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Sarcosaprophagous dipterans associated with differentially-decomposed substrates in Atlantic Forest environments

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Abstract

Sarcosaprophagous flies may show temporal variations in terms of their location and colonization in ephemeral resources, in addition to their richness and abundance changing according to the substrate age. The present study examined the influence of decomposition time on the composition of the sarcosaprophagous dipterofauna, sexual proportion of the collected specimens and the stage of sexual maturity of female flies (Calliphoridae and Sarcophagidae). For this, we used chicken liver with different decomposition times (0h, 24h, 48h, and 76h) for the collection of adult flies. We analysed some variables such as absolute and relative abundances, number of species, sex ratio and sexual maturity of female flies at each composition time tested. The assemblages were most diverse and abundant in more decomposed baits, with Calliphoridae being the most abundant taxa in all treatments. Reproductive stage varied according to the age of the substrate, with high rates of mature or pregnant females in the older substrates. Regarding the sex ratio, the number of males' blowflies was higher in older baits, but it did not vary for flesh flies. Thus, the substrate age is an influencing factor in the composition of sarcosaprophagous flies' assemblage, and it indication of the dipterans potential as primary or secondary colonizers throughout colonization of ephemeral resources.

Keywords: Blowflies; decomposition time; dipterans; flesh flies; rainforest.

Dípteros sarcosaprófagos associados a substratos diferencialmente decompostos em ambientes de Mata Atlântica

Resumo

As moscas sarcosaprófagas podem apresentar variações temporais quanto à sua localização e colonização em recursos efêmeros, além de sua riqueza e abundância variarem de acordo com a idade do substrato. O presente estudo examinou a influência do tempo de decomposição na composição da dipterofauna sarcosaprofauna, proporção sexual dos espécimes coletados e no estágio de maturidade sexual das moscas fêmeas (Calliphoridae e Sarcophagidae). Para isso, utilizou-se fígado de galinha com diferentes tempos de decomposição (0h, 24h, 48h e 76h) para a coleta de moscas adultas. As variáveis analisadas foram: as abundâncias absoluta e relativa, o número de espécies, a proporção sexual e maturidade sexual de moscas fêmeas em cada tempo de composição testado. As assembleias foram mais diversas e abundantes nas iscas mais decompostas, sendo Calliphoridae o táxon mais abundante em todos os tratamentos. O estado reprodutivo variou de acordo com a idade do substrato, com altas taxas de fêmeas maduras ou grávidas nos substratos mais velhos. Em relação a proporção sexual, o número de califorídeos machos foi maior nas iscas mais velhas, mas não variou para sarcofagídeos. Assim, a idade do substrato é um fator que influencia na composição da assembleia de moscas sarcosaprófagas, e indica o potencial dos dípteros como colonizadores primários ou secundários ao longo da colonização de recursos efêmeros.

Palavras-chave: Calliphoridae; dípteros; floresta tropical; Sarcophagidae; Tempo de decomposição.

Introduction

When an animal dies, its body becomes a temporary island of nutrients that can be explored by a variety of organisms, such as bacteria, arthropods and vertebrates (Barton et al., 2013). Arthropods that visit and use carrion as a resource are classified as generalists (omnivores) or specialists (scavengers) (Catts & Goff, 1992). The specialists are strongly dependent on the ephemeral resource, and use it as copula site, oviposition/ larviposition site, place of development of immature colonizers, or of feeding of adults, especially for females to complete ovarian development (oogenesis) (Mackerras, 1933).

Field studies commonly use animal baits or carcasses for necrophagous dipterans inventories (Vasconcelos & Araujo, 2012), and demonstrate a high richness of visiting species in the first 24h of exposure (Vasconcelos, Cruz, Salgado & Thyssen, 2013), which may be associated with the high volatile organic compound richness "VOCs" emitted at the beginning of decomposition (Recinos-Aguilar et al., 2020; Kotzé, Delclos, Knap, Wade, & Tomberlin, 2021). According to Kotzé et al. (2021) this high richness of volatile compounds even occurs in different conditions such as the presence and absence of colonization of the resource by blow flies.

