

Seasonal Abundance and Distribution of Necrophagous Diptera in Western São Paulo State, Brazil

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ABSTRACT

The diversity and abundance of necrophagous Diptera were investigated in urban, farm and wild areas in Botucatu, São Paulo State, Brazil, from March 2003 through February 2004, in order to evaluate the current distribution and abundance of flies important in a forensic context. Members of the family Sarcophagidae were most abundant, followed by Drosophilidae, Calliphoridae and Phoridae. Members of Muscidae were least abundant. Flies were more abundant in spring and summer than in fall and winter. Members of Sarcophagidae, Calliphoridae and Phoridae were most abundant in urban areas. *Chrysomya albiceps* was the most abundant calliphorid species, followed by *Lucilia eximia*, *Chrysomya megacephala*, *Cochliomyia macellaria* and *Lucilia cuprina*. The implications of these results for the necrophagous fauna structure are discussed.

Keywords: forensic entomology, necrophagous flies, seasonal abundance

INTRODUCTION

After death, animal tissues, including those of humans, are attractive to many kinds of organisms, especially insects. Hence, the decomposition of terrestrial vertebrates is characterised not only by the action of fungi and bacteria, but also by an ample number of arthropods, mainly necrophagous insects (Anderson 1995; Amendt *et al.* 2004). In addition, the kind of death markedly affects decomposition, because it determines how fast a corpse can reach putrefaction (Anderson 1995).

Season has an important impact on the weather and the flora and fauna of a region, which influence significantly the faunal colonisation of a body. Many fly species vary in abundance depending upon season. For example, in Mississippi, *Lucilia coeruleiviridis* and *Cochliomyia macellaria* were dominant in the warmer summer months, whereas *Calliphora livida* and *Cynomyopsis cadaverina* dominated in the winter months, with *Phormia regina* found throughout the year (Goddard and Lago 1985).

Studies of succession and decomposition in carcasses have been done mostly in temperate countries (Arnaldos *et al.* 2004). However, research programs have also been implemented in Brazil, in an attempt to understand the dynamics of these insects in tropical areas and their association with forensic studies (Von Zuben *et al.* 1996; Carvalho *et al.* 2000, 2004).

Population abundance in necrophagous flies has usually been estimated from periodic census by using traps (Martinez-Sanchez *et al.* 2000). The abundance of these species has been investigated by field succession experiments, which involved capture of flies from pigs in specific areas (Souza and Linhares 1997).

The relative abundance of certain insects and the potentially differing time of colonisation of the remains in different seasons are essential factors to understand the succession process in carcasses (Smith 1986). Studies of this nature should be performed throughout the year, in order to develop a valid database for specific areas. In Brazil, no systematic study has investigated the abundance of necrophagous flies in different places, such as urban, wild and

farm areas, especially in locations in which the environmental conditions differ in terms of altitude and temperature.

In this study, we investigated the diversity and abundance of necrophagous Diptera in Botucatu, São Paulo State, Brazil, from March 2003 through February 2004, in order to evaluate the current distribution of species. This study includes urban, wild and farm areas. We believe that this information can significantly increase the level of knowledge of fly diversity associated with carcasses, and consequently provides important information on flies as forensic indicators.

MATERIALS AND METHODS

Bimonthly collections were made during the course of one year, March 2003 through February 2004, in three different areas: urban, farm and wild. The urban traps were set in a town garden, near a residential area. The farm traps were set in the Experimental Farm of São Paulo State University, Botucatu, São Paulo, near the University campus. The wild area was a semi-deciduous forest near the farm.

Traps were made from plastic drinking bottles (2000 mL), each with a hole in its bottom (9 cm diameter × 30 cm length). Chicken viscera obtained from poultry house animals, especially gizzard (20 g), were placed in the bottles as bait and maintained during three days in the collecting area. Six traps were set in trees: three in the shade and three in the sun. Because the number of specimens found in the shade was not significantly different from individuals found in the sun ($p > 0.05$), all data were pooled for analysis. The traps were removed after 72 h and the flies identified and recorded. Except for members of the family Calliphoridae, the flies were identified only to family level.

