

Initial research on necrophagous true flies (Diptera) in Gryfino Commune

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Abstract As a result of research carried out in June and July 2017, at five sites in north-western Poland with different characteristics, 769 individuals belonging to three dipteran families of forensic significance were collected. The most abundant family was Calliphoridae, which was represented by 8 species (*Calliphora vicina*, *Calliphora loewi*, *Lucilia bufonivora*, *Lucilia caesar*, *Lucilia illustris*, *Lucilia sericata*, *Lucilia silvarum*, and *Lucilia richardsi*). The experiment examines differences in the dominance of individual species depending on the habitat type and the time of year, and also shows correlations between environmental parameters and individual taxa.

Wstępne badania nad fauna muchówek (Diptera) nekrofagicznych w gminie Gryfino

Słowa kluczowe entomologia sądowa, północno-zachodnia Polska, eksperyment terenowy, Calliphornidae, kryminalistyka

Streszczenie W wyniku badań przeprowadzonych w czerwcu i lipcu 2017 roku, na pięciu stanowiskach o zróżnicowanej charakterystyce w północno-zachodniej Polsce zebrano 769 osobników należących do 3 rodzin muchówek mających znaczenie w kryminalistyce. Najliczniej występującą rodziną była Calliphornidae, którą reprezentowało 8 gatunków (*Calliphora vicina*, *Calliphora loewi*, *Lucilia bufonivora*, *Lucilia caesar*, *Lucilia illustris*, *Lucilia sericata*, *Lucilia silvarum*, *Lucilia richardsi*). Doświadczenie sprawdza różnice w dominacji poszczególnych gatunków w zróżnicowaniu względem typu siedliska oraz w odstępstwie czasowym, a także pokazuje korelację między parametrami środowiskowymi, a poszczególnymi taksonami.

Introduction

The main goal of forensic entomology is to determine the time elapsed since death, i.e. the post mortem interval (PMI), by means of quantitative and qualitative analysis of necrophagous insects found on the body, especially when the use of other medico-legal methods is difficult (Włodarczyk, 2007). This science is also helpful in reconstructing the circumstances and place of death (when the body has been moved) and can provide information about the victim, such as health condition or medical negligence. The bodies of preimaginal stages of necrophages accumulate heavy metals, chemicals and toxic substances. They can also collect the spores of pathogenic microbes, thus indicating the cause of death.

Determination of the date of death is based on two main elements. First, insects colonize the body in a predictable and known order, and they appear on it in separate groups known as waves of succession. Secondly, the rate of development of the preimaginal stages of each insect species is also known, and is closely dependent on environmental conditions. In forensic entomology, all factors that may affect the decomposition rate of the body are taken into account, such as air temperature, humidity, insolation, light, and rain (Kaczorowska, 2014).

True flies (Diptera) are the first to arrive at the corpse, and among these, blow flies (Calliphoridae) play the most important role. Their larvae eat decaying organic matter and indicate the time elapsed from death to the discovery of the body (Merritt et al., 2000).

The classic method of species identification is assessment of the morphological features and anatomy of a preserved specimen using special identification keys. Preparation of the cephalopharyngeal apparatus can be used for this purpose. Unfortunately, this is a tedious and difficult task, even for an experienced entomologist. An additional difficulty is the lack of detailed morphological data for some insect families or the need to revise available data, especially in relation to eggs and preimaginal stages. Due to the high degree of similarity of many species, it is easy to make a mistake during identification, resulting in an erroneous estimate of the date of death (Skowronek, 2009).

An alternative method of identifying insects of forensic significance is currently being developed, using methods based on molecular biology and genetics. The material for analysis can be DNA located in the nucleus (nDNA) or in the mitochondria (mtDNA). Pioneer research is being carried out at the Museum and Institute of Zoology, Polish Academy of Sciences in Warsaw to implement the HRM-PCR method (high-resolution DNA melting analysis-polymerase chain reaction), based on analysis of high-resolution DNA denaturation curves in conjunction with PCR and preparation of reference DNA samples of blow flies (Calliphoridae) occurring in Poland (Skowronek, Chowaniec, 2010). Research is also being conducted on the barcoding method, which exploits the COI (encoding 1 subunit of cytochrome oxidase) specific for each species. This method enables rapid and efficient species identification (Bogdanowicz, Rogalla, 2009).

The numbers of taxa and specimens on the body, the rate of colonization, and the development time of species found on cadavers depends on multiple factors, both biotic and abiotic (Piotrowski, 1990). The most important factor of these include climatic conditions, i.e. air temperature and humidity. Other important factors for establishing the date of death include the geographic location and type of habitat where the body is located, its position relative to the sun, the cause of death, and where and how the body was concealed. Knowledge of the developmental biology and behaviour of animals found on cadavers is also very important (Kaczorowska, 2014).

