

On the influence of environmental factors on the oviposition activity of necrophagous flies

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Abstract

1. Locating an optimal oviposition site can be a challenging task for female insects, especially when dealing with a patchy, unpredictable and ephemeral food source such as carrion. Understanding the biotic and abiotic parameters that influence the oviposition behaviour of necrophagous flies is not just of great biological importance but also essential for their application in legal investigations.

2. In this study, we monitored the oviposition activity of necrophagous flies (Calliphoridae, Sarcophagidae) using mouse carcasses in an urban (city) and a rural (mixed forest) habitat in Frankfurt/Germany over a 2-year period.

3. Over 240 sampling days, 220,963 larvae of 4 blow fly species and 1 flesh fly were sampled. The most abundant species was the blow fly *Lucilia ampullacea*, followed by its family members *Calliphora vicina* and *Lucilia caesar*, the flesh fly *Sarcophaga caerulescens* and *Lucilia sericata*. Up to seven environmental parameters were statistically significant predictors for a colonisation of the carcasses, leading to unique patterns of seasonal and daily oviposition activity for all five species.

4. Overall, the analysis showed that the seasonal adaption (the phenology of each species), the habitat (rural vs. urban) as well as temperature are the most important factors influencing the oviposition behaviour and activity of necrophagous blow flies and flesh flies.

KEYWORDS

Calliphoridae, Central Europe, habitat, modelling, Sarcophagidae, seasonal adaption

INTRODUCTION

Oviposition substrate selection is a challenging but important task for female insects with far-reaching consequences for the life-history of their offspring (Janz, 2008). Decisions are always a complex trade-off between various, sometimes conflicting factors such as the suitability of the food source, predation risks to the offspring, microclimate, clutch size or intra- and inter-specific competition (Archer & Elgar, 2003; Baleba et al., 2020; Erzinçioğlu, 1996; Gião & Godoy, 2007; Janz, 2008; Reznik et al., 1992; Shah & Sakhawat, 2004; Wells & Greenberg, 1994). Especially for an unpredictable, patchy (Carter

et al., 2007) and ephemeral (Barton et al., 2013) food source like carrion, female necrophagous insects are strongly selected to detect nutritious diets (Archer & Elgar, 2003) and have to invest a large proportion of their energy in reproduction (Denno & Cothran, 1975).

Knowledge of the biotic and abiotic parameters influencing the oviposition behaviour of necrophagous insects can not only contribute to the understanding of key issues in ecology, such as dispersal, as a central process in metapopulation dynamics (Driscoll, 2007; Renault, 2020) or pest control (Aak et al., 2011; Esser, 1990) but is also essential for the application of these flies in legal investigations.

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The time of insect colonisation of a human cadaver is essential for estimating the minimum time since death or post mortem interval (PMI_{min}) (Amendt et al., 2004). Catts (1992) discussed the fact that even under optimal environmental conditions, there is not always a ready population of flies waiting to start oviposition. This assumption is supported by studies on the Australian Facility for Taphonomic Experimental Research, in which a delay of up to 3 days in colonisation on human cadavers even under optimal conditions was observed (Dawson et al., 2020; Skopyk et al., 2021). The factors responsible for delays in oviposition have hardly been investigated in detail so far but are of great importance, as testing the validity of a key assumption, like the timely colonisation of a body, would lead to a better case specific estimation of the PMI (Tarone & Sanford, 2017).

Some studies were performed to analyse relevant biotic factors like intra- and inter-specific competition (Denno & Cothran, 1976; Kotzé & Tomberlin, 2020; Prinkkila & Hanski, 1995), the previous presence of predator and prey larvae on the carrion (Brundage, 2012; Gião & Godoy, 2007), the presence of pheromones (Barton Browne et al., 1969; Lima & Von Zuben, 2016; Norris, 1965) but also physical barriers or other concealments (Bhadra et al., 2014; Charabidzé et al., 2015; Lutz, Moreau, et al., 2019; Scholl & Moffatt, 2017; Voss et al., 2008) and the habitat itself, for example, indoor or outdoor (Anderson, 2011; Cammack et al., 2016; Reibe & Madea, 2010).

Given that unfavourable parameters can completely prevent an oviposition, abiotic variables such as temperature, precipitation, relative humidity or season seem to have an even greater influence on the colonisation times and oviposition behaviour of flies (George et al., 2013). The most important factor is temperature (Campobasso et al., 2001; George et al., 2013; Hans et al., 2018; Mahon et al., 2004; Ody et al., 2017), and values below 12°C and above 30°C are generally accepted as a threshold for oviposition for most blow fly species (Gennard, 2007). However, due to species-specific temperature adaptations certain species are still able to oviposit at temperature as low as 4°C (Deonier, 1940; Faucherre et al., 1999). Rain can delay colonisation of up to 3 days (Mahat et al., 2009), but no study found a significant effect (George et al., 2013). Smith (1986) declared relative humidity to be a major factor controlling oviposition of necrophagous flies, nevertheless only one study estimated it as a negative predictor for colonisation probability (George et al., 2013). Variation in barometric pressure may herald changes in weather conditions that flies can detect with external baroreceptors on their antennae (Wellington, 1946), but whether such changes impact blow fly oviposition is still unclear. Wind provides important orientation information for flies, even over long distances (Spivak et al., 1991). For an oviposition, wind speeds below 10 km/h seem to be optimal for blow flies (Baldrige et al., 2006; George et al., 2013), whereas the absence of wind seems to have an inhibiting effect (George et al., 2013). Seasonality has not only a big impact on the abundance of flies (Davies, 1999; Lopes de Carvalho & Linhares, 2001; Lutz, Verhoff, et al., 2019; Moretti et al., 2013; Weidner et al., 2015), but also on their oviposition activity (Davies, 1999; Janz, 2008; Parry et al., 2016), due to the high correlation of season with other abiotic factors. Finally, the interrelation between season, environmental parameters and oviposition activity

must be considered at the level of individual species and populations (Tomberlin, Mohr, et al., 2011), as each species has seasonal and local adaptations (Brundage et al., 2011) and differences between populations may occur.

Overall, a number of biotic and abiotic factors influences the oviposition of necrophagous flies, and understanding the nature and extent of these effects is highly relevant when evaluating the PMI_{min} in an entomological report (Tarone & Sanford, 2017). It is important especially in court to show that you know and understand your target species, the assumptions for PMI_{min} estimations and their limitations in order to explain inconsistencies and possible contradictions (Kotzé et al., 2021).

While few studies analysed the effect of abiotic variables on the oviposition activity of necrophagous flies under laboratory conditions (Hans et al., 2018; Ody et al., 2017; Yang & Shiao, 2012), field studies are still rare (George et al., 2013; Mahon et al., 2004) and not in the focus of decomposition studies (Tomberlin et al., 2012). Therefore, in the present study, the oviposition activity of five necrophagous fly species (Calliphoridae, Sarcophagidae) was monitored using mouse carcasses placed at 10 sites in both an urban and a rural habitat in Frankfurt/Germany. We modelled the seasonal oviposition activity and the influence of environmental parameters on the egg-laying probability at the level of single species based on 240 sampling days over a 2-year period. The overall goal was to identify and understand possible species-specific climatic and seasonal adaptations of these taxa in one of the largest cities in Germany.

