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Evaluation of housing as a means to protect cattle from *Culicoides* biting midges, the vectors of bluetongue virus

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Abstract. The housing of animals at night was investigated as a possible means of protecting them from attack by *Culicoides* biting midges (Diptera: Ceratopogonidae), the vectors of bluetongue. Light-trap catches of *Culicoides* were compared inside and outside animal housing, in the presence and absence of cattle. A three-replicate, 4 × 4 Latin square design was used at four farms in Bala, north Wales, over 12 nights in May and June 2007, and the experiment repeated in October. In the two studies, respectively, >70 000 and >4500 *Culicoides* were trapped, of which 93% and 86%, respectively, were of the *Culicoides obsoletus* group. Across the four farms, in May and June, the presence of cattle increased catches of *C. obsoletus* by 2.3 times, and outside traps caught 6.5 times more insects than inside traps. Similar patterns were apparent in October, but the difference between inside and outside catches was reduced. Catches were strongly correlated with minimum temperature and maximum wind speed and these two variables explained a large amount of night-to-night variation in catch. Outside catches were reduced, to a greater extent than inside catches, by colder minimum temperatures and higher maximum wind speeds. These conditions occur more frequently in October than in May and June, thereby suppressing outside catches more than inside catches, and reducing the apparent degree of exophily of *C. obsoletus* in autumn. The results suggest that the risk of animals receiving bites from *C. obsoletus* is reduced by housing at both times of year and the benefit would be greatest on warm, still nights when outside catches are at their greatest.

Key words. *Culicoides*, *Culicoides obsoletus*, animal bait, bluetongue, control, stabling.

Introduction

Bluetongue (BT), a viral disease of ruminants transmitted by biting midges of the genus *Culicoides* (Diptera: Ceratopogonidae), emerged in southern Europe towards the end of the 1990s and has continued to occur throughout the current decade (Mellor & Wittmann, 2002; Purse *et al*., 2005; Mellor *et al*., 2008). Bluetongue virus (BTV) serotypes 1, 2, 4, 9 and 16 have been detected in the region and well over 1 million sheep have died as a direct or indirect consequence of the disease. Since 2006, BTV serotype 8 has affected at least 11 countries in northern Europe and tens of thousands of cattle or sheep farms have been affected in the subsequent 3 years. Bluetongue virus serotypes 6 and 11 have also been detected recently in northern Europe.

Transmission of BTV in Europe is associated with several species of *Culicoides*, including the Afro-Asiatic *Culicoides*

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imicola (Kieffer), which has a distribution that extends into southern Europe, and at least some members of the Palaearctic *Culicoides obsoletus* and *Culicoides pulicaris* species groups (Meiswinkel *et al*., 2007a). In northern Europe to date, polymerase chain reaction (PCR)-positive pools have been recorded from undifferentiated specimens of the *C. obsoletus* group in Germany (Mehlhorn *et al*., 2007) and two individual species within this group: *Culicoides dewulfi* (Goetghebuer) in the Netherlands (Meiswinkel *et al*., 2007a) and *Culicoides chiopterus* (Meigen) in the Netherlands (Dijkstra *et al*., 2008) and France (Balenghien, 2008). Separately, laboratory studies have demonstrated the vector competence of British *Culicoides scoticus* (Downes & Kettle) (another species within the *C. obsoletus* species group) for BTV serotypes 8 and 9 (Carpenter *et al*., 2008a), and additionally of *Culicoides impunctatus* (Goetghebuer) and *C. pulicaris* (Linnaeus) for BTV serotype 9 (Carpenter *et al*., 2006).

The stabling of horses at night in Cape Colony (now the Republic of South Africa) was identified, historically, as a protective measure against African horse sickness (Paton, 1863), a disease of equids closely related to BT and also transmitted by *Culicoides* vectors. Recent research in South Africa has shown that this protection may be attributed to the fact that horses in stables receive fewer bites from vector *Culicoides*. Experimental studies using traps sited inside and outside stables showed that *C. imicola* was strongly exophilic ('outdoor-loving') and that closing doors and gauzing windows led to a further 14-fold reduction in numbers caught inside (Meiswinkel *et al*., 2000).

