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Effects of burned liver on necrophagous flies in southern Brazil

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ABSTRACT

This study investigated the effects of burning on the richness, abundance, and community structure of necrophagous flies in southern Brazil. Flies were collected over a period of seven days in the field, and were subsequently observed for 60 days in the laboratory. There were three bovine liver treatments: unburnt, burnt, and gasoline-treated (control). A total of 2,501 flies, representing 14 families and 41 species, were identified, including *Chrysomya albiceps*, *Fannia* subgroup *pusio*, *Synthesiomyia nudiseta*, and *Fannia femoralis*. While the abundance of flies was similar across the three treatments, exhibiting a quadratic distribution, species richness displayed a quadratic trend with a significant temporal shift; peak richness occurred 20 hours earlier in the unburnt liver than in the burnt or the control treatments. Both total abundance and patterns of community composition in our analysis indicated important factors to take into account. Total abundances were similar between the burnt and unburnt treatments, demonstrating a uniform ecological response to burning, irrespective of the condition of the liver. In contrast, the community composition of these treatments showed a significant divergence from the control treatment, which was distinctively dominated by Drosophilidae and Phoridae. These findings are crucial for accurate postmortem Interval (PMI) estimations in forensic entomology, particularly in cases involving burnt remains, and highlight the significant impact of fire on necrophagous fly communities.

Introduction

The colonization patterns of animal cadavers vary distinctly between burnt and unburnt corpses (Catts and Goff, 1992). Additionally, there is a delay in the colonization of burnt compared to unburnt cadavers.

In forensic entomology, the late arrival of colonizers at a corpse may cause bias in the estimate of *postmortem* Interval (PMI) (Campobasso et al., 2001). Insects, especially flies, are employed in the estimation of PMI in two ways: (i) since flies often lay eggs within minutes or hours after death, the biological cycles of necrophagous Diptera (Byrd and Castner, 2010) can be used to estimate the time-since-death by ascertaining the degree of development of the oldest maggots feeding on the corpse (Smith, 1986; Catts, 1992); (ii) if the local successional patterns are known, it is possible to infer the PMI by ascertaining the wave of colonization depending on the stage of decomposition (Battán Horenstein et al., 2010; Tomberlin et al., 2011).

However, several factors can introduce bias in estimating the PMI using insect biology and succession data. One significant source of bias is the reliance on assumptions that are not always valid (Tomberlin et al., 2011), and for this reason, understanding these assumptions and their potential impact on PMI estimations is crucial.

Another source of bias is the influence of external factors on insect colonization and development. Temperature, humidity, and environmental conditions can affect the rate of insect colonization and rate of development on a corpse, influencing the estimation of the PMI (Acikgoz, 2016; Franceschetti et al., 2021). The presence of drugs, toxins, or conditions that deeply affect the body (e.g., burning, wrapping in clothing) can also influence the timing of insect arrival and succession, further complicating PMI estimations (Amendt et al., 2011).

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Moreover, bias in PMI calculations may be introduced by the absence of standard research procedures and the variation in experimental designs used in forensic entomology investigations. To guarantee the accuracy and consistency of the entomological data utilized for PMI assessment, research techniques must be standardized (Tomberlin et al., 2012). A lack of standardized protocols can lead to inconsistencies in PMI estimations based on insect biology and succession data.

While extensive research has been conducted to understand the factors influencing estimations using insect biology, significant gaps remain, particularly concerning the effects of fire on necrophagous communities. The existing literature, such as studies by Avila and Goff (1998), Pai et al. (2007), Gruenthal et al. (2012), and Oliveira-Costa et al. (2014), underscores the complexity of assessing PMI in cases involving burnt remains.

Different animal models have been utilized to observe the effect of burning on necrophagous fauna. For instance, although it is widely accepted that the decomposition of domestic pig bodies weighing more than 23 kg follows similar stages to that of human bodies (Catts and Goff, 1992), caution is advised when extrapolating findings from experiments with bovine liver (McIntosh et al., 2017). While useful for determining richness and abundance patterns in ephemeral systems, these studies have limitations when applied to scenarios involving burnt substrates.

Biases in PMI estimations are notably pronounced when dealing with burnt substrates due to the altered necrophagous communities and the atypical environmental conditions they introduce. This highlights the need for standardized research methods to better account for these unique variables, ultimately improving the accuracy of PMI estimations in forensic entomology.

This study explored the impact of burnt substrates on the community structure of necrophagous flies. The following hypotheses were tested: (i) Species richness and abundance are greater in unburnt animal remains than in burnt remains when the latter have a repulsive effect on fly species. This can happen for example when gasoline is used in the burning process (Charabidze et al., 2009; Rumiza et al., 2010). (ii) If the repulsive effect is not significant, the necrophagous communities in burnt and unburnt remains are expected to be similar. (iii) The temporal patterns of species richness and abundance differ between unburnt and burnt remains. Burning creates a thin and rigid layer over the carcass, preventing the volatilization of gases that attract necrophagous flies (Tomberlin et al., 2011).

Methods

Study area

This study was carried out in the "Horto Botânico Irmão Teodoro Luis" (HBITL), "Universidade Federal de Pelotas" (UFPEL), in the municipality of Capão do Leão, geographical coordinates: 31º48'49" S and 52º25'53" W (Fig. 1 in the supplementary material). It is an area of 25 ha and border of 3,315 meters. The vegetation there is part of the Pampa Biome, characterized by shrub and herbaceous pioneer formations typical of the lagoon complex (Venzke, 2012). The subtropical climate is strongly influenced by the proximity to the ocean and by high levels of humidity (average 80.7%) and moderate temperature fluctuations throughout the year. The average temperature ranges between 12.3º and 23.2°C, with the lowest temperatures occurring in July and the highest occurring in January and February (Estação Agroclimatológica de Pelotas, 2013). The average rainfall is well distributed throughout the year (106 mm/month) (INMET, 2018).

Manufacture and installation of traps

The trap model used was modified from the one proposed by Hwang and Turner (2005). Plastic buckets with a capacity of 20 liters were perforated at the bottom to prevent water accumulation. Two holes of 6.5 cm in diameter each were made at 2 cm from the top edge of the bucket to serve to couple the collector bottles. Two other openings with exact measurements were made in the bucket cover. They were subsequently bonded fabric voile type, allowing air circulation, light entry, and preventing visitor insect escape. Tipper-like openings 15 cm wide by 4 cm high were made on the sides of the bucket, 12 cm from the bottom. These bottom apertures were above those made at the top and were intended to allow colonizers of the systems to enter. The collector bottles were made from two plastic jars with threaded caps and 700 ml capacity each. The caps of the jars were screwed together in the inner portion, thereby creating a hole for the passage of insects. This opening was fitted and fixed to the lateral orifice of the bucket so that the two holes remained attached, forming a corridor from one container to the other (Fig. 2 in the supplementary material).

