Necrophagous Diptera associated with wild animal carcasses in southern Brazil

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ABSTRACT. Necrophagous Diptera associated with wild animal carcasses in southern Brazil. The aim of this study was to acquire a better knowledge concerning the diversity of necrophagous Diptera that develop on wild animal carcasses. For this purpose, the decomposition of six wild animal carcasses was observed in order to collect and identify the main species of necrophagous flies associated with the decomposition process. The carcasses were found on highways near the cities of Pelotas and Capão do Leão in the initial stage of decomposition, with no significant injuries or prior larval activity. Four wild animal models were represented in this study: two specimens of Didelphis albiventris Lund, 1840; two Tupinambis meriana Linnaeus, 1758; one Nothura maculosa Temminck, 1815; and one Cerdocyon thous Linnaeus, 1766. A total of 16,242 flies from 14 species were reared in the laboratory, where Muscidae presented the greatest diversity of necrophagous species. Overall, (i) carcasses with larger biomass developed a higher abundance of flies and (ii) the necrophagous community was dominated by Calliphoridae, two patterns that were predicted from published literature; and (iii) the highest diversity was observed on the smaller carcasses exposed to the lowest temperatures, a pattern that may have been caused by the absence of the generalist predator Chrysomya albiceps (Wiedemann, 1819). (iv) An UPGMA analysis revealed a similar pattern of clusters of fly communities, where the same species were structuring the groupings.

KEYWORDS. Calliphoridae; community, diversity; flies; Insecta.

Wild animal carcasses do not remain exposed to the environment for a long time because they are rapidly decomposed. Decomposition is a natural process that is important for ecosystem nutrient cycling (Hanski 1987). Among organisms that contribute to the biomass cycle, the order Diptera is highly important because it is the first to reach carcasses, where the adult stage will feed and oviposit minutes after an animal’s death. In addition, the immature stages of many species use carcasses as a development site (Smith 1986). These biological characteristics make necrophagous Diptera important in applied sciences because they are vectors of pathogens that may cause myiasis and are useful in forensic entomology. Furthermore, knowledge of the ecological dynamics of necrophagous communities is very important to establish the role of necrophagous species in animal resources (Zumpt 1965; Greenberg 1971; Catts & Goff 1992; Watson & Carlton 2003; Byrd & Castner 2010).

Thus, knowledge of necrophagous communities is often applied in forensic entomology, with pig carcasses being the animal model used in the majority of the studies. However, other animal models have been studied, such as chickens (Hall & Dotsy 1993), rats (Monteiro-Filho & Penerere 1987), dogs (Johnson 1975), frogs and lizards (Cornaby 1974), rabbits (Souza et al. 2008), elephants (Smith 1986), bears (Anderson 1999; Watson & Carlton 2003, 2005), and alligators (Watson & Carlton 2003, 2005). Thus, the diversity of animal models studied raises the following question: Can the use of non-traditional forensic models (different classes: Mammalia, Aves, Reptilia) influence the diversity of necrophagous Diptera?

Considering the variety of models, one hypothesis is that the diversity of necrophagous insects found may be influenced by the histological constitution of the carcasses used. Therefore, the aim of the present study was to contribute to a clearer understanding of the necrophagous Diptera diversity that develops on wild animal carcasses in southern Brazil.

MATERIAL AND METHODS

The field site of exposure was located in a semi-urban area (31°48’00.31”S, 52°25’04.66”W) of the town Capão do Leão, and is characterized by pasture with grasses, herbs, and shrubs surrounded by woods of Eucalyptus spp.

Six carcasses were collected on the highways BR-392 and BR-116 near the access road to UFPel (Universidade Federal de Pelotas) and were donated to the Laboratory of Insect Biology and Taxonomy. These carcasses were two white-eared opossums, Didelphis albiventris Lund, 1840 (Mammalia, Didelphidae), weighing 0.68 kg (exposed in June 2008) and 3.00 kg (November 2009); two lizards, Tupinambis merianaæ

Linnaeus, 1758 (Reptilia, Teiidae), weighing 1.56 kg (November 2009) and 1.69 kg (December 2008); one spotted nothura, *Nothura maculosa* Temminck, 1815 (Aves, Tinamidae) weighing 0.29 kg (September 2008); and one crab-eating fox, *Cerdocyon thous* Linnaeus, 1766 (Mammalia, Canidae), weighing 5.60 kg (November 2009). All models used in the experiments arrived at the laboratory in the early stages of decomposition, apparently intact, and without serious injuries or prior larval activity. The white-eared opossum (November 2009) arrived frozen at the laboratory and had external bruises or prior larval activity. The white-eared opossum from November 2009. Table II shows the abundance of Diptera specimens collected in Pelotas, Rio Grande do Sul, Brazil.

