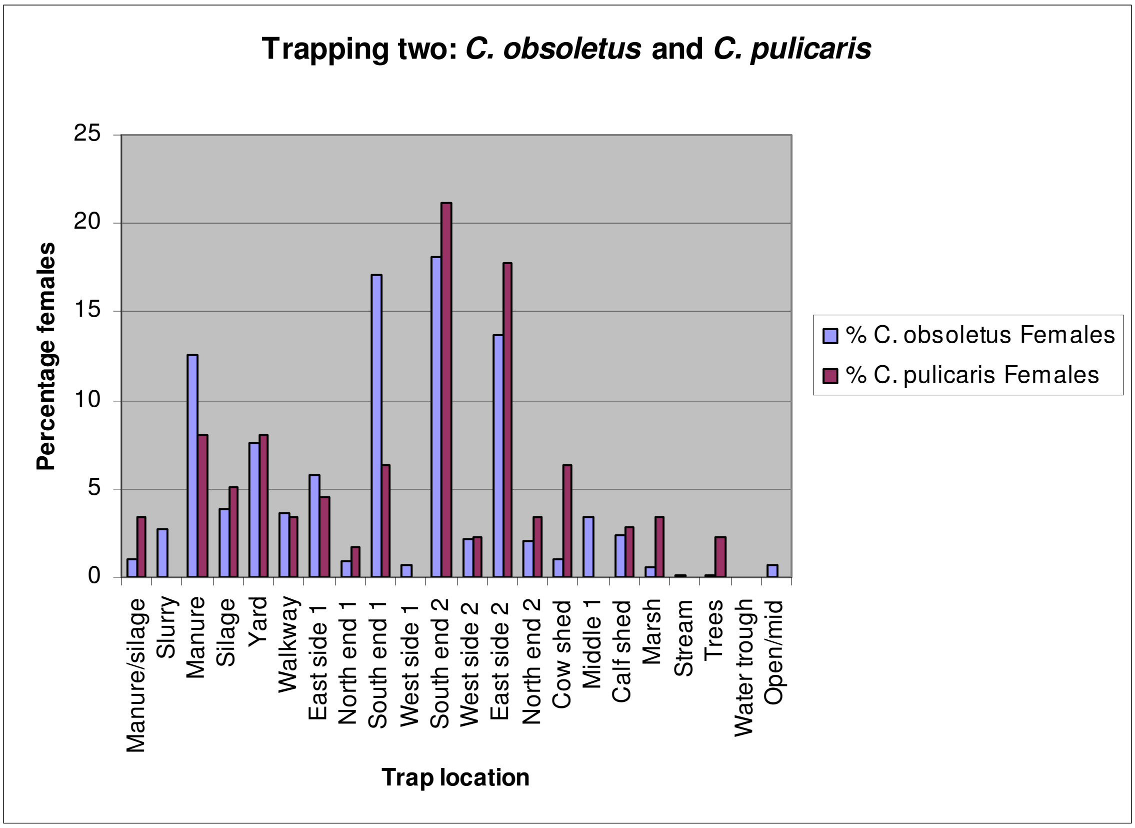

Figure 6 ***Cullicoides obsoletus* and *C. pulicaris* catches from each of the trap sites during trapping 2**

The *C. pulicaris* catch sizes had also all reduced during trapping 2, apart from the south end of shed 2, where the catch size increased. The *C. pulicaris* numbers had reduced by between 15% (Cow shed) and 96% (North end 1) at the farm holding sites, resulting in catches of between 0 and 37. The field trap *C. pulicaris* catch sizes had also reduced, with the most dramatic reductions seen at the Trees trap (from 763 to 4) and the Open/mid field (from 144 to 0).

The highest catches for both species during trapping 2 were from the southern and eastern sides of the sheds, but with only relatively low numbers at the west and northern shed trap sites. Moderate catches were associated with the Manure, Silage and Yard sites. The field catches were all very low.

3.1.4.3 Trapping 3

The catch results varied widely for both *C. obsoletus* and *C. pulicaris* during trapping 3, when compared to trapping 2 (Figure 7). The *C. obsoletus* catches from the farm holding ranged from 30 (Manure/silage) to 821 (Calf shed) and from the field sites from 0 (Stream and Water trough) to 19 (Trees). The Calf shed catch size was particularly high. The *C. obsoletus* catch sizes were similar to trapping 2 at almost half of the sites, numbers had reduced in several sites and increased at almost half of the trap sites. The field sites all had catches of less than 19. Although catches of *C. pulicaris* also varied widely from the catch sizes during trapping 2, the only site that reduced similarly to *C. obsoletus* was the catch at the Yard site (the *C. pulicaris* catch had reduced by 57%, compared to 60% for *C. obsoletus*).

The farm *C. pulicaris* catches during trapping 3 ranged from 0 (West side 1) to 43 (North end 1) and the field catches ranged from 0 to 10. The *C. obsoletus* and *C. pulicaris* distributions were again similar during this trapping. The results were the highest from the north, east and south sides of shed 1 and the south and east sides of shed 2 for both species. The Manure, Silage and Slurry sites also had relatively high catches as well as the Walkway. Field catches were again relatively low. Although the catch of *C. pulicaris* at the Calf shed was relatively high, the *C. obsoletus* numbers from this site were also particularly high. The percentage distribution at each trap site are given in Figure 7.

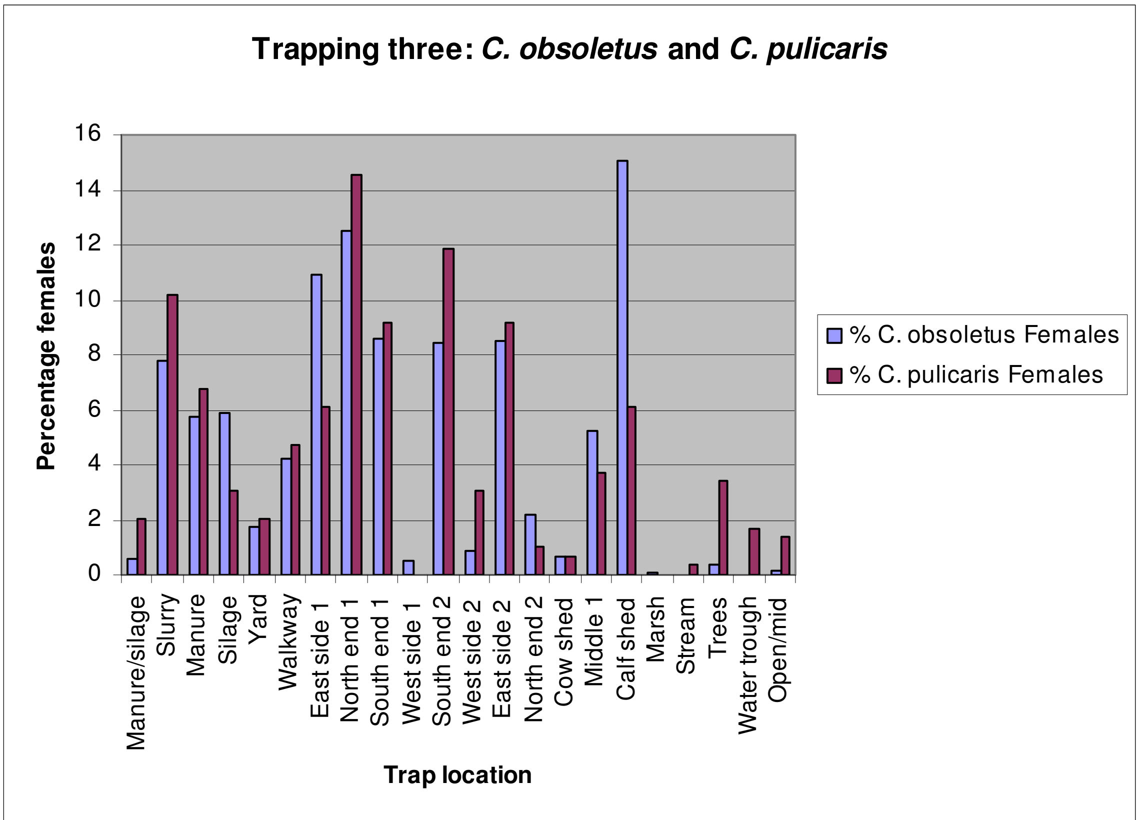

Figure 7 ***Cullicoides obsoletus*** **and** ***C. pulicaris*** **catches from each of the trap sites during trapping 3**

3.1.4.4 Trapping 4

The *C. obsoletus* numbers increased at all of the trap sites by between 1.02 times (Calf shed) and 1.26 times (Middle 1) and up to 9.9 times (Silage), when compared to numbers trapped during trapping 3 at the farm holding (Figure 8). More dramatic increases occurred at the field trap sites, with catches of between 6 (Stream) and 597 (Trees). The catches at the farm sites ranged between 70 (West side 1) and 3190 (Silage). Nine of the sites had catches of above 1000, and four catches were higher than the catch from that site during trapping 1. Six of the sites had catches above 1000 during trapping 1 as well as trapping 4, these were the north, east and south sides of shed 1, the south and east sides of shed 2 as well as the Manure site.

Culicoides pulicaris numbers increased by between 1.5 times (Manure/Silage) and 13 times (Silage) during trapping 4 when compared to catch numbers during trapping 3, in most but not all of the farm trap sites. The catches at the farm sites ranged between 0 (West side 1) and 114 (Silage). The field site catches ranged from 1 (Stream) to 108 (Trees).

The *C. pulicaris* distributions were similar to *C. obsoletus*, but with a higher relative catch at the Trees and Open/mid field sites and a lower relative number at the Manure site. The catches inside the animal housing, at the Yard site and at most of the field sites had relatively low catch sizes for both species. The percentage distribution at each trap site are given in Figure 8.

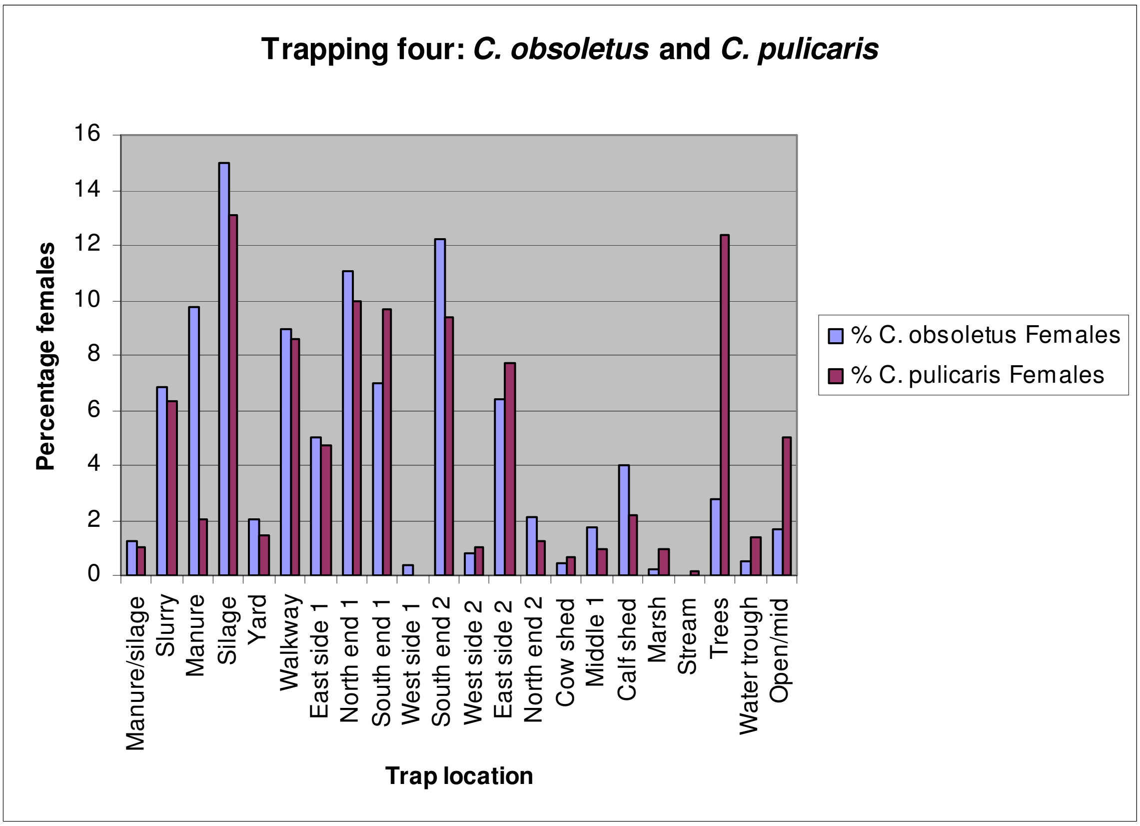

Figure 8 ***Cullicoides obsoletus*** **and** ***C. pulicaris*** **catches from each of the trap sites during trapping 4**

3.1.4.5 Trapping 5

The catches during trapping 5 were associated with reduced catches at all of the farm sites when compared to results from trapping 4, for both *C. obsoletus* and *C. pulicaris* (Figure 9). The *C obsoletus* numbers decreased by only 8% in the Cow shed catch, but otherwise the catch sizes reduced by between 79% (West side 1) and 98% (Manure/silage) at the other trap sites. The farm catch sizes ranged between 5 and 290.

The peak catch numbers of *C. obsoletus* at the field site during trapping 5 was 65 from the Trees site and from the other four sites counts ranged from 1 to 15. Catches from the field sites had reduced compared to the trapping 4 catches by between 79% (Marsh) and 99% (Water trough). This is consistent with the reduction of numbers from the farm sites.

The catch sizes of *C. pulicaris* from the farm sites had reduced by between 37% (Calf) and 90% (North end 1) during trapping 5 when compared to trapping 4. The *C. pulicaris* numbers ranged from 0 to 19. There were two sites with a catch of zero, the south and west sides of shed 1. The field site catches reduced by between 90% and 100% between the two trapping periods.

The sites with relatively high *C. obsoletus* numbers during this trapping were the north end of shed 1, the east side of shed 2 and the Manure, Slurry and Silage sites. The highest catches for *C. pulicaris* were the south and east sides of shed 2 and the Silage, Slurry and Trees trap sites. The Calf shed catch was also relatively high for both *C. obsoletus* and *C. pulicaris*. The percentage distributions at each trap site are given in Figure 9.

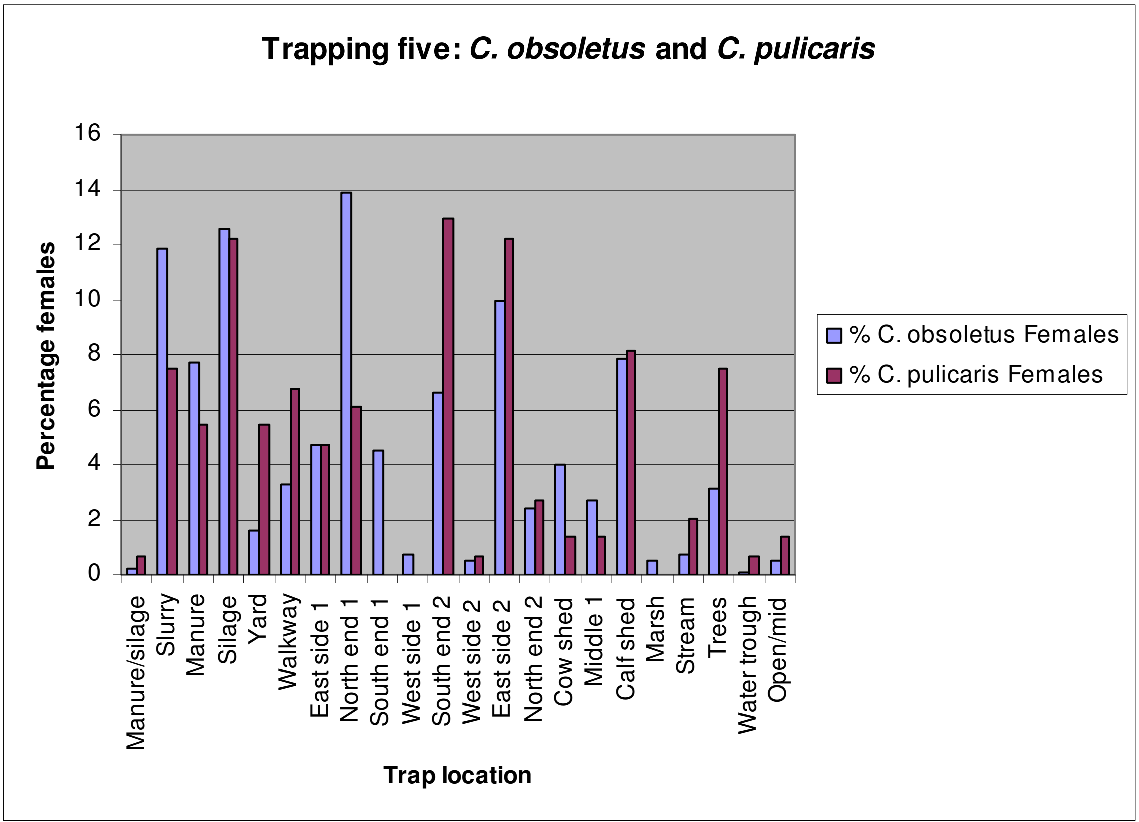

Figure 9 ***Cullicoides obsoletus* and *C. pulicaris* catches from each of the trap sites during trapping 5**

3.1.4.6 Trapping 6

The *C. obsoletus* catches had reduced to less than 10 from all of the trap sites, apart from the Slurry site (10), the middle of shed 1 (47) and the Calf shed (56). No *Culicoides* were caught from three of the farm sites, which were the Yard and the west sides of sheds 1 and 2 (Figure 10). The Trees and Open/Mid field traps also had catches with no *C. obsoletus* and the Water trough trap only caught 2 *C. obsoletus*. The Marsh and Stream traps had blown over. The percentage distributions at each trap site are given in Figure 10.

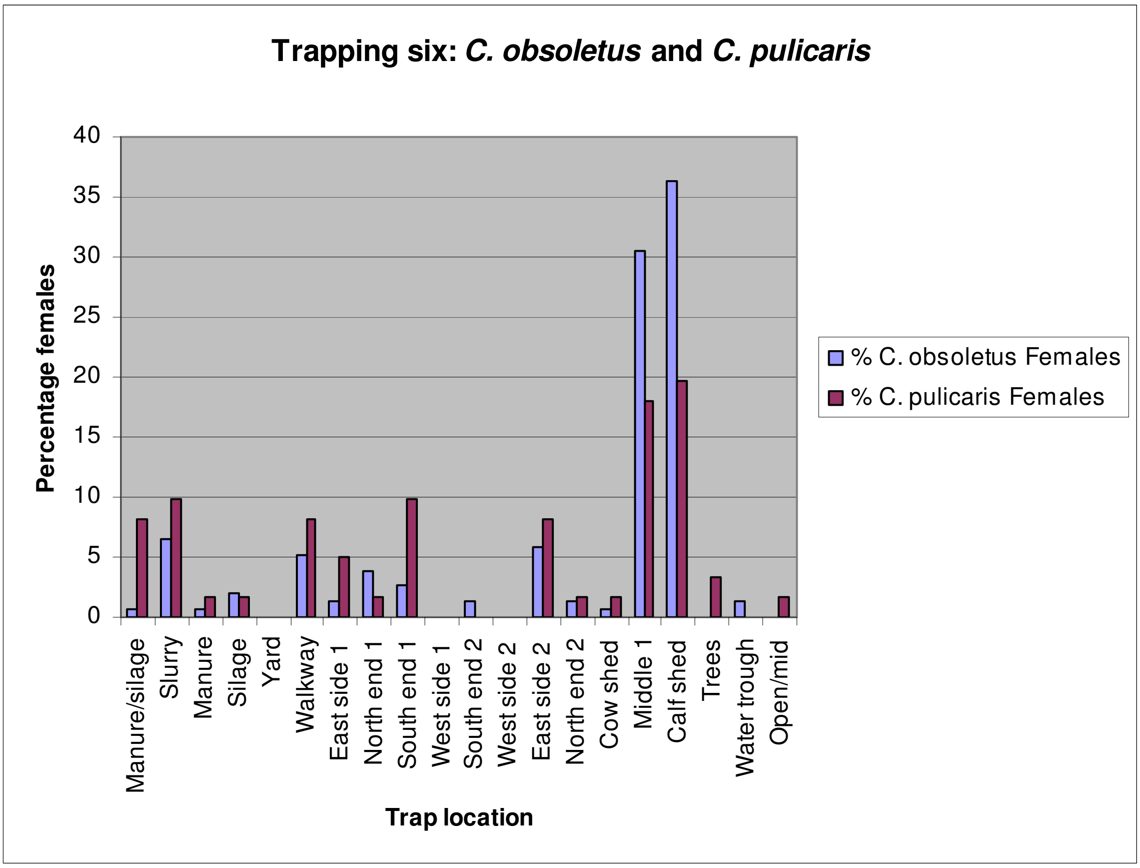

Figure 10 ***Cullicoides obsoletus*** **and** ***C. pulicaris*** **catches from each of the trap sites during trapping 6**

The *C. pulicaris* numbers at all of the trap sites had reduced from the trapping 5 catch sizes apart from the Manure/silage site (increased from 1 to 5), the south end of shed 1 (increased from 0 to 6), the middle of shed 1 (increased from 2 to 11) and the Calf shed catch remained at 12. The catch sizes reduced at the other sites by between 45% (Slurry) and 94% (Silage) and one catch reduced from 19 to 0 (South end 2) and from 1 to 0 (West side 1). The *C. pulicaris* catches ranged from 0 (Yard, West side 1, South end 2, West side 2 and Water Trough) to 12. Only two sites were above 10, Calf shed (12) and Middle 1 (11).