In the field, traps were filled with 8 cm of soil and the substrates were placed on the soil. The soil was used to maintain moisture and for insect pupariation sites. The trap was closed on top and sealed with tape; then, it was suspended by a wire holder and secured with nylon rope 1.5 m from the soil. The traps were kept at a minimum distance of 50 m from each other (Fig. 2 in the supplementary material).

Field experiment

Four sites representing analytical blocks were selected. Three traps were installed per block. Each trap was filled with 1000 g of one of the tree baits: (i) unburnt bovine liver (unburnt); (ii) bovine liver burnt for five minutes with 500 mL of gasoline (burnt) (Fig. 1); and (iii) unburnt bovine liver with 500 mL of gasoline (control).

Figure 1 Animal remains used in the traps for the collection of insects in the Horto Botânico Irmão Teodoro Luis (HBITL), Universidade Federal de Pelotas (UFPEL), in the municipality of Capão do Leão. Unburnt, unburnt bovine liver; Burnt, burnt bovine liver; Control, bovine liver with gasoline.

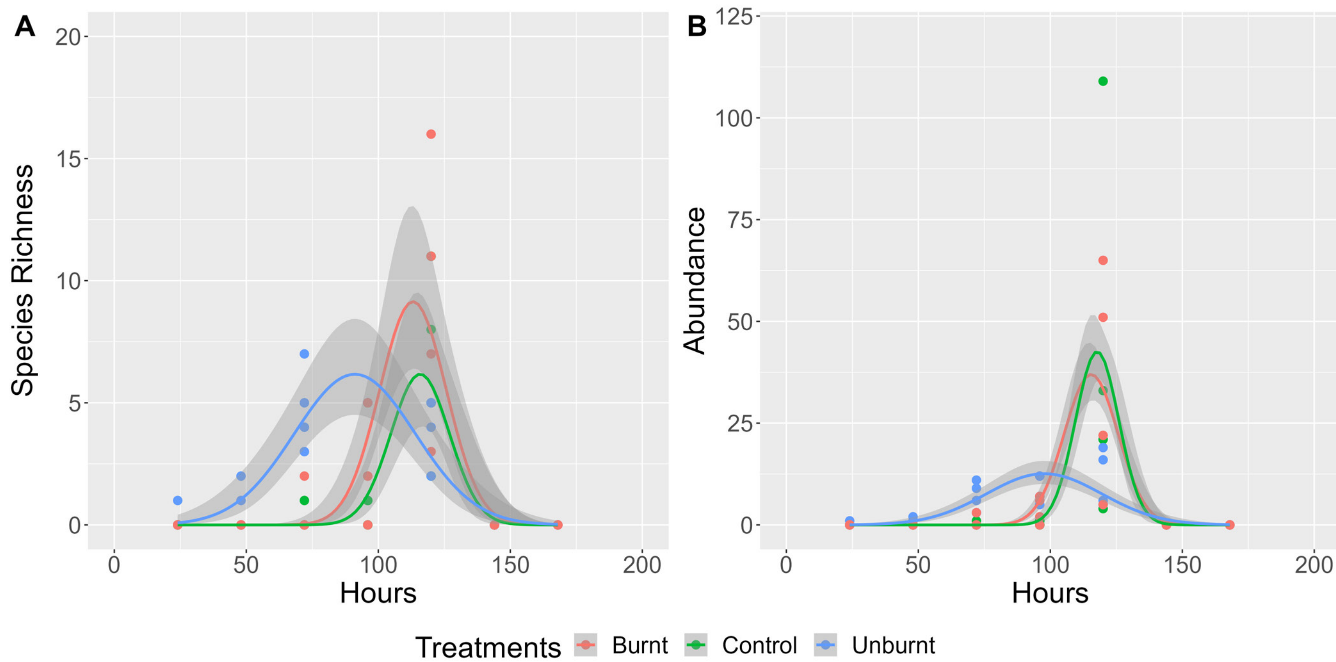

Figure 2 Generalized linear models with Poisson distribution and Quasipoisson correction Species Richness (A) and Abundance (B) of visitor necrophagous species community at HBITL, Rio Grande do Sul (Brazil). Burnt, burnt bovine liver; Control, bovine liver with gasoline; Unburnt, unburnt bovine liver.

Traps were installed on January 14, 2013 (summer), and they remained in the field for seven days. The traps were observed twice daily: at 10 a.m., when the bottles were filled with 70% alcohol, and at 2 p.m., when the bottles were removed and the flies were collected. The relative humidity (RH) ranged from 73 to 82%, and the mean temperature ranged from 25.5 to 29.6°C.

After the 7th day, the traps were transferred from the field to the laboratory under controlled conditions (25°C and 75% RH).

Fly species determination

Most of the identification of adult flies followed Albuquerque et al. (1981), Carvalho and Ribeiro (2000), Carvalho and Couri (2002), Couri and Carvalho (2002), Brown and Kung (2007, 2010), Carvalho and Mello-Patiu (2008), Disney (2008), Wendt and Carvalho (2009), Vairo et al. (2011), and Carvalho et al. (2012). The Rhinotoridae family was determined by Dra. Júlia Calhau Almeida (Universidade Federal do Espirito Santo), Mycetophilidae by Dra. Sarah Siqueira Oliveira (Universidade Federal de Goiás), Fanniidae by Dra. Lisiane Dilli Wendt (Universidade Federal do Paraná), and Drosophilidae by Dr. Carlos Ribeiro Vilela (Universidade de São Paulo).

Data Analysis

The data matrix was divided into the richness and abundance of necrophagous adult fly visitors and the colonizers.

The data matrix on necrophagous adult flies was built based on the adults of each species, which were collected daily using the field traps. A data matrix colonizer was constructed containing information on the species that developed from each experiment outlined in methods. This distinction is significant because some species visit carcasses solely to feed and are not ready for oviposition or to face high competition in a homogeneous and limiting space. In contrast, species ready for oviposition will lay eggs or larvae that will develop within this system, thereby altering its dynamics.