The emerged adult insects were killed and preserved in 70% alcohol for later identification. The adult dipteran identification followed the taxonomic keys of Carvalho & Ribeiro (2000), Carvalho (2002), Carvalho & Mello-Patiu (2008), Wendt & Carvalho (2009), and Vairo et al. (2011). The voucher specimens were deposited in the Diptera collection of the Laboratory of Parasite and Vectors Ecology (UFPe) and in the Padre Jesus Santiago Moura entomological collection of the Federal University of Parana (UFPR).

An agglomerative hierarchical classification multivariate analysis with UPGMA (Unweight Pair-Groups Method using Arithmetic Averages) was used to verify the relationship between the dipteran communities among carcasses models and the connection between dipteran communities among times of year using the dissimilarity coefficient of Bray-Curtis such as in Sneath & Sokal (1973). The statistical software R (R Development Core Team 2013) with *vegan* and *stats* packages was used for the analysis of the co-relationship matrix with the *vegdist* function and the dissimilarity between the groups with the *hclust* function (Borcard et al. 2011). A so-called heatmap analysis was also applied to the Spearman correlation matrix. A heatmap is a two-dimensional visualization technique for high-dimen-

### RESULTS

A total of 16,242 adult specimens of 14 species (Table II) were reared from the six carcasses (Table I). The relationship between vertebrate carcass mass and the abundance of specimens was directly proportional except for one lizard model (Table I), which had few Diptera specimens. The number of species varied according to the carcass model, and the greatest number of species was observed in the opossum weighing 0.68 kg that had been exposed to the lowest average temperature registered (12.7°C).

<table>
<thead>
<tr>
<th>Vertebrate carcass</th>
<th>Biomass</th>
<th>Month/Year</th>
<th>Average Temperature</th>
<th>Total Abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cerdocyon thous</em> (C1)</td>
<td>5.60 Kg</td>
<td>Dec/09</td>
<td>21.6°C</td>
<td>8,583</td>
</tr>
<tr>
<td><em>Didelphis albiventris</em> (D1)</td>
<td>0.69 Kg</td>
<td>Jun/08</td>
<td>12.7°C</td>
<td>2,273</td>
</tr>
<tr>
<td><em>Didelphis albiventris</em> (D2)</td>
<td>3.00 Kg</td>
<td>Nov/09</td>
<td>21.6°C</td>
<td>3,383</td>
</tr>
<tr>
<td><em>Nothura maculosa</em> (N1)</td>
<td>0.29 Kg</td>
<td>Sep/09</td>
<td>15.0°C</td>
<td>1,267</td>
</tr>
<tr>
<td><em>Tupinambis merianae</em> (T1)</td>
<td>1.56 Kg</td>
<td>Nov/09</td>
<td>21.6°C</td>
<td>563</td>
</tr>
<tr>
<td><em>Tupinambis merianae</em> (T2)</td>
<td>1.69 Kg</td>
<td>Dec/09</td>
<td>22.6°C</td>
<td>173</td>
</tr>
</tbody>
</table>

The four Diptera families were present in all carcasses, except for Sarcophagidae, which was not observed in the opossum from November 2009. Table II shows the abundance of necrophagous species reared on vertebrate carcasses, where Calliphoridiae was the most frequent family found, followed by Sarcophagidae, Muscidae, and Fanniidae.

The Calliphoridae species *Chrysomya albiceps* (Wiedemann, 1819), *Hemilucilia semiadiaphana* (Rondani, 1850), and *Lucilia eximia* (Wiedemann, 1819) were numerically dominant, representing 92.8% of Diptera collected in this study. The blowfly *C. albiceps* occurred exclusively on the crab-eating fox from December 2009 and the opossum from November 2009. These carcasses shared two variables: (i) a mass greater than 3.00 kg and (ii) the same average temperature during exposure (21.6°C) (Figs. 1, 2). *Hemilucilia semiadiaphana* predominated in carcasses below 3 kg and *L. eximia* in carcasses below 1 kg. These associations favored the clusters observed (Fig. 1).

The second most abundant family was Sarcophagidae, surpassing Calliphoridiae only in the lizard from December
Necrophagous Diptera associated with wild animal carcasses in southern Brazil


Microcerella halli (Engel, 1931) was observed exclusively in the lizard from November 2009, with two specimens.

Sarcophaga (Liopygia) crassipalpis (Macquart, 1839) was observed only in the opossum from June 2008, with an abundance of 267 specimens.

Peckia (Patonella) resona (Lopes, 1935) occurred in multiple carcasses at distinct abundances: two specimens in the crab-eating fox, 10 in the spotted nothura, one in the lizard from November 2009, and 126 from the other lizard (December 2008).