There was therefore a distinct shift during trapping 6 in the relative populations of both *C. obsoletus* and *C. pulicaris* to the middle of shed 1 and the Calf shed, both sites within the animal housing.

3.1.4.7 Trapping 7

Due to strong winds at the Manure and Manure/silage sites and the field sites, traps were not set in these locations during trapping 7. The catches of *C. obsoletus* on the farm ranged from 3 to 224 and had increased from between 1.5 times (south end of shed 2 from 2 to 3) and 99 times (east side of shed 1 from 2 to 198), when compared to the catch sizes from trapping 6, at all of the trap sites (Figure 11).

During trapping 7 the *C. pulicaris* catches at the majority of the sites remained the same or increased relative to the catch sizes from trapping 6. The *C. pulicaris* numbers had increased by between 1.3 times (South end 1) and 13 times (Silage). The south and west sides of shed 2 both failed to catch any *C. pulicaris* females, as in trapping 6. The catches ranged between 0 and 37.

The relative distributions of the *C. obsoletus* and *C. pulicaris* populations were again similar, with notable peaks for both species at the Walkway, the east side of shed 1 and the Calf shed. There were also relatively high numbers caught in the middle of shed 1. There were moderate sized catches from the north end of shed 1 for *C. obsoletus* and the Slurry and Silage catches for *C. pulicaris*. The percentage distributions at each trap site are given in Figure 11.

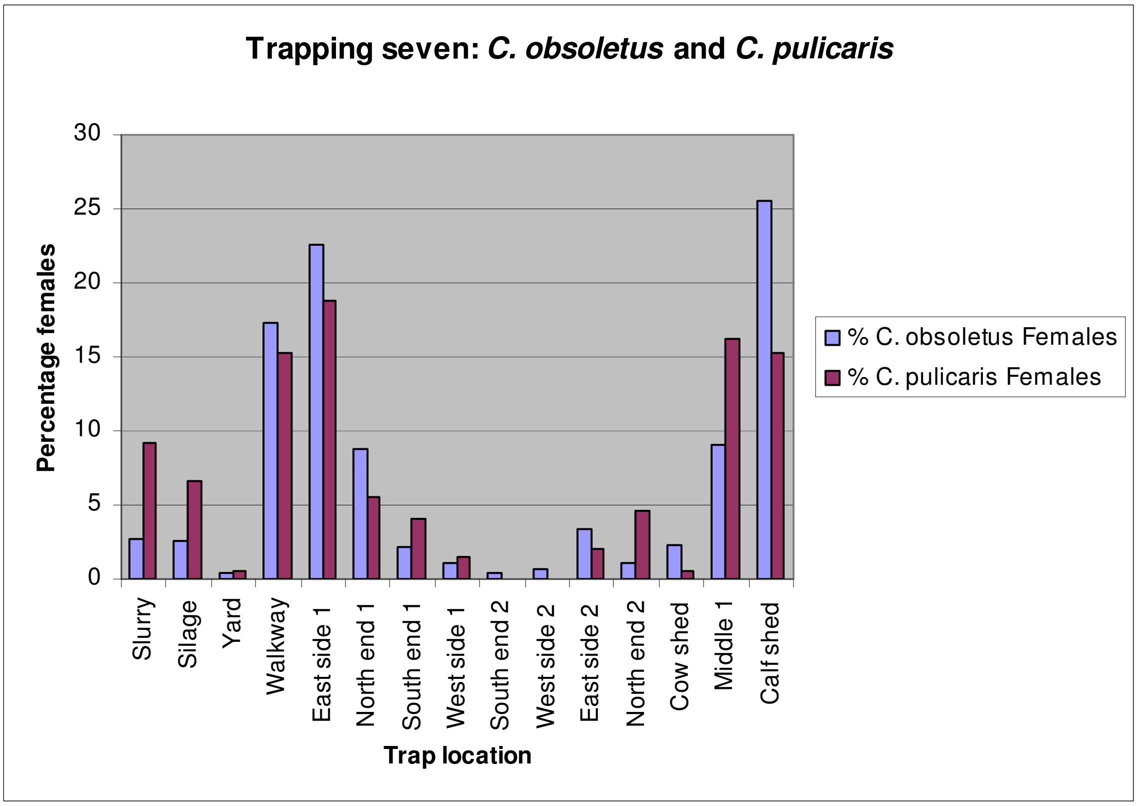

Figure 11 ***Cullicoides obsoletus* and *C. pulicaris* catches from each of the trap sites during trapping 7**

3.1.4.8 Trapping 8

During trapping 8 the trap on the east side of shed 2 was disconnected and the Trees trap and Water trough traps had both blown over during the night. The *C. obsoletus* catch numbers at the remaining traps were reduced when compared to catches from trapping 7, apart from the catches from the south ends of sheds 1 and 2, which remained the same, and two sites that had marginal increases (Figure 12). All of the other catches had decreased by between 40% (West side 1) and 93% (Walkway). The range of catches from the farm sites was between 0 (Manure/silage and Yard) and 85 (Middle 1). Only 3 (Stream), 1 (Marsh) and 0 catches (Open/mid field) were obtained at the three functioning field sites

The majority of the sites had reduced *C. pulicaris* catches when compared to trapping 7, with reductions of between 23% (North end 2) and 97% (Walkway). The Yard site catch reduced from 1 to 0. Five sites remained the same or marginally increased when compared to trapping 7. The catches during trapping 8 ranged from 0 (Manure/silage, Yard, West side 2, Marsh, Stream and Open/mid field) to 15 (East side 1). Only one other site had a catch of greater than 10 (Middle 1, 13). The north, south and west sides of shed 1 as well as the north end of shed 2 had relatively high *C. pulicaris* catch numbers. No *C. pulicaris* were caught at the three field sites during this trapping.

Trapping 8 was associated with relatively high catches from the middle of shed 1 and the Calf shed for both species, but particularly *C. obsoletus*. The north, east and south sides of shed 1 had relatively high catches for both species. The percentage distributions at each trap site are given in Figure 12.

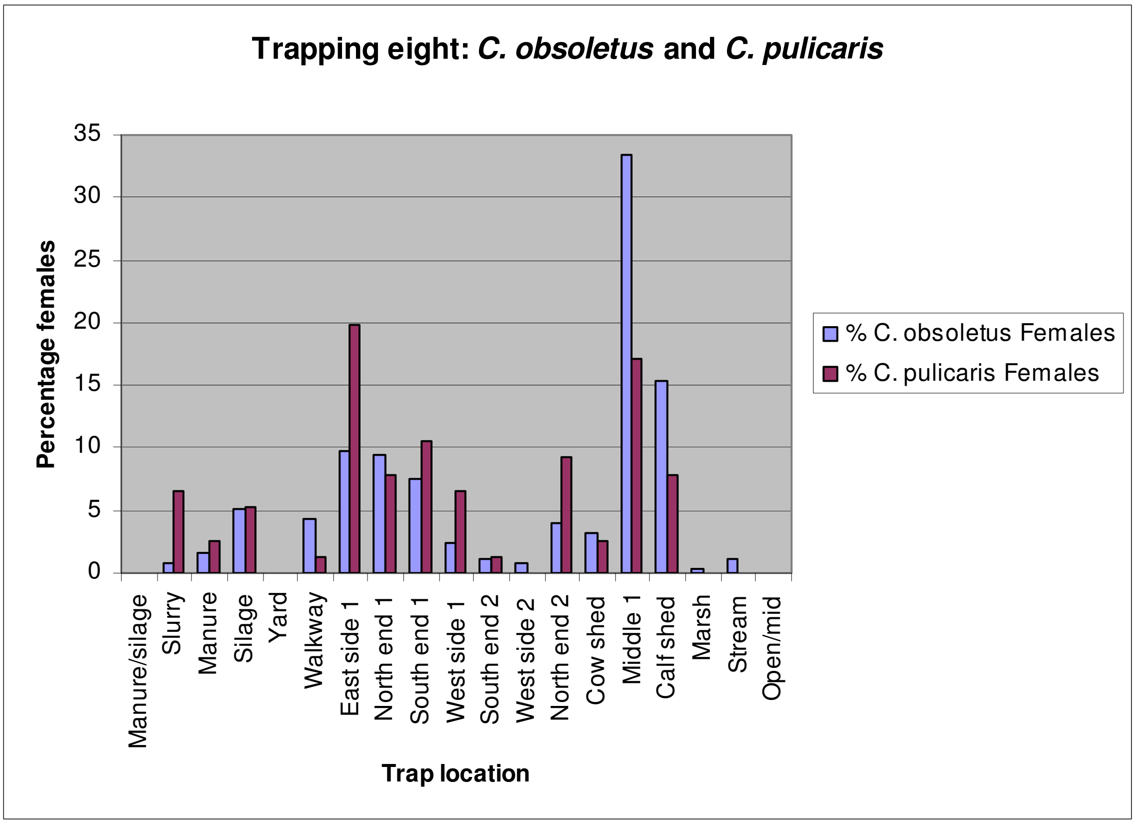

Figure 12 *Cullicoides obsoletus* and *C. pulicaris* catches from each of the trap sites during trapping 8

3.1.4.9 Summary of trapping distributions

The highest overall *Culicoides* numbers were achieved during trapping 1, with trapping 4 having the next highest catches. The scale of the total catch size during trapping 1 was largely influenced by the catches from the Trees site. The catches during trapping 2 and 3 were all consistently reduced when compared to both trappings 1 and 4, but still with some moderate to high *Culicoides* numbers. Trapping 3 catches were variable when compared to trapping 2 with some sites increasing in number and some decreasing. Trapping 5 had a general reduction in the catch sizes from all of the trap sites, which continued to very low numbers during trapping 6, which produced the smallest total catch during the study. Trapping 7 had reasonably low catch numbers but with an increase relative to trapping 6. Trapping 8 produced catches of a magnitude equivalent to or less than the catch sizes collected during trapping 7. The variations in the total number of female *Culicoides* collected between trappings are illustrated in Figure 13.

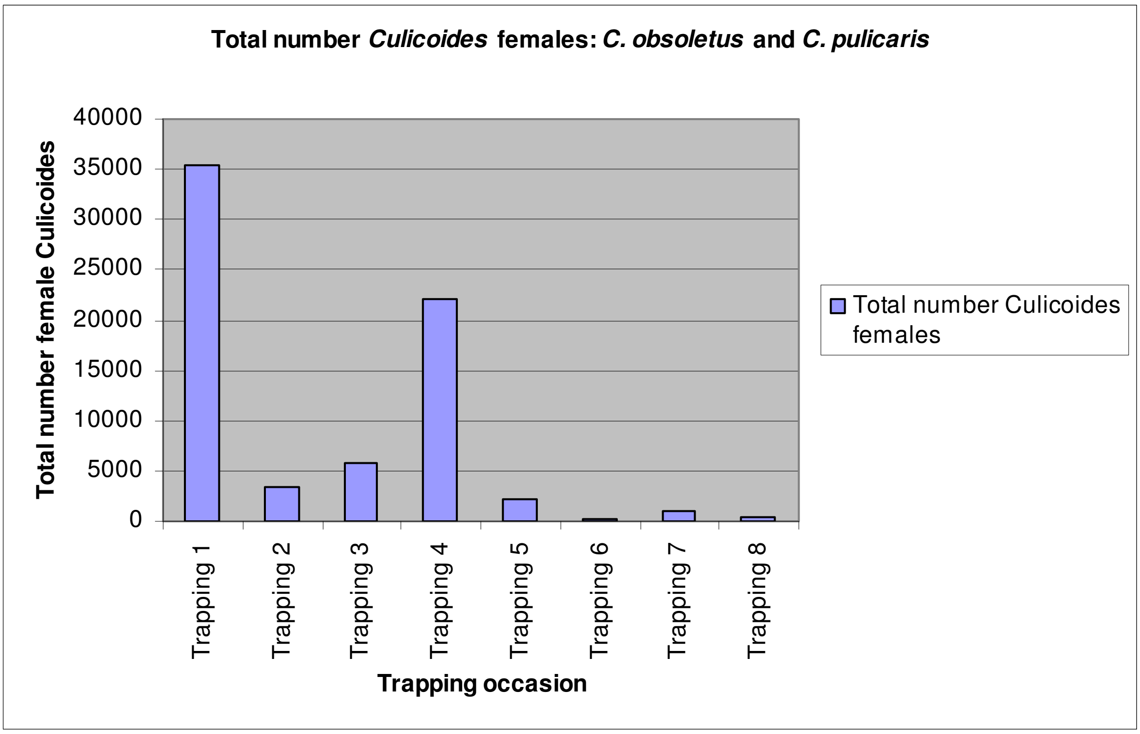

Figure 13 Total numbers of female *C. obsoletus* and *C. pulicaris* per trapping from all sites

3.1.5 *Culicoides* total female catch sizes comparing farm and field trap location data

The total catches from the all of the farm sites and all of the field sites from each trapping were examined to determine any variations between trappings associated with these two groups of trap locations. The results are shown in Tables 5 and 6.

Table 5 Total, mean number and percentage of female *C. obsoletus* collected from the farm and field trap locations

Location	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 6	Tr 7	Tr 8	Total
Farm total no's	25970	3148	5434	21287	1984	152	877	252	58001
Farm mean per catch	1527.65	185.18	319.65	1252.18	116.71	8.94	58.47	15.75	436.10
Farm % of total	44.78	5.43	9.37	36.70	3.42	0.26	1.51	0.43	
Field total no's	8051	48	28	1104	101	2		4	9338
Field mean Per catch	2012.75	9.60	5.60	221.80	20.20	0.67		1.33	311.27
Field % of total	86.22	0.51	0.30	11.82	1.08	0.02		0.04	

Table 6 Total, mean number and percentage of female *C. pulicaris* collected from the farm and field trap locations

Location	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 6	Tr 7	Tr 8	Total
Farm total no's	491	165	278	698	130	58	197	75	2092
Farm mean per catch	28.88	9.71	16.35	41.06	7.65	3.41	13.13	4.69	15.73
Farm % of total	23.47	7.89	13.29	33.37	6.21	2.77	9.42	3.59	
Field total no's	926	10	20	173	17	3		0	1149
Field mean per catch	231.50	2.0	4.0	34.60	3.40	1.0		0	38.30
Field % of total	80.59	0.87	1.74	15.06	1.48	0.26		0	

The total numbers of *C. obsoletus* from the farm trap sites were similar during trappings 1 and 4, but with a slightly larger catch during trapping 1 (Table 5). *Culicoides pulicaris* numbers, however, were higher from the farm sites during trapping 4 (Table 6). The fluctuations in catch sizes were otherwise consistent with those described in section 3.1.4.9 for the overall total of *C. obsoletus* and *C. pulicaris* between trappings.

The field sites had a bias towards high catch sizes, of both *C. obsoletus* and *C. pulicaris*, during trapping 1 (Table 5 and 6). During trapping 1, 86% of the total catch from all trappings of *C. obsoletus* and 81% of the total *C. pulicaris* were collected during this trapping. This was due to particularly high numbers of both species that were collected from the Trees trap location during trapping 1. Otherwise the variations in catch size between trappings were similar to the farm sites.

3.1.6 *Culicoides obsoletus* males

3.1.6.1 Prevalence of *C. obsoletus* males at the different trapping sites

Males of the *C. obsoletus* group complex were trapped at all of the sites apart from the Water trough and Marsh sites. The total catches from the trap sites ranged from three (Open/mid field) to 86 (East side 2). Only the east side of shed 2 and the Calf shed traps caught males at every trapping. Otherwise individual catches ranged from 1 (majority of the traps during at least one trapping) to the highest individual catch of 42 (east side of shed 2 during trapping 4). The highest overall numbers of males were from the east side of shed 2 (86), the Walkway (63), the east side of shed 1 (56), the middle of shed 1 (52) and the Calf shed (47). There were a total of between 20-33 trapped from the Manure/silage, the north and south ends of shed 2, the Yard, Manure, Silage sites, and the north end of shed 1. The west side of shed 2, Cow shed 2, Trees and Stream traps caught between 10-19 males and only a total of 3-8 males were trapped by the Open/mid field traps and the west and south sides of shed 1.

Taken as a percentage of all *Culicoides* trapped, including males and females, the middle of shed 1 had the highest percentage of *C. obsoletus* males from the farm sites, of 2.76%. The percentage males from all of the farm sites ranged from 0.13% (South end 1) to 2.76% (Middle 1). The percentage of the total female and the total male *C. obsoletus* collected at each trapping, taken as a percentage of the totals collected during the entire study, were then compared to identify differences between the relative proportions between trappings, as shown in Table 7.

Table 7 Percentage of female and male *C. obsoletus* collected during each trapping

Trapping number (Tr)	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 6	Tr 7	Tr 8
Percentage of total females trapped	50.5	4.5	8.0	32.0	3.0	0.20	1.0	0.35
Percentage of total males trapped	20.0	4.0	9.0	32.0	2.0	2.0	23.0	8.0

The ratios between trappings were similar for both the male and female catches between the first five trappings, except during trapping 1 where the females were proportionally higher during this trapping. The males were proportionally higher in trappings 6, 7 and 8.

The numbers of males trapped at each site during each tapping were compared to the percentage of *C. obsoletus* females in that catch (as a percentage of all of the *C. obsoletus* females within that trapping). The percentage females were listed as females above or below the percentage mean (Table 8).

Table 8 Correlation of male and female *C. obsoletus* catch sizes

Numbers of males	Number of catches with % females above the % mean	Number of catches with % females below the % mean
>20	7	0
10-19	6	1
5-9	12	11
<5	22	39
0	8	50

The higher percentage female catches (those above the mean) were associated with larger male catches. The numbers of *C. obsoletus* group males were examined to indicate the potential proportions relative to the female population, for potential extrapolation of the *C. obsoletus* group sibling species data. Overall the numbers of males correlated with the numbers of females in individual catches, suggesting extrapolation of the sibling species data could be representative for females of the *C. obsoletus* group.

3.1.6.2 *Cullicoides obsoletus* group male speciation

Speciation of the *C. obsoletus* group was performed by examination of the genitalia of males from this group. All four sibling species within the *C. obsoletus* group were found during the study, which are *C. obsoletus*, *C. chiopterus*, *C. scoticus* and *C. dewulfi*. From the farm holding sites, using males collected from all of the trappings, from all of the sites, the proportions of the different species found were 42% *C. obsoletus*, 11% *C. chiopterus*, 10% *C. scoticus* and 37% *C. dewulfi*. From the field trap sites no *C. dewulfi* were trapped and the percentages of the other species were 62% *C. obsoletus*, 14% *C. chiopterus* and 24% *C. scoticus*. Overall from all the sites the proportions were 43% *C. obsoletus*, 11% *C. chiopterus*, 11% *C. scoticus* and 35% *C. dewulfi*. As the numbers of males were generally low, the varying proportions of the different species within individual trappings were difficult to assess. No *C. obsoletus* group males were found at the Marsh and Water trough sites.

3.1.6.3 *Cullicoides obsoletus* sibling species

Culicoides obsoletus: This species was consistently identified at all but one of the farm sites during the majority of the trappings. Individual sites had no *C. obsoletus* for occasional trappings. Only the south end of shed 1 had no *C. obsoletus* identified throughout the study. *Culicoides obsoletus* was found at only the Stream, Trees and Open/mid field sites of the field traps.

Culicoides chiopterus: This species was identified at most of the farm sites apart from the Slurry site, Silage store and the middle of shed 1. All of the farm sites had this species identified during trapping 1. No males of this species were identified at any of the farm or field sites during trappings 5 and 6 and only single sites were positive during trapping 7 and 8. Otherwise *C. chiopterus* was found in from one to four trappings, for all of the positive sites. This species was only identified at the Stream and Open/mid field sites out of the field sites.

Culicoides scoticus: *Culicoides scoticus* was identified at all of the farm trap sites, apart from the Cow shed trap in shed 2. *Culicoides scoticus* was also found at the farm sites during all of the trappings apart from trapping 2. Only a single site however was positive during each of trappings 6 and 8. Otherwise this species was found at each site in between one and four trappings. This species was found only at the Stream and the Trees field sites.

Culicoides dewulfi: This species was found at all of the farm sites, for between one and seven trappings. This species was not identified at any of the field sites.

The proportions of the different male *C. obsoletus* sibling species found at each of the trap sites from all of the trapping occasions are shown in Figure 14. The males identified as "No tail" are those with missing genitalia which prevented accurate speciation of these particular males.

