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Does covering of farm-associated *Culicoides* larval habitat reduce adult populations in the United Kingdom?

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ABSTRACT

Culicoides biting midges (Diptera: Ceratopogonidae) are the biological vectors of a range of internationally important arboviruses of livestock, including bluetongue virus (BTV) and the recently emerging Schmallenberg virus (SBV). *Culicoides* species in the subgenus *Avaritia* (in the UK: *Culicoides obsoletus* Meigen, *Culicoides scoticus* Downes & Kettle, *Culicoides dewulfi* Goetghebuer and *Culicoides chiopterus* Meigen) have been implicated in BTV transmission in northern Europe and to a varying degree utilise cattle dung as a larval development substrate. The collection of cattle dung into heaps on farms provides a localised source of *Culicoides* emergence in close proximity to livestock. This study assesses the impact of covering dung heaps prior to the onset of adult *Culicoides* activity with the aim of reducing recruitment to the local adult populations at four livestock farms in England. Light suction trap catches of adult *Culicoides* from these farms were compared with those from four untreated control farms from a wide geographic range across the UK. It was demonstrated that implementing control of emergence from dung heaps did not have a significant impact upon the local adult subgenus *Avaritia* abundance at the treated farm holdings and that the onset of *Culicoides* activity was similarly unaffected. Use of this method in isolation is unlikely to have an effect in reducing the risk of BTV and SBV transmission. The implications of these results for control of farm-associated *Culicoides* in Europe are discussed.

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1. Introduction

Culicoides biting midges (Diptera: Ceratopogonidae) are the biological vectors of a range of internationally important arboviruses of livestock, including bluetongue virus (BTV), Schmallenberg virus (SBV) and African horse sickness virus (AHSV) (Elbers et al., 2013; Mellor et al., 2000). In northern Europe, putative BTV and SBV vector species have been identified in the *Avaritia* subgenus, represented in the UK by *Culicoides obsoletus* (Meigen), *Culicoides scoticus* Downes & Kettle, *Culicoides dewulfi* (Goetghebuer) and *Culicoides chiopterus* (Meigen). Within the subgenus, *C. dewulfi* and *C. chiopterus* develop directly in cattle dung (Campbell and Pelham-Clinton, 1960; Kettle and Lawson, 1952; Kremer, 1965), although other alternative habitats including bogs rich in decaying vegetation (Dzhafarov, 1964; Goetghebuer, 1936) and sap running from wounds in elm trees (Edwards et al., 1939) require further confirmation. In contrast, larvae of *C. obsoletus*

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and *C. scoticus* have been frequently recorded as occupying a wide-range of habitats including marshes, swamps, acid grassland, leaf litter, rotting vegetable matter, maize silage residues, organically enriched soil and fungi (Boorman, 1986; Buxton, 1960; Campbell and Pelham-Clinton, 1960; Dzhafarov, 1964; Glushchenko and Mirzaeva, 2008; Goetghebuer, 1936; González et al., 2012; Harrup et al., 2013; Hill, 1947; Kettle and Lawson, 1952; Kremer, 1965; Trukhan, 1975; Zimmer et al., 2008, 2012). The relative contribution of each of these habitats to emerging adult populations of *C. obsoletus* and *C. scoticus* is currently unknown.

Control measures aimed at reducing or destroying available larval *Culicoides* habitats may be broadly divided into three main categories: (1) conventional larvicidal applications; (2) biorational applications and (3) habitat modification and destruction (see Carpenter et al., 2008a for review). All of these measures require detailed knowledge of the distribution and abundance of *Culicoides* larval habitat, which to a great degree determines the efficacy of procedures applied (Kettle, 1962). Larval habitat modification and eradication has historically been most effective when practiced against *Culicoides* with a localised distribution inhabiting areas that can be straightforwardly manipulated in a cost-effective manner. A key example is *Culicoides sonorensis* Wirth and Jones, the principle vector of BTV in the USA, which primarily develops in dairy wastewater lagoons (Mullens, 1989; O Rourke et al., 1983; Schmidtmann et al., 1983, 1998). Waste and water management strategies, focusing on the efficacy of draining water trough overflows and dairy waste water evaporation beds, have been shown to be effective for controlling *C. sonorensis* in certain contexts (Jones, 1977; Mullens and Rodriguez, 1988).

