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Succession Patterns and Species Composition of Forensically Important Insects During Animal Decomposition in Ardabil, Northwest Iran

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ABSTRACT

Background: Accurate estimation of the post-mortem interval (PMI) is a critical aspect of forensic investigations. Forensic entomology, which studies insect colonization and development on decomposing remains, contributes significantly to PMI determination. This study aimed to explore the succession patterns and species composition of insects of forensic importance during carcass decomposition in Ardabil City, northwest Iran.

Methods: Over one year (autumn 2021 to summer 2022), rabbit carcasses were used as decomposition models across different seasons. Adult and immature insects were collected daily throughout the five recognized stages of decomposition. Collected specimens were preserved, mounted, and identified using standard entomological keys. Seasonal diversity and species abundance were assessed using the Shannon-Wiener index, while inter-seasonal similarities were measured with the Jaccard index.

Results: Twenty insect species were identified, with Diptera predominating in the early decomposition stages. The highest species richness (18 species) and diversity occurred in summer, where *Lucilia sericata* and other Calliphoridae were most common. Spring followed with 16 species, also dominated by *L. sericata*. Winter yielded only three species, mainly *Calliphora vicina*, and no insects were observed in autumn due to cold and snowfall.

Conclusion: Insect succession patterns vary by season and location, influencing PMI estimations. Species-level patterns are likely specific to local ecological conditions, emphasizing the importance of regional studies in forensic entomology.

Keywords: Forensic entomology, Insect succession, Carrion insects, Post-mortem interval, Ardabil, Iran

Introduction

Forensic entomology is the application of insect biology in legal investigations, especially for estimating the post-mortem interval (PMI) based on insect activity on decomposing remains (1). Insects are usually the first organisms to colonize a corpse and play a key role in decomposition. The main value of entomological evidence lies in analyzing insect succession and developmental stages to estimate the minimum time since death (2). Accurate PMI estimation depends on identifying and aging immature insect stages found on corpses. Necrophagous insects, mainly Diptera and Coleoptera, are primary decomposers and are closely associated with corpses. Forensic entomology examines this relationship, focusing on how insects contribute to decomposition and how their presence provides forensic clues (3). The use of arthropods in criminal cases dates back centuries. One of the earliest documented examples occurred in 13th-century China, where flies were attracted to a seemingly clean weapon due to blood traces, helping identify the killer (4). Forensic entomology bridges natural ecological processes with legal investigations. It is particularly useful in homicide, accidents, and long-term decomposition (5). Since the late 19th century, arthropods have been used in hundreds of forensic cases (6). Entomological evidence serves multiple purposes: estimating time of death, determining whether a body was moved, detecting trauma, and even revealing exposure to toxins or drugs (6–8). Among these, PMI estimation is the most critical and widely applied function. Studies show that entomological methods provide more accurate and statistically reliable PMI estimates than traditional pathology, especially in advanced decomposition when soft tissues are lost (9). Two main entomological strategies are used for PMI estimation. First, many flies lay eggs shortly after death, so the developmental stage of the oldest larvae can indicate the minimum time since death (10). Second, insect succession follows a fairly predictable pattern, allowing PMI estimation based on the sequence of insect arrivals (11). However, many factors affect decomposition and insect activity. These include the deceased's physical condition, environmental features of the site, and weather conditions like temperature, humidity, rainfall, and light (12, 13). Other variables such as age, health status, body exposure, insect accessibility, and elevation also influence decomposition and insect diversity (12–14). Due to such variability, insect communities differ across regions, and local faunal data are essential for accurate PMI estimation. So far, no comprehensive study has examined insect succession patterns in northwestern Iran. Therefore, this study aims to identify key insect species involved in carcass decomposition across four seasons (autumn, winter, spring, and summer) in Ardabil County, northwest Iran, during 2021–2022. These findings will serve as baseline data for future regional forensic entomology research.

