

Temporal distribution of ten calyptrate dipteran species of medicolegal importance in Rio de Janeiro, Brazil

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*Brazilian studies involving entomological succession patterns in carcasses have been used to describe the necrophagous entomofauna of a determined municipality or region with forensic objectives. Following the same objectives, an ecological study with 10 calyptrate dipterans was carried out during the winter of 2007 and the summer of 2008 in the metropolitan region of the municipality of Rio de Janeiro. The aim of this study was to describe several aspects of the phenology of these species in three neighbouring areas. Carcasses of three domestic pigs (*Sus scrofa* L.) were used in each season as models for forensic and legal medicine investigations in the region. Temperature, relative humidity and rainfall were measured daily and their relations with population abundance of the colonising species and the decomposition stages were analysed. Ten fly species were recorded to be colonising the carcasses, five of which belonged to the Calliphoridae family, three to the Muscidae, one to the Fanniidae and one to the Sarcophagidae family. Data show preferences of these species for climatic season and decomposition stage, as well as for the studied area and suggest that short distances can significantly influence the abundance of some species.*

Key words: calyptrate dipterans - domestic pigs - decomposition - Brazil - forensic entomology

Knowledge of the necrophagous entomofauna is one of the bases of forensic entomology. In fact, it contributes to the understanding of the temporal distribution and behaviour of many species that are also active in the decomposition of human corpses (Smith 1986, Catts & Haskell 1991, Byrd & Castner 2001). A large number of worldwide studies have been carried out to identify the insects that are associated with the degradation of corpses; these studies have mainly aimed to observe the ecology of the species involved (Benecke 2001). The results of these studies are used as a tool for bioecological, morphological and molecular studies of potential importance for forensic entomology. Through these studies, entomological methods may also be applied as an auxiliary tool for criminal investigations (Oliveira-Costa 2007).

Calyptrate flies [e.g., the blowflies (Calliphoridae) and the domestic flies (Muscidae)] have an extensive variety, different strategies of survival and very diverse bionomic characteristics, including hematophagy, viviparity and endoparasitism (McAlpine 1989, Yates & Wiegmann 1999). Among them, four families are of great forensic importance throughout the world and also in the municipality of Rio de Janeiro: Calliphoridae, Sarcophagidae, Muscidae and Fanniidae (Salviano 1996, Barbosa et al. 2009).

Two main groups of necrophagous calyptrate dipterans can be collected in association with carcasses; the first looks for animal protein for ovarian development and the second uses it as temporary resource for larvae

breeding. In the latter case, these insects normally have a preference for certain stages of carcass decomposition, thereby allowing the forensic entomologist to determine the postmortem interval (PMI) (Kamal 1958, Smith 1986, Greenberg 1991). Additionally, other important aspects in a criminal investigation can be considered, such as determining the cause of death, confirming the hypothesis of neglect of children or the elderly and investigating the possibility that a corpse was moved from the crime scene (Benecke 2001, Benecke & Lessig 2001).

In Brazil, the number of studies involving necrophagous entomofauna has increased in recent years. However, considering the country dimensions and its megadiversity of species, there is a need for additional studies (Pujol-Luz et al. 2008).

The present paper aims to identify, quantify and analyse the ecological aspects of ten calyptrate dipterans with forensic entomological importance, in order to improve the possibility of estimating the minimal PMI and to evaluate the existence of population variations between three neighbouring areas in the Rio de Janeiro municipality.

MATERIALS AND METHODS

Study location - The study was carried out in the north side of Rio de Janeiro, a municipality in Southeast Brazil. Three neighbouring locations with different physiographic characteristics within the large area of the Fundação Oswaldo Cruz-Fiocruz were chosen for the experiments and were named area 1 (22°52'33"S 43°14'47"W), 2 (22°52'21"S 43°14'49"W) and 3 (22°52'27"S 43°14'40"W) (Supplementary data).