Members of Calliphoridae were identified to species because of the need to record the current status of the group in terms of abundance and distribution of native and exotic species. The structure of the Brazilian calliphorid fauna has changed since the biological invasion of species of *Chrysomya* about 30 years ago (Guimarães *et al.* 1978).

One-way Anova was employed to compare the difference in terms of abundance among areas, families and species, in the case

of Calliphoridae. Mean monthly temperatures for the Botucatu area were obtained from the Meteorological Station of São Paulo State University in Botucatu, which is located near the three experimental areas. All traps were placed at a distance of 3 kilometres from the Meteorological Station.

The frequency distribution of flies in traps was fitted to the Negative binomial and Poisson distributions, in order to determine whether the number of adults found among traps was clumped or random. The k parameter in the Negative binomial distribution was estimated by the maximum likelihood method (Ludwig and Reynolds 1988). The fits of the Negative binomial and Poisson distributions were tested by the Pearson χ^2 statistic (Ludwig and Reynolds 1988). In the Negative binomial distribution, the null hypothesis was that the frequency distribution of adults exhibited a clumped distribution pattern. The parameter k is a measure of the degree of clumping, and tends toward zero at maximum clumping. In the Poisson distribution, the null hypothesis was that the number of adults found follows a random distribution.

RESULTS AND DISCUSSION

The collections resulted in 1,503 specimens, members of five families: Sarcophagidae, Calliphoridae, Drosophilidae, Phoridae and Muscidae (Table 1). Members of Sarcophagidae were most abundant, with 590 specimens. There were 533 individuals of Drosophilidae, 227 of Calliphoridae and 140 of Phoridae. Only 13 individuals of Muscidae were collected over the 1-year period (Table 1).

The Sarcophagidae, commonly called flesh-flies, is a large family, with over 2000 species of cosmopolitan distribution (Smith 1986). Sarcophagids occur in tropical and warm-temperature regions, with adults often observed on flowers, feeding on sweet substances, including sap and honeydew (Smith 1986). In addition to carrion, they also may feed on excrement or exposed meat (Smith 1986; Wolff *et al.* 2001). Flesh-flies are attracted to carrion under most conditions, including sun, shade, dry, wet indoors, and outdoors (Wolff *et al.* 2001). Some sarcophagids have evolved into parasitoids, attacking live insects, with Orthoptera as particularly common hosts; other species live in nests of hymenopterans and termites, eating the food stored for the original insect larva, and often the larva itself (Ferrar 1987).

Members of the family Drosophilidae were the second most abundant group collected. Species of Drosophilidae are attracted to practically any fermenting substance, with more than 2000 known species, widely distributed by commercial traffic (Smith 1986). Drosophilids are commonly found in breweries, public houses, pickling factories, fruit and vegetable canneries, canteens and restaurants; some species are found on carrion, principally when putrid liquids exude (Atkinson 1985; Smith 1986). High variability in terms of relative abundance seems common for some species of fruit flies (Beaver 1977; Atkinson 1985).

We can suggest no clear reason for the higher abundance of Sarcophagidae and Drosophilidae than Calliphoridae found during this study. Most time-series studies of necrophagous Diptera suggest that the calliphorids are the

most abundant family of flies captured (Carvalho *et al.* 2000; Carvalho and Linhares 2001; Carvalho *et al.* 2004). One question arising from these results, is whether the trap design can influence the abundance and diversity of flies captured. Several trap designs are employed in studies of this nature (Hall 1995). However, the trap used in our investigation is very similar to the trap employed by Hwang and Turner (2005), who developed a bottle trap made from soft plastic drinking bottles. They observed that the Calliphoridae was the most abundant family captured in the London area (Hwang and Turner 2005).

