Impact of flooding on the immature stages of dung-breeding *Culicoides* in Northern Europe

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**Abstract**

In Northern Europe, dung-breeding *Culicoides* (e.g., *Culicoides chiopterus* (Meigen 1830) and *Culicoides dewulfi* (Goetghebuer 1936)) are considered to be important vectors of the Bluetongue virus and Schmallenberg virus. The interpretation of their distribution is difficult due to the lack of knowledge about their ecology. Previously, soil moisture and especially flooding were identified as important factors that influence the development of several biting-midge species. Therefore, this experimental study addressed the question whether flooding has a negative impact on the development of immature stages of Obsoletus group species. Ten cowpats were collected, and each was divided into four quarters and kept at different moisture regimes in a greenhouse: (1) “dry” (no water added), (2) “control” (regularly moistened), (3) “alternately flooded” and (4) “permanently flooded”, to compare *Culicoides* emergence. Flooding had a significant negative impact on the emergence of *Culicoides*. No individuals emerged from the “permanently flooded” treatment and only two individuals were sampled from the “alternately flooded” treatment. In contrast, the total emergence from the non-flooded samples in the “dry” (96 individuals, 38.6% of all *Culicoides*) and “control” (151 individuals, 60.6% of all biting midges) treatments was considerably higher. Biting midges were predominantly identified as *C. dewulfi* (161 individuals, 64.7% of all *Culicoides*) and *C. chiopterus* (63 individuals, 25.3% of all *Culicoides*). There were no significant differences in emergence between the “dry” and “control” treatments. Our results highlight the importance of soil moisture on the distribution of *C. chiopterus* and *C. dewulfi*. Regarding physiological and behavioural adaptations of other *Culicoides* species, we argue that pupae of *C. chiopterus* and *C. dewulfi* are in danger of drowning when breeding sites are flooded as they cannot float. On the contrary, our results indicate that desiccation might not be harmful to these species.

1. Introduction

Several *Culicoides* species are vectors of pathogens (e.g., Bluetongue virus (BTV), Schmallenberg virus (SBV), or African Horse Sickness virus (AHSV)), among which BTV has received the greatest attention in Northern Europe. Bluetongue virus can cause a non-contagious disease of ruminants, resulting in huge economic losses, e.g., the BTV epidemic 2006–2010 led to costs of more than 250,000,000€ in Germany alone (Conraths et al., 2012). Indigenous biting midges of the Obsoletus and Pulicaris group are considered to be the most important vectors (Ander et al., 2012). The recent epidemic caused by SBV...
again emphasised the importance of biting midges as vectors of pathogens and once again, members of the Obsoletus group were suspected to be the main vectors (De Regge et al., 2012; Rasmussen et al., 2012).

Due to their veterinary importance, many studies have attempted to identify the environmental factors that drive the occurrence of Culicoides species (Baylis et al., 1998; Conte et al., 2003). A variety of data types (e.g., temperature, landscape, land cover) have been used to develop species distribution models, however, because of the lack of basic knowledge concerning the ecology of biting midges (e.g., breeding or resting sites), a comprehensive interpretation of the modelling results is often subject to uncertainties. Several studies have demonstrated the need for information on the species to interpret distribution models for Culicoides. The interpretation of models for Culicoides imicola led to the prediction that areas with an annual rainfall greater than 1000 mm might be unsuitable for C. imicola (Wittmann et al., 2001), because the pupae drown when breeding sites are flooded (Nevill, 1967). Another study revealed modelling results that appear contradictory at first sight. Here, a negative impact of cattle density and a positive impact of pasture cover on the species abundance of Culicoides impunctatus were found (Purse et al., 2011), however, due to the knowledge of this species’ preference for rush pasture cover (Blackwell et al., 1994, 1999), Purse et al. (2011, p. 174) concluded that the correlations “are probably related to the association of this species in the larval stage with rush pasture cover that arises from light grazing and high soil water content”.

Larvae of biting midges belonging to the subgenus Avaritia are generally expected to have a slow head-to-tail flexion, in contrast to the serpentine swimming movements of other Culicoides species. Furthermore, the pupae are not able to float (Cannon and Reye, 1966). This is interpreted as an adaptation to breeding sites with a relative high viscosity (e.g., dung) and might also explain their breeding site preferences in comparison to other Culicoides species. Members of the Pulicaris group are tolerant of, or might even prefer, waterlogged breeding sites, because the pupae can float on the water surface (Nevill et al., 2007; EFSA, 2007). Additionally, there was no negative impact of flooding on the eggs or larvae of seven South African Culicoides species and only a negative impact on the pupae of C. imicola, which drown under waterlogged conditions (Nevill, 1967).