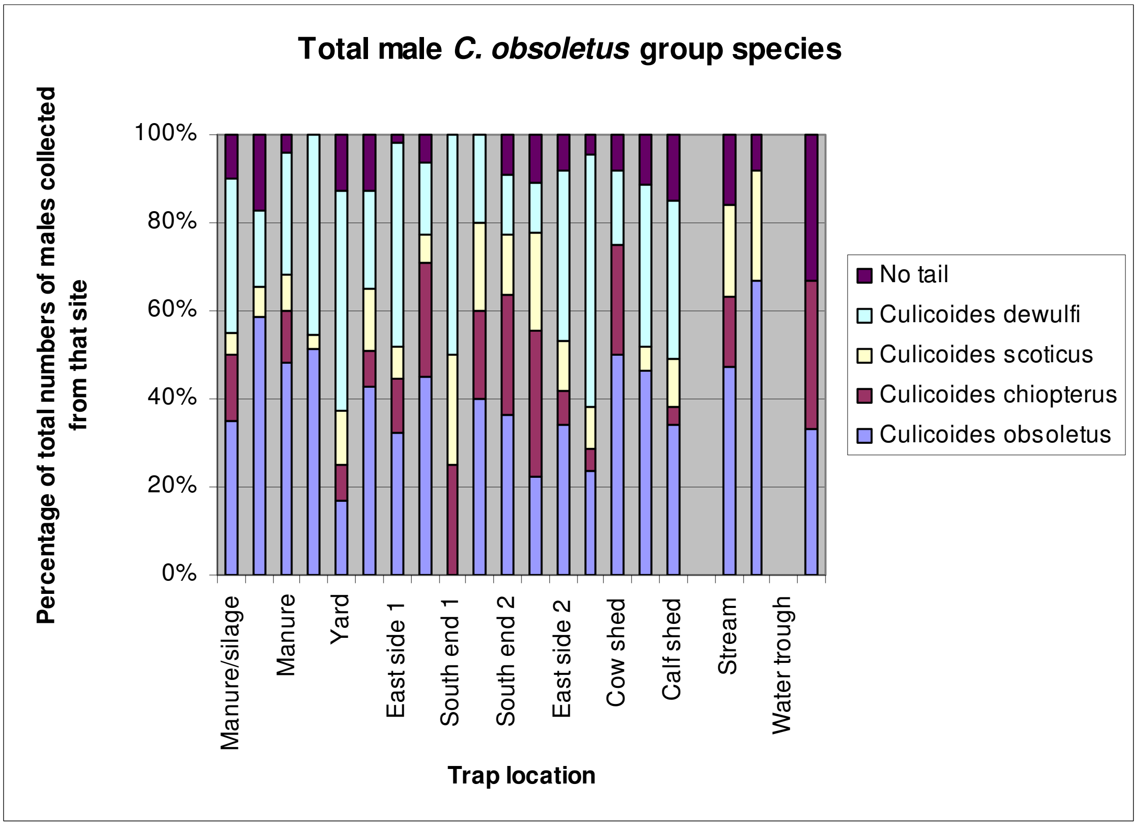

Figure 14 Proportions of male *Culicoides obsoletus* sibling species at each of the trap sites, using the total catches from all trappings

3.1.7 *Culicoides pulicaris* males

3.1.7.1 Prevalence of male *C. pulicaris*

The numbers of *C. pulicaris* males did not necessarily correlate with the magnitude of female numbers, with some very high catches having no males (including female catches of up to 114). The numbers of males ranged between 0 and 17 per trap site per trapping, with the east side of shed 1 having the highest overall number of *C. pulicaris* males, caught during one trapping. The highest total number of males trapped was from the east side of shed 2, with a total of 31 from seven trappings. The highest number of *C. obsoletus* males was also found at this site. A total of 96 trappings caught no male *C. pulicaris*, including all eight of the Cow shed trappings. This also included seven out of the eight trappings from the Calf shed and the west side of shed 1, and six out of the eight trappings for the east side and middle of shed 1 and the west side of shed 2. Using a percentage prevalence of male *C. pulicaris* within the total counts of *C. obsoletus* and *C. pulicaris* males and females, *C. pulicaris* males from the farm sites ranged from 0 (Cow shed) to 1.12% (Manure/silage). Sites other than the Manure/silage trap site had catches containing less than 1% *C. pulicaris* males. The sites above 0.5% were the middle of shed 1, Walkway, the east side of shed 2 and the Yard. Of the field sites the Stream site trapped relatively high numbers of male *C. pulicaris* (2.67%) with the other field sites catching between 0.11% (Trees) and 1.87% (Marsh).

3.2 Analysis of the reproductive stages of the trapped *Culicoides obsoletus* and *Culicoides pulicaris* females

The *Culicoides* females collected during the study were assessed for reproductive stage by examining the colour of the abdomen. The nulliparous females had a largely colorless abdomen and parous females were identified by a red colouration to the abdomen. The presence of eggs differentiated the gravid from the non-gravid females and the presence of blood within the abdomen indicated the recently blood-fed females. Each catch was examined and the numbers of each stage, including numbers of nulliparous relative to parous and the presence of gravid and of blood fed females, were all recorded.

The nulliparous females represent the young pre-breeding females in the population and the parous females are the mature females that have bred. As the numbers of nulliparous females represent the newly emerged female population increased numbers of nulliparous females can be used as an indicator of nearby breeding sites. Similarly gravid females may also provide an indicator of breeding sites in the vicinity.

Blood fed and gravid females can be classed as parous or nulliparous depending on the maturity of these females. During this study the maturity of the blood fed and gravid females was not assessed, and these females were only recorded as blood fed or gravid.

3.2.1 *Culicoides obsoletus* female reproductive stages

The results at each site varied between trappings. Nulliparous and parous females were found at all of the sites. The individual catches could have greater numbers of nulliparous than parous females, equal proportions of nulliparous and parous recorded as "50:50" (within five midges) or greater numbers of parous than nulliparous females. The individual catches were therefore recorded according to the relative proportions of nulliparous and parous females within that catch. The results for *C. obsoletus* are shown in Table 9 and for *C. pulicaris* in Table 11. The catches from each of the trap sites were also classed as either negative or positive for gravid and blood fed females, and the percentage of positive catches was calculated for *C. obsoletus* (Table 10) and for *C. pulicaris* (Table 12).

Table 9 The number of nulliparous and parous *Culicoides obsoletus* females at different trappings

Trap location	No of catches assessed	% *C. obsoletus* catches N>P (n)	% *C. obsoletus* catches N:P= 50:50(n)	% *C. obsoletus* catches P>N (n)	% *C. obsoletus* catches 0 (n)
Farm trap sites					
Manure/ Silage	7	28.6 (2)	14.3 (1)	42.9 (3)	14.3 (1)
Slurry	8	0	25.0 (2)	75.0 (6)	0
Manure	6	16.7 (1)	0	66.7 (4)	16.7 (1)
Silage	7	0	14.3 (1)	85.7 (6)	0
Yard	8	25.0 (2)	12.5 (1)	37.5 (3)	25.0 (2)
Walkway	8	12.5 (1)	25.0 (2)	62.5 (5)	0
East side 1	8	12.5 (1)	0	87.5 (7)	0
North end 1	8	50.0 (4)	0	50.0 (4)	0
South end 1	8	12.5 (1)	12.5 (1)	75.0 (6)	0
West side 1	8	0	25.0 (2)	62.5 (5)	12.5 (1)
South end 2	7	14.3 (1)	28.6 (2)	42.9 (3)	14.3 (1)
West side 2	7	14.3 (1)	0	71.4 (5)	14.3 (1)
East side 2	7	0	0	100.0 (7)	0
North end 2	8	0	0	100.0 (8)	0
Cow shed	8	37.5 (3)	12.5 (1)	50.0 (4)	0
Middle 1	8	25.0 (2)	25.0 (2)	50.0 (4)	0
Calf shed	7	14.3 (1)	14.3 (1)	71.4 (5)	0
Field trap sites					
Marsh	6	66.7 (4)	16.7 (1)	16.7 (1)	0
Stream	6	33.3 (2)	16.7 (1)	33.3 (2)	16.7 (1)
Trees	5	20.0 (1)	0	60.0 (3)	20.0 (1)
Water trough	5	40.0 (2)	0	20.0 (1)	40.0 (2)
Open/mid field	7	42.9 (3)	0	28.6 (2)	28.6 (2)

N = Nulliparous
P = Parous
Gr = Gravid
BF = Blood fed
n = number of catches

There were typically only low numbers of collections at the farm sites, only one (12.5 to 16.7%) or two of the collections (25.0 to 28.6%), where the numbers of nulliparous exceeded the parous numbers. There were no collections where nulliparous numbers exceeded parous from the Slurry, Silage, West side 1, East side 2 and North end 2. In between three and eight of the catches from other farm sites (37.5 to 100.0% of the catches) parous female numbers exceeded the numbers of nulliparous. All of the catches from the north and east sides of shed 2 had populations consisting of more parous than nulliparous midge. Eleven of the sites had more catches with parous numbers exceeding nulliparous, than with nulliparous exceeding parous (Table 9).

The field sites, apart from the Trees site, had more collection with nulliparous exceeding parous. The Trees site had a catch ratio similar to the farm site results. The remaining field sites still had catches of between one and two containing more parous than nulliparous. The catches with zero *C. obsoletus* catches are also indicated (Table 9).

Table 10 The number of trappings positive for gravid and blood fed *Culicoides obsoletus* females

Trap location	No of catches Assessed for gravid and blood fed (minus zero catches)	% catches with gravid	% catches with blood fed
Farm trap sites			
Manure/Silage	6	83.3 (5)	50.0 (3)
Slurry	8	87.5 (7)	87.5 (7)
Manure	6	100.0 (6)	66.7 (4)
Silage	8	62.5 (5)	75.0 (6)
Yard	6	83.3 (5)	83.3 (5)
Walkway	8	87.5 (7)	87.5 (7)
East side 1	8	87.5 (7)	87.5 (7)
North end 1	8	87.5 (7)	87.5 (7)
South end 1	8	87.5 (7)	75.0 (6)
West side 1	7	100.0 (7)	42.9 (3)
South end 2	7	71.4 (5)	57.1 (4)
West side 2	7	42.9 (3)	42.9 (3)
East side 2	7	85.7 (6)	100.0 (7)
North end 2	8	75.0 (6)	75.0 (6)
Cow shed	8	50.0 (4)	62.5 (5)
Middle 1	8	100.0 (8)	87.5 (7)
Calf shed	8	100.0 (8)	87.5 (7)
Field trap sites			
Marsh	6	50.0 (3)	16.7 (1)
Stream	5	40.0 (2)	40.0 (2)
Trees	5	80.0 (4)	40.0 (2)
Water trough	3	66.7 (2)	33.3 (1)
Open/mid field	5	60.0 (3)	40.0 (2)

The numbers of collections containing gravid females at the farm trap sites ranged from 71.4% to 100.0% (5 to 8), for fourteen of the trap sites. The remaining three sites, consisting of the west side of shed 2, the Cow shed 2 and the Silage site had only between 42.9% (3) and 62.5% (5) of the collections containing gravid females (Table 10).

The field sites had lower numbers of gravid and blood fed females when compared to the farm sites, apart from the Trees site where 80.0% (4) of the collections were positive for gravid females, similar to the farm sites. Between 40.0% and 66.7% of the collections at the other field sites were positive for gravid females (Table 10).

The positive blood fed females collections ranged between 75.0% and 100.0% (east side of shed 2) at the farm sites. The lowest numbers of positive collections for blood fed females were from the west sides of shed 1 and 2, the south end of shed 2, the Cow shed 2 and Manure site with only between 42.9% and 66.7% of the collections positive for blood fed females. The numbers of collections positive for blood fed females were low at all of the field sites (Table 10).

3.2.2 *Culicoides pulicaris* female reproductive stages

Nulliparous and parous females were found at all of the trap sites and similar to the *C. obsoletus* data the individual catches were recorded according to the relative proportions of nulliparous and parous females within that catch (Table 11).

Table 11 The number of nulliparous and parous *Culicoides pulicaris* females at different trappings

Trap location	No of catches assessed	% *Culicoides pulicaris* catches N>P (n)	% *Culicoides pulicaris* catches N:P=50:50 (n)	% *Culicoides pulicaris* catches P>N (n)	% *Culicoides pulicaris* catches 0 (n)
Farm trap sites					
Manure/Silage	7	28.6 (2)	14.3 (1)	42.9 (3)	14.3 (1)
Slurry	8	37.5 (3)	12.5 (1)	37.5 (3)	12.5 (1)
Manure	6	33.3 (2)	16.7 (1)	50.0 (3)	0
Silage	7	14.3 (1)	0	85.7 (6)	0
Yard	8	37.5 (3)	0	37.5 (3)	25.0 (2)
Walkway	8	37.5 (3)	25.0 (2)	37.5 (3)	0
East side 1	8	37.5 (3)	0	62.5 (5)	0
North end 1	8	62.5 (5)	0	37.5 (3)	0
South end 1	8	25.0 (2)	12.5 (1)	50.0 (4)	12.5 (1)
West side 1	8	12.5 (1)	12.5 (1)	12.5 (1)	62.5 (5)
South end 2	7	57.1 (4)	0	14.3 (1)	28.6 (2)
West side 2	7	0	14.3 (1)	28.6 (2)	57.1 (4)
East side 2	7	28.6 (2)	14.3 (1)	57.1 (4)	0
North end 2	8	12.5 (1)	12.5 (1)	75.0 (6)	0
Cow shed	8	25.0 (2)	12.5 (1)	50.0 (4)	12.5 (1)
Middle 1	8	37.5 (3)	25.0 (2)	25.0 (2)	12.5 (1)
Calf shed	7	57.1 (4)	28.6 (2)	14.3 (1)	0
Field trap sites					
Marsh	6	33.3 (2)	16.7 (1)	0	50.0 (3)
Stream	5	40.0 (2)	0	0	60.0 (3)
Trees	5	20.0 (1)	40.0 (2)	20.0 (1)	20.0 (1)
Water trough	5	40.0 (2)	20.0 (1)	0	40.0 (2)
Open/mid field	7	28.6 (2)	0	28.6 (2)	42.9 (3)

There were fewer collections from the farm trap sites with a greater proportion of parous than nulliparous *C. pulicaris* than were found for *C. obsoletus*. The catches with parous exceeding nulliparous numbers were found in between 14.3% and 75.0% (1 and 6) of the catches, compared to 37.5% to 100.0% of the catches for *C. obsoletus*. Seven of the 22 trap sites had a greater proportion of catches with parous numbers exceeding nulliparous, compared to eleven sites of *C. obsoletus*. Only the west side of shed 2 had no catches where nulliparous numbers exceeded parous. The trap sites with more catches where nulliparous numbers exceeded parous were the north end of shed 1, the south end of shed 2, the middle of shed 1 and the Calf shed (Table 11).

The Trees and Open/mid field sites had equal numbers of catches with nulliparous and parous numbers predominating. The remaining field sites had more catches where nulliparous numbers exceeded parous. There were more catches without any *C. pulicaris* than there were catches without *C. obsoletus*. All of the field sites had at least one catch without any *C. pulicaris* females (Table 11).

The catches from each of the trap sites were classed as either negative or positive for gravid and blood fed females, and the percentage of positive catches was calculated for *C. pulicaris* (Table 12).

Table 12 The number of collections positive for gravid and blood fed *Culicoides pulicaris* females

Trap location	No of catches Assessed for gravid and blood fed	% catches with gravid	% catches with blood fed
Farm trap sites			
Manure/Silage	6	16.7 (1)	50.0 (3)
Slurry	7	42.9 (3)	42.9 (3)
Manure	7	0	42.9 (3)
Silage	8	25.0 (2)	37.5 (3)
Yard	6	33.3 (2)	16.7 (1)
Walkway	8	75.0 (6)	62.5 (5)
East side 1	8	37.5 (3)	37.5 (3)
North end 1	8	37.5 (3)	37.5 (3)
South end 1	7	28.6 (2)	14.3 (1)
West side 1	3	0	33.3 (1)
South end 2	6	50.0 (3)	16.7 (1)
West side 2	4	50.0 (2)	50.0 (2)
East side 2	7	42.9 (3)	57.1 (4)
North end 2	8	25.0 (2)	25.0 (2)
Cow shed	7	0	42.9 (3)
Middle 1	7	28.6 (2)	28.6 (2)
Calf shed	8	12.5 (1)	37.5 (3)
Field trap sites			
Marsh	3	66.7 (2)	33.3 (1)
Stream	3	33.3 (1)	0
Trees	5	60.0 (3)	60.0 (3)
Water trough	3	66.7 (2)	33.3 (1)
Open/mid field	4	75.0 (3)	50.0 (2)

The numbers of positive gravid females in catches was less than for *C. obsoletus*. Thirteen sites had positive catches of between 12.5% and 50.0% (1 to 3 catches). The Walkway had a particularly high number of positive catches of 75% (6). The Manure site, west side of shed 1 and Cow shed all had no gravid females (Table 12).

The numbers of blood fed female positive catches were also low when compared to *C. obsoletus*. Fifteen of the farm sites had between 14.3% and 50.0% (1 to 3) positive catches. The Walkway and east side of shed 2 were comparatively high, with positive catches of 62.5% (5 catches) and 57.1% (4 catches), respectively. The numbers of gravid and blood fed positive catches at the field sites were comparable to the farm site results (Table 12).

To determine the potential influence of the proximity of livestock on the numbers and distribution of gravid and blood fed *C. obsoletus* and *C. pulicaris* at the field sites, the numbers collected during each trapping were examined (Table 13).

3.2.3 The numbers of gravid and blood fed *Culicoides* females collected at the field sites

Table 13 The numbers of gravid and blood fed *Culicoides* females collected at the field sites

Trap location	Tr 1	Tr 2	Tr 3	Tr 4	Tr 5	Tr 6	Tr 7	Tr 8
Marsh: *C. obsoletus*	7 Gr	0	0	5 Gr 1 BF	1 Gr			0
Marsh: *C. pulicaris*	2 Gr	2 Gr	0	1 BF	0			0
Stream: *C. obsoletus*	1 Gr 1 BF	0	0	1 Gr 1 BF	0			0
Stream: *C. pulicaris*	0	0	0	1 Gr	0			0
Trees: *C. obsoletus*	130 Gr 19 BF	0	3 Gr	21 Gr 7 BF	1 Gr	0		
Trees: *C. pulicaris*	43 Gr 18 BF	0	2 Gr	12 Gr 8 BF	0	1 BF		
Water trough: *C. obsoletus*		0	0	14 Gr 1 BF	0	1 Gr		
Water trough: *C. pulicaris*		0	2 Gr	2 Gr 1 BF	0	0		
Open/mid field: *C. obsoletus*	43 Gr 1 BF	4 Gr	0	5 Gr 1 BF	7 Gr	0		0
Open/mid field: *C. pulicaris*	9 Gr 3 BF	0	1 Gr	4 Gr 1 BF	0	0		0

Tr = Trapping
Gr = Gravid females
BF = Blood fed females

Numerous gravid and blood fed *C. obsoletus* and *C. pulicaris* were found at the field trap sites during trapping 1 and relatively high numbers were also found during trapping 4. Gravid *C. obsoletus* females were found at every site during both trappings, with a peak number of up to 130 at the Trees site during trapping 1. Gravid *C. pulicaris* females were also found at every site except the Stream trap site, with a peak number of 43 again at the Trees site during trapping 1. There were blood fed female *Culicoides* found at all of the trap sites, apart from the Marsh site during trapping 1 and the Stream site for *C. pulicaris* during trappings 1 and 4. Low numbers of gravid females of both species were found at occasional sites during trappings 2, 3 and 5. A single blood fed and single gravid female were found during trapping 6 (Table 13).

The animal field movements prior to the first trapping were uncertain and the numbers of gravid and blood fed found during trapping 1 may suggest recent livestock contact. Sheep were moved two days before trapping 4 to a field at least 10 m from the Marsh site and at least 55 m from the Open/mid field, which appeared to coincide with increased numbers of gravid and blood fed females of both *C. obsoletus* and *C. pulicaris* during this trapping. When sheep were located at least 100 to 180 m from the trapping fields there appeared to be a limited affect with only a few gravid females found during trapping 3, 5 and 6. Although sheep were moved to a field 65 m from the Marsh site and 65 m from the Open/mid field two days before trapping 8, there were no gravid or blood fed females found in the catches during trapping 8, although only low numbers of *Culicoides* were trapped at the field sites during this trapping (Table 13).

3.2.4 Ranges for the reproductive stages of the *Culicoides obsoletus* and *Culicoides pulicaris* females

The mean percentages of nulliparous (Table 14), parous (Table 15), gravid (Table 16) and blood fed (Table 17) stages were calculated for each of the trap sites, using the results collected from all of the trappings for both *C. obsoletus* and *C. pulicaris*. The total female catch contributed to by each reproductive stage as a percentage during each trapping was used to calculate the mean value for each trap site.

Table 14 Mean values of nulliparous *Culicoides* females

Range (means)	Trap sites: *C. obsoletus*	Range (means)	Trap sites: *C. pulicaris*
43-78%	Stream, Marsh, Water Trough, Open/mid field, Trees	42-53%	North end 1, Stream, Trees, Calf shed, Yard, Walkway
36-37%	Middle 1, Cow shed	36-41%	South end 2 South end 1 Middle 1 Slurry, Manure Water Trough
30-36%	North end 1, Yard, Calf shed, South end 2	30-35%	Manure/silage, Silage East side 2, East side 1, Cow shed, Open/mid field, Marsh
22-27%	Walkway, East side 1, West side 1, South end 1, North end 2, West side 2, Manure/silage		
13-22%	Slurry, Silage, Manure East side 2,	12-25%	West side 1 North end 2, West side 2

C. obsoletus: All five field sites had relatively high mean percentages (43-78%) of nulliparous females. All of the sites within the animal housing (Cow shed 2, the middle of shed 1 and the Calf shed) had means of between 30 and 37%. The Yard, Walkway and most of the external shed sites had mean percentages of between 22 and 36%. The lowest range of mean percentages was 13-22%, which included three of the four manure and forage sites (the Slurry, Silage and Manure sites) as well as the east side of shed 2 (Table 14).