Materials and Methods

Study site and animal sample

Ardabil City (Fig. 1) was the site of the study in 2021–2022. The city of Ardabil experiences four distinct climates: cold mountainous, temperate, warm Mediterranean, and moderate. Five to eight months of the year are cold in this city, which is located in one of Iran's coldest locations. Although it falls during all seasons, spring and autumn have the most substantial precipitation. Summers are moderate, and winters are extremely cold in the city. Around 7 degrees Celsius is the typical temperature there. The Sabalan, Talesh, and Bozghoush mountains, the Caspian Sea's vapours, the cold northern winds, and the forest areas to the north and east of it all have a major effect on Ardabil City's rainfall and temperature fluctuations. The remaining portion is made up of low-lying, flat regions, while the remaining two-thirds have a mountainous character with significant elevation variation. With an elevation of roughly 4811 meters, Sabalan Peak is the province's highest peak. The altitude, which is frequently covered in snow, has a significant impact on the temperature (15). Over a year, from early fall 2021 to late summer 2022, rabbit carcasses were used in all four study seasons. After being purchased, live rabbits were sacrificed, and their carcasses were positioned in designated bases. As corpses, 1.5–2 kg rabbits were used. The animal was promptly taken to the field after being put to death by electric shock while being watched over by a veterinarian who was a project participant. The corpse was put in a cage with a 1.27 cm mesh that was 92 x 92 x 153 cm in size. The cages were securely fastened to the ground to keep the carcass from moving and becoming ingested by scavenging organisms. The bottom of the cage was open to allow the carcass to be in direct contact with the earth. At a distance of 8 cm from the carcass, two insect net traps (12 mm in diameter and 7 mm deep) and a cup filled with water and liquid soap for crawling insects were constructed. The sample procedure was followed at each time by the carcass decomposition stages because the carcass's decomposition changes depending on the season.

Sampling Protocol of Adult and Larvae Insects

Samples were collected every 12 to 14 hours for 21 days in spring, 10 days in summer, and intermittently in autumn and winter, covering a total of 28 days per season. During each visit, the physical condition of the carcass—including discoloration, bruising, bloating, and the degree of decomposition—was recorded. Photographs and videos were taken daily. Adult insects were collected using sweep nets above and around the carcass, following a standardized protocol. Specimens were anesthetized with ethyl acetate in a collection container, then mounted using entomological pins and stored in insect boxes for identification. Larval sampling was performed twice daily, each session lasting 30 minutes,

Fig. 1. Map of the study area in Ardabil Province

using brushes and forceps. Collected larvae were killed and fixed in boiling water to preserve morphological features. They were then transferred into glass jars containing 70% ethanol, glycerin, and KAA (cresol) solution, and transported to the laboratory. Additionally, several live third-instar larvae were brought back for rearing under controlled conditions to complete their development. Identification was carried out using standard taxonomic keys and reference specimens from the Entomology Museum of the School of Public Health. All identifications were verified by expert taxonomists (16–18).

Shannon diversity index and Jaccard Similarity

To assess species diversity, we utilize the Shannon diversity index calculator.

H=−∑[(pi)×log⁡(pi)]H = - \sum \left [(p_i) \times \log(p_i) \right]H=−∑[(pi)×log(pi)]

Where:

H - Shannon diversity index

p_i - proportion of individuals of the ithi^{th}ith species in a whole community

∑ - sum symbol

log - usually the natural logarithm, but the base of the logarithm is arbitrary (10 and 2 based logarithms are also used).

pi=niNp_i = \frac{n_i}{N}pi=Nni

Where:

n_i - individuals of a given type/species

N - total number of individuals in a community

We employed the Jaccard similarity criteria to compare the similarity of samples.

Results

Insect species observed on rabbit carcasses that were collected and identified in northwestern Iran (Ardabil City) during the autumn and winter of 2021 and the spring and summer of 2022 are listed in Table 1. In this study, eighteen species were found over the summer. The Calliphoridae family, which was observed 24 hours after the carcass was placed, included two species: *Chrysomya albiceps* and *L. sericata*. These species were observed during the fresh, swollen, and early active decay stages. About forty-five hours after the carcass was placed, the larvae of both species were found, indicating that they had laid eggs on the carcass while it was still fresh. Nine days after the carcass was deposited at the location, the first pupa was also found during the summer. Additionally, species from the Sarcophagidae family, including *Sarcophaga argyrostoma*, *S. crassipalpis*, and *S. africa*, were collected in the fresh, swollen, and early active decay stages. Two species, *Musca domestica* and *Muscina stabulans*, were identified across three stages of carcass decomposition—fresh, swelling, and decay—during the summer months. In the final stages of decomposition, species such as *Necrobia rufipes*, *Cotinis mutabiis*, and *Gonocephalum sp.* were identified. These species belonged to the beetle families Cleridae, Scarabaeidae, and Tenebrionidae, respectively. Some specimens from the family Carabidae were identified in the active decay, final decay, and dry stages. The species *Creophilus maxillosus*, from the Staphylinidae family, was identified in both the first stage of active decay and the final decay stage. Additionally, some specimens from the families Nitidulidae and Gryllidae were identified

