

VARIATIONS IN COLONIZATION AND SUCCESSION PATTERN OF DIPTERAN FLIES OF FORENSIC IMPORTANCE ON INDIAN MOLE-RAT CARCASSES IN URBAN AND SUBURBAN LOCALITIES OF KOLKATA, WEST BENGAL: IMPLICATIONS IN CORPSE RELOCATION STUDIES.

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ABSTRACT

Necrophagous diptera form the first wave of arthropod colonization of carrion, facilitating microbial decomposition and helping maintain the balance of the ecosystem, thus are of considerable importance both from ecological and forensic entomological perspective. The present study indicates the difference in dipteran species composition and its succession patterns observed in colonising Indian mole-rat (*Bandicota bengalensis*) carcasses in two different localities, an urban and a suburban region of Kolkata and its adjoining areas in West Bengal during the month of April. It was observed that from the urban locality, a total of eight dipteran species belonging to three different families were recorded from the rat carrion, whereas, six dipteran species from four families were recorded from the suburban region. Moreover, in the urban habitat, it was noticed that the muscids were the predominant colonizers both with respect to species richness, abundance and frequency of colonization. The suburban area showed overall uniformity in terms of species richness and abundance in all the families. The two carcasses were seen to differ in dipteran succession patterns as well, with muscids, *Synthesiomyia nudiseta* being the first and *Atherigona orientalis*, the last to colonize in the urban region, in contrast to the suburban region with *Chrysomya rufifacies* (Calliphoridae), the first and *Megaselia scalaris* (Phoridae), the last species to colonize the carcass. *Synthesiomyia nudiseta* and *Chrysomya rufifacies* were the most abundant species found in the urban and suburban areas respectively. The study, first of its kind in India, will aid in future studies on carrion ecology and forensic entomological research of this region.

Keywords: Diptera, Carcass, Forensic entomology, Succession.

INTRODUCTION

Necrophagy is the act of feeding on dead or decaying animal flesh. Decaying carrion provides a transient, rapidly changing resource which supports a large, dynamic fly community thus aiding in estimation of PMI and criminal forensic investigations. Necrophagous insects, often called carrion insects, are key players in the decomposition process which is associated with decaying human and animal remains and utilized by insects as their micro-niches, thus, forming diverse

micro-communities (Allee *et al.* 1949; Kuusela and Hanski, 1982). Necrophagous dipterans are the initial wave of insects to colonize carcasses, commencing the process of decomposition. Though the adults feed on the fluids of the corpse, the larvae are the true decomposing organisms, secreting enzymes directly into the carrion and helping with the liquefaction of the corpse tissues while assisting the increase of microbial activity, thus playing a crucial role in nutrient cycling and maintaining

homeostasis of the ecosystem (Galante and Marcos-Garcia, 2004).

Forensic entomology is the science, which applies knowledge of insect and other arthropods to civil proceedings and criminal trials in homicide cases and has its applications in wildlife crime control as well. These insect colonizers of carrion can be used to estimate the time of death, that is, the time interval between death and corpse discovery, also called postmortem index (PMI), movement of the corpse, manner and cause of death and association of suspects at the death scene (Sukontason *et al.*, 2007).

The arthropods involved in succession vary according to geographical areas, even in places with similar climates. Also, the number of individuals and the species colonizing these microhabitats vary enormously from one patch to another, and also through time (Galante and Marcos-Garcia, 2004). Hence, for attaining accurate results in forensic entomological studies, it is utmost crucial that the study of insect fauna in each region is essential for interpreting differences in decomposition of carrion in relation to biogeography and ecology of necrophagous insect communities. Forensic entomological studies in India has gained importance in recent years as seen in related studies (Bharti and Singh, 2003; Sinha, 2009; Bharti, 2012; Jadav and Sathe, 2015; Singh, Kumari & Singh, 2016).

Studies on filth-inhabiting flies in Kolkata, West Bengal have been initiated by Joseph and Parui (1980). Diversity pattern of carrion flies using various parameters in three districts of West Bengal, India has been studied by Majumdar *et al.*, (2008). But till date, no such detailed studies based on dipteran colonisation and succession patterns of animal carcasses in different habitats have been carried out and thus there remains a gap area in forensic entomological studies from India.