MATERIALS AND METHODS

Study site

Sampling was conducted at five urban and five rural sites (Table 1) in the area of Frankfurt am Main Germany (50°6'39.32"N 8°40'55.656"E). The urban sites were situated within a radius of 0.59 km² and were at least 299 m apart, with the greatest distance being 1041 m. All sites were characterised by a high density of streets and houses and were similarly structured. The rural sites were located in a municipal forest 1.14 km away from the urban sites. It was a mixed deciduous and coniferous forest with oak (*Quercus* sp.), beech (*Fagus* sp.), pine (*Pinus silvestris*) as well as birch (*Betula* sp.) trees. Minimum distance between each site was 100 m.

Sampling parameters

Thawed mouse carcasses with a weight of 20–30 g were used as oviposition substrate. They were surplus mice (*Mus musculus musculus* Linnaeus, 1758) from the breeding of the Central Research Facility of the Department of Medicine of the University Hospital, Frankfurt. Most of them were unusable siblings of mouse lines of different background strains. On each sampling day, carcasses were placed on the ground at the different sampling sites between 6 and 7 AM and

TABLE 1 List of the 10 sampling sites

Location	Latitude (+50°)	Longitude (+8°)	Habitat type	Description
1	5°43.59"N	40°9.93"E	Urban	Small park behind an apartment building
2	5°38.09"N	39°57.26"E	Urban	Garden of the Institute of Legal Medicine
3	5°28.44"N	39°43.16"E	Urban	Grass strip next to a street
4	5°24.05"N	40°1.04"E	Urban	Grass strip next to a street
5	5°42.62"N	40°45.10"E	Urban	Grass strip under trees in an estate
6	4°55.22"N	39°50.31"E	Rural	Strip at a trail in a mixed deciduous and coniferous forest
7	4°54.56"N	39°50.08"E	Rural	Strip at a trail in a mixed deciduous and coniferous forest
8	4°53.89"N	39°49.96"E	Rural	Strip at a trail in a mixed deciduous and coniferous forest
9	4°53.26"N	39°49.86"E	Rural	Strip at a trail in a mixed deciduous and coniferous forest
10	4°52.76"N	39°50.08"E	Rural	Strip at a trail in a mixed deciduous and coniferous forest

collected at sunset (+1 h). They were secured with wire mesh and tent pegs to prevent scavenging and/or removing. After collection, the carcasses were transferred to closed but ventilated plastic buckets (1 L, Bürkele, Germany) and kept under room temperature $23 \pm 2^\circ\text{C}$ for further rearing of larvae. When the larvae reached the post-feeding stage, they were killed with almost boiling water and then stored in 96% ethanol until identification. All larvae were morphologically identified to species level using the keys of Szpila (Szpila, 2010; Szpila et al., 2015) and voucher specimens from the Institute of Legal Medicine Frankfurt am Main before being counted.

Oviposition monitoring took place over a 2-year period from 1 April 2019 to 25 March 2021. Ten days were randomly selected each month, resulting in a total of 240 sampling days over the entire study. With 10 sites, 10 sampling days per month and a 2-year period, this sampling design resulted in 2400 samples (mouse carcasses).

Environmental parameters

On each sampling day, mean day temperature ($^\circ\text{C}$), maximum and minimum day temperature ($^\circ\text{C}$), precipitation (amount in mm and duration in h), wind speed (m/s), barometric pressure (h Pa), relative humidity (%) and number of hours of sunshine for each day (h) were recorded. A 'day' lasted from 7 AM until sunset. The sunset times were obtained from the national weather service (Deutscher Wetter Dienst). On days when sunrise was after 7 AM, the actual time of sunrise was defined as the beginning of the day. Hourly values during daylight were used to calculate all environmental parameters for the analysis. The day of the year (DoY) was used as a temporal variable. All data were collected from 3 local weather stations as none of the stations measured all of the required parameters. They were approximately 2–8 km from the sampling localities.

Statistical analysis

One hundred and thirty-seven cadavers disappeared from the sampling sites during the study and were therefore excluded from the

analysis. In total, the data used for the analysis included 240 sampling days and 2263 samples. A canonical correlation analysis using CCorA function from the package VEGAN (Oksanen et al., 2018) was computed to examine the relationships between the abundance of necrophagous fly species and the environmental parameters as well as the habitat (urban vs. rural). A series of generalised additive models (GAMs) were used to identify how abiotic variables affecting the probability of an oviposition by the species in question. The dependent variable was the daily presence/absence of each species fitted using a binomial distribution. For the independent variables, all abiotic factors were used at the start for each model because they were identified as important predictors for the flight activity of necrophagous flies in a previous study at the same location (Lutz, Verhoff, et al., 2019). The habitat (urban, rural) was added as a fixed effect to the model, to identify species-specific habitat preferences. Correlation between the independent variables was tested using Pearson linear correlation and collinearity with the variance inflation factor, and variables with values lower than 3 were used for the models (Zuur et al., 2010). All models accounted for the random effect of sampling sites and the year. The models were performed for each species separately, starting with the before-mentioned set of abiotic variables and compared using the Akaike information criterion (AIC) to identify the best model. Final models were those with the lowest AIC value, selected with a threshold value of 2. Abiotic variables were removed when not improving the AIC value of the model, resulting in different sets of important abiotic parameters for each species. All analyses were conducted using RSTUDIO version 4.0.5 (RStudio Team, 2020).

RESULTS

During the 240 sampling days, a total of 220,963 larvae were sampled from the mouse carcasses and identified; 98.7% of these were necrophagous blow flies (Diptera: Calliphoridae) and 1.3% necrophagous flesh flies (Diptera: Sarcophagidae). The most abundant species was by far *Lucilia ampullacea* Villeneuve, 1922 ($n = 126,145$), followed by *Calliphora vicina*, Robineau-Desvoidy, 1830 ($n = 81,137$), *Lucilia*

