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Research

Population and Community Ecology

Calliphoridae (Diptera) Associated With *Rattus rattus* Carcasses in the Tijuca National Park, Rio de Janeiro, Brazil

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Abstract

Forensic entomology is a complementary tool for penal procedures, mainly on estimating postmortem interval. Study of cadaveric fauna in various environments is primary as source of information to support this science. This study collected information about the fauna of Calliphoridae associated to carcasses of *Rattus rattus* in the Tijuca National Park, RJ. Four collections were conducted, one for each season of 2015, exposing six carcasses at georeferenced points in each collection. The carcasses were placed 550 m from the boarder and equidistant by 100 m. Five decomposition stages were identified, and 10,559 individuals of Calliphoridae belonging to 10 species were collected. The most abundant species were *Hemilucilia semidiaphana* (Rondani) (Diptera: Calliphoridae) and *Lucilia eximia* (Wiedemann) (Diptera: Calliphoridae). *L. eximia* was the most abundant species during the Swelling and Black Putrefaction stages, succeeded in the next stages by two species of the genera *Hemilucilia*. *H. semidiaphana* was the dominant species in the last two stages, followed by *Hemilucilia segmentaria* (Fabricius) (Diptera: Calliphoridae). The genus *Mesembrinella* (Diptera: Calliphoridae) occurred mainly during the Black putrefaction stage. *Mesembrinella bellardiana* (Aldrich) was more abundant, with higher occurrence during the Black putrefaction and Dry decay stages. *Mesembrinella peregrina* (Aldrich) occurred in the two last stages with low abundance. *Huascaromusca aeneiventris* (Wiedemann) (Diptera: Calliphoridae) occurred during all the observed stages, mainly during the Butyric fermentation stage. *Huascaromusca purpurata* (Aldrich) (Diptera: Calliphoridae) occurred only during the Dry decay stage and in low abundance. A succession pattern in the carcasses colonization was observed, providing relevant information for the resolution of criminal investigations in this environment.

Key words: decay stage, forensic entomology, insect succession, rainforest

Forensic entomology is the science that applies knowledge about insects or other arthropods as a contribution in penal processes or police procedures through the interpretation of the entomological data found in the crime scene that originated these legal processes (Oliveira-Costa 2011, Ururahy-Rodrigues et al. 2013). One possible application is related to murder investigations, providing decisive elements for estimating the postmortem interval (PMI; Marchenko 2001, Oliveira-Costa 2011, Vasconscelos et al. 2013), which is calculated based on the larval and pupal development information collected on the body or its surroundings to determine arthropod developmental time (Ururahy-Rodrigues et al. 2013). After 72 h, forensic entomology becomes the most precise method to determine the PMI. (Oliveira-Costa 2011). Nowadays, entomological evidence in medicolegal procedures can be used in studies of body displacement, entomotoxicology, cases of negligence, victim–suspect association by DNA extracted from insects, and sexual abuse (Wells and Stevens 2008, Grisales et al. 2010, Oliveira-Costa 2011, Vasconscelos and Araújo 2012).

Throughout the world, pig carcasses are used as an experimental model for the study of forensic entomology (Souza 2009, Biavati et al. 2010, Rosa et al. 2011); however, other animal carcasses have been used, such as rats (Monteiro-Filho and Penereiro 1987, Moura and Monteiro-Filho 1997, Moretti et al. 2008, Silva et al. 2014), rabbits (Souza et al. 2008, Corrêa et al. 2014), dogs (Barbosa 2013, Martins et al. 2013), skunks (Krüger et al. 2010), and animal parts including those from beef, chicken, and fish (Leandro and D'Almeida 2005; Marinho et al. 2006; Souza et al. 2008; Ferraz et al. 2010a,b; Gonçalves et al. 2011; Koller et al. 2011).