Prior to the incursion of BTV serotype 8 into northern Europe, little was known regarding the likelihood of northern Palaearctic *Culicoides* species entering stables; only a single, preliminary study from Canada had reported exophilic behaviour in *C. obsoletus* (Anderson *et al*., 1993). Shortly after the incursion, the competent authorities recommended the housing of animals as a means of protecting livestock in the region from BT. It was subsequently reported, however, that at least some individuals of northern Palaearctic species freely enter animal housing (Meiswinkel *et al*., 2007b). Because of difficulties in providing detailed information to farmers regarding the value of housing to mitigate against transmission according to season, degree of enclosure and host species, stabling was largely withdrawn from the recommended methods of controlling the spread of the virus (Defra, 2007).

Although northern European *Culicoides* certainly enter animal houses and feed on livestock within, what may matter more in some situations is whether an animal housed inside will receive fewer bites from potentially infected vectors than the same animal housed outside: in other words, can the risk of virus transmission be reduced by housing, even if it cannot be eliminated? Recent evidence remains equivocal. A study in the Netherlands found that twice as many *Culicoides* were caught in a trap outside animal housing compared with a trap operated simultaneously inside (Meiswinkel *et al*., 2008). By contrast, in Belgium *Culicoides* catches inside animal accommodation were 15–22 times greater than catches in the immediate surroundings outside (Zimmer *et al*., 2008).

Both of these studies suffer from a methodological problem: the difference between indoor and outdoor trappings is obscured by the greater proximity of animals to one trap compared with the other. On the cattle farms in both studies, cattle were returned to housing at night and were therefore likely to be in closer proximity to the inside trap than the outside one.

In May–June 2007, an experimental study was conducted to quantify the relative *Culicoides* trap catches both inside and outside animal housing. Importantly, this was conducted in both the presence and absence of cattle, resulting in a balanced design and allowing the quantification of the effect of the presence or absence of cattle on trap catches. The findings of this study, in agreement with those of Baldet *et al*. (2008), showed preliminary evidence for an influence of weather on the relative exophily of *Culicoides* vectors: therefore the study was repeated during the colder season in October 2007.

Materials and methods

Experimental design

The studies were conducted over 12 nights each during the periods 21 May to 8 June 2007, and 19–31 October 2007. For each study a three-replicate, 4×4 Latin square design was used to investigate four treatments at four sites over three sets of 4 nights. The purpose of the Latin square design was to allow for the confounding effect of two variables, differences between sites and differences between nights, to be controlled for in the analysis. The four treatments were (a) trap outside/cattle absent; (b) trap outside/cattle present; (c) trap inside/cattle absent, and (d) trap inside/cattle present.

Farms

Four mixed farms (beef cattle and sheep) within a 5-km radius of Bala in north Wales (52°54′39.35″ N, 3°35′46.67″ W; altitudes 226–270 m above sea level) agreed to participate in the study. Each farm provided five to 10 Welsh Black or Limousin-cross beef cattle for the nights when the presence of animals was required. The housing facilities were those used by the farmers for their cattle at night and varied between the farms in terms of size and age but also in the degree to which they were 'enclosed', defined here as the proportion of the surface area of the housing that consisted of open door, window, hole or more general opening. We estimated this proportion by taking measurements of walls and openings. Each farmer also provided an area (e.g. a walled yard) outdoors in which the same cattle could be kept at night. The same housing and trapping positions were used in the May–June and October studies.

Traps and midge collection

Four Onderstepoort-type down-draught black light traps with 8-W ultraviolet (UV) light bulbs were used to collect the *Culicoides*; one trap was placed on each farm (Venter & Hermanides, 2006). Mosquito netting installed around the light source prevented entry of larger insects. A beaker containing 100–200 mL water with two to three drops of detergent, added to reduce surface tension, was attached to the net funnel of the trap to collect the *Culicoides*. The traps were hung, either inside the animal housing or outside, at heights of 1.5–2.0 m and within 2–10 m of cattle when animals were present. Traps were operated from before dusk to well after dawn. On collection, the insects were filtered out of the water and stored in 70% ethanol until further analysis.

Three of the traps were of the mains/battery type produced by Onderstepoort Veterinary Institute until recently. The fourth, used at Farm 2, was an older, mains-only trap. All traps were operated on mains electricity; nevertheless, in the mains/battery traps, the 240-V input is reduced to 12 V to power the fan. Recent results suggest that mains/battery traps are less effective at catching *Culicoides* than the latest mains-only Onderstepoort traps (M. Baylis & H. Guis, 2008, unpublished data), but the former type is no longer in production (G. Venter, 2008, personal communication). Although the use of one older mains and three mains/battery traps does not affect our ability to test for treatment differences, we consider that it may contribute to any Farm effect.

