Diversity of Forensically-Important Dipteran Species in Different Environments in Northeastern Brazil, with Notes on the Attractiveness of Animal Baits

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Diversity of forensically-important dipteran species in different environments in northeastern Brazil, with notes on the attractiveness of animal baits

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Abstract

The distribution and habitat preferences of necrophagous Diptera in northeastern Brazil is poorly known despite the medical and forensic relevance of species in the Families Calliphoridae, Sarcophagidae, and Muscidae. We performed a survey on the diversity of necrophagous species in 4 types of environments: rainforest, agroecosystem, beach, and urban areas. Adult flies were collected by using suspended traps containing decomposing animal tissue (chicken liver, sardine, or pork) as baits. A diverse assemblage of necrophagous Diptera was registered in all environments, consisting of 20 species from 7 families: Calliphoridae, Fanniidae, Muscidae, Phoridae, Piophilidae, Sarcophagidae, and Ulidiidae. *Megaselia scalaris* (Loew) (Phoridae), *Chrysomya albiceps* (Wiedemann) (Calliphoridae), and *Tricharaea* sp. (Muscidae) were the most abundant species. The rainforest fragment and the sugarcane plantation were the environments with the highest degree of species similarity. The type of bait did not significantly influence the number of species captured. The invasive species *Chrysomya megacephala* (Fabricius) and *C. albiceps* were present in high abundance in all environments, especially at the sandy beach, where they corresponded to 100% of all Calliphoridae specimens.

Key Words: forensic entomology; Calliphoridae; blow flies; Sarcophagidae; *Chrysomya*

Resumen

La distribución y la preferencia por hábitat de moscas necrófagas en el Noreste de Brasil son poco conocidas a pesar de la importancia médica y forense de muchas especies, particularmente de las familias Calliphoridae, Sarcophagidae and Muscidae. Un levantamiento de corta duración de especies necrófagas fue conducido en 4 tipos de ambiente: bosque atlántico, agroecosistema, playa y área urbana. Moscas adultas fueran colectadas con trampas conteniendo tejido animal (hígado de pollo, sardina o cerdo) como atractivos. Una comunidad diversa de Diptera necrófagos fue registrada en todos los ambientes, consistiendo de 20 especies de 7 familias Calliphoridae, Fanniidae, Muscidae, Phoridae, Piophilidae, Sarcophagidae y Ulidiidae. *Megaselia scalaris* (Loew) (Phoridae), *Chrysomya albiceps* (Wiedemann) Calliphoridae) y *Tricharaea* sp. (Muscidae) fueran las especies más abundantes. El bosque atlántico y la plantación de cana-de-azúcar compartieron lo más alto grado de similaridad. El tipo de atractivo no influenció significativamente en el número de especies capturadas. Las especies invasoras *Chrysomya megacephala* y *C. albiceps* fueran registradas en alta abundancia en todos los ambientes, especialmente en la playa, donde correspondió a 100% de todos los Calliphoridae, lo que despierta preocupaciones acerca de la resiliencia de las asambleas nativas.

Palabras Clave: entomología forense; Calliphoridae; moscardones; Sarcophagidae; *Chrysomya*

Necrophagous Diptera play a key role in nutrient cycling in terrestrial ecosystems as they accelerate the breakdown of animal tissues, which facilitates the action of decomposing microorganisms (Savage 2002). Species from at least 23 dipteran families exploit carrion as a food source, of which Calliphoridae, Sarcophagidae, Muscidae and Fanniidae are the most important (Savage 2002). Additionally, species of Piophilidae, Phoridae, Stratiomyiidae, Ulidiidae, Sphaeroceridae, Sepsidae, and Syrphidae have been recorded to feed on carcasses and cadavers (Catts & Goff 1992; Vasconcelos et al. 2013).