Following the incursion of BTV serotype 8 (BTV-8) into northern Europe some eighteen months passed before the implementation of inactivated vaccination schemes (Carpenter et al., 2009). During this time a range of *Culicoides* control techniques were recommended across affected countries as mitigation against infection with BTV (Carpenter et al., 2008a). In the UK the traditional method for dealing with manure and waste bedding material from livestock farms is to store it in piles (Nicholson and Brewer, 1997), colloquially known as muck heaps (Fig. 1). Muck heaps are usually located at a designated point on the farm property, often close to livestock housing, before being spread on fields as a natural fertiliser.

Prior to the BTV-8 incursion, muck heaps had been suggested as a major development site of ruminant associated *Culicoides* (Campbell and Pelham-Clinton, 1960; Harrup et al., 2013; Kettle and Lawson, 1952; Kremer, 1965; Schwenkenbecher et al., 2009). Due to this, covering of muck heaps prior to *Culicoides* emergence in spring was recommended to farmers as a method to ameliorate potential BTV transmission (Defra, 2009). Little quantitative data, however, existed regarding the impact of covering muck heaps upon *Culicoides* abundance, although the technique has been employed with variable success in small scale field-trials to target larval development sites of stable flies (*Stomoxys calitrans* (L.)) (Meyer and Shultz, 1990; Todd, 1964), house flies (*Musca domestica* L.) (Gerry et al., 2005; Meyer and Shultz, 1990), coastal flies (*Fannia femoralis* (Stein)) (Gerry et al., 2005) and the black dump fly (*Hydrotaea aenescens* (Wiedemann)) (Gerry et al., 2005). This study therefore aimed to assess both the logistics and the impact of covering dung heaps on the local abundance of adult *Culicoides*.

2. Materials and methods

2.1. Study area and Culicoides collection

Using a trapping network run by volunteers at eight livestock farms in England between 2006 and 2009 (Fig. 2), estimates of *Culicoides* abundance were made using Onderstepoort Veterinary Institute (OVI) type 8W ultraviolet (UV) down-draught suction traps (Agricultural Research Council, South Africa). Traps were suspended at a height of 1.5–2.0 m above the ground and insects collected into a 500 ml beaker suspended below the trap that contained approximately 100 ml of water with a small drop of detergent (Hederol, Procter and Gamble Professional, UK). Traps were run for one night each week at each farm from dusk until dawn to coincide with crepuscular peaks in *Culicoides* activity (Hill, 1947; Kettle, 1957; Parker, 1949; Service, 1969). The contents of each collecting pot was passed through a fine mesh sieve (aperture of <0.25 mm) and the retained insects washed using 70% ethanol into a 250 ml straight-side wide-mouth polypropylene sample jar. Sufficient 70% ethanol was then added to cover the sample for storage prior to postage to The Pirbright Institute for identification. *Culicoides* were separated from other insects collected and identified to species or subgenus level (Campbell and Pelham-Clinton, 1960) using a stereomicroscope (10–40× magnification). Male subgenus *Avaritia* species were identified from their genitalia which is species diagnostic, while females were identified to subgenus level only.

2.2. Habitat modification control measure

All muck heaps present at farms one to eight were created by owners predominately from a mixture of cattle waste and straw bedding, with the exception of farm two where sheep rather than cattle waste formed the principle component. The muck heaps at the farms included in this study are not normally covered and range in volume from approximately 60 m^3 to 280 m^3, with new material added on average biweekly. During winter 2009, four of the eight farms (farms one, two, three and four: Fig. 2) from which weekly estimates of *Culicoides* abundance were available were randomly selected for implementation of the control measure. Farms one, two and four all had one muck heap each, while farm three had two muck heaps, each of these muck heaps were covered with a 200 g/m^2 (14 by 14 per square inch weave) green tarpaulin (Bradshaws Direct, York, UK), which excluded both light and water from the surface of the muck heap (Fig. 1). Tarpaulins were weighted and secured with 8 mm polypropylene rope (Wickes, Northampton, UK) and 440 mm by 215 mm by 100 mm medium density blocks (Wickes, Northampton, UK) (Fig. 1). The muck heaps at farms one, two, three

Fig. 1. Muck heaps before (uncovered) and after (covered) coverage at farms one, two, three and four.