Knowing the entomofauna associated to the decomposition of animal carcasses in rainforest areas of Rio de Janeiro will provide valuable information to be applied in forensic entomology. The Calliphoridae (Diptera) are the primary insects involved with the decomposition of exposed bodies because they are able to find and colonize carcasses within a few minutes following death and use the carcass as a food source for the development of their larvae. Thus, insects in this family are considered the most important in the medico-legal component of forensic entomology (Barbosa et al. 2010, Biavati et al. 2010, Krüger et al. 2010, Silva et al. 2010, Azmi and Lim 2013, Ururahy-Rodrigues et al. 2013). No such study has been conducted at Tijuca National Park (PARNATijuca), a secondary Atlantic Forest formation in the heart of the city of Rio de Janeiro, Brazil. This protected area is the largest urban forest in the world (3,953 ha) and is being considered by UNESCO as a World Heritage conservation site. This forest receives many visitors and can be accessed from many parts of the city (http://www.rio.rj.gov.br/web/irph/sitio-unesco). Anthropological pressure at Tijuca Forest is one of the main factors affecting the biodiversity of the area, as it is strongly associated with the invasion and establishment of invasive species (Gadelha et al. 2015a,b). Due to the constantly changing insect fauna, monitoring and surveying the forensic insects in this conservation unit is very important to provide reliable data for forensic investigations.

This study aims to evaluate the temporal presence and distribution of Calliphoridae associated with carcasses of rats in the PARNA Tijuca, Rio de Janeiro, Brazil. The collected information will be maintained in a database, thus assisting criminal investigators and forensic entomologists with relevant data to estimate the postmortem interval (PMI) or perhaps narrow the location of where an individual had died.

Materials and Methods

This project was approved by the Ethics Committee for Animal Research of the Universidade Federal do Estado do Rio de Janeiro, CEUA-UNIRIO/2015-1. The experiment was conducted in the PARNA Tijuca, Sector A, during the four seasons of 2015. The experimental models used were *Rattus rattus*, Wistar lineage, acquired from Fundação Oswaldo Cruz (FIOCRUZ), with body weight range between 200 and 400 g. Four repetitions were completed, with the use of six carcasses on each sampling interval (n = 24), using CO_2 chamber—from dry ice—as euthanasia method. Collections were conducted daily, between 9 a.m. and 11 a.m. Collections of both adult and immature Calliphoridae and environmental data (temperature, relative humidity, and rainfall) were registered, as well as the observation of the decomposition stages.

The animals were placed in traps developed by Azevedo (2016). The trap consists of a black polyethylene box (40 × 28 x 30 cm) containing six orifices with diameter of 3 cm around it for the insects to enter, and a 14 cm opening above it to connect a translucent polyethylene vessel (21 cm high x 14.5 cm diameter or 2,000 cm^3) to which the insects were attracted by phototropism. The carcass was exposed inside the trap within a metal cage (30 × 23 × 18 cm) that was placed on a polyethylene tray (30 × 22 × 6.5 cm) and held at ground level. One hundred grams of sawdust were put in the tray to support pupariation of the larvae leaving the carcass.

The six traps were placed along the Tijuca Peak trail, 550 m from the border area with Largo do Bom Retiro, separated by 100 m between traps (Lat/Long: Trap 1: −22.947417° −43.293631°; Trap 2: −22.946578° −43.293475°; Trap 3: −22.946414° −43.292925°; Trap 4: −22.945672° −43.292625°; Trap 5: −22.945408° −43.292031°; Trap 6: −22.944917° −43.291239°).