To test hypothesis (i), we ascertained the influence of the treatment (unburnt, control, and burnt) and days of colonization on the species richness and abundance. Due to the replicability (N=4 blocks or replicates), tests of normality were not performed for the colonizers, and the data were analyzed using generalized linear models (GLMs) with a Poisson distribution, considering the Quasi likelihood of abundance due to the overdispersion of data. To analyze the impact of different systems and exposure days on species richness and abundance, we used a GLM with a Poisson distribution. We accounted for data dispersion using a Quasi-likelihood approach for abundance.

To address potential differences in the composition of the necrophagous fly community, we assessed the similarity/dissimilarity of community composition across treatments using nonmetric multidimensional scaling (NMDS) on a Bray-Curtis similarity matrix followed by a PERMANOVA test for each matrix (visitors and colonizers). We performed the statistical analyses using the R program and the community analysis packages “vegan” and “MASS” (R Development Core Team, 2018; Oksanen et al., 2020).

Results

A total of 2.501 flies were collected, representing 14 families distributed in 41 species and morphospecies. Calliphoridae and Fanniidae were the most abundant, with 1.069 and 480 specimens, respectively. Among the colonizers, the most abundant species were *Chrysomya albiceps* (Wiedemann, 1819) (n=1006), *Fannia* subgroup *pusio* (n=272), *Synthesiomyia nudiseta* (Wulp, 1883) (n=188) and *Fannia femoralis* (Stein, 1898) (n=166) (Table 1).

Richness and abundance of necrophagous adult flies in the traps

The richness exhibited a quadratic statistical model relationship with time ($Chi_{1;79}$=174.217, P<0.001, Fig. 2A), with different patterns among the unburnt (P<0.001) and burnt plus control treatments

Table 1
Diptera collected in the “Horto Botânico” in the three systems: liver plus gasoline (LG), unburnt liver (LN), and burnt liver (LB). *NA: necrophagous flies adults. **C**: flies colonizers

Species	Systems						Σ
	LG		LN		LB		
	NA	C	NA	C	NA	C	
Calliphoridae							
Hemilucilia semidiaphana (Rondani, 1850)					1		1
Chrysomya putoria (Wiedemann, 1819)			31		3		34
Chrysomya megacephala (Lopes, 1935)		3			1		4
Chrysomya albiceps (Wiedemann, 1819)		2		565	1	438	1006
Lucilia eximia (Wiedemann, 1819)			12	7	5		24
Fanniidae							
Euryomma carioca (Albuquerque)	1		2	4	7		14
Fannia subgrupo *pusio* (Lopes, 1935)	1	3	29	145	38	56	272
Fannia obscurinervis (Stein)			1				1
Fannia femoralis (Wiedemann, 1819)		1		116		49	166
Fannia saprosky Seago, 1954						2	2
Fannia subgrupo *heydenii*			14		8		22
Muscidae							
Polietina orbitalis (Stein, 1904)			2				2
Neomuscina sp.			8		8		16
Neomuscina zosteris (Shannon & Del Ponte, 1926)			5		4		9
Hydrotaea aenescens (Wiedemann, 1830)				33			33
Neurotrixa sulina Costacurta and Carvalho, 2005					1		1
Myospila sp.			1				1
Philornis sp.					1		1
Synthesiomyia nudiseta (Wulp, 1883)			1	73		114	188
Sarcophagidae							
Peckia (Pattonella) resona (Lopes, 1935)	1		8	12	4	9	34
Sarcophaga crispina (Lopes, 1938)						4	4
Sarcophaga sp1			2				2
Sarcophaga sp2			3		2		5
Drosophilidae							
Drosophila fumipennis (Duda, 1925)	1						1
Drosophila fuscolineata (Duda, 1925)	1						1
Drosophila polymorpha (Dobzhansky & Pavan 1943)	9				2		11
Drosophila willistoni Gleason 1998	73	19				1	93
Drosophila griseolineata (Duda, 1927)	1						1
Drosophila repleta (Wollaston, 1858)	1						1
Drosophila simulans (Sturtevant, 1919)	3				19		22
Drosophila immigrans (Sturtevant, 1921)						1	1
Drosophila melanogaster (Sturtevant, 1927)		3			1		4
Drosophila mercatorum (Paterson & Wheller 1942)	1					1	2
Phoridae							
Megaselia sp.	11		7		5		24
Pulliciphora borinquenensis (Wheeler, 1906)	49				16		65
Dohrniphora sp.	8		7		40		55
Diplonevra sp.	2		4		7		13
Conocephalus sp.	2						2
Rhinotoridae							
Rhinotora sp.			2				2
Mycetophilidae							
Sciophila sp.	2						2
Stratyomidae							
Hermetia illuscens (Linnaeus, 1758)				1		26	26
Milichidae		4		73		242	319
Chloropidae					1		1
Psychodidae	3						3
Ulidiidae	1		3		2		6
Sciaridae	3				1		4
Total	209		1171		1121		2501

(P = 0.345). The highest richness occurred 20 to 24 h earlier in the unburnt treatment than in the burnt plus control treatments ($Chi_{2;77}$=50.451, P<0.001). The abundance differed among all the treatments ($Chi_{2;80}$=12.20, P=0.002), showing a quadratic model over time ($Chi_{1;79}$= 797.26, P<0.001, Fig. 2).

Richness and abundance of colonizers

The richness ($Chi_{2;9}$=9.061; P=0.011) and abundance ($Chi_{2;9}$ = 1244.9; p < 0.001) of colonizers differed between the treatments. According to the GLM showing the effect of the treatment on colonizer species richness, the control treatment (unburnt bovine liver with gasoline) differed from the other treatments (p = 0.017), exhibiting lower richness (Fig. 3A). There was no significant difference between the unburnt and burnt treatments (p = 0.773). According to the GLM for abundance, the three treatments were significantly different from each other, with unburnt showing the highest abundance (p = 0.013), followed by the burnt treatment (p = 0.001) and the control treatment (p = 0.001) (Fig. 3B).

Composition

The fly species composition differed between the control and unburnt plus burnt treatments in the visitor communities (PERMANOVA test, $F_{2;9}$ = 2.106; p = 0.021) and the colonizer communities (PERMANOVA test, $F_{2;8}$ = 2.011, p = 0.037). These differences were visualized using NMDS as a dimension reduction technique to illustrate the species composition among the three treatments (Fig. 4). The unburnt and burnt treatments shared the most abundant species (Table 1), such as *C. albiceps*, *F. pusio*, *F. femoralis*, *S. nudiseta*, and specimens of Milichidae (Table 1, Fig. 4). In contrast, the control treatment was colonized by *D. willistoni* and *D. melanogaster*. Drosophilids dominated the composition of the control treatment, while the scuttle flies did not colonize it.