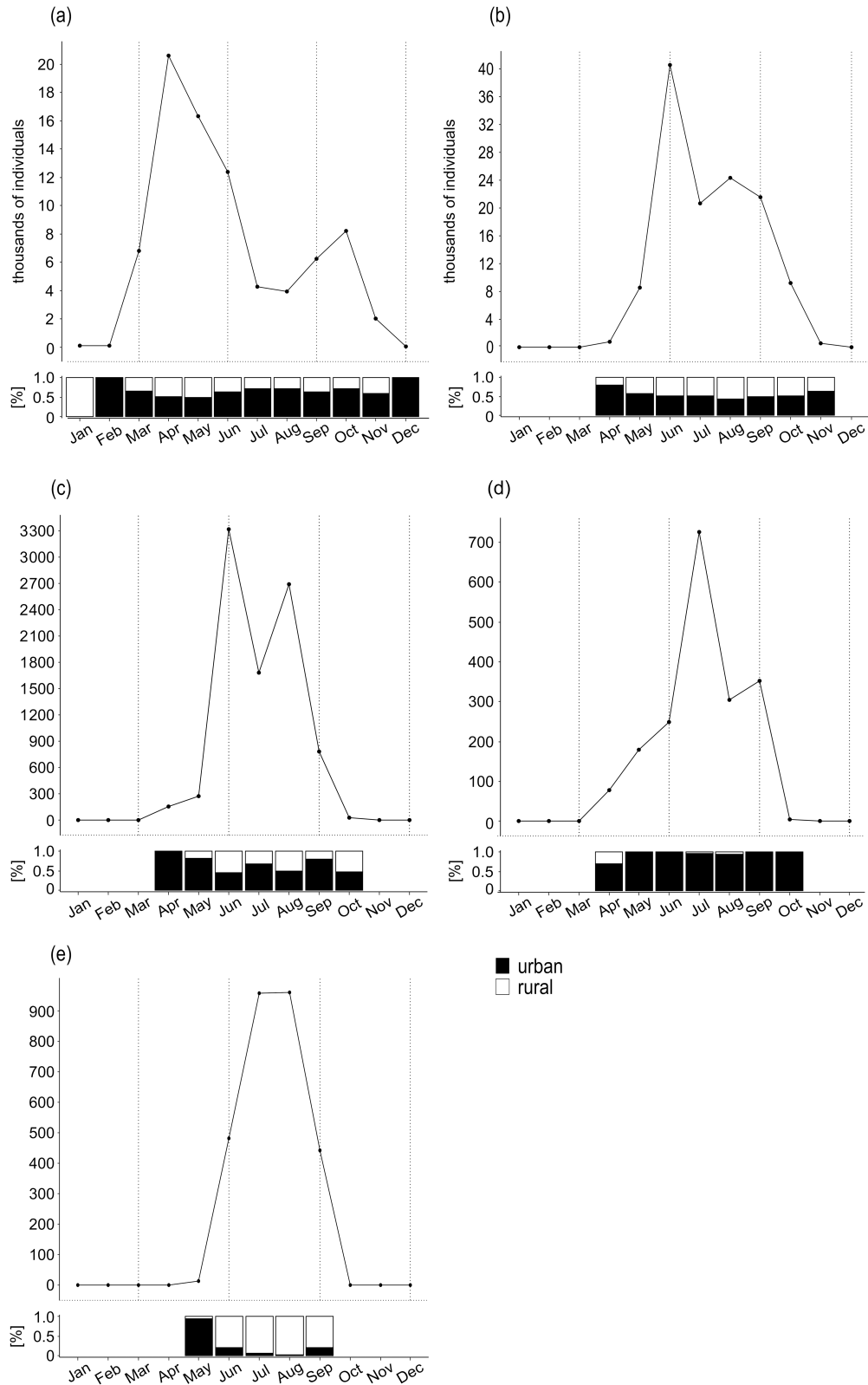


FIGURE 1 Seasonal oviposition activity on mouse carcasses of (a) *Calliphora vicina*, (b) *Lucilia ampullacea*, (c) *Lucilia caesar*, (d) *Lucilia sericata* and (e) *Sarcophaga caerulea*. The upper parts show the total number of specimens per month. Each month covers 20 sampling days 10 per year, the lower part shows the proportion of specimens (bars) found on the carcasses in an urban habitat (black) and in a rural (white) habitat. The dotted line splits the sampling period in spring, summer, autumn and winter

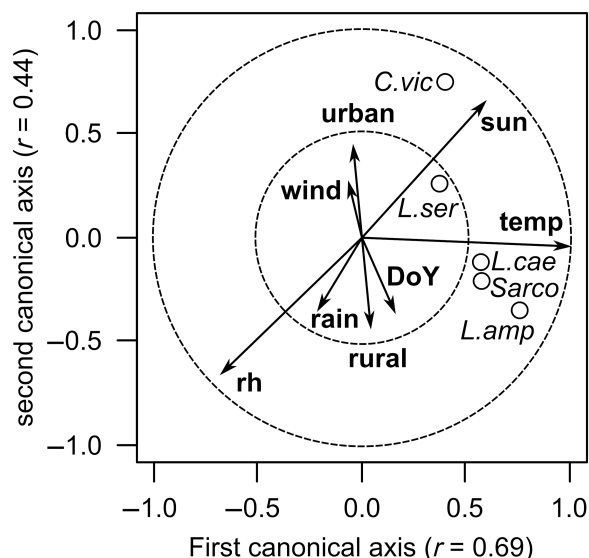


FIGURE 2 Canonical correlation analysis between environmental parameters (arrows) and abundance of juvenile stages on mouse carcasses (Pillai's trace = 0.77, $p < 0.001$, canonical correlations = 0.69 [axis 1] and 0.44 [axis 2], redundancy $r^2 = 0.48$)

caesar (Linnaeus, 1758) ($n = 8934$), *Sarcophaga caerulescens* Zetterstedt, 1838 ($n = 2852$) and *Lucilia sericata* (Meigen, 1826) ($n = 1895$).

Seasonal oviposition activity

C. vicina was the only species that oviposited on the carcasses throughout the year (Figure 1a) and was sampled on 65% ($n = 157$) of all sampling days. The earliest occurrence was on day 28 of the year, that is, 28 January and the latest on day 351, that is, 16 December. Oviposition activity was bimodal with peaks in spring (March, April) and autumn (September, October) and a steep decrease during the summer months.

L. ampullacea was sampled on 49.1% ($n = 118$) of all sampling days. The first oviposition was seen on day 111 of the year, that is, 21 April and the latest on day 310, that is, 6 November. Oviposition activity was characterised with a steep increase from spring to summer, a high peak in June and a slow decrease from summer to autumn from September onwards (Figure 1b). From December until April, no oviposition of this species took place.

L. caesar was less abundant than *L. ampullacea* but showed a similar oviposition activity (Figure 1c). It was sampled on 38.5% ($n = 93$) of all sampling days. The first occurrence was on day 105, that is, 14 April and the last occurrence on day 298, that is, 25 October. There was no oviposition from November to April, after which it was very active in the summer months (June, July, August) and decreased in activity again from September on.

L. sericata was the least abundant species and was sampled on 18.3% ($n = 44$) of all sampling days. Oviposition activity was almost restricted to spring and summer; first occurrence on day 108, that is,

TABLE 2 Explained variance by the generalised additive models (GAMs) for each species

Species	Explained variance (%)	Significant predictor variables
<i>Calliphora vicina</i> Robineau-Desvoidy, 1830	56.9	Seven
<i>Lucilia ampullacea</i> Villeneuve, 1922	64.5	Four
<i>Lucilia caesar</i> (Linnaeus, 1758)	45.9	Seven
<i>Lucilia sericata</i> (Meigen, 1826)	37.8	Five
<i>Sarcophaga caerulescens</i> Zetterstedt, 1838	44.2	Five

17 April, last occurrence 296, that is, 23 October. The species was absent from November until April (Figure 1d).

S. caerulescens was the only flesh fly species that colonised the mouse carcasses. It was sampled on 23% ($n = 56$) of all sampling days, all in the warmer months (May until September) with the peak from July to August. From October until May, the species was absent (Figure 1e).