However, the diversity of flies varies according to the substrate age, whereas more advanced resources attract more abundant and diverse assemblages (Oliveira, Soares, & Vasconcelos, 2016). This corroborates the observations of Smith (1986), who reported that families with high forensic potential such as Muscidae, Phoridae and Sarcophagidae are more abundant in more advanced stages (e.g. bloated and decay), although they still locate fresh carcasses.

These ecological parameters may be related to the species feeding preference, which may be specialists for fresh carcasses, or prefer older resources (Kotzé & Tomberlin, 2020a). In addition, recent studies have shown that the carcass colonizer *Cochliomyia macellaria* (Fabricius, 1775) prefers to oviposit on highly decomposed substrates (Kotzé & Tomberlin, 2020a). However, *Hermetia illucens* (Linnaeus, 1758) does not demonstrate discriminating between fresh and more decomposed substrates when looking for resources to lay their eggs (Kotzé & Tomberlin, 2020b).

Surprisingly, the empirical evaluation of the effect of substrate age on scavenger flies is more common in laboratory experiments and with specific species (Kotzé & Tomberlin, 2020a, b), and field studies are still scarce. The field study that aims to explain the effect of the substrate age on the assemblies of flies with forensic importance in rainforest in Brazil have focused on Calliphoridae species (Oliveira et al., 2016). However, other studies seek to know the diversity of volatile compounds that are released and attract insects in the first days of decomposition (Recinos-Aguilar et al., 2020; Kotzé et al., 2021).

The pioneering study by Oliveira et al. (2016) presented empirical data at the species level only for Calliphoridae, and do not evaluate how species from other families respond to substrates with different decomposition times, such as Sarcophagidae species, a family that has been colonizing corpses in the Northeast region (Oliveira & Vasconcelos, 2010; Meira, Barbosa, Jales, Santos, & Gama, 2020). Furthermore, the substrate age can influence sexual maturation (Mohr & Tomberlin, 2015), colonization rate (George, Archer, & Toop, 2012), and also the time of colonization – TOC (Kotzé & Tomberlin, 2020a), important information for medico-legal investigations (Sanford, 2015).

As far as we know, the present study is one of the few field studies that aims to understand the effects of the substrate age on dipterans assemblages, especially about species from Calliphoridae, Fanniidae, Muscidae, Phoridae and Sarcophagidae families. These taxa have several species with forensic potential in South America (Carvalho & Mello-Patiu, 2008), and are commonly found associated with carcasses and corpses (e.g. Oliveira & Vasconcelos, 2010; Vasconcelos et al., 2013; Meira et al., 2020). However, they are still poorly explored in ecological studies, and so, we were motivated to investigate how the assemblages of necrophagous dipterans are composed and structured in the face of exposure to baits with different decomposition times. We specifically aimed to assess whether the age of the baits can influence: (i) the composition of the sarcosaprophagous dipterofauna; (ii) the sexual proportion of the collected specimens; and (iii) the sexual maturity stage of female flies (Calliphoridae and Sarcophagidae), something important from a forensic point of view.

Materials and Methods

Area of Study

The experiment was performed in rainforest environments in the municipality of Natal, Northeastern Brazil (05°48'45"S; 35°11'35"W). Local climate is within the humid tropical range, with annual average temperatures ranging from 22.6 to 29.2°C and annual rainfall around 1,450 mm, with an average relative humidity of 80% (Secplan/RN, 1981; Freire, 1990). The average wind speed is 4.3 ± 0.7 m/s, with an average maximum speed of 7.7 ± 1.1 m/s (Espírito Santo & Silva, 2006). Additionally, the sampling points are localized in areas of the Atlantic Forest vegetation, with a high diversity of trees of the Passifloraceae, Myrtaceae, Moraceae and Fabaceae families, resulting in a closed canopy with low direct sunlight penetration and only a few gaps. Local fauna of vertebrates is mainly comprised of endemic reptiles and birds, which includes species of marmosets, bats, and opossums.

Insect Sampling and Determination of Reproductive Stage

Sampling was performed in July 2019 in five expeditions, with the replacement of traps in each sampling. During the experiments we registered an average temperature of 27 ºC ± 2ºC and relative humidity around 75% ± 5 %.