Discussion

Our findings indicate that burning did not directly affect the community composition of necrophagous flies in southern Rio Grande do Sul, Brazil. However, the timing of species colonization differed between the two treatments (burnt and unburnt). And while species richness was not affected by the treatment, abundance did, suggesting partial support for hypothesis (i). Furthermore, the timing of colonization was influenced by the treatments: there was greater species richness earlier in the unburnt than in the burnt and the control treatment, supporting hypothesis (iii). The community composition in the control treatment differed markedly from the other treatments, in agreement with hypothesis (ii). Both the unburnt and burnt shared many colonizing species, confirming hypothesis (ii)

Our data, derived from experiments using bovine liver, present specific challenges compared to other studies that utilized different animal models with various biomasses and often had low replication samples (Avila and Goff, 1998; Anderson, 2005; Chin et al., 2008; Vanin et al., 2009; Oliveira-Costa et al., 2014; Mashaly, 2016). The main challenges are to ensure consistent samples and to control the burning process. Variations in the decomposition and attraction of insects that feed on dead organisms can occur due to differences in biomass and tissue composition in different animal models. Additionally, studies with low replication can lead to increased variability and less reliable data.

Using bovine liver in our experiments promoted consistency, and allowed for better control of the burning process. This consistency ensured more reliable results by providing uniform samples, which helped reduce the error rates and variability previously noted by McIntosh et al. (2017). Moreover, the sample size in our study provided a robust data set that increased the reliability and validity of our findings, giving us a broader perspective on the community structure and colonization patterns of necrophagous flies. Our findings sometimes diverge from those of previous research. For example, while some studies reported earlier oviposition on burnt remains (Avila and Goff, 1998; Pai et al., 2007; McIntosh et al., 2017), others have observed it occurring later

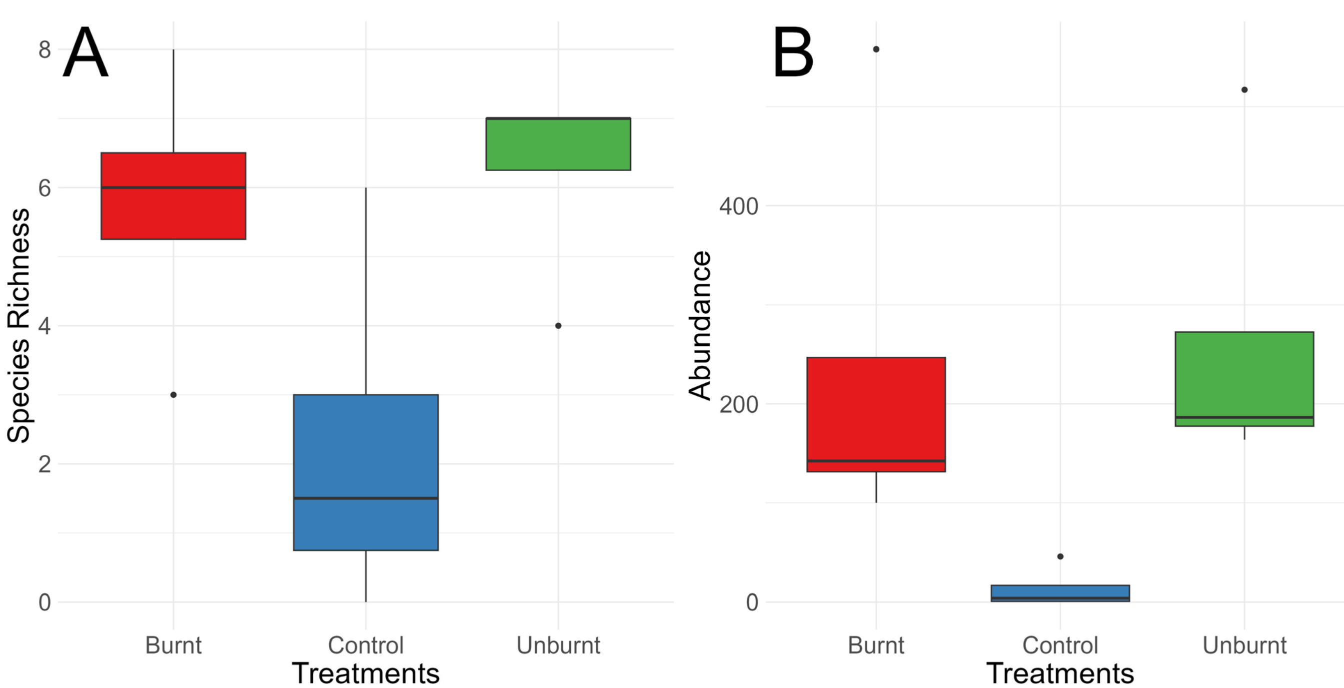

Figure 3 Generalized linear models with Poisson distribution to Species Richness (A) and Abundance (B) of colonizer necrophagous species community at HBITL, Rio Grande do Sul (Brazil). Burnt, burnt bovine liver; Control, bovine liver with gasoline; Unburnt, unburnt bovine liver.