Recent emphasis on forensic entomological studies like the ones carried out recently in Central Europe have been given on assessing the differences in dipteran species composition and succession patterns in different habitats, so as to obtain information to be used in homicide cases on corpse relocation (Grassberger and Frank 2004; Matuszewski *et al.*, 2008; Matuszewski, Szałałowicz & Jarmusz, 2013).

Such research is based on the hypothesis that if an

insect can be found exclusively in a rural or urban habitat, analysis of the carrion associated fauna may help determining whether the remains have been moved from an urban to a rural environment or vice versa (Erzinclioglu 1989; Catts and Haskell 1990).

Thus, the principal objective of our study was to assess which species may serve as forensic entomology indicators in postmortem interval estimations or proof of corpse displacement in the urban setting of Kolkata metropolitan area and its adjoining suburbs. So, a representative region of urban Kolkata, Bangur Avenue was chosen as an urban habitat for carrying out the research, whereas, a locality of Howrah, suburban Kolkata has been chosen as the semi-urban habitat for the study of assessing difference in dipteran species composition and succession patterns on Indian mole-rat carcasses in the month of April, 2017. Thus, the data obtained from this study will aid in future research on forensic entomology from this region.

MATERIALS AND METHODS

1. Study site and time: The study was carried out simultaneously in two localities-Bangur, urban locality in Kolkata and Ramrajatala, Howrah, a semi-urban (suburban) locality under Kolkata metropolitan area, West Bengal, India during the month of April, 2017. The urban study site is an area with high human population density is more congested than the suburban study site which has more greenery. Kolkata has a tropical wet-and dry climate; the study was conducted during April, early summer, with hot and humid weather. The study was carried on the backyards of houses. Research sites were located near about 20 km from each other.

2. Carcass: Three carcasses of common pest Indian mole-rat (*Bandicota bengalensis*) weighing approximately 100g each were used for each research site. The animals were killed with a blow on the head and the carcasses were immediately placed inside metal grated cages measuring 55cm × 55 cm × 45cm made with 5cm × 3 cm wire mesh. All the cages were fixed to the ground using sturdy wooden holders. This allowed insects to enter but prevented any disturbances from necrophagous vertebrate scavengers. The carcasses were placed in natural environmental conditions.

3. Collection of meteorological data: Daily weather

data were obtained from Meterological Department, Alipore, Kolkata. During the inspections of the carcasses, ambient air maximum and minimum temperature and relative humidity of the two research sites were measured using a hygrometer with in-built thermometer (model HTC-1).

4. Observation of the carcass and its successive colonization by dipteran species: The Indian mole-rat carcasses were observed for its successive colonisation by different dipteran species right from the fresh stage till the dry remains stage for both the sites. Each experiment was continued until the entire carcass was consumed.

The carcasses were observed for three times a day, in the morning, afternoon and evening. Adult flies were collected on a daily basis until apparent insect activity had ceased. The state of the carcasses were documented by means of both a digital camera (Nikon Coolpix camera model S3700) and written reports.

5. Collection and preservation:

a) Adults: Adult flies were collected with the help of an aerial sweep net. Live adult flies were killed in a killing-jar containing ethyl acetate and they were preserved dry in insect-envelope. The specimens were sorted out by basic body plan assessment and relaxed in a relaxing-chamber for about 24 hours. Then they were pinned and the specimens were kept in a dry chamber for at least 4-5 days before being dry-preserved in insect cabinet.

b) Immature stages - Dipteran larvae were collected with the help of forceps. Some of the dipteran larvae were kept alive and reared with goat liver in the laboratory for identification while a few were killed in boiling water and preserved with 70% ethyl alcohol for photo documentation purpose.

All specimens were labelled based on collection date and time. The taxonomic and morphological identification and quantification were carried out in the Laboratory of Diptera Section, Zoological Survey of India (ZSI), HQ, Kolkata, India.