In Poland, experimental field research is mainly conducted by teams of scientists from Poznań and Toruń, who have published many specialist articles and journals devoted to this field.

Research on the use of molecular biology and genetics to identify insects of forensic significance is carried out continually (Skowronek, 2012). This is a cause for optimism, although for a number of reasons it will be some time before specialized forensic entomological laboratories or ‘body farms’ appear in Poland (Mikołajczyk, 2009).

The aim of this study was to determine the composition of dipteran fauna feeding on pork liver, which mimicked a cadaver for the purposes of the experiment, taking into account different types of habitats and time intervals before the body was revealed.

Study area

The study area comprised fields and forests in the settlement of Szczawno, located in the Gryfino Commune in the West Pomeranian Voivodeship. The geographic location of the commune influences the local climatic conditions. The basic meteorological parameters of the area, such as the average level of precipitation, dominant wind directions and strength, and average temperatures, are representative of most of Western Pomerania. The growing season lasts on average about 210 days, the average yearly temperature ranges from 6.5 to 7°C, and precipitation varies between 600 and 650 mm. An important feature of the local climate is variable and irregular weather, associated with both the easy movement of large masses of air and the complex relief of the terrain (Korzeń et al., 2007).

Five stations were established in the field, differing in surrounding vegetation, insolation, and distance from households and water bodies. The sites are shown on the map and designated with symbols (Figure 1).



Figure 1. Study area with sites indicated

The first station (A) was located directly next to a household, between a garden and a wheat field. The perceived temperature was 22–25.9°C on June 6 and 29.6–29.9°C on July 19. The sun

exposure at this site was estimated to be high. During the day, swallow activity was observed over the field, which may have influenced further results. The distance from water was about 200 m.

The second site (B) was located directly by a fishing pond, about 200 m away from the nearest household. There was lush vegetation around the pond, composed mainly of grasses and herbaceous plants. The site has strong sun exposure and the perceived temperature was 25.7–28.2°C on June 6 and 27.3–29.3°C on July 19.

The third station (C) was set up at the edge of a coniferous forest. The tree density here was small, and the forest was directly adjacent to a field. Insolation was estimated as medium, and the perceived temperature was 25.3–25.7°C on June 6 and 22.2–25.5°C on July 19. The station was about 300 m from the nearest household and nearly 100 m from a nearby pond. During the July trial, all of the material had been plundered by scavengers. The remaining fragments of the bloodied, plastic bag in which the livers were kept served as a lure enabling insect collection.

The fourth station (D) was set up on fallow land, in the shade of a single small tree. Sunlight exposure and perceptible air temperature during the day were the highest of all sites: 28.1–31.4°C on June 6 and 26.5–30°C on July 19. Considerable ant activity was also observed during the day, in both the June and July trials. The vegetation was dominated by grass with an admixture of herbaceous plants, and there was a wheat field nearby. The site was located about 420 m from the nearest household and about 200 m from a body of water.

The fifth station (E) was located in a coniferous forest with broadleaf undergrowth. This site had the least sunshine, being protected from the sun by dense tree crowns and shrubs. The litter here was also densely overgrown with grasses. The temperature was 26.7–26.9°C on June 6 and 24.3–25.1°C on July 19. The site was about 380 m from the nearest household and about 200 m from the nearest water body.

Material and methods

The study was based on material collected on June 6 and July 19, 2017. In order to effectively carry out the experiment, the stations were secured against scavengers and the equipment needed to carry out the field experiment was assembled (nets and collecting bottles).

The material for the study comprised 800 g portions of pork liver that were frozen until needed for the experiment. Two days before the field experiment, the meat was thawed and exposed to outdoor weather conditions to enhance the properties making it attractive to necrophages. The material was then placed at each of the previously selected sites. Necrophages foraging on the livers were caught repeatedly throughout the day using a net, and then placed in previously prepared collecting bottles containing ethyl acetate. At each site the daytime temperature was measured and a GPS device was used to obtain the geographic coordinates. The sites were then marked on a map of the area and the distance from water bodies and houses was determined. The level of insolation at each site was estimated as well, using a three-point scale.

The collected material was transported to the laboratory for analysis. Flies were identified to family and species using a stereoscopic microscope and keys by Draber-Mońko (2014). The results were analysed by creating a database in Microsoft Excel 2010 and calculating the dominance index. Then the statistical significance of differences in the abundance of fauna collected in June and July was calculated using the Mann-Whitney U test. The Kruskal-Wallis test was used to verify the statistical significance of differences in the dominance of individual species. Spearman correlation and correspondence analysis were used to analyse the effect of environmental parameters on the abundance of necrophagous fauna.

