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Research Article

Dipterofauna Associated with *Sus scrofa* Linné, 1758, Carcasses in Urban and Coastal Regions of São Paulo State, Brazil

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Cadaverous entomofauna successions vary according to the region, environment, and climate, and such differences may occur within the same country due to seasonal variations. The present study aimed to analyze and compare the dipterofauna that visit or colonize carcasses in the urban and coastal areas of São Paulo, Brazil, during summer and winter seasons. Four swine (*Sus scrofa* Linné, 1758) carcasses of approximately 12 kg were used. The animals were previously euthanized and then placed in metal cages covered with a flight intercept trap (Shannon, modified). In total, 10,495 flies from 39 families were collected, with 15 species belonging to the Calliphoridae family, 14 species belonging to the Fanniidae family, 43 species belonging to the Muscidae family, and 22 species belonging to the Sarcophagidae family. Flies from these four families visited all carcasses; however, they did not show the highest visitation frequencies in all of the trials. Species variations occurred between the experiments that were performed at different locations and in different seasons. Furthermore, difference in the number of insects attracted to each stage of decomposition was observed. In addition to the four families highlighted above, the families Phoridae, Sepsidae, Otitidae, and Piophilidae were observed in all carcasses.

1. Introduction

Forensic entomology is the study of insects and arthropods in relation to legal issues, particularly death investigations [1]. When combined with a criminal investigation, the analysis of arthropods associated with corpses can add information, help solve crimes and elucidate their circumstances, and determine the postmortem interval (PMI), thus linking suspects to crime scenes, demonstrating the movement of corpses, or determining the levels of drugs consumed by the deceased [2]. Adult or immature insect specimens should be considered physical evidence that is as important as the victim's own biological material [3]; therefore, it is necessary to handle these specimens with the appropriate techniques to prevent the destruction and contamination of insects and preserve the collection [1].

The majority of invertebrate fauna associated with carcasses consist of dipteran and coleopteran insects that are attracted by different decomposition stages occurring in the body, and their presence generates a succession of complex communities composed of scavenger species and their predators and parasites [4]. The duration of each phase or stage of putrefaction during cadaver decomposition can differ depending on the location and conditions of the carcass/corpse; however, the order of the events remains constant [5]. In tropical countries, the high typical temperatures shorten the time between death and skeletonization relative to what occurs in temperate regions and result in a shorter

period for data and material collection [6]. In addition to variations in decomposition duration, the variety of species involved in the decomposition process can vary according to climate [4], region [7], altitude [8], and vegetation [9], and variations may occur in the same location during different seasons [10, 11].

In South America, the literature on cadaverous entomofauna is relatively limited [12], especially in Brazil, which has limited available published entomological data related to carcasses in different regions of the country. Papers have been published on studies in Curitiba [13], Campinas [14], and Rio de Janeiro [15], among others.

To increase the knowledge of insect fauna that visit and/or colonize carcasses, two locations that have been previously unexplored by forensic entomological studies were chosen. The present study aimed to compare the cadaverous entomofauna in the city of São Paulo and the town of Peruíbe over two seasons (summer and winter). This research focused on the Diptera order and the Calliphoridae, Muscidae, Fanniidae, and Sarcophagidae families because the species attract greater forensic interest.

2. Materials and Methods

2.1. Location of Experiments. The experiments were performed in two separate locations:

(i) Coastal and forest regions of the Juréia-Itatins Ecological Station in the town of Peruíbe in pristine sites unaffected by environmental changes caused by urbanization and located at sea level at 24°22′S latitude and 47°01′W longitude: its predominant vegetation is dense rain forest with humid subtropical climate without dry season. The average annual temperature is 20,5°C and average annual rainfall is recorded in 2277.8 mm.

(ii) Extremely urbanized region in São Paulo City at a site on the campus of the School of Medicine, University of São Paulo, at an altitude of approximately 868 m at 23°33′S latitude and 46°40′W longitude: the climate is temperately humid with average annual temperature of 18°C and average annual rainfall of 1340 mm.

Ignoring differences in altitude and field routes, the straight-line distance between the two experimental deployment points was approximately 98 km.

2.2. Experimental Model. The experimental model was composed of swine carcasses (*Sus scrofa* Linné, 1758) weighing approximately 12 kg. The animals were previously euthanized by spinal concussion using a pneumatic hammer (humane method), which causes instantaneous death without a great leakage of blood and does not induce chemical changes related to stress that could interfere in the analysis process. This animal model has the highest acceptance rate for studies related to decomposition because the decomposition pattern is similar to that of human body decomposition, the carcasses are relatively easy to obtain and cheap, and the practice does not tend to inspire public objections [5].

Authors have used different names to identify each phase, and in the present study, the division suggested by Catts and Goff that consists of five stages was used [5]: fresh, bloat, active decay, advanced decay, and skeletal remains (skeletonization).

2.3. Experimental Deployment. The carcasses were placed into 70 × 50 × 50 cm cages directly on the ground in approximately 10 cm deep shallow graves. This methodology was applied to protect the carcasses from vertebrate predators and allow adequate colonization by arthropods.