The adult insects were collected daily and transferred to polyethylene bags (3,000 cm^3) and taken to the Laboratório de Estudo de Diptera (LED), UNIRIO, where they were killed in a freezer (−10°C) prior to taxonomic identification. The sawdust was collected daily and taken to LED for the collection of larvae, and kept in a climatic chamber (Quimis, Q315F16; T = 28°C day, 26°C night, RU = 70 + 10%, 12 h of light) until adult emergence. The stage related to the presence of maggots was the day they left the carcass. For the identification process, Calliphorid adults were pinned and observed with a stereoscope microscope, following the taxonomic keys of Mello (2003) and Kosmann et al. (2013). The individuals are stored in the entomological collection of LED of University of State of Rio de Janeiro, in Rua Frei Caneca, 94, Centro, Rio de Janeiro, Brazil, CEP 20211-040.

Statistical analyses were performed using the R software (version 3.2.3); Canonical Correlation and Redundancy analyses were conducted using the Vegan package (developed by Oksanen et al.); a posttest, adapted for the Kruskal function of the Agricolae package (developed by Mendiburu) in R was employed. Kruskal–Wallis and ANOVA tests (α = 0.05) were used to compare the environmental data throughout the seasons. To analyze the relationship between species (daily abundance of individuals, including maggots and adults) and abiotic factors (temperature, relative humidity, and rainfall), redundancy (RDA) and Canonical Correlation (CCA) analyses were conducted; and Spearman correlation was employed to compare their influence on the species. Data on temperature, rainfall, and relative humidity were acquired from the Weather Station 83473, located 13.6 km from the study site, available on the website Banco de Dados Meteorológicos para Ensino e Pesquisa—(http://www.inmet.gov.br/projetos/rede/pesquisa).

Results

In total, 10,559 individuals of Calliphoridae belonging to 10 species were collected (Table 1). The most abundant species were *Hemilucilia semidiaphana* (Rondani) and *Lucilia eximia* (Wiedemann), respectively. Five decomposition stages were identified (Fig. 1), following the classification proposed by Bornemissza (1957): Initial or fresh; Early putrefaction or Swelling, Black putrefaction, Butyric fermentation, and Dry decay. The process of decomposition of *R. rattus* carcasses in this study lasted longer in the winter (12 d), and it was shorter in the summer (8 d). During the spring and autumn, the carcass took 10 d to complete the decomposition process (Table 2). It was considered the final stage of decomposition when the carcasses reached the last stage of decomposition, called Dry decay, which was identified when there was only skin, bone, and cartilage. At this stage, there was no odor, and larval activity had ceased.

Analysis of the weather conditions, recorded during the experiment showed that temperature was statistically different among the seasons (P = 5.498e−5), according to results of the Kruskal–Wallis test (α = 0.05). The lowest temperatures occurred in autumn and winter with the lowest temperature at 19.8°C, in May and August 2015, whereas the highest temperatures were recorded in spring and summer where the highest temperature recorded was 37.1°C in January 2015 (Table 3). However, no correlation was found between any species occurrence or weather data (Table 3). Relative humidity ranged from 61.3% in the summer to 88.7% in the spring; however, average relative humidity did not differ significantly among the seasons. Precipitation was higher in the spring (81.9 mm^3) and summer (68.2 mm^3), but very low in the winter, and was zero in the autumn.

Table 1. Abundance of Calliphorid species collected from 24 *Rattus rattus* carcasses placed in Tijuca National Park, RJ, Brazil, 2015

Species	Life stage	Decomposition stage				Total
		Swelling	Black putrefaction	Butyric fermentation	Dry decay	
Lucilia eximia	Adult	1,244	1,127	634	271	3,330
	Maggot	2	2	47	3	
Chrysomya megacephala	Adult	0	0	3	0	3
	Maggot	0	0	0	0	
Cochliomyia hominivorax	Adult	0	0	0	1	1
	Maggot	0	0	0	0	
Mesembrinella bellardiana	Adult	1	40	156	96	302
	Maggot	0	0	8	1	
Mesembrinella peregrina	Adult	0	1	39	44	89
	Maggot	0	0	3	2	
Hemilucilia segmentaria	Adult	163	232	518	171	2,677
	Maggot	0	8	1,200	385	
Hemilucilia semidiaphana	Adult	72	214	2,402	607	3,680
	Maggot	0	8	232	145	
Huascaromusca aneiventris	Adult	5	11	60	20	99
	Maggot	0	0	3	0	
Huascaromusca purpurata	Adult	0	0	0	2	2
	Maggot	0	0	0	0	
Laneela nigripes	Adult	9	99	171	93	376
	Maggot	0	0	2	2	
Total		1,496	1,742	5,488	1,841	10,559

Six animals in each season, 2 at each collection point/totaling 24.