Results

During the analysis of the collected material, we identified three families of necrophagous flies of forensic significance: Calliphoridae, Sarcophagidae and Muscidae. Seven species from the family Calliphoridae were found in the June trial (*Calliphora vicina* Robineau-Desvoidy, *Lucilia sericata* Meigen, *L. bufonivora* Moniez, *L. silvarum* Meigen, *L. illustris* Meigen, *L. caesar* Linnaeus, and *L. richardsi* Collin) and seven species in the July trial (*Calliphora vicina*, *C. loewi* Enderlein, *Lucilia sericata*, *L. silvarum*, *L. illustris*, *L. caesar*, and *L. richardsi*). The results are presented in Table 1 below.

Table 1. Total material collected in the study

Species	6 June 2017						19 July 2017						total
	A	B	C	D	E	subtotal	A	B	C	D	E	subtotal	
<i>Calliphora loewi</i> Enderlein, 1903									1			1	1
<i>Calliphora vicina</i> Robineau-Desvoidy, 1830	2		2			4			2			2	6
<i>Lucilia bufonivora</i> Moniez, 1876	1	1				2							2
<i>Lucilia caesar</i> Linnaeus, 1758	26	37	54	10	45	172	93	127	38	22	102	382	554
<i>Lucilia illustris</i> Meigen, 1826	1	1	7		4	13	10	13			2	25	38
<i>Lucilia richardsi</i> Collin, 1926				1		1				2		2	3
<i>Lucilia sericata</i> Meigen, 1826	4	1		3		8	14	9	2	15		40	48
<i>Lucilia silvarum</i> Meigen, 1826	12	11				23	11	16		2		29	52
Muscidae	1	2				3					1	1	4
Sarcophagidae	6	10	1	4	3	24	7	5	15	3	7	37	61
Total	53	63	64	18	52	250	135	170	58	44	112	519	769

The table presents the numbers of taxa identified in the June and July trials. The species *Lucilia bufonivora* was found in the June material, with one specimen each at sites A and B. This species did not appear at any site in the July trial. The material collected in July contained one individual of the species *Calliphora loewi* at station C, which did not appear anywhere else. The later material was also more abundant in terms of quantity. We caught and identified 250 dipterans in June and 519 in July, from the families Calliphoridae, Sarcophagidae and Muscidae, for a total of 769 insects. The statistical significance of differences in the abundance of fauna collected in June and July was calculated using the Mann-Whitney U test, with a result of $Z = -1.25336$; $p = 0.2$. Thus the test showed that the differences in numbers between the two samples were not statistically significant.

The dominance structure is presented in the form of pie charts, separately for each station and each trial (June and July) (Figures 2–6).

The highest dominance index at station A for both trials was obtained for *Lucilia caesar*, with values of 49.1% in the June and 68.9% in the July. The values for other species were lower, e.g. *L. silvarum* with 22.64% in the first trial and 8.2% in the second trial, and *L. sericata* with 7.6% in the June trial and 10.4% in the July trial. Flies of the Sarcophagidae family attained dominance indices of 11.3% and 5.2%, respectively. The last species in both samples was *L. illustris*, which accounted for 1.9% and 7.4% of the material at the first site respectively. In addition, the material

from the first trial included the species *Calliphora vicina* (3.8%) and *L. bufonivora* (1.9%), as well as flies of the family Muscidae (1.9%).