Table 1. Insects collected and identified from rabbit carcasses in Ardabil city, 2021-2022

Family	Species	Summer	Spring	Winter	Fall
CalliPhoridae	*L.sericata*	✓	✓	-	-
Calliphoridae	*C.vicina*	✓	✓	✓	-
Calliphoridae	*Ch.albiceps*	✓	✓	-	-
Sarcophaghidae	*S.argyrostoma*	✓	✓	-	-
Sarcophaghidae	*S.crassipalpis*	✓	✓	-	-
Sarcophaghidae	*S.africa*	✓	-	-	-
Muscidae	*M.domestica*	✓	✓	-	-
Muscidae	*M.stabulans*	✓	✓	-	-
Formicidae	*Pheidole sp.*	✓	✓	-	-
Formicidae	*Messor sp.*	✓	✓	-	-
Formicidae	*Cataglyphis*	✓	✓	-	-
Vespidae	*Vespa germanica*	✓	✓	-	-
Cleridae	*Necrobia rufipes*	✓	✓	-	-
Scarabaeidae	*Cotinis caenosus*	✓	✓	-	-
Staphilinidae	*Creophilus maxillosus Creophilus*	✓	✓	-	-
Tenebrionidae	*Gonocephalum sp.*	✓	✓	-	-
Carabidae	-	✓	✓	-	-
Gryllidae	*Gryllus domesticus*	✓	-	-	-
Nitidulidae	-	✓	-	-	-
Hysteridae	*Hyster sp.*	-	-	✓	-

exclusively in the dry stage. Formicidae family, including *Pheidole sp.*, *Messor sp.*, and *Cataglyphis sp.*, were observed around the eyes of the carcass. These ants were present throughout all phases of decomposition, indicating their early and sustained colonization. In the spring, a total of sixteen species were identified. From the Calliphoridae family, only *Lucilia sericata* was recognized and collected during the fresh, swollen, and early active decay stages. Two species from the Muscidae family, *Musca domestica* and *Muscina stabulans*, were observed during the active decay and late swelling phases. During the final stage of decay, the species *Necrobia rufipes* (Cleridae), *Cotinis mutabilis* (Scarabaeidae), and *Ocephalum sp.* (Tenebrionidae) were collected and identified. The rove beetle *Creophilus maxillosus* (Staphylinidae) was found in both the initial and final stages of active decay. Furthermore, the social wasp *Vespa germanica* (Vespidae) was present and collected during the fresh, swollen, active, and final stages of decomposition. As in summer, the ant species *Pheidole sp.*, *Messor sp.*, and *Cataglyphis sp.* were again observed in all decomposition stages. During the winter of 2021, only one species, *Calliphora vicina* from the Calliphoridae family, was found on the carcass in the fresh, swollen, and early active decay stages. This species had laid eggs on the carcass. No adult specimens from the Muscidae and Sarcophagidae families were captured during this season, and no larvae were successfully reared to adulthood. Insect activity was minimal during winter due to temperatures falling below 14°C. Only *C. vicina* (Diptera) and *Hister sp.* (Coleoptera) showed activity when the temperature exceeded 14°C. In the autumn of 2021, daily inspections of the carcass revealed that it did not progress beyond the fresh stage. One week after placement, temperatures dropped below freezing, with an average of 7.1°C in November and 6.9°C in December. This declining temperature trend likely inhibited insect activity, as no adult insects were collected, and no eggs or larvae were observed during the observation period.

Stages of Carcass Decomposition Across Different Seasons

Fresh Stage

In summer, the fresh stage lasted for two days. The first day featured clear skies, with an average temperature of 22.5°C and a relative humidity of 47%. On the second day, the weather was partly cloudy, with an average temperature of 19.7°C and 48% humidity. No odor was detected during this stage (Fig. 2). In spring, this stage also spanned two days. On the first day, the average temperature was 17.2°C, with 76% humidity, while the second day recorded 16°C and 70% humidity. The carcass remained intact, with no odor or visible decomposition. Within the first few hours, adult flies from the Calliphoridae and Sarcophagidae families were observed near the natural openings of the body. On the second day, ant activity around the eyes was noted (Fig. 3). During winter, the fresh stage persisted for 23 days, with average ambient conditions of 11.2°C and 68.5% humidity. No insect activity or odors were noted during daily inspections. According to local meteorological data, March temperatures fluctuated between a high of 7°C and a low of -2°C (Fig. 4). In autumn, the average temperatures in November and December were 7.1°C and 6.9°C, respectively, with humidity around 66.8%. A week after carcass placement, temperatures dropped below freezing, likely halting insect colonization and egg-laying (Fig. 5).