Area 1 was situated 20 m from a place with moderate movement of vehicles and people; its vegetation was mostly composed by *Eugenia jambolana* (*jamelão*) and *Panicum maximum* (*capim-colonião*) and the carcass

Financial support: PAPES V/CNPq (403482/2008-9)

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Received 16 October 2009

Accepted 3 February 2010

was placed in a location that was partially protected from direct sunlight. Area 2 was located about 100 m from movement of vehicles and people; its hard vegetation was mainly composed by *Caesalpinia ferrea* (*pau-ferro*) and the carcass was totally protected from the sun. Area 3 was positioned 10 m from a place with abundant movement of people and vehicles; its vegetation was composed by *E. jambolana*, *Lecythis* sp. (*sapucaia*) and *P. maximum* and the carcass was exposed to the sun for almost the entire day. The experiments were carried out during the winter of 2007 (from August 15 2007-August 27 2007) and the summer of 2008 (from January 16 2008-January 30 2008).

Experimental model - For each study season, three domestic pigs (*Sus scrofa* L.) weighing 15 kg were used, one for each area. They were obtained from a pig farm, killed on location by a blow to the skull at 10 am and immediately transported in sealed plastic bags to the study area, where they were set out one hour later.

Model of traps - The traps model and the field protocols were the same as those proposed by Salviano (1996). The adults were collected daily between 12-2 pm, at which time the collector was replaced by a new one. In the laboratory, the collector tube was placed in a freezer at -17°C for 40 min. After freezing, the insects were transferred to test tubes that were labelled with the date, the hour, the carcass number and the locality. The material was returned to the freezer for posterior sorting, identification and counting of specimens. To confirm which species had colonised the carcasses, egg samples were collected and placed on filter paper and after eclosion, the first instar larvae were placed on decomposing flesh to obtain the adults. Larvae and pupae from the carcasses and the immediate surroundings were also collected for the same reason.

Abiotic variables register - The local temperature and the relative humidity of air (RH) were measured daily at the collection time with a Thomas Scientific® thermal hygrometer.

Identification of specimens - The Calliphoridae were identified using the key proposed by Mello (2003). The Muscidae and Fanniidae were identified using the keys proposed by Carvalho and Couri (2002), Couri and Carvalho (2002) and Carvalho et al. (2002). The male Sarcophagidae were identified using the key proposed by Carvalho and Mello-Patiu (2008) and the females were identified by comparison with the Sarcophagidae collection of the Entomology Department of Museu Nacional, Universidade Federal do Rio de Janeiro.

The decomposition stages - To evaluate the decomposition, stages were classified using the terminology of Tullis and Goff (1987) and the morphological modifications of the carcass were adjusted to decomposition time in our climatic conditions and subdivided into days.

Statistical analyses - To evaluate the relative abundance of the species and their possible preference for the area studied (area 1, 2 or 3), carcass decomposition stages and seasons, the analysis of variance (ANOVA) (Sokal & Rohlf 1995) of the three factors (area of study,

stage of decomposition and season) was carried out and the relative abundance was the variable response. Significances were determined by the Tukey test ($p \leq 0.05$). The species that were not found in their immature stages on the carcass during the decomposition process were excluded from the statistical analyses, due to their lesser importance as forensic indicators. The immature samples were qualitative and were not included in the statistical analyses.

RESULTS

Decomposition process - The following five decomposition stages were observed: fresh, bloated, decay, post-decay and skeletonisation. Differences in the duration of decomposition stages at each area and between the seasons studied were only observed in the summer. Each stage was divided into a number of days according to the morphological changes observed (Fig. 1).

Meteorological data - For both seasons, the ambient temperature and RH were recorded daily at the location of each carcass (Figs 2-5).

Species composition - The following 10 species were collected as adults and also as larvae colonising the carcasses: *Chrysomya albiceps* (Wiedemann 1819), *Chrysomya megacephala* (Fabricius 1794), *Chrysomya putoria* (Wiedemann 1818), *Hemilucilia segmentaria* (Fabricius 1805), *Lucilia eximia* (Wiedemann 1819), *Ophyra aenescens* (Wiedemann 1830), *Ophyra chalcogaster* (Wiedemann 1824), *Synthesiomyia nudiseta* (Wulp 1883), *Fannia pusio* (Wiedemann 1830) and *Peckia (Pattonella) intermutans* (Thomson 1869). Table I shows the absolute and relative abundance of each species in the two studied seasons.