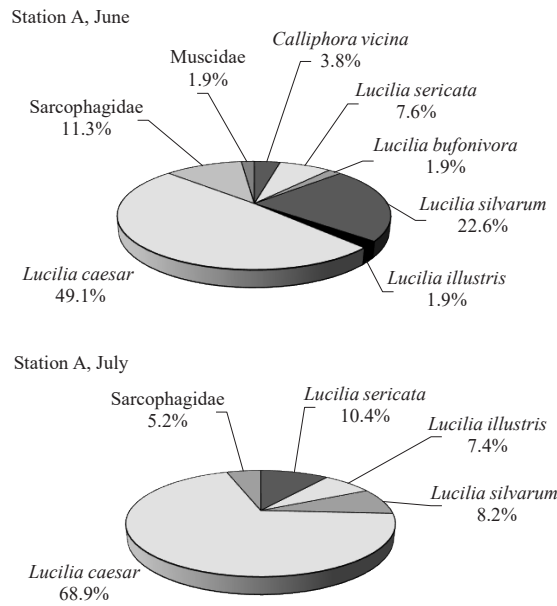


Figure 2. Dominance indices at station A in June and July

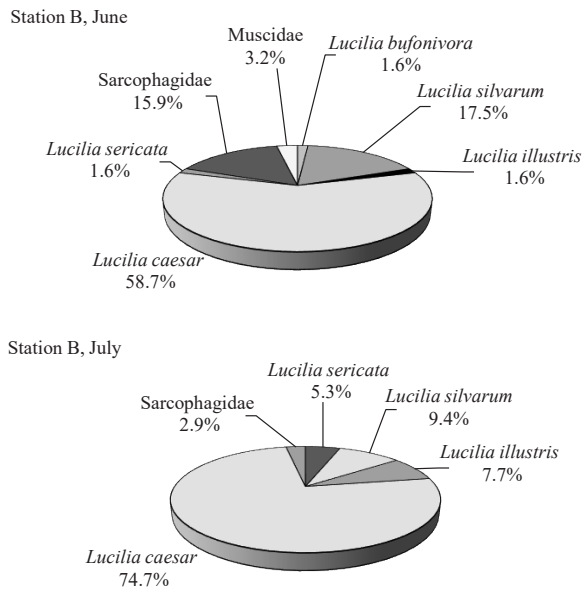


Figure 3. Dominance indices at station B in June and July

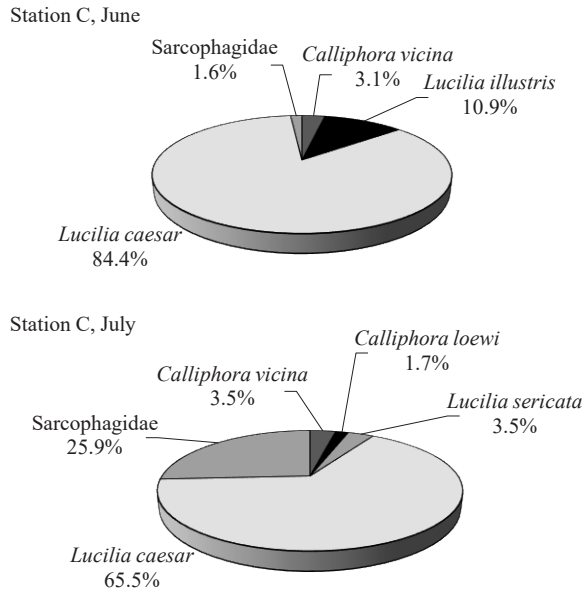


Figure 4. Dominance indices at station C in June and July

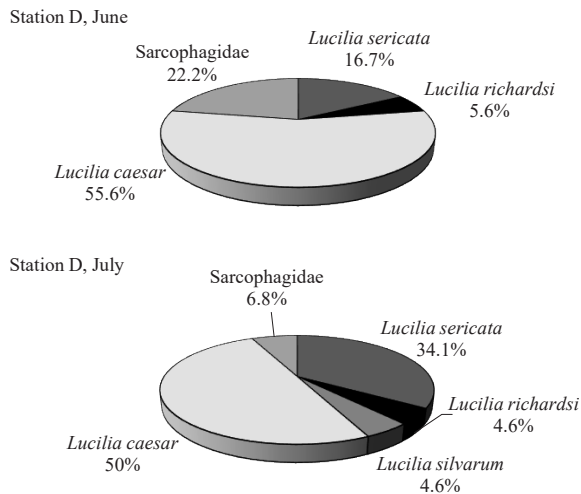


Figure 5. Dominance indices at station D in June and July

Station B was also the most attractive site for flies of the species *Lucilia caesar*, which accounted for as much as 58.7% of the June material and 74.7% of the July material among all specimens. The second highest value here was noted for *L. silvarum*, accounting for 17.5% and 9.4% of flies caught respectively. *L. illustris* (1.6% and 7.7%) and *L. sericata* (1.6% and 5.3%) were

more active in July. Activity of dipterans from the family Sarcophagidae was also noted at station B, with a dominance index of 15.9% for the June trial and 2.9% for the July trial. In addition, the first sample contained flies from the family Muscidae (3.2%) and the species *L. bufonivora* (1.6%).

Station C was also dominated by the species *Lucilia caesar*, which accounted for 84.4% of the material from the first trial and 65.5% of the material from the second trial. *Calliphora vicina* attained a similar dominance index in the two samples, with values of 3.1% for June and 3.5% for July. Flies from the family Sarcophagidae accounted for 1.6% of the material from the first trial but as much as 25.9% in the second. In addition, the first sample included the species *L. illustris* (10.9%), which was not detected in the second trial. On the other hand, the second sample had the species *L. sericata* (3.5%) and *C. loewi* (1.7%), which were not found in the first trial.