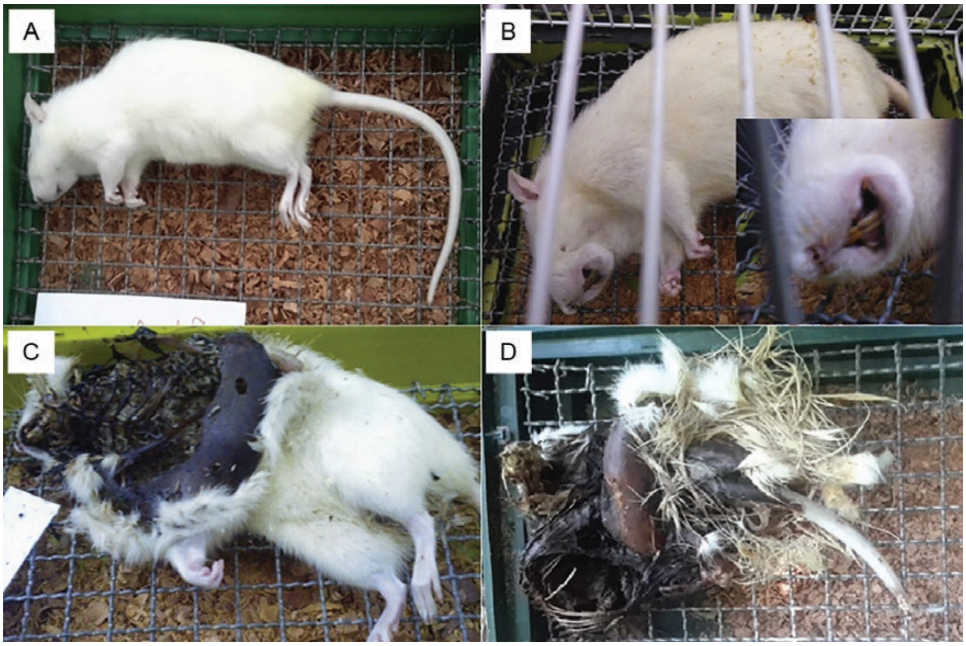

Fig. 1. Decomposition stages of *Rattus rattus* carcasses exposed in the Tijuca National Park, RJ, Brazil using stages determined by Bornemissza (1957). (A) Swelling stage; (B) Black putrefaction stage, detail of the beginning of larval activity in the oral cavity; (C) Butyric fermentation stage, with rupture of the abdomen; (D) Dry decay stage, when only bones, dry skin, and hair remain, without larval activity.

Canonical Correspondence and Redundancy of environmental factors analyses indicated no relationship between the environmental factors analyzed and the occurrence of Calliphoridae species in this study. In addition, no correlation was found between the species abundance and the environmental conditions when analyzed using the Spearman correlation (Table 4).

Analysis of diversity indices (Table 5) showed that the highest richness (Richness and Margaleff indexes) occurred in the spring and winter. The highest diversity (Shannon–Wienner index) was registered in the summer, while the lowest diversity corresponded to the winter. The Pielou and Simpson indexes show the highest equitability during the summer and the lowest during the Autumn.