Fig. 2. Rabbit carcass in the fresh stage of decomposition in Ardabil city, summer 2022

Fig. 3. Rabbit carcass, fresh stage of decomposition in Ardabil city, spring 2022

Fig. 4. Rabbit carcass, fresh stage of decomposition in Ardabil city, winter 2021

Fig. 5. Rabbit carcass, fresh stage of decomposition in Ardabil city, winter 2021

Bloated Stage

In summer, the bloated stage occurred on days three and four post-placement. On the third day, the temperature reached 19.7°C with 42% humidity and was bright and breezy. By day four, average temperature and humidity were 18.4°C and 37%, respectively. Mild bloating and a faint odor were present, and first-instar larvae and eggs were visible beneath the carcass. On the second day of bloating, the carcass was severely swollen, emitted a strong odor, and larval masses were evident in the oral cavity. The temperature within the larval mass reached 30°C (Fig. 6).

Spring showed a similar two-day bloated phase. On the first day, ambient temperature and humidity were 19°C and 70%. Swelling was most evident in the head and abdomen. Egg masses and first-instar larvae were abundant, and a strong odor permeated the area. Calliphoridae and Sarcophagidae flies were present in large numbers, alongside Myrmicinae ants (Fig. 7).

In winter, bloating began 23 days after carcass placement and lasted for 10 days. Snow gradually melted, and temperatures briefly rose above freezing, averaging 16.7°C with 33% humidity. Despite visible swelling, no odor was detected. The temperature steadily declined throughout this phase (Fig. 8).

Decay Stage

The decay stage lasted six days in summer. Daily temperatures and humidity were as follows: 17.2°C and 62% (Day 1), 16.1°C and 57% (Day 2), 16.1°C and 53% (Day 3), 15°C and 6% (Day 4), 16.2°C and 77% (Day 5), and 16.5°C and 70% (Day 6) (Fig. 9). The carcass had ruptured, releasing abdominal gases and producing a strong

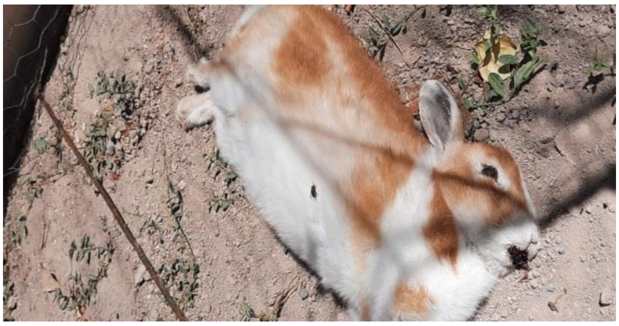

Fig. 6. Bloated carcass (third day), adult larvae laying in the mouth of the carcass, in Ardabil city, summer 2022

Fig. 7. Rabbit carcass in the bloat stage, attracting a lot of insects in Ardabil city, spring 2022

Fig. 8. Rabbit carcass in the bloated stage, after the snow melted in Ardabil city, winter 2021

Fig. 9. Rabbit carcass, active decay with the presence of diatoms and active decomposition of the carcass in Ardabil city, summer 2022

Fig. 10. Rabbit carcass, active decay stage, increase in 3rd instar larvae, rainy weather, in Ardabil city, spring 2022

Fig. 11. Rabbit carcass, active decay after 33 days, larval activity in the lower part of the carcass in Ardabil city, winter 2022

odor. Tissue decomposition progressed rapidly. Dipteran activity was high early on, with later appearances of Staphylinidae beetles. Third-instar larvae dominated during this phase, eventually giving way to pupation, and fly activity diminished significantly.

In spring, decay spanned five days. On the first day, rainfall occurred, with an average temperature of 15.3°C and 78% humidity. The carcass had ruptured, and a strong odor was present. Egg masses and third-instar larvae increased across various regions of the body. Temperatures and humidity remained high throughout this phase. By the end, the carcass had begun to desiccate, and adult fly presence decreased. Cleridae and Staphylinidae beetles became more active (Fig.10).