Muscidae was the most diverse family, predominantly species with facultative predatory larvae in the third instar. Ophyra aenescens (Wiedemann, 1830) and O. albuquerquei Lopes,

Figs 1–2. Heatmaps. 1. Heatmap showing abundances within the 14 species among the carcasses communities. The species heatmap displays raw species counts per carcass, where the counts are colored based on the contribution of each species to the total species count present in that carcass, weak colored shades indicates contributions with low percentage of species to sample, whereas strong colored shades contributes for a high percentage of species. D1, Didelphis albiventris (Mammalia, Didelphidae), weighing 0.68 kg; D2, Didelphis albiventris, weighing 3.00 kg; T1, Tupinambis merianae (Reptilia, Teiidae), weighing 1.56 kg; T2, Tupinambis merianae, weighing 1.69 kg; N1, Nothura maculosa (Aves, Tinamidae) weighing 0.29 kg; C1 Cerdocyon thous (Mammalia, Canidae) weighing 5.60 kg. 2. Heatmap showing abundances within the 14 species among the carcasses exposure month of the year. The species heatmap displays raw species counts per carcass, where the counts are colored based on the contribution of each species to the total species count present in that carcass, weak colored shades indicates contributions with low percentage of species to sample, whereas strong colored shades contributes for a high percentage of species.

Table II. Necrophagous Diptera species and their abundance in vertebrates carcasses, in Pelotas, Rio Grande do Sul, Brazil. See Table I for codes of carcasses.

<table>
<thead>
<tr>
<th>Diptera</th>
<th>Vertebrate carcasses</th>
<th>CI</th>
<th>D1</th>
<th>D2</th>
<th>N1</th>
<th>T1</th>
<th>T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calliphoridae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysomya albiceps (Wiedemann, 1819)</td>
<td></td>
<td>8177</td>
<td>0</td>
<td>237</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemilucilia segmentaria (Fabricius, 1805)</td>
<td></td>
<td>0</td>
<td>401</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hemilucilia semidiaphana (Rondani, 1850)</td>
<td></td>
<td>307</td>
<td>0</td>
<td>3057</td>
<td>0</td>
<td>541</td>
<td>0</td>
</tr>
<tr>
<td>Lucilia exigua (Wiedemann, 1819)</td>
<td></td>
<td>0</td>
<td>1473</td>
<td>0</td>
<td>1230</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td>Fanniidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fannia femoralis (Stein, 1897)</td>
<td></td>
<td>64</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Muscidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Musca domestica Linnaeus, 1758</td>
<td></td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Musca stabulans (Fallén, 1817)</td>
<td></td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Ophyra aenescens (Wiedemann, 1830)</td>
<td></td>
<td>33</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Ophyra albuquerquei Lopes, 1985</td>
<td></td>
<td>0</td>
<td>0</td>
<td>88</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Psilochaeta pampiana (Shannon &amp; Del Ponte, 1926)</td>
<td></td>
<td>0</td>
<td>65</td>
<td>0</td>
<td>24</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Stomoxys calcitrans (Linnaeus, 1758)</td>
<td></td>
<td>0</td>
<td>50</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sarcophagidae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microcerella hallii (Engel, 1931)</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Peckia (Patonella) resona (Lopes, 1935)</td>
<td></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>1</td>
<td>126</td>
</tr>
<tr>
<td>Sarcophaga (Liopygia) crassipalpis (Macquart, 1839)</td>
<td></td>
<td>0</td>
<td>267</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

2008. Microcerella hallii (Engel, 1931) was observed exclusively in the lizard from November 2009, with two specimens. Sarcophaga (Liopygia) crassipalpis (Macquart, 1839) was observed only in the opossum from June 2008, with an abundance of 267 specimens. Peckia (Patonella) resona (Lopes, 1935) occurred in multiple carcasses at distinct abundances: two specimens in the crab-eating fox, 10 in the spotted nothura, one in the lizard from November 2009, and 126 from the other lizard (December 2008).

Muscidae was the most diverse family, predominantly species with facultative predatory larvae in the third instar. Ophyra aenescens (Wiedemann, 1830) and O. albuquerquei Lopes,
1985 were observed in the crab-eating fox, the opossum from November 2009, and both lizards (December 2008 and November 2009). The most abundant Muscidae was Psilochaeta pampiana (Shannon & Del Ponte, 1926), with 65 specimens in the opossum from June 2008, 24 specimens in the spotted nothura, and two specimens in the lizard from November 2009. The house fly Musca domestica (Linnaeus, 1758) was reared in the opossum from June 2008 and the spotted nothura, the two smaller carcasses exposed to the lowest temperatures (Table I). Two specimens of M. domestica completed their development, one in the opossum from June 2008 and the other in the spotted nothura. The false stable fly Muscina stabulans (Fallén, 1817) was found 10 times: nine adults in the opossum from June 2008 and one in the spotted nothura. There were 50 specimens of Stomoxys calcitrans (Linnaeus, 1758) found exclusively in opossum from June 2008, where they were the second most abundant Muscidae in the carcass.