The insects were collected using traps adapted from Oliveira et al. (2016), suspended ca. 1.5 m above the ground. Traps consist of a conical tube, with approximately 50 cm high, 10 cm in diameter, and composed of two Polyethylene Terephthalate (PET) bottles (2 L). The upper part was transparent and open at the base, while the lower part was painted black. The lower part supports a recipient containing the bait - in this case, chicken liver (50 g) with different decomposition times (0 h, 24 h, 48 h and 76 h), and then exposed in the field for 24 h to avoid the influence of crowding in the choice of the different treatment. The use of 50 g of substrate was based on studies that used 19g or 50g of decomposed baits and collected high abundance and diversity of necrophagous flies (Moretti & Godoy, 2013; Barbosa, Jales, Vasconcelos, & Gama, 2020). The baits were randomly distributed in four treatments according to the substrate age (decomposition time): fresh baits (0 h), initial decomposition (24 h) and baits in more advanced stages of decomposition with 48 h and 76 h at room temperature (26 °C) in the laboratory.

The sampling methodology was determined in order to mitigate the effect of spatio-temporal dynamics observed by Moretti et al., (2013) in blow flies species' preference for the age of the baits. For this, sampling was performed in the rainy period and in an Atlantic Forest environment, in five expeditions with the use of 20 traps (5 per treatment) in each expedition. We designed five sampling grids, with one trap per treatment, totaling four traps per grid. Intra-grid, the traps remained 20 m from each other, with 250 m of inter-grid distance. In total, 25 replicates of each treatment were obtained. All captured insects were killed in ethyl acetate vapors, preserved in 70% ethanol, and identified using taxonomic keys (Carvalho & Mello-Patiu, 2008; Disney & Franquinho Aguiar, 2008). Voucher specimens were deposited in the entomological collection of the Federal University of Rio Grande do Norte, Natal.

The ovarian development stage associated with sexual maturity, was inferred for female of Calliphoridae and Sarcophagidae specimens, which had their abdomen's dissected for ovaries analysis. For this, 30 female Calliphoridae/treatment and 20 female Sarcophagidae/treatment were used. These taxa were selected because they are common in human cadavers, including in the studied region (Andrade, Varela-Freire, Batista, & Medeiros, 2005; Oliveira & Vasconcelos, 2010; Cherix, Wyss, & Pape, 2012; Thyssen et al., 2018; Meira et al., 2020). The ovaries were removed by incision of the abdomen followed by direct observation under the stereoscope (40x magnification). We adapted the classification of ovarian development proposed by Chaiwong et al. (2012), in which the following stages are described: to Calliphoridae (*Chrysomya megacephala* (Fabricius, 1794) and *Lucilia eximia* (Wiedemann, 1819)), the most representative species in all treatments: (i) initial, when the oocytes are not differentiated or visible and (iii) mature, in which the oocytes fully occupy the follicle as a mature egg; Sarcophagidae: (i) initial, when the oocytes are not differentiated or visible; (ii) premature, in which the oocytes are differentiated or visible; and (iii) mature or pregnant, when it is possible to see the pre-larvae.

Data Analysis

We analyzed the species' richness and specific abundance values, related to the substrate age (decomposition times): fresh baits (0 h), baits in initial decomposition (24 h) and baits in more advanced stages of decomposition with 48 h and 76 hours. Differences in species' richness and in the abundance of adult flies captured in traps with different decomposition time were assessed by ANOVA, with Tukey posttest, after log (X + 1) transformation. For ANOVA, the number of individuals per treatment in each grid (replicates) was considered to determine the abundance of specimens, species, and families per treatment. A dominance ranking of the species in each treatment was also performed. To test for similarities in the assemblages of each decomposition time, we did a similarity matrix, through the index of Bray Curtis, after data transformation log (x + 1). Finally, we quantified the mean number of female and male specimens in each treatment and differences were assessed by ANOVA. All data were tested for normality using Shapiro-Wilk's. We used the software BioEstat 5.0 and Primer 6.0 with 5% significance level.