Oviposition activity and influence of environmental parameters

In order to assess the relationship between the environmental parameters, the habitat features and the oviposition activity of the aforementioned fly species, a canonical correlation analysis was performed. On the first horizontal axis, the analysis shows that all species were positively influenced by mean daytime temperature, the hours of sunshine on a day and negatively influenced by relative humidity (Figure 2). On the second vertical axis, day of the year was shown to have a strong negative effect on *C. vicina* and a minor impact on *L. sericata* but a positive effect on *L. caesar*, *L. ampullacea* and *S. caerulescens*. Furthermore, wind speed had a positive effect on *L. sericata* and *C. vicina* and a negative effect on all other species. *C. vicina* and *L. sericata* seem to be associated with the urban sites, whereas *S. caerulescens* seems to be associated with the rural sites.

The GAMs explained 37.8% up to 64.5% of the variability in the species presence on the carcasses (Table 2). The number of significant predictors for an oviposition differed between the species from four (*L. ampullacea*, Tables 2, 3) up to seven abiotic variables for *L. caesar* and *C. vicina* (Tables 2, 3).

Effect of site and habitat

For each species, the probability for an oviposition differed significantly among sampling sites (Table 3, Figure 3). For *C. vicina*, *L. sericata* and *S. caerulescens*, there was a significant difference between the habitats,

TABLE 3 Approximate significance of smooth terms in the additive models of the oviposition activity of necrophagous flies

Figure	Environmental parameter	Chi-square value of parameters included in the models (<i>edf</i> value)				
		<i>Calliphora vicina</i>	<i>Lucilia ampullacea</i>	<i>Lucilia caesar</i>	<i>Lucilia sericata</i>	<i>Sarcophaga caerulescens</i>
Figure 5a–d	Day of the year	187.60 (10.26)***	185.28 (11.1)***	50.97 (6.0)***	-	41.27 (3.9)***
	Year	4.69 (1.0)*	37.27 (0.9)***	28.48 (1.0)***	3.42 (1.0)*	6.70 (0.87)*
Figure 4a–e	Mean day temperature	98.95 (4.6)***	74.37 (4.7)***	55.99 (4.24)***	32.02 (2.0)***	-
Figure 6a,b	Barometric pressure	-	-	18.20 (1.0)***	2.27 (1.0) ^{ns}	-
Figure 6c–e	Relative humidity	-	-	34.69 (1.0)***	3.94 (2.7) ^{ns}	18.56 (3.6)**
Figure 6f,g	Wind speed	-	-	8.41 (1.7)*	2.66 (1.5) ^{ns}	-
Figure 6h	Hours of sunshine	47.01 (7.4)***	-	-	-	-
Figure 6i	Precipitation × hours of rain	45.29 (10.5)***	-	-	-	-
Figure 6j	<i>L. caesar</i>	-	-	-	16.56 (3.09)**	-
Figure 3a–e	Sites	19.12 (5.7)***	28.18 (6.9)***	11.63 (4.9)*	17.26 (7.0)***	6.74 (3.7)*

Note: *p* value ***<0.0001; **<0.001; *<0.05. ns, not significant; -, not included in the model.

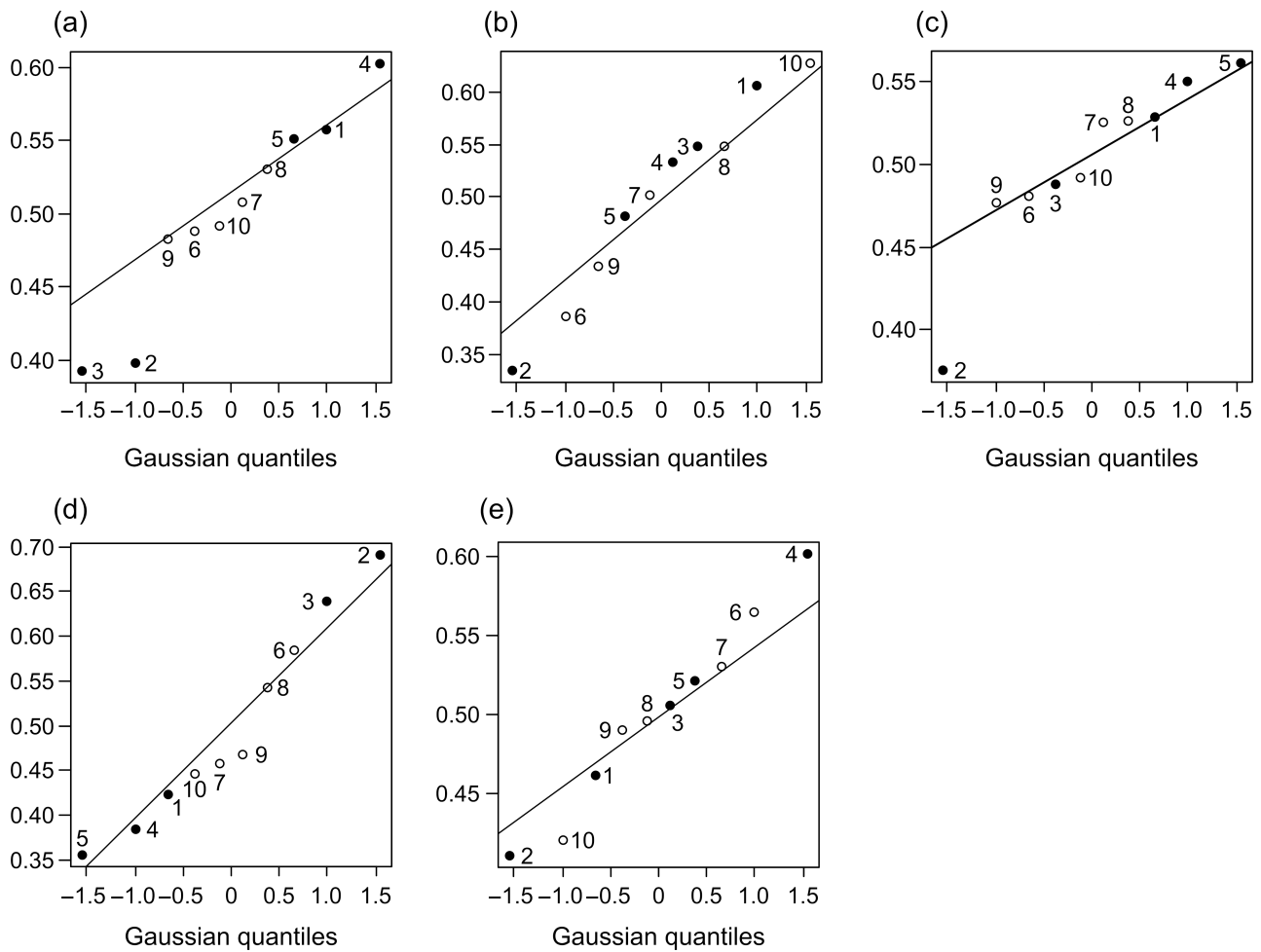


FIGURE 3 The effect of site on the probability of an oviposition by (a) *Calliphora vicina*, (b) *Lucilia ampullacea*, (c) *Lucilia caesar*, (d) *Lucilia sericata* and (e) *Sarcophaga caerulescens*. The figure presents the value of the smooth parameter function associated with the random effect of site. Detailed information on the sampling sites can be found in Table 1. The filled circles representing the sampling sites in an urban habitat and the open circles representing the sampling sites in a rural habitat