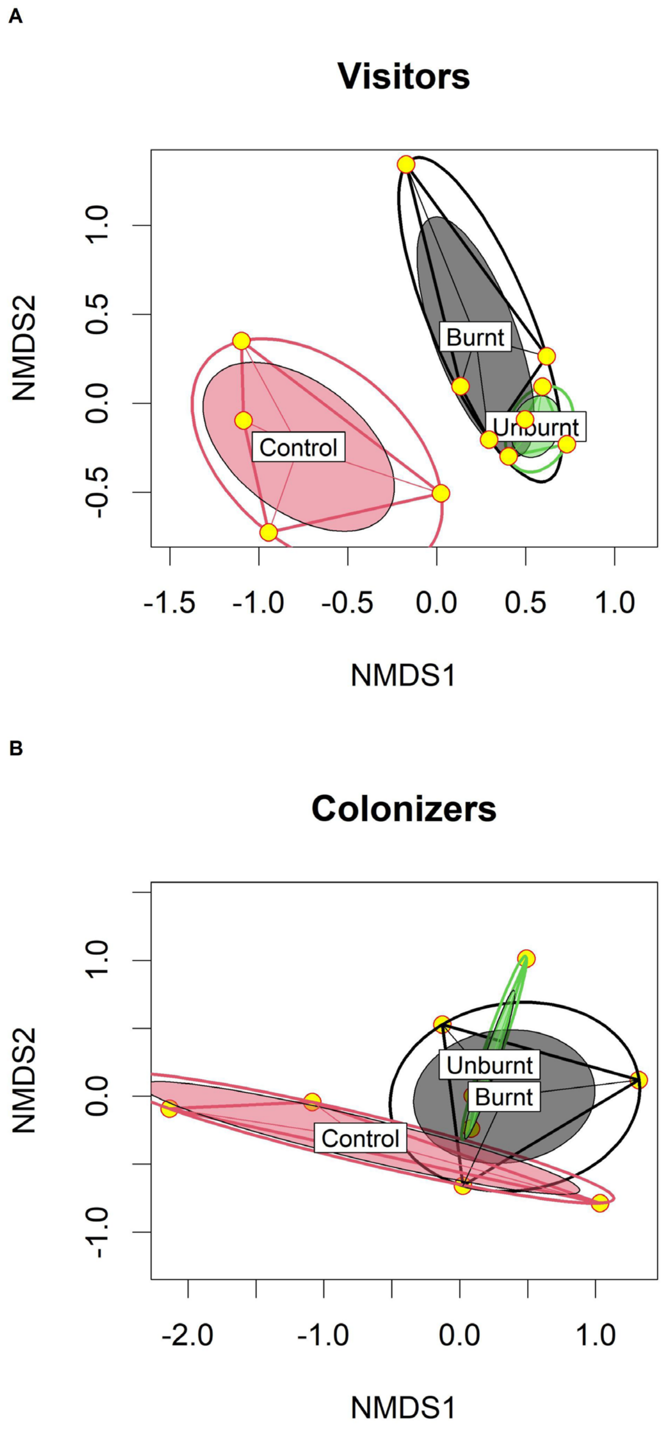

Figure 4 Nonmetric multidimensional scaling (NMDS) ordination map of similarity/dissimilarity for species composition of (A) visitor necrophagous adult flies and (B) colonizer communities at HBITL, Rio Grande do Sul (Brazil) in the three different systems. Burnt, burnt bovine liver; Control, bovine liver with gasoline; Unburnt, unburnt bovine liver.

(Chin et al., 2008; Oliveira-Costa et al., 2014; Mahat et al., 2016). These discrepancies likely stem from variations in the burning intensity and the experimental models employed. In our experiments, burning caused the external surface of the bovine liver to dry out and form a crust.

This crust potentially prevents the release of chemical attractants, as suggested by Wardle (1921) and Oliveira-Costa et al. (2014). The formation of a crust on burnt corpses may impede the release of chemical attractants during decomposition, as observed in the burnt liver. This crust could act as a barrier, hindering the diffusion of volatile organic compounds (VOCs) that attract insects (Dekeirsschieter et al., 2009; Schotsmans et al., 2012; Pascual et al., 2017; Boros et al., 2020; Newsome et al., 2021). Tomberlin et al. (2011) highlighted the pre-colonization interval, which begins at death and extends until arthropods colonize the remains, suggesting that factors affecting this interval (such as microbial presence inhibiting oviposition) are crucial. This interval includes the detection and acceptance phases, where flies respond to stimuli from the remains.

In this context, the crust formed on burned corpses could impact the attraction phase by limiting the dispersal of VOCs and chemical signals. The attraction of necrophagous insects and vertebrate scavengers to carrion is primarily driven by cadaveric odor, which plays a crucial role in this process (Cláudia-Ferreira, 2023). The presence of a crust may interfere with the normal progression of decomposition by affecting the release of these crucial attractants, potentially delaying the arrival of insects as observed in the analysis of visitors. During decomposition, the attraction phase involves the release of chemical signals that draw insects in. If a crust forms, it could impede the diffusion of these attractants, altering the natural course of decomposition.

The release of these cadaveric VOCs influences the succession of insects visiting the carcass, with different species being attracted to the cadaver at various stages of decomposition (Michaud et al., 2010; Johansen et al., 2013). This was not observed in the similarity analyses of the communities in the burnt and unburnt treatments. Furthermore, the decomposition of carrion not only attracts insects but also influences the demography of scavenger populations, interactions, and ecosystem services such as nutrient cycling and disease moderation (Walker et al., 2021). The process of decomposition creates well-defined cadaver decomposition islands, with microbial activity contributing to the attraction of scavengers and the recycling of nutrients (Boros et al., 2020).

However, at higher levels of burning on this scale, where organs and viscera are exposed, a greater attraction of flies to burnt carcasses is generally expected, as reported by Campobasso et al. (2001) and Vanin et al. (2009). Nonetheless, observations by Mahat et al. (2016) at level 3 of the CG scale challenge this assumption. *Chrysomya megacephala* and *Chrysomya rufifacies* were found to delay oviposition by one day in corpses burnt to CGS level 3 when compared to control carcasses in the study done by Mahat et al. (2016). CGS levels 1 and 2 showed no signs of this delay. Comparatively speaking, the CGS level 3 burnt carcasses were drier and had less bodily fluids that were leaking than the control and CGS levels 1 and 2. The decomposing smell coming from the carcass, which is one of the main things that draws flies, was probably lessened by the increased charring of tissues at CGS level 3. These results show the variety of parameters impacting fly attraction and oviposition on burnt corpses, challenging the notion that higher burning levels inevitably result in increased attraction of flies to carcasses.

Our study corroborates the critical role of burning in influencing colonization and abundance in forensic entomology, consistent with previous work by Catts and Goff (1992), Introna et al. (1998), Anderson (2010), and Amendt et al. (2007). Despite conflicting results regarding the impact of burning on early colonization stages, our research supports the hypothesis that fire does not significantly prevent fly colonization, as similar richness and abundance patterns were observed across both burnt and unburnt treatments.

Our comparative analysis revealed that both burnt and unburnt bovine liver provide more favorable habitats for necrophagous species such as blowflies, little houseflies, and houseflies than does unburnt liver treated with gasoline, where the community composition shifted toward drosophilid and scuttle flies. This pattern of similarity in the communities between burnt and unburnt (untreated) liver corroborates earlier observations reported by Avila and Goff (1998) and Chin et al. (2008) and highlights the adaptability of certain species to environments after burning.

