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Short Communication

Impact of freezing on the emergence of *Culicoides chiopterus* and *Culicoides dewulfi* from bovine dung

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ABSTRACT

The emergence of *Culicoides chiopterus* (Meigen), 1830 and *C. dewulfi* Goetghebuer, 1936 (Diptera: Ceratopogonidae) from cowpats in northwestern Germany was investigated. In order to investigate the survival of both species at low temperatures, cowpat subsamples were frozen for 48 h at −18 and −21 °C. Emergence from frozen and non-frozen samples was compared. The number of emerging adults of *C. chiopterus* from samples frozen at −18 °C was greatly reduced and no emergence was observed from samples frozen at −21 °C. No adult *C. dewulfi* emerged from frozen samples, suggesting this species is less resistant to these temperatures, compared to *C. chiopterus.*

1. Introduction

Several species of the genus *Culicoides* (Diptera: Ceratopogonidae) are known to transmit pathogens that infect cattle and other livestock (Mellor et al., 2000). Members of the Obsoletus group belong to the main vectors for the bluetongue virus (BTV) and Schmallenberg virus (SBV) in central and northern Europe. For BTV, *Culicoides obsoletus* sensu stricto has been identified as a main vector, but also *C. chiopterus* and *C. dewulfi* are among the potential vectors for this virus (Carpenter et al., 2009 (review); Hoffmann et al., 2009). Furthermore, there is evidence that they are potentially involved in the transmission of SBV (De Regge et al., 2012; Elbers et al., 2013).

While the veterinary importance of the genus is beyond question, knowledge of the ecology of *Culicoides*, e.g., on overwintering mechanisms, is still fragmentary. Many species in temperate regions overwinter in the larval stage (Becker, 1960; Jones, 1967; Szadziewski et al., 1997). Concluding from the emergence of *C. obsoletus* in the spring, dung heaps are assumed to be habitats for overwintering individuals of this species in the United Kingdom (Harrup et al., 2013). Larvae of *C. chiopterus* and *C. dewulfi* are known to develop in cowpats (Kettle and Lawson, 1952), and can be found overwintering in this habitat on cattle pastures (Steinke et al., 2014).

The present study focused on the survival of *C. chiopterus* and *C. dewulfi* at very low temperatures to investigate the potential impact of severe winters on the population. For this purpose, overwintering larvae within cowpats were frozen in the laboratory to investigate subsequent emergence. A preliminary study demonstrated that larvae of *C. chiopterus* can survive freezing at −15 °C (data not shown). Thus, we investigated emergence after freezing at −18 °C and −21 °C to determine the temperature level precluding survival.

2. Methods

Sampling was performed on a pasture (GPS coordinates: N53 9.985 E8 8.771) belonging to an organic cattle farm in northwestern Germany in January 2014. Pasturing

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was stopped in the preceding autumn. Thus, cowpats used for the experiments had a minimum age of 3–4 months. Several days prior to the main study, small subsamples (approximately 10 g each) were collected from the edges of 15 cowpats. *Culicoides* larvae were extracted from the samples with an adapted Berlese method (Steinke et al., 2014) to ensure that only colonised cowpats were used in the experiment. In 12 cowpats, *Culicoides* larvae were found and 10 of these "positive" cowpats were randomly selected for further use. These cowpats were removed whole along with the soil layer (down to 3–4 cm) underneath and transported to the laboratory. Each pat was divided into three triangular pieces of approximately equal size. One subsample of each cowpat was frozen for 48 h at −18 °C and another one at −21 °C in two separate deep freezers. The third subsample served as the control and was stored outdoors. During freezing, the temperature within the cowpats was monitored with miniature data loggers (iButton DS1921G, Maxim Integrated) that were carefully inserted into the centre of the subsamples. Additionally, the temperature in the deep freezers was monitored (HOBO Pendant® Temperature/Alarm Data Logger 8 K, ONSET). The time between sampling and the initiation of freezing was approximately 1 h. After 48 h, all samples were placed in emergence traps in a greenhouse under natural daylight. Rearing temperature (Hobo Pro v2, ONSET [in combination with solar radiation shield RS3]/Resolution: 0.02 °C at 25 °C, Bourne, MA, USA) was 16.0 ± 2.9 °C with a relative air humidity of 68.1 ± 12.6%. The traps were composed of plastic buckets with two lateral aeration windows and a transparent top collection container. Emerging insects fly into the top container and are preserved in salt solution. Trapped insects were removed every second or third day and cowpat samples were moistened with tap water. *Culicoides* biting midges were sorted out after emergence and identified to the species level following the key of Campbell and Pelham-Clinton (1960).