Weather data

In May, PC-linked professional wireless weather stations (model WMR928NX) (Oregon Scientific™, Maidenhead, U.K.) were installed on three farms (the fourth station malfunctioned), within 10 m of the animal housing and outside yard. The weather stations measured temperature (±0.1 °C) and relative humidity (RH) (±1%) both inside and outside, and rainfall (±1 mm) and wind speed (±0.2 m/s) outside. Readings were taken every minute; the loggers, however, only store maxima and minima of temperature, RH and wind speed, and total rainfall, since the memory was last cleared. We collected these readings each morning and evening and thus obtained data on total daily rainfall, maximum temperature during the day, minimum temperature during the night, minimum RH and maximum wind speed. Manual maximum/minimum thermometers and a rain gauge were installed on the fourth farm, and also read twice daily. In October, Oregon Scientific™ weather stations were installed on all four farms.

Analysis of catches

Using a binocular microscope, all *Culicoides* spp. were first separated from other insects. All *Culicoides* were then identified to group or species level by their characteristic wing patterns. Female *Culicoides* were grouped into six species categories: *C. obsoletus* group; *C. pulicaris*; *C. punctatus* (Meigen); *C. pulicaris* group (comprising specimens with wing characteristics of both *C. pulicaris* and *C. punctatus*); *C. impunctatus*, and ‘Other *Culicoides*’. The first five species groups were further separated by their physiological state according to whether they were nulliparous, parous, gravid or blood-engorged. The ‘total’ female catch is defined as the sum of these four groups. Any females damaged beyond accurate recognition were discounted. Male *Culicoides* within the *C. obsoletus* group were identified to species level using a key (Downes & Kettle, 1952) as *C. obsoletus* Meigen, *C. chiopterus*, *C. scoticus* or *C. dewulfi*. Catches of *Culicoides* larger than about 3000 were subsampled and catch size was estimated (Van Ark & Meiswinkel, 1992).

After both trials, a sample of ∼100 female *C. obsoletus* group from each farm were identified to species level by multiplex PCR (Mathieu *et al*., 2007; Carpenter *et al*., 2008b).

Statistical analysis of data

For the May–June study, the distributions of $\log_{10}(n+1)$-transformed catches of *C. obsoletus* females (Total, Nulliparous, Parous, Gravid, Blood-fed) were not significantly different from normal (Anderson-Darling Test for Normality, $P > 0.4$). Catches of the *C. pulicaris* group were smaller and sometimes zero; the distributions were significantly different from normal, even after transformation ($P < 0.05$). In the October study, catches of both species groups were low and were, in most cases, not normally distributed even after transformation.

For normally distributed datasets, analysis of variance (ANOVA) was used to investigate the effects of Farm, Night and Treatment on transformed catch. The four treatments were considered as factorial combinations of two treatments: the presence/absence of cattle (hereafter Animals) and inside/outside (hereafter In/Out). Explanatory variables in the ANOVA model were Farm ($n = 4$), Block (the blocks of 4 nights) ($n = 3$), Night ($n = 4$, nested within Block), Animals ($n = 2$) and In/Out ($n = 2$), and included the following interactions: Animals × In/Out; Farm × Animals; Farm × In/Out, and Farm × Animals × In/Out. Farm, Block and Night were entered as random factors; Animals and In/Out represented fixed effects. Interactions that were not significant were removed from the model, which was then re-run until a final model was assembled that comprised all the main effects and any significant interactions. Means of transformed catches were detransformed for presentation. Model fit is described by values of adjusted R^2.

Data that were not normally distributed were analysed using non-parametric tests. Friedman’s method for randomized blocks (Sokal & Rohlf, 1981) was used in lieu of two-way ANOVA. Night was used as the blocking factor, and the effects of Farm and Treatment were tested separately. The presented test statistics are adjusted for ties.

Differences between farms in weather variables were tested by two-way ANOVA, with Farm and Night as main effects.

Results

May–June

A total of 71 729 *Culicoides* were caught in the 48 collections (Table 1). The *C. obsoletus* group accounted for 93% of female and 50% of male *Culicoides*. More than 75% of

Table 1. Total catches of *Culicoides* from the May–June and October trials.

