



OPEN Dipteran succession on decomposing domestic pig carcasses in a rural area of southeastern Brazil

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Diptera plays a pivotal role in forensic investigations, particularly in estimating the post-mortem interval. This can be achieved by analyzing the succession patterns of species on decomposing bodies, which requires detailed knowledge of the local fauna. In rural areas of the Rio de Janeiro state, Brazil, forensic entomological succession remains understudied. This study aimed to evaluate the ecological succession of adult and immature dipterans on decomposing pig carcasses in rural areas of Seropédica municipality during dry and rainy seasons, through the threshold indicator taxa analysis (TITAN). For adult dipterans during the rainy season, we identified 25 'Z-' taxa, with change points (cp) between days 4 and 14, while one 'Z+' taxon showed a cp at day 16. During the dry season, 22 'Z-' taxa exhibited cp between days 4 and 1, and one 'Z+' taxon was identified on day 13. Among immature dipterans, we identified 3 'Z-' taxa with cp between days 9 and 16, while 2 'Z+' taxa showed cp. at days 5 and 21.5. Our findings provide valuable insights for forensic professionals by offering continuous ecological change point analysis, which help address the challenges of interpreting heterogeneous decomposition stages, which are often difficult to delineate in forensic casework.

The alarming rate of violent deaths in Brazil has caused distress among the population, a problem made worse by the lack of resolution in many cases¹. Between 2012 and 2022, the homicide rate fluctuated between 25 and 32 homicides per 100,000 inhabitants². Notably, since 2023, Rio de Janeiro has experienced a marked increase in murders, making it the state with the third-highest absolute number of homicides nationwide. Much of this violence is attributed to territorial disputes involving various criminal factions and paramilitary groups, commonly known as narco-militias^{3,4}. The West Zone of Rio de Janeiro's metropolitan area, particularly the municipality of Seropédica, experiences some of the highest levels of violence related to these territorial conflicts. This region is considered strategically important due to Brazil's major BR-101 highway and the port of Itaguaí⁵. The high number of abandoned corpses in rural landscapes and remnants of forested land underscores the pressing need for forensic expertise, including the application of forensic entomology as a tool in criminal investigations^{6,7}.

Forensic entomology is a valuable tool that aids various aspects of investigations into violent deaths, particularly by helping to estimate the colonization time of necrophagous insects, especially Calliphoridae and Sarcophagidae dipterans^{8–10}. Depending on the conditions the body is exposed to, this information can be used to estimate the minimum post-mortem interval (minPMI). Additionally, data on the ecological succession of these insects throughout the decomposition process can provide insights into the maximum post-mortem interval (maxPMI)^{11–13}. Dipterans play a crucial role in animal organic matter decomposition, including corpses, as part of their detritivorous activities^{8,14}. Understanding the colonizing species—those whose females lay eggs or larvae on remains, allowing their immatures to develop in this environment—and the specific abiotic variables of each area is fundamental for calculating the colonization time¹². In contrast, the visiting fauna consists of adults that interact with the carcass only for feeding or as an extension of their habitat^{15–18}. Analyzing the succession

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dynamics of this fauna is key to estimating maxPMI, as it allows inferences about species that may serve as indicators of seasonality, geographic location, and specific decomposition stages^{19–24}.

Forensic studies using animal models have provided valuable preliminary data on the ecological succession of insects on cadavers and the development of colonizing species throughout the decomposition process^{19,23–32}. The domestic pig *Sus scrofa* Linnaeus, 1758 is commonly used in these studies due to its similarities to humans, including omnivorous feeding habits, microbiota composition, coat characteristics, rib cage size, and cadaveric fauna³³. Considering the numerous factors that influence faunal composition and succession patterns—such as temperature, humidity, photoperiod, altitude, topography, phytophysiognomy, and seasonality—it is essential to thoroughly understand the local fauna. This knowledge should be acquired through experiments conducted across a variety of environments (e.g., urban, rural, forest, coastal, mountainous), biomes, and seasons, allowing for a more accurate extrapolation of data to forensic practice¹⁰.

A recent study on insect succession in Brazil's Cerrado biome recommended evaluating species that indicate specific stages of decomposition for each season³². Several authors have highlighted and postulated the influence of seasonality on faunal composition and entomological succession in carcasses and corpses. Following this recommendation, we aimed to enhance the precision of expert conclusions by focusing on visiting fauna. Notably, this study is the first to apply TITAN analysis to immature stages of colonizing species, which has significant implications for understanding early colonization dynamics. While the colonizing fauna exhibited similarities across seasons, differences in specimen abundance prompted us to conduct combined succession analyses.

In the Rio de Janeiro state, the fauna and ecological succession associated with the primary and secondary Atlantic Forest, as well as urban environments, have been well documented^{14,17,27,34–37}. In contrast, rural regions like Seropédica remain relatively understudied. Given the high rate of violence and the increasing demand for forensic expertise in this municipality, conducting forensic entomology studies in the area is imperative. We aimed to address this gap by providing an analytical approach to the ecological succession of visiting and colonizing Diptera during the decomposition process of *S. scrofa* carcasses exposed in rural areas of the Atlantic Forest biome in Rio de Janeiro's metropolitan region.

Methods

Experiment location and period

The experiment was conducted during the dry season (October 2022) and the rainy season (April and May 2023) in Seropédica, located within the Atlantic Forest biome. This region is characterized by diverse rural landscapes and forest remnants. Seropédica lies in the metropolitan area of Rio de Janeiro State, approximately 75 km from the state capital, at an altitude of 26 m above sea level. It covers an area of about 265 km² and has a population of 83,841 inhabitants³⁸.

The average temperature in the region is 24 °C, with annual precipitation totaling 1,260 mm. The climate is classified as Aw according to the Köppen system, indicating a subhumid tropical climate with limited water deficit and mesothermal conditions, which ensures even heat distribution throughout the year³⁹. In spring (the dry period), maximum temperatures reach 31 °C, with a minimum of 22 °C and an average of 26.5 °C. The average relative humidity is 54%, with a total precipitation of 106.5 mm. In autumn (the rainy period), maximum temperatures are around 27 °C, with a minimum of 17 °C and an average of 22 °C. The relative humidity averages 66%, with a total precipitation of 97 mm⁴⁰.

Three rural areas were selected to perform the experiment, with each location at least 400 m apart (A – 22°46'24" S; 43°41'20" W, B – 22°42'32" S; 43°43'45" W and C – 22°42'36" S; 43°43'31" W) (Fig. 1). It is noteworthy that domestic animals, including canines, felines, bovines, and equines, were present in all three areas.

Experiment model and description of traps

A domestic pig (*S. scrofa*) carcass weighing approximately 12 kg was placed in each study area during both seasons to attract dipterans, indicating the use of pseudoreplicates. The pigs used in the experiment were humanely slaughtered at a licensed slaughterhouse authorized to sell animals for human consumption. The carcasses were promptly transported to the experimental sites, arriving approximately one hour post-slaughter. For the experiment, the carcasses were placed into modified Shannon traps (Fig. 2A), which featured a metal grid allowing direct contact with the ground and a protective metal cage to prevent access by large scavengers (Fig. 2B). The trap design included a tent suspended about 20 cm above the ground, with opaque white fabric on the sides and an opening at the base. This setup enabled insects to detect the decomposition odor, while those attracted by positive phototropism were subsequently captured actively, using an entomological net, in the upper section of the tent.