Results and Discussion

A total of 4,209 adult flies belonging to five families and 24 species were captured. Calliphoridae was the most abundant family, with a total 3,137 individuals captured and identified, representing 74,5% of the total specimens. Muscidae was the second most representative family, with 8.3% of the captured flies, followed by Phoridae (7.7%), Sarcophagidae (6.0%) and Fanniidae (3.5%). Sarcophagidae was the most diverse family, with at least 9 spp. recorded, followed by Calliphoridae (6 spp.), Muscidae (5 spp), Phoridae and Fanniidae with two species each (Table 1). The number of species of sarcosaprophagous flies observed in the present study was similar to other studies carried out in Brazil (Carvalho, Thyssen, Linhares, & Palhares, 2000; Moretti & Godoy, 2013; Vasconcelos et al., 2013; Barbosa et al., 2020), which experimentally demonstrates that baits represent an efficient methodology for field studies.

Of the blowflies, the most common species were *C. megacephala* (51.0%) and *L. eximia* (32.7%). *Atherigona orientalis* (Schiner, 1868) represented 93.7% of the Muscidae specimens, while *Fannia* sp. composing 99.3% of the captured Fanniidae. *Megaselia scalaris* (Loew, 1866) was the most abundant phorid, with 99.1% of the specimens, and *Oxysarcodexia amorosa* (Schiner, 1868), 44.0% of the identified flesh flies. It should be noted that for flesh flies, 85.0% of all the collected flies were female, which were not identified at the species level.

The effect of substrate age on the structure of necrophagous flies' assemblages, especially for Calliphoridae species, has already been mentioned in another fragment of the Atlantic Forest (Oliveira et al., 2016). In the present study, Calliphoridae was the most abundant taxon in all treatments, especially in the older baits. This frequency of blow flies' specimens throughout the decay of animal organic matter is a pattern that is repeated in several studies (Rodriguez & Bass, 1983; Oliveira et al., 2016; Vasconcelos, Salgado, Barbosa, & Souza, 2016).

There was statistical difference in abundance between Calliphoridae ($F_{3;\,16} = 5.17$; p = 0.01) and Muscidae ($F_{3;\,16} = 4.80$; p = 0.01) treatments, especially in treatments with 0h and 76h ($p < 0.05$) and 24h and 76h of decomposition ($p < 0.05$), with a preference for more decomposed substrates in both families. Differences were also found for Fanniidae ($F_{3;\,16} = 2.62$; p = 0.05), where baits with 76h of decomposition were more attractive than 24h and 48h ($p < 0.05$). However, no differences in abundance were observed for Phoridae ($F_{3;\,16} = 1.81$; p = 0.18) and Sarcophagidae ($F_{3;\,16} = 0.28$; p = 0.18).

According to Oliveira et al. (2016), the Calliphoridae family also showed a preference for highly decomposed substrates, including the exotic species of the *Chrysomya* genus, while Sarcophagidae species was more generalist, corroborating our findings. The preference of blow flies for more advanced stages such as bloated and active decay was also recorded in carcasses and human corpses (Rodriguez & Bass, 1983; Alves, Santos, Farias & Creão-Duarte, 2014; Vasconcelos et al., 2016). The Calliphoridae species preference for older substrates may be associated with the fact that many species, e.g. *C. macellaria*, had a tendency towards greater oviposition in aged baits (Kotzé & Tomberlin, 2020a), which may be the case of *C. megacephala* and *C. albiceps* in the present study.