Controlled experiments of environmental (e.g., soil characters, type of vegetation) and management factors (e.g., manure storage) can help to understand the ecological processes that affect Culicoides species distribution in and around farms (Scolamacchia et al., 2013). There still is a deficit of experiments to close the broad gaps of knowledge on the ecology of biting midges. Even the breeding ecology of the exhaustively investigated species C. imicola, which is known as the main vector of BTV and AHSV in Southern Europe, the Mediterranean region, and Africa (Mellor et al., 2000), is not fully understood and laboratory studies are particularly lacking (Peters et al., 2013). Soil moisture is expected to be an important factor influencing the occurrence of Culicoides species, but the connection between soil moisture and Culicoides larval development has not yet received sufficient experimental attention (Mellor et al., 2000), although a deeper understanding would help to interpret different patterns of species distribution.

Due to the current state of knowledge on breeding sites of Culicoides species in Europe, members of the Obsoletus group are thought to avoid waterlogged habitats (González et al., 2013). It was supposed that the dung-breeding members of the Obsoletus group (Culicoides chiopterus and Culicoides dewulfi) are able to colonise the driest habitats, which are occupied by immature Culicoides (Kettle and Lawson, 1952). However, this was not deduced from experiments on the impact of flooding on immature stages, but from studies on their breeding habitats (Hill, 1947; Zimmer et al., 2008). Therefore, this study aimed to evaluate the impact of flooding on the development of immature C. chiopterus and C. dewulfi in cowpats.

2. Materials and methods

On 4 April 2013, we selected 10 cowpats on a farm close to the city of Oldenburg (Lower Saxony, Germany). This farm represents a typical dairy farm situated in rural regions in Northern Germany, with a total area of 195 ha and 100 ha grassland, which is used as pasture or meadow. The pasture from which we collected the cowpats is located at a distance of approximately 250 m from the cowshed and is surrounded by forest, a residential area and a small stream. The pasture had not been used for grazing during the winter, i.e., from October until we took the cowpat samples. Therefore, it is reasonable to assume that the cowpats were approximately 5–6 months old.

From the centre of each cowpat, an area of 14 cm × 14 cm was sampled together with approximately 3 cm of the adjacent soil and was transported to a greenhouse. Preliminary studies demonstrated that cowpats can differ strongly in the abundance of Culicoides. Therefore, the sampled cowpat areas were divided into four equally sized, quadratic samples (7 cm × 7 cm) and a different treatment was applied to each sample: (1) “dry”: no water was added to the samples, (2) “control”: each sample was moistened with a pressure spray device every three days (ca. 10 ml tap water per quarter), (3) “alternately flooded”: each sample was alternately flooded with tap water (for 24 h, water level ca. 11.5 cm) or not flooded (for 48 h), and (4) “permanently flooded”: each sample was permanently flooded with tap water (water level ca. 11.5 cm). Water loss due to evaporation was replaced daily with tap water.

Samples were placed under emergence traps and were covered with a collecting jar. The collecting jars were filled with saturated salt solution to catch and preserve the emerging insects (Fig. 1). The emergence traps of the “alternately flooded” and “permanently flooded” treatments were placed in plastic trays (60 cm × 40 cm × 40 cm, five emergence traps per tray, two trays per treatment). Four small holes (0.1 cm) were drilled into the base of the emergence traps and were covered with gauze (mesh size: 125 μm). These holes allowed the filling and draining of the emergence traps with tap water, but the gauze prevented the cowpat material and its coloniser to be washed out.

Collecting jars of the emergence traps were emptied daily. Sampling, refilling and draining were conducted at
Further 63 individuals (25.3%) as C. chiopterus. Eleven male individuals (4.4%) were identified as Culicoides scoticus. Three female individuals (1.2%) were identified as either Culicoides obsoletus or C. scoticus (Fig. 2), as the morphological differentiation of these two species is difficult. A further 11 male individuals (4.4%) were not determined to species level because their hypopygium were destroyed.

Flooding proved to have a significant impact on the development of Culicoides from the samples (Fig. 2). Most Culicoides emerged from samples in the “dry” (96 individuals, 38.6%) and “control” (151 individuals, 60.6%) treatments, whereas two individuals (0.8%) emerged from the “alternately flooded” treatment and none from the “permanently flooded” treatment. There were no significant differences between the numbers of Culicoides emerging from the “dry” and the “control” treatment (U-test, \( p > 0.05 \); Figs. 2 and 3). All of the three detected Culicoides species emerged from the “dry” and “control” treatment, but only C. chiopterus emerged from the “alternately flooded” treatment. There were no significant differences in C. chiopterus and C. dewulfi emergence between the “dry” and “control” treatment (U-tests, \( p > 0.05 \)) (Figs. 2 and 3). Due to the low number of C. scoticus, we did not apply a statistical test to compare “dry” and “control” treatments for this species.