Field procedures

The carcasses were monitored daily to track the decomposition process, which was recorded by day. We chose to classify decomposition phases according to the most widespread terminology in Brazilian legal medicine institutes^{41–43}. However, caution is recommended in this separation into well-defined stages, for several reasons, as follows: (1) in forensic practice, a clear division of the various phases of decomposition is rarely observed, with most cases showing a “mosaic decomposition,” where different body parts may be at different stages; (2) several abiotic and biotic factors directly influence the occurrence and duration of cadaveric transformation phenomena; (3) decomposition is a continuous process, and dividing it into stages serves primarily didactic purposes^{43–45}.

Daily collections began at 9:00 am during the dry (October 2022; spring) and rainy (April–May 2023; autumn) seasons, starting 24 h after the initial exposure of the carcasses. Abiotic variables, such as temperature

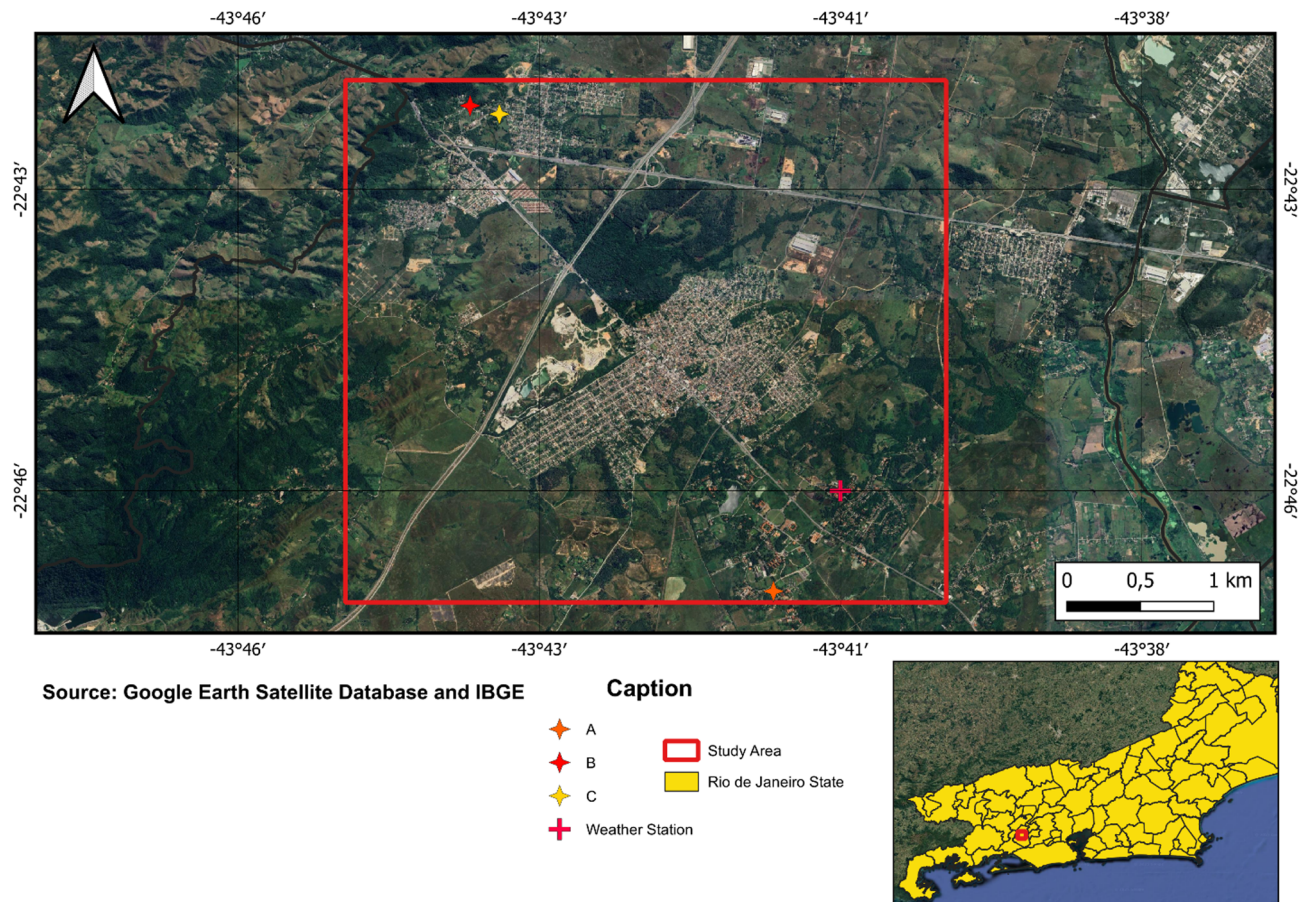


Fig. 1. Map of the study area with collection points (A–C), weather station, and the location in Rio de Janeiro State (QGIS Software) Author: Lucas Barbosa Cortinhas.



Fig. 2. (A) Modified Shannon-type trap in which *Sus scrofa* carcasses were placed to attract dipterans during the dry and rainy seasons in Seropédica, Rio de Janeiro, Brazil. (B) Carcass of *Sus scrofa* exposed inside the trap, positioned on an iron grid in direct contact with the ground and protected by a metal cage to prevent access by large scavengers in Seropédica, Rio de Janeiro, Brazil.

and relative humidity, were recorded daily using a J-Prolab thermo-hygrometer, including on day 0, when the traps were set up and the carcasses were exposed. Precipitation data were collected from “Seropédica Ecologia Agrícola” (22°45′36″ S; 43°40′48″ W) (Fig. 2), the nearest meteorological station to the experimental sites.

Adult specimens (visiting fauna) were actively captured from the traps using an entomological net and euthanized in chloroform death chambers. The insects were then transferred to labeled Falcon tubes containing information on the trap number, date, time, and collection area. To ensure the preservation of specimens, a camphor stone wrapped in voile was placed in each tube lid to absorb moisture until the end of the collection period.

Egg and larval masses (colonizing fauna) were observed in natural body cavities (e.g., mouth, eyes, ears, and anus), as well as in the armpits, neck, back, and legs of the carcasses, and in the surrounding soil. Approximately 10% of the immatures observed on the carcass were collected using a brush and placed directly into ground beef with a decomposition time equivalent to that of the carcasses, following the Brazilian Standard Operating Procedures for transporting entomological evidence from crime scenes to the laboratory when the distance is significant^{13,43}. The masses were stored in plastic containers sealed with organza fabric to ensure aeration and labeled with date, time, and collection area. Both eggs and larvae were reared to adulthood under uncontrolled environmental conditions to be identified using taxonomic keys.

Dispersing larvae and pupae (colonizing fauna) with distinct morphotypes were collected through five-minute random soil sampling. These immatures were sorted, categorized by morphotype, and placed in containers filled with vermiculite. Each container was labeled with the date, developmental stage, and collection area and left under uncontrolled environmental conditions until the emergence of adults. The specimens were subsequently sent to the Integrated Laboratory: Simuliids and Onchocerciasis and Medical and Forensic Entomology (LSOEMF) at the Oswaldo Cruz Institute, Oswaldo Cruz Foundation (IOC/FIOCRUZ) for identification and further analysis.