The most prevalent species in our study, such as *C. albiceps*, *F. pusio*, *F. femoralis*, and *S. nudiseta*, along with other members of the Calliphoridae, Muscidae, Fanniidae, and Sarcophagidae families, have previously been associated with decomposing animal remains in southern Brazil (Vianna et al., 1998; Souza et al., 2008; Azevedo and Krüger, 2013; Zafalon-Silva et al., 2014). Notably, *C. albiceps* is recognized as the most important forensic indicator in southern Brazil, Uruguay, and parts of Argentina (Souza et al., 2008; Aballay et al., 2008, 2012; Krüger et al., 2010; Patitucci et al., 2015; Mulieri et al., 2015; Castro et al., 2019; Remedios-DeLeon et al., 2019). These findings reinforce that these species are also crucial forensic indicators for burned organic remains.

In conclusion, this study clearly demonstrated how fire influences the colonization patterns and community dynamics of necrophagous flies, which are crucial elements in forensic entomology. While fire affects the timing of colonization and can change habitat suitability, it does not significantly reduce fly colonization in terms of species richness and abundance. Importantly, the observed variability in the responses of flies to burnt, unburnt, and gasoline-treated liver underscores their adaptability to various postfire conditions. The consistent findings across different experimental conditions underscore the potential of these results to refine methods for estimating the postmortem interval (PMI) in forensic cases involving fire-damaged remains. Therefore, it is imperative to conduct further research to uncover the biochemical and ecological mechanisms driving the observed patterns of colonization and community structure. Such studies will deepen our understanding of forensic entomology and improve the precision of forensic investigations in scenarios impacted by fire.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

RSM conceived the project, collected the data, reared the flies in the laboratory, analysed the data, and wrote the final version of the manuscript. MGMP contributed to species identification and the writing of the final manuscript. LSF assisted with data analysis and writing the final version of the manuscript. JLPD collected and identified the material and contributed to writing the preliminary versions of the manuscript. AZS contributed to species identification, data analysis, and writing the final version of the manuscript. RM collected the data, contributed to the trap design, and assisted with data analysis. FDK contributed to the experimental design, data analysis, and writing of the final version of the manuscript. RFK coordinated the work, conceived the sampling design and trap for field collection, analysed the data, and contributed to writing the final version of the manuscript.

References

Aballay, F.H., Murua, A.F., Acosta, J.C., Centeno, N.D., 2008. Primer registro de artropodofauna cadavérica em sustratos humanos y animales en San Juan, Argentina. Rev. Soc. Entomol. Argent. 67, 157-163.

Aballay, F.H., Murua, A.F., Acosta, J.C., Centeno, N.D., 2012. Succession of carrion fauna in the arid region of San Juan Province, Argentina and Its Forensic Relevance. Neotrop. Entomol. 41, 27-31. https://doi.org/10.1007/s13744-011-0005-9.

Acikgoz, H.N., 2016. Malpractices concerning collection of forensic entomological evidence and importance of standardization in Turkey. Forensic Res. Criminol. Int. J. 3 (1), 237-241. https://doi.org/10.15406/frcij.3.1.

Albuquerque, D.D.O., Pamplona, D., Carvalho, C.J.B., 1981. Contribuição ao conhecimento dos *Fannia* R-D, 1830 da região neotropical (Diptera, Fanniidae). Arch. Mus. Nac. 56, 9-34.

Amendt, J., Campobasso, C.P., Gaudry, E., Reiter, C., LeBlanc, H.N., J.R. Hall, M., 2007. Best practice in forensic entomology - Standards and guidelines. Int. J. Legal Med. 121, 90-104. https://doi.org/10.1007/s00414-006-0086-x.

Amendt, J., Richards, C., Campobasso, C., Zehner, R., Hall, M., 2011. Forensic entomology: applications and limitations. Forensic Sci. Med. Pathol. 7 (4), 379-392. https://doi.org/10.1007/s12024-010-9209-2.

Anderson, G.S., 2005. Effects of arson on forensic entomology evidence. J. Can. Soc. Forensic Sci. 38, 49-67. https://doi.org/10.1080/00085030.2005.10757584.

Anderson, G.S., 2010. Factors That Influence Insect Succession on Carrion. In: Byrd J.H., Castner J.L. (Eds.), Forensic Entomology: The Utility of Arthropods in Legal Investigations. CRC Press, Boca Raton, pp. 143–176.

Avila, F., Goff, M.L., 1998. Arthropod succession patterns onto burnt carrion in two contrasting habitats in the Hawaiian Islands. J. Forensic Sci. 43, 581-586. https://doi.org/10.1520/jfs16184j.

Azevedo, R.R., Krüger, R.F., 2013. The influence of temperature and humidity on abundance and richness of Calliphoridae (Diptera) | A influência da temperatura e umidade na abundância e na riqueza de Calliphoridae (Diptera). Iheringia Ser. Zool. 103, 145-152. https://doi.org/10.1590/S0073-47212013000200010.

Battán Horenstein, M., Linhares, A.X., Rosso De Ferradas, B., García, D., 2010. Decomposition and dipteran succession in pig carrion in central Argentina: ecological aspects and their importance in forensic science. Med. Vet. Entomol. 24, https://doi.org/10.1111/j.1365-2915.2009.00854.x.

Boros, G., Czeglédi, I., Erős, T., Preiszner, B., 2020. Scavenger-driven fish carcass decomposition and phosphorus recycling: laboratory experiments with freshwater fish and crayfish. Freshw. Biol. 65 (10), 1740-1751. https://doi.org/10.1111/fwb.13576.

Brown, B., Kung, G.A., 2007. Revision of the New World *Dohrniphora* Dahl species with hind tibial setae (Diptera: phoridae). Arthropod Syst. Phylogeny 65, 155-235. https://doi.org/10.3897/asp.65.e31675.

Brown, B.V., Kung, G.A., 2010. Revision of the New World *Dohrniphora* Dahl species lacking large hind tibial setae (Diptera: phoridae). Zootaxa 2699, 1-142. https://doi.org/10.11646/zootaxa.2699.1.1.

Byrd, J.H., Castner, J.L., 2010. Forensic Entomology: The Utility of Arthropods in Legal Investigations Forensic Entomology, 2nd ed. CRC Press, Boca Raton, 681pp.

Campobasso, C.P., Di Vella, G., Introna, F., 2001. Factors affecting decomposition and Diptera colonization. Forensic Sci. Int. 120, 18-27. https://doi.org/10.1016/S0379-0738(01)00411-X.