Species vs. season - According to ANOVA and Tukey tests, five species showed significant differences in their abundances and averages. *F. pusio* was more abundant in the winter; in fact, 81.4% ($p < 0.01$) of the specimens were collected in this season. On the other hand, some species were more abundant in the summer, such as *O. aenescens*, with 88.1% ($p < 0.01$), *C. megacephala*, with 96.3% ($p < 0.01$), *C. putoria*, with 93.6% ($p < 0.01$), and *H. segmentaria*, with 62.7% ($p < 0.05$).

In the winter, *F. pusio* had a significant relation to area 2 when compared with area 3 only, where 56.9% ($p < 0.01$) of the specimens were collected. The other studied species showed variations of their abundances, but there was no significant difference between the areas (Supplementary data).

When the average number of individuals from each species was compared in the summer, most of the significant differences occurred between areas 2-3. Note that 70.8% of the specimens of *O. aenescens* in this season were collected from area 2 ($p < 0.01$); *C. megacephala* also had a preference for this area, with 55.6% of the specimens ($p < 0.05$) and the same occurred with *C. albiceps*, which made up 48.1% ($p < 0.05$). However, area 2 had significant differences with the other areas when considered simultaneously: *O. chalcogaster* had 69.7% ($p < 0.01$) and *H. segmentaria* had 57.5% ($p < 0.01$) (Supplementary data).

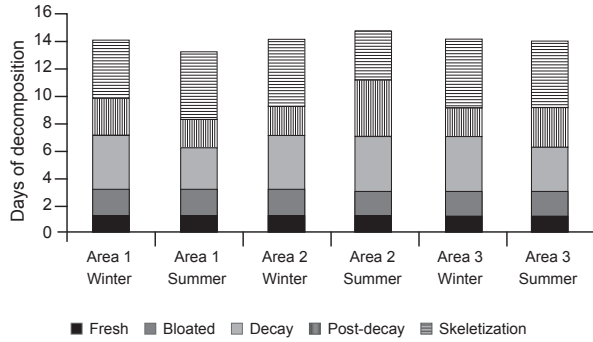


Fig. 1: duration of the carcass decomposition stages in each study area.

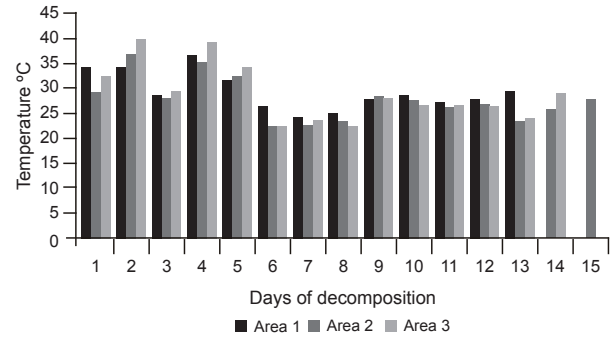


Fig. 3: ambient temperature (°C) data for each study area in summer 2008.

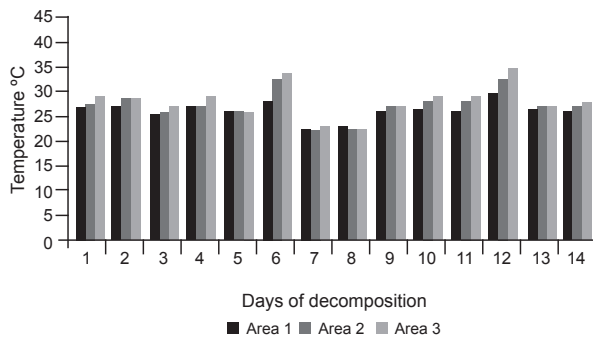


Fig. 2: ambient temperature (°C) data for each study area in winter 2007.