By relating the occurrence of species to the decomposition stages, it was possible to determine the temporal distribution of these species and identify succession patterns (Fig. 2). The Fresh stage began in the moment of death and lasted until the moment when the body

Table 2. Duration of the decomposition stages of *Rattus rattus* carcasses placed in Tijuca National Park RJ, Brazil during 2015

	Seasons/months**			
Decomposition stages*	Summer Jan/Feb	Autumn May	Winter Jul/Aug	Spring Nov
Fresh	2 h	2 h	2 h	2 h
Swelling	48 h	72 h	48–72 h	48–72 h
Black putrefaction	24 h	24–48 h	48–72 h	24–48 h
Butyric fermentation	24—48 h	48—96 h	96 h	48—72 h
Dry decay	48—72 h	48—96 h	48—72 h	48—96 h
Total	146–192 h	194–218 h	242–314 h	170–290 h
	6- 8 d	8–10 d	10–12 d	7- 10 d

*Decomposition stages as determined using Bornemisszа (1957).
**Six animals in each season, 2 at each collection point/totaling 24.

Table 3. Maximum, minimum, and mean temperature, relative humidity, and precipitation accumulation measured in 2015, in Tijuca National Park, RJ, obtained from Meteorological Station 83743, Rio de Janeiro, RJ, Brazil—BDMEP/INMET

	Temperature (°C)			Relative humidity (%)			
Season	Minimum	Maximum	Mean*	Minimum	Maximum	Mean*	Accumulated precipitation
Summer	23.5	37.1	27.5a	61.3	83.5	75.6a	68.2
Autumn	19.8	30.9	24.2b	64.3	80.5	74.2a	0.0
Winter	19.8	32.0	24.0b	72.3	84.8	77.2a	0.1
Spring	21.0	33.6	26.5a	67.3	88.7	79.9a	81.9

*Different letters represent statistical difference according to Kruskal–Wallis test (α = 0.05%).

Table 4. Spearman correlation value (ρ) and respective significance (p) between the abundance of the Calliphoridae species and the environmental factors analyzed for Calliphoridae collected on *Rattus rattus* carcasses in Tijuca National Park, RJ, Brazil in 2015

	Temperature		Humidity		Rainfall	
Species	ρ	*P*	ρ	*P*	ρ	*P*
Lucilia eximia	−0.2602	0.1503	−0.1605	0.3802	−0.255	0.159
Mesembrinella bellardiana	0.3839	0.0301*	0.0682	0.7106	0.283	0.1165
Mesembrinella peregrina	0.0085	0.9634	−0.1807	0.3222	−0.1619	0.3759
Hemilucilia segmentaria	0.0579	0.753	−0.1245	0.4972	0.0898	0.6249
Hemilucilia semidiaphana	0.2785	0.1227	0.0313	0.865	0.1517	0.4071
Huascaromusca aneiventris	0.1756	0.3364	0.1716	0.3477	0.2913	0.1057
Huascaromusca purpurata	NA	NA	NA	NA	NA	NA
Laneela nigripes	0.237	0.1915	0.1752	0.3373	0.1845	0.3122
Chrysomya megacephala	NA	NA	NA	NA	NA	NA
Cochliomyia hominivorax	NA	NA	NA	NA	NA	NA

NA, correlation not analyzed due to low number of specimens collected.
*Difference due to outliers.

Table 5. Analysis of the diversity indices of the Calliphoridae collected on *Rattus rattus* carcasses in Tijuca National Park, RJ, Brazil in 2015

Diversity index	Total	Summer	Autumn	Winter	Spring
Richness	10	7	7	9	8
Margalef	0.9714	0.7628	0.7668	0.9555	0.9812
Shannon–Wiener	1.3768	1.6253	0.9823	1.2581	1.4915
Pielou	0.5979	0.8352	0.5048	0.5726	0.7173
Simpson	0.2904	0.2179	0.4680	0.3569	0.2850

started to bloat. This stage lasted about 2 h in all repetitions. The putrefaction odor was not noticeable in this stage. No samples were made in this stage due to its short period.