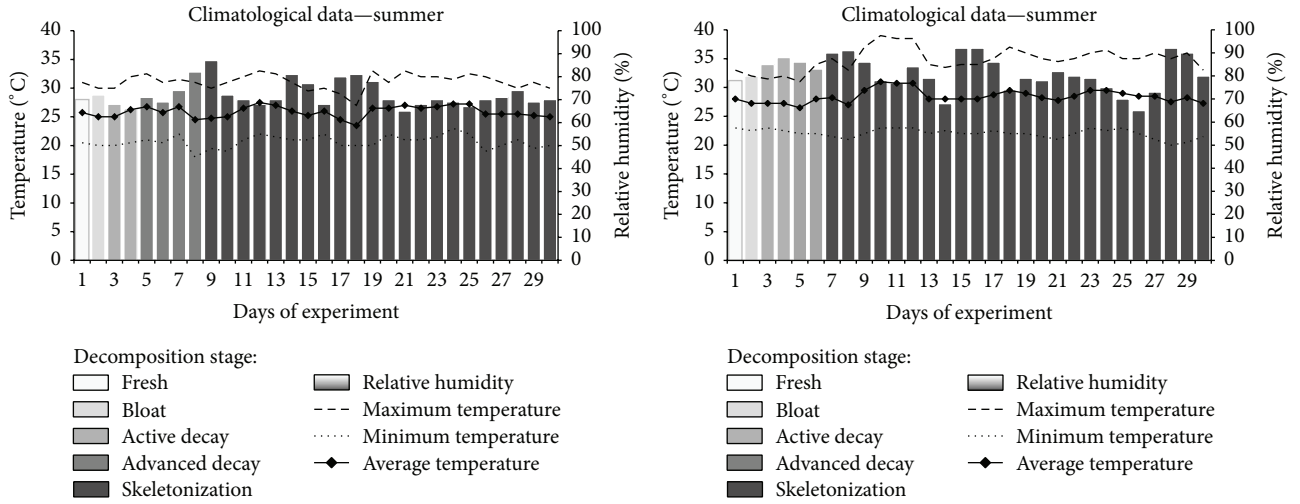
Modified Shannon traps were used as described by Cavallari et al. [16], and they were characterized by a white voile tent fabric properly positioned and fixed at the upper portion (2 m in diameter × 2 m in length) to cover the entire carcass and maintain raised areas above the soil for insect transit and ventilation. The tissue ends were trapped using soil cuttings. This light-colored tent acts to direct insects to the collector bottle, which is located along the upper part, where the insects remain trapped and fixed in alcohol gel until collection. This trap can be deployed indefinitely and provides insect capture over the entire period.

2.4. Experiment Duration. This study was conducted in two stages, with the initial experiments performed in both locations during summer (Jan/2011) and subsequent experiments performed during winter (Aug/2011). Regardless of the duration of carcass decomposition, a thirty-day period for each experiment was implemented to maintain a standard for data collection.

2.5. Collection, Assembly, and Identification. Samples were collected daily during the first two weeks and after this period the collection was made every two days during the last two weeks between 12 pm and 2 pm, a period with an increased photoperiod. The specimens that were captured alive were euthanized with ethyl acetate and placed in 70°GL alcohol for transportation to the laboratory.

The preparation of insects for analysis consisted of washing with distilled water to remove any impurities and traces of alcohol gel. This step was followed by submersion in modified Dietrich's solution, which consists of a solution prepared with distilled water, 95°GL alcohol, formaldehyde, acetic acid, and glycerin. This solution is a special fixative designed to maintain the integrity of stored insects as well as the colors and structures of the specimens [17]. After storage in the solution, the dipterans were pinned and identified at the laboratory using dichotomous keys [17–31].

2.6. Climatic Data. The measurements to obtain climatological data were performed using thermohygrometers (model SH122, J. Prolab Industry and Commerce Products for Laboratory Ltd., São Paulo, SP, Brazil), which were left at the experimental sites and performed daily recordings. The pluviometric indexes were not obtained; however, the maximum and minimum temperature and relative humidity data should be sufficient to correlate the duration of



(a) SP-S, São Paulo City experiment

(b) JIES-S, Peruíbe experiment

Figure 1: Climatological data for the summer experiments.

the decomposition phases with the incidence of the cadaveric fauna succession.

2.7. Statistical Analysis. To identify and compare the fauna from the two different locations, a statistical analysis was performed that included calculating two faunal diversity indexes:

(i) The Simpson diversity and dominance index, which reflects the probability of two randomly chosen individuals from the same community belonging to the same species.

(ii) The Shannon-Wiener index, which measures the degree of uncertainty when predicting the species that belong to a randomly chosen specimen: smaller index values indicate a lower degree of uncertainty and represent a sample with low diversity and higher index values indicate a greater degree of uncertainty and represent a sample with greater diversity.

3. Results and Discussion

Two experiments were conducted in each location and season. According to Hanski [32], environmental changes can result in large variations of the species that visit and colonize a carcass. Thus, the insect fauna composition of contrasting habitats must be evaluated at the same location and at different times of the year to establish a forensic entomology baseline that contains information on insect colonization and succession and can be used for future applications and criminal forensic operations [7].

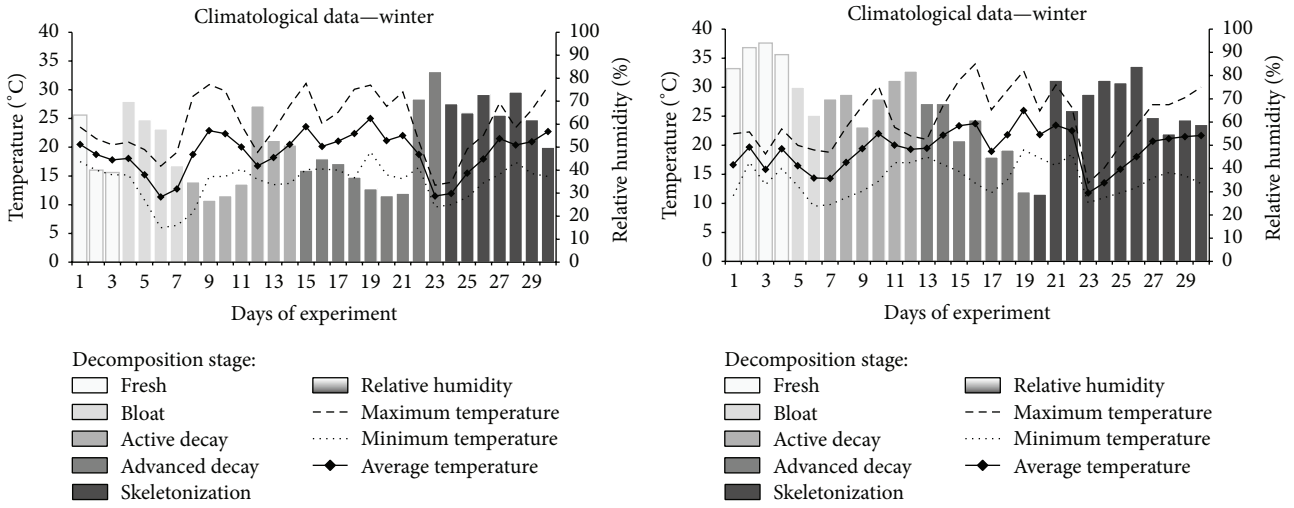
Similar to the methods used by Eberhardt and Elliot [9], the fresh phase was calculated from the moment of swine death until the early bloat phase. As expected, less than a month was required for the carcasses to reach total decomposition, which differs from the results found by Martinez et al. [8], who found that the carcass decomposition process required 83 days, and those of Lopes de Carvalho and Linhares [33], who conducted a study during the wet season and found that casting decomposition required 45 days. To verify the decomposition phases and climatological monitoring, a standard 30 days of observation was followed in the present study. However, the collections ended after the skeletonization stage was reached, which was when the last evaluation was conducted. A total of 10,495 specimens (Table 1) were collected, and they were all identified and associated with the decomposition stage at which they were captured.