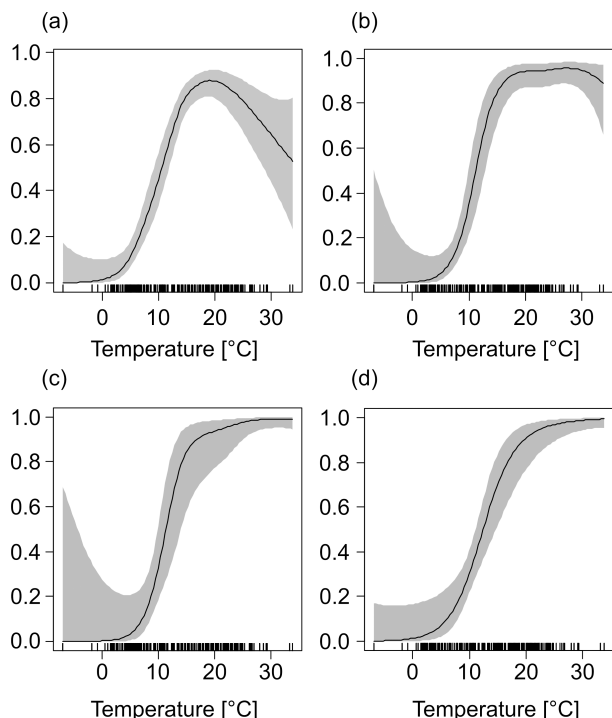


FIGURE 4 The effect of mean day temperature on the probability of an oviposition by (a) *Calliphora vicina*, (b) *Lucilia ampullacea*, (c) *Lucilia caesar* and (d) *Lucilia sericata*. The figure presents the model visualisation of the generalised additive model (GAM) predicting the probability of the presence of eggs associated with the independent variable mean day temperature. Shaded areas represent the 95% confidence intervals associated with the given smooth function

with a higher probability of an oviposition at the urban sites for *C. vicina* ($z = -3.63$, $p < 0.001$) and *L. sericata* ($z = -2.84$, $p < 0.001$) and a higher probability of an oviposition at the rural sites for *S. caerulescens* ($z = 5.37$, $p < 0.001$). No significant difference between the habitats was identified for *L. ampullacea* and *L. caesar*.

Effect of temperature

For all species, except *S. caerulescens*, temperature was one of the most important variables affecting the probability of an oviposition (Table 3, Figure 4) and had a strong positive effect. For *C. vicina*, the probability increased at low temperatures from 5°C upwards and reached the optimum at 20°C. Temperatures above 20°C had a negative effect (Figure 4a, Table 3). For *L. ampullacea*, temperature had a positive effect and the probability strongly increased at temperatures above 10°C. An oviposition was most likely at temperatures above 18°C and below 30°C. *L. caesar* had a similar trend and was active at temperatures above 10°C, but only reached the plateau at 25°C. For *L. sericata*, temperature had a strong positive effect and oviposition probability increased at temperatures from 12°C upwards and reached the plateau at 28°C.

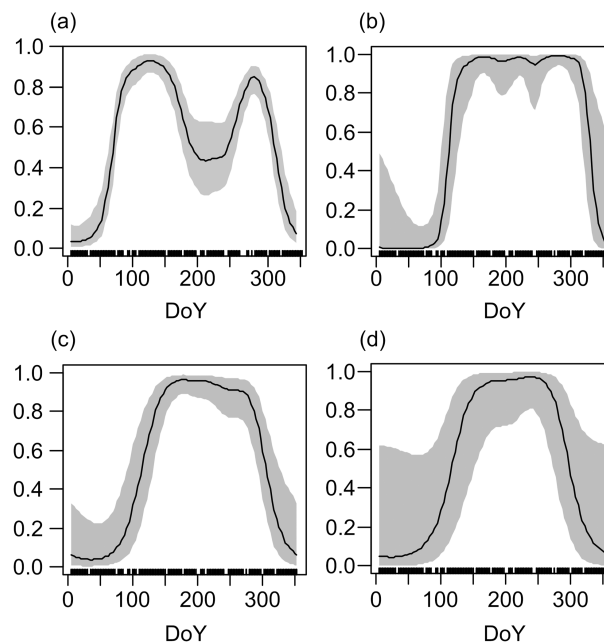


FIGURE 5 The effect of day of the year (DoY) on the probability of an oviposition by (a) *Calliphora vicina*, (b) *Lucilia ampullacea*, (c) *Lucilia caesar* and (d) *Sarcophaga caerulescens*. The figure presents the model visualisation of the GAM predicting the probability of the presence of eggs associated with the independent variable DoY. Shaded areas represent the 95% confidence intervals associated with the given smooth function

Effect of day of the year

For all species, except *L. sericata*, day of year was the most important variable affecting oviposition, emphasising the phenology of each species (Figure 5, Table 3). For *C. vicina*, the probability was low just at the beginning of the year, high in spring and autumn and decreased strongly in summer (Figure 5a). *L. ampullacea* was absent only at the beginning of the year until day 100, from then on, the probability of oviposition was high until day 300, after which a steep decrease was observed until the end of the year (Figure 5b). *L. caesar* and *S. caerulescens* showed a similar trend, with a slow increase in activity from spring until summer, high activity throughout the summer and a steep decrease from autumn to winter (Figure 5c,d).

Further parameters

For both, *L. caesar* and *L. sericata* increasing barometric pressure had a significant positive effect (Figure 6a,b). Relative humidity affected *L. caesar* significant positively (Figure 6c), whereas for *L. sericata*, values between 50% and 70% relative humidity are optimal for an oviposition (Figure 6d). For *S. caerulescens*, relative humidity above 70% had a negative effect (Figure 6e). Wind speed had a positive effect on *L. caesar* and *L. sericata* (Figure 6f,g). *C. vicina* was the only species significantly affected by sunshine hours and the amount and duration of rain on a day. Whereas the hours of sunshine had an overall positive