	May–June ($n = 48$)		October ($n = 48$)	
Species trapped	Female	Male	Female	Male
C. obsoletus group total	66 159	192	3967	62
By parous stage				
Nulliparous	76%	—	46%	—
Parous	13%	—	33%	—
Gravid	6%	—	20%	—
Blood-fed	5%	—	1%	—
By species				
C. chiopterus	2%	0%	7%	13%
C. dewulfi	1%	6%	8%	22%
C. obsoletus s.s.	73%	89%	47%	12%
C. scoticus	23%	5%	37%	53%
C. pulicaris group	2586	12	45	3
C. pulicaris	604	44	598	24
C. punctatus	399	0	8	3
C. impunctatus	505	81	4	1
Other *Culicoides*	1092	55	3	1
Total *Culicoides*	71 345	384	4625	94

The four species of the *Culicoides obsoletus* group were identified by multiplex polymerase chain reaction (females) or morphology (males).

female *C. obsoletus* insects were nulliparous. The *C. obsoletus* group catch was dominated by *C. obsoletus s.s.* Estimates for the proportion of each species were broadly similar for males and females, although *C. scoticus* appears to have been under-represented in the male catch.

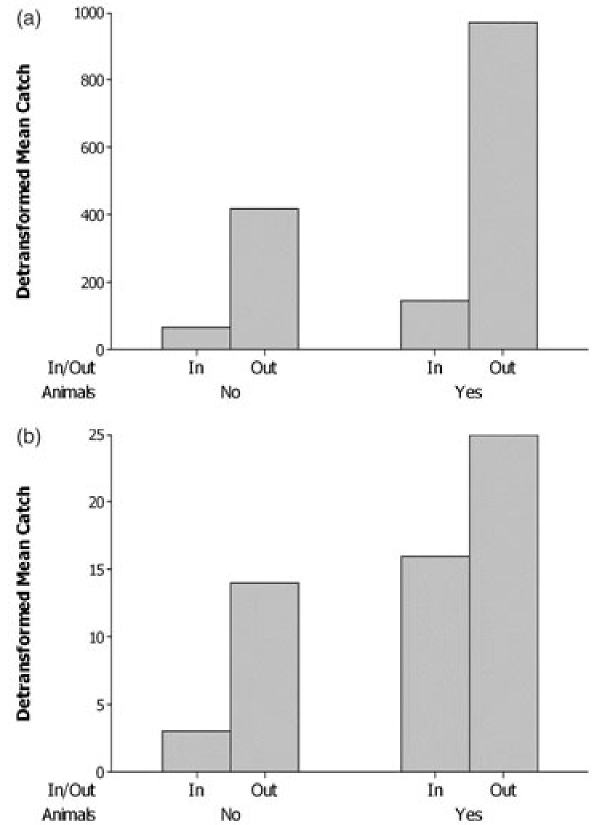

Fig. 1. Detransformed mean catches of (a) total female *Culicoides obsoletus* in May–June and (b) nulliparous female *C. obsoletus* in October.

Culicoides obsoletus *females*

There was significant variation between nights (Night: $F_{9,28} = 6.7$, $P < 0.001$) in the total catch of female *C. obsoletus*. Across all farms, the presence of cattle increased detransformed mean catches by a factor of 2.3 (Animals: $F_{1,28} = 9.9$, $P < 0.005$) and positioning traps outside increased catches by a factor of 6.5 (Fig. 1A). However, the effect of positioning a trap outside was dependent upon farm (Farm × In/Out: $F_{3,28} = 5.3$, $P < 0.01$), with two-, 60-, six- and three-fold increases on Farms 1–4, respectively (Fig. 2). No other interactions were significant. The R^2 of the final model was high (77%).

Identical models to that above were reached for nulliparous ($R^2 = 80\%$) and parous ($R^2 = 71\%$) females. The presence of cattle increased catches by similar amounts (2.7 and 2.0 times, respectively). Positioning traps outside increased catches of nulliparous females to a greater extent than catches of parous females (13.3 and 3.4 times, respectively).

For gravid females the Farm × In/Out interaction approached, but did not reach, significance ($F_{3,28} = 2.7$, $P < 0.07$). Without the interaction in the model ($R^2 = 58.5\%$), there were highly significant effects of Farm ($F_{3,28} = 11.8$, $P < 0.001$) and Night ($F_{9,28} = 5.1$, $P < 0.001$), but no effects of In/Out ($P < 0.5$) or Animals ($P < 0.5$).