6. Morphological identification of specimens collected: Certified reference materials, Senior-White *et al.*, (1940); Emden (1965); Mercedes and Hardy (1977); Nandi (2002) were utilized for further identification of specimens.

7. Observing stages of decomposition: The carcasses of the Indian mole-rat were observed thrice daily, once in the morning, afternoon and late in the evening to assess the changes and duration of each decompositional stage. A slightly modified version of the stages of decomposition proposed by Wolff *et al.*, (2001) and Martinez, Duque & Wolff, (2006) was used.

8. Assessing dipteran successional patterns: The chronological successional waves of dipteran species colonizing the carcasses were assessed by combining data from specimens collected by sweeping nets and also from larval collections.

RESULTS

1) Climatic conditions (ambient air temperature and relative humidity) of the two research sites:

Urban locality- The maximum air temperature of the study site during the study period of 15 days ranged from 31 to 38°C with an average of 35.30±0.4°C whereas the minimum temperature ranged from 24 to 28°C, average 26.36±0.3°C. The relative humidity ranged from 52.25 to 78.5% with an average of 65.40±2.25%.

Suburban locality- The maximum air temperature of the study site during the study period of 18 days ranged from 30 to 37.5°C with an average of 34.35±0.44°C whereas the minimum temperature ranged from 24.5 to 29°C, average 26.26±0.31°C. The relative humidity ranged from 52 to 78.5% with an average of 68.80±1.88%.

There were no significant differences observed between research sites in ambient air temperature and relative humidity for the entire study period as shown in Figure 1.