Laboratory procedures

The collected specimens were sorted, separated by family, and identified to the species level whenever possible under a Zeiss® stereoscopic microscope, using taxonomic keys^{46–57} and relevant literature^{13,43,58}. It is important to note that only males were identified at the species level for the Sarcophagidae due to the lack of sufficient taxonomic information available for females. A portion of the specimens was deposited in the collection of the Carlos Tokarnia Pathological Anatomy Museum (MAPCT) at the Rural Federal University of Rio de Janeiro (UFRRJ); the remaining specimens were deposited in the Didactic Entomological Collection of LSOEMF.

Statistical analysis

Spearman’s rank correlation test was applied to examine the relationship between the decomposition duration and environmental variables, including temperature, humidity, and precipitation. Adult and immature specimens were collected separately during the two periods, however, the statistical analysis of immatures was performed as a single sample. This approach was based on preliminary studies conducted by our group in primary and secondary Atlantic Forest areas which found no significant differences in the composition and abundance of colonizing fauna (immatures), with significant variation observed only in visiting fauna (adults)^{27,35,37}.

Threshold Indicator Taxa Analysis (TITAN) was employed to evaluate changes in the distribution patterns of visiting and colonizing dipterans and to detect change points (cp.) and trends over the timeline corresponding to the decomposition days of pig carcasses. This analysis identifies change points based on insect abundance along an environmental gradient, which in our study is represented by the decomposition timeline. Additionally, it determines the direction of change using scores derived from the individual indicator value (IndVal) index. Species that are more abundant after the change point, during the later stages of decomposition, are classified as Z+, while those that are more abundant leading up to the change point, during the early stages, are classified as Z-. For the classification, both purity and reliability levels must meet or exceed 90%, and the p-value must be ≤ 0.05. These values are derived from IndVal scores at each change point, utilizing bootstrap and permutation procedures. Threshold Indicator Taxa Analysis (TITAN) was employed to evaluate changes in the distribution patterns of visiting and colonizing dipterans and to detect change points (cp.) and trends over the timeline corresponding to the decomposition days of pig carcasses. This analysis identifies change points based on insect abundance along an environmental gradient, which in our study is represented by the decomposition timeline. Additionally, it determines the direction of change using scores derived from the individual indicator value (IndVal) index. Species that are more abundant after the change point, during the later stages of decomposition, are classified as Z+, while those that are more abundant leading up to the change point, during the early stages, are classified as Z-. For the classification, both purity and reliability levels must meet or exceed 90%, and the p-value must be ≤ 0.05. These values are derived from IndVal scores at each change point, utilizing bootstrap and permutation procedures.

Taxa sampled from less than 5% of our sites were excluded from the TITAN analysis^{59,60}, to minimize analytical bias, reduce random errors, and avoid insufficient data for threshold identification along disturbance gradients. Species included in the TITAN analysis met data quality criteria for purity and reliability (filter > 0). Species failing these criteria (filter = 0) were excluded. The included species were assigned to either the Z- group (filter = 1) or the Z+ group (filter = 2). All analyses were performed in R (R Core Team 2024) using the “TITAN2” package.

Results

Abiotic factors associated with study periods

The average environment temperature during the dry period was 29.6 °C, with a relative humidity of 60.3% and accumulated precipitation of 9.6 mm. In contrast, the average ambient temperature during the rainy period was

27.3 °C, with relative humidity at 63.4% and accumulated precipitation measuring 36.8 mm. Table S1 shows daily records of temperature (°C), relative humidity (%), and precipitation (mm) during the experiments.

Decomposition process

Four stages were discerned based on changes in the external morphology of the carcasses: fresh (0 to 2 days in both seasons), indicated by an appearance similar to that of a live animal, presence of *livor mortis* and abdominal green spot; bloated (2 to 4 days in both seasons), recognized by an enlargement in volume caused by the accumulation of gases produced internally by bacterial action, as well as protrusion of the eyes and rectum, and progressive bad odor; colliquative (4 to 12 days in both seasons), distinguished by the collapse of carcasses due to gas pressure, with associated evisceration and progressive darkening of exposed areas, a creamy consistency, and exudate releases; and skeletonization (12 to 15 days in dry season and 12 to 30 days in rainy season), characterized by the period between the exposure of the first bones and the absence of any soft tissue.

The decomposition process lasted an average of 15.3 ± 0.6 days during the dry period and 22.7 ± 7 days during the rainy period. A negative correlation was observed between decomposition duration and both temperature ($r = -0.40$) and humidity ($r = -0.21$). In contrast, a positive correlation was found between decomposition duration and precipitation ($r = 0.67$).

Visiting dipterans

We collected 41,731 Diptera specimens, representing 13 families and 65 species (Tables 1 and 2, Supplementary Table S1). The families with the highest number of species were Sarcophagidae ($n = 31$), Muscidae ($n = 14$), and Calliphoridae ($n = 8$). Of the total specimens, 29,353 (70.33%) were collected during the dry season, while 12,378 (29.66%) were collected during the rainy season. Calliphoridae was the most abundant family in this experiment, with *Chrysomya albiceps* (Wiedemann, 1819) as the most prevalent species in both studied seasons (dry season: $n = 4,628$; rainy season: $n = 2,783$). In the dry season, *Cochliomyia macellaria* (Fabricius, 1775) followed with $n = 3,798$, while in the rainy season, *Chrysomya megacephala* (Fabricius, 1794) had an abundance of $n = 1,578$. Muscidae was the second most abundant family, with a notable abundance of *Morellia humeralis* (Stein, 1918) (dry season: $n = 2,339$; rainy season: $n = 1,115$), *Hydrotaea aenescens* (Wiedemann, 1830) (dry season: $n = 2,076$; rainy season: $n = 856$), and *Biopyrellia bipuncta* (Wiedemann, 1830) (dry season: $n = 1,652$; rainy season: $n = 117$).