Station D had relatively similar fauna in the two trials. The largest share at this site fell to *Lucilia caesar*, reaching values of 55.6% and 50%. Ranking second were flies of the species *L. sericata*, with a dominance index of 16.7% in the June trial and as much as 34.1% in July. Flies of the family Sarcophagidae accounted for 22.2% of the flies in the June material for this site and 6.8% in the July trial. *L. richardsi* had a similar share in both trials, with a dominance index of 5.6% for the first and 4.6% for the second. In the July material, *L. silvarum* was detected as well, constituting 4.6% of the total number of individuals.

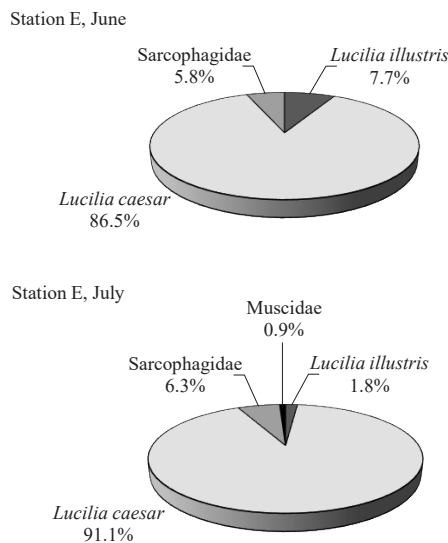


Figure 6. Dominance indices at station E in June and July

Station E had the highest proportion of flies of the species *Lucilia caesar*. The dominance index for this species reached 86.5% for the first trial and 91.1% for the second trial. Flies of the family Sarcophagidae had a similar share in both trials (5.8 and 6.3%). *L. illustris* was more active in June and accounted for 7.7% of flies at this site, as compared to July, when its dominance index was 1.8%. Flies of the Muscidae family were only detected at this site in July, in the amount of 0.9% of all specimens.

The results presented above show that the largest share at each station, in both June and July, fell to the species *Lucilia caesar*. The dominance index for this species ranged from 49.1% to 91.1%. Other important species in terms of quantity were *L. sericata* and *L. silvarum*, whose proportions at each station were strongly linked to the location and the month when the material was collected.

The Kruskal-Wallis test was used to test differences in the dominance of each species, resulting in statistically significant data. The result of the Kruskal-Wallis test was $H(9, N = 100) = 57.78997$; $p \leq 0.02$.

This was followed by Spearman correlation analysis, which showed strong correlations between individual species and environmental parameters, such as the distance from the site to water bodies and buildings, temperature, insolation and vegetation type (Table 2).

Table 2. Spearman's rank order correlation for individual species

	Distance from water	Distance from buildings	Temp.	Sunlight	Coniferous forest	Mixed forest	Fields	Fallows
<i>C. loewi</i>	-0.26352	0.00000	-0.52543	-0.26352	0.52705	-0.16667	0.00000	-0.37268
<i>C. vicina</i>	-0.17252	-0.30861	-0.72616	-0.17252	0.51755	-0.32733	0.34503	-0.48795
<i>L. bufonivora</i>	-0.19764	-0.53033	-0.26271	0.39528	-0.39528	-0.25000	0.39528	0.27951
<i>L. caesar</i>	-0.30277	-0.36927	-0.08537	-0.38534	0.30277	0.34816	-0.22019	-0.38925
<i>L. illustris</i>	-0.23758	-0.51250	0.18577	-0.11180	0.02795	0.17678	0.02795	-0.17788
<i>L. richardsi</i>	0.39284	0.70273	0.50477	0.39284	-0.39284	-0.24845	0.00000	0.55556
<i>L. sericata</i>	0.13932	-0.19938	0.51853	0.80805	-0.72446	-0.61679	0.66873	0.66989
<i>L. silvarum</i>	-0.27990	-0.69834	0.25130	0.73657	-0.73657	-0.46585	0.61872	0.60417
Muscidae	-0.10206	-0.31950	-0.36177	0.05103	-0.20412	0.16137	0.00000	0.07217
Sarcophagidae	-0.24923	-0.44584	-0.26994	0.08308	-0.05538	-0.08757	0.19385	-0.01958

Positive correlations were obtained for the following:

- the species *Lucilia richardsi* with distance from buildings,
- the species *Lucilia sericata* with insolation and open areas such as fields or fallows,
- the species *Lucilia silvarum* with insolation.