The ecological importance of necrophagous dipterans has been strengthened in the last few decades by their use (as entomological evidence) in cases of homicide to provide information about the time and site of death and the presence of incriminating substances (e.g., drugs, poison) (Carvalho et al. 2001). Similarly, discrepancies between the insects found on a body and the composition of insect species at the site of discovery of the body may suggest a post-mortem transfer of the corpse (Moretti & Godoy 2013). This is because calliphorid species often differ in habitat preference. *Cochliomyia macellaria* (Fabricius, 1775) and *Lucilia eximia* (Wiedemann, 1819), for example, appear to be synanthropic (i.e., are associated with human-impacted environments [Montoya et al. 2009]), whereas *Mesembrinella* spp. thrive in preserved forest fragments (Sousa et al. 2011; Cabrini et al. 2013).

The association of a species to protected environments may help in quantifying the degree of conservation of an area and in predicting the impact of invasive species. In the last few decades, Old World species such as *Chrysomya albiceps* (Wiedemann, 1819), *C. megacephala* (Fabricius, 1974), and *C. putoria* (Wiedemann, 1818) (Calliphoridae) have been detected on the American continent in environments that include the Amazon forest (Sousa et al. 2011; Ururahy-Rodrigues et al. 2013), the savannah-like cerrado (Biavati et al. 2010; Rosa et al. 2011),

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rainforests (Vasconcelos et al. 2013), seasonally dry forests (Vasconcelos & Salgado 2014), and oceanic islands (Carmo & Vasconcelos 2014). Additionally, considering that several Calliphoridae, Muscidae, and Sarcophagidae species cause myiasis and can transmit pathogens to man and other vertebrates (Guimarães & Papavero 1999), the need for field surveys to fully establish the distribution of these species becomes clear.

This study aimed at performing a short-term survey on the diversity of necrophagous Diptera species in 4 environments in northeastern Brazil, a region that harbors one of the highest homicide rates on the American continent (Waiselfisz 2013). Specifically, we aimed to (1) detect habitat overlap of necrophagous dipteran species in urban areas, rainforest fragments, agricultural sites, sandy beaches, and other environments typical of the region; (2) compare the attractiveness of animal baits (chicken liver, pork, and sardine) to different species; (3) describe local assemblages of necrophagous species in terms of richness, similarity, dominance, and equity among environments; and (4) detect the presence of invasive species of Calliphoridae.

We tested the following hypotheses: (1) asynanthropic species are dominant in forested environments; (2) urban areas harbor a more diverse assemblage of necrophagous Diptera due to the abundant offer of alternative sources of food (e.g., litter); (3) the relative frequency of invasive species is higher in urban areas that are exposed to a more intense flow of biological material; (4) environments with a low diversity of microhabitats, such as beaches, would be associated with a correspondingly low richness and abundance of Diptera species; and (5) environments with low vegetation diversity, such as monocultures, will harbor simple species assemblages compared with areas with complex vegetation, such as forest fragments.

Materials and Methods

STUDY AREAS AND EXPERIMENTAL DESIGN

Field experiments were carried out in 4 environments in the state of Pernambuco, Brazil. The environments represent the diversity of landscape in northeastern Brazil and include an urban area, a rainforest fragment, a sugarcane plantation, and a sandy beach. All of the locations have the same type of weather according to the Köppen climate classification. The urban area comprised an intensely populated neighborhood in the municipality of Recife (08°20ˈ44"S, 34°57ˈ10"W), in which the presence of residences, shops, schools, and restaurants is associated with deficient hygiene conditions and frequent litter accumulation. The agroecosystem consisted of an extensive monoculture of sugarcane (*Saccharum officinarum* L.; Poales: Poaceae), a crop that has been cultivated in the region for the past 5 centuries. The plantation is located in the municipality of Goiana (07°36'05"S, 35°01'00"W). The rainforest fragment, adjacent to the sugarcane plantation, is an area protected by environmental law and harbors a high diversity of native herbaceous and arboreous plant species. The rainforest is located 10 km from the sugarcane plantation. The coastal environment (Carne de Vaca) is a sandy beach under low anthropogenic impact and is located in the municipality of Goiana (07°34'40"S, 34°02'08"W). Carne de Vaca is used mainly as a weekend tourism site and has few permanent residences.