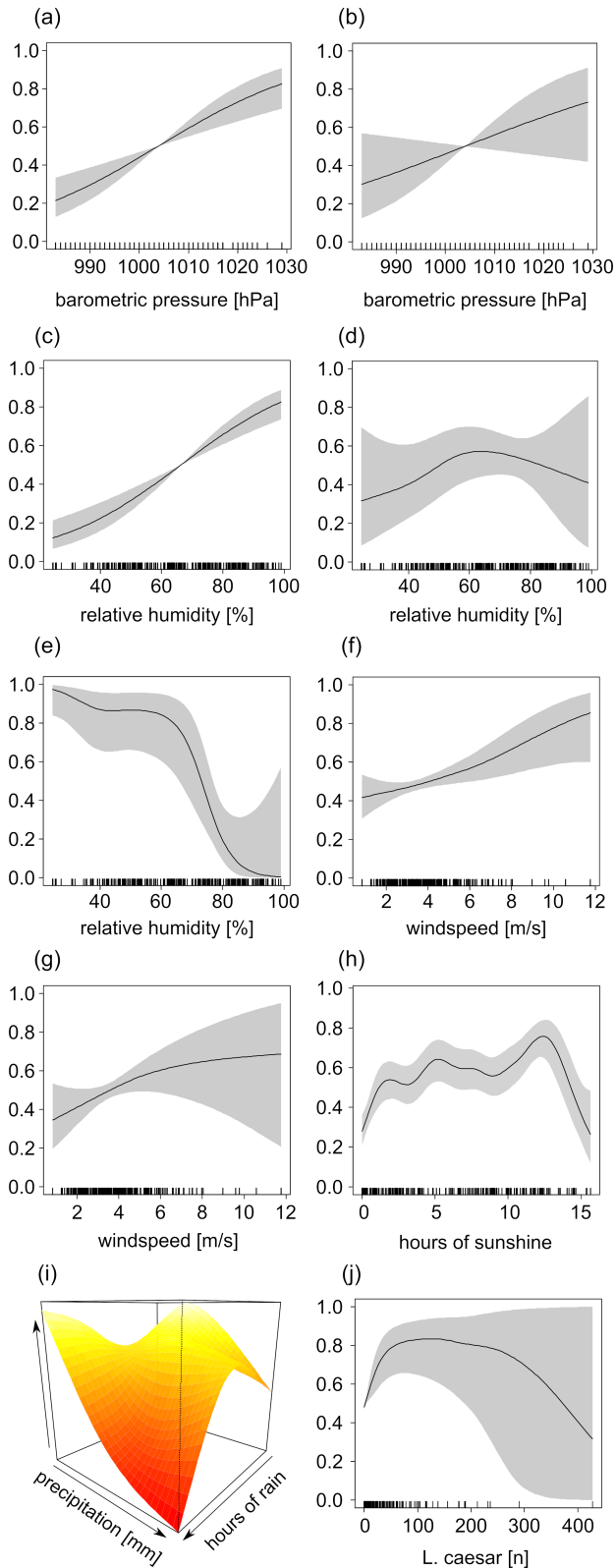


FIGURE 6 Variables affecting the probability of an oviposition by (a) *Lucilia caesar*, (b) *Lucilia sericata*, (c) *L. caesar*, (d) *L. sericata*, (e) *Sarcophaga caerulescens*, (f) *L. caesar*, (g) *L. sericata*, (h,i) *Calliphora vicina*, (j) *L. sericata*. The figure presents the model visualisation of the GAM predicting the probability of the presence of eggs associated with each independent variable. Shaded areas represent the 95% confidence intervals associated with the given smooth function

effect (Figure 6h) for values below 12 h a day, rain had a largely negative effect (Figure 6i). For *L. sericata*, the presence of *L. caesar* on the carcasses had an overall significant negative effect on the probability of an oviposition (Figure 6j). Furthermore, for all species, the oviposition activity differed between the years of the experiment (Table 3).

DISCUSSION

This study is the first attempt to understand and quantify the oviposition behaviour and activity of wild necrophagous blow flies and flesh flies in Central Europe and model their dependence on season, habitat and environmental parameters. Furthermore, this is an extension of a study conducted in the same location (Lutz, Verhoff, et al., 2019) in which a similar approach was used to analyse flight activity of necrophagous female blow flies and its relationship to environmental parameters. A limitation mentioned in that study was that 'not every female fly on the wing with access to carrion will necessarily guarantee an oviposition', a point already highlighted by Catts (Catts, 1992). To understand/assess delays in colonisation of cadavers or even no colonisation (Wells et al., 2021), it is essential to monitor not only flight activity but quantify the oviposition activity.

The high percentage (98.7%) of blow fly species is not surprising and underlines their verified dominant role as primary colonisers of carrion (Amendt et al., 2004; Blackith & Blackith, 1990; Lutz, Verhoff, et al., 2019; Wall & Warnes, 1994). By far the most abundant species, accounting for 57% of all specimens, was *L. ampullacea*, making it an important species in forensic entomology especially in Germany. It is also abundant in bait traps (Fremdt & Amendt, 2014; Lutz, Verhoff, et al., 2019; Zabala et al., 2014) and frequently colonises human (Bernhardt et al., 2018; Vanin et al., 2008) and animal remains (Hwang & Turner, 2005; Matuszewski et al., 2008; Smith & Wall, 1997a). Despite its abundance, there are no published developmental data for this species, which hampers its use in forensic science. The two other dominant species in this study, *C. vicina* and *L. caesar*, are well known as first colonisers of human bodies (Bernhardt et al., 2018) and frequently found on mouse carcasses (Blackith & Blackith, 1990; Isische et al., 1992) and in baited traps (Fremdt & Amendt, 2014; Lutz, Verhoff, et al., 2019).

The biggest discrepancy to other studies on the activity of necrophagous blow flies was the low occurrence/abundance of *L. sericata* with <1% of all specimens. Usually, *L. sericata* is the dominant taxon on carrion (Bernhardt et al., 2018; Lutz et al., 2021; Rose & Wall, 2011) and in baited traps (Fremdt & Amendt, 2014) in summer and was the most abundant species in a monitoring study in the same location (Lutz, Verhoff, et al., 2019). Reasons for the low presence in this study can be the oviposition substrate used, as a rare occurrence of this species on mouse carcasses was observed (Blackith & Blackith, 1990; Davies, 1999; Isische et al., 1992; Lane, 1975). In addition to the type of carcass, size/weight of the carrion has been shown to have a major influence on the decision for an oviposition by blow flies (Hewadikaram & Goff, 1991; Matuszewski et al., 2014) and is a dimension of colonisation choice as well as food source partitioning

among coexisting necrophagous species (Denno & Cothran, 1975). For *L. sericata*, this was demonstrated in an experiment (results not shown) using rat instead of mouse carcasses, which resulted in an overall higher number of juvenile *L. sericata*. For smaller carcasses, a main driver for species limitations is intra- and inter-specific competition for the limited food source (Atkinson & Shorrocks, 1981; Beaver, 1977). *L. sericata* performs poorly in interspecific competition (Kheirallah et al., 2007; Prinkkila & Hanski, 1995; Smith & Wall, 1997b), which can reduce the specimen number on a cadaver and the body size and fecundity of the surviving specimens (Holdaway, 1930). Our results provide support for this trend (Figure 6j), as the presence of *L. caesar* is a significant negative predictor for the oviposition by *L. sericata*. These two species share their seasonal niche and temperature adaptation and maybe cannot coexist together on smaller carcasses. Overall, there appears to be a complex interaction of various factors, such as interspecific competition, carrion type/weight, that influences the oviposition activity of *L. sericata*, but this needs to be investigated in detail in further studies.