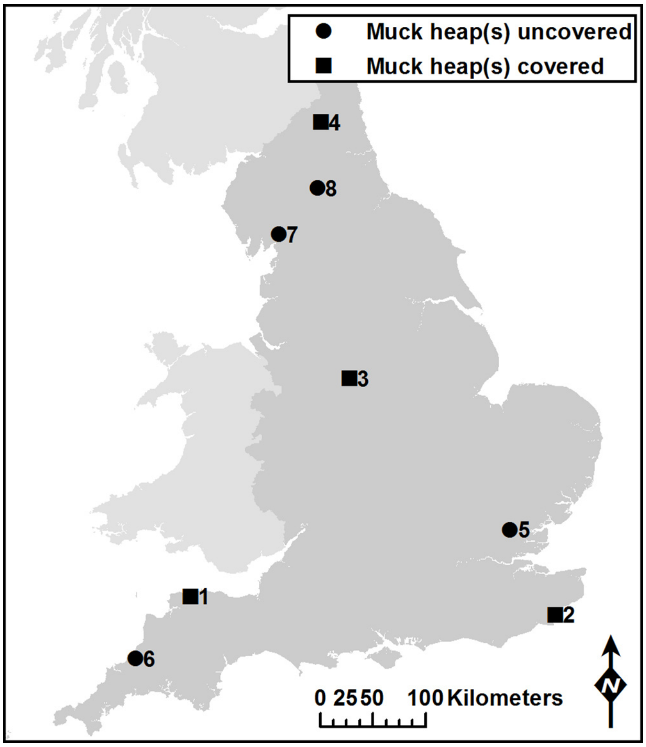

Fig. 2. Spatial distribution of treatment farms (muck heap(s) covered – farms one, two, three and four) and control farms (muck heaps uncovered – farms five, six, seven and eight) (ESRI basemap data (ESRI, 2006)).

and four were covered from early March 2009 during the seasonal vector-free period (European Commission, 2007), until the end of May 2009 following the spring peak in *Culicoides* emergence (Sanders et al., 2011). Farming activities on the farms prevented the muck heaps from remaining covered for a longer time period. The muck heaps at the remaining four farms (farms five, six, seven and eight, Fig. 2) remained uncovered throughout and were used as controls, to allow an assessment of the overall trend in *Culicoides* subgenus *Avaritia* populations for the 2009 season when compared to previous seasons (2006–2008).

Light suction traps were located within 100 m of muck heaps, livestock housing and grazing pasture at all farms and greater than 5 km from the muck heaps of any neighbouring farms. Although seasonal variation in the number of livestock located in close proximity (<100 m) to the light suction trap did occur within farms, this variation was consistent between years and was primarily associated with the variation between livestock being housed during winter and grazed at pasture during spring, summer and autumn.

2.3. Data analysis

To assess the effect of covering muck heaps on the first generational peak in 'local' adult populations of the *Culicoides* subgenus *Avaritia*, trap catches were analysed using generalised linear models assuming Poisson errors and a log link function. Furthermore, the models included overdispersion (to allow for the high week-to-week variability in catches), temporal autocorrelation amongst the observations (to allow for dependence between observations) and hierarchical structure in the model parameters (to allow for between-farm differences) (Sanders et al., 2011).

The number of female subgenus *Avaritia Culicoides* collected (y_{jk}) at the jth observation on farm k (collected on day t_{jk}) was assumed to follow a Poisson distribution, that is,

$$y_{jk} \sim \text{Poisson}(\mu_{jk}), \quad (1)$$

with the expected trap catch, μ_{jk}, given by,

$$\begin{aligned} log(\mu_{jk}) = {} & c_{jk}\left[b_{0k}^{(C)} + b_{1k}^{(C)} \sin\left(\frac{2\pi}{365}(t_{jk} - \phi_{jk})\right)\right] \\ & + (1 - c_{jk})\left[b_{0k}^{(U)} + b_{1k}^{(U)} \sin\left(\frac{2\pi}{365}(t_{jk} - \phi_{jk})\right)\right] \\ & + \sigma_{jk} + \varepsilon_{jk}. \end{aligned} \quad (2)$$