Another reason for our result may be the degree of humidity of the bait, chicken viscera, especially gizzard. Certainly the humidity degree, age of the bait and its stage of decomposition are also important factors capable of affecting the number, sex and age composition of blowfly populations (Vogt and Woodburn 1994). Other baits have been used in previous blowfly studies, such as rodent carcasses, chicken faeces and fish, suggesting that the type of bait can be associated to the Diptera fauna (Mendes and Linhares 1993; Oliva 2001; Wall *et al.* 2001; Kozlov and Whitworth 2002). In this study we decided to include gizzard to investigate also the influence of this substrate. Responses of blowflies to odor appear to involve distinct sets of semiochemical cues (Ashworth and Wall 1994). Attraction, orientation and landing in blowflies apparently occur in response to putrefactive sulphur-rich volatiles originating from decomposition products and, the oviposition is influenced by the presence of ammonia-rich compounds (Ashworth and Wall 1994). However, oviposition is also significantly influenced by moisture (Ashworth and Wall 1994) and this factor can have been decisive for the differences found in terms of collecting succession in the current study.

During the period of time when the traps were maintained in the field, we observed that the bait was dried by the wind, which in this area has a mean velocity of 14 km/h. This drying may have contributed to make the bait less attractive to calliphorids, which are usually the first species to arrive in carcasses (Smith 1986). In addition, the local altitude is 840 m, making possible differences in terms of abundance and diversity of flies compared to lower areas (Mani 1968). As altitude increases, conditions for life become more rigorous, with food becoming scarce, humidity and temperature falling, and the temperature oscillating much more (Mani 1968).

Calliphoridae, a family with over 1000 described species that are widely distributed in all zoogeographical regions (Smith 1986), was the third most abundant taxonomic group. Of all the calliphorids collected, the highest abundance was recorded for *Chrysomya albiceps*, with 136 specimens, followed by *Lucilia eximia* with 80 specimens, *Chrysomya megacephala* with 20 specimens, *C. macellaria* with 8 specimens and *Lucilia cuprina* with only 1 individual (Table 2). However, *C. albiceps* was not recorded during four months, whereas *L. eximia* was observed all year (Table 2). The highest abundance of *C. albiceps*, *L. eximia* and *C. megacephala* was recorded in the urban area.

Table 1 Abundance and distribution of individual flies, classified by family and area.

Month	Calliphoridae			Sarcophagidae			Drosophilidae			Phoridae			Muscidae			Σ
	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	
Mar	18	1	1	38	16	5	65	346	10	0	3	28	1	2	0	534
Apr	19	0	2	49	11	13	0	0	0	0	0	0	0	0	0	94
May	7	0	1	11	12	6	0	0	0	0	0	0	0	0	1	38
Jun	21	0	2	48	15	15	1	0	0	0	0	0	0	0	0	102
Jul	0	2	0	6	1	1	0	0	2	13	0	19	0	0	0	44
Aug	0	0	1	1	4	20	1	2	18	0	0	1	0	1	0	49
Sep	1	0	0	2	7	20	0	0	1	3	0	10	0	0	0	44
Oct	3	0	1	21	14	0	0	0	1	0	0	1	0	5	2	48
Nov	106	21	1	104	30	42	47	7	6	24	4	1	0	0	0	393
Dec	12	0	1	29	5	16	8	7	0	16	1	0	0	0	0	95
Jan	3	0	1	8	4	13	0	3	7	6	6	0	1	0	0	52
Feb	2	0	0	3	0	0	0	1	0	2	2	0	0	0	0	10
Σ	192	24	11	320	119	151	122	366	45	64	16	60	2	8	3	1503

Table 2 Abundance and distribution of calliphorid flies, by species and area.