C. pulicaris: The mean percentages of nulliparous females found in the Yard and Walkway catches were in the high, 42-53% range. The Manure/silage, Manure, Slurry and Silage sites all had mean percentages within the 30-41% range. The shed sites included the west sides of sheds 1 and 2 within the 12-25% range, the east sides of shed 1 and 2 in the 30-35% range and the south sides of sheds 1 and 2 in the 36-41% range. The mean from the north end of shed 1 was in the highest 42-53% range and the north end of shed 2 was in the 12-25% range. The field site mean values were anywhere between 30% and 53% and varied widely (Table 14).

Table 15 Mean values of parous *Culicoides* females

Range (means)	Trap sites: *C. obsoletus*	Range (means)	Trap sites: *C. pulicaris*
62-66%	East side 2, West side 2, North end 2, South end 1, Slurry	60-77%	North end 2, Silage
50-58%	East side 1, North end 1, West side 1, Calf shed, Cow shed, Manure, Manure/silage, Silage, Yard, Trees	48-56%	East side 1, East side 2, Manure, Cow shed
45-49%	Walkway, Middle 1, Open/mid field, South end 2		
30-33%	Marsh, Water Trough	30-43%	South end 1, North end 1 Middle 1, Manure/silage, Slurry, Walkway, Trees
		9-24%	Calf shed, Yard, West side 2, South end 2, West side 1, Open/mid field, Water, Trough, Marsh
7%	Stream		
		0	Stream

C. obsoletus: Three of the shed 2 sites had mean parous percentages of between 62-66%. Of the shed 1 sites only the south side was in this range. The remaining shed 1 sites had mean percentages in the 50-58% range. The south side of shed 2 was in the 45-49% range. The Yard site mean percentage was in the 50-58% range and the Walkway mean was in the 45-49% range. The manure and forage sites consisted of the Slurry mean in the 62-66% range and the Manure, Manure/silage and Silage sites were in the 50-58% range. The internal shed sites consisted of the Cow shed 2 and Calf shed sites with mean percentages within the 50-58% range, and the middle of shed 1 within the 45-49% range.

The field sites ranged from the Trees site mean within the 50-58% range, the Open/mid field within the 45-49% range and the remaining three sites with low means of 33% and less. The Stream site mean was very low at 7% (Table 15).

C. pulicaris: The highest mean parous percentage was higher for *C. pulicaris* than for *C. obsoletus*; however, the distribution of mean values was wider for *C. pulicaris*, with more sites having mean values in the lower ranges. The sites within the very high mean percentage range of 60-77% were the north end of shed 2 and the Silage site. The east side of shed 1 and 2 were both in the 48 to 56% range. The south and north sides of shed 1 were in the 30-43% range. The west side of shed 1 and the west and south sides of shed 2 mean values were only in the low range of 9-24%. The Walkway site was in the 30-43% range and the Yard site was in the 9-24% range. The manure and forage sites, other than the Silage site (in the 60-77% range), consisted of the Manure site with a mean in the 48-56% range and the Manure/silage and Slurry site mean values in the 30-43% range. Inside the animal housing the mean values ranged from the Cow shed 2 mean of 48-56%, the middle of shed 1 with a mean between 30-43% and the Calf shed mean in the low range of 9-24%. Of the field sites the Trees site mean was in the 30-43% range and the Marsh, Water trough and Open/mid field sites were in the 9-24% range. There were no *C. pulicaris* females trapped at the Stream site (Table 15).

Table 16 Mean values of gravid *Culicoides* females

Range (means)	Trap sites: *C. obsoletus*	Range (means)	Trap sites: *C. pulicaris*
16-21%	West side 1, Manure/silage, Walkway	11-17%	Stream, Water Trough
11-14%	Water Trough, East side 1, Slurry		
6-9.5%	Yard, South end 1, North end 1, East side 2, Manure, Silage, Middle 1, Cow shed, Marsh, Open/mid field	5.5-9.5%	Walkway, Marsh, Trees, Open/mid field
2-5.5%	Calf shed, South end 2, West side 2, North end 2, Stream, Trees	2-4%	East side 1, North end 1 West side 2, North end 2 Middle 1, Yard, Slurry
		0.1-1.9%	Manure/silage, Silage East side 2, South end 2, South end 1, Calf shed
		0	Cow shed, Manure, West side 1

C. obsoletus: The mean percentages of gravid females varied widely between the different trap sites. The mean percentage ranged between 2 and 21% from all sites. The manure and forage stores included the Manure/silage mean in the 16-21% range, the Slurry site mean in the 11-14% range and the Manure and the Silage site means were both in the low 6-9% range. The shed 1 site means ranged from the west side with a mean in the 16-21% range, the east side mean was in the 11-14% range and the north and south means were between 6-9%. The east side of shed 2 was in the 6-9% range and the remaining shed 2 sites had mean percentages between 2-5.5%. The Walkway mean was in the high 16-21% range and the Yard was in the 6-9% range. The Cow shed 2 and the middle of shed 1 both had means in the 6-9 percentage range and the Calf shed mean was 2-5.5%. Of the field sites the Water trough mean was in the 11-14% range, the Marsh and Open/mid field means were between 6-9% and the Trees and Stream site means were in the 2-5.5% range (Table 16).

C. pulicaris: The mean percentages of gravid females were high in the field site catches, with the Stream and Water trough mean percentages in the 11-17% range and the remaining three field sites had means within the 5.5-9.5% range. The Walkway mean was within the 5.5-9.5% range and the Yard mean was in the 2-4% range. The mean percentages from the east and north sides of shed 1 and the west and north sides of shed 2 were in the 2-4 % range. The east and south sides of shed 2 and the south side of shed 1 were in the 0.1-1.9% range. There were no gravid *C. pulicaris* females in the catches from the west side of shed 1. Of the manure and silage storage sites the Slurry site percentage mean was in the 2-4% range, the Manure/silage and Silage means were between 0.1-1.9% and no gravid *C. pulicaris* were found at the Manure site. Inside the animal housing the middle of shed 1 mean was in the 2-4% range, the Calf shed mean was in the 0.1-1.9% range and no gravid *C. pulicaris* were found in the Cow shed 2 catches (Table 16).

Table 17 Mean values of blood fed *Culicoides* females

Range (means)	Trap sites: *C. obsoletus*	Range (means)	Trap sites: *C. pulicaris*
		11-21%	Cow shed, Calf shed, Trees, Walkway, East side 2
		7-9%	North end 1, East side 1 South end 2 Middle 1 Manure/silage, Manure
6.3-7.8%	Silage, Middle 1, Walkway		
3.9-4.9%	Calf shed, Cow, East side 2, West side 2, Manure/silage	3.5-5.5%	Yard, South end 1, West side 1, Slurry, Silage, West side 2, North end 2
2.8-3.8%	North end 2, North end 1, East side 1, Slurry, Stream		
1.1-2.4%	Yard, Manure, South end 1, West side 1, South end 2	0.5-2.1%	Marsh, Water Trough, Open/mid field
0.1-1.0%	Water Trough, Marsh, Trees, Open/mid field		
		0	Stream

C. obsoletus: The manure and forage stores sites included the Silage site with a mean blood fed percentage in the highest 6.3-7.8% range. The distribution of blood fed females did not necessarily equate with trap location around the animal housing, with other locations not adjacent to the animal housing such, as the Walkway and Manure/silage sites, also having reasonably high mean percentages of blood fed. The sites within the animal housing, however, included the middle of shed 1 in the highest 6.3-7.8% range and the Cow and Calf shed sites were in the 3.9-4.9% range, along with external shed sites for shed 2. Relatively low percentages were associated with the south ends of sheds 1 and 2, the west side of shed 1 and the Manure store. Of the field site the Stream mean was in the 2.8-3.8% range and the Marsh, Open/mid field, Trees and Water trough were in the lowest 0.1-1% range (Table 17).

C. pulicaris: The trap sites located within the animal housing consisted of the Cow shed 2 and Calf shed traps with percentage means in the 11-21% range and the middle of shed 1 with a mean value in the 7-9% range. The east side of shed 2 was in the 11-21% range but otherwise the percentage means from catches around the animal sheds equated with the manure and forage store and Yard trap means. Of the field sites the mean percentage of blood fed females was particularly high from the Trees site (11-21%) but otherwise the field sites were associated with very low mean percentage values (Table 17).

The Walkway had a relatively high mean percentage of blood fed females, of both *C. obsoletus* and *C. pulicaris*. The east side of shed 2 also had a reasonably high mean percentage of blood fed females for both species (Table 17).

3.3 Weather data

The ambient temperature, relative humidity (%) and wind speed (m/s) was measured and recorded at the time of setting and collection at each of the trapping sites, at trap level. The minimum and maximum temperatures and the wind gust speed overnight and wind direction were also monitored and recorded on each trapping night. The minimum and maximum temperature values recorded during setting and collection for each trapping were selected for inclusion in Table 18. The overnight minimum and maximum temperature values were recorded at three sites during the study: the Cow shed 2; west side shed 2 (farm sites) and the Open/mid field (field site). Again the minimum and maximum recordings from these sites have been included in Table 18.

Table 18 Minimum and maximum ambient temperature measurements recorded at the farm and field trapping locations, during each trapping occasion

Trapping time	Setting-Lowest temp all sites (°C)	Setting-Highest temp all sites (°C)	Overnight Lowest temp all sites (°C)	Overnight Highest temp all sites (°C)	Collection-Lowest temp all sites (°C)	Collection-Highest temp all sites (°C)
Trapping 1-farm	15.4	20.2	9.4	19.2	11.5	14.8
Trapping 1-field	11.3	14.5	8.0	16.4	14.7	16.4
Trapping 2-farm	14.9	19.2	5.3	20.7	11.5	14.8
Trapping 2-field	10.6	12.5	7.8	18.5	14.7	16.4
Trapping 3-farm	13.8	18.8	4.8	20.0	10.5	16.6
Trapping 3-field	17.2	19.4	3.1	20.6	9.1	10.6
Trapping 4-farm	14.1	18.2	5.8	19.6	11.4	20.0
Trapping 4-field	16.3	16.9	3.7	17.7	9.1	10.8
Trapping 5-farm	14.0	18.4	2.6	22.6	8.8	16.9
Trapping 5-field	10.5	13.7	1.8	24.0	13.6	14.7
Trapping 6-farm	10.5	12.7	5.2	12.5	8.5	16.0
Trapping 6-field	7.9	8.4	3.5	17.1	10.3	11.6
Trapping 7-farm	13.3	14.1	11.0	18.0	11.0	16.9
Trapping 7-field						
Trapping 8-farm	14.4	18.2	11.7	22.4	16.3	21.8
Trapping 8-field	13.2	13.8	11.2	20.2	16.4	16.7

Humidity measurements were taken at each trap site during setting and collection and were monitored overnight using a weather station. The minimum, maximum and overnight recordings from all of the sites are shown in Table 18.

Table 19 Minimum and maximum humidity (%) measurements recorded at the farm and field trapping locations, during each trapping occasion

Trapping time	Setting-Lowest humidity all sites (%)	Setting-Highest humidity all sites (%)	Overnight Lowest humidity all sites (%)	Overnight Highest humidity all sites (%)	Collection-Lowest humidity all sites (%)	Collection-Highest humidity all sites (%)
Trapping 1-farm	81.6	96.6	68.6	98.4	68.6	98.4
Trapping 1-field	83.3	90.1			76.0	82.0
Trapping 2-farm	62.0	77.0	62	96	75.3	88.8
Trapping 2-field	85.3	94.0			76.5	82.2
Trapping 3-farm	59.1	79.7	59.1	95	69.5	86.6
Trapping 3-field	60.5	69.1			88.0	93.4
Trapping 4-farm	71.7	88.7	66.0	100	66.0	100.0
Trapping 4-field	73.8	77.4			94.2	100.0
Trapping 5-farm	69.4	89.5	69.4 (foggy)	98.9 (foggy)	73.0	98.9
Trapping 5-field	88.3	95.6			72.0	78.9
1.4mm rain 24 hours before Trapping 6						
Trapping 6-farm	57.5	69.8	50.1	88	50.1	79.3
Trapping 6-field	85.1	87.5			60.3	70.1
14.8mm rain during 24 hours before trapping 7						
Trapping 7-farm	91.3	95.5	83	100	92.5	100.0
Trapping 7-field						
Trapping 8-farm	65.4	85.3	65.4	87	65.9	75.4
Trapping 8-field	84.1	87.4			74.3	76.3

The variation in minimum and maximum ambient temperatures (°C) and humidity (%) between trappings is shown in Figure 15.

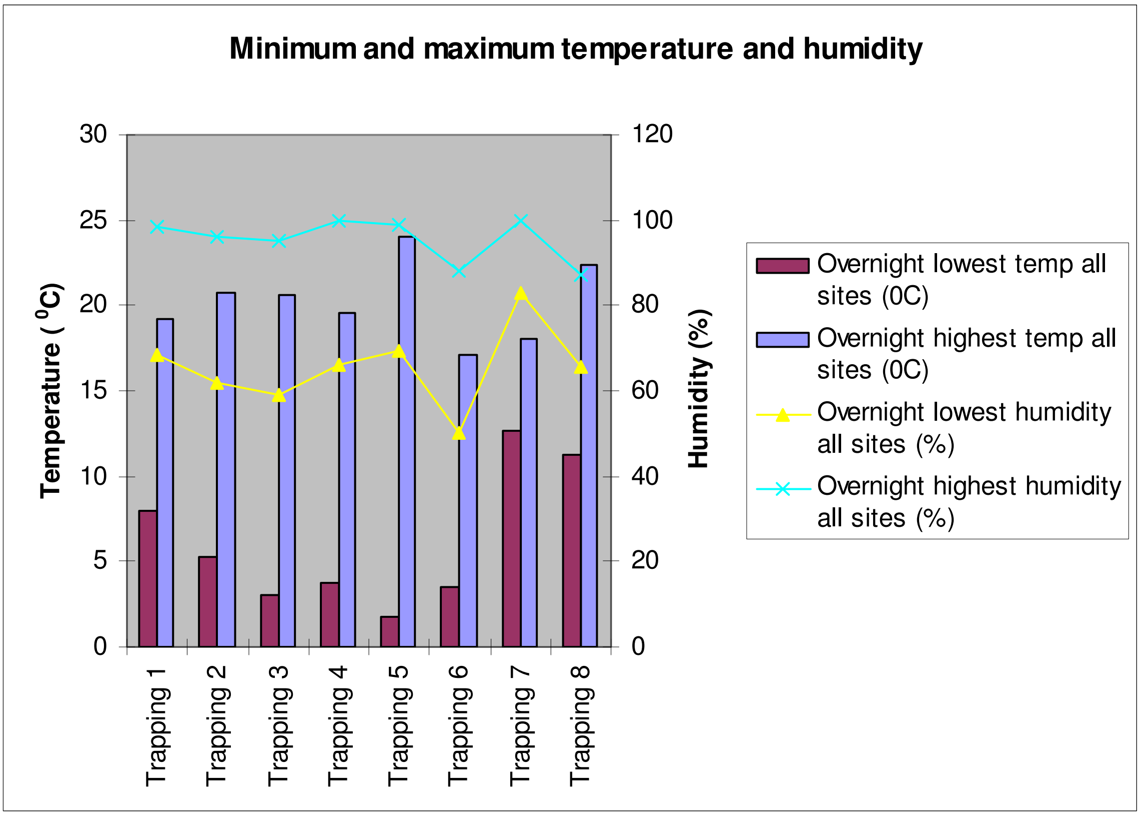

Figure 15 Variations in the temperature and humidity (minimum and maximum measurements) on each trapping occasion

The minimum and maximum wind speeds recorded during setting and removal/collection of the traps are shown in Table 19. The overnight wind gust and wind direction was also monitored during each trapping using a weather station at one location at the farm holding.

Table 20 Minimum and maximum wind speed (m/s) and wind direction measurements recorded at the farm and field trapping locations, during each trapping occasion

Trapping time	Setting-Lowest wind speed all sites (m/s)	Setting-Highest wind speed all sites (m/s)	Maximum gust (m/s)	Collection-Lowest wind speed all sites (m/s)	Collection-Highest wind speed all sites (m/s)	Wind direction (direction from)
Trapping 1-farm	0	2.0	2.0	0	0	SE-NE
Trapping 1-field	0	1.2		0	3.5	
Trapping 2-farm	0	0	7.6	0	0	NE/NW -SE
Trapping 2-field	0	0		0	1.9	
Trapping 3-farm	0	0	8.9	0	6.3	SE-SW
Trapping 3-field	0	1.4		0	5.7	
Trapping 4-farm	0	0	6.9	0	4.2	SW
Trapping 4-field	0	3.5		0	4.3	
Trapping 5-farm	0	2.4	4.8	0	2.8	NE/NW-SE
Trapping 5-field	0	0		0	4.4	
Trapping 6-farm	1.9	8.8	16.6	0	3.5	NE-W
Trapping 6-field	0	6.1		1.2	4.3	
Trapping 7-farm	0	8.7	8.7	0	8.0	NE-SW
Trapping 7-field						
Trapping 8-farm	0	4.4	13.0	0	8.9	NE-W-S
Trapping 8-field	0	5.5		0.9	9.2	

The variation in the maximum wind speed (m/s) recorded at the farms sites between trappings are shown in Figure 16, and for the field sites in Figure 17.

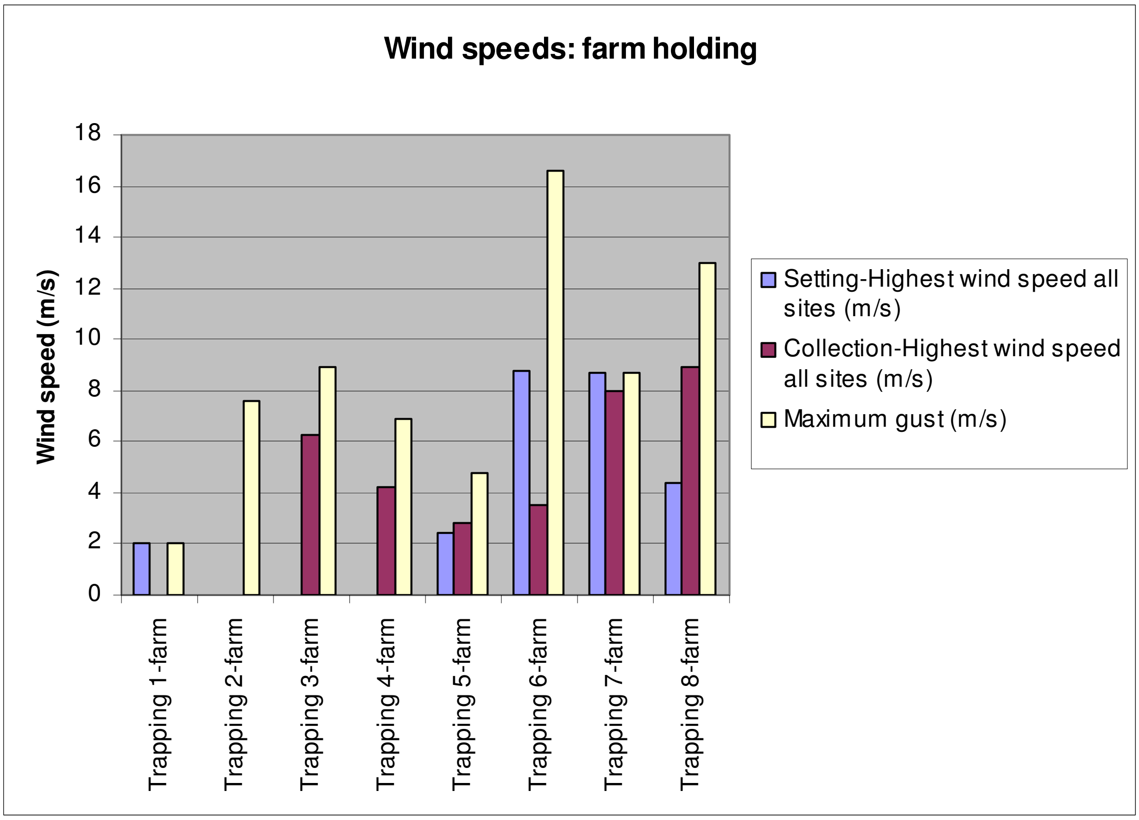

Figure 16 Maximum wind speed recorded at setting and collection and the maximum gusts recorded at the farm holding on each trapping occasion

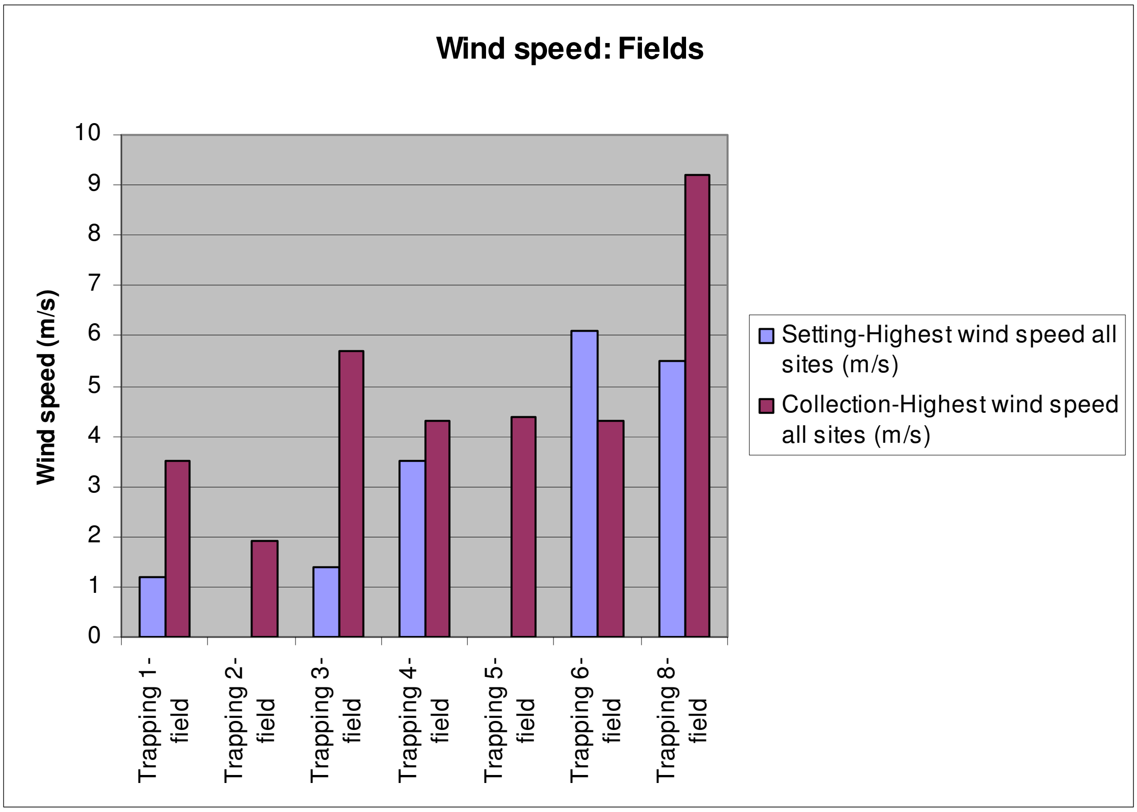

Figure 17 Maximum wind speeds recorded at setting and collection at the field sites on each trapping occasion

3.4 Ranking of results based on a female *Culicoides* scoring system

A ranking system, using six determinants, was used for assessing the risk of exposure to *C. obsoletus* and *C. pulicaris* females. The method used is described in Chapter 2 "Materials and Methods", section 2.5.1. The higher score for a trap site, on a relative scale, appeared associated with a higher exposure rate using the above determinants. The scoring and subsequent ranking of the trap sites based on the scoring system are included in Tables 20 to 22 for *C. obsoletus*, Tables 23 to 25 for *C. pulicaris* and Tables 26 to 28 for the combined *Culicoides* species.