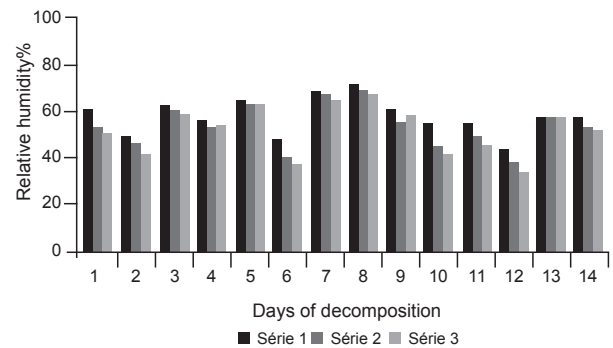


Fig. 4: relative humidity (%) data for each study area in winter 2007.

Relation between coloniser species vs. decomposition stages - Table II shows the absolute and relative number of adults captured during the experiment, statistically correlated to the stages of carcass decomposition. *C. megacephala* showed a preference for the bloating and decay stages of summer and more than 80% occurred in these two stages alone. *H. segmentaria* had a significant preference for the bloating stage in both seasons; this species was considered to be one of the pioneers in the decomposition process.

O. aenescens was highly abundant in the decay stage of the summer, with 1,476 (66.3%) specimens captured. There was no significant difference between the other decomposition stages for this species. However, *S. nudiseta* was captured in greater numbers in the winter of 2007 and had a significant preference for the bloating stage in the winter, the stage in which it was considered to be a pioneer in the decomposition process, with 523 (51%) specimens captured. *F. pusio* was the most numerous insect in the whole experiment, but its appearance was only significant in the winter decay stage, when 1,305 (41.9%) specimens were collected.

DISCUSSION

Forty-five calyprate species were collected in the two experiments, although most of them probably went to the carcasses to feed and only 10 were found colonising the

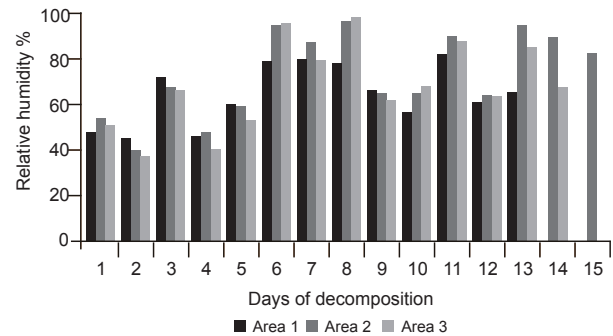


Fig. 5: relative humidity (%) data for each study area in summer 2008.

models. These results are in accordance with Johnson (1975) and Carvalho et al. (2000), who pointed out that few species actually have value for forensic entomology. Only the species that use the corpses as the substrate for development of the immature forms are important for determination of the PMI interval through assessment of their post-embryonic development (Erzinçlioglu 1985, Greenberg 1991, Goff 2000).

Succession patterns of the calyprate flies in these experiments were not clearly observed, a fact also reported by Salviano (1996) in the same studied area. Contrasting results have been observed in experiments

TABLE I
Absolute and relative abundance of colonizing species in each studied season