A greater number of species was recorded on substrates with 76h of decomposition (16 spp.), followed by 24 h (14 ssp.), and 0h and 48h (12 species each). However, there was no difference in richness between treatments ($F_{3;\ 16} = 3.76$; $p = 0.06$). The abundance of the most representative species in the study, *C. albiceps* ($F_{3;\ 16} = 6.12$; $p < 0.01$) and *C. megacephala* ($F_{3;\ 16} = 6.10$; $p < 0.01$) differed between the 76h and 0h treatments ($p < 0.05$) and 24h ($p < 0.05$), for both species. *Aterigona orientalis* abundance differed between the treatment of 76h and 0h ($F_{3;\ 16} = 6.12$; $p < 0.01$), 24h and 48h ($p < 0.05$). For *Fannia* sp ($F_{3;\ 16} = 5.77$; $p < 0.01$), 76h was more attractive than 24h and 48h ($P < 0.05$). However, no differences were observed for *L. eximia* ($F_{3;\ 16} = 0.17$; $p < 0.50$) and *M. scalaris* ($F_{3;\ 16} = 1.77$; $p < 0.19$). Those data also clarify that the food preference for a specific substrate age range may vary within the same family, since *C. megacephala* and *C. albiceps* were strongly attracted to more decomposed baits, while *L. eximia* was more associated with fresh ones (24h). It is possible that the difference between blow flies' species may be due the reduced competitive ability from *L. eximia* (Recinos-Aguilar et al. 2020; Barbosa, Jales, Medeiros, Vasconcelos, & Gama, 2021), although both species occur at all stages of decomposition.

Table 1 - Abundance by families and species of dipterans in the different decomposition times in a rainforest fragments in Brazil.

Family/species	Substrate age (h)					
	0	24	48	76	Absolute (N)	Relative (%)
Calliphoridae						
Chrysomya albiceps	27	2	86	380	495	11.76
Chrysomya megacephala	113	16	305	1,165	1,599	37.99
Chrysomya putoria	0	0	1	1	2	0.05
Cochliomyia macellaria	1	0	2	11	14	0.33
Lucilia cuprina	1	0	0	1	2	0.05
Lucilia eximia	139	396	263	227	1,025	24.35
Fanniidae						
Fannia pusio	0	1	0	0	1	0.02
Fannia sp	21	13	19	95	148	3.52
Muscidae						
Atherigona orientalis	19	4	58	245	326	7.75
Musca domestica	0	9	0	0	9	0.21
Ophyra aenescens	1	0	0	4	5	0.12
Helina sp	0	1	1	0	2	0.05
Synthesiomyia nudiseta	0	0	2	4	6	0.14
Phoridae						
Megaselia scalaris	51	33	64	171	319	7.58
Megaselia sp	0	0	0	3	3	0.07
Sarcophagidae						
Oxysarcodexia amorosa	13	3	0	1	17	0.40
Oxysarcodexia backeri	0	1	0	0	1	0.02
Oxysarcodexia intona	0	1	0	0	1	0.02
Oxysarcodexia timida	0	0	0	2	2	0.05
Peckia chrysostoma	3	0	3	4	10	0.24
Peckia lambens	0	1	0	2	3	0.07
Peckia pexata	1	0	0	0	1	0.02
Tricharaea canuta	0	1	1	0	2	0.05
Tricharaea occidua	0	2	0	0	2	0.05
Sarcophagidae sp*	53	20	83	58	214	5.08
Total by decomposition time	443	504	888	2,374	4,209	100.00

*unidentified females.

The theoretical framework built over the years in forensic entomology shows that successive waves of insects that reach carcasses and corpses vary according to the decomposition stage (Oliveira & Vasconcelos, 2010; Matuszewsk, Szafalowicz, & Jarmusz, 2013; Vasconcelos et al., 2016; Thyssen et al., 2018; Meira et al., 2020), with blowflies arriving in the initial phase and remaining present throughout the degradation of the resource. However, in this study we show that many Diptera taxa seem to prefer more decomposed baits, probably due to the release of more complex odor plumes, a consequence of the number of volatile compounds released by the baits according to the stage of decomposition (Vass et al., 2008).

Throughout the study, it was also observed that the assemblages of baits in the earliest stages of decomposition were dominated by *L. eximia*, while in more advanced stages there was dominance of *C. megacephala* (Figure 1). Besides, there was a high similarity between the 76h samples (Table 2), with the lowest similarity found between the 24h and 76h samples (Table 2). The high number of specimens of *L. eximia* at the beginning of the decomposition process may be associated with the ability of this specie to identify the VOCs emitted in the first hours of exposure and avoid competition with other colonizers, as observed by Recinos-Aguilar et al. (2021). This specie is still adapted to locate baits with difficult access or small carcasses (Moretti, Ribeiro, Thyssen, & Solis, 2008; Barbosa et al., 2020). On the other hand, the high similarity of the assemblages present in the oldest baits is due to the fact that it concentrates a greater number of individuals and species, since these stages concentrate a high abundance of dipterans (Rodriguez & Bass, 1983).