Fanniidae was reared in lower abundances, F. femoralis being the only species represented in the present study, although it was found in all carcasses.

**DISCUSSION**

Carcasses with larger biomass developed a higher abundance of flies, as predicted by other authors (Moura et al. 2005; Simmons et al. 2010), except for the lizard carcasses. In ephemeral substrates, the majority of species occur at low density, presenting few species with elevated abundance, which is a common pattern in nature (Preston 1962). The communities were dominated by species with low competitiveness, except C. albiceps in carcasses with a biomass above 1 kg (Moura et al. 2005; Salazar 2006; Souza et al. 2008), where this species was a very good competitor. There was also no relationship between the size of the carcass and the structure of the necrophagous insect community (Moura et al. 2005). Differences in the structure of these communities in ephemeral resources are probably attributable to seasonal variations in temperature and relative humidity (Campobasso et al. 2001) (Fig. 2).

However, two consistent patterns of occurrence have been observed for species of necrophagous flies in southern Brazil, the first being the pattern of synchronic behavior between C. albiceps and L. eximia. The blowfly L. eximia shows a regular occurrence during the whole year; however, in cold seasons C. albiceps visits carcasses only at a low abundance (Souza et al. 2008). When the temperature rises above 20°C there is an increase in the abundance of C. albiceps and a reduction or disappearance of L. eximia from ephemeral resources (Vianna et al. 2004; Souza et al. 2008; Azevedo & Krüger 2013). The coexistence of both species did not occur, probably because L. eximia presents generalist behavior in the exploitation of organic matter in decomposition. On the other hand, C. albiceps demonstrates facultative predation behavior over other Diptera larvae that are not specialized in resource exploitation, such as L. eximia, causing competitive exclusion (Faria et al. 2004).

The second pattern is the occurrence of species of Muscidae that are facultative predators of third-instar larvae (Skidmore 1985). In ephemeral resources, it is common to find these species in the process of intraguild competition, particularly when resources are scarce (Revilla 2002; Duarte et al. 2013). Muscidae species that occurred in this study exhibited this behavior. However, they differed in their temperature and relative humidity requirements. While O. aeneascens and O. albuquerquei occurred in carcasses exposed to temperatures above 21°C, as observed by Ribeiro et al. (2000), M. stabulans occurred on substrates that were exposed to lower temperatures (Souza et al. 2008). Similar to species of Ophyra Robineau-Desvoidy, 1930 and M. stabulans, Psilochaeta pampiana has facultative predatory behavior. Although P. pampiana was considered only a rat carcass visitor in other studies (Moura et al. 1997), in the present study it developed in three different models (Table II), and these represent new records of this species developing in carcasses, at least in the Pelotas region.

The Sarcophagidae species M. halli developed in the reptile carcass from November 2009 and was also observed in other studies, such as snake carrion (Bothrops alternatus Duméril, Bibron & Duméril, 1854) (Moretti et al. 2009), small rodents (Moretti et al. 2008), and rabbits (Vairo et al. 2011). These facts about M. halli are in agreement with those discussed by Moretti et al. (2009) for the data of Kneidel (1984), stating that many species reared on small carcasses of non-mammals are apparently opportunistic users of a wide variety of organic substances in decomposition, as commonly observed in other generalist necrophagous species. Other Sarcophagidae species, such as S. (L.) crassipalpis and P. (P.) resona, have also been observed in others carcasses (Nishida 1984; Monteiro-Filho & Peneireiro 1987; Moura et al. 1997), including in the Pelotas region (Krüger et al. 2003; Souza et al. 2008).

Fanniidae was previously reported in carcasses in other studies, and adult emergence from the carcass is usually noticed at low abundances (Carvalho et al. 2000; Souza & Linhares 1997). This pattern is probably caused by the pressure of other competing dipteran larvae on Fanniidae larvae, and only a small part of the population manages to avoid predation and develop to the adult stage. In this study, F. femoralis was the only Fanniidae found. It presented generalist behavior in its exploitation of resources due to its development in all carcass models, and it was found in carcasses exposed to both high and low temperatures. Fannia femoralis was observed in other carcass experiments, presenting a similar aseasonal pattern of occurrence as in our results (Aballay et al. 2012; Horenstein et al. 2010; Moura et al. 2005).

This study contributes to our knowledge of the necrophagous fauna associated with wild animals, allowing us to infer that the most important factors in structuring necrophagous communities are environmental factors such as climatic conditions (seasonality) and the site of carcass site exposure rather than the species of animal carcass. These patterns may be evidenced by the well-known biological characteristics of
necrophagous species and by the clusters of similarity (UPGMA analysis; Figs. 1, 2) regarding the similar structuring pattern of dipteran communities independent of the carcass model.

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