The temperature within cowpats was monitored in the same cattle pasture for 2 months prior to the experiment (20/11/13–23/02/14) in order to determine the temperature range overwintering larvae were exposed to naturally in advance of the freezing treatment. Three cowpats of similar size and height were chosen (diameter: 30 ± 4 cm, height: 4 ± 1 cm). The temperature was again measured with miniature data loggers that were inserted in the top half and the bottom half of each pat. Air temperature was measured at the edge of the pasture (10–20 m from the cowpats) at a height of 1 m (Hobo Pro v2, ONSET [in combination with solar radiation shield RS3]/Resolution: 0.02 °C at 25 °C, Bourne, MA, USA). The temperature of the air and cowpats was measured hourly. All statistical analyses were carried out with the program R (R Development Core Team, 2014). A confidence level of 5% was used to define statistically significant differences. Numbers of emerged individuals from different treatments were compared with the Wilcoxon signed-rank test.

3. Results

Minimal temperatures within cowpat subsamples (−18.2 ± 0.9 °C [mean ± SD]) frozen at −18 °C were reached towards the end of the 48 h period (46.3 ± 1.9 h). Subsamples frozen at −21 °C reached a minimal temperature of −23.4 ± 2.4 °C after 42.7 ± 4.8 h. From samples frozen at −21 °C, no *Culicoides* emerged, whereas a total of 33 *C. chiopterus* (20 females/12 males) emerged from 3 of the 10 subsamples frozen at −18 °C (Table 1). A total of 1361 *Culicoides* midges eclosed from the control samples with a high variation of individual numbers among the cowpats (62.1 ± 55.5 per subsample). Of these individuals, 621 (45.6%) were identified as *C. chiopterus* (331 females/290 males) and 711 (52.2%) individuals as *C. dewulfi* (148 females/563 males). In comparison with the control samples, the number of *C. chiopterus* individuals that emerged from subsamples frozen at −18 °C was significantly lower (Wilcoxon signed-rank test, $n = 10$, $p < 0.001$).

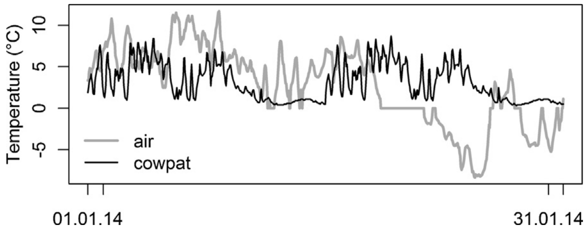

Fig. 1. Hourly measured air temperature (°C) (grey thick line) on a pasture in northwestern Germany (measured at a height of 1 m) in comparison to the average temperature measured within cowpats on the pasture (black thin line) from 1st to 31st January 2014 (temperature was measured within three cowpats, in the top half and the bottom half of each sample).

According to the temperature monitoring on the pasture, it is unlikely that the overwintering larvae were confronted with freezing in advance of the main experiment. When the air temperature fell below 0 °C, cowpat temperatures did not follow but remained close to the freezing point (Fig. 1). The average temperature measured within cowpats in the period from 20/11/13 to 23/02/14 was 3.5 ± 2.3 °C.

4. Discussion

Larvae of *C. variipennis*, the primary vector of the bluetongue virus in North America (reviewed by Tabachnick, 1996), are able to survive freezing at a temperature of −2.2 °C over a period of 6 weeks (62% of exposed larvae; Rowley, 1967). Icebound larvae of the same species collected from frozen pond mud were alive and active after thawing (Vaughan and Turner, 1987). The present study demonstrates the competence of larvae of another veterinary important species, *C. chiopterus*, to survive temperatures far below the freezing point. The lowest temperature in the subsamples from which emergence was observed was −18 °C, while no emergence could be observed after freezing at −21 °C. The lower threshold for survival of *C. dewulfi* was assumed to be at a higher temperature, as this species was abundant in the control samples, but did not emerge from any samples after freezing, indicating a lower physical resistance towards the low temperatures tested in the present study. *Culicoides* are generally able to survive in severe climates.