We also noted that the ovaries reproductive stage and development varied according to the substrate age. The number of mature or pregnant females of *C. megacephala*, *L. eximia* and Sarcophagidae spp. was higher in baits with advanced decomposition stage (48 and 76h) (Table 3). For *C. megacephala* and *L. eximia*, 80% of the females analyzed for the least decomposed substrates (0h and 24h) had poorly developed ovaries (Figure 2a). In contrast, in the most decomposed substrates (48h and 76h) the ovaries were mature, with oocytes occupying the entire follicle and looking like an egg (Figure 2b), besides the females had an expanding abdomen.

In the substrates with initial decomposition (0h and 24h), 60% of the Sarcophagidae females had poorly developed ovaries and without differentiation between the oocytes (Figure 2c), while 37.5% were in an intermediate stage (premature), (Figure 2d) and 2.5 % in advanced stage (pregnant), with oocytes looking like first instar larvae (Figure 2e). In baits with advanced decomposition, only two development stages were observed, of which 27.5% were with intermediate stage (premature) (Figure 2d) and 72.5% of the analyzed Sarcophagidae females were pregnant (Figure 2e).

The results of the ovarian development observed for Calliphoridae and Sarcophagidae, families commonly found associated with carcasses and corpses (Andrade et al., 2005; Oliveira & Vasconcelos 2010; Matuszewsk et al., 2013; Vasconcelos et al., 2016; Thyssen et al., 2018; Meira et al., 2020), reveal that these taxa can direct fresh resources for feeding, using them as a protein source, while aged baits are more preferentially used as copulation or breeding sites for the offspring. Their use as a copulation site is suggested due to the fact that Calliphoridae males are more common in aged baits.