In winter, active decay was brief, lasting three days when average temperatures were around 10.7°C and humidity was 68.8%. The carcass ruptured, releasing a putrid odor, and larvae were concentrated in the lower portions. Only Calliphoridae flies contributed to decomposition. The Sarcophagidae and Muscidae families were absent. A subsequent temperature drop below freezing for five days halted visible activity, and the carcass began drying (Fig. 11).

Dry Stage

In summer, the dry stage persisted for approximately two weeks. The average temperature was 17.8°C with 71% humidity. At this point, the carcass had fully decomposed, leaving only bones, dry skin, and hair. The site was predominantly inhabited by ants and beetles, with minimal larval presence and no adult flies (Fig. 12). In spring, this stage began about three weeks after placement. The mean temperature was 21.9°C and the humidity averaged 69.2%. The remaining tissue had dried completely, and odors had dissipated. Scarabaeidae and Cleridae beetles, along with scavenger ants, were observed feeding on residual tissues (Fig. 13, 14).

Fig. 12. Rabbit carcass, drying stage, exterior and interior view in Ardabil city, spring summer 2022

Fig. 13. Rabbit carcass, drying stage, exterior and interior view in Ardabil city, spring 2022

Fig. 14. Carcass of a winter rabbit specimen, dry stage, exterior and interior views, in Ardabil city, spring 2021

Biodiversity Indicators

A total of seven Dipteran species (122 samples) were collected, including *L. sericata*, *Chrysomya albiceps*, *Sarcophaga crassipalpis*, *S. africa*, *S. argyrostoma*, *Musca domestica*, and *Muscina stabulans*. Of these, four species were successfully reared in laboratory conditions, totaling 473 individuals. The Shannon-Wiener diversity index for collected specimens was highest in summer (1.38), followed by spring (1.28). For reared specimens, summer yielded a value of 0.4, while spring showed a much lower diversity (0.07). As expected, winter presented the lowest biodiversity due to reduced insect activity. Index values for both seasons are shown in Tables 2 and 3.

Table 2. Shannon-Weiner index for summer-caught and raised specimens in Ardabil city, 2022

Species	**Frequency**	**Proportion (Pi)**	**Loge pi**	**Piloge pi**
L.sericata	58	0/4754098361	-0/7435780341	-0/3535043113
Ch.albiceps	9	0/0737704918	-2.6067964674	-0/0548541173
S.crassipalpis	17	0/1393442623	-1/9708077006	-0/2746207452
Sarcophaga.africa	2	0/0163934426	-4/1108738656	-0/0673913748
S.argyrostoma	18	0/1475409836	-1/9136492869	-0/2823416981
Musca domestica	11	0/0901639344	-2/4061257722	-0/2169457663
Muscina stabulans	5	0/0409836066	-3/1945831313	-0/1309255383
Total	122		1/3880583	
Species	Frequency	Proportion (Pi)	Loge pi	Piloge pi
L.sericata	426	0/9006342495	-0/1046560422	-0/094256816
Ch.albiceps	30	0/0634249471	-2/7578980076	-0/1749195352
S.crassipalpis	8	0/0169133192	-4/0796538491	-0/0690004878
S.argyrostoma	7	0/0147991543	-4/2131852417	-0/0623515785
Total	473		0/400528415	

Table 3. Shannon-Weiner index for spring-caught and raised specimens in Ardabil city, 2022

Species	Frequency	Proportion (Pi)	Loge pi	Piloge pi
L.sericata	34	0.435897	-0.8303493021	-0/3619467697
S.crassipalpis	7	0.0897435	-2.4107996776	-0/2163536009
S.argyrostoma	9	0.1153846	-2/1594843827	-0/2491712417
Musca domestica	26	0.333333	-1/0986132887	-0/3662040634
Muscina stabulans	2	0.0256410	-3/6635626461	-0/0939374098
Total	78		1/2876130855	
Species	Frequency	Proportion (Pi)	Loge pi	Piloge pi
L.sericata	317	0/9875389408	-0/0125393493	-0/0123830957
S.argyrostoma	2	0/0062305296	-5/0782939418	-0/0316404607
S.crassipalpis	2	0/0062305296	-5/0782939418	-0/0316404607
Total	321		0/0756640171	

Jaccard Similarity Analysis

Jaccard similarity analysis revealed the greatest overlap between summer and spring samples, with a similarity coefficient of 0.84, indicating high species continuity between these seasons. The lowest similarity (0.09) was observed between summer and winter, reflecting stark differences in species presence and abundance due to seasonal climatic variation. High dissimilarity coefficients suggest pronounced species turnover across seasons.