3.4.1 Ranking for *Culicoides obsoletus* females

Table 21 Female measurements used for scoring trap sites for female *C. obsoletus*

Trap location	Mean	Mean %	Peak %	Peak catch	Total no's all catches	No of % above the mean
Trees	1349.50	4.70	21.79	7412	8097	1
Manure	862.0	6.71	12.52	3082	6034	5
North end 1	797.75	8.63	13.91	2924	6382	6
East side 2	775.0	8.04	13.67	2920	5425	6
South end 1	742.88	7.39	17.12	3298	5943	5
East side 1	665.75	8.68	22.58	3144	5326	7
South end 2	628.88	6.48	18.05	2606	5031	4
Silage	612.25	6.23	14.99	3190	4898	4
Calf shed	463.75	13.84	36.36	1487	3710	5
Walkway	432.75	6.22	17.33	1896	3462	3
Slurry	312.75	5.00	11.89	1462	2502	4
North end 2	256.75	2.37	3.98	1355	2054	0
Yard	223.38	2.04	7.54	975	1787	1
Middle shed 1	216.38	11.01	33.33	703	1731	4
Manure/ silage	164.86	0.88	2.43	827	1154	0
Open/mid field	130.0	0.63	1.65	521	910	0
Cow shed	120.38	1.78	4.03	692	963	0
West side 1	108.38	0.98	2.35	715	867	0
West side 2	91.50	0.89	2.16	421	732	0
Marsh	30.0	0.34	0.59	100	180	0
Water trough	21.0	0.37	1.30	102	105	0
Stream	7.67	0.35	1.18	18	46	0

Table 22 Ranking following initial scoring for *C. obsoletus*

Rank	Trap location	Mean	Mean %	Peak %	Peak	Total no's	Total score
1	Trees	22	11	19	22	22	96
2	East side 1	17	20	20	19	17	93
3	North end 1	20	19	14	17	21	91
4	South end 1	18	17	16	21	19	91
5	Manure	21	16	12	18	20	87
6	Calf shed	14	22	22	13	14	85
7	East side 2	19	18	13	16	18	84
8	South end 2	16	15	18	15	16	80
9	Silage	15	14	15	20	15	79
10	Walkway	13	13	17	14	13	70
11	Middle shed 1	11	21	21	7	9	69
12	Slurry	12	12	11	12	12	59
13	North end 2	10	10	8	11	11	50
14	Yard	9	9	10	10	10	48
15	Manure/ silage	8	5	7	9	8	37
16	Cow shed	6	8	9	6	7	36
17	West side 1	5	7	6	8	5	31
18	Open/mid field	7	4	4	5	6	26
19	West side 2	4	6	5	4	4	23
20	Water trough	2	3	3	3	2	13
21	Marsh	3	1	1	2	3	10
22	Stream	1	2	2	1	1	7

Table 23 Adjusted rank for frequency of high percentage trappings of *C. obsoletus*

Rank	Trap location	Total rank score from above criteria	Number of female catches above the mean	Number of catches above the mean multiplied by score per catch	Rank score including score for catches above the mean	New ranking with number of high percentage catch scores included
2	East side 1	93	7	19.25	112.25	1
3	North end 1	91	6	16.50	107.50	2
4	South end 1	91	5	13.75	104.75	3
7	East side 2	84	6	18.84	102.84	4
5	Manure	87	5	15.70	102.70	5
1	Trees	96	1	3.67	99.67	6
6	Calf shed	85	5	13.75	98.75	7
8	South end 2	80	4	11.0	91.0	8
9	Silage	79	4	11.0	90.0	9
11	Middle shed 1	69	4	11.0	80.0	10
10	Walkway	70	3	8.25	78.25	11
12	Slurry	59	4	11.0	70.0	12
14	Yard	48	1	2.75	50.75	13
13	North end 2	50	0	0	50.0	14
15	Manure/ silage	37	0	0	37.0	15
16	Cow shed	36	0	0	36.0	16
17	West side 1	31	0	0	31.0	17
18	Open/mid field	26	0	0	26.0	18
19	West side 2	23	0	0	23.0	19
20	Water trough	13	0	0	13.0	20
21	Marsh	10	0	0	10.0	21
22	Stream	7	0	0	7.0	22

3.4.2 Ranking for *Culicoides pulicaris* females

Table 24 Female measurements used for scoring trap sites for female *C. pulicaris*

Trap location	Mean	Mean %	Peak %	Peak catch	Total no's all catches	No of % above the mean
Trees	149.50	13.78	53.85	763	897	5
East side 2	35.0	9.08	17.71	93	245	6
North end 1	29.25	6.59	14.58	87	234	6
Open/mid field	27.71	2.80	10.16	144	194	2
Silage	23.63	6.06	15.23	114	189	5
South end 2	22.63	7.14	21.14	82	181	4
South end 1	22.25	6.49	10.53	84	178	5
Walkway	22.0	6.35	15.23	75	176	5
East side 1	21.75	8.35	19.74	45	174	7
Slurry	15.75	6.20	10.17	55	126	6
Calf shed	13.50	7.81	19.67	30	108	5
Manure	13.29	4.11	8.00	31	93	3
Middle shed 1	11.25	7.29	18.03	32	90	3
Manure/silage	9.29	2.58	8.20	38	65	3
Yard	8.88	2.44	8.00	29	71	2
North end 2	8.38	3.21	9.21	26	67	2
Marsh	5.0	0.78	3.43	16	30	0
Cow shed	4.75	1.84	6.29	13	38	1
West side 2	4.75	1.01	3.05	15	38	0
Water trough	3.60	0.75	1.69	12	18	0
West side 1	2.25	1.10	6.58	10	18	1
Stream	1.33	0.39	2.04	3	8	0

Table 25 Ranking after initial scoring for female *C. pulicaris*

Rank	Trap location	Mean	Mean %	Peak %	Peak	Total no's	Total score
1	Trees	22	22	22	22	22	110
2	East side 2	21	21	17	19	21	99
3	North end 1	20	16	14	18	20	88
4	South end 2	17	17	21	16	17	88
5	Silage	18	12	16	20	18	84
6	East side 1	14	20	20	13	14	81
7	Open/mid field	19	10	11	21	19	80
8	South end 1	16	15	13	17	16	77
9	Walkway	15	14	16	15	15	75
10	Calf shed	12	19	19	9	12	71
11	Middle shed 1	10	18	18	11	10	67
12	Slurry	13	13	12	14	13	65
13	Manure	11	11	8	10	11	51
14	Manure/slurry	9	9	9	12	7	46
15	Yard	8	8	8	8	9	41
16	North end 2	7	7	10	7	8	39
17	Cow shed	5	6	5	4	6	26
18	West side 2	5	4	3	5	6	23
19	Marsh	6	3	4	6	4	23
20	West side 1	2	5	6	2	3	18
21	Water trough	3	2	1	3	3	12
22	Stream	1	1	2	1	1	6

Table 26 Adjusted rank for frequency of high percentage trappings of *C. pulicaris*

Rank	Trap location	Total rank score from above criteria	Number of female catches above the mean	Number of catches above the mean multiplied by score per catch	Rank score including score for catches above the mean	New ranking with number of high percentage catch scores included
1	Trees	110	5	18.35	128.35	1
2	East side 2	99	6	18.84	117.84	2
3	North end 1	88	6	16.5	104.50	3
6	East side 1	81	7	19.25	100.25	4
4	South end 2	88	4	11	99.0	5
5	Silage	84	5	13.75	97.75	6
8	South end 1	77	5	13.75	90.75	7
9	Walkway	75	5	13.75	88.75	8
7	Open/mid field	80	2	6.28	86.28	9
10	Calf shed	71	5	13.75	84.75	10
12	Slurry	65	6	16.5	81.50	11
11	Middle shed 1	67	3	8.25	75.25	12
13	Manure	51	3	9.42	60.42	13
14	Manure/slurry	46	3	9.42	55.42	14
15	Yard	41	2	5.5	46.50	15
16	North end 2	39	2	5.5	44.50	16
17	Cow shed	26	1	2.75	28.75	17
18	West side 2	23	0	0	23.0	18
18	Marsh	23	0	0	23.0	18
20	West side 1	18	1	2.75	20.75	19
21	Water trough	12	0	0	12.0	21
22	Stream	6	0	0	6.0	22

3.4.3 Ranking for combined *Culicoides obsoletus* and *Culicoides pulicaris* females

Table 27 Female measurements used for scoring trap sites for female *C. obsoletus* and *C. pulicaris*

Trap location	Mean	Mean %	Peak %	Peak catch	Total no's all catches	No of % above the mean
Trees	1499.17	5.22	23.07	8175	8995	1
Manure	875.43	6.66	12.28	3113	6128	5
North end 1	827.0	8.37	13.40	2998	6616	6
East side 2	810.0	8.16	13.88	3013	5670	6
South end 1	765.13	7.65	16.55	3332	6121	6
East side 1	687.50	8.94	21.88	3189	5500	7
South end 2	651.50	6.49	18.21	2688	5212	4
Silage	635.88	6.28	14.91	3304	5087	4
Calf shed	477.25	12.72	31.63	1493	3818	5
Walkway	454.75	6.22	16.95	1971	3638	3
Slurry	328.50	5.38	11.60	1517	2628	4
North end 2	265.13	2.60	5.14	1381	2121	1
Yard	232.25	2.07	7.56	1004	1858	1
Middle shed 1	227.63	10.21	29.61	716	1821	4
Manure/silage	174.14	1.22	2.79	865	1219	0
Open/mid field	157.86	0.78	1.88	665	1105	0
Cow shed	125.13	1.77	3.85	705	1001	0
West side 1	110.63	1.09	3.32	725	885	0
West side 2	96.25	0.87	2.17	436	770	0
Marsh	35.0	0.30	0.74	116	210	0
Water trough	24.60	0.32	0.93	114	123	0
Stream	9.0	0.35	0.91	21	54	0

Table 28 Ranking after initial scoring for female *C. obsoletus* and *C. pulicaris*

Rank	Trap location	Mean	Mean %	Peak %	Peak	Total no's	Total score
1	Trees	22	11	20	22	22	97
2	East side 1	17	20	19	19	17	92
3	South end 1	18	17	16	21	19	91
4	North end 1	20	19	13	16	21	89
5	Manure	21	16	12	18	20	87
6	East side 2	19	18	14	17	18	86
7	Calf shed	14	22	22	12	14	84
8	South end 2	16	15	18	15	16	80
9	Silage	15	14	15	20	15	79
10	Walkway	13	13	17	14	13	70
11	Middle shed 1	9	21	21	7	9	67
12	Slurry	12	12	11	13	12	60
13	North end 2	11	10	9	11	11	52
14	Yard	10	9	10	10	10	49
15	Manure/slurry	8	7	6	9	8	38
16	Cow shed	6	8	8	6	6	34
17	West side 1	5	6	7	8	5	31
18	Open/mid field	7	4	4	5	7	27
19	West side 2	4	5	5	4	4	22
20	Marsh	3	1	1	3	3	11
21	Water trough	2	2	3	2	2	11
22	Stream	1	3	2	1	1	8

Table 29 Adjusted rank for frequency of high percentage trappings of *C. obsoletus* and *C. pulicaris*

Rank	Trap location	Total rank score from above criteria	Number of female catches above the mean	Number of catches above the mean multiplied by score per catch	Rank score including score for catches above the mean	New ranking with number of high percentage catch scores included
2	East side 1	92	7	19.25	111.25	1
3	South end 1	91	6	16.50	107.50	2
4	North end 1	89	6	16.50	105.50	3
6	East side 2	86	6	18.84	104.84	4
5	Manure	87	5	15.70	102.70	5
1	Trees	97	1	3.67	100.67	6
7	Calf shed	84	5	13.75	97.75	7
8	South end 2	80	4	11.0	91.0	8
9	Silage	79	4	11.0	90.0	9
10	Walkway	70	3	8.25	78.25	10
11	Middle shed 1	67	4	11.0	78.0	11
12	Slurry	60	4	11.0	71.0	12
13	North end 2	52	1	2.75	54.75	13
14	Yard	49	1	2.75	51.75	14
15	Manure/silage	38	0	0	38.0	15
16	Cow shed	34	0	0	34.0	16
17	West side 1	31	0	0	31.0	17
18	Open/mid field	27	0	0	27.0	18
19	West side 2	22	0	0	22.0	19
20	Marsh	11	0	0	11.0	20
21	Water trough	11	0	0	11.0	21
22	Stream	8	0	0	8.0	22

3.4.4 High, moderate and low risk ranking for *Culicoides* exposure for each of the trap sites

To enable a theoretical assessment of the risk of exposure to female *C. obsoletus* and *C. pulicaris,* the above scores were used to allocate exposure categories. As the maximum potential score would be 22 x 6, this value was evenly divided into tertiary ranges. The trap sites were then listed in tables according to their rank score in three categories labelled as low (Table 32), mid (Table 31) and high (Table 30) range categories.

Table 30 High range category with a rank score of between 90-132 for *C. obsoletus*, *C. pulicaris* and both species combined

***C. obsoletus* trap scores**		***C. pulicaris* trap scores**		***C. obsoletus* and *C. pulicaris* combined assessment scores**	
East side 1	112.25	Trees	128.35	East side 1	111.25
North end 1	107.50	East side 2	117.84	South end 1	107.50
South end	104.75	North end 1	104.50	North end 1	105.50
East side 2	102.84	East side 1	100.25	East side 2	104.84
Manure	102.70	South end 2	99.0	Manure	102.70
Trees	99.67	Silage	97.75	Trees	100.67
Calf shed	98.75	South end 1	90.75	Calf shed	97.75
South end 2	91.0			South end 2	91.0
Silage	90.0			Silage	90.0

Table 31 Mid range category with a rank score of between 45-89 for *C. obsoletus*, *C. pulicaris* and both species combined

***C. obsoletus* trap scores**		***C. pulicaris* trap scores**		***C. obsoletus* and *C. pulicaris* combined assessment scores**	
Middle 1	80.0	Walkway	88.75	Walkway	78.25
Walkway	78.25	Open/mid field	86.28	Middle 1	78.0
Slurry	70.0	Calf shed	84.75	Slurry	71.0
Yard	50.75			North end 2	54.75
North end 2	50.0			Yard	51.75

Table 32 Low range category with a rank score of between 0-44 for *C. obsoletus*, *C. pulicaris* and both species combined

***C. obsoletus* trap scores**		***C. pulicaris* trap scores**		***C. obsoletus* and *C. pulicaris* combined assessment scores**	
Manure/ silage	37	Cow shed	28.75	Manure/slurry	38
Cow shed	36	West side 2	23.0	Cow shed	34
West side 1	31	Marsh	23.0	West side 1	31
Open/mid field	26	West side 1	20.75	Open/mid field	27
West side 2	23	Water trough	12.0	West side 2	22
Water trough	13	Stream	6.0	Marsh	11
Marsh	10			Water trough	11
Stream	7			Stream	8

Ranking of the results using the scoring system allowed categorization of the trap sites according to the level of exposure to *C. obsoletus* and *C. pulicaris* females, and the relative rates of exposure to the combined species. This was used to determine the relative risk of exposure of livestock at varying locations to the potential vectors of BT.

In the high score range for the individually assessed *C. obsoletus* and *C. pulicaris* there were three shed 1 sites, the east, north and south sides of the shed, followed by the east side and south end of shed 2. The only field site in this range was the Trees site, which achieved the highest score for *C. pulicaris*. There was the Silage store site for both *C. obsoletus* and *C. pulicaris*. The Calf shed and the Manure sites were in this category for *C. obsoletus* and for the combined species scores (Table 30).

In the mid-score category the middle of shed 1, the Walkway and Yard sites, the Slurry site and the north end of shed 2 scored within this range for both *C. obsoletus* and *C. pulicaris*. There were more sites and the sites varied significantly in this score range for *C. pulicaris*, which also included the Manure, Manure/silage, Calf shed and Open/mid field sites (Table 31).

In the low-score range the Cow shed 2 score was high in the list for this category, for both *C. obsoletus* and *C. pulicaris*. The west sides of both sheds 1 and 2 as well as three field sites, consisting of the Marsh, Water trough and Stream sites were within this category for both species. The Manure/silage and Open/mid field sites were within the low-score category for *C. obsoletus*, but had scored within the mid-score range for *C. pulicaris*. The combined species range was consistent with the *C. obsoletus* range, but the order in the list was slightly altered by the influence of the *C. pulicaris* data (Table 32).

3.5 Multilevel modelling analysis of the data

Multilevel modelling was used to analyze the data, to examine the likely influence of climatic, topographical and animal management factors on the *Culicoides* distribution and activity. The following variables (predictor variables) were used in the model, to assess the individual and then the overall effect of each predictor variable on the numbers of *C. obsoletus* and *C. pulicaris* females collected.

Weather variables:

▪ T set	Temperature °C at the time of the trap setting
▪ T coll	Temperature °C at the time of the trap removal
▪ T min	Minimum temperature °C during the trapping period
▪ T max	Maximum temperature °C during the trapping period
▪ Hum set	Humidity reading at the time of the trap setting (%)
▪ Hum coll	Humidity reading at the time of the trap removal (%)
▪ Wind sp. Set	Wind speed at the time of the trap setting (m/s)
▪ Wind sp. Coll	Wind speed at the time of the trap collection/removal (m/s)

Management and topographical variables:

▪ Prox cattle	Proximity of cattle (metres)
▪ Prox sheep	Proximity of sheep (metres)
▪ Prox manure	Proximity of manure/slurry store (metres)
▪ Prox bedding	Proximity of straw bedding (metres)
▪ Prox forage	Proximity of forage store/silage (metres)
▪ Prox water	Proximity of water source (metres) (includes water trough, stream, ditch)
▪ Prox trees	Proximity of trees (metres)
▪ TSCC (days since contact cattle)	Time, in days, since contact with cattle (within 100 metres of the trap location)
▪ TOCC (days of contact cattle)	Time, in days, of contact with cattle overnight (within 100 metres of the trap location)
▪ TSCS (days since contact sheep)	Time, in days, since contact with sheep (within 100 metres of the trap location)
▪ TOCS (days of contact sheep)	Time, in days, of contact with sheep overnight (within 100 metres of the trap location)

A parameter estimate value was determined by the model, the magnitude of which reflected the effect of each predictor variable on the numbers of *C. obsoletus* and *C. pulicaris* females. A negative parameter estimate value indicated a reduced number of *Culicoides* and positive value indicated an increased number of *Culicoides* associated with the predictor variable measurement. Standard errors were used to examine the significance of each of the parameter estimates, and hence the likely significance of the individual predictor variable on *Culicoides* numbers. The parameter estimate was considered significant if the standard error was less than half the value of the parameter estimate.