Negative correlations were obtained for the following:

- the species *Calliphora vicina* with high temperature,
- the species *Lucilia sericata* with coniferous forests,
- the species *Lucilia silvarum* with distance from buildings and coniferous forests.

DCA analysis for the abundance of individual taxa of necrophagous fauna showed that the length of the gradient represented by the first ordinate axis was below 3.0, so that direct RDA analysis could be performed to determine the relationship between the abundance of individual taxa and environmental parameters (ter Braak, 1986; ter Braak et al., 1988). The RDA analysis (Figure 7) showed a strong correlation for the species *Lucilia sericata* with insolation and open areas, such as fields and fallows. The species *L. silvarum* and *L. bufonivora* showed a correlation with decreasing distance from households, while the reverse was true of *Calliphora loewi* (correlation with increasing distance from buildings and coniferous forest). *L. richardsi* was the only species associated with an increase in perceived temperature. *L. illustris* was linked to the proximity of a water body. No clear results were shown for the remaining taxa.

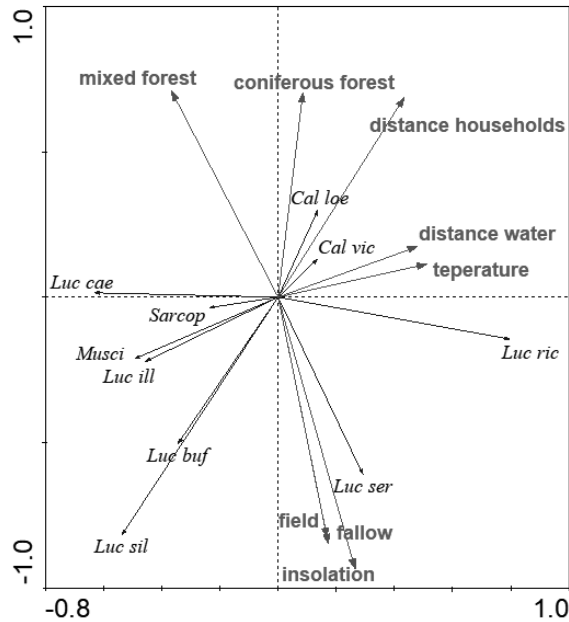


Figure 7. RDA analysis of relationships between abundance of individual taxa and environmental parameters

Discussion

The analysis of the material collected in the field revealed the presence of three families of forensic significance: Calliphoridae, Sarcophagidae and Muscidae. The most abundant was the Calliphoridae, in which the following species were identified: *Calliphora vicina*, *Calliphora loewi*, *Lucilia caesar*, *Lucilia illustris*, *Lucilia silvarum*, *Lucilia bufonivora*, *Lucilia sericata* and *Lucilia richardsi*. The most abundant species was *Lucilia caesar*, accounting for 49.06% to 91.07% of all the material, depending on the site and trial (June or July).

In temperate climate conditions, eight waves of succession of insects are observed on unburied carcasses. The aforementioned arthropod families and species represent the first and second waves of succession. The first wave of succession comprises flies of the family Calliphoridae, such as *Calliphora vicina*, *Calliphora vomitoria* and *Lucilla* spp., as well as *Musca domestica*, *Musca autumnalis* and *Muscina stabulans* of the family Muscidae. The appearance of these insects signifies the initiation of autolysis in the body. The second wave of succession of unburied carcasses consists of flies of the genera *Lucilia* and *Protophormia* and the species *Cynomya mortuorum* (Calliphoridae), as well as *Sarcophaga* spp. (Sarcophagidae), which arrive at the start of putrefaction. Both waves occur within the first three months after death (Kaczorowska et al., 2002).

Comparison of the first and second trials, bearing in mind the time interval separating them, indicates that June favours greater diversity of necrophages, which was particularly evident at sites A and B. This may be influenced by the lower perceptible temperature at these sites in June, as some species are negatively phototropic and avoid flying in full sunlight and high temperatures. Among dipterans, species of the genus *Calliphora* prefer shade, while *Lucilia* and *Sarcophaga* prefer light (Smith, 1986). Knowledge of the preferences of insects and their classification

according to preference for light or shade is useful in establishing whether a body has been moved or how long it has been exposed to sunlight.