During the dry period, the Z-peak change occurred on day 7, with a 5 th – 95 th percentile confidence interval of 6.5–8.5 days. Twenty-three taxa indicative of carcass decomposition time were identified using TITAN (Fig. 3, Supplementary Table S2). The following species were classified as Z-, reflecting an increase in occurrence and abundance in the earlier days of decomposition: *Hemilucilia segmentaria* (Fabricius, 1805) (cp. = 4.5 days); *Synthesiomyia nudiseta* (Wulp, 1883) (cp. = 5 days); *Peckia (Euboettcheria) collusor* (Curran and Walley, 1934) (cp. = 5 days); *Oxysarcodexia xanthosoma* (Aldrich, 1916) (cp. = 5 days); *Oxysarcodexia avuncula* (Lopes, 1933) (cp. = 5 days); *Musca domestica* (Linnaeus, 1758) (cp. = 5.5 days); *Lucilia eximia* (Wiedemann, 1819) (cp. = 5.5 days); *Atherigona orientalis* Schiner, 1868 (cp. = 6 days); *Oxysarcodexia culmiforceps* Dodge, 1966 (cp. = 6.5 days); Ulidiidae sp.2 (cp. = 7 days); *Oxysarcodexia fringidea* (Curran and Walley, 1934) (cp. = 7 days); *Chrysomya megacephala* (Fabricius, 1794) (cp. = 7 days); *Ravinia belforti* (Prado and Fonseca, 1932) (cp. = 7.5 days); *Oxysarcodexia parva* Lopes, 1946 (cp. = 7.5 days); *Oxysarcodexia amorosa* (Schiner, 1868) (cp. = 7.5 days); *Biopyrellia bipuncta* (Wiedemann, 1830) (cp. = 7.5 days); Sarcophagidae spp. (cp. = 8 days); *Cochliomyia macellaria* (Fabricius, 1775) (cp. = 8 days); *Morellia humeralis* (Stein, 1918) (cp. = 8.5 days); *Hydrotaea aenescens* (Wiedemann, 1830) (cp. = 8.5 days); *Oxysarcodexia fluminensis* Lopes, 1946 (cp. = 9.5 days); Ulidiidae sp.1 (cp. = 11.5 days). In contrast, the Z+ peak change occurred on day 13, with a 5 th – 95 th percentile confidence interval of 1.5–13 days. Only *Stomoxys calcitrans* (Linnaeus, 1758) (cp. = 12.5 days) was classified as Z+, signifying an increase in occurrence and abundance toward the later stages of decomposition.

In the rainy period, the Z- peak change occurred on day 12, with a 5 th – 95 th percentile confidence interval of 8–13.5 days. Twenty-six taxa indicating carcass decomposition time were identified from TITAN (Fig. 4, Supplementary Table S3). The following species were classified as Z-: *Cardiophaga* sp. (cp. = 4 days); *L. eximia* (cp. = 5 days); *Peckia (Sarcodexia) lambens* (Wiedemann, 1830) (cp. = 7.5 days); *Peckia (Euboettcheria) anguilla* (Curran and Walley, 1934) (cp. = 8 days); *M. domestica* (cp. = 8 days); *C. megacephala* (cp. = 8.5 days); *C. macellaria* (cp. = 8.5 days); *Peckia (Peckia) chrysostoma* (Wiedemann, 1830) (cp. = 9 days); *H. aenescens* (cp. = 9 days); *Cyrtoneuropsis* sp. (cp. = 9 days); *B. bipuncta* (cp. = 9 days); *A. orientalis* (cp. = 9.5 days); Muscidae spp. (cp. = 10.25 days); *O. fringidea* (cp. = 11 days); *Graphomya maculata* (Scopoli, 1763) (cp. = 11.25 days); *M. humeralis* (cp. = 11.5 days); *Xanthacrona bipustulata* Wulp, 1899 (cp. = 12 days); Ulidiidae sp.2 (cp. = 12 days); *Oxysarcodexia thornax* (Walker, 1849) (cp. = 12 days); *Cyrtoneurina* sp. (cp. = 12 days); *O. fluminensis* (cp. = 12.5 days); *R. belforti* (cp. = 13 days); *Fannia* sp. (cp. = 13 days); Sarcophagidae spp. (cp. = 13.5 days); and *O. amorosa* (cp. = 13.5 days). In contrast, the Z+ peak change occurred at 14.5 days, with a 5 th – 95 th percentile confidence interval of 2–22.5 days. Only *H. segmentaria* (cp. = 15.5 days) was classified as Z+.

Colonizing dipterans

We collected a total of 19,273 individuals, representing 13 species from six families. The most abundant dipterans in both periods were *Chrysomya albiceps* (Wiedemann, 1819) (Calliphoridae; $n = 8,744$), *H. aenescens* (Muscidae; $n = 7,835$), and *H. segmentaria* (Calliphoridae; $n = 1,235$), which together constituted 91% of the collected fauna (Supplementary Table S4).

Among the immature calliphorids, *C. albiceps* and *H. segmentaria* were classified as Z- according to TITAN, with change points of 16 and 13.5 days, respectively. The sarcophagid *P. (P.) intermutans* was also classified as Z-, with a change point at 8.5 days. *Hydrotaea aenescens* and *Piophilha casei* (Linnaeus, 1758) were classified as

Family	Species	nDry	nRain	nT
Calliphoridae	<i>Chloroprocta idioidea</i> (Robineau-Desvoidy, 1830)	49	16	65
	<i>Chrysomya albiceps</i> (Wiedemann, 1819)	4,628	2,783	7,411
	<i>Chrysomya megacephala</i> (Fabricius, 1794)	507	1,578	2,085
	<i>Chrysomya putoria</i> (Wiedemann, 1818)	0	21	21
	<i>Cochliomyia macellaria</i> (Fabricius, 1775)	3,798	116	3,914
	<i>Hemilucilia segmentaria</i> (Fabricius, 1805)	84	329	413
	<i>Hemilucilia semidiaphana</i> (Rondani, 1850)	42	30	72
	<i>Lucilia eximia</i> (Wiedemann, 1819)	50	23	73
Sarcophagidae	<i>Argoravinia rufiventris</i> (Wiedemann, 1830)	2	0	2
	<i>Dexosarcophaga</i> (<i>Farrimyia</i>) <i>carvalhoi</i> (Lopes, 1980)	1	0	1
	<i>Engelmyia inops</i> (Walker, 1849)	0	1	1
	<i>Helicobia aurescens</i> (Townsend, 1927)	3	0	3
	<i>Helicobia morionella</i> (Aldrich, 1930)	1	0	1
	<i>Oxysarcodexia adunca</i> Lopes, 1975	1	0	1
	<i>Oxysarcodexia amorosa</i> (Schiner, 1868)	568	161	729
	<i>Oxysarcodexia avuncula</i> (Lopes, 1933)	13	8	21
	<i>Oxysarcodexia culmiforceps</i> Dodge, 1966	22	8	30
	<i>Oxysarcodexia fluminensis</i> Lopes, 1946	102	55	157
	<i>Oxysarcodexia fringidea</i> (Curran & Walley, 1934)	93	95	188
	<i>Oxysarcodexia parva</i> Lopes, 1946	65	7	72
	<i>Oxysarcodexia riograndensis</i> Lopes, 1946	2	0	2
	<i>Oxysarcodexia simplicoides</i> (Lopes, 1933)	1	3	4
	<i>Oxysarcodexia thornax</i> (Walker, 1849)	465	265	730
	<i>Oxysarcodexia timida</i> (Aldrich, 1916)	0	1	1
	<i>Oxysarcodexia xanthosoma</i> (Aldrich, 1916)	9	5	14
	<i>Peckia</i> (<i>Euboeettcheria</i>) <i>anguilla</i> (Curran & Walley, 1934)	5	18	23
	<i>Peckia</i> (<i>Euboeettcheria</i>) <i>collusor</i> (Curran & Walley, 1934)	21	5	26
	<i>Peckia</i> (<i>Pattonella</i>) <i>intermutans</i> (Walker, 1861)	15	9	24
	<i>Peckia</i> (<i>Peckia</i>) <i>chrysostoma</i> (Wiedemann, 1830)	12	62	74
	<i>Peckia</i> (<i>Peckia</i>) <i>pexata</i> (Wulp, 1895)	4	3	7
	<i>Peckia</i> (<i>Sarcodexia</i>) <i>lambens</i> (Wiedemann, 1830)	8	13	21
	<i>Peckia</i> (<i>Squamatodes</i>) <i>ingens</i> (Walker, 1849)	5	4	9
	<i>Ravinia advena</i> (Walker, 1853)	53	10	63
	<i>Ravinia belforti</i> (Prado & Fonseca, 1932)	79	65	144
	<i>Sarcofahrtiopsis cuneata</i> (Townsend, 1935)	1	0	1
	<i>Titanogrypa</i> (<i>Cucullomyia</i>) <i>larvicida</i> (Lopes, 1935)	0	3	3
	<i>Titanogrypa</i> (<i>Cucullomyia</i>) <i>luculenta</i> (Lopes, 1938)	0	1	1
	<i>Titanogrypa</i> (<i>Sarconeiva</i>) <i>fimbriata</i> (Aldrich, 1916)	0	1	1
	<i>Tricharaea</i> (<i>Sarcophagula</i>) <i>occidua</i> (Fabricius, 1794)	18	4	22
	Sarcophagidae spp.	3,078	1,958	5,036