For blood-fed *C. obsoletus*, Night ($F_{9,21} = 4.0$, $P < 0.01$) and the three-way interaction of Farm × Animals × In/Out ($F_{3,21} = 7.4$, $P < 0.001$) were both significant and so all variables remained in the final model. The Farm effect in the three-way interaction arose because in one farm, inside, more fed *C. obsoletus* were caught when cattle were absent than when they were present; whereas in all other instances more fed *C. obsoletus* were caught when animals were present than absent. Across all farms, the presence of animals increased the detransformed mean catch of blood-fed *C. obsoletus* nine-fold (present = 9.9, absent = 1.1), whereas outside catches were about twice as great as those inside.

Culicoides pulicaris *group females.* There was a significant effect of treatment on the catch of *C. pulicaris* group (Friedman's method for randomized blocks; $S_3 = 24.1$, $P < 0.001$); detransformed median catches were: Out/No animals, 14.4; Out/Animals, 24.1; In/No animals, 0.8, and In/Animals, 1.6. Outside catches were 15–20 times greater than inside catches and the presence of animals nearly doubled catches. Similar results were obtained for nulliparous and parous females except that, as for *C. obsoletus*, the effect of positioning traps outside was greater for nulliparous (34–50 times) than parous (12–14 times) females. Similar results were also obtained for

C. pulicaris and *C. punctatus*; however, there was no effect of treatment on the catch of *C. impunctatus*.

Males. There was no effect of treatment (Friedman's method) on males of any *Culicoides* species within the *obsoletus* or *pulicaris* groups.

October

A total of 4719 *Culicoides* were caught in the 48 collections (Table 1). *Culicoides obsoletus* accounted for 86% of female and 66% of male *Culicoides* caught. Nearly half of the *C. obsoletus* group catch was nulliparous and a third was parous. The *obsoletus* group catch was dominated by *C. obsoletus s.s.* and *C. scoticus*. Estimates for the proportion of each species were broadly similar for males and females, although *C. obsoletus s.s.* appeared to be under-represented in the male catch. Differences between May–June and October included an increase in the parity rate (proportion of catch that was parous) of *C. obsoletus* group females and a reduction in the catch proportion that were *C. obsoletus s.s.*, with a corresponding increase in the proportions of the other three species. Given the small numbers caught in October, further analysis was restricted to female *C. obsoletus* and *C. pulicaris* only.

Culicoides obsoletus *females.* There was a significant effect of treatment on nulliparous *C. obsoletus* (Friedman, $S_3 = 8.5$, $P < 0.05$). Detransformed median catches were: Out/No animals, 13.7; Out/Animals, 24.5; In/No animals, 2.9, and In/Animals, 16.1 (Fig. 1B). In both the absence and presence of animals, the outside catch was larger than the inside catch, but the magnitude of the difference was less than in May–June (Fig. 1A). Both inside and outside, catches were larger when animals were present.

The effect of treatment was not significant for parous or total female *C. obsoletus* catches, although in both cases the treatment rankings were identical to that for nulliparous females. There was a significant effect of treatment on the number of blood-fed *C. obsoletus* ($S_3 = 8.1$, $P < 0.05$); the highest numbers of blood-fed midges were for the two treatments with animals present.

Culicoides pulicaris *females.* There was no effect of farm or treatment on the catches of female *C. pulicaris* in October.

Openness of stables

We estimated that the percentage of the surface of the stables open to the outside was 5.5%, 2.5%, 5.3% and 6.3% on Farms 1–4, respectively. It may be noteworthy that the farm with the most enclosed stable, Farm 2, was also the farm which recorded the greatest difference between catches inside and outside in May–June (Fig. 2) and October.

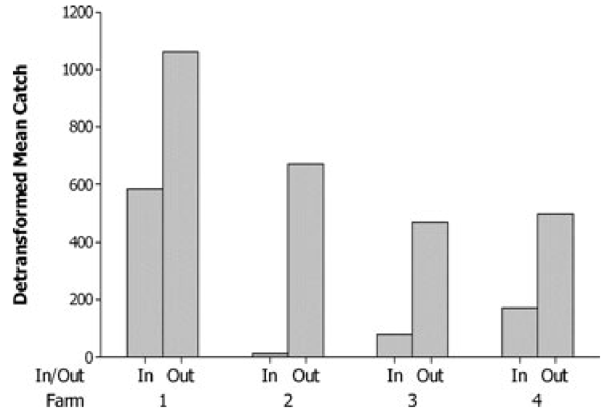

Fig. 2. Detransformed mean catches of *Culicoides obsoletus* females inside and outside animal housing on four farms in May–June.