There were over twice as many individuals in the second (July) samples, irrespective of the type of habitat, except for the site where scavengers had removed the food source, leaving only fragments of the bloodied bag. Inadequate protection of the sample contributed to the negative U Mann-Whitney test result for the statistical significance of differences in the abundance of collected fauna. Without the third site, the result would certainly have been statistically significant. This may have been influenced by the seasonal activity of necrophagous insects, observed mainly in temperate climate zones. This is obviously an individual characteristic of the species, but in the local weather conditions, fauna is richest in terms of quantity in late spring and summer. Insects found on the body represent multiple generations in the season and fly from early spring to late autumn, but are not able to reproduce during the entire period (Smith, 1986).

The most similar results between the two months in which the field experiment was conducted were obtained at sites D and E. The fourth site (D) was the furthest removed from any households, as well as from water bodies. The liver was exposed in the field, in the shade of a single small tree. The perceived temperature in both trials exceeded 30°C. The conditions prevailing at the fourth site were reflected in the number of insects arriving at the food source, limiting their number (Nuotreva, 1959). While *Lucilia caesar* was the most abundant species, a high dominance index was also noted here for *L. sericata*. Cragg (1956) describes this species as flying only in spring and summer, on hot days, laying eggs on surfaces heated to 30°C or with strong sunlight. The Spearman correlation analysis and RDA analysis revealed a clear, strong correlation of this species with insolation and open areas such as fields and fallows. Site D was therefore an ideal place for laying eggs, which contributed to the significant percentage share of this species.

Site E was a mixed forest. In theory, this site should have a large diversity of arthropod species, due to the abundant vegetation and favourable conditions for shade-loving insects. Research on the succession of insects on unburied carcasses in Polish forests has been conducted by Matuszewski et al. (2008 and 2010a), in the forests of Wielkopolska in 2005–2007. They recorded 14 species of flies and 16 species of beetles on the body of a domestic pig (Matuszewski, 2010a). Another example of research in this area is an experiment conducted by Bourel et al. (1999), who studied the fauna of unburied carcasses in the dune zone of the coast of northern France. The greatest diversity of taxa was observed on the bodies lying in the mixed forest: 42 arthropod species, of which 18 were of the order Diptera. The food source in their case consisted of fresh rabbit carcasses, which may have additionally contributed to differences in the results (Smith, 1986). In the experiment carried out for the present study, the station in the mixed forest attracted the lowest diversity of species and proved to be an ideal site for feeding by flies of the species *Lucilia caesar*. This species has been described by Matuszewski et al. (2010b) as the most common species in forests, appearing at every site during their experiment. The dominance index for this species was 86.54% in June and increased to 91.07% in July, marginalizing the activity of other species.

The greatest differences between the June and July trials were obtained at site C, located at the edge of a forest, which could have resulted in forest fauna mixing with open area species (Fiedler, 2008). In the June sample, 10.94% of the dipterans collected here were of the species *Lucilia illustris*, which was not found in the July sample. In July, we found flies of the species *L. sericata* and *Calliphora loewi*, described by Günther Enderlein as a Holarctic species, attracted by the smell of stinkhorns. Differences in the results could in this case again be due to the

disappearance of the intended food source in the July trial, so that dipterans were attracted only by the bloody fragments of the bag in which the liver was stored.

The Spearman correlation and RDA analyses showed several connections between individual taxa and environmental parameters. Particularly noteworthy is the correlation of the previously mentioned species *Lucilia sericata* with high insolation and open habitats, such as fields and fallows, and its negative correlation with mixed and coniferous forests. Another species with unambiguous results in both analyses is *L. silvarum*, which was linked to the proximity of households. The results of the Spearman correlation analysis also indicate several other links between taxa and environmental parameters, but these were not reflected in the RDA analysis. The second analysis, in turn, showed correlations that were not found in the results of the first analysis. Apart from the sensitivity and specificity of each of the analyses, the ambiguity of the results was additionally due to the small number of samples, i.e. replications, and the small number of specimens representing individual taxa collected during the field experiment, sometimes limited to a few individuals.

The field experiment was not without flaws, which should be avoided by those repeating the experiments carried out in this study or planning similar ones. In the future, the number of trials should be increased and spread out over time and the research area should be expanded. In addition, each individual sample should contain necrophages caught over several consecutive days. The means of constructing the trap itself should also be improved so that scavengers will not be able to disturb the food source. Adherence to these suggestions should improve the research in terms of both quantity and quality.

Conclusions

1. The date of exposure (June or July) of pork liver does not significantly affect the qualitative differences in the taxa present on the food source, but has a significant effect on quantitative differences.
2. Some taxa show a strong correlation with specific environmental parameters:
 - *Lucilia sericata* with strong insolation and open areas such as fields and fallows,
 - *Lucilia silvarum* with proximity to buildings.
3. The species described as *Lucilia caesar* was the most common species in the study area, irrespective of the date and the environment where the food source was exposed.