Table 1. Occurrence by season and absolute abundance (n) of Diptera species (Calliphoridae and Sarcophagidae) associated with the decomposition of *Sus scrofa* carcasses exposed in dry and rainy seasons, in seropédica, Rio de Janeiro, Brazil. *nDry* absolute abundance in the dry season. *nRain* absolute abundance in the rainy season. *nT* total absolute abundance.

Z+, with change points at 11 and 21.5 days, respectively. These data are presented in Fig. 5, and Supplementary Table S5.

Discussion

The longer duration of the decomposition process observed in the rainy season contradicts findings from other studies conducted in southeastern Brazil^{19–22,35,61,62}. Previous research has indicated that the low humidity typical of the dry season can lead to rapid carcass desiccation, ultimately reducing food availability for insect larvae. Furthermore, low humidity negatively impacts the growth and survival of insect larvae; for instance, larvae of species such as *L. eximia* and *C. megacephala* require moisture for optimal development. In dry conditions, rapid moisture loss can result in decreased feeding and increased mortality rates, suggesting that rainy conditions may be more conducive to faster decomposition^{19–22,35}. It is important to note that, in our study, relative humidity values were similar in both seasons. Instead, temperature appears to have been the primary factor, as values were

Family	Species	nDry	nRain	nT
Muscidae	<i>Atherigona orientalis</i> Schiner, 1868	759	50	809
	<i>Biopyrellia bipuncta</i> (Wiedemann, 1830)	1,652	117	1,769
	<i>Cyrtoneurina</i> sp.	42	430	472
	<i>Cyrtoneurosis</i> sp.	98	34	132
	<i>Graphomya maculata</i> (Scopoli, 1763)	82	116	198
	<i>Graphomya</i> sp.	0	1	1
	<i>Hydrotaea aenescens</i> (Wiedemann, 1830)	2,076	856	2,932
	<i>Hydrotaea solitaria</i> (Albuquerque, 1958)	2	0	2
	<i>Morellia humeralis</i> (Stein, 1918)	2,339	1,115	3,454
	<i>Morellia</i> sp.	32	9	41
	<i>Musca domestica</i> (Linnaeus, 1758)	61	57	118
	<i>Muscina stabulans</i> (Fallén, 1817)	1	0	1
	<i>Stomoxys calcitrans</i> (Linnaeus, 1758)	10	4	14
	<i>Synthesiomia nudiseta</i> (Wulp, 1883)	23	3	26
Fanniidae	<i>Fannia</i> spp.	321	170	491
Stratiomyidae	<i>Hermetia illucens</i> (Linnaeus, 1758)	47	7	54
	Stratiomyidae sp.	2	1	3
Piophilidae	<i>Piophila casei</i> (Linnaeus, 1758)	19	7	26
Syrphidae	<i>Eristalis</i> sp.	3	2	5
	<i>Ornidia obesa</i> (Fabricius, 1775)	22	55	77
Sepsidae	Sepsidae spp.	2,605	435	3,040
Anthomyiidae	<i>Anthomyia</i> sp.	22	3	25
Micropezidae	<i>Cardiophala</i> sp.	37	134	171
Drosophilidae	Drosophilidae spp.	123	4	127
Ulidiidae	<i>Euxesta</i> spp.	2,345	518	2,863
	Ulidiidae sp.1	226	14	240
	Ulidiidae sp.2	192	61	253
	<i>Xanthacrona bipustulata</i> Wulp, 1899	77	15	92

Table 2. Occurrence by season and absolute abundance (n) of Diptera species (other families) associated with the decomposition of *Sus scrofa* carcasses exposed in dry and rainy seasons, in seropédica, Rio de Janeiro, Brazil. *nDry* absolute abundance in the dry season. *nRain* absolute abundance in the rainy season. *nT* total absolute abundance.

higher during the dry season compared to the rainy season. Temperature significantly influences decomposition by affecting microbial activity and insect behavior. Warmer temperatures can enhance the metabolic rates of decomposers, including insects, which facilitates faster colonization and resource utilization, thereby accelerating the overall decomposition rate^{6–8,10}. Notably, the abundance of Diptera in the rainy season accounted for less than half of the total number of specimens collected in the dry season. These findings align with those of other studies focusing on forensic-relevant Diptera fauna surveys also in southeastern Brazil, which reported higher abundances and richness during the warmer months of the year^{16,20,22}.

In the visiting fauna, of the 65 taxa collected, 34 were identified as indicators of specific stages in the decomposition process using the TITAN approach. All of these taxa are widely distributed throughout the country^{65,66}. They hold particular significance for forensic applications since several of them, such as *H. segmentaria* (cp. = 4.5 days) and *M. domestica* (cp. = 5.5 days), show notable increases in both occurrence and abundance during the early stages of decomposition. Their presence enables forensic entomologists to estimate PMI more accurately, thereby facilitating criminal investigations by precisely identifying the timeline of events. However, caution should be exercised when extrapolating these findings to other regions, particularly those with distinct biomes, such as the Amazon rainforest or the semi-arid Caatinga. Variations in humidity, temperature, and available resources could significantly impact dipteran community composition and decomposition dynamics. Additionally, climatic factors like seasonal rainfall patterns and temperature fluctuations can further influence the behavior and life cycles of the identified taxa. Ideally, each region and environment should have its own dedicated succession study to enhance the accuracy and applicability of such data¹⁰.