INSECT COLLECTION AND IDENTIFICATION

Adult flies were collected by using traps described by Oliveira & Vasconcelos (2010). Each trap consists of a black conic tube open at both ends, on the top of which a transparent vial closed at the top is attached. The trap was suspended 75 cm above the soil and contained decomposing animal tissue (100 g, after 24 h exposure to 25 °C) as bait. Three types of bait were used: chicken liver, sardine, and pork meat. Traps were exposed in the field for 48 h in each environment and were positioned 25 m apart. Three traps (each trap containing one type of bait) arranged in this manner and exposed for the specified length of time were considered a single sample. Samples were replicated 6 times between Oct 2008 and Jan 2009. Insects were identified using the taxonomic keys of Carvalho & Ribeiro (2000), Carvalho et al. (2002), Mello (2003), and Carvalho & Mello-Patiu (2008). Only males of the sarcophagid species we collected were identified owing to the reliance of taxonomic keys on the morphology of the aedeagus.

ECOLOGICAL AND STATISTICAL ANALYSES

The species assemblage of necrophagous dipterans in each environment was characterized by the following variables: richness, abundance, relative frequency, diversity (estimated by Shannon–Wiener index), and equity (Pielou index [Magurran 2004]). We performed a Chi-square test to compare species richness and abundance in each environment according to the type of bait. Further analysis of variance (2-way ANOVA) was performed to test for differences among the abundance of each species in each environment and the influence of the type of substrate (bait). To test for similarities in the assemblages of necrophagous dipterans between different environments, we built a similarity matrix, through the index of Bray Curtis, after data transformation log(x + 1). The statistical packages Primer 5.0 (Clarke & Gorley 2001) and Biostat 5.0 (Ayres et al. 2007) were used, with a significance level of 5% throughout the analysis.

Results

When all samplings were combined, a total of 3,434 adult dipterans of 20 species belonging to 7 families were collected in the 4 environments (Table 1). Overall, the most abundant species were *Megaselia scalaris* (Loew, 1866) (22.1%), *C. albiceps* (21.3%), and *Tricharaea* sp. (18.2% of adults). In terms of overall abundance, the urban area and the sugarcane plantation harbored 35.9% and 33.8%, respectively, of all collected individuals.

Five species were recorded in only one environment: *Mesembrinella bellardiana* (Séguy, 1925), *Hemilucilia segmentaria* (Fabricius, 1805) and *Chloroprocta idioidea* (Robineau-Desvoidy, 1830) (Calliphoridae) were collected only in the rainforest; *Ophyra chalcogaster* (Wiedemann, 1824) (Muscidae) was trapped only on the beach, and *Peckia* (*Sarcodexia*) *lambens* (Wiedemann, 1830) (Sarcophagidae) only in the urban area.

There was no significant difference in species richness ($F_{3;6}$ = 3.811; P = 0.076) or in the abundance of individuals collected among the environments ($F_{3;6}$ = 3.513; P = 0.089). However, when the quantities of the most abundant species were analyzed (2-way ANOVA with Tukey test), the abundance of *C. albiceps* varied among the environments ($F_{3;6}$ = 6.429; P = 0.027) and was significantly higher in the sugarcane plantation when compared with the beach (P < 0.05). The abundance of *Fannia pusio* (Wiedemann, 1830) (Fanniidae) also differed among the environments ($F_{3;6}$ = 9.283; P = 0.012) and was lower on the beach when compared with the forest fragment, the sugarcane plantation, or the urban area (P < 0.05, for all comparisons). The abundance of *M. scalaris* also varied across the environments ($F_{3;6}$ = 6.799; P = 0.024) and was higher in the urban area when compared with the plantation and with the beach (P < 0.05, for both comparisons). In contrast, no significant differences

Table 1. Abundance (A) and relative frequency (RF) of forensically-important Diptera species in 4 types of environment in northeastern Brazil. Shaded cells indicate the 3 most abundant species in each environment.