Influence of weather

Weather records across the four farms were averaged for each night in May–June and October; the means and ranges over the 12 days of each trial are shown in Table 2. The trial in May–June was undertaken during a period of relatively warm and dry weather. The weather in October was colder, wetter, more humid and windier. Generally, temperatures inside stables were 1–2 °C less extreme than those recorded outside; it was also more humid inside than outside.

Despite the relatively close proximity of the four farms, certain weather variables differed between them. In May–June, there were significant effects of Farm on mean maximum temperature ($F_{3,27} = 12.3$, $P < 0.001$), mean minimum temperature ($F_{3,33} = 37.2$, $P < 0.001$) and mean maximum wind speed ($F_{2,22} = 18.5$, $P < 0.001$). In October there were significant differences in mean minimum temperature ($F_{3,33} = 3.9$, $P < 0.05$) and mean maximum wind speed ($F_{3,33} = 15.4$, $P < 0.001$). Across both seasons, Farm 1 tended to be the coldest at night, whereas Farm 3 was the windiest at night.

Influence of weather on catches of C. obsoletus. There were significant associations between certain weather variables and catches of *C. obsoletus* (Table 3). In May–June, the logged total catch of *C. obsoletus* females was most significantly correlated negatively with wind speed (Fig. 3A); a positive correlation with minimum temperature approached but did not reach significance (Fig. 3B). A multiple regression of maximum wind speed and minimum temperature on logged catch was significant (overall: $F_{2,9} = 7.4$, $P < 0.02$, $R^2 = 54\%$; maxWind, $T = -2.89$, $P < 0.02$; minT, $T = 2.14$, $P = 0.06$). In May–June, therefore, windier, and possibly colder, nights were less favourable for *C. obsoletus*.

By contrast, in October, wind speed was not correlated with *C. obsoletus* catch; rather, catches were reduced at lower minimum temperature and RH.

Interaction of weather and housing. As wind speed must be lower inside stables than outside, and minimum temperatures

Table 2. Mean (range) temperature, relative humidity and wind, and total rainfall, over the 12 nights in May–June and October 2007.

	May–June		October	
Weather variable	Inside	Outside	Inside	Outside
Max temp, °C	21.1 (14.4–26.9)	21.7 (14.8–27.0)	12.3 (10.1–14.5)	13.8 (10.1–17.1)
Min temp, °C	9.5 (6.7–13.1)	7.8 (4.8–13.0)	6.2 (1.4–12.2)	5.5 (0.4–11.5)
Min RH, %	56 (44–74)	44 (37–59)	78 (62–95)	64 (38–91)
Max wind speed, m/s	NA	3.6 (2.3–5.6)	NA	4.5 (1.7–10.1)
Total rainfall, mm	NA	1.4	NA	37.5

Maximum temperature was obtained from readings taken during the day. Minimum temperature, RH and wind speed were obtained from readings taken at night. Rainfall is the total for the previous 24 h. Raw data represent averages across the four farms.
RH, relative humidity.

Table 3. Pearson correlation coefficient matrix of key weather variables with $\log_{10}$ total female catch of *Culicoides obsoletus*.

	C. obsoletus	Max temp	Min temp	Min RH	Max wind
C. obsoletus	—	−0.222	0.676†	0.653†	0.410
Max temp	0.525	—	−0.072	−0.048	0.173
Min temp	0.522*	0.124	—	0.796‡	0.646†
Min RH	0.125	−0.322	0.679†	—	0.833‡
Max wind	−0.657†	−0.842‡	−0.134	0.224	—

*$P < 0.1$.
†$P < 0.05$.
‡$P < 0.01$.
Raw data represent the 12 nightly averages across the four farms. The bottom/left half of the table refers to May–June; the top/right half refers to October.
RH, relative humidity.

are higher inside than outside (Table 1), it is possible that the effects of weather differ for inside and outside traps.