Table 1: Number of specimens collected.

Experiment	Collected insects
Summer	
JIES	3123
SP	1705
Winter	
JIES	4095
SP	1572
Total	**10495**

3.1. Summer Experiments

3.1.1. SP-S. During the experiment in São Paulo City, the average temperature was 25.8°C and the average relative humidity was 72%. The average maximum and minimum temperatures were 31°C and 20.6°C, respectively (Figure 1(a)). The early colonization of the carcass occurred immediately after placement. The weather at placement was hot and humid, which are ideal conditions for the visitation/colonization of a large number of insects. Although storms occurred during the experimental period, especially in the afternoon, decomposition was severe, and on the 9th day, the carcass was already skeletonized. The number of



(a) SP-W, São Paulo City experiment

(b) JIES-W, Peruíbe experiment

Figure 2: Climatological data for the winter experiments.

specimens collected was considered satisfactory and unaffected by the rains during the period of experimentation, which differs from the results found by Eberhardt and Elliot [9], who noted the lowest visitation rates to open-air exposed carcasses and a subsequent deceleration of decomposition during the rainy season. Similar results were published by Lopes de Carvalho and Linhares [33], whose study also coincided with greater rainfall and higher temperatures; thus the decomposition was rapid and intense. The flies were classified as belonging to 11 families, and 27 species were identified from the families with the greatest forensic interest (Calliphoridae, Muscidae, Fanniidae, and Sarcophagidae) (Table 2).

3.1.2. JIES-S. In the experiment inside the Juréia-Itatins Ecological Station in the town of Peruíbe, the average temperature was 28.4°C and the average relative humidity was 81%. The average maximum and minimum temperatures were 34.7°C and 22°C, respectively. The period was characterized by high temperatures and humidity, and the daily maximum recorded temperature exceeded 30°C. Carcass decomposition occurred in 7 days; therefore, it was not possible to differentiate between the bloat and active decay steps among the collections, and these steps were assumed to have occurred on the same day. The collected dipterans were classified as belonging to 16 families, and 39 distinct species were collected from the families with the greatest forensic interest (Table 2).

Figure 1 graphically represents the temperatures and relative humidity measured during the two summer experiments as well as the cadaverous decomposition stages observed during the collection, which were categorized according to changes in the carcasses' appearance [8]. The decomposition stages did not occur in isolation, which is consistent with Freire's [34] assertions that putrefaction stages do not occur simultaneously in all parts of the body or carcass but rather overlap. In the present study, these phases were estimated according to the predominant appearance at the moment of collection, and the hotter and more humid climates promoted faster decay, which was observed in the experiment at JIES-S. This rapid succession of putrefaction stages can hamper the analysis of cadaverous entomofaunal succession, especially the identification of insects according to the decomposition phase. Therefore, because of rapid putrefaction, the only observations were of the arthropod families or species' preferences for each putrefaction stage; however, the same type of insect may have been present at different phases or even at all phases.

3.2. Winter Experiments

3.2.1. SP-W. At the São Paulo City experimental site, the average temperature was 19.2°C and the average relative humidity was 51%. The average maximum and minimum temperatures were 24.3°C and 14°C, respectively (Figure 2(a)). The low temperatures and humidity likely influenced the period of time required for carcass decomposition because, among the four experiments, this experiment presented the longest time interval until the skeletonization phase. This result corroborates the data published by Wang et al. [35], who reported that, during winter, carcass decomposition was longer relative to other seasons. Among the 19 collected Diptera families, 38 species were collected from the families with the greatest forensic interest (Table 3).

3.2.2. JIES-W. At the experimental site in the town of Peruíbe, the average temperature was 19.5°C and the average relative humidity was 67%. The average maximum and minimum temperatures were 24.8°C and 14.3°C, respectively. The extended range of temperatures and relative humidities during the trial and the sequence of cadaverous decomposition stages over time are shown in Figure 2(b). After observing and comparing the periods of decomposition of the four carcasses during the experiments (Figures 1 and 2), our conclusions are consistent with those of Segura et al.

Table 2: Identification of Diptera collected during summer.