Species		Season		Total
		Winter	Summer	
<i>Chrysomya albiceps</i>	n	362	636	998
	%	36.3	63.7	100
	\bar{X}	3.580 ^a	4.686 ^a	-
<i>Chrysomya megacephala</i>	n	62	1,617	1,679
	%	3.7	96.3	100
	\bar{X}	1.714 ^b	7.623 ^a	-
<i>Chrysomya putoria</i>	n	13	191	204
	%	6.4	93.6	100
	\bar{X}	1.035 ^b	2.690 ^a	-
<i>Hemilucilia segmentaria</i>	n	56	94	150
	%	37.3	62.7	100
	\bar{X}	1.559 ^b	2.150 ^a	-
<i>Lucilia eximia</i>	n	191	223	414
	%	46.1	53.9	100
	\bar{X}	2.292 ^a	3.054 ^a	-
<i>Ophyra aenescens</i>	n	264	1,962	2,226
	%	11.9	88.1	100
	\bar{X}	3.332 ^b	8.018 ^a	-
<i>Ophyra chalcogaster</i>	n	97	66	163
	%	59.5	40.5	100
	\bar{X}	2.239 ^a	1.815 ^a	-
<i>Synthesiomyia nudiseta</i>	n	743	282	1,025
	%	72.5	27.5	100
	\bar{X}	4.960 ^a	3.808 ^a	-
<i>Fannia pusio</i>	n	2,534	580	3,114
	%	81.4	18.6	100
	\bar{X}	10.360 ^a	5.404 ^b	-
<i>Peckia intermutans</i>	n	13	4	17
	%	76.5	23.5	100
	\bar{X}	1.071 ^a	0.845 ^a	-

a, b: averages for each species are not significantly different at 5% ($p < 0.05$). The averages were compared using the Tukey test; n: absolute abundance of species; %: relative abundance of species; \bar{X} : average values.

carried out in temperate countries, where the necrophagous insects clearly obeyed an arrival order (succession) on the models (Bornemissza 1957, Rodriguez & Bass 1983). However, the seasonal variation significantly influenced the abundance of some studied species. In summer, with higher average temperatures, there was a large increase in the species abundance, as observed by Tullis and Goff (1987), Tomberlin and Adler (1998) and Oliveira-Costa et al. (2001a).

Family Calliphoridae - The Calliphoridae, including species whose larvae are voracious and competitive, were the most important group in the decomposition process (Smith 1986, Greenberg 1991). Many of the species are indicators of PMI or can be used for the detection of

drug/chemical residuals (Zuben et al. 1996, Carvalho et al. 2001, Introna et al. 2001).

C. megacephala was the most abundant Calliphoridae in the whole experiment, with 96.3% of the individuals collected in the summer. Larvae were also found. This result corroborates the findings of Early and Goff (1986) and Tullis and Goff (1987), who found the same pattern in cat and pig carcasses in Hawaii. In Brazil, Oliveira-Costa et al. (2001b) found this species of blowfly colonising human corpses in Rio de Janeiro. Andrade et al. (2005) also collected maggots of this species in the decay stage in human corpses. However, according to Souza and Linhares (1997), this species could not be fundamentally necrophagous because collected few individuals on pig carcasses in Campinas, state of São Paulo (SP).

TABLE II
Absolute and relative abundance of the colonizing species divided by decomposition stage and season