Month	<i>C. albiceps</i>			<i>L. eximia</i>			<i>L. cuprina</i>			<i>C. megacephala</i>			<i>C. macellaria</i>			Σ
	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	Urban	Farm	Wild	
Mar	10	1	0	9	0	1	0	0	0	0	0	0	1	0	0	22
Apr	3	0	0	16	0	2	0	0	0	0	0	0	0	0	0	21
May	5	0	0	2	0	0	0	0	0	0	0	1	0	0	0	8
Jun	7	0	0	13	0	1	1	0	0	0	0	1	0	0	0	23
Jul	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	2
Aug	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1
Sep	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1
Oct	1	0	0	2	0	1	0	0	0	0	0	0	0	0	0	4
Nov	86	13	3	6	3	1	0	0	0	13	0	1	1	5	1	133
Dec	4	0	1	8	0	0	0	0	0	3	0	0	0	0	0	16
Jan	2	0	0	8	0	1	0	0	0	1	0	0	0	0	0	12
Feb	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	2
Σ	118	14	4	67	5	8	1	0	0	17	0	3	2	5	1	245

The farm area was where *C. macellaria* was the most abundant (Table 2). The abundance among calliphorid species was significantly different only in the wild area (Table 2).

The structure of the Brazilian necrophagous fauna, particularly Calliphoridae, has been influenced by the abundance of exotic blowflies such as species of *Chrysomya*, which were introduced into the Americas about 30 years ago (Guimarães *et al.* 1978). Four species of *Chrysomya* have been introduced into the New World (Guimarães *et al.* 1978): *Chrysomya megacephala* (F.), *Chrysomya putoria* (Wiedemann), *C. albiceps* (Wiedemann) and *C. rufifacies* (Wiedemann). These species originally occurred in Australia, the Oriental Region, and Africa, and were first detected in South America around 1975, except *C. rufifacies* which has been found only in North America (Guimarães *et al.* 1978). The successful biological invasion, colonisation and persistence of *Chrysomya* species in different regions of the world can be explained by their short life cycle and high growth rate (Smith 1986; Godoy *et al.* 1993). Particularly in tropical areas such as Brazil, introduced blowflies found a suitable environment to maintain their populations at high levels (Guimarães *et al.* 1978; Smith 1986; Souza and Linhares 1997).

Of the Calliphoridae, *C. albiceps* was the most abundant species in the urban and farm areas. This may be attributed to its predatory habit on other species and its rapid development (Faria *et al.* 1999). The conspicuous abundance of *C. albiceps* was also observed in urban areas in Campinas, São Paulo, Brazil; Rio de Janeiro; Goiás; and Curitiba, Paraná, Brazil (Moura *et al.* 1997; Souza and Linhares 1997; Carvalho *et al.* 2004).

In spite of its lower abundance compared to *C. albiceps*, *L. eximia* was collected during all seasons, differing in this regard from *C. albiceps*. *L. eximia* is able to maintain high abundances in both urban and wild areas during all seasons (Moura *et al.* 1997), which could explain the persistence of this species over the course of the year. The least abundant species were *L. cuprina* and *C. macellaria*. The low abundance of *C. macellaria* is easily explained, because it has been strongly influenced by the invasion of *Chrysomya* species about 30 years ago (Guimarães *et al.* 1978; Faria *et al.* 1999).

Most species of Phoridae were collected in the urban and wild areas (Table 1). This is a large family of flies, with some 3000 species (Smith 1986). Phorids breed in a wide variety of decaying organic material, and several genera are regularly found in vertebrate carrion (Smith 1986). The variety of substrates utilised by the species explains their presence in the traps, but we can suggest no specific reason to find them more abundantly in the urban and wild areas than in the farm area. The family Muscidae exhibited the lowest abundance. This result was not expected, because muscids have often been abundant in studies performed in different areas (Smith 1986; Axtell and Arends 1990). We believe that the principal reason for this result is the presence of a poultry house near the farm area, which may

have attracted the flies to the high concentration of chicken excrement, compared to the bait used in our investigation.

The urban area was where the highest abundance was recorded for Calliphoridae, but also for Sarcophagidae and Phoridae (Table 1). Drosophilidae and Muscidae were most abundant in the farm area (Table 1). The difference in terms of abundance of flies was significant among families in the urban ($p < 0.05$) and wild areas ($p < 0.05$). Excluding Muscidae from the wild-area comparison, the same result was found. However, in the farm area, no significant difference was found in terms of abundance of flies ($p > 0.05$).