Here, c_{jk} indicates whether ($c_{jk} = 1$) or not ($c_{jk} = 0$) the muck heap was covered, the terms including sine functions describe seasonality in the *Culicoides* population when the muck heap is covered (C) or uncovered (U) (here b_0 is the log mean population, b_1 is the amplitude and ϕ is the phase), while σ_{jk} allows for overdispersion in the data and ε_{jk} allows for temporal autocorrelation between observations. Between-site variation was incorporated by assuming the parameters for each site are drawn from higher-level distributions, so that,

$$b_{ik}^{(\bullet)} \sim N(\mu_{b_i^{(\bullet)}}, \sigma^2_{b_i^{(\bullet)}}),$$
$$\phi_k = 182.5 + 182.5\phi'_k, \quad \phi'_k \sim \text{Beta}(a_\phi, b_\phi),$$

while overdispersion in the data was modelled as,

$$\sigma_{jk} \sim N(0, \sigma_d^2).$$

Finally, temporal autocorrelation was described by a stationary AR(p) process (Diggle et al., 2002), so that,

$$\varepsilon_{jk} = \sum_{i=1}^{p} \rho_i \varepsilon_{j-i,k} + z_{jk},$$
$$z_{jk} \sim N(0, \sigma_\varepsilon^2).$$

This approach implicitly assumes the observations are regularly spaced, though because of missing data they are not quite so. Parameters in the model were estimated using Bayesian methods in OpenBUGS (version 3.2.2 (Lunn et al., 2009)). Non-informative priors were used for the higher-order parameters: diffuse normal for the μs; diffuse gamma for the σs; and uniform (with appropriate ranges) for the ρs. Two chains, each of 150,000 iterations, were run with the preceding 50,000 iterations discarded to allow burn-in of the chain. Each chain was subsequently thinned (selecting every twentieth iteration) to reduce autocorrelation amongst the samples. Convergence of the chains was assessed visually and using the Gelman–Rubin diagnostic provided in OpenBUGS. Selection of the number of seasonality components n and the number of lags, p, for the AR(p) model was based on the deviance information criterion (DIC; (Spiegelhalter et al., 2002)). This indicated that an AR(1) model was adequate to describe the data (increase in DIC of 22.9 for the AR(2) compared with the AR(1) model).

The model was assessed using posterior predictive checking (Gelman et al., 2004). More specifically, the posterior predictive distribution was used to generate replicated data by sampling parameter sets from the posterior distribution and using the sampled parameters to simulate data-sets using the model, (1) and (2). These were compared to the observed data using four measures: (i) χ^2 goodness-of-fit statistic (as a measure of overall fit); (ii) total annual catch; (iii) maximum daily catch each year; and (iv) time of first appearance each year (defined as >5 individuals caught). If the observed data generate a more extreme value of the measures than the replicate data (as judged by the proportion of replicates which generate a value of the measure less than the observed data; equivalent to a classical (i.e. non-Bayesian) *P*-value), this provides an indication that the model does not adequately capture the data.

A model was parameterised only for the abundance of females of the subgenus *Avaritia*. Trap data for males, which are not consistently collected at light suction traps, were excluded because of low sample sizes and because male *Culicoides* do not take blood meals from vertebrates and, consequently, do not transmit BTV, or other arboviruses, between hosts.

Table 1
Summary statistics (mean, median and 95% credible limits) for the marginal posterior densities for parameters in the model for the seasonal dynamics of *Culicoides* biting midges and the impact of covering muck heaps.

Parameter	Mean	Median	95% credible limits	
			Lower	Upper
Hierarchical parameters, phase				
a_ϕ	22.87	18.87	0.77	69.46
b_ϕ	95.01	78.60	1.66	287.20
Hierarchical parameters, log mean and amplitude when muck heaps covered				
$\mu_{b_0^{(C)}}$	1.56	1.61	−0.59	3.39
$\mu_{b_1^{(C)}}$	−8.86	−8.82	−11.92	−5.96
$\sigma_{b_0^{(C)}}$	0.96	0.16	0.01	6.82
$\sigma_{b_1^{(C)}}$	2.71	0.26	0.01	17.69
Hierarchical parameters, log mean and amplitude when muck heaps uncovered				
$\mu_{b_0^{(U)}}$	0.33	0.29	−0.65	1.39
$\mu_{b_1^{(U)}}$	−5.08	−5.20	−5.83	−3.48
$\sigma_{b_0^{(U)}}$	0.96	0.16	0.01	6.82
$\sigma_{b_1^{(U)}}$	2.71	0.26	0.01	17.69
Overdispersion parameter				
σ_d	3.20	3.21	2.41	3.98
Autocorrelation parameters				
ρ	0.67	0.66	0.49	0.88
σ_ε	1.72	1.65	1.03	2.79