The beginning of the Swelling stage (Fig. 1A) was not easily noticeable, but the abdominal, facial, and genital areas did show increased distention. Fluids expelled by the natural orifices caused a fetid odor, which increased the attraction of cadaveric insects. In this stage, the first necrophagous insects arrived at the carcasses. The Black putrefaction stage (Fig. 1B) was distinguished by the rupture of the skin tissue, increasing the available surface for

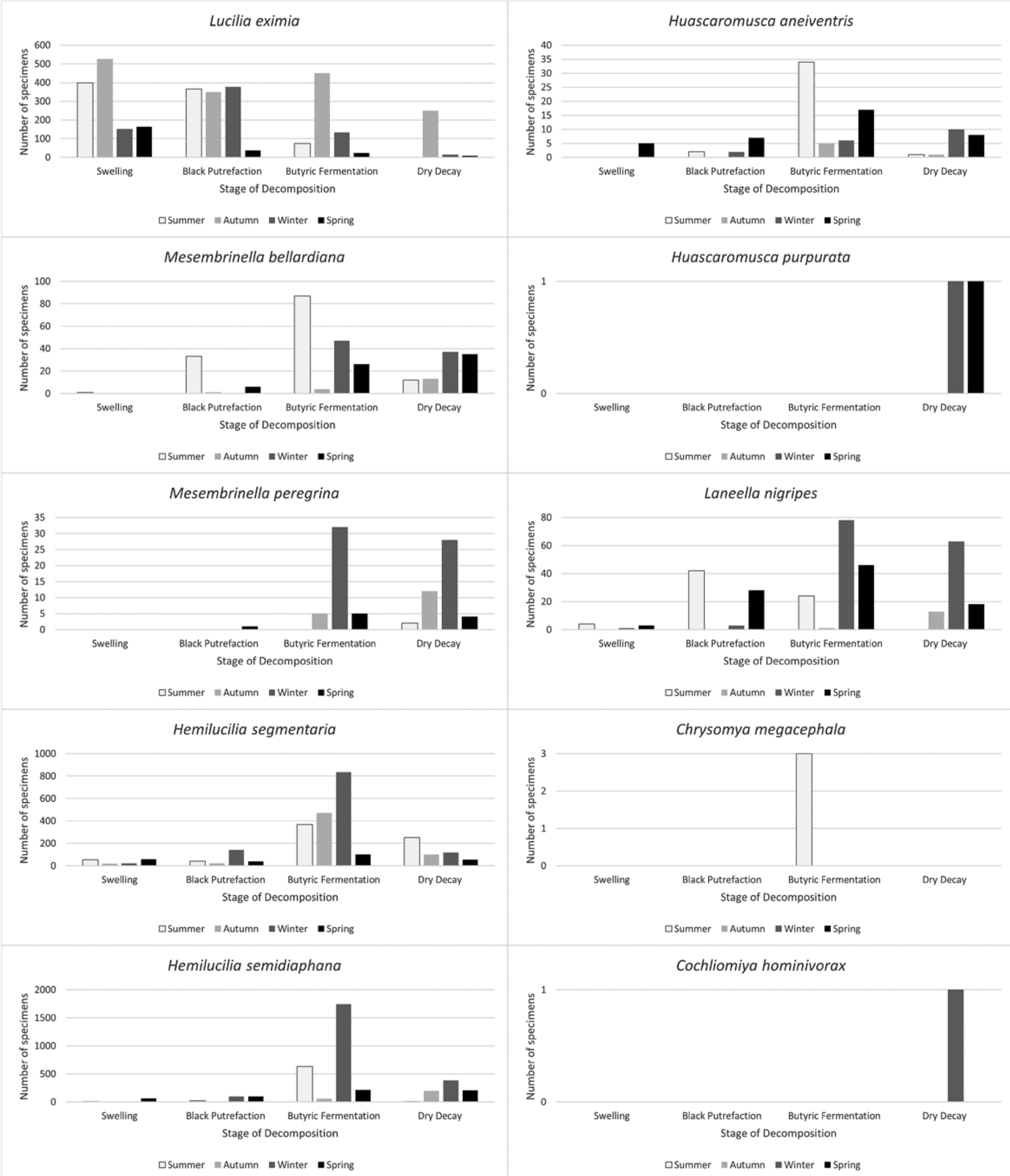

Fig. 2. Temporal distribution of Calliphorid species: presence or absence and abundance using collections from 24 *Rattus rattus* carcasses in the Tijuca National Park, RJ, Brazil. Collections completed from six rats at all decomposition stages in four seasons during 2015.

larvae to feed. This rupture started from natural orifices, mainly the oral one. The highest larval activity was observed in this stage, besides high fetid odor and the presence of coleopteran individuals. Oviposition events were rare at this stage, and it lasted 2 to 3 d. *L. eximia* was prevalent during Swelling and Black putrefaction stages with 84 and 65% of individuals collected, respectively (Table 1, Fig. 2).

Few carcass resources remained at the start of the Butyric fermentation stage (Fig. 1C), and it was possible to observe the migration of larvae to the sawdust to prepare for pupariation. Despite the low resources available, attractiveness of adults was still high. Odor was noted to be reduced, and this stage lasted 1 to 4 d, with a faster progression in summer and slower in winter. The Dry decay stage (Fig. 1D) was achieved when only skin, bones, hair, and cartilage remained. Odor was no longer noticeable in this stage, and larval activity was highly decreased. This stage was observed over 4 d, and then the carcasses were removed from the experimental site. *H. semidiaphana* was prevalent during the Butyric fermentation and Dry decay stages with 47 and 42%, respectively, of collected specimens of this species, followed by *Hemilucilia segmentaria* (Fabricius) with 32 and 28%, respectively (Table 1).