Species	Winter										Summer										Total
	Decomposition stage					Decomposition stage					Decomposition stage					Decomposition stage					
	F	B	D	PD	S	F	B	D	PD	S	F	B	D	PD	S	F	B	D	PD	S	
<i>Chrysomya albiceps</i>	n	211	147	4	0	0	162	422	31	21	0	0	0	0	0	0	42.3	42.3	3.1	2.1	998
	%	21.1	14.7	0.5	0	0	16.2	42.3	3.1	2.1	0	0	0	0	0	0	4.3	4.3	0.3	0.2	100
	\bar{X}	8.129 ^{ab}	7.015 ^{a,b,c}	1.344 ^{b,c}	0.707 ^c	0.707 ^c	7.191 ^{a,b,c}	10.687 ^a	2.342 ^{b,c}	2.505 ^{b,c}	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	10.687 ^a	10.687 ^a	2.342 ^{b,c}	2.505 ^{b,c}	-
<i>Chrysomya megacephala</i>	n	31	30	1	0	0	632	866	62	56	0	1	1	1	1	1	51.6	51.6	3.7	3.3	1,679
	%	1.8	1.8	0.1	0	0	37.6	51.6	3.7	3.3	0	0.1	0.1	0.1	0.1	0.1	5.2	5.2	0.4	0.4	100
	\bar{X}	3.268 ^b	3.008 ^b	0.880 ^b	0.707 ^b	0.707 ^b	13.760 ^a	15.713 ^a	3.741 ^b	4.019 ^b	0.707 ^b	0.880 ^b	0.880 ^b	0.880 ^b	0.880 ^b	0.880 ^b	15.713 ^a	15.713 ^a	3.741 ^b	4.019 ^b	-
<i>Chrysomya putoria</i>	n	4	9	0	0	0	32	146	9	4	0	0	0	0	0	0	14.6	14.6	0.7	0.4	204
	%	2.0	4.4	0	0	0	15.6	71.6	4.4	2.0	0	0	0	0	0	0	15.6	71.6	4.4	2.0	100
	\bar{X}	1.268 ^b	1.785 ^b	0.707 ^b	0.707 ^b	0.707 ^b	3.209 ^b	6.628 ^a	1.616 ^b	1.290 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	6.628 ^a	6.628 ^a	1.616 ^b	1.290 ^b	-
<i>Hemilucilia segmentaria</i>	n	47	9	0	0	0	49	34	7	4	0	0	0	0	0	0	34	34	7	4	150
	%	31.3	6.0	0	0	0	32.7	22.7	4.7	2.6	0	0	0	0	0	0	22.7	22.7	4.7	2.6	100
	\bar{X}	3.944 ^a	1.729 ^{b,c}	0.707 ^c	0.707 ^c	0.707 ^c	4.038 ^a	3.281 ^{a,b}	1.544 ^{b,c}	1.179 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	3.281 ^{a,b}	3.281 ^{a,b}	1.544 ^{b,c}	1.179 ^c	-
<i>Lucilia eximia</i>	n	174	15	2	0	0	133	60	26	3	0	1	1	1	1	1	60	60	26	3	414
	%	42.1	3.6	0.5	0	0	32.1	14.5	6.3	0.7	0	0.2	0.2	0.2	0.2	0.2	14.5	14.5	6.3	0.7	100
	\bar{X}	6.690 ^a	2.303 ^{a,b}	1.052 ^{a,b}	0.707 ^b	0.707 ^b	6.337 ^{a,b}	4.001 ^{a,b}	2.883 ^{a,b}	1.171 ^{a,b}	0.707 ^b	0.880 ^{a,b}	0.880 ^{a,b}	0.880 ^{a,b}	0.880 ^{a,b}	0.880 ^{a,b}	4.001 ^{a,b}	4.001 ^{a,b}	2.883 ^{a,b}	1.171 ^{a,b}	-
<i>Ophyra aenescens</i>	n	64	177	18	5	5	179	1,476	224	83	0	0	0	0	0	0	1,476	1,476	224	83	2,226
	%	2.9	7.9	0.8	0.2	0.2	8.0	66.4	10.1	3.7	0	0	0	0	0	0	66.4	66.4	10.1	3.7	100
	\bar{X}	4.594 ^b	7.607 ^b	2.401 ^b	1.351 ^b	1.351 ^b	7.024 ^b	20.228 ^a	7.290 ^b	4.840 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	20.228 ^a	20.228 ^a	7.290 ^b	4.840 ^b	-
<i>Ophyra chalcogaster</i>	n	33	51	10	3	3	17	36	9	4	0	0	0	0	0	0	36	36	9	4	163
	%	20.3	31.3	6.1	1.8	1.8	10.5	22.1	5.5	2.4	0	0	0	0	0	0	22.1	22.1	5.5	2.4	100
	\bar{X}	3.353 ^{a,b}	4.173 ^a	1.793 ^{b,c}	1.171 ^c	1.171 ^c	2.129 ^{a,b,c}	3.383 ^{a,b}	1.676 ^{b,c}	1.179 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	3.383 ^{a,b}	3.383 ^{a,b}	1.676 ^{b,c}	1.179 ^c	-
<i>Synthomyia nudiseta</i>	n	523	196	19	5	5	87	91	68	36	0	0	0	0	0	0	91	91	68	36	1,025
	%	51.0	19.1	1.9	0.5	0.5	8.5	8.9	6.6	3.5	0	0	0	0	0	0	8.9	8.9	6.6	3.5	100
	\bar{X}	12.116 ^a	8.055 ^{a,b}	2.536 ^{b,c}	1.386 ^{b,c}	1.386 ^{b,c}	4.969 ^{a,b,c}	5.314 ^{a,b,c}	4.606 ^{b,c}	3.448 ^{b,c}	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	0.707 ^c	5.314 ^{a,b,c}	5.314 ^{a,b,c}	4.606 ^{b,c}	3.448 ^{b,c}	-
<i>Fannia pusio</i>	n	812	1,305	206	211	211	157	270	85	68	0	0	0	0	0	0	270	270	85	68	3,114
	%	26.1	41.9	6.6	6.8	6.8	5.0	8.7	2.7	2.2	0	0	0	0	0	0	8.7	8.7	2.7	2.2	100
	\bar{X}	15.873 ^{a,b}	20.456 ^a	7.140 ^{c,d}	7.623 ^{c,d}	7.623 ^{c,d}	7.247 ^{c,d}	9.062 ^{b,c}	5.247 ^{c,d}	4.757 ^{c,d}	0.707 ^d	0.707 ^d	0.707 ^d	0.707 ^d	0.707 ^d	0.707 ^d	9.062 ^{b,c}	9.062 ^{b,c}	5.247 ^{c,d}	4.757 ^{c,d}	-
<i>Peckia intermutans</i>	n	2	2	1	8	8	1	1	0	2	0	0	0	0	0	0	1	1	0	2	17
	%	11.8	11.8	5.9	46.9	46.9	5.9	5.9	0	11.8	0	0	0	0	0	0	5.9	5.9	0	11.8	100
	\bar{X}	1.052 ^{a,b}	1.052 ^{a,b}	0.880 ^{a,b}	1.666 ^a	1.666 ^a	0.880 ^{a,b}	0.880 ^{a,b}	0.707 ^b	1.052 ^{a,b}	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.707 ^b	0.880 ^{a,b}	0.880 ^{a,b}	0.707 ^b	1.052 ^{a,b}	-