In this study, only a small percentage (1.3%) of larvae were flesh flies, which is consistent with other studies, stating that flesh flies are outcompeted by blow flies on a cadaver (Denno & Cothran, 1976) as a result of two different strategies of carrion exploitation. The only species was *S. caerulescens*, an early coloniser of human and animal carrion (Cherix et al., 2012; Matuszewski et al., 2013; Pohjoismäki et al., 2010). Unlike blow flies, flesh flies are currently neglected in forensic entomology and often not even identified to species level in field studies (Cherix et al., 2012). This is not just because of their rarer occurrence on human cadavers but also due to the difficult identification of the juvenile stages (Cherix et al., 2012). Thanks to the present survey, we were able to analyse the phenology and seasonal adaptation of this species, as well as to model its dependence on habitat and other environmental parameters, which highlights the potential of this species in forensic science (Matuszewski, 2021).

Seasonal oviposition activity

According to Moreau et al. (2015), the goal of a seasonal study is to extrapolate the findings, here: species-specific oviposition activity, to the same seasons but to different years. Therefore, it is necessary to replicate experiments in at least 2 years. In the current study, the oviposition probability varied significantly among years for all species, suggesting the importance to consider such variation when interpreting the results and to understand and quantify the variables that are responsible for these differences. The two-year period allowed profound statements about the phenology of the species and their seasonal adaptations with regard to oviposition in a small geographical area. For all species, except *L. sericata*, *day of the year* was the most or second most important variable influencing the oviposition activity, and all species showed species-specific seasonal adaptations. *C. vicina* had the widest seasonal niche, with ovipositions throughout the year, confirming it as a cold-adapted species with a year-round activity (Fremdt & Amendt, 2014; Lutz, Verhoff,

et al., 2019; Samuel, 1997) and occurrence on human remains (Bernhardt et al., 2018; Lutz et al., 2021; Schröder et al., 2003). Our results classify *L. ampullacea* as a summer species, being highly active in June, July and August and absent from December until April. Such seasonal preference for summer was also observed in other studies (Fischer, 2000; Greco et al., 2014; Hwang & Turner, 2005; Lutz, Verhoff, et al., 2019). The phenology of *L. caesar* was characterised by a slow increase in oviposition activity from April to May and high activity peaks in June and August, as well as an absence of this species from November until April. These findings mirror the observed seasonal flight activity of adult *L. caesar* in the monitoring study conducted in 2017 (Lutz, Verhoff, et al., 2019) and are in line with other studies regarding the ecology of this species (Samuel, 1997; Townsend, 1928). The phenology of *L. sericata* was characterised by a high activity in summer and no activity from November until April. Due to the overall low abundance of *L. sericata*, the statistical power of the model was low (*day of the year* not significant); nevertheless, the observed phenology fits the one described in other studies (Bernhardt et al., 2018; Fremdt & Amendt, 2014). The flesh fly *S. caerulescens* appears to be a specialist having a narrow seasonal niche with an oviposition restriction to the summer months (Cherix et al., 2012; Denno & Cothran, 1975; Fremdt & Amendt, 2014). The high seasonal adaptation of this species is also supported by the fact that the variable *Day of the year* was the most important predictor for an oviposition by this species.

Habitat

The results of the GAMs as well as the canonical correlation showed that for each species, the probability for an oviposition differed significantly between the sampling sites. For *L. sericata*, it was even one of the most important factors influencing its oviposition (Table 3). This indicates that even in a radius of ~3 km, the microclimate of each site has a major effect on the oviposition probability as well as species distribution (Fenton et al., 1999; Janz, 2008; Lane, 1975; Lembrechts et al., 2019). For three species, *C. vicina*, *L. sericata* and *S. caerulescens*, also a significant species-specific habitat preference was observed in the models. *C. vicina* revealed a positive correlation of the oviposition probability with the urban habitat, confirming this species as a primary urban fly with synanthropic behaviour (Greco et al., 2014; Hwang & Turner, 2005; Smith, 1986). The fact that almost all larvae were sampled from the carcasses at the urban sites (Figure 1d) and *site* was one of the most important factors influencing the oviposition behaviour of *L. sericata* underscores the highly synanthropic nature of this species (Fremdt & Amendt, 2014; Greco et al., 2014; Isische et al., 1992; Vanin et al., 2008). For *S. caerulescens*, a clear preference for the rural sites was observed (Figure 1e). Fremdt and Amendt (2014) also found a correlation of the occurrence of this species and rural habitats, and Cherix et al. (2012) reported two cases of colonisation on human carcasses, both found in a natural environment similar to our rural sampling sites. The two remaining species, *L. ampullacea* and *L. caesar*, showed no clear habitat preference. While this was observed for *L.*

ampullacea, *L. caesar* is usually classified as a woodland species (Hwang & Turner, 2005; Lutz, Verhoff, et al., 2019), associated with rural areas, which could not be proven with our analysis.

Temperature

Temperature controls the behaviour, physiology and fitness of organisms and consequently drives species distribution and diversity patterns of ectotherms (Angilletta, 2009). In our study, *mean day temperature* was a highly important variable influencing the oviposition behaviour of almost all species. Despite the overall positive effect of temperature, each species, and here especially *C. vicina*, showed specific adaptations. The lowest mean day temperature at which this species colonised the mouse carcasses was 5.1°C, confirming the findings by Faucherre and Cherix (Faucherre et al., 1999), who observed oviposition by *C. vicina* at temperatures as low as 4°C. For all other species, an overall positive effect of temperature was observed up to values of 30°C, indicating that with increasing temperature the probability of an oviposition increases likewise. *L. ampullacea* was the only *Lucilia* species that colonised the mouse carcasses at a mean day temperature of 6.72°C. Above this value, the oviposition probability increased strongly and was close to 1 at values above 17°C and below 30°C. *L. caesar* and *L. sericata* showed a similar response to temperature, with no oviposition below 10°C and a high probability at values above 20°C. The upper threshold for an oviposition could not be estimated in this study because even at temperatures of 33.8°C, these species still colonised the carcasses. This was also demonstrated by Ody et al. (2017), who observed that *L. sericata* continued to lay eggs at temperatures as high as 40°C. The species with the lowest temperature tolerance was *S. caerulescens*. This species did not oviposit on the mouse carcasses at temperatures below 13.3°C, which is in line with its observed phenology and the strong association to summer months and high temperatures. Developmental thresholds of other flesh flies like 11.0°C for *S. africa* (Al-Misned, 2004) and 7.0°C for *Sarcophaga argyrostoma* (Grassberger & Reiter, 2002) confirm our findings.

Despite its adaptation to cold conditions, 20°C turned out to be the optimal temperature for an oviposition by *C. vicina* in the field. This is consistent with Ody et al. who observed a decrease in the probability of an oviposition at values above 20°C in the laboratory (Ody et al., 2017), and Hwang & Turner who reported 20°C as the optimal growth temperature of *C. vicina* (Hwang & Turner, 2009).