For May–June the inside catch was significantly correlated with minimum temperature recorded indoors (c = 0.486, $P < 0.02$), whereas correlations with wind speed and minimum temperature recorded outdoors approached but did not reach significance (respectively, c = −0.445, $P = 0.064$; c = 0.390, $P = 0.06$). Two-variable models were no better than minimum indoor temperature alone; for example, the regression of wind speed and minimum temperature indoors on inside catch approached but did not reach significance ($F_{2,15} = 3.3$, $P < 0.07$). By contrast, the regression of wind speed and minimum temperature outdoors on outside catch was significant (overall: $F_{2,15} = 6.5$, $P < 0.01$; $R^2 = 39\%$; maxWind, T = −3.16, $P < 0.01$; minT, T = 3.04, $P < 0.01$). The inclusion of Animal in this model improved fit even further ($F_{3,14} = 16.3$, $P < 0.001$, $R^2 = 73\%$). Indeed, this three-variable model (Animal, Wind speed and Minimum temperature outdoors) was remarkably effective at explaining variation in (logged) outside catches (R^2: *C. obsoletus* nulliparous, 71.5%; parous, 77.3%; blood-fed, 79.6%; gravid, 43.3%; *C. pulicaris* group, 64.3%; *C. pulicaris*, 66.3%; *C. impunctatus*, 39.0%; Other *Culicoides*, 76.9%).

As minimum temperature and maximum wind speed appear to affect catches of *C. obsoletus*, they were added as covariates to the model described earlier of Farm, Block, Night (within Block), In/Out and Animals, and interactions, on the total catch of female *C. obsoletus* both indoors and outdoors. For this model, the minimum temperature used (recorded inside or

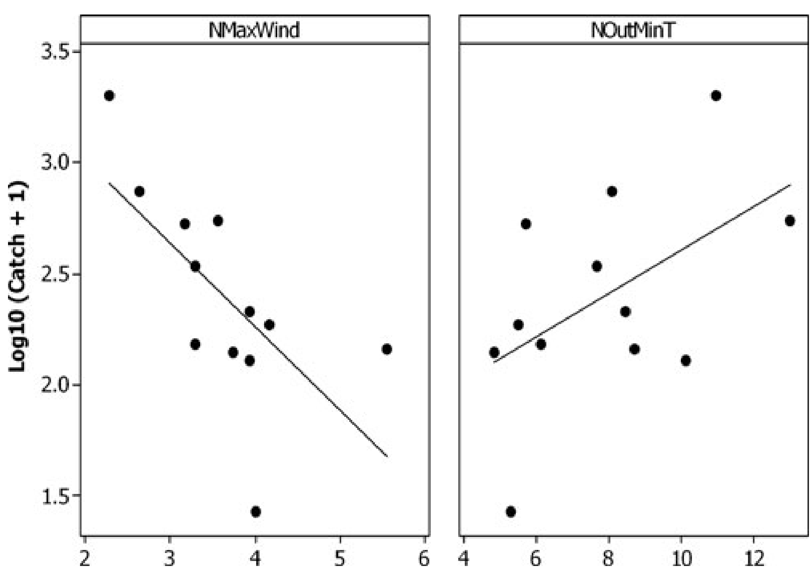

Fig. 3. The relationship between maximum wind speed recorded at night (NMaxwind, m/s) and minimum temperature recorded outdoors at night (NOutMinT, °C) on the log-transformed catch of *Culicoides obsoletus* females in summer. Each point is the average, across four study farms, for one study night.

outside) was determined by the trap position on that farm on that particular night. The inclusion of wind speed in the model prevented the use of data for Farm 4. Farm, Animals, In/Out, the Farm × In/Out interaction, and both weather variables were all highly significant in the final model. Block and Night were not significant. In other words, the inclusion of key weather variables led to the removal of Night as a significant variable in the final model. The R^2 of this final model was very high, at 83.4%.

It is inappropriate to explore the relationship between weather and catch of *C. obsoletus* in October in as much detail as for May–June because the log-transformed inside catch data depart from normality. Both inside and outside catches were correlated with minimum temperature (respectively, Spearman's rho = 0.433, $P < 0.05$; Pearson's c = 0.382, $P < 0.07$) and minimum RH (rho = 0.452, $P < 0.05$; c = 0.455, $P < 0.05$). Inside catch was not correlated with wind speed. However, for outside catch, wind speed was significant in a two-variable model with minimum RH (T = −2.15, $P < 0.05$) and approached significance in a two-variable model with minimum temperature (T = −1.95, $P < 0.07$). In other words, the evidence indicates that outside catches in October were reduced at low RH, low minimum temperature and at high wind speed. The addition of Animal to these models did not improve fit, and Animal was not significant as a variable.

