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Short communication

Effects of temperature and photoperiod on the development of overwintering immature *Culicoides chiopterus* and *C. dewulfi*

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ABSTRACT

In areas with harsh winters, several studies have recorded low numbers – or even zero – adult *Culicoides* during the winter period followed by a population peak in the subsequent spring. This experimental study determined whether temperature, photoperiod, or the combination thereof affect the development of overwintering immature dung breeding *Culicoides*, resulting in this peak. Temperature had a significant impact on the development period of *Culicoides chiopterus* (Meigen), 1830 and *C. dewulfi* Goetghebuer, 1936. In treatments with constant high temperature, emergence occurred shortly after the beginning of the experiment (mean = day 9). In contrast, no individuals emerged from the other two treatments, as long as the temperatures were below 10 °C. In these treatments, the emergence of *Culicoides* started when the temperature exceeded 20 °C for some days (mean = day 33). There was no significant difference between the two photoperiods (February or April day length; 9 h:15 h [light:dark] vs. 13 h:11 h). Our results highlight the importance of temperature on the spring emergence of *C. chiopterus* and *C. dewulfi*, but the response to the four temperature-photoperiod treatments did not differ between the two species.

1. Introduction

Several *Culicoides* species all over the world are known to be vectors of a variety of pathogens (e.g., bluetongue virus (Mellor et al., 2000), African horse sickness virus (Mellor et al., 2000) and Schmallenberg virus (De Regge et al., 2012; Rasmussen et al., 2012). At least in the temperate climate zone, a recurrent phenological pattern can be observed, with low numbers or a total absence of *Culicoides* adults during the winter followed by a peak of emergence in the spring (e.g., Hoffmann et al., 2009). However, it is unclear what factor or combination of factors (e.g., temperature and photoperiod) regulates the development of the overwintering stages, resulting in this first population peak of the year. Understanding the factors that regulate the development of overwintering, immature *Culicoides* could help to predict the vector phenology and the associated virus epidemiology more precisely, e.g., by adding the factors of temperature and photoperiod into models of vector phenology (Searle et al., 2012).

It has been known for a long time that photoperiod, temperature or the combination of both affects the induction, maintenance and termination of the diapause in insects (e.g., Tauber and Tauber, 1976). This also applies to different *Culicoides* species, whose diapause is controlled by temperature (reviewed by Alekseev et al., 2007). Whereas, to our knowledge, only Isaev (1985) found a combined impact of warm temperature and long photoperiod on the pupation rate of *Culicoides odibilis* Austen, 1921, for other families of the order Diptera (e.g., Chironomidae or Culicidae), there are several examples of species where the photoperiod, alone or in combination with temperature, was found to induce or terminate diapause (reviewed by Alekseev et al., 2007).

Cowpats in northern Germany are predominantly colonized by two *Culicoides* species: *Culicoides chiopterus* (Meigen), 1930 and *C. dewulfi* Goetghebuer, 1936 (Lühken et al., 2015), which are expected to breed exclusively in dung (Kettle and Lawson, 1952), while the differences between the ecological niches are unknown (e.g., Lühken et al., 2015). Furthermore, both species are considered to be potential vectors of important veterinary pathogens (e.g., De Regge et al., 2012). This study aims to compare the emergence patterns of *Culicoides* from cowpats that were experimentally exposed to different temperature-photoperiod treatments. According studies reviewed by Alekseev et al. (2007), which predominantly detected an impact of temperature, it was assumed that high

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Table 1
Temperature-photoperiod treatments exposed to the samples from each cowpat.

Treatment	Temperature	Photoperiod
A	Constant high temperature (20–25 °C)	Long day photoperiod, April day, 13 h:11 h [light:dark]
B	Constant high temperature (20–25 °C)	Short day photoperiod, February day, 9 h:15 h [light:dark]
C	Constant low temperature (<10 °C) at the beginning of the experiment and variable higher temperatures (>15 °C) at the end of the experiment	Long day photoperiod, April day, 13 h:11 h [light:dark]
D	Constant low temperature (<10 °C) at the beginning of the experiment and variable higher temperatures (>15 °C) at the end of the experiment	Short day photoperiod, February day, 9 h:15 h [light:dark]

temperatures, but not photoperiod, triggered the emergence of *Culicoides* from the collected cowpats.

2. Materials and methods

On 3 February 2014, 15 cowpats were selected on a farm close to the city of Oldenburg (Lower Saxony, Germany; latitude 53.1, longitude 8.1). This farm represents a typical dairy farm situated in rural regions in northern Germany, with 195 ha of total land area and 100 ha of grassland used as pasture or meadow. The pasture where the cowpats were collected is located approximately 250 m from the cowshed, surrounded by a small forest, a residential area, and a small stream. As cattle had not grazed on this pasture during the winter, i.e., from October until February, and based on our previous experiences with *Culicoides* breeding sites, it was assumed that the sampled cowpats were between four and six months old. We carefully took into consideration that the cowpats bore the same visual appearance (e.g., same height of grass around the cowpats).