In the dry season, four species of Calliphoridae emerged as potential indicators of decomposition time, classified as Z-: *H. segmentaria*, *L. eximia*, *C. megacephala*, and *C. macellaria*. The first two species actively seek out carcasses to lay their eggs during the initial days of decomposition, with their abundance gradually decreasing afterward. Notably, the change points for *H. segmentaria* and *L. eximia* (4.5 and 5.5 days, respectively) coincide with the population peak of *C. albiceps*, a voracious and competitive blowfly known for its predatory larvae^{16,27,61,62}. In contrast, *C. megacephala* and *C. macellaria* showed change points on days 7 and 8. The genus

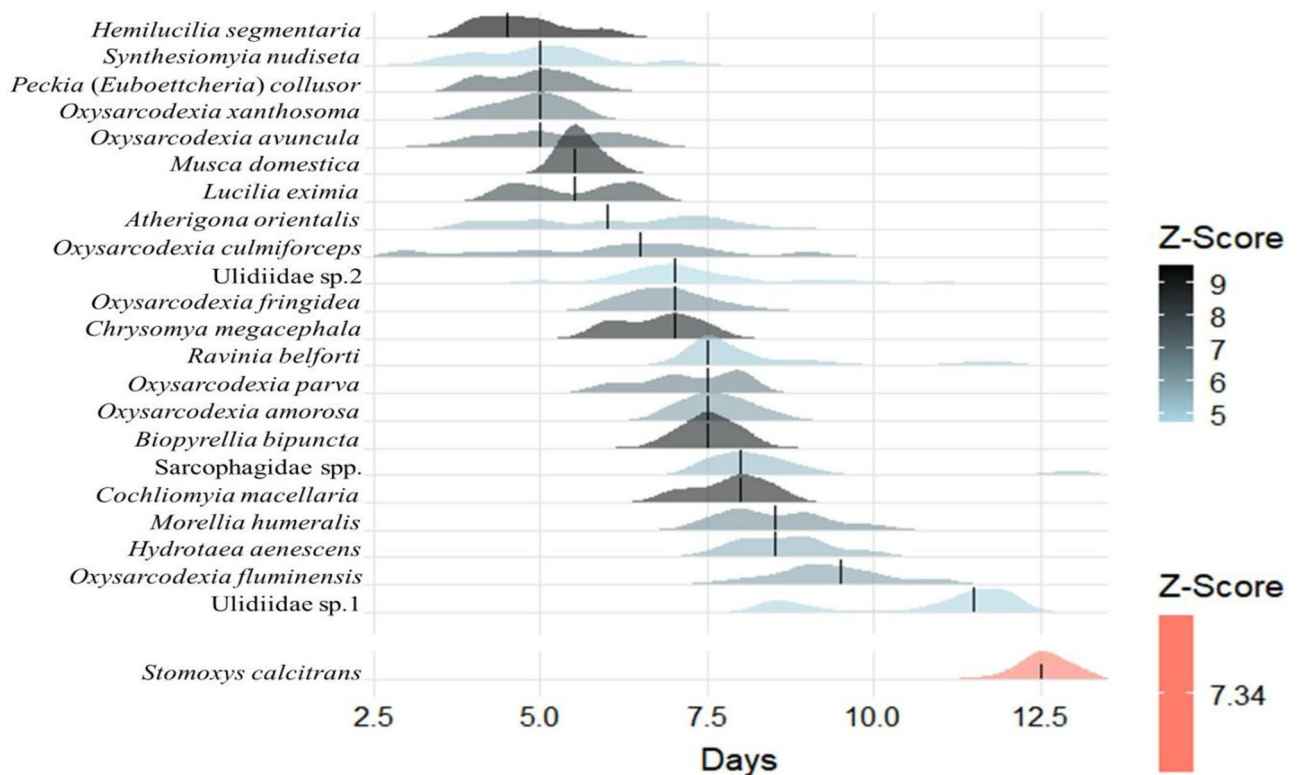


Fig. 3. Change points and distribution of visiting dipterans with positive (Z+) and negative (Z-) responses to the decomposition time gradient of pig carcasses in rural areas during the dry season in Seropédica, Rio de Janeiro, Brazil. Red symbols, lines, or dots represent Z+ species values, while blue symbols, lines, or dots denote Z- species values. The Z score indicates the level of association of each species with the temporal gradient of decomposition days. A higher score reflects a stronger association with the gradient, resulting in a more intense color for each species on the graph. Conversely, a lower score indicates a weaker association, leading to a fainter color for each species.

Chrysomya is particularly drawn to carcasses, feces, and exudates released during the bloated stage, which likely explains their presence until the 7th day.

Interestingly, the change point for *C. macellaria* indicates that its population dynamics may be affected by the exotic *Chrysomya* species introduction, which generally reduces its abundance at carcasses^{16,27,29,67}. However, the fact that *C. macellaria* was still present until the 8th day indicates a potential for coexistence in rural environments with minimal human impact. This observation invites further investigation to determine whether *C. macellaria* is migrating to less anthropized regions with a lower presence of highly synanthropic *Chrysomya* species. A similar pattern was noted by researchers^{14,27} studying pig carcasses in secondary forest areas of the metropolitan region of Rio de Janeiro, highlighting the need for more in-depth studies across various ecological contexts.

In the rainy season, the same Calliphoridae species also demonstrated their potential to indicate specific stages of decomposition, suggesting that they can be useful across different seasons. However, *H. segmentaria*, previously classified as Z-, was categorized as Z+ (with a change point at 15.5 days). This change is associated with the emergence of the adults from the 1st generation of eggs laid by females at the beginning of the decomposition process. It is only when this new generation emerges that an accurate estimation of the PMI can be made based on the succession pattern¹³. Notably, the change points for *L. eximia* (5 days), *C. megacephala* (8.5 days), and *C. macellaria* (8.5 days) exhibited slight variations compared to those observed during the dry season. This indicated the direct influence of abiotic factors, such as temperature, relative humidity, and precipitation, on the dipteran community.

During the dry season, the Sarcophagidae were represented by nine Z- species: *P. (E.) collusor*, *O. xanthosoma*, *O. avuncula*, *O. culmiforceps*, *O. fringidea*, *R. belforti*, *O. parva*, *O. amorosa*, and *O. fluminensis*. It is well established that different stages of decomposition provide specific resources that various species prefer based on their physiological needs, and flies respond rapidly to these changing conditions^{66,69,70}. Previous studies in secondary forest areas of Rio de Janeiro^{14,27,36} reported higher abundances of *R. belforti* and *O. amorosa* in the intermediate stages of decomposition and the persistence of *O. fluminensis* into more advanced stages, which is consistent with our observations.