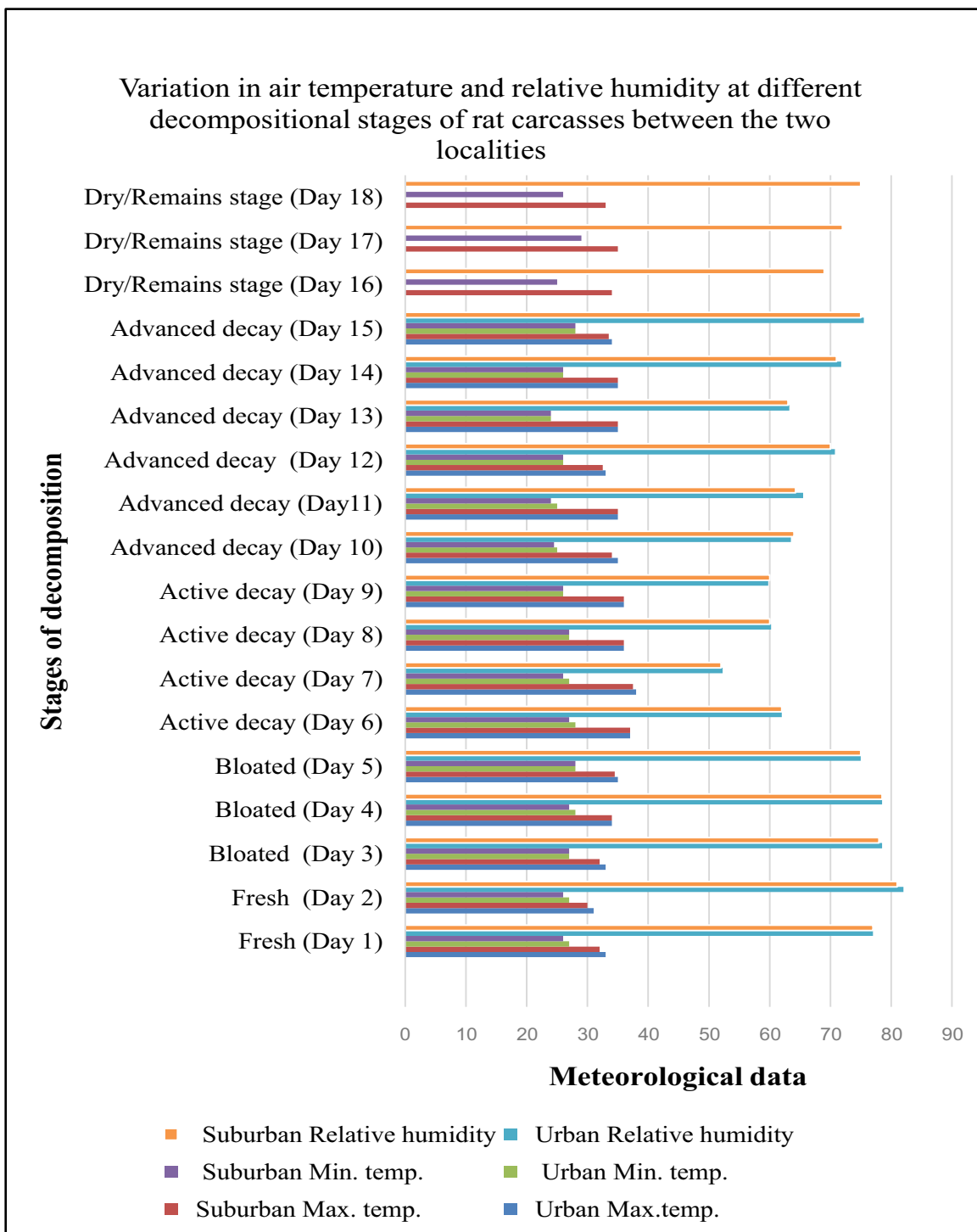


Figure 1: Graphical representation of ambient air temperature (maximum and minimum) and relative humidity during the different stages of decomposition of rat carcasses at an urban and a suburban region of Kolkata, West Bengal. Insignificant changes in ambient air temperature and relative humidity was observed between the two research sites during the study period.

2) Stages and rate of decomposition:

There were no differences between research sites in the number and type of decompositional stages. Five stages of decomposition were observed in both the sites

and they are- fresh stage, bloated stage, active-decay stage, advanced decay stage and lastly the dry or remains stage. The structural changes of the carcasses as observed during the five decompositional stages are briefly described in Table 1.

Table 1: Descriptions of the different decompositional stages as observed from the Indian mole-rat carcasses in both the study sites.

Stages of decomposition	Defining characteristics of decompositional stages
Fresh stage	Starts from minutes after death and lasts for 1-2 days with no notable differences in appearance of the carcass. Dipteran adults start visiting the carrion. No decompositional odour noticed.
Bloated stage	Starts right after the fresh stage with inflation of the abdomen along with odour of putrefaction. Dipteran adults are noticed to colonize the carcass in large numbers. Dipteran larvae also start to appear.
Active decay stage	Carcass appears deflated in appearance; high number of maggot masses observed feeding on soft tissues; strong odour of decay. Dipteran adults also observed visiting the carcass. Liquefaction was observed with fluids oozing out of the carcass.
Advanced decay stage	Intense migration of larvae is observed; decrease in decay odour; most of the flesh has been removed at the end of this stage.
Dry/Remains stage	Carcass dry in appearance, consists of only bones, skin and fur; little to no odour is observed.

However, substantial differences were observed in the rate of decomposition of the carcasses in the two habitats. In the urban region, the decomposition was faster with the total number of days recorded for reaching the dry stage was found to be fifteen, whereas in the suburban site it reached up to eighteen days. The differences in the rate of decomposition were observed only in the last two decompositional stages, namely, the advanced decay and the remains stage. The advanced decay and dry remains stage of the carcass kept in the urban site lasted for four and two days respectively, in contrast to the suburban region, where advanced decay lasted for six days and dry remains stage for three days as shown in Figure 2.

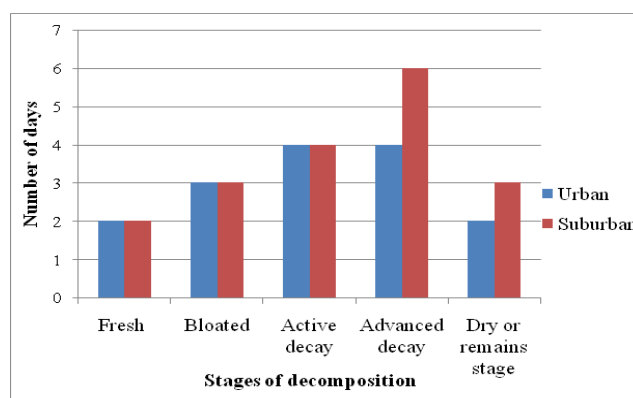


Figure 2: Graphical representation of the difference in rate of decomposition of the Indian mole-rat carcasses in the two different habitats.