Remaining species collected were few in number. The genus *Mesembrinella* occurred, mainly, during the Black putrefaction stage, with *Mesembrinella bellardiana* (Aldrich) most abundant, during Black putrefaction and Dry decay stages. *Mesembrinella peregrina* (Aldrich) occurred in the final two stages with low abundance. *Huascaromusca aeneiventris* (Wiedemann) occurred during all the decomposition stages, but was most abundant during the Butyric fermentation stage. *Huascaromusca purpurata* (Aldrich), on the other hand, occurred only during Dry decay stage and with low abundance. Peaks of *Laneella nigripes* (Guimarães) (Diptera: Calliphoridae) were also observed in the Black putrefaction stage. *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) and *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae) adults were collected during Butyric fermentation and Dry decay stages, respectively, in small number.

Discussion

The faunistic composition can vary in several scales depending mainly on topography and environmental factors (Silva et al. 2010, Al-Mesbah 2012, Silva 2012). Interspecific competition is commonly reported in necrophagous communities and species distribution studies because the co-inhabiting species are in constant competition. Dominance of two or three species has been reported in necrophagous communities (Oliveira-Costa et al. 2013). The fluctuation of environmental conditions, mainly temperature, is another factor identified as influent to the development of calliphorid species (Krüger et al. 2010). Due to these variations, it is necessary to collect data specific to each environment and infeasible to use data from differing environments (Al-Mesbah 2010). Human activity also may influence the distribution and diversity of Calliphoridae (Marinho et al. 2006, Mello et al. 2007). The Tijuca National Park is the largest urban forest in the world and, despite its preserved status, is constantly under influence of human activity because it is utilized by the surrounding community.