Discussion

This study is the first to systematically investigate the effects of positioning a light trap inside or outside animal holdings, in the presence or absence of animals, on *Culicoides* catches in northern Europe. The results demonstrate that in May–June catches in light traps are several times greater outside stables than inside, irrespective of the presence or absence of cattle. The implication is that cattle housed inside may gain a degree of protection from the bites of *Culicoides* vectors of BTV, compared with cattle kept outside. This was evident in the fact that when animals were present, the catch of blood-engorged *C. obsoletus* was three to four times higher outside stables than inside.

This apparently simple picture is less clear in the study carried out in October. The reduced abundance of *Culicoides* in autumn lessened our ability to tease apart the impact of different factors; nevertheless, catches in light traps outside were again greater than those inside. The difference between outside and inside catches was, however, reduced compared with that in the May–June study.

Why is there a difference between the two seasons? One possibility relates to seasonal changes in the species composition or age structure of the *C. obsoletus* group. In summer, the *C. obsoletus* group comprised a higher proportion of *C. obsoletus s.s.* and a higher proportion of younger, nulliparous individuals, than in autumn. Higher levels of exophily in *C. obsoletus s.s.* or in nulliparous females, than in other species or in parous females, could therefore lead to seasonal changes in the level of exophily.

However, the current data provide strong evidence for additional effects of weather. In both seasons, low catches were associated with colder nights and stronger winds. These weather variables differ between the two seasons and had more marked effects on catches outside than inside. These observations may explain the seasonal change. In May–June, when nights are warm and calm, outside catches of *C. obsoletus* exceed those inside. In October, nights are both colder and windier; this weather suppresses the outside catch to a greater extent than that inside, and leads to an apparent reduction in exophily by autumn.

The observation that the weather is more strongly associated with outside catch than inside catch is interesting: if all *Culicoides* caught inside stables have recently entered from outside, then the weather might be expected to affect both equally. Therefore, this observation suggests that at least some of the inside catch may have originated inside the housing. However, there is a complicating factor. The weather is known to affect the efficiency with which light traps catch *Culicoides*; in particular, at higher wind speeds light traps catch a smaller proportion of the *Culicoides* that are active (Baylis *et al.*, 2004). Consequently, some of the differences between inside and outside trap catches may relate to the operation of the traps themselves, rather than influences of the weather on *Culicoides*.

Culicoides catches can vary hugely from one night to the next; indeed, Night was a highly significant variable in our initial models. Interestingly, however, by including minimum temperature and wind speed in the models, the effect of Night was removed, suggesting that minimum temperature and wind speed play a large role in driving nightly variation in catch. An inference is that on any one farm and night, if it is warm and calm then cattle will be attacked by *Culicoides* more if kept outside than inside; on colder or windier nights, the difference between outside and inside catches will be much reduced.

It has been demonstrated that screening the windows and doors of a stable dramatically reduces the number of *Culicoides* that enter (Meiswinkel *et al.*, 2000). In the current study, there was no screening of the animal housing, and on all four farms there was opportunity for *Culicoides* to enter the housing. Nevertheless, there was large variation in the relative catches outside vs. inside (from two to 60 times), and the greatest difference corresponded to the animal housing with the least amount of open door and window. A previous study has reported that entry of *Culicoides* spp. into horse stables is proportionate to the size of the entrance to the stable (Barnard, 1997). The current result is preliminary, given the small number of animal housings tested, but merits further investigation in case there is a level of enclosedness which affords good protection against *Culicoides* without requiring the expense of screening or possible health risks associated with lack of ventilation or access to grazing.

The degree of protection from attack by *Culicoides* afforded by the stabling of animals at night is dependent upon the species of midge: in South Africa, *C. imicola* was more exophilic than *Culicoides bolitinos* (Meiswinkel). The current study adds to a body of evidence that northern Palaearctic *C. obsoletus* are exophilic (Anderson *et al.*, 1993; Meiswinkel *et al.*, 2008), although there are exceptions (Zimmer *et al.*, 2008). The current results show, however, that exophily is far from absolute and many *C. obsoletus* still enter animal housing; the degree of exophily varies from farm to farm (or, at least, from animal house to animal house) and varies seasonally, possibly because of differential effects of unfavourable weather on outdoor catches. Stabling is therefore one of a suite of mitigating measures that could be usefully employed to reduce BTV transmission, but it is unlikely in itself to provide complete protection.

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