Diptera family	Genus/species	São Paulo-summer (SP-S) Decomposition stage					Total	Peruíbe-summer (JIES-S) Decomposition stage				Total
		F	B	AcD	AdD	S		F	B/AcD	AdD	S	
Anthomyiidae	*Hylemyioide plurinervis*	—	—	—	—	—	**—**	1	—	—	—	**1**
Calliphoridae	*Chrysomya albiceps*	—	7	12	18	5	**42**	5	10	9	3	**27**
	Chrysomya megacephala	—	1	2	—	—	**3**	2	6	6	—	**14**
	Chrysomya putoria	—	2	1	5	3	**11**	—	—	—	—	**—**
	Cochliomyia macellaria	—	—	—	—	—	**—**	—	1	—	—	**1**
	Hemilucilia segmentaria	—	—	—	—	—	**—**	1	3	3	1	**8**
	Hemilucilia semidiaphana	—	—	1	—	—	**1**	12	55	51	48	**166**
	Lucilia eximia	11	6	9	2	2	**30**	—	2	1	—	**3**
	Lucilia purpurascens	—	2	2	1	1	**6**	5	12	31	10	**58**
	Paralucilia xanthogeneiates	—	—	—	—	—	**—**	—	5	4	—	**9**
Dolichopodidae	sp.	—	—	—	—	—	**—**	—	—	6	8	**14**
Drosophilidae	sp.	—	—	—	—	—	**—**	—	3	5	5	**13**
Fanniidae	*Euryomma carioca*	—	5	13	20	8	**46**	—	—	—	—	**—**
	Fannia canicularis	—	—	—	1	—	**1**	—	—	—	—	**—**
	Fannia femoralis	—	6	9	39	11	**65**	31	44	117	29	**221**
	Fannia flavicincta	—	—	—	—	—	**—**	—	—	5	—	**5**
	Fannia heydenii	—	—	—	1	1	**2**	13	24	8	—	**45**
	Fannia penicilaris	—	—	—	—	—	**—**	—	1	7	3	**11**
	Fannia pusio	2	3	3	32	11	**51**	12	31	109	7	**159**
	Fannia trimaculata	—	—	—	—	—	**—**	7	12	52	4	**75**
	**Fannia* sp.	5	10	34	87	47	**183**	111	215	519	40	**885**
Lonchaeidae	sp.	—	—	—	—	—	**—**	—	—	1	—	**1**
Micropezidae	sp.	2	—	—	—	—	**2**	1	4	4	3	**12**
Milichiidae	sp.	—	—	—	—	—	**—**	—	3	19	68	**90**
Muscidae	*Atherigona orientalis*	—	—	—	—	—	**—**	2	1	8	6	**17**
	Biopyrellia bipuncta	—	—	—	1	—	**1**	3	7	2	—	**12**
	Brontaea delecta	—	—	—	1	—	**1**	—	—	—	—	**—**
	Brontaea normata	—	—	1	—	—	**1**	—	2	—	2	**4**
	Correntosia bicolor	—	—	—	—	—	**—**	1	—	—	—	**1**
	Cyrtoneurina crispaseta	—	—	—	—	—	**—**	4	12	—	—	**16**
	Cyrtoneurina sp.	—	—	—	—	—	**—**	—	1	4	2	**7**
	Cyrtoneuropsis dubia	—	—	—	—	—	**—**	9	22	3	1	**35**
	Cyrtoneuropsis gluta	—	—	—	—	—	**—**	—	—	—	1	**1**
	Cyrtoneuropsis maculipennis	—	—	—	—	—	**—**	1	3	—	—	**4**
	Cyrtoneuropsis pararescita	—	—	—	—	—	**—**	—	—	—	2	**2**
	Cyrtoneuropsis similata	—	—	—	—	—	**—**	—	—	1	—	**1**
	Cyrtoneuropsis veniseta	—	—	—	—	—	**—**	—	6	3	1	**10**
	Morellia humeralis	—	—	—	—	—	**—**	2	7	1	—	**10**
	Musca domestica	1	5	7	4	1	**18**	3	8	5	—	**16**
	Mydaea nubivena	—	—	—	—	—	**—**	1	—	—	—	**1**
	Neomuscina inflexa	—	—	—	—	—	**—**	1	—	—	—	**1**
	Ophyra aenescens	—	9	18	7	7	**41**	19	29	73	3	**124**
	Ophyra albuquerquei	—	2	3	—	4	**9**	—	—	—	—	**—**
	Ophyra chalcogaster	—	3	8	1	5	**17**	—	—	—	—	**—**
	Parapyrellia maculipennis	—	—	—	—	—	**—**	—	3	2	—	**5**
	Sarcopromusca pruna	—	—	—	—	—	**—**	—	1	—	—	**1**
	Synthesiomyia nudiseta	—	—	1	1	1	**3**	—	—	—	—	**—**

Table 2: Continued.

Diptera family	Genus/species	São Paulo-summer (SP-S) Decomposition stage					Total	Peruíbe-summer (JIES-S) Decomposition stage				Total
		F	B	AcD	AdD	S		F	B/AcD	AdD	S	
Otitidae	sp.	—	—	—	3	2	**5**	30	119	130	76	**355**
Phoridae	sp.	1	2	1	10	9	**23**	21	36	124	40	**221**
Piophilidae	sp.	—	4	4	7	1	**16**	3	3	17	5	**28**
Sarcophagidae	*Microcerella halli*	—	—	—	2	1	**3**	—	—	7	38	**45**
	Oxysarcodexia amorosa	—	—	—	—	—	**—**	1	6	4	1	**12**
	Oxysarcodexia diana	1	1	5	2	—	**9**	—	—	—	—	**—**
	Oxysarcodexia paulistanensis	—	—	—	2	1	**3**	—	—	—	—	**—**
	Oxysarcodexia thornax	—	—	3	1	1	**5**	—	1	—	—	**1**
	Oxysarcodexia timida	—	—	—	—	—	**—**	—	1	—	—	**1**
	Peckia australis	1	—	1	2	1	**5**	—	—	—	—	**—**
	Peckia collusor	—	1	1	2	—	**4**	—	—	—	—	**—**
	Peckia intermutans	—	—	—	—	—	**—**	—	2	—	—	**2**
	Ravinia belforti	—	1	3	2	1	**7**	—	—	—	—	**—**
	Sarcodexia lambens	—	—	4	2	1	**7**	—	—	—	1	**1**
	**sp.	—	3	22	18	6	**49**	1	6	8	41	**56**
Sepsidae	sp.	10	200	208	424	189	**1031**	20	26	118	106	**270**
Stratiomyidae	*Auloceromyia* sp.	—	—	—	—	—	**—**	1	1	—	—	**2**
	Hermetia illucens	—	—	—	—	—	**—**	3	5	9	9	**26**
	Hermetia sp.	—	—	—	—	—	**—**	—	—	1	1	**2**
	Merosargus sp.	—	—	—	—	—	**—**	—	—	2	4	**6**
	Sargus sp.	—	—	—	—	—	**—**	—	1	—	—	**1**
Syrphidae	*Eristalis transversa*	—	—	—	—	—	**—**	—	—	2	1	**3**
	Ornidia obesa	—	—	2	1	—	**3**	—	2	—	—	**2**
	sp.	—	—	—	—	—	**—**	—	1	2	—	**3**
Tephritidae	sp.	1	—	—	—	—	**1**	—	—	—	—	**—**
Total of collected insects		**35**	**273**	**378**	**699**	**320**	**1705**	**327**	**743**	**1483**	**570**	**3123**

F: fresh; B: bloat; AcD: active decay; AdD: advanced decay; S: skeletonization.
*_Fannia_ sp.: female specimens belonging to the *pusio* subgroup.
**sp.: females specimens not identified to a genus or species.

[36], who stated that the duration of the decay process is dependent on the particular environmental conditions as well as the activity of the insects associated with the carcass. Of the 4,095 flies collected, 35 Diptera families were identified, and 73 species were collected from the families with the greatest forensic interest (Table 3).

Variations in the insects collected throughout the study are shown in Figure 3, and the highest visitation period to the carcasses occurred during the phases of active and advanced decay, which was also noted by Valdes-Perezgasga et al. [37]. In both seasons, visitations were greater at the dense forest location compared with that of the urban area, which indicates that there are greater numbers of dipterous insects in the forest regions relative to the amount of effectively synanthropic insects.