a, b, averages for each species are not significantly different at 5% (p < 0.05); B: bloated; D: decay; F: fresh; n: absolute abundance of species; P: post-decay; S: skeletonization; \bar{X} : average values; %: relative abundance of species.

The second most abundant Calliphoridae species was *C. albiceps*, with 998 specimens; this species appeared mainly in the summer (63.7%) and a large number of immature specimens were collected in both seasons. This fact is not surprising, because *C. albiceps* is one of the most synatropic flies in Brazil (d'Almeida & Lopes 1983). The immature stages of *C. albiceps* have a predatory behaviour mainly when food resources are scarce (Faria & Godoy 2001). According to Oliveira-Costa (2007), *C. albiceps* is considered to be a forensic indicator species in Southeast Brazil, indicating that this species arrives on the carcass immediately after its exposure. Accordingly, many authors reported its occurrence and importance in the degradation of human corpses in Brazil and throughout the world (Monteiro-Filho & Penereiro 1987, Salviano 1996, Carvalho et al. 2000, Marchiori et al. 2000, Arnaldos et al. 2001, Oliveira-Costa et al. 2001b, Wolff et al. 2001, Grassberger et al. 2003).

L. eximia was the third most collected Calliphoridae species. It was most abundant in the first days of decomposition stages (fresh and bloating), in accordance with other works involving entomological succession in carcasses (Salviano 1996, Carvalho et al. 2000).

In relation to *C. putoria*, few specimens were collected compared to *C. albiceps*, although immature specimens were collected in the carcass in winter and summer. Other authors have also found immature stages of this species in carcass models and human corpses (Salviano 1996, Carvalho et al. 2000, Oliveira-Costa 2001b). However, Souza and Linhares (1997) suggested that this species visits the carcass only to feed because its ovarian follicles appear in the process of vitellogenesis.