3) Species composition of carrion dipterofauna:

Substantial differences were observed both in terms of species composition and successional patterns on the Indian mole-rat carcasses in the urban and suburban locality.

Regarding the composition of carrion dipteran species that colonized the carcasses in the urban region, muscids were the most diverse and dominant group, with six muscid species recorded out of a total of eight species of diptera as shown in Table 2.

One species each of Calliphoridae and Sarcophagidae were recorded from the urban region. In contrast to this, the species composition in the suburban region showed more or less uniformity in terms of diversity and dominance, with two species each of Calliphoridae and Muscidae and one species of Sarcophagidae recorded from the carcasses. One species of Phoridae, another dipteran family was recorded from the carcasses of the suburban area which was absent in the urban locality as shown in Table 3.

4) Successional patterns of carrion dipterofauna:

Variation in successional patterns of dipteran species

was observed from the carcasses in the two habitats.

Dipteran succession in the urban habitat: Muscids were the first to arrive and colonize the carcass, with the successional wave starting with *Synthesiomyia nudiseta* (Wulp, 1883) and continued with *Ophyra capensis* (Wiedemann, 1818) and *Musca domestica* (Linnaeus, 1758) in the fresh stage. Calliphorid species *Chrysomya rufifacies* (Macquart, 1843) was the next species to colonize the carcass followed by Sarcophagidae *Sarcophaga albiceps* (Meigen, 1826). No other species was recorded in the fresh stage and only adults were present. In the bloated stage, all five species recorded in the fresh stage were present along with adults of three other muscid species, namely, *Musca sorbens* (Wiedemann, 1830), *Hydrotaea chalcogaster* (Wiedemann, 1824) and *Atherigona orientalis* (Schiner, 1868). Larval stages of *Chrysomya rufifacies*, *Musca domestica* and *Sarcophaga albiceps* were also observed in this stage. In the active decay stages, adults and larvae of different dipteran species were observed which was gradually reduced in the advanced decay stage as shown in Table 2. The remains stage showed the presence of only adult *muscid Atherigona orientalis*.

Table 2: Succession of a total of eight species of dipterofauna at different decompositional stages on Indian mole-rat carcasses in an urban locality of Kolkata, West Bengal

URBAN LOCALITY						
Family	Species	Presence of dipterofauna during the five stages of decomposition				
		Fresh	Bloat	Active decay	Advanced decay	Dry remains
Calliphoridae	<i>Chrysomya rufifacies</i> (Macquart, 1843)	A	A,L	L	L	-
Muscidae	<i>Musca domestica</i> (Linnaeus, 1758)	A	A,L	A,L	A,L	-
	<i>Musca sorbens</i> (Wiedemann, 1830)	-	A	A	-	-
	<i>Ophyra capensis</i> (Wiedemann, 1818)	A	A	A	-	-
	<i>Hydrotaea chalcogaster</i> (Wiedemann, 1824)	-	A	A	-	-
	<i>Synthesiomyia nudiseta</i> (Wulp, 1883)	A	A	A	A	-
	<i>Atherigona orientalis</i> (Schiner, 1868)	-	A	A,L	A,L	A
Sarcophagidae	<i>Sarcophaga albiceps</i> (Meigen, 1826)	A	A,L	A,L	L	-

Abbreviations used: A- Adult flies; L- larvae.

Dipteran succession in the suburban habitat: In contrast to the urban region, Calliphoridae was the first family to colonize the carcass in the suburban region. *Chrysomya rufifacies* was the first to arrive, followed by *Chrysomya megacephala* (Fabricius, 1794). *Musca domestica* and *Sarcophaga ruficornis* (Fabricius, 1794) arrive next in the fresh stage. As in the case of urban region, only adults were observed in the fresh stage.