3.5.1 Example of the individual predictor variable analysis

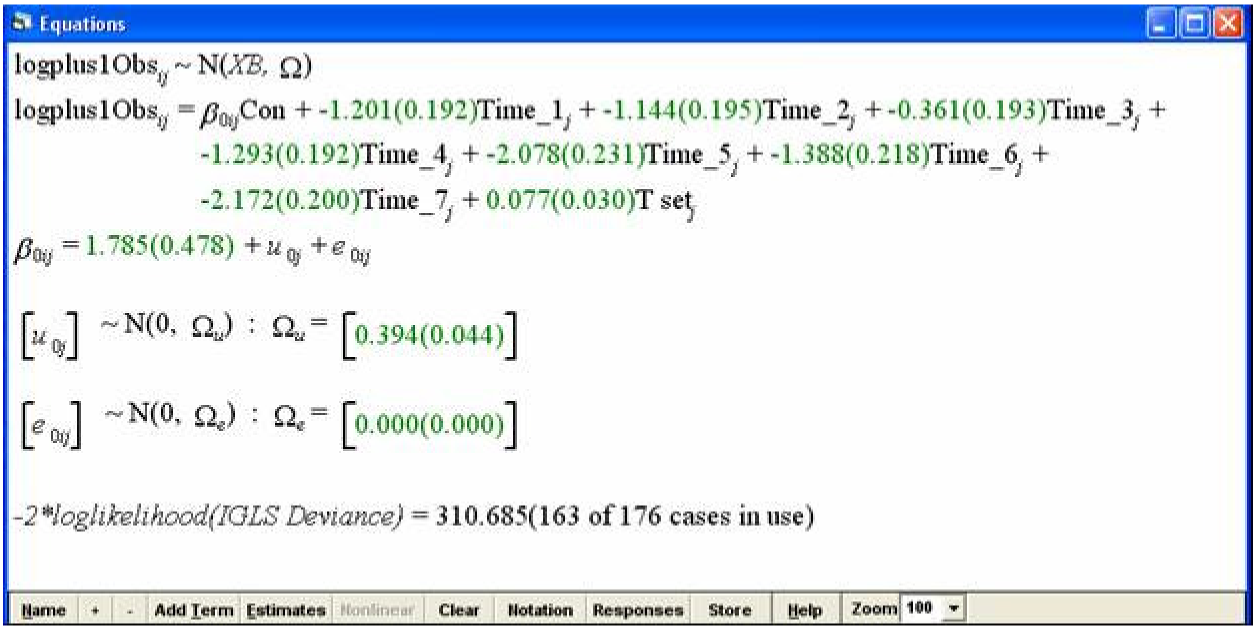

Figure 18 Multilevel model for the individual predictor variable analysis (example T set for *C. obsoletus*)

3.5.2 Multilevel model results

Each of the predictor variable numerical figures, measured in °C, % humidity, time in days, wind speed in m/s and distance in metres, were added to the multilevel model. The parameter estimates determined for each predictor variable are recorded in Table 33 for *C. obsoletus* and Table 34 for *C. pulicaris*. Standard errors were then used to determine the likely significance of the variable's influence on the *Culicoides* numbers and the significant results were highlighted, along with an interpretation.

3.5.2.1 Effect of individual predictor variables on *C. obsoletus* female numbers

Table 33 Significance of individual predictor variables on *C. obsoletus* female numbers

Predictor variable	Parameter estimate	Standard error	Significance level	Interpretation: Predictor variable association with *C. obsoletus* numbers
T set	+0.077	0.03	$p<0.05$ Significant	Higher temperature at setting, higher *C. obsoletus*
T coll	+0.033	0.025	$p>0.05$ Not Significant	
T min	+0.319	0.048	$p<0.05$ Significant	Higher minimum temperature, higher *C. obsoletus*
T max	-0.153	0.041	$p<0.05$ Significant	Higher maximum temperature, lower *C. obsoletus*
Hum set	-0.020	0.008	$p<0.05$ Significant	Higher humidity at setting, lower *C. obsoletus*
Hum coll	-0.010	0.007	$p>0.05$ Not Significant	
Wind speed set	-0.115	0.036	$p<0.05$ Significant	Higher wind speed at setting, lower *C. obsoletus*
Wind speed coll	-0.067	0.031	$p<0.05$ Significant	Higher wind speed at collection, lower *C. obsoletus*
Prox cattle	+0.247	0.045	$p<0.05$ Significant	Increased distance from catte, higher *C. obsoletus*
Prox sheep	-0.203	0.022	$p<0.05$ Significant	Increased distance from sheep, lower *C. obsoletus*
Prox manure	-0.203	0.022	$p<0.05$ Significant	Increased distance from manure, lower *C. obsoletus*
Prox bedding	-0.201	0.022	$p<0.05$ Significant	Increased distance from bedding, lower *C. obsoletus*
Prox forage	-0.241	0.388	$p>0.05$ Not Significant	
Prox water	+0.346	0.262	$p>0.05$ Not Significant	
Prox trees	-3.692	0.487	$p<0.05$ Significant	Increased distance from trees, lower *C. obsoletus*
TSCC	-0.037	0.005	$p<0.05$ Significant	Increased time since cattle contact, lower *C. obsoletus*
TOCC	+0.004	0.001	$p<0.05$ Significant	Increased time of cattle contact, higher *C. obsoletus*
TSCS	+0.007	0.001	$p<0.05$ Significant	Increased time since contact sheep, higher *C. obsoletus*
TOCS	-0.716	0.162	$p<0.05$ Significant	Increased time of contact sheep, lower *C. obsoletus*

The model results for *C. obsoletus* identified numerous predictor variables that appeared to have a significant effect on the *C. obsoletus* female numbers when examined individually.

3.5.2.2 Effect of individual predictor variables on *C. pulicaris* female numbers

Table 34 Significance of individual predictor variables on *C. pulicaris* female numbers

Predictor variable	Parameter estimate	Standard error	Significance level	Interpretation: Predictor variable association with *C. pulicaris* numbers
T set	+0.039	0.023	p>0.05 Not Significant	
T coll	+0.014	0.019	p>0.05 Not Significant	
T min	+0.097	0.039	p<0.05 Significant	Higher minimum temperatures, higher *C. pulicaris*
T max	-0.063	0.031	p<0.05 Significant	Higher maximum temperatures, lower *C. pulicaris*
Hum set	-0.013	0.006	p<0.05 Significant	Higher humidity at setting, lower *C. pulicaris*
Hum coll	-0.009	0.005	p>0.05 Not Significant	
Wind speed set	-0.066	0.027	P<0.05 Significant	Higher wind speeds at setting, lower *C. pulicaris*
Wind speed coll	+0.001	0.023	p>0.05 Not significant	
Prox cattle	+0.097	0.035	p<0.05 Significant	Increased distance from cattle, higher *C. pulicaris*
Prox sheep	-0.060	0.020	p<0.05 Significant	Increased distance from sheep, lower *C. pulicaris*
Prox manure	-0.060	0.020	p<0.05 Significant	Increased distance from manure, lower *C. pulicaris*
Prox bedding	-0.058	0.020	p<0.05 Significant	Increased distance from bedding, lower *C. pulicaris*
Prox forage	+0.166	0.290	p>0.05 Not Significant	
Prox water	+0.131	0.197	p>0.05 Not Significant	
Prox trees	-1.355	0.409	p<0.05 Significant	Increased distance from trees, lower *C. pulicaris*
TSCC	-0.014	0.004	p<0.05 Significant	Increased time since cattle contact, lower *C. pulicaris*
TOCC	+0.001	0.001	p>0.05 Not Significant	
TSCS	+0.003	0.001	p<0.05 Significant	Increased time since contact sheep, higher *C. pulicaris*
TOCS	-0.364	0.125	p<0.05 Significant	Increased time of contact sheep, lower *C. pulicaris*

The final model results for both *C. obsoletus* and *C. pulicaris* were then determined by looking at the likely significance of each of the predictor variables when examined in combination (stepwise addition). An example of the equation used is given in Figures 19 and 20.

3.5.2.3 Final model results identifying the significant predictor variables affecting *C. obsoletus* numbers when examined in combination

Equations

$$\text{logplus1Obs}_{ij} \sim \text{N}(XB, \Omega)$$

$$\text{logplus1Obs}_{ij} = \beta_{0ij}\text{Con} + -1.212(0.150)\text{Time\_1}_j + -0.768(0.203)\text{Time\_2}_j + 0.180(0.282)\text{Time\_3}_j + -0.822(0.268)\text{Time\_4}_j + -1.705(0.289)\text{Time\_5}_j + -1.132(0.235)\text{Time\_6}_j + -1.801(0.225)\text{Time\_7}_j + -0.315(0.060)\text{Prox manure}_j + 0.096(0.043)\text{Prox cattle}_j + 0.032(0.013)\text{TSCC}_j + -0.087(0.028)\text{Wind sp. set}_j$$

$$\beta_{0ij} = 2.498(0.370) + u_{0j} + e_{0ij}$$

$$\left[u_{0j}\right] \sim \text{N}(0, \Omega_u) : \Omega_u = \left[0.238(0.026)\right]$$

$$\left[e_{0ij}\right] \sim \text{N}(0, \Omega_e) : \Omega_e = \left[0.000(0.000)\right]$$

$-2*loglikelihood(IGLS Deviance)$ = 228.883(163 of 176 cases in use)

Name + - Add Term Estimates Nonlinear Clear Notation Responses Store Help Zoom 100

Figure 19 Multilevel model for the final model for *C. obsoletus*, including all of the predictor variables

When all of the predictor variables were examined in stepwise combination, the remaining significant factors identified were: reduced numbers of *C. obsoletus* associated with an increased distance from the manure stores; increased time since cattle contact and higher wind speeds at setting. An increase in *C. obsoletus* numbers was associated with an increased distance from cattle.

3.5.2.4 Final model results identifying the significant predictor variables affecting *C. pulicaris* numbers when examined in combination

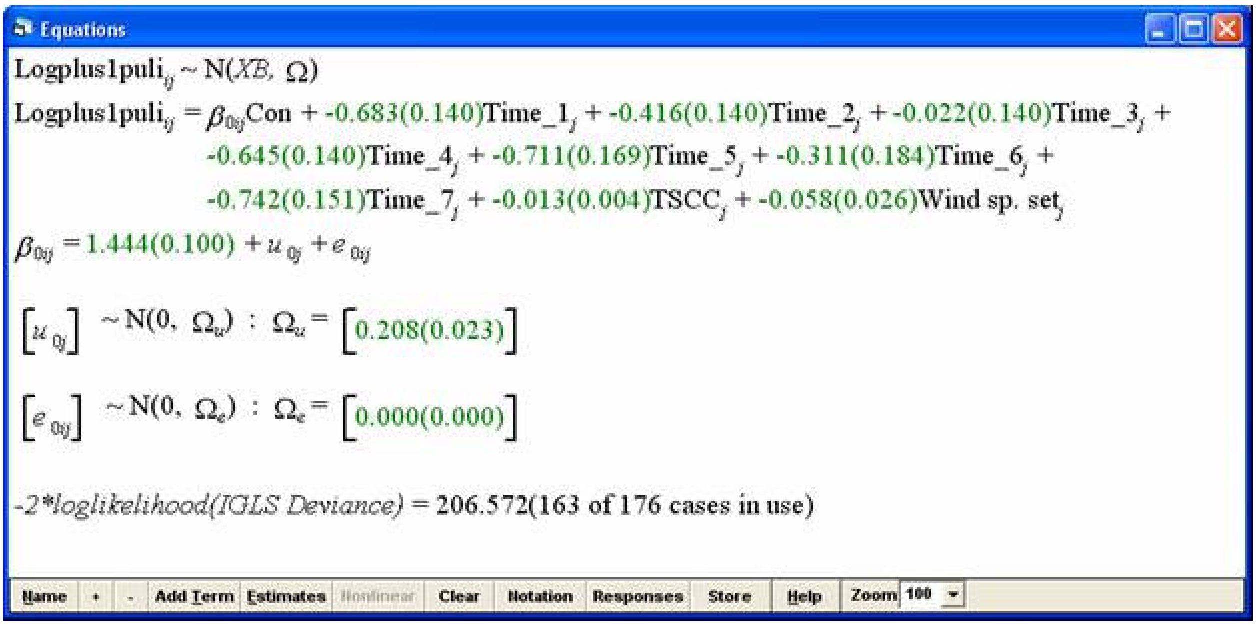

Figure 20 Multilevel model for the final model for *C. pulicaris*, including all of the predictor variables

After examination of all predictor variables in combination, the remaining significant factors identified were an increased time since contact with cattle and higher wind speed at setting resulting in reduced numbers of *C. pulicaris*.

Chapter 4

Discussion

Culicoides midges were trapped at 22 sites on 8 occasions between the 12th September and the 10th October 2008, to coincide with the usual autumnal seasonal peak of northern European *Culicoides* populations and the time of peak bluetongue transmission in northern Europe. During 2006, BT cases were initially detected in Germany during late August, with a peak incidence during mid-October and with a slow decline in cases until the end of the year (Conraths *et al.*, 2009). Baldet and Delecolle (2007) described an epidemiological study in France in which they found the midge population had rapidly increased three fold during early September 2006 compared to midge numbers at the end of August 2006.

The study farm used for sample collection was selected after fulfilling the criteria of an average sized dairy farm that had typical management practices for a farm in the U.K.. The farm also had an average sized sheep flock, again managed typically for a sheep flock in the U.K.. By using a farm typical for the U.K. information gained during the study could potentially be extrapolated to similar farm types.

4.1 *Culicoides* species found during the study

Only two *Culicoides* species groups were identified on the study farm during the project. These consisted of members of the *C. obsoletus* and *C. pulicaris* groups. The females were identified only to species group level for both. The proportions of females of *C. obsoletus* and *C. pulicaris* group were compared at all of the trap sites, during all trappings. The *C. obsoletus* group females contributed to between 94.67% and 98.46% (mean of 96.29%) of the total catch at the farm sites and between 82.35% and 90.02% (mean of 85.73%) at the field sites. *Culicoides pulicaris* made up the rest of the catch, consisting of between 1.54% and 5.33% (mean 3.71%) of the total farm site catches and 9.98% and 17.65% (mean 14.27%) of the field site catches. The results indicated that there were consistent proportions of *C. obsoletus* and *C. pulicaris* within the group of farm trap sites and within the group of field trap sites. Previous studies have identified similar relative proportions of *C. obsoletus* and *C. pulicaris* (Carpenter *et al.,* 2008). These authors, using light-traps, found *C. obsoletus* constituted 86.6% and *C. pulicaris* 5.2% of the total catch. Baldet and Delecolle (2007) found *C. obsoletus/scoticus* contributed to 88.6% of the catch population (94.6% when *C. dewulfi* and *C. chiopterus* are included) and *C. pulicaris* usually made up 5% (>4%) of the catch. Meiswinkel *et al.* (2007) found 68.5% of the catch population consisted of *C. obsoletus/scoticus*, which increased to 86.3% when *C. dewulfi* and *C. chiopterus* were included. *Culicoides pulicaris* (including *C. punctatus*) made up 12.9% of the total catch population. Savini *et al.* (2005) found 90% of the *Culicoides* caught during three trappings were made up of members of the *C. obsoletus* complex. Mehlhorn *et al.* (2007) found 97% of the midges caught during the study were *C. obsoletus.* Of these studies only the study by Carpenter *et al.* (2008) was within the U.K., the others were carried out in other European countries, although similar proportions of the different species groups were identified in these studies.

4.1.1 Speciation of the sibling species within the Obsoletus group using male specimens

Speciation of members of the *C. obsoletus* group requires either visual examination of the male genitalia or PCR testing (which was outside the scope of this project), consequently all speciation within the current work involved male specimens only. All four sibling species of the *C. obsoletus* group, that occur within the U.K., namely *Culicoides obsoletus* (Meigen), *Culicoides chiopterus* (Meigen), *Culicoides scoticus* (Downes and Kettle) and *Culicoides dewulfi* (Goetghebuer) were identified during the study. The relatively low numbers of males trapped at individual sites limited the interpretation of sibling species distribution at each site, during each trapping. The overall numbers of the *C. obsoletus* group sibling species was therefore examined from the farm sites as a whole. From the males, the mean numbers of the *C. obsoletus* group sibling species at the farm holding were in the relative proportions of 42% *C. obsoletus*, 11% *C. chiopterus* 10% *C. scoticus* and 37% *C. dewulfi*. The distribution of *C. obsoletus* group males at the field sites was variable and overall only low numbers were trapped (a total of 34 were trapped at the field sites compared to a total of 545 from the farm sites). The proportions of the *C. obsoletus* group sibling species at the field sites were 62% *C. obsoletus*, 14% *C. chiopterus* and 24% *C. scoticus*. As the results were based on only low numbers from the field sites these proportions may not reflect the true species distribution from these sites. In comparison Baldet and Delecolle (2007) identified species proportions of 88.6% *C. obsoletus/scoticus*, 5% *C. pulicaris*, 4% *C. dewulfi* and 2% *C. chiopterus* along with numerous other *Culicoides* species during a study in France.

Culicoides obsoletus was isolated from all but one of the farm trap sites, and was consistently found during the study on the farm holding. Although *C. obsoletus* itself constituted the highest percentage of the *C. obsoletus* sibling species found at the field sites, it's occurrence across the sites was erratic. *Culicoides scoticus* was found at all of the farm sites apart from inside the Cow shed 2, and was found only at the Trees and Stream sites in the field group. The potential breeding sites for this species on this particular farm holding were uncertain, with suggested breeding sites in the literature stated as maize silage residues (Zimmer *et al*., 2008) and certain fungi (Kettle, 1990). *Culicoides dewulfi* was frequently and regularly found at all of the farm sites, in one to seven of the trappings, and was the second most prevalent species trapped. The *C. dewulfi* numbers collected were only marginally lower than *C. obsoletus*. No *C. dewulfi* were found in any of the field catches. Baldet and Delecolle (2007) suggested cattle densities, soil types and varying cattle management could influence *C. dewulfi* numbers and distribution. *Culicoides chiopterus* was found at all of the farm sites apart from the slurry site, silage site and the middle of shed 1. At the field sites this species was found only at the Stream and Open/mid field sites. Both *C. dewulfi* and *C. chiopterus* breed in dung and will preferentially breed in cattle dung (Kettle, 1990) and larvae of both species have been found over-wintering in cow dung (Zimmer *et al*., 2008). The dependence on cattle dung may influence the distribution of both of these species, and indicated the presence of cattle on this farm was likely to have significantly influenced the occurrence of these two species. The relative proportions of the different sibling species collected by light-trapping have to be interpreted cautiously however as Carpenter *et al*. (2008) suggested light-trapping may result in an underestimation of the prevalence of *C. chiopterus*, *C. dewulfi* and possibly of *C. scoticus*. An overestimation of *C. obsoletus* numbers was also thought likely. This is due to suspected variances in the relative tendencies of the different sibling species to approach light-traps. During this study the relative proportions of these species could have been affected by the use of light-traps, as the *C. obsoletus* numbers were proportionally higher.

The numbers of *C. obsoletus* group males collected in each catch were compared to the relative number of *C. obsoletus* group females, to determine if the proportions of males to females were consistent for extrapolation of the sibling species data to the female populations. Overall, the proportions of males collected correlated with the proportions of females in individual catches.

4.1.2 Species distribution

A higher proportion of the catches consisted of midges from the *C. obsoletus* group, rather than *C. pulicaris*, from both the farm and field trap sites. The field catches, however, contained a higher relative proportion of *C. pulicaris* when compared to the farm holding catches. Of the *C. obsoletus* sibling species, *C. dewulfi* males were only found in the farm holding catches and not at any of the field sites, and in numbers only marginally less than the most prevalent sibling species, *C. obsoletus*. *Culicoides obsoletus* was found at all sites in both the farm and field groups. *Culicoides scoticus* was identified from at least one site of every environmental type on the farm holding (for example manure stores, forage stores, yard areas, external cow shed sites and internal cow shed sites) but was absent from the Marsh, Open/mid field and Water trough sites. *Culicoides chiopterus* was also collected from every farm environmental area, but was not found at the Marsh, Trees or Water trough sites in the field group.

The potential factors affecting the prevalence and the distribution of the different species includes the availability of suitable breeding sites; the lifespan of the individual species; the reproductive rate of different species; the availability of a blood source; the varying tendencies to disperse from the breeding site and the activity rates of different species under varying climatic conditions. The varying tendency of species to approach a light-trap is a sampling factor that could also affect catch results as discussed above. *Culicoides dewulfi* may have been limited to the farm holding sites due to a requirement for regular contact with cattle, a need for sheltered conditions or a specific requirement for a particular type of breeding site. *Culicoides dewulfi* has been shown to breed in cow dung (Kettle, 1990) and the type or nature of the dung may be important and have influenced the distribution of this species during this study. Further research would be necessary to determine the breeding and survival requirements of *C. dewulfi*.

Culicoides scoticus was only found at the Tree and Stream sites in the field group, possibly reflecting a need for a relatively sheltered environment, reasonably close to trees and close to a natural water site (such as a stream and ditch), all of which were features of the Tree and Stream sites and absent from the other field sites. *Culicoides chiopterus* is believed to breed preferentially in cow dung. Detection of *C. chiopterus* from only the Stream and Open/mid field sites may indicate the presence of breeding sites for this species close to these sites.