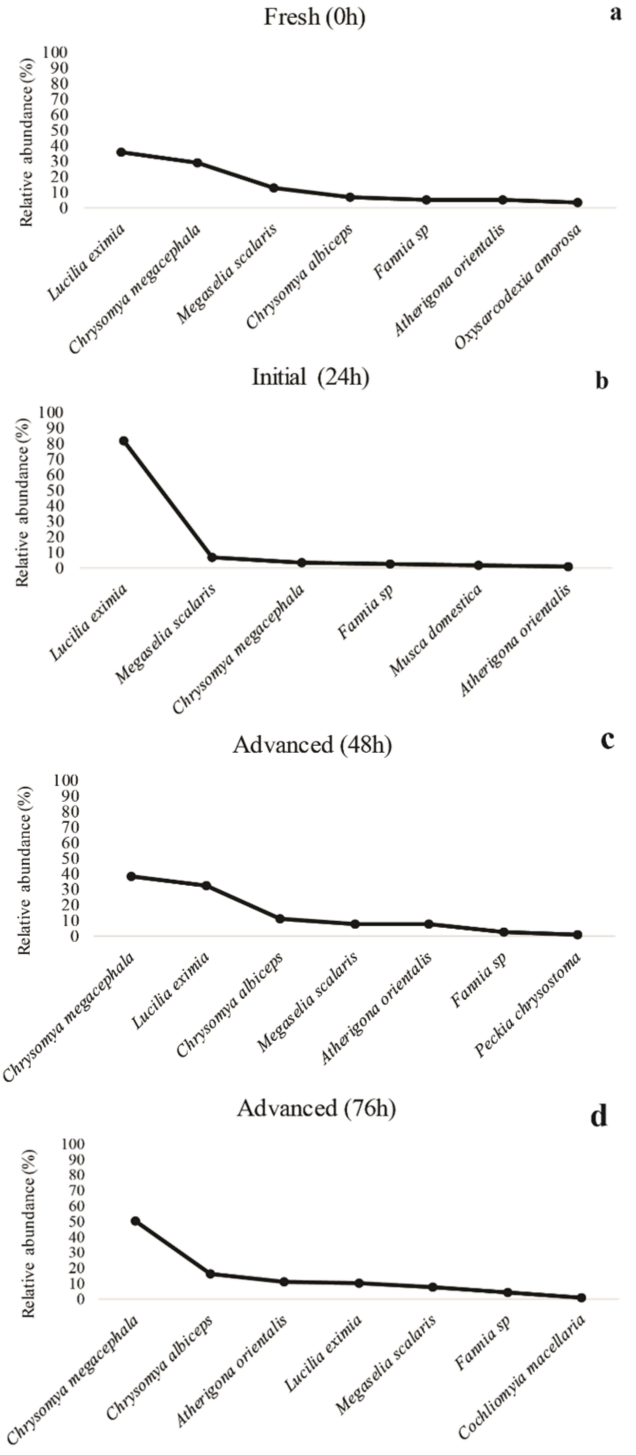

Figure 1. Diptera species dominance ranking by decomposition times. (a) fresh (0h); (b) Initial (24h); (c) advanced (48h) and (d) advanced (76h).

These ephemeral resources range of use further strengthens the forensic potential of dipteran families, especially the fact that flesh flies' females appeared to be pregnant and about to undergo larviposition, which are the important specimens for calculating the post-mortem interval (Catts & Goff, 1992).

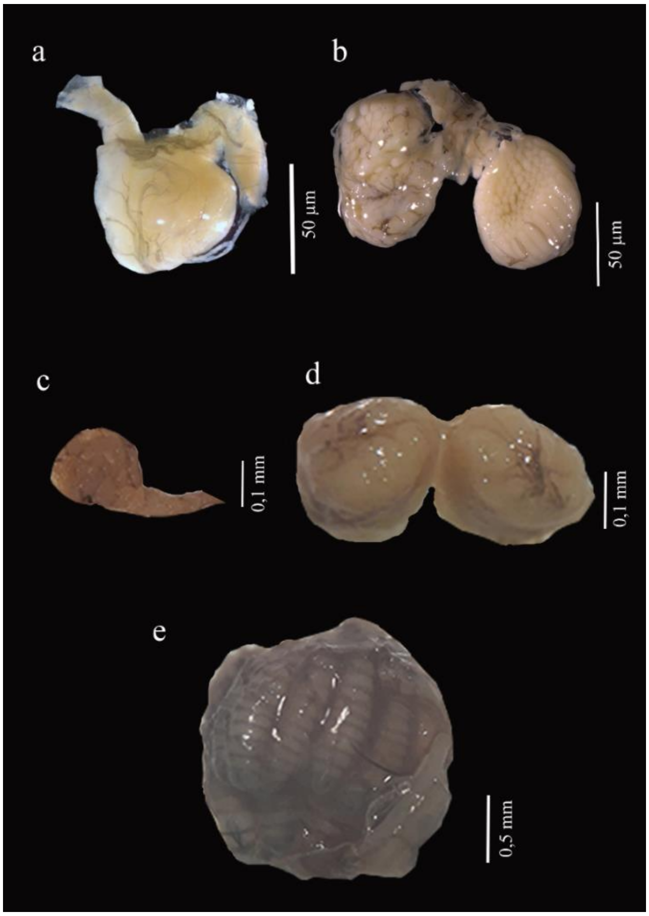

Figure 2 - Ovarian stage of Calliphoridae and Sarcophagidae specimens, with classification adapted from Chaiwong et al. (2012). a and b - Calliphoridae: (a) Initial and (b) mature - oocyte fully occupying the follicle as a mature egg; c-e - Sarcophagidae: (c) Initial; (d) premature ovaries and (e) mature or pregnant, it is possible to see the pre-larvae.

Another curious fact was the strong relationship of *M. scalaris* to the most decomposed baits, contrasting other studies carried out with baits of different decomposition stage (Oliveira et al., 2016). This result reflects on the ability of the species to use the resource for a longer time, although *M. scalaris* being considered an initial colonizer (Disney & Franquinho Aguiar, 2008). It should also be noted that although *A. orientalis* has a preference for decomposing plant substrates (D'Almeida, 1989), this species was more common on substrates with 76h of decomposition, which may reveal a possible food adaptability of the species.