The daily mean temperatures in the emergence traps fluctuated between 15 and 20 °C (mean = 18.5 °C, SD = ±2.2 °C), but were similar between the four treatments and were close to the room temperature. Slightly higher daily mean temperatures occurred in the “permanently flooded” treatment (Fig. 4). Despite high fluctuations in oxygen saturation in the “permanently flooded” treatments, the oxygen saturation differed between the traps, where we placed the emergence traps to achieve flooding. Oxygen saturation in one of the traps dropped significantly from about 100% to approximately 50% during the first few days, but increased during subsequent days and often exceeded 100% (Fig. 5). In the other traps, oxygen saturation decreased much more strongly and did not exceed 80% maximum oxygen saturation until the last day of biding emergence (Fig. 5).

4. Discussion

One main outcome of the present study was that C. chiopterus and C. dewulfi were not able to survive flooding of their breeding substrate. Even alternate flooding of these substrates every 24 h almost completely prevented the emergence of Culicoides biting midges. From our experiment, we cannot conclude whether the strong reduction in emergence is caused by a high mortality of larvae, pupae or both. However, the inability of pupae to float is considered to be the most important reason for the breeding-site selection by Culicoides species of the subgenus Avaritia, habitats of which are described as moist but not waterlogged (Nevill et al., 2007). This agrees with a literature review according to which the members of the Obsoletus group (C. chiopterus, C. dewulfi, C. obsoletus and C. scoticus) in the subgenus Avaritia (Borkent, 2013) do not occur in waterlogged habitats (González et al., 2013).

The oxygen saturation in the “permanently flooded” treatment strongly decreased at the beginning of the

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**Fig. 1.** Schematic drawing of an emergence trap [area = 256 cm²]. Emerging insects fly towards the light and are trapped in a saturated salt solution. Each emergence trap has two aeration windows covered with gauze (mesh size = 125 μm) to ensure gas exchange and prevent warming within the trap. Indications of size are given in cm. At the bottom of the emergence traps, four small holes (Ø 1 cm) were drilled to facilitate filling and draining and covered with gauze (mesh size = 125 μm) to prevent the cowpat material and its coloniser to be washed out.

the same time (ca. 14–16 h) for all treatments. Oxygen and water temperature in the water were measured daily in the “permanently flooded” treatment and after refilling and before draining in the “alternately flooded” treatment (WTW Oxi 330, Sensor CellOx 325, Germany). One emergence trap in each treatment was randomly selected to record temperature data (logger: HOBO Pendant® Temperature/Alarm Data Logger 8K, ONSET, Bourne, MA, USA). Another data logger recorded the room temperature. The temperature was measured every 4 h. After 31 days from when the last insect emerged from the cowpats (28 May 2013), the experiment was stopped. The samples were sorted and biting midges were determined to the group level (Obsoletus group, Pulicaris group or other Ceratopogonidae). Males and females of the Obsoletus group were determined to the species level based on morphological characters (Campbell and Pelham-Clinton, 1960). Data analysis was conducted with R (R Development Core Team, 2011), using the package ggplot2 (Wickham, 2009) for graphs.

3. Results

We collected a total of 249 Culicoides biting midges belonging exclusively to the Obsoletus group. All except for one cowpat (90%) were colonised by biting midges. The emergence per cowpat showed a high variability (mean = 24.9, max = 123, min = 0). The majority of the 161 individuals (64.7%) were determined as C. dewulfi and a
experiment, which might explain the failure of development. However, it can be expected that dung-breeding Culicoides biting midges are able to tolerate hypoxic or hypercapnic conditions, because the oxygen content within cowpats can also be low (1–2%) and that of carbon dioxide can be high (25–30%) (Holter, 1991). One counter-argument is the vertical distribution of immature stages of Culicoides. Eggs are laid on top of the cowpat (Bishop et al., 1996) and larvae/pupae prefer the top layer of breeding substrates (Blackwell and King, 1997; Kettle, 1977; Mullens and Rodriguez, 1992; Zimmer et al., 2008), where the oxygen concentration is higher (Holter, 1991). Additionally, while the cowpats were ageing, the oxygen concentration quickly increased, whereas that of carbon dioxide decreased (Holter, 1991). Thus, dung-breeding Culicoides do not have to deal with very low levels of oxygen or very high levels of carbon dioxide during immature development.