No significant correlation was found between temperature and abundance of flies, and rainfall and abundance. However, during summer and spring, flies were more abundant than during fall and winter. The absence of a significant correlation between weather conditions and abundance has also been observed in other geographic areas, for example Malaysia. A study in Malaysia showed that the number of specimens of *C. bezziana* caught was unaffected by weather conditions at the time of trapping, but was positively correlated with the total rainfall (Mahon *et al.* 2004).

Comparing the average climatic conditions recorded during the collecting over 2003/2004 with data from previous and subsequent years, 2001 and 2005 respectively, no significant variation between averages was detected, suggesting that our findings can express a typical necrophagous Diptera time series pattern in this municipality. In this case, the best explanation for the contrasting results with other studies in terms of blowfly diversity and abundance could be the type of bait employed.

The frequency-distribution analysis of adults revealed that the clumped pattern of distribution, described by the Binomial negative model, was the most prevalent pattern of distribution (Table 3). A few areas within families showed a random pattern characterised by the Poisson model (Tables 3, 4), probably as a function of the low abundance recorded. Calliphoridae exhibited the closest value to zero for the k parameter among the families, indicating the highest degree of clumping. This pattern of distribution was observed because blowflies usually tend to search for substrates previously visited by other individuals of the same family. Adult aggregation in blowflies has been frequently documented (Cruickshank and Wall 2002), and this behaviour has been understood as a strategy to increase egg crowding, promoting proteolytic enzyme production by larvae after they hatch (Smith 1986).

The approach taken here to analyse frequency distribution of flies has been often employed in studies to search for spatial patterns in the distribution of invertebrates, particularly parasites and insects (Sréter *et al.* 1994; Reigada and Godoy 2005). Most of these studies have investigated the effect of distribution patterns of eggs and larvae among discrete patches on the coexistence of competing species. These analyses have also been used to investigate aggregated patterns as a consequence of post-feeding larval dispersal in three blowfly species, *C. macellaria*, *C. megace-*

Table 3 Frequency distribution of flies among traps, by family and area.

Family	Area	Mean	s ²	K	χ ²	df	Test
Calliphoridae	Urban	8.92	75.35	0.07	14.04	17	**
	Farm	2	36.18	0.09	2.44	11	**
	Wild	0.92	0.45		3	2	*
Sarcophagidae	Urban	21.92	373.57		1.52	48	*
	Farm	9.91	69.9	1.06	31.55	29	**
	Wild	12.58	140.45	0.57	50.68	36	**
Drosophilidae	Urban	10.16	478.15	0.12	9.99	19	**
	Farm	2.25	9.11	0.34	1.53	3	**
	Wild	3.75	31.47	0.36	24.48	17	**
Phoridae	Urban	5.33	64.42	0.21	23.40	21	**
	Farm	1.33	4.06	0.34	5.43	5	**
	Wild	5	86.18	0.22	24.73	20	**
Muscidae	Urban	0.16	0.15		0.21	1	*
	Farm	0.66	224	0.19	6.39	4	**
	Wild	0.25	0.38	0.30	1.21	1	**

* Poisson distribution (P < 0.001)

** Negative binomial (P < 0.05)

Table 4 Frequency distribution of flies among traps, by species and area.

Species	Area	Mean	s ²	K	χ ²	df	Test
<i>C. albiceps</i>	Urban	9.83	585.42	0.31	12.46	64	**
	Farm	1.16	13.96	0.06	2.03	8	**
	Wild	0.33	0.78	0.16	3.72	2	**
<i>L. eximia</i>	Urban	5.58	28.08	0.91	22.59	15	**
	Farm	0.42	0.99	0.12	4.85	2	**
	Wild	0.66	0.42		1.55	2	*
<i>C. megacephala</i>	Urban	1.42	14.08	0.11	3.85	10	**
	Wild	0.25	0.20		0.52	1	*
<i>C. macellaria</i>	Urban	0.16	0.15		0.21	1	*
	Farm	0.42	2.08	0.03	1.54	2	**

* Poisson distribution (P < 0.001)

** Negative binomial (P < 0.05)

phala and *C. putoria*, and recently to analyse the influence of larval predation on the dispersal of blowfly larvae (Reigada and Godoy 2005).