Carvalho, C.J.B., Ribeiro, P.B., 2000. Chave de identificação das espécies de Calliphoridae (Diptera) do sul do Brasil. Rev. Bras. Parasitol. Vet. 9, 169-173.

Carvalho, C.J.B., Couri, M.S., 2002 Part I. Basal groups. In: Carvalho, C.J.B. (ed.). Muscidae (Diptera) of the Neotropical Region: taxonomy. Editora Universidade Federal do Paraná, Curitiba, Brazil, pp. 17-132.

Carvalho, C.J.B., Mello-Patiu, C.A., 2008. Key to adults of the most common forensic species of Diptera in South America. Rev. Bras. Entomol. 52, 390-406. https://doi.org/10.1590/S0085-56262008000300012.

Carvalho, C.J.B., Rafael, J.A., Couri, M.S., Silva, V.C., 2012. Diptera. In: Rafael, J.A., Melo, G.A.R., Carvalho, C.J.B., Casari, S.A., Constantino, R. (eds.). Insetos do Brasil. Holos, Ribeirão Preto, pp. 701–744.

Castro, M., Centeno, N., González-Vainer, P., 2019. An initial study of insect succession on pig carcasses in open pastures in the northwest of Uruguay. Forensic Sci. Int. 302, 109837. https://doi.org/10.1016/j.forsciint.2019.05.053.

Catts, E., 1992. Problems in estimating the postmortem interval in death investigations. J. Agric. Entomol. 9, 245-255.

Catts, E.P., Goff, M.L., 1992. Forensic entomology in criminal investigations. Annu. Rev. Entomol. 37, 253-272. https://doi.org/10.1146/annurev.en.37.010192.001345.

Charabidze, D., Bourel, B., Hedouin, V., Gosset, D., 2009. Repellent effect of some household products on fly attraction to cadavers. Forensic Sci. Int. 189, 28-33. https://doi.org/10.1016/j.forsciint.2009.04.009.

Chin, H.C., Marwi, M.A., Salleh, A.F.M., Jeffery, J., Kurahashi, H., Omar, B., 2008. Study of insect succession and rate of decomposition on a partially burned pig carcass in an oil palm plantation in Malaysia. Trop. Biomed. 25, 202-208.

Cláudia-Ferreira, A., 2023. The future is now: unraveling the expanding potential of human (necro) microbiome in forensic investigations. Microorganisms 11 (10), 2509. https://doi.org/10.3390/microorganisms11102509.

Couri, M.S., Carvalho, C.J.B., 2002. Part II. Apical groups. In: Carvalho, C.J.B. (Ed.), Muscidae (Diptera) of the Neotropical Region: Taxonomy. Editora Universidade Federal do Paraná, Curitiba, pp. 133-262.

Dekeirsschieter, J., Verheggen, F., Gohy, M., Hubrecht, F., Bourguignon, L., Lognay, G., Haubruge, É., 2009. Cadaveric volatile organic compounds released by decaying pig carcasses (*Sus domesticus* l.) in different biotopes. Forensic Sci. Int. 189 (1-3), 46-53. https://doi.org/10.1016/j.forsciint.2009.03.034.

Disney, R.H.L., 2008. Review of neotropical *Chonocephalus* Wandolleck (Diptera: phoridae). Zootaxa 1772, 1-54. https://doi.org/10.11646/zootaxa.1772.1.1.

Estação Agroclimatológica de Pelotas, 2013. Available in: http://www.cpact.embrapa.br/agromet/estacao/boletim.html/ (accessed 20 July 2013).

Franceschetti, L., Pradelli, J., Tuccia, F., Giordani, G., Cattaneo, C., Vanin, S., 2021. Comparison of accumulated degree-days and entomological approaches in post mortem interval estimation. Insects 12 (3), 264. https://doi.org/10.3390/insects12030264.

Gruenthal, A., Moffatt, C., Simmons, T., 2012. Differential decomposition patterns in charred versus un-charred remains. J. Forensic Sci. 57, 12-18. https://doi.org/10.1111/j.1556-4029.2011.01909.x.

Hwang, C., Turner, B.D., 2005. Spatial and temporal variability of necrophagous Diptera from urban to rural areas. Med. Vet. Entomol. 19, 379-391. https://doi.org/10.1111/j.1365-2915.2005.00583.x.

INMET, 2018. Available in: http://www.inmet.gov.br/projetos/rede/pesquisa/gera_serie_txt_mensal.php?&mRelEstacao=83985&btnProcesso=serie&mRelDtInicio=01/01/2013&mRelDtFim=31/12/2013&mAtributos=,1,/ (accessed 11 April 2018).

Introna, F., Campobasso, C., Di Fazio, A., 1998. Three case studies in forensic entomology from Southern Italy. J. Forensic Sci. 43, 210-214. https://doi.org/10.1520/jfs16117j.

Johansen, H., Solum, M., Knudsen, G., Hågvar, E., Norli, H., Aak, A., 2013. Blow fly responses to semiochemicals produced by decaying carcasses. Med. Vet. Entomol. 28 (1), 26-34. https://doi.org/10.1111/mve.12016.

Krüger, R.F., Kirst, F.D., de Souza, A.S.B., 2010. Rate of development of forensically-important Diptera in southern Brazil. Rev. Bras. Entomol. 54, 624-629. https://doi.org/10.1590/S0085-56262010000400014.

Mahat, N.A., Zainol-Abidin, N.L., Nordin, N.H., Abdul-Wahab, R., Jayaprakash, P.T., 2016. Patterns of oviposition and development of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) on burned rabbit carcasses. Forensic Sci. Int. 260, 9-13. https://doi.org/10.1016/j.forsciint.2015.12.047.

Mashaly, A.M.A., 2016. Entomofaunal succession patterns on burnt and unburnt rabbit carrion. J. Med. Entomol. 53, 296-303. https://doi.org/10.1093/jme/tjv202.

McIntosh, C.S., Dadour, I.R., Voss, S.C., 2017. A comparison of carcass decomposition and associated insect succession onto burnt and unburnt pig carcasses. Int. J. Legal Med. 131, https://doi.org/10.1007/s00414-016-1464-7.

Michaud, J., Majka, C., Privé, J., Moreau, G., 2010. Natural and anthropogenic changes in the insect fauna associated with carcasses in the north american maritime lowlands. Forensic Sci. Int. 202 (1-3), 64-70. https://doi.org/10.1016/j.forsciint.2010.04.028.