	Rainforest fragment		Sugarcane plantation		Sandy beach		Urban area	
Family / Species	A	RF %	A	RF %	A	RF %	A	RF %
Calliphoridae								
Chrysomya albiceps (Wiedemann, 1830)	166	23.99	548	47.28	3	0.85	13	1.06
Chrysomya megacephala (Fabricius, 1805)	29	4.19	35	3.02	4	1.14	51	4.14
Chrysomya putoria (Wiedemann, 1830)	8	1.16	22	1.90	—	—	—	—
Chloroprocta idioidea (Robineau-Desvoidy, 1830)	1	0.14	—	—	—	—	—	—
Cochliomyia macellaria (Fabricius, 1805)	1	0.14	3	0.26	—	—	—	—
Hemilucilia segmentaria (Fabricius, 1805)	1	0.14	—	—	—	—	—	—
Lucilia eximia (Wiedemann, 1819)	—	—	4	0.35	—	—	3	0.24
Mesembrinella bellardiana Aldrich, 1922	2	0.29	—	—	—	—	—	—
Fanniidae								
Fannia pusio (Wiedemann, 1830)	123	17.77	146	12.60	16	4.56	167	13.56
Muscidae								
Atherigona orientalis Schiner, 1868	15	2.17	8	0.69	1	0.28	16	1.30
Musca domestica Linnaeus, 1758	5	0.72	35	3.02	25	7.12	19	1.54
Ophyra chalcogaster (Wiedemann, 1824)	—	—	—	—	2	0.57	—	—
Synthesiomyia nudiseta (Wulp, 1883)	—	—	8	0.69	2	0.57	39	3.17
Ulidiidae								
Ulidiidae sp.1	5	0.72	5	0.43	1	0.28	v	—
Ulidiidae sp.2	8	1.16	2	0.17	—	—	—	—
Phoridae								
Megaselia scalaris (Lowe, 1866)	190	27.46	52	4.49	67	19.09	450	36.53
Piophilidae								
Piophila casei Linnaeus, 1758	3	0.43	35	3.02	160	45.58	293	23.78
Sarcophagidae								
Ravinia belforti (Prado & Fonseca, 1932)	—	—	5	0.43	—	—	4	0.32
Peckia (*S.*) *lambens* (Wiedemann, 1830)	—	—	—	—	—	—	7	0.57
Tricharaea sp.	135	19.51	251	21.66	70	19.94	170	13.80
Total	692	100	1,159	100	351	100	1,232	100

in the abundance of *C. megacephala* ($F_{3;6}$ = 1.551; *P* = 0.295), *P. casei* ($F_{3;6}$ = 4.668; *P* = 0.052), and *Tricharaea* sp. ($F_{3;6}$ = 1.895; *P* = 0.231) were observed among the environments.

The Shannon–Wiener diversity indices were overall low and similar across the different environments (Fig. 1). Pielou's equity indices were also very similar across the environments. According to the dendrogram built by the Cluster analysis, the assemblages from the sugarcane and the rainforest were the most similar of all environments, with a similarity above 70% (Fig. 2). When the geographical origin of the species was taken into consideration, the proportion of invasive blow fly species (genus *Chrysomya*) was significantly higher than that of native species in all environments (Table 1) and was 100% of all Calliphoridae specimens collected on the beach.

The type of bait did not influence the species richness ($F_{2;6}$ = 1.043; *P* = 0.409) or abundance ($F_{2;6}$ = 3. 986; *P* = 0.079), despite the typically high number of flies in traps containing either pork or sardine (Table 2). Regarding the differential attractiveness of baits in each of the 4 environments, chicken liver attracted fewer adults when compared with pork and sardine in all environments except the beach (*P* < 0.001 for all) (Table 3). When the abundances of the 6 most common species were analyzed (2-way ANOVA) followed by a Tukey test for multiple comparison, the species did not discriminate between the types of bait (*C. albiceps*: $F_{2;6}$ = 0.375; *P* = 0.705; *C. megacephala*: $F_{2;6}$ = 0.080; *P* = 0.923; *F. pusio*: $F_{2;6}$ = 1.704; *P* = 0.259; *M. scalaris*: $F_{2;6}$ = 0.243; *P* = 0.792; *P. casei*: $F_{2;6}$ = 0.343; *P* = 0.724; and *Tricharaea* sp.: $F_{2;6}$ = 0.577; *P* = 0.593).