3.3. Faunistic Indexes. The Simpson diversity and dominance index (Figure 4) indicates that diversity and dominance are inversely proportional [38]; thus, diversity tends to increase and dominance tends to decrease during active and advanced

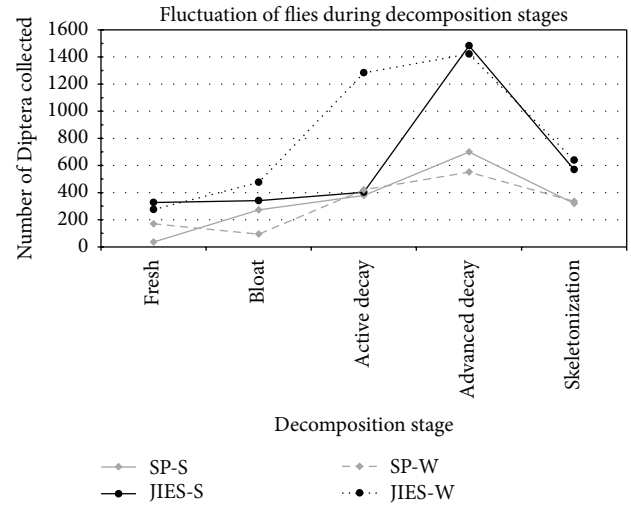


Figure 3: Graphical comparison of dipteran fluctuations. SP-S: summer experiment, São Paulo. SP-W: winter experiment, São Paulo. JIES-S: summer experiment, Peruíbe. JIES-W: winter experiment, Peruíbe.

Table 3: Identification of Diptera collected during winter.

Diptera family	Genus/species	São Paulo-winter (SP-W) Decomposition stage					Total	Peruíbe-winter (JIES-W) Decomposition stage					Total
		F	B	AcD	AdD	S		F	B	AcD	AdD	S	
Anisopodidae	sp.	—	—	—	—	—	**—**	—	—	—	—	1	**1**
Anthomyiidae	*Anthomyia* sp.	3	2	—	1	—	**6**	—	—	—	—	—	**—**
Apioceridae	sp.	—	—	—	—	—	**—**	—	—	—	—	1	**1**
Calliphoridae	*Chrysomya albiceps*	—	—	34	138	77	**249**	—	2	2	3	2	**9**
	Chrysomya megacephala	—	—	1	9	5	**15**	—	—	—	—	—	**—**
	Chrysomya putoria	—	—	8	28	14	**50**	—	—	—	—	—	**—**
	Cochliomyia macellaria	—	—	—	1	—	**1**	—	—	—	—	1	**1**
	Compsomyiops fulvicrura	—	—	—	—	—	**—**	4	3	5	1	—	**13**
	Hemilucilia segmentaria	—	—	—	—	—	**—**	1	—	17	4	5	**27**
	Hemilucilia semidiaphana	—	—	—	—	—	**—**	5	4	33	48	15	**105**
	Lucilia cuprina	—	—	—	—	1	**1**	—	—	—	—	—	**—**
	Lucilia eximia	38	22	31	21	8	**120**	9	1	—	—	—	**10**
	Lucilia purpurascens	102	47	94	87	33	**363**	1	—	4	5	1	**11**
	Lucilia sp.	—	2	4	1	—	**7**	—	—	—	—	—	**—**
	Mesembrinella bellardiana	—	—	—	—	—	**—**	—	1	6	3	4	**14**
	Mesembrinella sp.	—	—	—	—	—	**—**	—	—	2	4	3	**9**
	Paralucilia fulvinota	—	—	—	—	—	**—**	—	—	29	20	6	**55**
	Paralucilia xanthogeneiates	—	—	—	—	—	**—**	22	84	214	165	18	**503**
Ceratopogonidae	sp.	—	—	—	—	—	**—**	1	—	—	—	—	**1**
Chloropidae	sp.	—	—	12	2	3	**17**	—	3	8	34	26	**71**
Chyromyidae	sp.	—	—	—	—	—	**—**	—	1	—	—	—	**1**
Clusiidae	sp.	—	—	—	—	—	**—**	1	—	—	—	—	**1**
Conopidae	sp.	—	—	—	—	—	**—**	1	—	—	—	—	**1**
Culicidae	sp.	—	—	—	—	—	**—**	1	—	—	—	—	**1**
Dolichopodidae	sp.	—	—	—	—	—	**—**	—	1	9	11	8	**29**
Drosophilidae	sp.	1	4	37	13	24	**79**	3	10	18	42	18	**91**
Empididae	sp.	—	—	—	—	—	**—**	—	—	—	5	—	**5**
Fanniidae	*Euryomma carioca*	—	3	3	7	2	**15**	—	—	1	1	1	**3**
	Euryomma peregrinum	—	—	—	—	—	**—**	—	—	—	1	1	**2**
	Fannia canicularis	—	—	—	—	—	**—**	2	—	—	—	—	**2**
	Fannia femoralis	—	—	6	7	2	**15**	1	10	13	9	3	**36**
	Fannia flavicincta	—	—	—	—	—	**—**	1	1	4	12	5	**23**
	Fannia heydenii	—	—	1	—	—	**1**	12	30	109	122	22	**295**
	Fannia obscurinervis	—	—	—	—	—	**—**	—	—	20	15	3	**38**
	Fannia penicilaris	—	—	—	—	—	**—**	—	—	—	59	21	**80**
	Fannia punctipennis	—	—	—	—	—	**—**	1	—	—	—	—	**1**
	Fannia pusio	—	—	—	1	1	**2**	—	1	5	1	1	**8**
	Fannia sabroskyi	—	—	—	4	—	**4**	—	—	—	2	—	**2**
	Fannia snyderi	—	—	—	—	—	**—**	—	—	—	5	2	**7**
	Fannia trimaculata	—	—	—	2	1	**3**	—	—	1	5	3	**9**
	Fannia yenhedi	—	—	—	—	—	**—**	—	—	—	6	1	**7**
	**Fannia* sp.	6	—	45	65	40	**156**	27	77	126	199	65	**494**
Heleomyzidae	sp.	—	—		1	—	**1**	—	—	—	—	—	**—**
Lauxaniidae	sp.	—	—	—	—	—	**—**	1	1	4	—	—	**6**
Lonchaeidae	sp.	—	—	—	—	—	**—**	1	—	3	16	8	**28**
Micropezidae	sp.	—	—	—	—	—	**—**	15	7	35	24	33	**114**
Milichiidae	sp.	—	1	1	4	—	**6**	—	9	33	10	29	**81**

Table 3: Continued.