Mulieri, P.R., Patitucci, L.D., Olea, M.S., 2015. Sex-biased patterns of saprophagous Calyptratae (Diptera) collected with different baits of animal origin. J. Med. Entomol. 52, 1-8. https://doi.org/10.1093/jme/tjv031.

Newsome, T., Barton, B., Buck, J., DeBruyn, J., Spencer, E., Ripple, W., Barton, P., 2021. Monitoring the dead as an ecosystem indicator. Ecol. Evol. 11 (11), 5844-5856. https://doi.org/10.1002/ece3.7542.

Oksanen, J.F., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2020. vegan: Community Ecology Package. R package version 2.5-7. Available in: https://CRAN.R-project.org/package=vegan (accessed 11 April 2018).

Oliveira-Costa, J., Lamego, C., Couri, M., Mello-Patiu, C., 2014. Differential Diptera succession patterns onto partially burned and unburned pig carrion in southeastern Brazil. Braz. J. Biol. 74, 870-876. https://doi.org/10.1590/1519-6984.06113.

Pai, C.Y., Jien, M.C., Li, L.H., Cheng, Y.Y., Yang, C.H., 2007. Application of forensic entomology to postmortem interval determination of a burned human corpse: A homicide case report from Southern Taiwan. J. Formos. Med. Assoc. 106, 792-798. https://doi.org/10.1016/S0929-6646(08)60043-1.

Pascual, J., Hoermann, C., Rottler-Hoermann, A., Nevo, O., Geppert, A., Sikorski, J., Overmann, J., 2017. Function of bacterial community dynamics in the formation of cadaveric semiochemicals during in situ carcass decomposition. Environ. Microbiol. 19 (8), 3310-3322. https://doi.org/10.1111/1462-2920.13828.

Patitucci, L.D., Mulieri, P.R., Do Mínguez, M.C., Mariluis, J.C., 2015. An inventory of saprophagous Calyptratae (Insecta: Diptera) in urban green spaces of Buenos Aires City. Rev. Mus. Argent. Cienc. Nat., 17, 97–107. https://doi.org/10.22179/revmacn.17.385

R Development Core Team, 2018. R: a language and environment for statisti- cal computing. Available in: http://www.R-project.org (accessed 11 April 2018).

Remedios-DeLeon, M., Castro, M., Morelli, E., 2019. Artropodofauna cadavérica associada a *Sus scrofa* L. en el sur de Uruguay. Bol. Soc. Zool. Urug. 28, 21-28.

Rumiza, A.R., Khairul, O., Zuha, R.M., Heo, C.C., 2010. An observation on the decomposition process of gasoline-ingested monkey carcasses in a secondary forest in Malaysia. Trop. Biomed. 27 (3), 373-383.

Schotsmans, E., Denton, J., Dekeirsschieter, J., Ivaneanu, T., Leentjes, S., Janaway, R., Wilson, A., 2012. Effects of hydrated lime and quicklime on the decay of buried human remains using pig cadavers as human body analogues. Forensic Sci. Int. 217 (1-3), 50-59. https://doi.org/10.1016/j.forsciint.2011.09.025.

Smith, K.G.V.A., 1986 Manual of Forensic Entomology. Cornell University Press, Ithaca, USA, 205 pp.

Souza, A.S.B., Kirst, F.D., Krüger, R.F., 2008. Insects of forensic importance from Rio Grande do Sul state in southern Brazil. Rev. Bras. Entomol. 52, 641-646. https://doi.org/10.1590/S0085-56262008000400016.

Tomberlin, J.K., Benbow, M.E., Tarone, A.M., Mohr, R.M., 2011. Basic research in evolution and ecology enhances forensics. Trends Ecol. Evol. 26, 53-55. https://doi.org/10.1016/j.tree.2010.12.001.

Tomberlin, J.K., Byrd, J.H., Wallace, J.R., Benbow, M.E., 2012. Assessment of decomposition studies indicates need for standardized and repeatable research methods in forensic entomology. J. Forensics Res. 3, 147.

Vairo, K.P., de Mello-Patiu, C.A., de Carvalho, C.J.B., 2011. Pictorial identification key for species of Sarcophagidae (Diptera) of potential forensic importance in southern Brazil. Rev. Bras. Entomol. 55, 333-347. https://doi.org/10.1590/S0085-56262011005000033.

Vanin, S., Caenazzo, L., Arseni, A., Cecchetto, G., Cattaneo, C., Turchetto, M., 2009. Records of *Chrysomya albiceps* in Northern Italy: an ecological and forensic perspective. Mem. Inst. Oswaldo Cruz 104, 555-557. https://doi.org/10.1590/S0074-02762009000400003.

Venzke, T.S., 2012. Florística de comunidades arbóreas no Município de Pelotas, Rio Grande do Sul. Rodriguésia 63, 571-578.

Vianna, E.E.S., Brum, J.G.W., Ribeiro, I.B., Berne, M.E.A., Silveira Junior, P., 1998. Synanthropy of Calliphoridae (Diptera) in Pelotas, Rio Grande do Sul state, Brazil. Rev. Bras. Parasitol. Vet. 7, 141-147.

Walker, M., Uribasterra, M., Asher, V., Getz, W., Ryan, S., Ponciano, J., Blackburn, J., 2021. Factors influencing scavenger guilds and scavenging efficiency in southwestern Montana. Sci. Rep. 11 (1), https://doi.org/10.1038/s41598-021-83426-3.

Wardle, R.A., 1921. The protection of meat commodities against blowflies. Ann. Appl. Biol. 8, 1-9.

Wendt, L.D., Carvalho, C.D., 2009. Taxonomia de Fanniidae (Diptera) do sul do Brasil–II: Novas espécies e chave de identificação de *Fannia* Robineau-Desvoidy. Rev. Bras. Entomol. 53, 171-206.

Zafalon-Silva, Â., Hoffmeister, C.H., Anjos, V.A., Ribeiro, P.B., Krüger, R.F., 2014. Necrophagous Diptera associated with wild animal carcasses in southern Brazil. Rev. Bras. Entomol. 58, 337-342.

Supplementary material

The following online material is available for this article:

Figure S1 The “Horto Botânico Irmão Teodoro Luis (HBITL)”, Universidade Federal de Pelotas (UFPEL), in the municipality of Capão do Leão.

Figure S2 Trap used for the collection of insects in the Horto Botânico Irmão Teodoro Luis (HBITL), Universidade Federal de Pelotas (UFPEL), in the municipality of Capão do Leão.