Initially, a small substrate sample (5 cm × 5 cm) was collected from the margins of each of the 15 cowpats, while the rest remained in the field. These rim-samples were analyzed using the Berlese method (Steinke et al., 2014) to evaluate whether or not the cowpats were colonized by *Culicoides* larvae. Four days later (7 February 2014), an area of 14 cm × 14 cm was sampled from the center of each of the nine cowpats that had been found to contain *Culicoides* larvae. These core-samples were taken, along with approximately 3 cm of the soil underneath for moisture-regulation, and then transported to the laboratory. Preliminary studies demonstrated that cowpats could differ strongly in the density of immature *Culicoides*. Therefore, the sampled cowpat areas were divided into four equally sized, quadratic samples (7 cm × 7 cm), which were randomly exposed to a different temperature-photoperiod treatment (Table 1). The two "constant high temperature" treatments, A and B were kept at room temperature between 20 °C and 25 °C, while the two treatments, C and D were kept in a climate chamber. The climate chamber was switched off after 27 days and the temperature in the climate chamber was then influenced by the outside temperature, resulting in "variable higher temperatures (>15 °C) at the end of the experiment". The temperatures studied in this experiment are in the range of soil temperatures that can be measured in the field during February and May.

Samples were placed underneath emergence traps and covered with collecting jars (Steinke et al., 2014). Collecting jars were filled with a saturated salt solution to collect and preserve the emerging insects. The emergence traps allocated to one treatment were placed in one separate lightproof wooden box (length 118.5 cm × width 51.5 cm × depth 50 cm). Each of the wooden boxes was closed with a wooden cover, and a fluorescent lamp (Osram®, Biolux T8 36W, length 121 cm) was included inside the box. Boxes were opened only to empty the collecting jars and moisturize the samples.

The collecting jars of the emergence traps were emptied three days a week (Monday, Wednesday and Friday), resulting in sampling intervals of two and three days. Once a week, the samples were moistened with dechlorinated tap water at room temperature (approximately 20 ml per sample) to prevent drought-induced pupation or emergence. Sampling and moistening was conducted at the same time around noon for all treatments. A data logger (Hobo U23 Pro v2 Data Logger, Bourne, MA, USA) recorded the air temperature in each wooden box at 4 h intervals. Twelve days after the last *Culicoides* emergence was recorded (9 April 2014), the experiment was terminated. Samples were sorted and *Culicoides* were identified to the group level (Obsoletus group, Pulicaris group, other Ceratopogonidae). Males and females of the Obsoletus group were determined to the species level based on morphological characters (Campbell and Pelham-Clinton, 1960; Glukhova, 1989; Delécolle, 1985).

Data analysis was conducted using R (R Development Core Team, 2010). A recently proposed method was applied to analyze the phenology of insects, which was used to identify the timing of the peak of emergence (Searle et al., 2012). The mean number of emerged individuals was calculated for each day of the sampling interval and a three-day moving average of emergence was calculated for each sample and for each species and sex. A separate generalized additive model with a poisson distribution, log link and spline smoothing was fit for each sample using the R package, mgcv (Wood, 2011). Therefore, emergence data were rounded to integer values in order to provide whole the number necessary for the poisson models. The degree of smoothing was selected automatically using generalized cross-validation. The findPeaks function from the quantmod package (Ryan, 2013) was used to identify emergence peaks in the predicted values. Mean and 95% confidence intervals were calculated for the first emergence, the last emergence, the peak of emergence and the total number of emerging individuals for each sex of each species using the summarySEwithin function from the R misc package (Hope, 2013), with the cowpats as subjects and the treatment as a within-subjects variable. Non-overlapping 95% confidence intervals were considered a sign of significant difference. The package, ggplot2 (Wickham, 2009) for graphs and gridExtra (Auguie, 2012) was used for multi-panel graphs.

3. Results

A total of 765 *Culicoides* emerged from cowpats that were exposed to the four temperature-photoperiod treatments. All adults belonged to the Obsoletus group. The majority of the 564 individuals (73.7%) were determined to be *C. chiopterus* and a further 201 individuals (26.3%) were *C. dewulfi*.

Temperature proved to have a significant impact on the development period of both species. In the constant warm environment at temperatures around 22 °C (treatments A and B), the first specimens of *C. chiopterus* and *C. dewulfi* emerged between day 5 and day 18 (mean = day 9) of the experiment, while the last individuals emerged between day 11 and day 31 (mean = day 17) (Fig. 1). The peak of emergence occurred between day 9 and day 20 (mean = day 13) (Fig. 2).

In contrast, no *Culicoides* emerged from the samples in treatments C and D, as long as the temperatures were below 9 °C. Emergence started in these treatments between day 30 and day 39 (mean = day 33), when temperatures rose above 24 °C around day 30, and emergence finally ceased between day 37 and day 60 (mean = day 45) (Fig. 1). The first peak of emergence occurred