Diptera family	Genus/species	São Paulo-winter (SP-W) Decomposition stage					Total	Peruíbe-winter (JIES-W) Decomposition stage					Total
		F	B	AcD	AdD	S		F	B	AcD	AdD	S	
Muscidae	*Atherigona orientalis*	—	—	1	—	—	**1**	—	—	—	1	—	**1**
	Biopyrellia bipuncta	—	—	—	—	—	**—**	1	—	—	2	—	**3**
	Brontaea debilis	—	—	—	—	—	**—**	—	1	—	—	—	**1**
	Brontaea delecta	—	—	—	1	—	**1**	—	2	—	—	—	**2**
	Brontaea normata	—	—	2	—	—	**2**	1	—	—	—	—	**1**
	Brontaea sp.	—	—	—	—	—	**—**	—	—	—	—	1	**1**
	Cyrtoneurina cf. *alifusca*	—	—	—	—	—	**—**	—	—	1	3	1	**5**
	Cyrtoneurina sp.	—	—	—	—	—	**—**	3	2	3	18	5	**31**
	Cyrtoneurina varicolor	—	—	—	—	—	**—**	—	—	—	1	—	**1**
	Cyrtoneuropsis dubia	—	—	—	—	—	**—**	19	23	13	36	4	**95**
	Cyrtoneuropsis incognita	—	—	—	—	—	**—**	—	—	1	1	1	**3**
	Cyrtoneuropsis maculipennis	—	—	—	—	—	**—**	—	—	—	24	8	**32**
	Cyrtoneuropsis varicolor	—	—	—	—	—	**—**	—	—	19	10	—	**29**
	Cyrtoneuropsis veniseta	—	—	—	—	—	**—**	2	4	8	19	4	**37**
	Graphomya analis	—	—	—	1	—	**1**	—	—	—	—	—	**—**
	Limnophora deleta	—	—	—	—	—	**—**	—	—	1	2	1	**4**
	Morellia humeralis	—	—	—	—	—	**—**	1	—	—	—	—	**1**
	Morellia violacea	—	—	—	—	—	**—**	1	—	—	—	—	**1**
	Musca domestica	—	—	2	1	2	**5**	—	—	—	—	—	**—**
	Muscina stabulans	—	1	1	1	1	**4**	—	—	—	—	—	**—**
	Mydaea nubivena	—	—	—	—	—	**—**	—	—	—	—	1	**1**
	Mydaea plaumanni	1	—	—	—	—	**1**	—	—	—	—	1	**1**
	Myospila fluminensis	—	—	2	—	—	**2**	—	—	2	—	—	**2**
	Neomuscina currani	—	—	—	—	—	**—**	—	—	2	4	—	**6**
	Neomuscina inflexa	—	—	—	—	—	**—**	—	—	1	1	—	**2**
	Neomuscina sp.	—	—	—	—	—	**—**	1	—	—	—	—	**1**
	Neomuscina tinctinervis	—	—	—	—	—	**—**	—	—	—	2	—	**2**
	Ophyra aenescens	—	—	5	63	37	**105**	4	60	31	21	15	**131**
	Ophyra albuquerquei	—	—	1	2	2	**5**	—	3	3	3	3	**12**
	Ophyra capensis	—	—	—	10	2	**12**	—	—	—	—	—	**—**
	Ophyra chalcogaster	—	1	3	3	—	**7**	—	—	1	1	1	**3**
	Ophyra solitaria	—	—	—	—	—	**—**	2	3	3	3	2	**13**
	Ophyra sp.	—	—	—	2	—	**2**	—	—	—	—	—	**—**
	Phaonia sp.	—	—	—	—	—	**—**	—	1	—	—	—	**1**
	Pseudoptilolepis	—	—	—	—	—	**—**	—	—	5	3	—	**8**
	Synthesiomyia nudiseta	10	2	35	—	8	**55**	2	2	7	1	2	**14**
Mycetophilidae	sp.	—	1	—	—	—	**1**	—	—	1	5	1	**7**
Neriidae	sp.	—	—	—	1	—	**1**	—	—	—	1	1	**2**
Otitidae	sp.	—	—	8	3	12	**23**	15	24	77	9	3	**128**
Phoridae	sp.	5	3	34	20	21	**83**	54	53	191	215	140	**653**
Piophilidae	sp.	—	—	3	3	9	**15**	1	—	1	13	34	**49**
Pompilidae	sp.	—	—	—	—	—	**—**	—	—	—	1	—	**1**
Psilidae	sp.	—	—	—	6	—	**6**	—	—	—	—	—	**—**
Richardiidae	sp.	—	—	—	—	—	**—**	—	—	2	—	—	**2**
Ropalomeridae	sp.	—	—	—	—	—	**—**	—	—	—	—	2	**2**