For *P. (E.) collusor*, *O. xanthosoma*, *O. avuncula*, *O. culmiforceps*, *O. fringidea*, and *O. parva*, the change points ranged from 5 to 7.5 days, offering new insights into their behavior in the Rio de Janeiro state. These outcomes

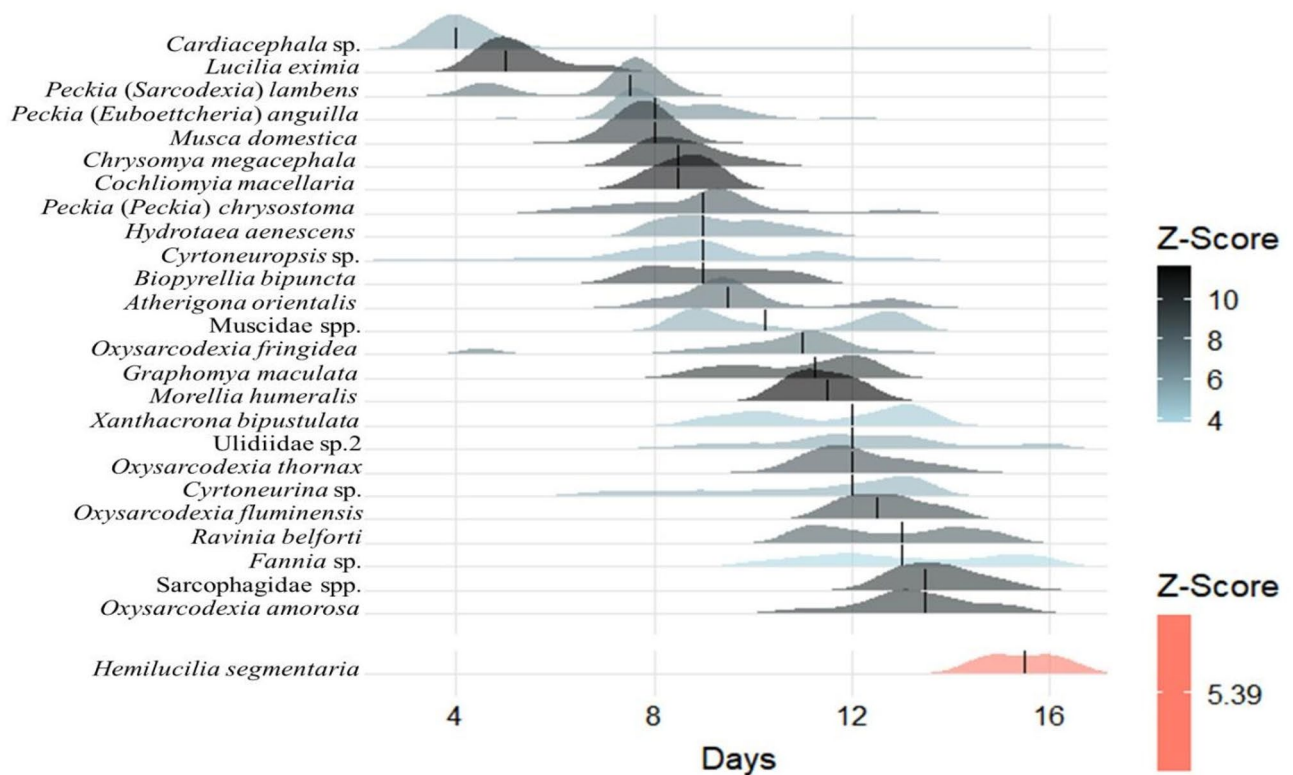


Fig. 4. Change points and distribution of visiting dipterans with positive (Z+) and negative (Z-) responses to the decomposition time gradient of pig carcasses in rural areas during the rainy season in Seropédica, Rio de Janeiro, Brazil. Red symbols, lines, or dots are the Z+ species values, and blue symbols, lines, or dots are the Z- species values. The Z score indicates the level of association of each species with the temporal gradient of decomposition days. A higher score reflects a stronger association with the gradient, resulting in a more intense color for each species on the graph. Conversely, a lower score indicates a weaker association, resulting in a fainter color for each species.

indicate a preference for intermediate stages of decomposition before their abundance begins to decline. In the rainy season, the Sarcophagidae was enriched by the addition of three new representatives from the genus *Peckia* - (*P. (S.) lambens*, *P. (E.) anguilla*, and *P. (P.) chrysostoma*) - along with a new representative of *Oxysarcodexia* (*O. thornax*), all identified as potential indicators of decomposition. Five taxa were observed in both seasons, although their change points varied: *O. fringidea*, *O. fluminensis*, *R. belforti*, Sarcophagidae spp., and *O. amorosa*. The increased precipitation, combined with lower temperatures likely facilitated the prolonged presence of sarcophagids throughout the decomposition process. This phenomenon may be attributed to the enhanced moisture content of food resources, which sustained their availability and continued to attract adult flies.

In the present study, Muscidae was represented by six species classified as Z- in the dry season: *S. nudiseta*, *M. domestica*, (*A. orientalis*, (*B. bipuncta*, *M. humeralis*, and *H. aenescens*). The succession patterns observed for these species were consistent with findings from previous studies conducted in primary and secondary forest environments in Rio de Janeiro^{14,27,35–37}. This reinforces their potential as indicators of intermediate decomposition stages, after which their representation declines. In contrast, *S. calcitrans* was classified as Z+. Its presence may be linked to hematophagy, likely influenced by the nearby presence of horses and cattle. Additionally, its occurrence during the decomposition process may be associated with blood and other body fluids released in cadaveric exudates, which are attractive to this species⁶³. During the rainy season, new representatives of Muscidae were categorized as possible indicators: *Cyrtoneuropsis* sp., Muscidae spp., *G. maculata*, and *Cyrtoneurina* sp., classified as Z-. Other species, also classified as Z-, were common to both seasons: *M. domestica*, *H. aenescens*, *B. bipuncta*, *A. orientalis*, and *M. humeralis*. These species remained on the carcass longer than in the dry season, which can be attributed to the enhanced moisture content of food resources that sustained their availability.

Two Ulidiidae taxa were classified as Z-, although their forensic relevance is limited. Their presence during the decomposition of carcasses is primarily associated with the presence of leaf litter, leaves, branches, fruits, and other decomposing plant material^{47,61}. Some authors classify these species as accidental visitors, indicating that they are not primarily attracted to carcasses but rather to the surrounding organic matter^{16,25,47,61}. The representation of species of Fanniidae, Micropezidae, and Ulidiidae highlights the diversity of dipteran taxa associated with different stages of decomposition. While *Fannia* sp. has established its role as a later-stage indicator in forensic contexts, the exclusivity of *Cardiacephala* sp. and *X. bipustulata* to the rainy season suggests potential environmental adaptations influencing their visiting patterns.