H. segmentaria was less abundant when compared to the other species; however, it was a pioneer as much in winter as in summer, appearing and ovipositing immediately after the exposition of the carcasses. A similar result was reported by Salviano (1996), who pointed out that this species was one of the most important ones in the decomposition process for the municipality of Rio de Janeiro. Souza and Linhares (1997) also captured few specimens of *H. segmentaria* in pig carcasses in Campinas-SP and they also noted the presence of immature specimens in all studied seasons.

Family Muscidae - The most abundant species among the Muscidae was *O. aenescens*, with a total of 2,226 specimens (42%) collected. A large number of immature specimens were collected on the carcasses in the decay stage thereby suggesting that *O. aenescens* was the most important species in this family. This result corroborates that of Salviano (1996), who reported that *O. aenescens* was the second most abundant Muscidae species and also found a large number of immature specimens in the bloated and decay stages.

O. aenescens were significantly abundant during the decay stage in summer and a large number of larvae were observed and collected on the carcasses in this stage. Early and Goff (1986) and Tullis and Goff (1987) also noted that this species had a preference for more advanced stages of decomposition (decay and post-decay). In Brazil, Salviano (1996) observed the same occurrence pattern for this species.

S. nudiseta was the third most abundant Muscidae species (20%) and 72% were collected in winter; a similar result for this season was found by Salviano (1996). Immature specimens of *S. nudiseta* were found colonising the carcasses in the two studied seasons and, according to many authors, this species is frequently associated with the decomposition of carcasses and human corpses (Jirón et al. 1983, Lord et al. 1992, Omar & Marwi 1994). Besides the apparent preference for winter, *S. nudiseta* also had a significant abundance in the bloated stage during this season. Different results were observed by Tullis and Goff (1987), who found adults and immature specimens only in the decay and post-decay stages.

Our study was the first time that *O. chalcogaster* was collected colonising pig carcasses in Rio de Janeiro state. Only immature forms of *O. aenescens* have been collected in experiments involving the decomposition of carcasses and human corpses (Salviano 1996, Oliveira-Costa 2007). Conversely, in Campinas-SP, Carvalho et al. (2000) collected immature specimens as much in human corpses as in pig carcasses and thus considered this species to be a PMI indicator.

Family Fanniidae - *F. pusio* was the most abundant species of this family, but the colonisation occurred only in the winter season. These results disagree with Salviano (1996), who stated that this species was more abundant in spring in the municipality of Rio de Janeiro. In the Rio de Janeiro Zoo, d'Almeida (1989) reported the Fanniidae as the most abundant of calyptrate dipterans collected on a variety of animal and vegetable substrates. In Campinas, SP, Souza and Linhares (1997) indicated the preference of the species for the fall season. In the same study, the author reported that *F. pusio* has little forensic potential because immature forms were not found in any of his experiments with pig carcasses in that state. Smith (1986) affirmed that the species of the genus *Fannia* had preference for the warmer seasons and were found colonising carcasses and human corpses.

In this study, *F. pusio* was more abundant in the decay stage in winter, in agreement with Tullis and Goff (1987). On the other hand, Salviano (1996) observed that this species has a preference for the final stages of decomposition (post-decay and skeletonisation).

Family Sarcophagidae - Despite the higher diversity observed in this family, only *P. intermutans* was observed colonising the carcass during the experiment in both studied seasons. This species was also found by both Salviano (1996) and Souza and Linhares (1997) in domestic pig carcasses and by Carvalho et al. (2000) in human corpses. Although this species was found colonising the carcasses, it appeared in relatively small numbers in comparison to the other species during this experiment.

Our results show that the seasons can influence the composition of necrophagous dipterans. The summer seems to be the season where the majority of species were more active, except for *F. pusio*. The study also suggests that the spatial distribution of species can be influenced by physiographic differences. This observation is supported by the higher attraction exerted on the species by area 2 and also by the differences in the chrono-

logy of decomposition stages that occurred in this area. Another important point on the attraction of the species is the influence of decomposition stages. The bloated stage and the decay stage were the most attractive to the dipterous colonisers in this study.

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