Discussion

In recent years, a database on the diversity of necrophagous Diptera species has been built in Brazil based on field surveys using animal carcasses as baits (e.g., Barbosa et al. 2009; Rosa et al. 2011; Vasconcelos et al. 2013). Species richness in the study reported here is similar to that observed in other studies in the Neotropical Region, including coffee plantations (Grisales et al. 2010), southern grasslands (Souza et al. 2008), rural areas (Faria et awl. 2013), oceanic island (Couri et al. 2008), urban areas, rainforest fragments (Moretti & Godoy 2013), and areas of the Amazon forest (Amat 2010).

The overlap observed among habitats in this study seems indicative of the environmental plasticity of necrophagous Diptera species given the similarity in species assemblages from the various environments. Our initial hypothesis that urban areas harbor a more diverse assemblage of necrophagous Diptera was therefore rejected. The rainforest fragment and the sugarcane plantation, particularly, shared virtually all species, despite significant differences in the vegetation composition and food resources in the areas. We believe that short distances between environments combined with the strong flight capacity of *Muscomorpha* species may be the main reasons for the similarity of these assemblages.

Alternatively, the high number of species in the rainforest fragment reflects the importance of refuges, because it was the only environment where typically asynanthropic species such as *M. bellardiana*

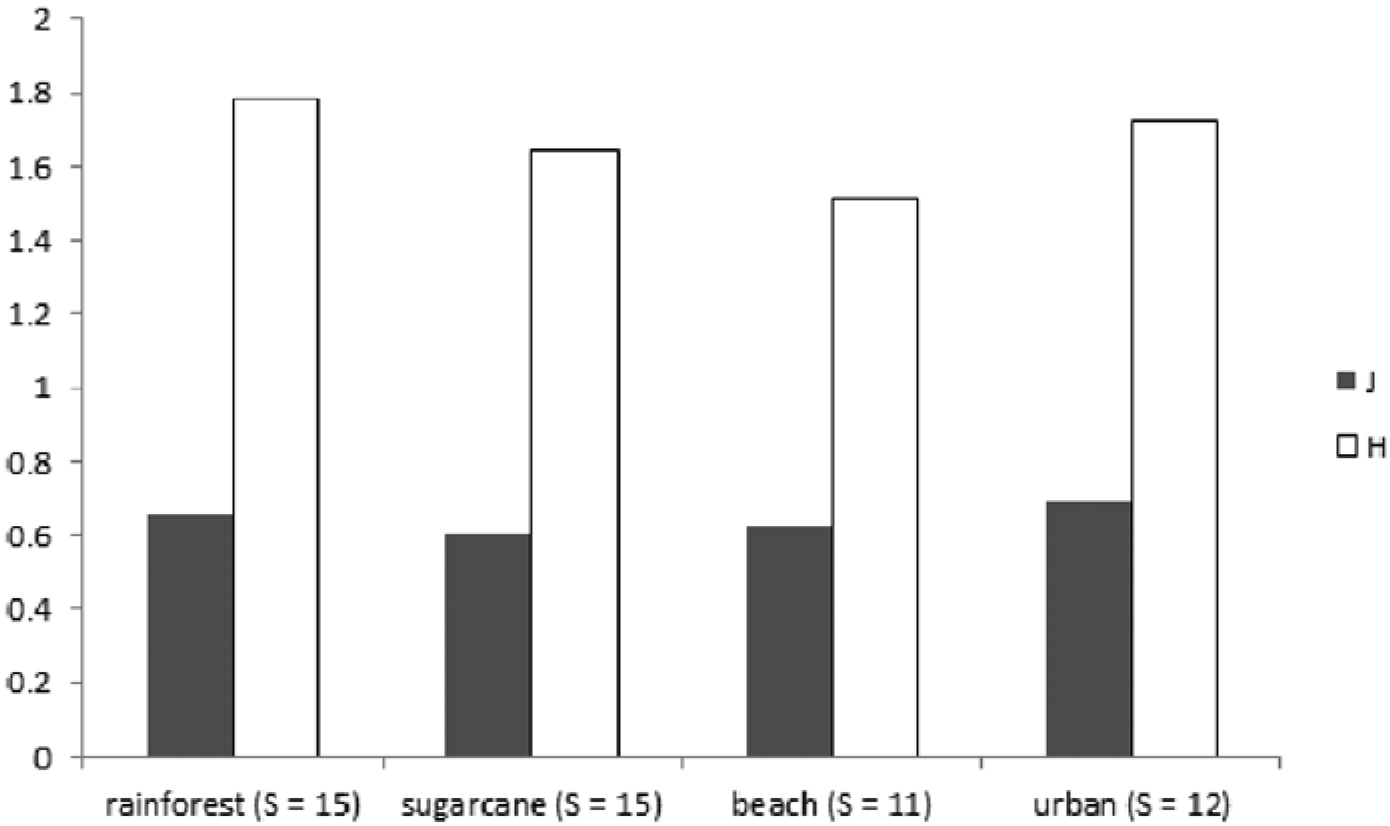

Fig. 1. Indices of Shannon–Wiener's diversity (H') and Pielou's Equity (J) of 4 types of environment in northeastern Brazil, regarding necrophagous Diptera species.

and *H. segmentaria* were found. Both species have been associated with coastal rainforest (Cabrini et al. 2013) and the Amazon forest (Montoya et al. 2009). These species have potential use as indicators of conservation status of forest fragments (Cabrini et al. 2013), which increases their forensic relevance as entomological evidence in cases of illegal deforestation. Their association with other types of rural en-