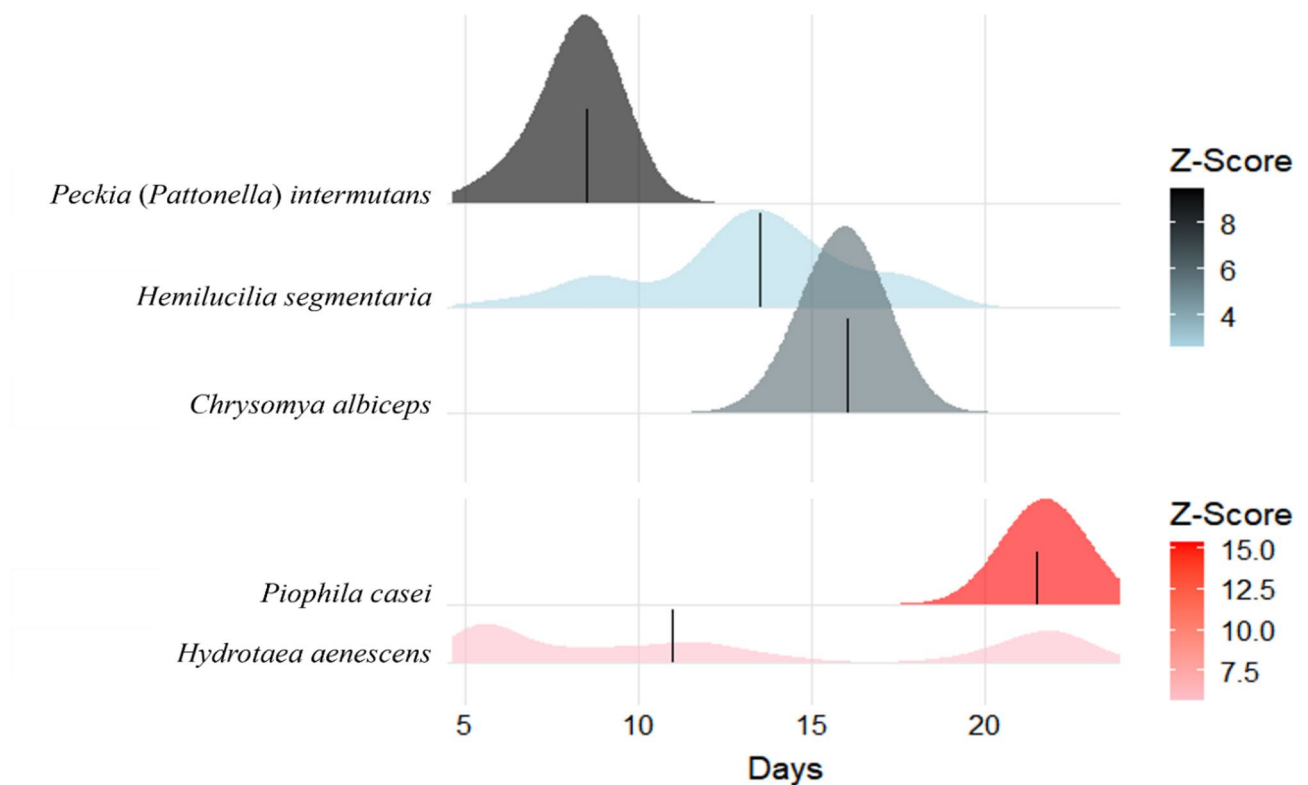


Fig. 5. Change points and distribution of colonizing dipterans exhibiting positive (Z+) and negative (Z-) responses to the decomposition time gradient of pig carcasses in rural areas in Seropédica, Rio de Janeiro, Brazil. Red symbols, lines, or dots represent the Z+ species values, while blue symbols, lines, or dots represent the Z- species values. The Z score indicates the level of association of each species with the temporal gradient of decomposition days. A higher score reflects a stronger association with the gradient, resulting in a more intense color for each species on the graph. Conversely, a lower score indicates a weaker association, resulting in a fainter color for each species.

The TITAN analysis provided valuable insights into the successional dynamics of colonizing species, identifying specific change points that correspond to key stages of decomposition. For instance, *Piophilidae casei* (Z+) associated with advanced decay, showed a change point at 21.5 days, confirming its role in late-stage decomposition^{61,62,64}. In addition, *Hydrotaea aenescens* (Z+) showed a change point at 11 days (ending of colliquative stage), which correspond to the peak third-instar larval dispersion for pupation, aligning with its early oviposition and larval feeding on carcass exudates in previous studies^{19,21,22,27}. Among the Z- species, *Peckia (Pattonella) intermutans* showed a change point at 8.5 days, decreasing in abundance until the final stages of decomposition. This species is frequently reported in Brazil as an early colonizer, with larviposition typically occurring during the fresh stage and larval abundance peaking after carcass rupture, when viscera and body fluids are released^{18,19,21,22,27,50,51}. Similarly, *Chrysomya albiceps* (Z-) showed a change point at 16 days, were collected in large quantities (specifically dispersing larvae and pupae) from day 13 onwards, coinciding with the end of the colliquative period, when the amount of soft tissue had already decreased considerably, which is consistent with studies across Brazil^{29,36,62,68}. *Hemilucilia segmentaria* (Z-), showing a change point at 13.5 days, acted as a pioneer colonizer, with immatures observed from day 2 (feeding larvae) and abundance peaking by day 13 (dispersing larvae and pupae), aligning with previous studies, in which the activity of its immatures was observed since the initial stages^{16,19,21,27,61,64,68}. By focusing on these species, we can better understand their ecological roles and how they contribute to the decomposition timeline, which is crucial for forensic applications.

A recent study employing the TITAN approach to assess the succession of the Sarcophagidae in pig carcasses in Brazil's Cerrado³² emphasized the importance of the Niche Theory, which links species distribution to their physiological needs and resource availability^{69–71}. According to this theory, transitions in species presence occur gradually over time and space rather than abruptly. Thus, TITAN provides a significant methodological advantage by promoting continuous data evaluation rather than confining decomposition into rigid phases, offering forensic entomologists a more nuanced approach. The change points provided in this analysis are especially valuable as they establish a continuous timeline of species succession, enabling more precise PMI estimates^{10,31}. For instance, by identifying change points for both visiting and colonizing species, TITAN offers a robust framework for interpreting complex successional patterns, especially in cases where decomposition stages overlap or are difficult to delineate^{32,60}, as well as to understand the role of these species at different

times of decomposition. Moreover, this approach enhances the accuracy of PMI estimates by accounting for the dynamic interactions between species and environmental factors, while also emphasizing the importance of localized succession studies in refining forensic methodologies. Furthermore, the recommendation³² to use a combination of identified indicators for more accurate PMI estimates aligns with our findings.

One of the study's limitations is the biological model used. The domestic pig was used as an animal model because it is considered most similar to humans in terms of thoracic cavity size, body hair composition, intestinal microbiota, omnivorous eating habits and decomposition rate^{72,73,33}, considering the many ethical, bureaucratic and legal restrictions on the use of human cadavers in research⁷⁴.

There is no doubt that the acceptance of forensic entomology in courts is conditional on the adoption of rigorous standards⁷⁵, and therefore, pseudoreplication is a limitation of this study^{76,77}. On the one hand, the use of only one carcass for each area and the non-repetition of the study in consecutive years compromise the detection of natural variations in the fauna found. On the other hand, the results obtained provide insights, direct new studies and can improve the estimation of PMI when combined with other techniques³². It is also worth noting that adequately replicated entomological succession studies require at least three carcasses for each treatment, which implies the need for many pig slaughters, a robust collection team, very well-defined geographic logistics and a significant execution time⁷⁸, making it often impractical to reach an impeccable level.

However, despite this point, our study supports these statistical recommendations and is congruent with prior studies in the Atlantic Forest biome of Rio de Janeiro, affirming the utility of the reported species as reliable forensic indicators. This research contributes valuable insights for forensic entomologists, enhancing their ability to inform investigations of violent deaths through precise PMI estimations.

Data availability

All data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Author contributions

C. H. G. N. and M. M. C. Q. planned the experiments. C. H. G. N., M. M. C. Q., and M. L. D. collected and reared the insects. C. H. G. N., R. T. M., and M. M. C. Q. collected and analyzed the data. C. H. G. N. and L. B. C. identified the specimens. C. H. G. N., M. M. C. Q., L. B. C., and P. M. M. prepared the images, tables, and the final manuscript. C. H. G. N., M. M. C. Q., L. B. C., and P. M. M. reviewed the final manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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