Regarding sex ratio, there was a high number of females in all treatments (Table 4).

Table 2. Analysis for the similarity between dipterans assemblages, according to the decomposition time. Bold - higher similarity value and Gray - lower similarity value.

Substrate age	Fresh (0h)	Initial (24h)	Advanced (48h)	Advanced (76h)
Fresh (0h)	58.006	-	-	-
Initial (24h)	58.49	59.317	-	-
Advanced (48h)	55.268	50.315	50.506	-
Advanced (76h)	56.685	46.336	56.886	74.792

Table 3. Abundance of Calliphoridae and Sarcophagidae females by ovarian stage, with classification adapted from Chaiwong et al. (2012).

Time	Ovarian stage			Total females
	Inicial	Premature	Mature or pregnant	
Chrysomya megacephala				
0 h	29	0	1	30
24 h	24	0	6	30
48 h	0	0	30	30
76 h	0	0	30	30
Lucilia eximia				
0 h	30	0	0	30
24 h	25	0	5	30
48 h	0	0	30	30
76 h	0	0	30	30
Sarcophagidae spp.				
0 h	16	3	1	20
24 h	8	11	1	20
48 h	0	7	13	20
76 h	0	4	16	20

Table 4. Mean number (+/- SD) of females and males of the families by decomposition time.

	Fresh (0h)		Initial (24h)		Advanced (48h)		Advanced (76h)	
Families	Female	Male	Female	Male	Female	Male	Female	Male
Calliphoridae	52 ± 22	4.2 ± 3.2	81 ± 25	1.8 ± 0.7	122 ± 57.1	9.8 ± 4.3	324.6 ± 88.6	36.4 ± 8.8
Fanniidae	4.4 ± 2.1	0	2.6 ± 2.4	0.2 ± 0.4	3.4 ± 1.9	0.4 ± 0.5	18.4 ± 7.4	0.6 ± 0.5
Muscidae	4 ± 3.2	0	3 ± 3.3	0.4 ± 0.5	11.4 ± 13.1	0.8 ± 0.7	49.4 ± 41	1.2 ± 0.4
Phoridae	10.2 ± 51	0	5.8 ± 3.3	0.8 ± 0.8	12.8 ± 13.9	0	33.4 ± 33.2	1.6 ± 1.1
Sarcophagidae	10.6 ± 4	3.4 ± 2.2	6.1 ± 2.7	2.1 ± 0.9	17.8 ± 7.9	0.5 ± 0.2	6.6 ± 2.9	1.8 ± 0.8

Regarding sex ratio, there was a high number of females in all treatments (Table 4). However, the number of Calliphoridae males was higher in the older baits ($F_{3;\,16} = 3.66$; $p < 0.05$), with greater number in 76h than 0h and 24h. The number of Sarcophagidae males did not differ between treatments ($F_{3;\,16} = 0.68$; $p = 0.57$). The high number of females in older substrates is explained by the fact that they use the decomposed resource as an oviposition site and, in many cases, have a greater number of eggs after 48 hours of resource exposure (Anderson & VanLaerhoven, 1996).

Conclusion

The results of this study reinforce the feasibility of using animal baits as an important resource for behavioral studies with sarcosaprophagous dipterans. Additionally, we corroborate that bait age is an influencing factor in the composition and structure of assemblages of sarcosaprophagous flies, as previously observed for Calliphoridae species, in which species respond differently with species being attracted quickly by fresher baits, while the greatest abundance of specimens and species richness are concentrated in the most decomposed resources.

Besides, the different stages of ovarian development associated with decomposition times may be an indication of the species potential to be primary or secondary colonizers throughout cadaveric colonization. Studies like this should be repeated in a wide variety of environments, also focusing on species as primary colonizers. Thus, it can also assist in the comprehension of the insect' activity period of sarcosaprophagous flies in the process of ephemeral resources degradation (e.g. carrion and corpses).

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