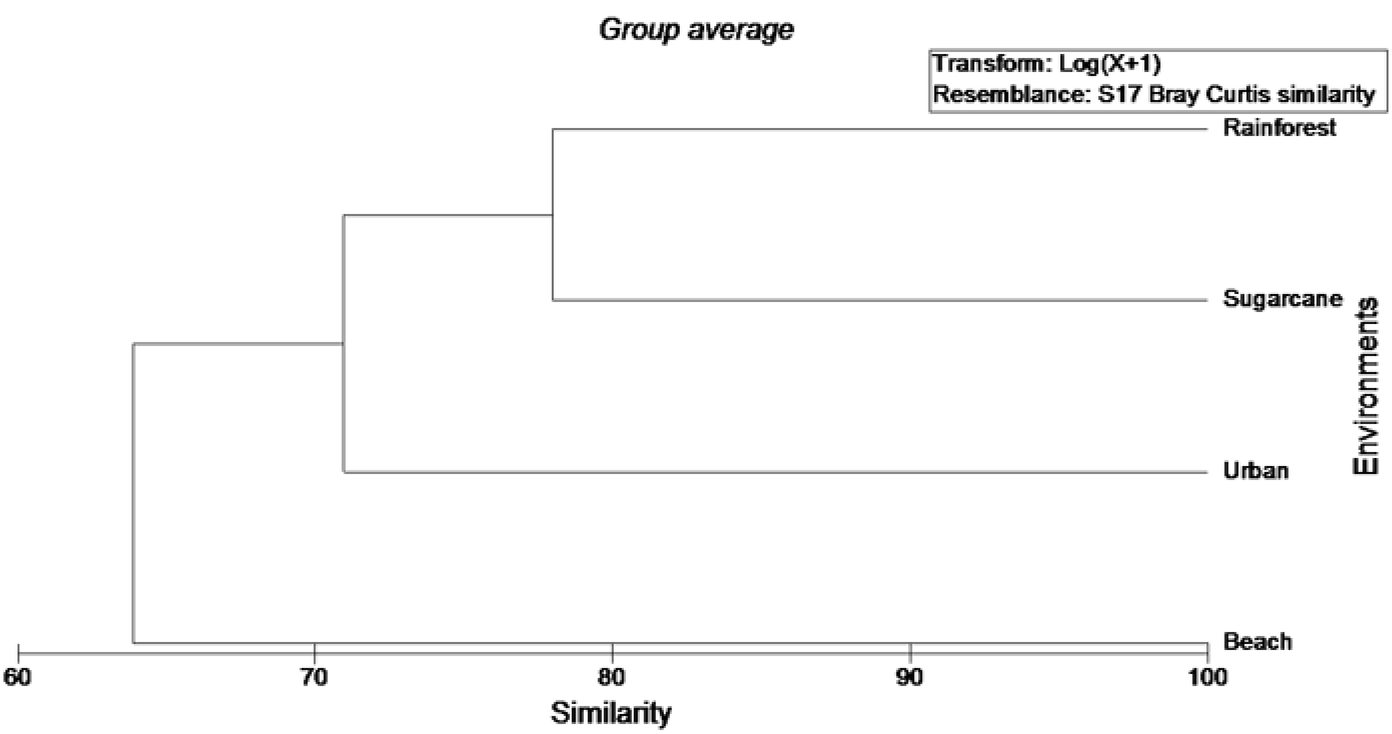

Fig. 2. Similarity analysis (Cluster's dendrogram) of the diversity of necrophagous Diptera species in 4 types of environment in northeastern Brazil.

Table 2. Abundance (A) and relative frequency (RF) of forensically-important Diptera species according to different types of animal baits. Shaded cells indicate the 3 species with the greatest abundances per type of bait.

	Chicken		Pork		Sardine	
Family/Species	A	RF %	A	RF %	A	RF %
Calliphoridae						
Chrysomya albiceps	35	6.25	371	24.82	324	23.50
Chrysomya megacephala	49	8.75	43	2.88	27	1.96
Chrysomya putoria	1	0.18	11	0.74	18	1.31
Chloroprocta idioidea	—	—	—	—	1	0.07
Cochliomyia macellaria	—	—	3	0.20	1	0.07
Hemilucilia segmentaria	1	0.18	—	—	—	—
Lucilia eximia	2	0.36	4	0.27	1	0.07
Mesembrinella bellardiana	1	0.18	1	0.07	—	—
Fanniidae						
Fannia pusio	93	16.61	150	10.03	209	15.16
Muscidae						
Atherigona orientalis	10	1.79	17	1.14	13	0.94
Musca domestica	20	3.57	36	2.41	28	2.03
Ophyra chalcogaster	—	—	—	—	2	0.15
Synthesiomyia nudiseta	23	4.11	14	0.94	12	0.87
Ulidiidae						
Ulidiidae sp.1	—	—	5	0.33	6	0.44
Ulidiidae sp.2	—	—	6	0.40	4	0.29
Phoridae						
Megaselia scalaris	147	26.25	319	21.34	293	21.25
Piophilidae						
Piophila casei	50	8.93	227	15.18	214	15.52
Sarcophagidae						
Ravinia belforti	4	0.71	2	0.13	3	0.22