Other environmental parameters

Blow flies like to oviposit in moist places because they offer the perfect condition for their offspring and prevent the juvenile stages, especially eggs, from drying out (Barton Browne, 1962; Cragg & Ramage, 1945; Smith, 1986). In this study, just *L. caesar* and *S. caerulescens* were significantly influenced by relative humidity. For *L. caesar*, it was a significant positive predictor for a colonisation. It is known (personal experience in breeding *L. caesar*) that the juvenile stages of this species need a high

relative humidity, especially for pupation in, for example, the soil. Therefore, the adult female is active when relative humidity increases, as this ensures optimal condition for her offspring. For *S. caerulescens*, values above 70% had a strong negative effect. For *L. sericata* no significant effect was observed, but values between 50% and 70% seem to be optimal. Just two species were significantly influenced by relative humidity, indicating that this parameter is probably not of too great importance for the oviposition activity of necrophagous flies (Berry & Kunz, 1978). This is in contrast to the study by George et al. (2013) who stated relative humidity as a negative predictor for a colonisation by blow flies or the assumption of Smith (Smith, 1986) that relative humidity is a major factor controlling oviposition of necrophagous flies. Further research is needed to analyse this effect.

Changes in barometric pressure can forecast changes in weather conditions, like an approaching thunderstorm or a dry period with sunshine (Barry & Chorely, 2003). To date, hardly any study has been conducted to analyse the effect of barometric pressure on the oviposition activity of necrophagous flies, and when they have, effects were not significant (George et al., 2013). In our study, just *L. caesar* and *L. sericata* were influenced by barometric pressure, and for both it was a positive predictor for an oviposition. Since both species are most active in summer at higher temperatures, an increase in barometric pressure forecasts optimal conditions for them.

Wind speed is central among the environmental parameters influencing the flight and oviposition activity of insects (Digby, 1958). Especially, for necrophagous flies, which depend on olfactory cues to detect relevant food and oviposition sites, wind serves as an important carrier of information (Aak et al., 2010). So far, wind speeds below 10 km/h have been stated as optimal for oviposition and the absence of wind as an inhibiting factor (George et al., 2013). In our study, again *L. caesar* and *L. sericata* were the only species that were influenced by wind speed. For both species, the oviposition probability increased with increasing wind speed, underlining the assumption that wind carries important olfactory cues.

For *C. vicina*, two further environmental parameters were significant predictors for an oviposition. The hours of sunshine on a day positively influenced the oviposition activity. However, days with more than 12 h of sunshine seem to have a lower probability for an oviposition. Especially, for cold-adapted species with a year-round occurrence, sunshine has a big impact on days with low mean temperatures. On such days in winter, *C. vicina* heats up its body on sunny spots on walls, which enables the adult females to be active even when the temperature is close or below their threshold (Greenberg, 1991). The slightly negative influence of values above 12 h can be explained by the phenology of the species. Days with 12 or even more hours of sunshine occur only in summer and are associated with high temperatures, both of which have a negative effect on the oviposition activity of *C. vicina*. Several studies highlighted rain as an important factor for insect live and observed an inhibitory effect resulting in a colonisation delay of up to 3 days (Mahat et al., 2009). However, in our study, just *C. vicina* was negatively influenced by the amount and duration of rain on a day. This can be explained with the seasonal activity of this species. Unlike all

other species, *C. vicina* is active all year round, including days with a higher probability of rain such as in winter and fall. Therefore, this factor is more decisive for the model of *C. vicina*.

Limitations of the study

The present study provides basic ecological information on the oviposition activity of necrophagous flies based on an extensive monitoring over a 2-year period. However, it has some limitations, which are particularly important for the transferability of the results. First, the choice of mouse carcasses as oviposition substrate maybe limited the species diversity and abundance sampled, as carcass mass affects species composition, with larger cadavers having a more complex and abundant insect assemblage (Matuszewski et al., 2014). In addition, our carcasses were frozen and then thawed. While one study reported that the freezing process prevented certain beetle species from colonising a rabbit carcass (Mise et al., 2013), other studies suggest that freezing has no effect on colonisation times (Bugajski et al., 2011) or the development of blow flies (Day & Wallman, 2006).

Another limitation is the use of nearby weather stations instead of on-site measurements, a problem that is widely discussed both in forensic entomology (Charabidze et al., 2018) and ecology (Maclean et al., 2021). Since each site has its own microclimate, using the same measurements for all sites cannot cover this variation. Measuring microclimates and the pitfalls associated with them is a very hot topic in ecology (Maclean et al., 2021) and needs to be considered in future studies, to provide a better understanding of the biology of necrophagous species on a smaller scale.

Overall, it is important to keep in mind that biogeographic variation and population-specific adaptations are common in forensically important species (Tomberlin, Mohr, et al., 2011). These parameters can strongly regulate/influence the number of generations per year (Vinogradova & Reznik, 2016), the induction, duration and intensity of larval diapause (Vinogradova, 1986) or the development time (Gallagher et al., 2010; Hwang & Turner, 2009; Tarone et al., 2011). They must be considered when experimental results, collected at a small local scale, are used to answer general questions in forensic entomological casework. This also applies to predictions based on field data, as the use of environmental parameters to predict oviposition assumes that these variables actually define the ecological limits of an oviposition (Williams et al., 2014). Other variables are not considered, resulting a prediction that may not reflect the reality.

CONCLUSION

This study is the first attempt to understand and quantify the oviposition of wild necrophagous blow flies and flesh flies in Central Europe and model their dependence on season, habitat and other environmental parameters. We emphasise the importance of analysing this type of data at the individual species level, as each taxon has a unique

set of adaptations. While seasonal adaptation (the phenology of each species), the habitat (rural vs. urban), as well as temperature are the most important factors, the effect of different resource sites definitely needs further investigation. Our results showed that oviposition differed significantly among the sites even within a radius of only ~3 km for all species. Therefore, the microclimate, for example, sun exposure, vegetation, infrastructure, human impact, etc., of each site has a major effect on the oviposition probability as well as species distribution. Reliable estimations of microclimatic conditions are therefore the key to understand how insects interact with their environment and is crucial for addressing applied challenges such as predicting the ecological consequences of climate change or resource limitations (Potter et al., 2013).

Tomberlin and colleagues (Tomberlin, Benbow, et al., 2011, Tomberlin, Mohr, et al., 2011) highlighted that basic ecological and evolutionary experiments are the key for understanding the concept of carrion biology and especially for improving the accuracy and reliability of forensic case work. We believe that the results of our study can likely shed some light on the interpretation of rare cases with delayed colonisation or cases where exposed cadavers are uncolonised even under optimal conditions (Wells, 2019; Wells et al., 2021), but more importantly serve as a grounding for further understanding and quantification of the oviposition behaviour of necrophagous flies in their natural environment.

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CONFLICT OF INTEREST

The authors declare there are no conflicts of interest.

AUTHOR CONTRIBUTION

The study was designed by Jens Amendt and Lena Lutz. Data were collected by Lena Lutz and Tabea Rosenbaum. Statistical analyses were performed by Lena Lutz. The manuscript was written by Lena Lutz with editorial help from all authors especially Jens Amendt.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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