The higher relative proportion of *C. pulicaris* at the field sites as compared to farm sites was possibly influenced by an increased availability of breeding sites for this species in the pasture setting. These include drainage ditches, poorly drained soil and shallow standing water, a stream, leaf litter and hedge field boundaries (Carpenter *et al*., 2008; Tweddle, 2002; Kettle, 1990). Other suggested reasons for the higher proportion of *C. pulicaris* at the field sites may be a longer survival rate or reduced rates of dispersal associated with this species (particularly at the field sites, where there was no regular blood source for the females).

The higher proportion of *C. obsoletus* at individual sites such as the Tree site, relative to the other field sites, could be due to the availability of breeding sites close to this point, such as a ditch, decaying leaf litter (Carpenter *et al*., 2008; Tweddle, 2002; Kettle, 1990) and manure from livestock congregating under the shelter of the trees. Other factors could include persistence of a population in this particular location due to the relative shelter at this site, allowing for an increased survival time or reducing wind dispersal of the population.

4.2 Female *Culicoides* populations and distribution on the study farm

4.2.1 *Culicoides* catch rates

The numbers of *Culicoides* trapped varied overall between trapping times and at individual sites between trapping occasions. The overall catch following eight trappings consisted of 67339 *C. obsoletus* females with 578 males, and 3241 *C. pulicaris* females with 188 males. The peak individual *C. obsoletus* catch from one site was 34021 with 120 males and 1417 *C. pulicaris* females with 55 males, both acquired during the first trapping. This ranged to the lowest catch for *C. obsoletus* consisting of 154 females with 11 males, and for *C. pulicaris* 61 females with 5 males, both from trapping six. The peak catch from an individual site was during trapping 1 at the Trees trap site for both *C. obsoletus* and *C. pulicaris*. From this one site, 7412 *C. obsoletus* females and 763 *C. pulicaris* females were trapped during one night. Illustrating the potential variation of catches from individual sites, the catch from the Trees site had reduced to zero during trapping 6. Except for individual high catches from the Trees and Open/mid field sites during trapping 1 and moderately high catches at these sites during trapping 4, the farm-group trap catches were generally significantly higher than the field-group catches.

Comparing these results to data from other studies indicated catch rates can vary widely between studies. De Liberato *et al.* (2005) surveyed 32 sites in Italy and had catch results of between 11 and 18277 *C. obsoletus* from individual sites. During a study by Savini *et al.* (2005) 10000 mixed *Culicoides* species were trapped during three trappings, at three different locations in Italy. Carpenter *et al.* (2008), in a study in the U.K. during June and July, trapped a total of 8973 *Culicoides* after 23 days of light-trapping. Baldet and Delecolle (2007) during a study in France trapped an average of 115.5 *Culicoides*/trap/night during September. A study by Meiswinkel *et al.* (2007) in the Netherlands trapped a total of 120001 *Culicoides* from 35 light-trap collections during mid-September. The catches acquired during this study appear large in comparison to the catch rates in the studies described above.

The high catch rates during this study suggest the dairy farm holding maintained high *C. obsoletus* and *C. pulicaris* populations. These populations appeared to be dispersed around the holding, with relatively high catches of both species collected from sites widely distributed around the holding, on repeated occasions. Significantly higher *Culicoides* numbers were consistently trapped from the farm holding sites than from the field sites, with just individual field sites having single large *Culicoides* catches. The farm holding may sustain higher populations of *Culicoides* because of the potential constant or regular contact with cattle for blood feeding and possibly the number and range of suitable breeding sites. The sheds may also provide a sheltered environment from weather extremes, potentially increasing *Culicoides* survival times. It is possible that in the farm setting there may be a tendency for *Culicoides* to disperse only to sites around the farm holding itself. The above factors could all potentially result in enhanced breeding rates and extended life-spans for *C. obsoletus* and *C. pulicaris*, further increasing the local population.

4.2.2 Variations in female *Culicoides* numbers between trappings

Trapping 1 was associated with the highest total catch sizes of both *C. obsoletus* and *C. pulicaris* females when compared to all of the trappings. The magnitude of the total was largely due to high catch numbers of both *C. obsoletus* and *C. pulicaris* collected from the Trees site. There was generally a reducing trend in catch sizes from trapping 1 over the duration of the study, but with a peak in the catch size during trapping 4. The catches had reduced to 9.5% of the first catch during trapping 2 and to 16% of the trapping 1 catch during trapping 3. The overall catch during trapping 4 was 62.5% of the catch size from the first trapping and the catch size reduced to 5.9% during trapping 5. The smallest catch sizes were then collected during the final three catches with the catch size reducing to 0.6% of the first catch size during trapping 6, 3% during trapping 7 and to 0.9% during trapping 8. Trapping 6 was associated with the smallest total catch size of both *C. obsoletus* and *C. pulicaris* during the study. A reducing catch size trend was apparent from the 27th September (trapping 5) onwards, possibly indicating a seasonal effect but also possibly reflecting weather conditions at this time. The possible weather effects on the *Culicoides* catch sizes during this study are discussed in section 4.3. The patterns of overall catch size variations, between each trapping, was consistent for both *C. obsoletus* and *C. pulicaris*, with similar trap site distributions for both species. The increasing or reducing trends in catch size between trappings were also similar when catches from the field-group and farm-group were compared.

A consistent trend affecting different locations and affecting different *Culicoides* species was suggestive of a common influencing factor, such as an environmental factor, as the cause of the variations between trappings. Environmental factors could include changing weather conditions.

4.2.3 Numbers of female *Culicoides* found within animal buildings compared to numbers collected outside the buildings

Three traps were situated within cattle housing during this study. One trap was located within the cubicle shed, housing the lactating cows (Cow shed trap- shed 2), a trap was also placed within a loose-house shed containing freshly calved cows and heifers (Middle 1 trap-shed 1) and a trap was set within one of the calf sheds (Calf shed trap) housing pre-weaned calves. The sheds varied in size with the Calf shed measuring approximately a third of the size of the loose-house, and the loose house measured just less than half the size of the cubicle shed. The Calf shed and loose-house both contained straw bedding and mature manure. The cubicle shed contained no straw bedding or mature manure and had concrete floors with sawdust bedding in the cubicle pens. The cubicle shed and loose-housing both housed mature animals, and the Calf shed usually housed only young (pre-weaned) calves. The animal numbers within the sheds also varied, with the loose-house containing less than half the number of animals housed in the cubicle shed, and the Calf shed contained half the number again. Shed 1 and Shed 2 both had two open sides and the Calf shed had one open side, meaning the light trap was visible from outside the shed from these directions. Due to the proximity of at least one external light trap to these open sides, the likelihood of midges entering the sheds due to attraction to the light trap itself was considered minimal.

The ranking tables used for assessing exposure to female *C. obsoletus* and *C. pulicaris* indicated the Calf shed fell within the high-score category for *C. obsoletus* exposure and was in the mid-score category for *C. pulicaris*. The middle of shed 1 was within the mid-score category and the Cow shed (shed 2) was within the low- score category for both *C. obsoletus* and *C. pulicaris*. The scoring system suggested significant *Culicoides* exposure was possible within the animal housing. The sheds with increasing risk (determined by rank scoring) of *Culicoides* exposure were in order of decreasing shed size, which also included an association with straw bedding for the high-score (calf shed) and mid-score (middle of shed) sites. It is uncertain if the age of the animals within the housing could be significant.

Catches of *C. obsoletus* and *C. pulicaris* from the internal shed traps could exceed catches from the external shed sites. The Calf shed more consistently had catch sizes that exceeded catches from external traps. The *C. obsoletus* catch sizes in the Calf shed ranged from 30 up to 1487. All three internal shed sites had catches that exceeded at least one and more usually several of the external trap catches during every trapping. Although *Culicoides* are believed to have a reduced tendency to enter animal housing, the results from this study suggest *Culicoides* numbers within animal housing can frequently be high, even during occasions with what appear to be ideal weather conditions for *Culicoides* activity, external to the housing. When high *Culicoides* numbers were collected at external sites around the animal housing, the internal shed catches also appeared large. The population found within the housing may therefore have originated from external sites, rather than representing a significant resident population occurring within the housing itself. Baldet and Delecolle (2007) suggested that productive breeding sites within livestock buildings are unlikely, as the indoor trapping rate appears to fluctuate relative to ambient temperature. The presence of cattle within the sheds during this study may have further encouraged high *Culicoides* numbers within the buildings (Meiswinkel *et al.,* 2000).

There was an increased relative distribution of both *C. obsoletus* and *C. pulicaris* into the animal housing, particularly into the Calf shed and shed 1, during trappings 6 and 8 and to a lesser degree during trapping 7. Low ambient temperatures have been associated with the movement of *Culicoides* into buildings. Baldet and Delecolle (2007) found that when the minimum ambient temperature was below 5 °C and the maximum temperature was below 10 °C, no midges were caught in outdoor midge traps but low numbers were found inside the buildings. Also when ambient temperatures reached a minimum temperature of less than 10 °C and a maximum of less than 15 °C, the *C. obsoletus* and *C. scoticus* numbers found inside the animal buildings could exceed outside numbers by up to four times. Meiswinkel *et al.* (2000) also found increased numbers of *Culicoides* within animal housing when outside temperatures reduced. The overnight minimum temperatures during trappings 6, 7 and 8 were 5.2 °C, 11.0 °C and 11.2 °C, respectively and a minimum collection temperature of 8.5 °C (<10 °C) was recorded at the farm holding during trapping 6. Contrary to Baldet and Delecolle's (2007) findings indicating ambient temperature as the main factor resulting in the movement of *Culicoides* into the animal housing, a relative shift of the population into the animal housing during trappings 7 and 8 occurred when the minimum temperatures were above 10 °C and the maximum temperatures were above 15 °C. During trapping 5 there was a minimum overnight temperature as low as 2.6 °C at the farm holding, but a maximum temperature measuring above 15 °C. Significant numbers of *Culicoides* were collected in the external catches during trapping 5 despite the low minimum temperature, and the internal trap *Culicoides* collections were consistent with or lower than many of the external trap catches. Low ambient temperatures did not appear to influence the distribution of *Culicoides* in buildings during this study.

Although the catch sizes from within the housing were high relative to the external sites during trappings 6 and 8, the catches themselves were low when compared to previous catches from the internal shed sites. The findings from this study suggest that factors reducing *Culicoides* numbers outside the animal housing will also significantly affect the numbers found inside the animal housing.

High numbers of both *C. obsoletus* and *C. pulicaris* were found within the animal housing throughout the study, even during conditions suitable for external *Culicoides* activity. The smaller sized shed (Calf shed) had proportionally higher numbers of *Culicoides* with decreasing numbers associated with increasing shed size. Smaller sized sheds possibly remain more sheltered potentially allowing for increased *Culicoides* activity, and have a smaller relative airspace perhaps resulting in more *Culicoides* in the vicinity of the light trap. The presence of straw bedding may also be important, and the potential for breeding sites associated with straw bedding requires further investigation. The shift of the *Culicoides* population into the housing appeared to be minimally associated with low ambient temperatures, as discussed above. A consistent finding during trappings 6, 7 and 8, however, were north-east to west winds and variable wind directions, with wind gusts of between 13.0 and 16.6 m/s during the night. Wind speeds of at least 4.3 m/s but ranging up to 9.2 m/s were recorded at both trap setting and collection during the three trappings. This raises the possibility that high winds speeds and possibly prolonged strong winds may result in higher *Culicoides* numbers within buildings when compared to external sites. A reduced ability for light-traps to effectively trap *Culicoides* in windy locations is also a possible cause for this finding. Further research would be needed to determine a possible association between a shift of the *Culicoides* population into the buildings and high wind speeds.

4.2.4 Female *Culicoides* distribution at different yard locations

The Yard and Walkway sites were both within the mid-range category using the ranking system for both *C. obsoletus* and *C. pulicaris*, with the Walkway scoring higher than the Yard site. The Walkway represented the furthest site from the livestock areas, in a location adjacent to trees, where cows would pass when moving from the grazing to the milking parlour during the summer. Moderate to high numbers of *Culicoides* were maintained at the Walkway site, even during conditions that had reduced numbers at other sites. The site was sheltered by trees and foliage and had cattle within 14 m, which were perhaps factors contributing to moderately high and persisting numbers of *Culicoides* at this site. Relatively high numbers of catches were positive for blood fed females at this site suggesting active dispersal of *Culicoides* to this site from the animal housing, and the high percentage of gravid females for both *C. obsoletus* and *C. pulicaris* could indicate breeding sites nearby. The Yard catches had some moderate sized catches but seemed significantly affected by high wind speeds during some trappings, catches reducing even to zero. The Yard site was situated in a non-livestock area and indicated the level of population dispersal from the sites around the animal housing. Moderate numbers trapped at the Yard and Walkway sites, with varying proportions of nulliparous, parous, gravid and blood fed midges, suggested there was dispersal of *Culicoides* even into the non-livestock areas.

4.2.5 Female *Culicodes* distribution relative to manure storage areas and water sources

The *Culicoides* populations associated with the manure and forage store sites varied greatly between trappings. The rank scoring of the manure sites for *C. obsoletus* indicated a high-range score for the Manure site, the Slurry site was in the mid-score category and the Manure/silage site was in the low-score category. The Manure site, Manure/silage and Slurry sites were all in the mid-range category for *C. pulicaris*. The Silage site was in the high-score range for both *C. obsoletus* and *C. pulicaris*.

The Manure site and Silage sites were both associated with high numbers of *C. obsoletus,* and the Manure site had the highest mean number of *C. obsoletus* females out of the farm sites. The nulliparous female numbers were relatively low from the Manure site and catches were associated with a high percentage of parous and reasonably high numbers of gravid females.

Gravid females were, however, found frequently in catches, possibly suggesting *C. obsoletus* breeding sites were associated with the Manure site, combined with a *Culicoides* distribution from the nearby cattle shed.

In contrast, the mean catch number of *C. pulicaris* at the Manure site was low and no gravid *C. pulicaris* females were found at this site. The Silage site had only very low levels of nulliparous *C. obsoletus* and a high mean percentage of parous females alongwith moderate numbers of gravid females and a high percentage of blood fed females. The *C. pulicaris* females present at this site were predominantly parous with very few gravid and moderate numbers of blood fed females. The relatively low numbers of gravid females and a very low nulliparous mean percentage suggested there was unlikely to be a nearby breeding site for either *Culicoides* species at the Silage site. The relatively high numbers of parous and blood fed females suggested the *Culicoides* population collected was more likely to be associated with the nearby cow shed and cow yard. The Slurry site was associated with catches containing a high mean percentage of parous, a low percentage of nulliparous, relatively high numbers of gravid and frequent blood fed *C. obsoletus*. The *C. pulicaris* parous, nulliparous, gravid and blood fed females were all in the mid range numbers for all reproductive stages. A mixed range of female reproductive stages for both *Culicoides* species were observed, suggesting there were significant contributions to the *C. obsoletus* population at this site from the animal housing 18 m away, and possibly also from breeding sites nearby, due to large numbers of gravid females.

Finally the Manure/silage site was in an open yard location close to manure and silage stores but away from animal housing. The catch sizes noticeably reduced from this trap during trappings with high wind speeds (>3 m/s). The mean catches of *C. obsoletus* and *C. pulicaris* were low and were similar to or less than those at the Yard site. The proportions of nulliparous and parous as well as the mean values were typical of the majority of the farm sites for both *C. obsoletus* and *C. pulicaris.* These values as well as moderate percentages of blood fed females, despite no close livestock housing, suggested the *Culicoides* population at this site was largely dispersal from other sites rather than from local breeding sites. A high mean percentage of *C. obsoletus* gravid females, however, could indicate nearby breeding sites for this species. The *C. pulicaris* mean gravid percentage conversely was very low suggesting the opposite for this species.

There was no apparent influence on either the *C. obsoletus* of *C. pulicaris* numbers associated with a close proximity to a Water trough, an observation further supported by the multilevel model results, which indicated proximity to water had no apparent significant effect on the numbers of either species. Water troughs may have a limited suitability as *Culicoides* breeding sites, due to disturbance from refilling and by drinking animals. A leaking pipe had resulted in pasture water-logging close to the west side of shed 2. This water-logged area appeared to have very little effect on *Culicoides* numbers, with the catches from the west side of shed 2 remaining within the low-range score category for both *C. obsoletus* and *C. pulicaris*. In a temperate climate with generalized damp soil, temporary sites resulting from leaking water pipes appear unimportant as *Culicoides* breeding sites, possibly due to the availability of other suitable damp areas for breeding. Other water sources such as streams and ditches remain potentially significant for providing breeding sites for both *C. obsoletus* and *C. pulicaris*.

4.2.6 Female *Culicoides* distribution at northern, southern, eastern and western locations around the holding

The north, east and south sides of shed 1 and the east and south sides of shed 2 were all within the high score range, using the ranking system for assessing the risk of exposure to *C. obsoletus* and *C. pulicaris* females. The north end of shed 2 was in the mid-score range, and the west sides of sheds 1 and 2 were both in the low score ranges. Possible factors affecting the populations in the high-score range include close contact with livestock at all of the sites, the sheltered location of the east side of shed 1 and a close proximity to manure stores and foliage at the south ends of both sheds. The shed 1 sites were also close to straw bedding within the shed. The east side of shed 2 consisted of a silage feed barrier with a high potential for livestock contact. The farm holding is potentially associated with a wide range of breeding sites, including manure stores. Due to the mixed distribution of the nulliparous, parous and gravid stages identified at the external shed trap sites, a wide dispersal of *Culicoides* from breeding sites around the farm holding appeared likely.

The west sides of both sheds 1 and 2 were frequently associated with very low catches, particularly during trappings with wind speeds of more than 3 m/s. All of the shed sites had dramatically reduced catch sizes during trappings 6 and 8, which was consistent with other farm and field sites during those trapping nights. There were no consistent trends identified relating to reduced catches at specific sites due to wind direction. In general the proximity of surrounding sheds appeared to provide protection from strong winds apart from on the west sides of the sheds, which were relatively exposed.

4.2.7 Differences in female *Culicoides* population densities between different pasture microenvironments and compared to the farm buildings and yards

Overall the field sites were associated with lower catches of both *C. obsoletus* and *C. pulicaris* females when compared to the farm sites, with two exceptions consisting of the Trees site and the Open/mid field catches during trappings 1 and 4. Particularly high numbers of *Culicoides* were collected at the Trees site during trapping 1, the catch from this one site contributed to 86% of the total *C. obsoletus* and 81% of the total *C. pulicaris* collected from the group of field traps during the entire study. Using the rank scoring system the Trees site was ranked in the high-range category for both *C. obsoletus* and *C. pulicaris*, based largely on the high numbers collected during trapping 1. Features of the Trees site that may have encouraged an increased *Culicoides* population in this location included the shelter provided by the trees and the location of the site in a boundary between two fields, increasing the potential for livestock contact. The presence of a ditch and leaf matter from the trees also may provide *Culicoides* breeding sites in that location (Carpenter *et al.*, 2008; Tweddle, 2002; Kettle, 1990). Increased livestock contact could occur in sheltered sites under trees, when livestock congregate for shelter during strong winds, heavy rain or to shade from the sun, a time when livestock could be particularly at risk of exposure to *Culicoides*.

The Open/mid field site was located within 65 m of a ditch, stream and tree boundaries. This site was ranked in the mid-score range for *C. pulicaris* but was in the low-score range for *C. obsoletus*. Dispersal from breeding sites, particularly when no livestock are present close to the breeding sites, may encourage population dispersal to sites such as the middle of a field. Alternatively, the *Culicoides* at this site could indicate a local population, even within the open field. The Marsh, Stream and Water trough sites were associated with low score ranges for both *C. obsoletus* and *C. pulicaris*.

It is possible that the relatively low catch sizes at the field sites, when compared to the farm holding sites, were due to a lower density of breeding sites (i.e. the breeding sites may be more widespread); less efficient breeding sites in the field areas or may result from an increased dispersal of the adult females, particularly when there is a lack of nearby blood sources. The field sites were also potentially more susceptible to weather changes, with less shelter particularly from the wind at many of the sites when compared to the farm sites. The Stream site was particularly associated with consistently low catches, despite the presumed suitability of the stream as a potential breeding site, as a slow-flowing shallow stream shaded by trees (Kettle, 1990; Tweddle, 2002; Carpenter *et al.*, 2008). This site was perhaps less suitable as a breeding site for *Culicoides* due to the use of water troughs in the field, resulting in reduced livestock contact in this part of the field. Running water also could have reduced the suitability of this site for *Culicoides* breeding.

A higher proportion of *C. pulicaris* were found in the field sites when compared to the farm catches, possibly reflecting a greater availability of breeding sites for this species in the field environment, or possibly simply reflecting a lower tendency for dispersal of this species from the breeding site.