Peckia (*S.*) *lambens*	5	0.89	—	—	2	0.15
Tricharaea sp.	119	21.25	286	19.13	221	16.03
Total	560	100	1,495	100	1,379	100

vironments, like the sugarcane plantation, may help in associating the entomological fauna on a cadaver to a certain environment when postmortem transfer of bodies is suspected.

The presence of synanthropic species in the urban environment reflects the availability of abundant food resources and also the poor local hygiene conditions because several necrophagous species can also feed on human excrement and garbage. This pattern is evidenced for *Lucilia eximia* (Wiedemann, 1819), *Cochliomyia macellaria* (Fabricius, 1775) (Calliphoridae), *Ravinia belforti* (Prado & Fonseca, 1932) (Sarcophagidae) and *Musca domestica* Linnaeus, 1758 (Muscidae) (Linhares 1981; Montoya et al. 2009). The composition and structure of necrophagous assemblages can be modified by human action because urbanization-related processes can favor exotic species (Kavazos & Wallman 2012). This study reveals that blow fly assemblages in all sampled environments are dominated by the invasive *C. albiceps, C. megacephala*, and *C. putoria*, especially in the sugarcane planation and rainforest fragment where the 3 species combined represented 99% and 98%, respectively, of all adults of Calliphoridae (Table 4).