The abundance recorded for flies over the study period confirms the results obtained in several fly censuses in Brazil (Carvalho *et al.* 2000, 2004). These studies also showed that members of Calliphoridae and Sarcophagidae were most abundant. Summer and spring were the seasons in which the highest abundance of flies was observed, as noted in several studies (Carvalho *et al.* 2000; Centeno *et al.* 2002).

Comparing the results found in this study with data from other regions such as Argentina, the United States, Australia, New Zealand, the Iberian Peninsula, Spain, Austria, Egypt and India, important differences in terms of diversity and abundance can be observed. The seasonal pattern of arthropods in Buenos Aires was favorable to the presence of *Calliphora vicina*, but *C. macellaria* and *Lucilia chuvia* were also recorded (Centeno *et al.* 2002).

A large-scale study of the patterns of neonatal piglet decomposition and carrion insect succession carried out in southern Victoria, Australia, revealed that *Calliphora augur*, *Chrysomya rufifacies* and *C. varipes* were the calliphorid species most abundant in 1999 and 2000, except during June and July (Archer and Elgar 2003). *Lucilia sericata* was the most commonly trapped calliphorid species in the South Island of New Zealand, followed by *Calliphora hilli*, *C. stygia*, *C. vicina*, *C. quadrimaculata*, *Chrysomya rufifacies* and *Xenocalliphora hortona* (Barratt *et al.* 2001).

A study of the sarcosaprophagous community in the southeastern Iberian Peninsula during the four seasons, evaluated different decomposition stages, fresh, decomposing and advanced decomposition. The investigation revealed that *L. sericata* was the most abundant species of calliphorid in all decomposition stages and seasons, followed by *C. vicina*, *C. albiceps*, *Pollenia* sp. and *C. vomitoria* (Arnaldos *et al.* 2004).

In Central Europe, *C. vomitoria* and *C. albiceps* have

been found in abundance, with larvae and adults of *C. vomitoria* outnumbering all other blowfly species, followed by *Protophormia terraenovae*, *C. vicina* and *L. sericata* (Grassberger and Frank 2004). *Chrysomya albiceps* has been found in Austria, monopolising carcasses probably as a consequence of its predatory behaviour during the larval stage (Verves 2004; Grassberger and Frank 2004). *Lucilia sericata* and *C. albiceps* were the principal species coexisting in carrion in fall and spring in Egypt (Adham *et al.* 2001). Comparing the results found in this study with previous investigations in Brazil and other geographic locations, it is possible to conclude that *C. albiceps* is consistently abundant. This is certainly associated with its predatory habit, experimentally confirmed (Faria *et al.* 1999).

However, in some areas in the Northern Hemisphere, the genera *Lucilia* and *Calliphora* apparently dominate the fauna, even when *C. albiceps* is present (Grassberger and Frank 2004). Differences in terms of ovipositional succession in response to carcass decomposition stage and temperature could explain the success of *Lucilia* and *Calliphora* in spite of the presence of *C. albiceps* (Grassberger and Frank 2004). The absence of *Calliphora* from our traps confirms that this species is not present in the western part of São Paulo State, as observed in previous studies (Moura *et al.* 1997; Souza and Linhares 1997; Carvalho *et al.* 2000), although it is present in the southern part of the country (Carvalho and Ribeiro 2000).

Abundance and distribution of necrophagous Diptera are essential factors to be considered in forensic studies, since the diversity and numbers of flies can improve comprehension of the fauna associated with the decomposition of corpses, and consequently clarify questions concerning criminal acts (Grassberger and Frank 2004).

ACKNOWLEDGEMENTS

The research was supported by a grant from the Fundação de Amparo à Pesquisa do Estado de São Paulo. Work by NMB was sup-

ported by a graduate scholarship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico. WACG has been partially supported by a research fellowship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico. The authors also thank Janet W. Reid for revising the English text.

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