4.3 Examination of female *Culicoides* population data and environmental factors (along with ambient air temperature, wind speed, wind direction and humidity measurements)

The trapping sites where winds from a north-easterly or westerly direction occurred appeared to be associated with lower catches overall (trappings 2, 5, 6, 7 and 8). In contrast, during trapping 1 and 4, high *Culicoides* catches were observed when the wind direction was predominantly from the south-east or south-west. Variable wind direction, such as winds varying from the north-east to north-west to south-east, were found during two trappings with reduced catch sizes (trappings 2 and 5). Wind gusts of 7.6 m/s and above were also found during five of the trappings with reduced sized catches (trappings 2, 3, 6, 7 and 8). The duration of high wind speeds overnight may inhibit *Culicoides* activity at some sites, although wind duration was not measured apart from with wind speed measurements at setting and collection. Trappings 6, 7 and 8 had reduced sized catches and high wind speeds at setting and collection. Trappings 1 to 5 were associated with raised wind speeds at a few trap sites during setting, and winds of >3 m/s at collection at numerous trap sites during trappings 3 and 4. Trappings 1 to 5 were associated with total *C. obsoletus* catches of 2085 and above and total *C. pulicaris* catches of 147 and above, despite the high wind speeds recorded during trappings 3 and 4 and at some locations during the other trappings. Trappings where gusts of up to 6.9 m/s occurred overnight, and collection wind speeds of up to a maximum of 4.3 m/s at most trap sites, could still be associated with a high catch size: for example during trapping 4 there was a catch size of 21287 *C. obsoletus* and 871 *C. pulicaris*. This catch size was obtained despite wind speeds of greater than 3 m/s, with wind recorded at most of the trap sites. A reduced wind effect on *Culicoides* numbers due to a short wind duration or fluctuating winds overnight could explain this result. Trappings where north-east to west winds occurred or a very variable wind direction; wind gusts of between 8.7 and 16.6 m/s overnight; wind speeds of between 4.3 m/s and 9.2 m/s and windy conditions (>3 m/s) at setting and collection, had reduced overall catch counts of between 154 and 877 for *C. obsoletus* and of 61 to 197 for *C. pulicaris* (during trappings 6, 7 and 8). Carpenter *et al.* (2008) found that high wind speeds of >3 m/s reduced *Culicoides* catch sizes, even to zero in some cases and a variable wind direction was also thought to have a significant effect. A maximum wind speed of 1.47 m/s was suggested preferable to allow maximum *Culicoides* activity.

Minimum temperatures of between 3.7 °C and 9.4 °C at the field and farm sites were recorded during trappings 1 and 4, associated with peak *Culicoides* catches. Only trapping 5, that had low minimum temperatures at the farm sites of 2.6 °C and at the field sites of 1.8 °C, had reduced catch sizes (see ambient temperature data recorded in Table 18 and the graphs in Figure 13 for catch size variations and Figure 15 for wind speed data). These results may suggest a low temperature threshold, somewhere between 3.7 °C and 2.6 °C, could have resulted in reduced *Culicoides* activity. The duration of low ambient temperatures overnight may also determine the significance of the lowest overnight ambient temperature. *Culicoides obsoletus* is thought to be most active at temperatures between 7 and 16 °C, but with some activity at temperatures as low as 3.5 °C (Savini *et al.*, 2005). Carpenter *et al.* (2008) suggested temperatures of 20 to 22 °C may be optimal for *Culicoides* activity. Using a model, lower biting rates were predicted at temperatures between 12 and 15 °C, and high biting rates were predicted at between 25 and 30 °C. Baldet and Delecolle (2007) identified minimum temperatures of below 5 °C and maximums below 10 °C resulted in no midges in outdoor traps. Low ambient temperatures are thought to possibly reduce the flight activity and survival of adult *Culicoides* (Wittmann and Baylis, 2000).

The humidity readings at the trap sites ranged between 50.1% and 100.0% during the study (see humidity data recorded in Table 19). A mid-range relative humidity, of between 75-85% is thought ideal for midge survival and activity (Wittmann *et al.,* 2002). At lower humidity levels there is a risk midges may desiccate, and at higher levels midge saturation may result. Humidity results during the study were frequently outside the suggested ideal range during all of the trappings. Catches above 85% humidity included catches during trapping 1 and 4 that achieved the peak *Culicoides* catches, possibly suggesting these values outside the ideal had a limited influence on the *Culicoides* catch sizes. Humidities below 66%, however, were found during trappings 2, 3, 6 and 8, all trappings with reduced catch sizes.

Other factors potentially having a negative effect on catch sizes included the moonphase during trapping 2, which at just past full-moon could have contributed to reduced catch sizes due to reduced light-trap efficacy. Fog at sunset, overnight and at sunrise during trapping 5 may have either reduced the efficacy of the light-traps or may have reduced *Culicoides* flight activity. These effects, if significant, would be in combination with the more significant effects associated with high wind speeds and low minimum temperatures.

Wind speed appeared to have the greatest influence on the numbers and distributions of *Culicoides* trapped during the study, with not just peak wind speeds but possibly also the duration of high wind speeds (>3 m/s) affecting *Culicoides* activity. Variable wind directions also may in some cases have affected the numbers of *Culicoides* trapped, particularly when combined with high wind speeds (>3 m/s). Even when trappings with wind speeds recorded above 3 m/s, *Culicoides* were still trapped, indicating even during wind conditions thought unsuitable for *Culicoides* activity, individuals can still be trapped. During windy conditions it is possible that *Culicoides* activity may still continue in sheltered locations or during periods of reduced overnight wind speeds/strength. A proportional increase in the numbers of *Culicoides* trapped inside the animal housing during nights with high wind speeds (of more than 3 m/s) at trap setting and collection and with high overnight gusts was previously discussed.

Significant *Culicoides* activity was still possible during the study, even during adverse weather conditions such as high wind speeds (above 3 m/s), low temperatures (below 3 °C) and changeable wind directions. The maintainence of *Culicoides* activity around the farm holding during these conditions may have been possible due to the shelter provided by the farm buildings.

4.4 Effects of management practices on the female *Culicoides* population and distribution

The regular or constant contact with cattle at the farm holding, typically found on a dairy farm, allows a constant source of blood for the local *Culicoides* population. This allows for regular female blood feeding and therefore egg development and breeding, potentially encouraging large populations of *Culicoides* around the farm holding. This was indicted by the high percentage of catches containing blood fed *C. obsoletus* females collected from most of the farm sites. The mean percentage of blood fed females found in catches ranged up to 7.8%. The sites with the highest mean percentages of blood fed females for both species were associated with the three sites within the animal housing, the Walkway and the Silage feed barrier on the east side of shed 2. The locations immediately surrounding the animal sheds had variable mean percentages ranging from high to low percentages. Birley and Boorman (1982) determined *C. obsoletus* will often have a four day interval between blood meals, therefore the proportion of blood fed females found could fluctuate with the size of emerging populations and possibly any variations in the flying activities of recently fed females.

The farm holding could potentially provide a varied range of effective breeding sites for *C. obsoletus* and *C. pulicaris*. Manure stores, damp vegetated areas, trees with ground leaf litter, sites of poorly drained soil and areas of shallow standing water, were widely distributed around the holding, all potentially providing *Culicoides* breeding sites (Kettle, 1990; Tweddle, 2002; Carpenter *et al.,* 2008). Composting and rotting vegetation was also available in the form of discarded forage (silage) and areas of water-logged soil at the farm holding. Management factors that could affect the suitability of potential *Culicoides* breeding sites include the regular movement of slurry from the cubicle cow housing and yards, potentially disturbing *Culicoides* breeding sites. Regular additions of manure and slurry to manure store areas could also interrupt *Culicoides* breeding cycles. The relatively high *Culicoides* numbers, particulary of *C. obsoletus,* in and around the buildings with straw bedding, also raises the question whether the straw bedding within buildings could provide breeding sites. The buildings at the farm holding, both inside the buildings and in the vicinity of the buildings potentially allow protection of *Culicoides* from adverse weather such as strong winds, heavy rain and of breeding sites from temperature extremes.

The influence of movement of sheep on the population of *Culicoides* was assessed by examining the numbers of blood fed and gravid females collected from the field sites. Numerous gravid and blood fed *C. obsoletus* and *C. pulicaris* were found in the field catches during trapping 1 and trapping 4, particularly at the Trees and Open/mid field sites during trapping 1. During trapping 4 high numbers of gravid *C. obsoletus* and *C. pulicaris* females were again found at the Trees site and variably at the other field sites, as well as blood fed females. The dates of livestock contact at the field sites prior to trapping 1 were uncertain, however, the high numbers of gravid and blood fed *Culicoides* in trapping 4 were associated with sheep, grazing at least 10 m from the Marsh site and 55 m from the Open/mid field site. Sheep grazing within at least 100 to 140 m of the trapping sites resulted in low numbers of gravid *C. obsoletus* and *C. pulicaris* females and a single blood fed female during trappings 2, 3, 5 and 6. *Culicoides* often remain within 70 m of the breeding site and usually travel no more than 500 m, unless carried by wind or if females disperse in search of a blood meal (Kettle, 1990). Low catches at the field sites could therefore occur when sheep are located further than 70 m from the trap sites, due to dispersal of females in search of blood meals. Wind dispersal could also result in gravid and blood fed females at the trap sites at times where there are no livestock within close proximity of the trap sites. The *C. obsoletus* and *C. pulicaris* populations rapidly increased during trapping 4, two days after sheep had moved to within 70 m of the trap sites. The rapid change may have resulted from a mobile population of *Culicoides* following the sheep or an increased tendency for the *Culicoides* to remain in the locality, due to the close proximity of a blood source.

4.5 Reproductive stages of female *Culicoides* and the identification of breeding sites

Suggested measures for the presence of breeding sites are high proportions of nulliparous females, gravid females and possibly the presence of large numbers of males. The assessment of nulliparous and parous stages is very subjective and therefore open to variation between different assessors.

The number of males of the *C. obsoletus* group typically correlated with the numbers of females, with catches containing large numbers of females often yielding relatively large numbers of males. Males of the *C. obsoletus* group were trapped at all of the field sites apart from the Water trough and Marsh sites. Only the east side of shed 2 and the Calf shed traps caught males at every trapping and the highest overall numbers of males were from the east side of shed 2, the Walkway, the east side of shed 1, the middle of shed 1 and the Calf shed. The lowest numbers of males were trapped at the Open/mid field site, the west side of shed 1 and the south end of shed 1.

The majority of the farm sites had high percentages of catches with parous numbers exceeding nulliparous. The trap sites with the lowest percentage of catches where parous numbers exceeded nulliparous, were the Manure/silage, Yard and the south end of shed 2, possibly indicating the presence of breeding sites, due to relatively frequent catches where nulliparous exceeded parous. The trap sites with the highest percentage catches with nulliparous numbers exceeding parous were the north end of shed 1 and the Cow shed 2. The percentage of catches involved, however, was just 50% or less, which was not clearly indicative of the presence of breeding sites nearby. These results were combined with most of the farm sites having gravid females between 71 and 100% of the catches, suggesting either widespread breeding sites or wide dispersal of the gravid females. The west side of shed 2 and the Cow shed 2 were associated with the lowest percentage of catches positive for gravid females, followed by the Silage site. This perhaps suggested a lack of breeding sites in these areas, supported also by the fact no catches were found at the Silage site where nulliparous numbers exceeded parous.

Other sites without catches where nulliparous exceeded parous were the Slurry store, the west side of shed 1, the east side of shed 2 and the north end of shed 2. The east side of shed 2 and the north end of shed 2 had only catches where parous numbers exceeded nulliparous, possibly indicating an absence of breeding sites near these sites. The percentage catches, positive for gravid females from these sites, however, ranged between 62.5% and 100% giving conflicting information on the likelihood of breeding sites close to these sites. The sites with the highest mean percentage of gravid *C. obsoletus* females, indicating a higher proportion of gravid females found within the catches from this site, were the west side of shed 1, the Manure/silage and the Walkway site with means of between 16 and 21%. The sites with the next highest mean percentages of gravid females, of between 11 and 14%, were the Water trough, the east side of shed 1 and the Slurry site which could also indicate breeding sites in these locations. There were high mean nulliparous percentages at all of the field sites and also a high percentage of catches with nulliparous numbers exceeding parous numbers, perhaps suggesting nearby breeding sites, or perhaps indicating dispersal of the majority of parous females.

Culicoides pulicaris male numbers did not necessarily correlate with the magnitude of female numbers, with some very high catches having no males. The east side of shed 1 had the highest number of males caught during a single trapping, followed by the Manure/silage site and the Walkway. The highest total numbers of males trapped were from the east side of shed 2. All of the sites had males in at least one catch except the Cow shed trap which caught no *C. pulicaris* after eight trappings. The Slurry, Yard, the middle of shed 1, Trees, Open/mid field and the Calf shed sites were also associated with moderate male numbers, but often from individual catches only. The highest percentage of catches with nulliparous numbers exceeding parous were from the north end of shed 1, the south end of shed 2 and the Calf shed possibly indicating the presence of breeding sites, but these sites were not associated with high gravid numbers.

Otherwise the percentage of catches where nulliparous numbers exceeded the parous numbers were generally low from most of the farm sites, involving less than 40% of the catches at the varying sites. Most of the trap sites had similar numbers of catches with parous exceeding nulliparous catches. The Silage site and north end of shed 2 were associated with the highest percentage of catches where parous numbers exceeded nulliparous suggesting breeding sites may be unlikely in these areas. The sites with the highest mean percentages of gravid *C. pulicaris* were the Stream and Water trough with a mean percentage of 11-17% and the Walkway, Marsh, Trees and Open/mid field sites with 5.5 - 9.5%. The field sites were again associated with more frequent catch rates with more nulliparous than parous or with 50:50 nulliparous to parous, suggesting the populations trapped at the field sites may be associated with breeding sites.

The distributions of peak male catches, of sites with higher proportions of nulliparous and with a higher mean percentage of gravid females varied widely for both *C. obsoletus* and *C. pulicaris*. The results suggested that the male distributions and the proportion of nulliparous females gave no clear indication of possible breeding sites. The males of both *C. obsoletus* and *C. pulicaris* were widely distributed and the males were found in some sites in higher numbers where there were higher numbers of females. The wide male distribution therefore may just mirror the wide female distribution or alternatively may indicate a wide distribution of breeding sites around the farm sites. The results for the nulliparous female distributions also did not clearly indicate specific locations of likely breeding sites. The only pattern identified was significantly high proportions of parous females of both *C. obsoletus* and *C. pulicaris* collected at the Silage and north end of shed 2 trap sites. There were also high proportions of parous *C. obsoletus* associated with the east side of shed 2 and the Slurry site. The sites with particularly high proportions of parous females may indicate an absence of breeding sites in these locations.

The mixed results could have resulted from the relative difficulty differentiating nulliparous and parous females. Alternatively the results may reflect a wide range of breeding sites around the farm holding or have resulted from a wide dispersal and therefore mixing of the different female stages. The maximum distance between trap sites was approximately 65 m, a distance less than the suggested common flight range of *Culicoides* from breeding sites (Kettle, 1990). The use of male distributions or nulliparous female numbers as measures of proximity to breeding sites may be unreliable indicators, particularly in a concentrated popualtion. High percentages of gravid females may be a better indicator of sites close to breeding sites. Sites with a high percentage of gravid *C. obsoletus* females were the west side of shed 1, the Manure/silage site, Walkway site, the Water trough, the east side of shed 1 and the Slurry site. The sites with the highest mean percentages of gravid *C. pulicaris* were the Stream, Water trough, the Walkway, Marsh, Trees and Open/mid field sites. In contrast to *C. obsoletus* the Manure site had only low numbers of *C. pulicaris* gravid females, highlighting a probable difference in the preferred breeding sites of the species.

4.6 Multilevel model findings

Numerous factors were highlighted, using multilevel modelling statistical analysis, as potentially important variables with potential influences on the numbers of *C. obsoletus* and *C. pulicaris*.

The model including all of the important predictor variables in combination was used to account for autocorrelation of predictor variables. The most significant factors from the one-by-one analysis were added in a step-wise fashion. The model indicated that higher wind speeds at setting were likely to be associated with reduced numbers of both *C. obsoletus* and *C. pulicaris*. One possible explanation for this is that duration of high speeds could be important as high winds were more frequently encountered during trap collection than at setting. An increased time since contact with cattle in areas without current cattle contact, was associated with reduced numbers of both *C. obsoletus* and *C. pulicaris*. An increased distance from manure resulted in reduced numbers of *C. obsoletus,* again highlighting manure stores as a high risk site for *C. obsoletus*. As previously stated, an increased distance from cattle was associated with increased *C. obsoletus* numbers. This result is possibly wrongly interpreting the relatively reduced populations of *Culicoides* found within animal housing as occurring due to a close association with cattle rather than due to a reduced tendency of *Culicoides* to enter buildings. The high population around the farm holding suggested that contact with cattle is more likely to be associated with high numbers of *Culicoides* in the surrounding area. As further confirmation of the positive effect of contact with cattle on the numbers of *Culicoides*, the final model identified that reduced *Culicoides* numbers were associated with increasing time since contact with cattle, in areas without current contact with cattle.

4.7 Determining risk of bluetongue virus transmission for designing on farm risk assessments

Culicoides are the main vectors of BTV and transmission of this virus is dependent on the availability of competent *Culicoides* vectors. The timing of disease outbreaks in Europe tends to coincide with the autumnal peak in the *Culicoides* population. Members of the two species groups identified on this farm, *C. obsoletus* and *C. pulicaris*, are both competent BTV vectors and therefore the population and the distribution of the two species on this farm could have a direct influence on disease transmission in the event of a BT outbreak in this region. Only the female *Culicoides* blood feeds and more specifically the nulliparous and parous stages are the host seeking stages, able to function as virus vectors after blood feeding (Carpenter *et al.,* 2008).

Although vaccination is still the recommended and the most effective way of controlling BT, this study aimed to determine if management changes could also be used alongside vaccination to further reduce the risk of disease transmission. If new BTV serotypes emerge in Europe that are not currently included in the BT vaccine licensed for use in the U.K., other means of control may become necessary.

Although laboratory trials suggest temperatures of 27-30 °C are optimal for BTV replication, temperatures of less than 9-15 °C are necessary before BTV replication in the midge is inhibited (Mellor, 2000; Wittmann *et al.*, 2002; Purse *et al.*, 2005; Paweska *et al.*, 2004). Biting rates were investigated by Carpenter *et al.* (2008), and the study results suggested that at a temperature of 20.4 °C, humidity of 59.1%, a maximum wind speed of 1.47 mps and minimal wind direction changes, biting rates within a 90 minute period were on average 38 and could be more than 200. The climatic conditions identified during this study therefore were not just suitable for *Culicoides* activity but would also be suitable for BTV replication and transmission, with ambient temperatures recorded of up to 24 °C. This indicated that the data collection was carried out at a time when BT cases and significant transmission could occur and when the *Culicoides* population data would be most significant.

Varying infection rates have been identified in *Culicoides* populations during BT outbreaks. Savini *et al.* (2005) reported infection rates of at least 0.02% for *C. obsoletus* complex midges and de Liberato *et al.* (2005) reported a rate of at least 0.22%, a result consistent with at least one infected specimen from 460 *C. obsoletus* females. *Culicoides pulicaris* infection rates of 0.51% were recorded by Caracappa *et al.* (2003) in midges trapped during a BTV outbreak in Italy. Even with relatively low BTV infection rates in the *Culicoides* vector, the large numbers of *Culicoides* populations compensate for this (or overcome this), resulting in effective BTV transmission (de Liberato *et al.*, 2005). The total *Culicoides* population therefore equates with a "relative risk" of transmission. A model has been designed by Gubbins *et al.* (2008) to calculate the relative risk of BTV transmission using population data.

4.8 Conclusion

Results from this study indicated there was a wide distribution of *C. obsoletus* and *C. pulicaris* around the farm holding, possibly suggesting a wide distribution of potential breeding sites or just a rapid distribution of *Culicoides* around the farm holding from specific breeding sites. This study suggests that methods of control based on reducing specific breeding sites may be of limited benefit due to the potential range of sites available around the average dairy farm holding. Using the ranking system, specific farm sites were identified as higher risk sites for contact with *C. obsoletus* and *C. pulicaris*. These sites were the east, south and north sides of shed 1; the east and south sides of shed 2; the Manure store; the Silage store; the Calf shed and the Trees trap site in the fields. The generalised high numbers identified around the farm holding however suggests the farm holding overall could be considered a high risk site for *Culicoides* contact. Although there were generally lower catches found at the field sites, significant movements of the field *Culicoides* populations relative to livestock were possible, indicating that low field catches may not indicate these sites are “safe” from infection during a BT outbreak. The study results also indicated that individual field sites could periodically become high risk sites.

A reduction in BTV transmission therefore is ideally based on protection of livestock by vaccination potentially combined with methods of reducing the biting rates on livestock, which may involve the use of repellents.

Other observations made during this study were that high numbers of *Culicoides* can occur within buildings, with potentially increased numbers in smaller sheds and a possible added risk associated with straw bedding. Also that *Culicoides* remained active during nights with minimum temperatures around or below 5 °C, but with apparent reduced numbers. High wind speeds, possibly with a long duration, changeable wind directions and winds in one particular direction appeared to have a reducing effect on *Culicoides* numbers. High wind speeds at trap setting specifically was indicated by multilevel modelling as having a reducing effect on *Culicoides* numbers. In general contact with cattle had a positive effect on *Culicoides* numbers but contact with sheep seemed to have a limited effect. The manure site was highlighted as a high-score category for *C. obsoletus* and *C. pulicaris* exposure using the ranking system and was also highlighted by the final model analysis as being significantly associated with increased numbers of *C. obsoletus*.

Further areas of study highlighted by this work are:

- A need for more detailed studies on the actual *Culicoides* breeding sites around a farm holding and in field sites.
- A more detailed study on *Culicoides* behaviour in the pasture setting and how this relates to distance from livestock.
- Further work examining the efficacy of repellents and ways of reducing the *Culicoides* biting rate for livestock.
- Investigating wind speeds and duration as a possible very significant factor influencing *Culicoides* population, activity and distribution.

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