3. Results

Posterior predictive checking indicated that the statistical model, (1) and (2), with an AR(1) model for auto-correlation adequately captured the data in terms of overall fit (Fig. 3), total annual catch, maximum daily catch each year and time of first appearance each year. There was no evidence that covering muck heaps significantly reduced the abundance of female *Culicoides* biting midges of the *Avarita* subgenus (Table 1), which made up 34.9% of the total *Culicoides* collected from the eight farms. More precisely, there was no significant difference in the log mean abundance ($Pr(\mu_{b_0}^{(U)} > \mu_{b_0}^{(C)}) = 0.12$) for farms with covered and uncovered muck heaps and, indeed, the estimates for the amplitude were significantly higher ($Pr(\mu_{b_1}^{(U)} > \mu_{b_1}^{(C)}) = 0.99$) for farms with covered muck heaps compared with those where they were covered.

Based on collection of males in 2008, prior to implementation of control measures, all four members of the subgenus *Avaritia* were present on the eight farms used in this study. Following the covering of muck heaps at farms one to four in 2009 males of the four subgenus *Avaritia* species were recovered in light suction trap collections suggesting that the control measure did not completely eliminate any one of the four subgenus *Avaritia* species. No difference was observed in the week number in which *Culicoides* subgenus *Avaritia* activity began (i.e. first collection of the year in the UV light suction trap), between 2009 (muck heaps at farms one, two, three and four: covered) and 2007/2008 (Table 2).