Discussion

This study presents the first comprehensive seasonal analysis of insect succession on animal carcasses conducted in northwestern Iran, covering all four stages of decomposition: fresh, bloated, decay, and dry. Through this investigation, a localized baseline dataset was established for forensic entomology, which can significantly contribute to estimating post-mortem intervals (PMI) and characterizing crime scenes in the region. The five-stage decomposition pattern observed in our study is consistent with previous literature that reports similar processes in comparable climatic zones, particularly in studies involving rabbit carcasses (19–21). Our findings reaffirm the value of arthropod succession patterns as reliable indicators for distinguishing decomposition stages in forensic investigations. Species richness varied across seasons, ranging from three in winter to sixteen and eighteen in spring and summer, respectively. These results are consistent with earlier studies that reported variation in species richness depending on factors such as climate, vegetation, carcass type, and sampling design (17, 22, 23). For instance, Watson and Carlton (2003) reported that only 19 out of 78 insect species were common across different vertebrate carcass types in Louisiana, emphasizing the influence of carcass-specific factors on insect colonization (6). As in earlier studies (13, 17, 20, 24-27), Calliphoridae and Sarcophagidae were the first necrophagous families to colonize carcasses during warmer seasons. While family-level succession patterns remained consistent across seasons, variation was observed at the species level. This supports the notion that genus- and species-level dominance is influenced by environmental conditions (10, 15, 18, 22). For example, *L. sericata* dominated in spring and summer, whereas *C. vicina* was prevalent in autumn and winter, aligning with previous reports on species-specific thermal preferences (13, 18). Compared with the findings of Parkhideh et al. (2018), who reported 13 and 22 species in summer and autumn in Shiraz, we observed 16 species in spring, 18 in summer, but only 3 in winter. This discrepancy highlights the significant impact of local climatic variations on species diversity (18). The absence of Silphidae and Phoridae in our samples may be related to habitat type, carcass exposure, or vegetation cover, as noted in previous research (13, 23). Our results further support findings that *L. sericata* is more active during warm and sunny conditions, while *C. vicina* prefers cooler, shaded environments and may even exhibit nocturnal oviposition behaviors (13, 18). These thermal preferences underscore the importance of considering environmental parameters such as temperature and light exposure when interpreting insect succession in forensic contexts. Seasonal variation in species richness was most pronounced in our study, with diversity peaking in summer and reaching its lowest point in winter. This pattern mirrors findings from a study in Lisbon, which also reported maximum insect diversity in summer and minimum diversity in winter (23). Notably, species richness and abundance were highest during the bloated stage, underscoring its ecological importance in carrion insect succession. While temperature was identified as a major driver of species activity and presence, accurate PMI estimation also requires knowledge of the timing, abundance, and succession order of dominant species. In our study, *L. sericata* and *C. vicina* were the dominant blowfly species, depending on the season. In contrast, *C. albiceps* was the main species observed during summer in the Parkhideh et al. (2018) study. This suggests that while succession patterns may remain relatively stable at the family level across regions, species-level dynamics are shaped by microclimatic and ecological conditions (28-38). The lack of collected specimens in autumn, along with peak diversity in summer and lowest richness in winter, indicates distinct seasonal succession trends. These findings differ from those of Parkhideh et al. (2017) in Kazerun, where autumn exhibited the highest diversity (18). Such discrepancies likely reflect regional climatic differences and emphasize the importance of conducting localized forensic entomology studies.

Conclusion

This study highlights that insect successional patterns during carcass decomposition vary significantly across different geographic locations and seasons. Such variations emphasize the importance of site-specific and time-specific data in accurately estimating the post-mortem interval (PMI). Our findings indicate that reliable predictions of insect succession can only be made at the genus or species level when the ecological context of the cadaver's discovery, such as location and environmental conditions, is carefully considered. Therefore, establishing regional baseline data is essential for the development of precise forensic entomology tools and for enhancing the accuracy of PMI estimations in medico-legal investigations.

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Ethical considerations

Our project was approved by the Tehran University of Medical Sciences (IR. TUMS. SPH. AEC. 1402.017).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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