Most of the collected species (8/10) are considered sylvan species in other studies, which indicates the preservation of the PARNATIJUCA. *H. semidiaphana* and *H. segmentaria* are considered sylvan species, endemic to South America (Cabrini et al. 2013), showing high preference for forest areas (Marinho et al. 2006). These species were collected during all the sampled decomposition stages, with highest abundance during the two final stages. Additionally, *H. segmentaria* had the third highest abundance in this study, suggesting it is a common species in the park. These results corroborate with several other studies (Calderón-Arguedas et al. 2005, Silva 2012, Oliveira-Costa et al. 2013) that also observed this species in advanced decomposition stages. Thus, *H. segmentaria* should be considered as a potential forensic indicator for these later stages.

The *Mesembrinellinae* are a small group of exclusively neotropical flies highly related to forest areas, presenting high abundance and diversity in these areas (Marinho et al. 2006; Mello et al. 2007; Gadelha et al. 2009, 2015a,b; Ferraz et al. 2010a). Therefore, they are considered biological indicators for preserved forest areas, responding to different kinds of environmental impacts (Marinho et al. 2006; Mello et al. 2007; Gadelha et al. 2009, 2015a,b; Ferraz et al. 2010a). In this study, five species of this group were collected: *M. peregrina*, *M. bellardiana*, *H. aeneiventris*, *H. purpurata*, and *L. nigripes*. It can be noticed that the *Mesembrinella* species are attracted to carcasses preferentially during the final stages (Butyric fermentation and Dry decay), and that larvae migration of these species was only observed during the winter and summer (Fig. 2). It is important to note that *M. peregrina*, *H. aeneiventris*, *H. purpurata*, and *L. nigripes* have never been associated with carcasses before, which will be discussed in a further publication.

However, *C. megacephala* (Fabricius) and *C. hominivorax* (Coquerel), two species highly related to urban areas (Ferraz et al. 2008; Coronado and Kowalski 2009; Ferraz et al. 2010a,b; Valviesse et al. 2014), were collected during the summer, period with highest anthropic activity in the park due to vacation and the hot weather. However, no larval migration of these species was observed, only adults in low abundance (Fig. 2). Their presence can be an alert for needed preservation of the area studied. *C. hominivorax* is considered a primary myiasis agent, and *C. megacephala*, a secondary myiasis agent (Guimarães et al. 1983, Ferreira 2012). For this reason, they are considered accidental species.

L. eximia is a neotropical and nearctic species considered synanthropic, which occurs in both forest and urban areas (Marinho et al. 2006, Mello et al. 2007, Biavati et al. 2010, Ferraz et al. 2010a). Observing this species as the first to visit the carcasses corroborates with other studies in other environments that list *L. eximia* as a pioneer species on colonization of pig carcasses (Souza 2009, Biavati et al. 2010, Grisales et al. 2010, Silva 2012, Oliveira-Costa et al. 2013). This species can be considered forensically important because it is present along all decomposition stages and all seasons (Fig. 2), as in this situation it is possible to apply the most accepted model of PMI, the linear model of accumulated degree days, in which are related the development time of the specimen and the temperature (Oliveira-Costa 2011). *L. eximia* abundance decreased only during the Dry decay stage, corroborating with other studies (Calderon-Arguedas et al. 2005, Silva et al. 2010, Silva 2012). Larval migration by *L. eximia* was observed during each season, although the number of specimens was low. Most migration occurred during the Butyric Fermentation stage in all seasons.

The ability to measure biodiversity is critically important, given the soaring rates of species extinction and human alteration of natural habitats. At the small scale, species richness is generally used as a measure of diversity within a single ecological community, habitat, or microhabitat, although the definition of small depends on the species in question. (Brown et al. 2007). The diversity indices of Simpson and Shannon incorporate species abundances in addition to species richness and are intended to reflect the likelihood that two individuals taken at random are of the same species. However, they tend to de-emphasize uncommon species (Brown et al. 2007), justifying the need to also evaluate the Pielou index. As our data indicate, despite the high richness in the winter, the dominance of *H. semidiaphana* decreases the Shannon–Wienner diversity index. However, in the summer, despite the lowest richness recorded, the distribution of the species was more uniform, consequently increasing the diversity index.

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