The diversity of Sarcophagidae was expected to be higher in all environments, given that high species richness but low species abundance is a pattern commonly observed in necrophagous assemblages in the Neotropical Region (Barbosa et al. 2009; Vasconcelos et al. 2013). However, the scarcity of male specimens collected here hinders their taxonomical identification, which is based largely on their genitalia. Surprisingly, species of Piophilidae and Phoridae were more abundant than Calliphoridae species in the urban zone, which leads to our refutation of the hypothesis tested in this study. *Piophila casei* can feed on a variety of substrates such as stored food, which explains their abundance in metropolitan areas (Martín-Vega 2011). *Megaselia scalaris* also has a wide plasticity in both food and environmental requirements and is commonly found as contaminant in housings (Disney 1983). The detection of Fanniidae species reinforces their potential use in forensic investigations, because recent studies have expanded the register of *Fannia* species associated with human cadavers (Vasconcelos et al. 2014).

The low abundance of dipterans on the beach can be a consequence of the trap design, which is negatively affected by strong winds. Extremely high solar radiation and salinity in the sediment may act as unfavorable factors to the development of the immatures; also, strong wind is known to prevent blow flies from flying (Mulieri et al. 2011). The prevalence of small flies such as scuttle flies and cheese skipper flies may also be a result of these conditions.

Other field surveys of forensically important Diptera have corroborated the utility of animal tissues as baits, given their low cost and the absence of ethical issues compared with the use of human cadavers or animal carcasses (Moretti & Godoy 2013). It is known that necrophagous Diptera species can feed on a variety of resources that include decomposing plant material, pollen and nectar, and even other insects (Savage 2002). This fact helps to explain differences in the diversity of species collected with either chicken, sardine, or pork baits in our study. The ubiquitous *M. scalaris*, for example, can be herbivorous, saprophagous, necrophagous, parasitic, or predatory (Disney 1983) which, along with a reduced lifespan and high fecundity, explains its abundance in all environments. As expected, polyphagous species such as *M. domestica* occurred indiscriminately in all environments.

Moretti & Godoy (2013), using a similar trap in southern Brazil, reported a marked preference of necrophagous insects for chicken baits when compared with pork. In this regard, the effects of collect-

Table 3. Richness (S) and abundance (A) of necrophagous Diptera in northeastern Brazil according to different environments and types of animal baits; df = 2.

Environment	Index	Chicken	Pork	Sardine	χ^2	*P*
Rainforest fragment	S	8	12	10	0.80	0.670
	A	106	349	237	128.2	0.001
Sugarcane plantation	S	11	15	14	0.65	0.722
	A	117	506	536	282.8	0.001
Sandy beach	S	6	5	11	2.82	0.244
	A	68	33	250	232.0	0.001
Urban area	S	12	10	11	0.18	0.913
	A	269	607	356	150.0	0.001

Table 4. Comparison of relative frequencies of native and exotic Calliphoridae species in 4 types of environment in northeastern Brazil; df = 2.

Environment	Exotic	Native	χ^2	*P*
Rainforest fragment	98%	2%	3.456	0.0001
Sugarcane plantation	99%	1%	7.988	0.0001
Sandy beach	100%	0	—	—
Urban area	96%	4%	2.666	0.0001

ing method and the climatic/environmental conditions peculiar to a given region must be examined carefully before any conclusion may be reached regarding the effectiveness of a baited trap.

In addition to their ecological significance, species such as *C. albiceps*, *C. megacephala*, *M. domestica, F. pusio*, *M. scalaris*, and *P. casei* should be carefully monitored by local sanitary agencies because of the importance of these flies as causal agents of myiasis and as vectors of viruses, bacteria, helminthes, and protozoans that are pathogenic to humans and animals (Guimarães & Papavero 1999). Lastly, based on the habitat overlap reported here, we suggest that the use of Dipteran species to indicate site of death (for forensic purposes) should be focused exclusively on endemic species.

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