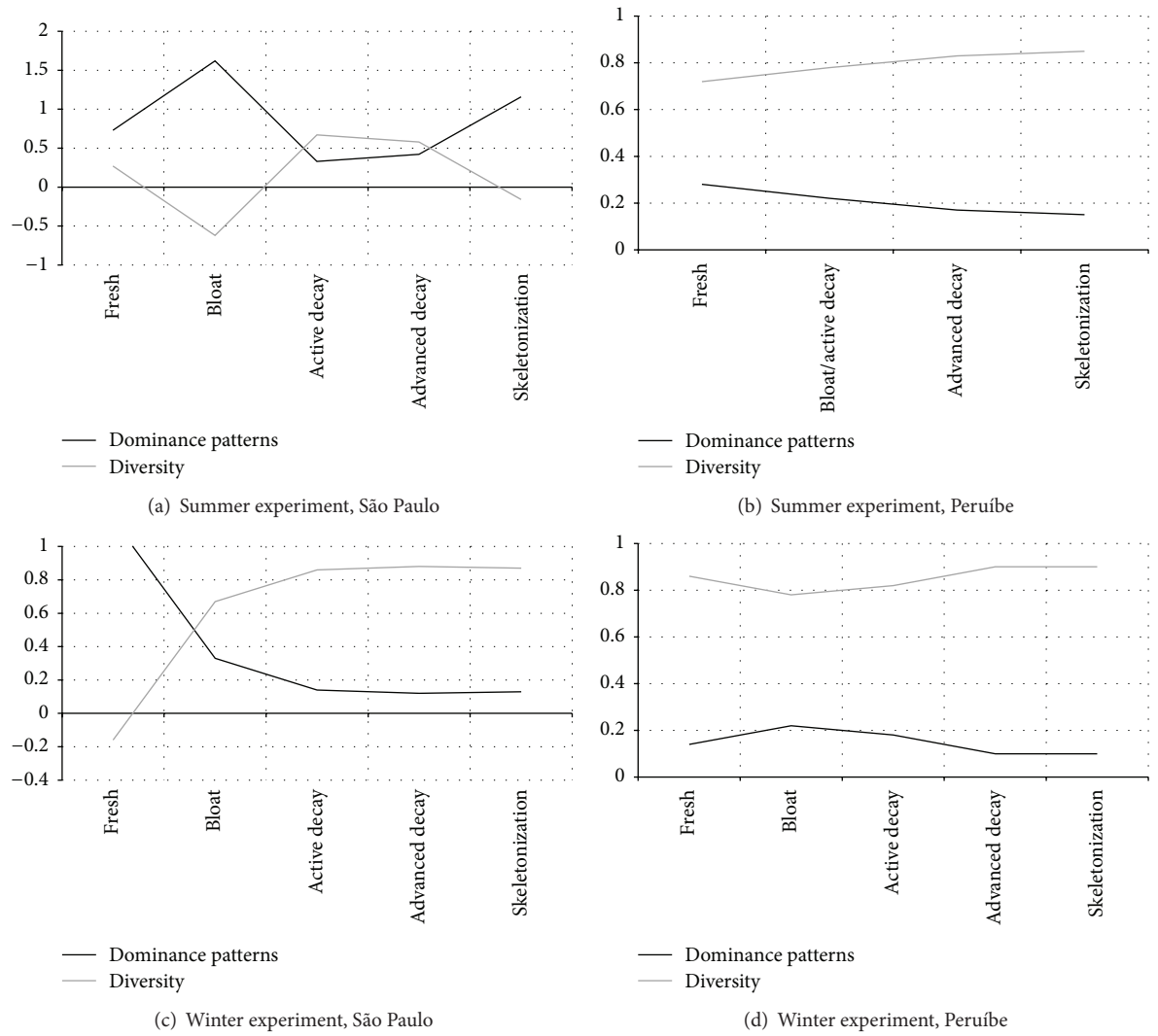
Table 3: Continued.

Diptera family	Genus/species	São Paulo-winter (SP-W) Decomposition stage					Total	Peruíbe-winter (JIES-W) Decomposition stage					Total
		F	B	AcD	AdD	S		F	B	AcD	AdD	S	
Sarcophagidae	*Boettcheria aurifera*	—	—	—	—	—	**—**	2	—	—	—	—	**2**
	Engelimyia inops	—	—	—	—	—	**—**	—	—	—	1	—	**1**
	Helicobia aurescens	—	—	1	—	—	**1**	—	—	—	—	—	**—**
	Microcerella halli	—	—	1	4	—	**5**	—	—	—	2	—	**2**
	Oxysarcodexia admixta	—	—	—	1	—	**1**	—	—	—	1	—	**1**
	Oxysarcodexia amorosa	—	—	—	—	—	**—**	—	2	3	2	2	**9**
	Oxysarcodexia angrensis	—	—	—	—	—	**—**	—	—	1	—	—	**1**
	Oxysarcodexia culmiforceps	—	—	—	—	—	**—**	—	—	1	—	—	**1**
	Oxysarcodexia diana	—	—	2	1	—	**3**	—	—	3	3	4	**10**
	Oxysarcodexia fluminensis	—	—	—	—	—	**—**	—	—	1	—	—	**1**
	Oxysarcodexia paulistanensis	—	—	2	3	—	**5**	—	—	—	—	—	**—**
	Oxysarcodexia thornax	—	—	1	4	—	**5**	—	—	—	—	—	**—**
	Oxysarcodexia timida	—	—	—	—	—	**—**	—	—	—	—	2	**2**
	Oxysarcodexia xanthosoma	—	—	—	—	—	**—**	—	—	—	—	1	**1**
	Peckia anguilla	—	—	—	—	—	**—**	—	—	1	1	—	**2**
	Peckia australis	—	—	2	—	—	**2**	—	—	—	—	—	**—**
	Peckia chrysostoma	—	—	—	—	—	**—**	—	—	1	—	—	**1**
	Peckia collusor	—	—	1	—	—	**1**	—	—	1	—	—	**1**
	Peckia intermutans	—	—	—	—	—	**—**	—	—	1	2	—	**3**
	Ravinia belforti	—	—	1	—	—	**1**	—	—	1	1	—	**2**
	Sarcodexia lambens	—	—	—	—	—	**—**	—	—	2	—	1	**3**
	Titanogrypa fimbriata	—	—	1	1	—	**2**	—	—	—	—	—	**—**
	[**] sp.	3	5	16	21	23	**68**	13	16	47	22	24	**122**
Sciaridae	sp.	1	—	—	—	—	**1**	5	3	—	—	—	**8**
Sepsidae	sp.	—	—	17	4	8	**29**	28	28	130	128	50	**364**
Sphaeroceridae	sp.	—	—	2	2	—	**4**	—	1	—	5	8	**14**
Stratiomyidae	*Sargus* sp.	—	—	—	—	—	**—**	1	—	—	—	2	**3**
Strongylophtalmyiidae	sp.	—	—	—	—	—	**—**	1	—	—	—	—	**1**
Syrphidae	*Ornidia obesa*	—	—	—	—	—	**—**	—	—	—	—	1	**1**
	Pyrirtis sp.	—	—	—	—	—	**—**	1	—	—	—	—	**1**
	Rhingia sp.	—	—	—	—	—	**—**	2	—	—	—	—	**2**
	Syrphus sp.	—	—	—	—	—	**—**	5	—	3	4	2	**14**
	sp.	—	—	—	1	—	**1**	—	—	1	—	1	**2**
Tethinidae	sp.	—	—	—	—	—	**—**	—	—	5	18	3	**26**
Tipulidae	sp.	—	—	—	—	—	**—**	—	2	7	—	—	**9**
Total of collected insects		**170**	**94**	**421**	**551**	**336**	**1572**	**275**	**476**	**1283**	**1422**	**639**	**4095**

F: fresh; B: bloat; AcD: active decay; AdD: advanced decay; S: skeletonization.
[*] *Fannia* sp.: female specimens belonging to the *pusio* subgroup.
[**] sp.: females specimens not identified to a genus or species.

decay phases. These results differ from those reported by Al-Mesbah et al. [39], who concluded that species variations decline over time, although, after 10 days, the population was relatively constant, a state that may have been influenced by habitat differences and the animal model used (rabbit).