References

- Bogdanowicz, W., Rogalla, U., (2009). Muchy i paskowe kody. *Genetyka i Prawo*, 7, 6–9.
- Bourel, B., Martin-Bouyer, L., Hédouin, V., Cailliez, J.-Ch., Derout, D., Gosset, D. (1999). Necrophilous insect succession on rabbit carrion in sand dune habitats in Northern France. *Journal of Medical Entomology*, 36, 420–425.
- Cragg, J.B. (1956). The olfactory behavior of *Lucilia* species (Diptera) under natural conditions. *Annals of Applied Biology*, 44, 467–477.
- Draber-Mońko, A. (2014). *Calliphoridae – plujki (Insecta: Diptera)*. Warszawa.
- Fiedler, A., Halbach, M., Sinclair, B., Benecke, M. (2008). What is the edge of a forest? A diversity analysis of adult Diptera found on decomposing piglets inside and on the edge of a western German woodland inspired by a courtroom question. *Entomologie Heute*, 20, 173.
- Kaczorowska, E., Draber-Mońko, A. (2014). *Wprowadzenie do entomologii sądowej*. Gdańsk.

- Kaczorowska, E., Pieśniak, D., Szczerkowska, Z. (2002). *Entomologiczne metody określania czasu zgonu*. Łódź.
- Korzeń, J., Korzeń, J., Róg, J., Omachel, A., Lewowski, W., Lichocki, J., Waliszewski, J., Korzeń, K. (2007). *Zmiany studium uwarunkowań i kierunków zagospodarowania przestrzennego Gminy Gryfino*. Jelenia Góra.
- Matuszewski, S., Bajerlein, D., Konwerski, S., Szpila, K. (2008). An initial study of insect succession and carrion decomposition in various forest habitats of Central Europe. *Forensic Science International*, 180, 61.
- Matuszewski, S., Bajerlein, D., Konwerski, S., Szpila, K. (2010a). Insect succession and carrion decomposition in selected forests of Central Europe. Part 1: Pattern and rate of decomposition. *Forensic Science International*, 194, 85.
- Matuszewski, S., Bajerlein, D., Konwerski, S., Szpila, K. (2010b). Insect succession and carrion decomposition in selected forests of Central Europe. Part 2: Composition and residency patterns of carrion fauna. *Forensic Science International*, 195, 42.
- Matuszewski, S. (2010). Katalog owadów przydatnych do ustalania czasu śmierci w lasach Polski. Część I: Wprowadzenie. *Problemy Kryminalistyki*, 267, 5–17.
- Merritt, R.W., Higgins, M.J., Wallace, J.R. (2000). Entomology. In: J. Singel, P. Saukko, G. Knupfer (eds.), *Encyclopedia of Forensic Sciences* (pp. 699–705). Waszyngton: Academic Press.
- Mikołajczyk, B. (2009). Czy Bill Bass zostałby w Polsce skazany? Art. 262 k.k. a rozwój kryminalistyki w kontekście działalności tzw. „farmy śmierci”. In: M. Zelek (ed.), *III Dni Kryminalistyki Wydziału Prawa i Administracji Uniwersytetu Rzeszowskiego. Materiały z konferencji* (pp. 301–313). Rzeszów.
- Nuotreva, P. (1959). Studies on the significance of flies in the transmission of poliomyelitis. III. The composition of the blowfly fauna and the activity of the flies during the epidemic season of poliomyelitis in South Finland. *Annales Entomologici Fennici*, 25, 121–136.
- Piotrowski, F. (1990). *Zarys entomologii parazytologicznej*. Warszawa.
- Skowronek, R. (2009). Owady na wokandzie. *Genetyka i Prawo*, 4–5.
- Skowronek, R. (2012). *Wykorzystanie entomologii w kryminalistyce i medycynie sądowej*. Katowice.
- Skowronek, R., Chowaniec, C. (2010). *Polska entomologia sądowa – rys historyczny, stan obecny i perspektywy na przyszłość*. Katowice.
- Smith, K.G.V. (1986). *A manual of forensic entomology*. Londyn: British Museum of Natural History.
- ter Braak, C.J.F. (1986). Canonical correspondence analysis: a new eigenvector technique for ultrivariate direct gradient analysis. *Ecology*, 67, 1167–1179.
- ter Braak, C.J.F., Prentice, I.C. (1988). A theory of gradient analysis. *Adv. Ecol. Res.*, 18, 271–317.
- Włodarczyk, R. (2007). *Kryminalistyczne ślady biologiczne „portretem” sprawców zabójstw i innych przestępstw*. Szczecin.

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