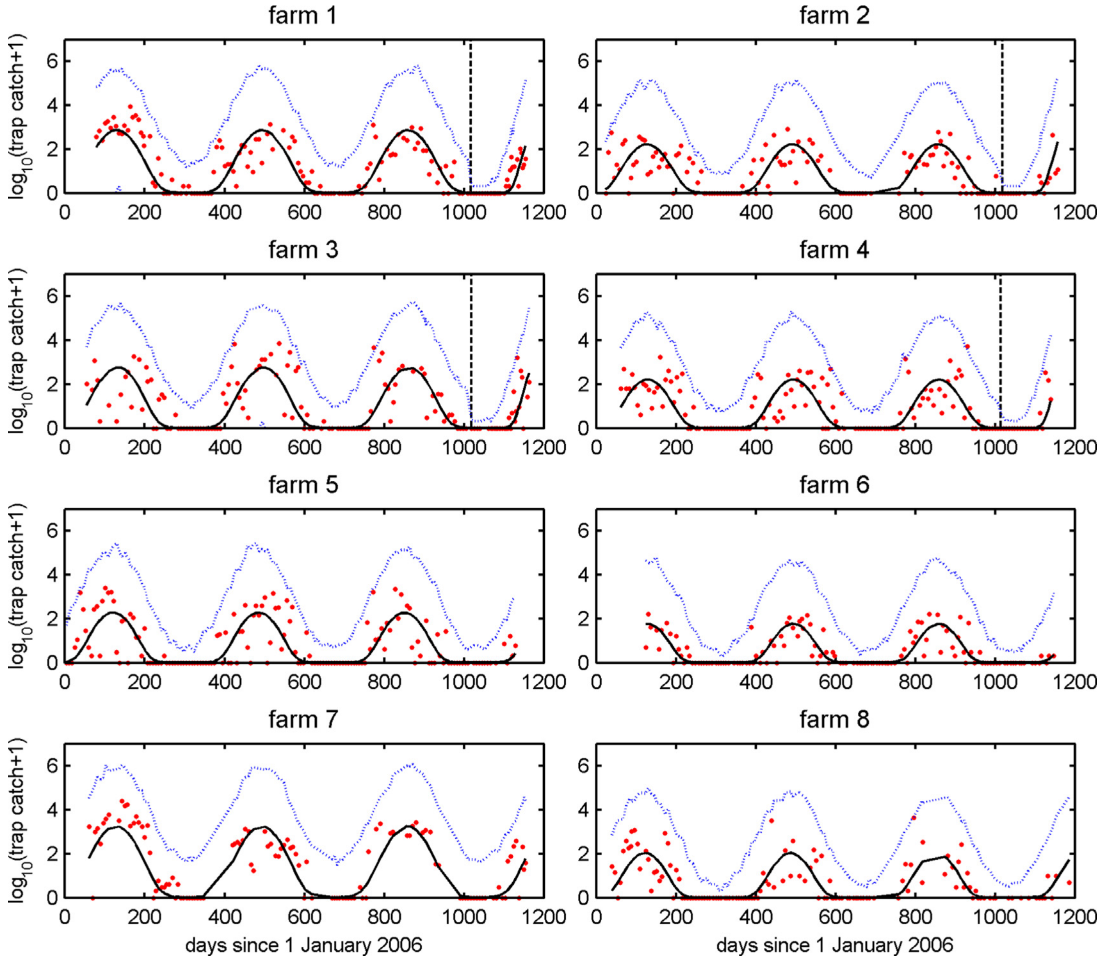

Fig. 3. Observed (circles) and expected (solid lines: posterior median; dotted lines: 2.5th and 97.5th percentiles for the posterior predictive distribution) weekly light trap catches of *Culicoides* (total female *Culicoides* subgenus *Avaritia*) collected between 2006 and 2009 on eight farms across the UK. The time at which muck heaps were covered (on farms one, two, three and four) is indicated by a vertical black dashed line: to the left of this the muck heaps at the farm were uncovered, to the right of it they were covered.

4. Discussion

Covering muck heaps with tarpaulins to prevent emergence of *Culicoides* was found to have no significant impact on adult abundance measured by light suction trap surveillance on four treatment and four control farms. Using knowledge of the probable emergence time of *Culicoides* in the UK, the covering of muck heaps was targeted at a period (early spring), prior to the likely onset of recorded adult *Culicoides* activity. While not confirmed directly through sampling of muck heaps, it is highly probable that *Culicoides* would have been present as over-wintering fourth instar larvae (Kettle, 1984). The failure of the method to eliminate any of the primary potential vector *Culicoides* species was most likely due to emergence of adults from other larval habitats across the farms in the study, as the overall timing of population activity did not differ between treatment and control farms. The study also anecdotally highlighted logistical difficulties in implementing covering of muck heaps in the field, including the limited time-span over which the covers could be applied to heaps due to the required addition and removal of manure to and from the muck heaps, raising the question of whether the method could be straightforwardly integrated into routine farming practice. On large-scale farms, this is likely to prevent user uptake, although the use of covers where *Culicoides* populations are limited and localised (for example in garden waste receptacles) may prove to be more easily targeted on smaller holdings.

The study further highlights the complexity of attempts to control multiple species of *Culicoides* with very different

Table 2
Week number in which first activity of *Culicoides* subgenus *Avaritia* was recorded in light trap collections.

Year	Farm number							
	1	2	3	4	5	6	7	8
2007	13	13	14	16	15	16	21	20
2008	14	19	18	17	17	18	15	18
2009	14[a]	15[a]	15[a]	15[a]	13[a]	13	13	16

[a] Muck heaps covered.

larval ecologies. No impact was recorded upon emerging populations of *C. obsoletus* and *C. scoticus*, which can be explained through their ability to exploit a wide range of larval development habitats. Areas of broadleaved woodland and marginal vegetation surrounding open water were present on each of the eight farms in close proximity (<500 m) to the light suction trap used for surveillance and may have significantly contributed to the local adult population. A similar dilution effect was observed by Todd (1964) during efforts to control *Stomoxys calcitrans* at a New Zealand dairy farm by covering larval development sites in 'ensilage stacks' (accumulations of decomposing organic matter including manure). Abundant and fragmented habitat types such as broadleaved woodland present particular problems with regard to targeting larval development sites as the potential for habitat modification is limited in comparison to other artificial/man-made habitats such as leaking taps or overflowing water troughs (Harrup et al., 2013).

The lack of effect recorded on populations of *C. chiopterus* and *C. dewulfi* was more disappointing, however, as these species were known to be restricted to cattle dung, the primary constituent of the heaps. This finding may reflect a lack of understanding of larval habitat requirements, as previous studies carried out in Australia have demonstrated moisture-associated vertical movement in dung associated *Culicoides brevitarsis* Kieffer larvae characterised emergence in detail (Bishop et al., 1996; Campbell and Kettle, 1976). Similar studies that further define the localisation of *C. chiopterus* and *C. dewulfi* in dung through direct sampling would therefore be useful in understanding the impact of dung disturbance not only by artificial collection into heaps but also in natural degradation by arthropod fauna (Bishop et al., 2005). The contribution to the local adult *Culicoides* population via dispersal from neighbourghing farms is also unknown and may act a significant confounding factor and limitation to the effectiveness of control measures if they are not uniformly employed across farms in an area.