Culicoides brevitarsis showed a vertical movement of larvae and pupae towards areas with higher moisture in cattle dung over time (Bishop et al., 1996). Furthermore, experimental studies identified a negative correlation between the water content of dung and the abundance of C. brevitarsis, but these results were not supported by field studies (Bishop et al., 2005; Campbell, 1974). The prevalence of this species was not limited by its moisture content (Campbell, 1974), which implies that C. brevitarsis is highly adapted to dry habitats. We also did not find any statistically significant differences between cowpats in the “dry” and the “control” treatment (regularly moistened). Therefore, besides the ability of short-range migration towards zones in the cowpat with higher moisture, we assume that coprophilic Culicoides possess physiological adaptations against desiccation (e.g., thickness of the cuticle), which otherwise might be disadvantageous (e.g., inhibiting oxygen uptake) under flooded conditions.

Dung-breeding Culicoides (e.g., C. brevitarsis) appear to have specific physiological and behavioural adaptations for breeding habitats that are not waterlogged: the pupae are not able to float and the larvae do not show a serpentine swimming characteristic (Cannon and Reye, 1966). Similarly, the eggs and larvae of C. imicola can survive flooding, but pupae drown (Nevill, 1967). In contrast, the pupae of aquatic species (e.g., Pulicaris group) are probably able to swim and do not drown when submerged and are therefore able to colonise waterlogged breeding sites (EFSA, 2007). Specific physiological or behavioural adaptations of immature stages might be the key factor in explaining the differences in the breeding site selection between

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**Fig. 2.** The number of individuals of Culicoides species emerging from samples in the four treatments. A random jitter was added to visualise the raw data.

**Fig. 3.** The number of individuals of Culicoides chiopterus and Culicoides dewulfi emerging from samples in the “dry” and “control” treatments. The lines connect samples from the same cowpat.
different *Culicoides* species (Nevill et al., 2007), e.g., the females might use volatile chemical cues to identify suitable breeding sites. Floating *Culicoides* pupae use gaseous inclusions within the cephalothorax for buoyancy (Dyce and Murray, 1966). Among the East Australasian *Culicoides* species studied by Dyce and Murray (1966), three types of pupal physiological and behavioural adaptations to different breeding sites were distinguished: pupae of Type A are able to float after flooding, but are not able to submerge again and breed on the margins of still and slow flowing waters, pupae of tree-hole breeding species of Type B can variably float or submerge, and pupae of Type C remain submerged and burrow in the substrate as adaptation for breeding sites in estuarine sands, which are regularly disturbed by flooding or desiccation depending on the tide. Additionally, a Type D was described for *C. imicola*, breeding in moist but not waterlogged soils (Foxi and Delrio, 2010), whose pupae cannot float or burrow and lie on the substratum and drown if flooded (Nevill, 1967). According to the results of this study, *C. chiopterus* and *C. dewulfi* also probably belong to Type D.

In lowland areas of Northern Europe, agricultural intensification is strongly connected to large-scale drainage via ditches (e.g., 300,000 km in the Netherlands) (Verdonschot et al., 2011). These ditches serve to drain rainwater or seepage from groundwater into rivers and lakes, thus resulting in a lower risk of flooding of farmland. Due to the sensitivity of *C. chiopterus* and *C. dewulfi* to flooding, recent agricultural practices might facilitate the populations of both species. Coprophilic *Culicoides* species are suspected to be common species and should be present if cow dung is available (Cannan and Reyne, 1966), thus, the species distribution is probably independent from land cover or edaphic conditions. In contrast, both species do not necessarily show an equal distribution (Nielsen et al., 2010) and edaphic variables were found to significantly affect the abundance of both species (Scolamacchia et al., 2013). Our study revealed a clear negative impact of flooding for *C. chiopterus* and *C. dewulfi*. As for *C. imicola*, it is therefore reasonable to expect that both species regularly avoid flooded breeding sites (Foxi and Delrio, 2010). Therefore, soil moisture probably is an important factor for species of the Obsoletus group and should be included in species distribution models, as it

**Fig. 4.** Daily mean temperature, with daily maximum and minimum values measured with data loggers in the emergence traps of the four treatments, and room temperature. Data from the beginning of the experiment until the day of the last emergence of *Culicoides* biting midges are shown.

**Fig. 5.** Oxygen saturation in the water. Daily measurements in both plastic trays of the “permanently flooded” treatment and both plastic trays of the “alternately flooded” treatment, where oxygen saturation was measured immediately after flooding and immediately before draining (24 h period). Data from the beginning of the experiment until the day of the last emergence of *Culicoides* biting midges are shown.
was successfully demonstrated for *C. imicola* (Peters et al., 2013).

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References