The Shannon-Wiener diversity index was calculated according to the experimental location: SP-S ($H' = 0.7543$), JIES-S ($H' = 1.1758$), SP-W ($H' = 1.1728$), and JIES-N ($H' = 1.3610$). The highest index corresponds to the experiment with the greatest species richness (JIES-W) and the largest number of collected insects, thus confirming the results obtained by Magurran [40], who assigned the highest index to the experiment with the highest number of identified species. The index values of the experiments at SP-W and JIES-S were similar, which shows that, despite differences in the number of collected specimens (1,572 and 3,123, resp.), the similar species richness values (55 and 59) approach the index value [38]. The lowest species richness



(a) Summer experiment, São Paulo

(b) Summer experiment, Peruíbe

(c) Winter experiment, São Paulo

(d) Winter experiment, Peruíbe

Figure 4: Graphical comparison of the Simpson dominance and diversity indexes.

was found in experiment SP-S, which also had the lowest Shannon-Wiener index, indicating that the most common species tend to have higher population levels and rare species have lower population levels [41].

3.4. Diptera. The analyses of the results obtained in the four experiments show that a large number of insects belonging to Calliphoridae, Muscidae, Fanniidae, and Sarcophagidae families visited all carcasses; however, these families did not show the highest visitation frequencies in all of the trials. Sarcophagidae, for example, were not always among the most abundant ones. Specimens belonging to the families Phoridae, Sepsidae, Otitidae, and Piophilidae appeared in all of the experiments and were abundant at times and less frequent at other times but always present. The same trend occurred for the families with forensic interest cited above (Figure 5). Several studies have reported correlations among at least one of these families [6–8, 11, 33, 35] and cadaverous decomposition. The forensic significance of these insects is likely related to their characteristics as local counters or indicators for different decomposition stages or the PMI.

The succession pattern of families observed in the samples during the decomposition process was similar to the results of the studies by Martinez et al. [8] and Tabor et al. [42]; however, significant variation occurred among the species. Compared with the results from studies performed in Brazil [6, 33], in which species such as *Chrysomya albiceps*, *Chrysomya putoria*, *Chrysomya megacephala*, *Lucilia eximia*, *Hemilucilia segmentaria*, *Hemilucilia semidiaphana*, *Cochliomyia macellaria*, *Mesembrinella bellardiana*, *Oxysarcodexia riograndensis*, *Peckia intermutans*, and *Ravinia belforti* were cited, greater species richness was observed in the present study.

Lopes de Carvalho and Linhares [33] considered *H. segmentaria* and *H. semidiaphana* to be forest area indicators; however, in the present study, a specimen was collected in the urban area, which suggests synanthropy in these insects because they can easily adapt to different environments as long as the necessary substrates for feeding and reproduction

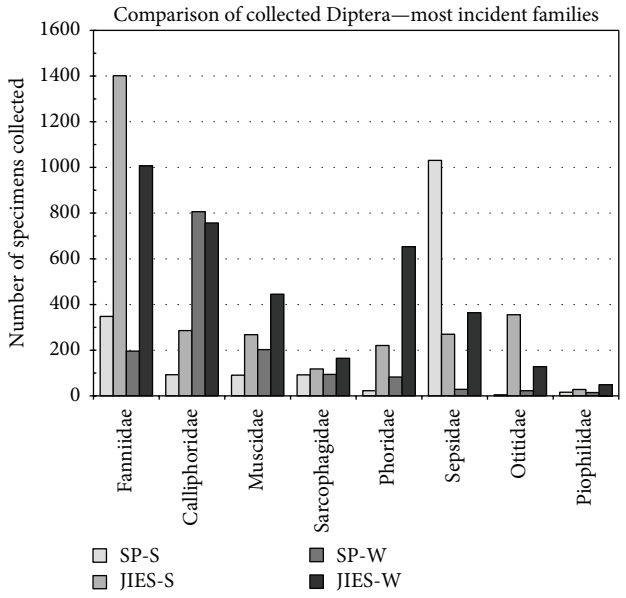


Figure 5: Graphical comparison of the collected Diptera from the families of forensic interest. SP-S: summer experiment, São Paulo. SP-W: winter experiment, São Paulo. JIES-S: summer experiment, Peruíbe. JIES-W: winter experiment, Peruíbe.

are present. *Cyrtoneurina* sp. and *Cyrtoneuropsis* sp. and two species of genus *Paralucilia* were reported in this study, and they can be considered forest indicators because they were only observed in the forest region. Moreover, species of the Stratiomyidae family were only found in the experiments located in the Juréia-Itatins Ecological Station, whereas *C. putoria* was only found in the urban areas, which indicates that it has adapted to coexist among people.

Fanniidae species were found in both locations, although a greater number of species from the genus *Fannia* were found in the forest region, whereas a greater number of species from the genus *Euryomma* were observed in the urban areas. Sarcophagidae specimens were found in all of the experiments; however, a greater specific variety was observed in the urban areas during summer, although a relatively small number of insects were found. This result corroborates the data published by Lopes de Carvalho and Linhares [33], who also found a large number of species represented by few specimens visiting the carcass.

4. Conclusions

The results obtained in the present study indicate that seasonal variations and differences in the environment influence the cadaverous decomposition process and the insects that colonize the carcass and their succession. The difference between putrefaction stages led to large variations in the number of attracted insects, and this result confirms the predilection of these insects for consuming resources offered by the carcass in each decomposition stage. Specimens belonging to the families Phoridae, Sepsidae, Otitidae, and Piophilidae were found in all of the experiments, which suggests greater forensic importance for these insects. Thus, it is recommended to conduct studies that emphasize these families to expand the range of available insects as forensic indicators, which would be beneficial for criminal forensic investigations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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