A second major difficulty in interpretation of the current study was the inability to identify females of the subgenus *Avaritia* to species level. These represented 88.0% of the total catch (266,148 individuals) collected across the eight farms used. Identification of this cryptic subgenus to species level is currently primarily based on multiplex PCR assays whose logistical and financial constraints limit the number of specimens which can be processed for the majority of studies. Advances in quantitative real-time PCR based assays of pools of *Culicoides*, however, will enable species-level characterisation of large multi-year datasets such as that included in this study (Mathieu et al., 2011).

In addition to failing to reduce local adult *Culicoides* abundance, no apparent change was observed on treatment farms in the onset of recorded female subgenus *Avaritia Culicoides* activity in 2009, when compared to 2007 and 2008. The speed of development of *Culicoides* larvae is in part determined by environmental temperature (Akey et al., 1978; Allingham, 1991; Bishop et al., 1996; Bishop and McKenzie, 1994; Kitaoka, 1982; Vaughan and Turner, 1987; Veronesi et al., 2009), hence it was hypothesised that the earliest emerging adults might originate from muck heaps which possess a higher than average temperature as a result of organic matter decomposition (Husted, 1994). This hypothesis could be tested by simultaneous measurement of the vertical distribution of larvae (Blackwell and King, 1997; Mullens and Rodriguez, 1992) and thermal regimes in muck heaps versus other available larval habitats.

Additional studies have also demonstrated that exposure of *Culicoides* larvae to high ambient temperatures can alter vector competence parameters in resulting adults (Mellor et al., 1998; Wittmann et al., 2002). Together with an increased interest in the potential role of environmental acquired microbiota in competence and/or survivorship of vector species of *Culicoides* (Campbell et al., 2004; Morag et al., 2012; Nakamura et al., 2009), microhabitat investigations of larvae are clearly of significant interest in the Palearctic region. Although a higher average temperature in the muck heap as a result of covering may increase vector competence if *Culicoides* were able to escape following emergence under the tarpaulin, it may also lead to a higher mortality rate in larval *Culicoides*. Todd (1964) hypothesised that the observed reduction in *S. calcitrans* larval numbers was as a result of the high temperatures observed and a lack of oxygen available under the plastic coverings used. The effect of covering muckheaps on the chemical composition of a muck heap, which has previously been shown to have a significant effect of the productivity of larval development habitats (Zimmer et al., 2012), and other factors such as the impact on dissolved oxygen content may also influence *Culicoides* larval development success and recruitment to the local adult population.

While the larval habitats of subgenus *Avaritia Culicoides* have been characterised in the UK, they are difficult to delineate precisely at the farm level. Our understanding of the relationship between available developmental habitat and the absolute adult abundance of these species within farms therefore remains extremely poor. In the case of *C. obsoletus* and *C. scoticus*, which are patchily distributed across a wide-variety of breeding habitats and across a wide geographic area, these studies would be challenging to perform. The localised larval development sites of *C. dewulfi* and *C. chiopterus*, however, represent a habitat the extent of which can be quantified accurately and may provide a highly malleable experimental system as has already been implemented with the Australian BTV vector *C. brevitarsis* (Bishop et al., 2005; Campbell and Kettle, 1976). The practical utility of such a system would in part be dependent upon future studies assessing the role of each of these species in the transmission of arboviruses, since these roles remain poorly defined at present (Carpenter et al., 2008b).

5. Conclusion

Covering muck heaps with tarpaulins to prevent the emergence of adult *Culicoides* on UK farms had no significant impact on local abundance. Bluetongue virus, and other *Culicoides* transmitted viruses, remain a threat to the European livestock, hence further work in understanding the relative contribution of different larval development habitats of species of the subgenus *Avaritia* to overall population abundance would assist in understanding transmission in the field. The results of a cost-benefit analysis for any proposed vector control measure must also be favourable and the measure itself must also be logistically feasible to be well received by farmers to ensure sufficient rates of uptake.

Conflicts of interest statement

The authors know of no financial or personal conflicts of interest with any person or organisation that could inappropriately influence this work. Funders had no role in study design or the collection, analysis and interpretation of data. Mention of proprietary products does not constitute an endorsement or a recommendation by the authors for their use.

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