Adults and larvae of all these four species were recorded in the bloated stage. In the decay stages, succession was almost similar to the urban region with only *Atherigona orientalis* arriving at the active decay stage and *Megaselia scalaris*, a phorid species arriving at the advanced decay stage as shown in Table 3. The remains stage showed the presence of only adult phorid *Megaselia scalaris*.

Table 3: Succession of a total of six species of diptero fauna at different decompositional stages on Indian mole-rat carcasses in a suburban locality of Kolkata, West Bengal

SUBURBAN LOCALITY						
Family	Species	Presence of diptero fauna during the five stages of decomposition				
		Fresh	Bloat	Active decay	Advanced decay	Dry remains
Calliphoridae	<i>Chrysomya megacephala</i> (Fabricius, 1794)	A	A,L	A,L	L	-
	<i>Chrysomya rufifacies</i> (Macquart, 1843)	A	A,L	A,L	A,L	-
Muscidae	<i>Musca domestica</i> (Linnaeus, 1758)	A	A,L	L	L	-
	<i>Atherigona orientalis</i> (Schiner, 1868)	-	-	A	A,L	A
Sarcophagidae	<i>Sarcophaga ruficornis</i> (Fabricius, 1794)	A	A,L	A,L	L	-
Phoridae	<i>Megaselia scalaris</i> (Loew, 1866)	-	-	-	A	A

Abbreviations used: A- Adult flies; L- larvae.

5) Comparative analysis of the relative abundance of dipteran families recorded from the two study sites:

As mentioned earlier, there is considerable amount of difference regarding species composition of carrion diptero fauna recorded from the two sites. Comparative analysis of the relative abundance of dipteran families recorded from the two habitats showed that the family Muscidae had the highest abundance in the urban region while family Calliphoridae was the most abundant in the suburban region as shown in Figure 3. Sarcophagidae was more abundant in the suburban region, while Phoridae was only recorded from the suburban region.

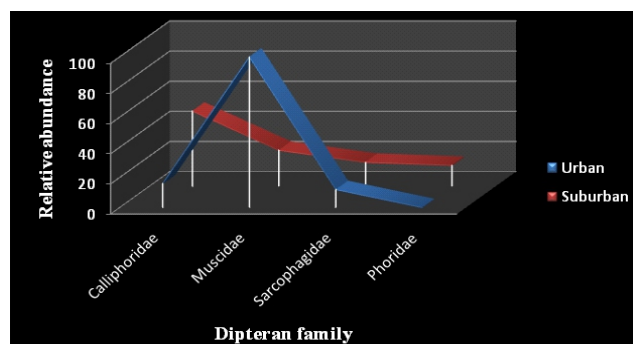


Figure 3: Graphical representation of the comparative analysis of the relative abundance of dipteran families recorded from the Indian mole-rat carcasses in the two study sites.

6) Species abundance at different decompositional stages in the urban and suburban region:

At the fresh and bloated stage, it was observed that, in the urban region muscid *Synthesiomyia nudiseta* was the most abundant, whereas, *Chrysomya rufifacies* was the most abundant species in the suburban region. At the active decay stage, in the urban region, *Ophyra capensis* was the most abundant, in contrast to *Chrysomya rufifacies* in the suburban region. Advanced decay stage showed the most abundance of *Synthesiomyia nudiseta* again as observed in the fresh and bloated stages in the urban region, whereas, *Megaselia scalaris* was the most abundant species at the advanced decay stage in the suburban region. At the dry remains stage, *Atherigona orientalis* was the only species recorded from the urban region, while *Megaselia scalaris* was the most abundant in the suburban region. The fly species abundance in different stages of decomposition of Indian mole-rat carcasses at the urban and suburban habitat is shown in Figure 4 and 5 respectively, whereas, comparative analysis of relative abundance among the fly species on Indian mole-rat carcasses in two different localities is shown in Figure 6.

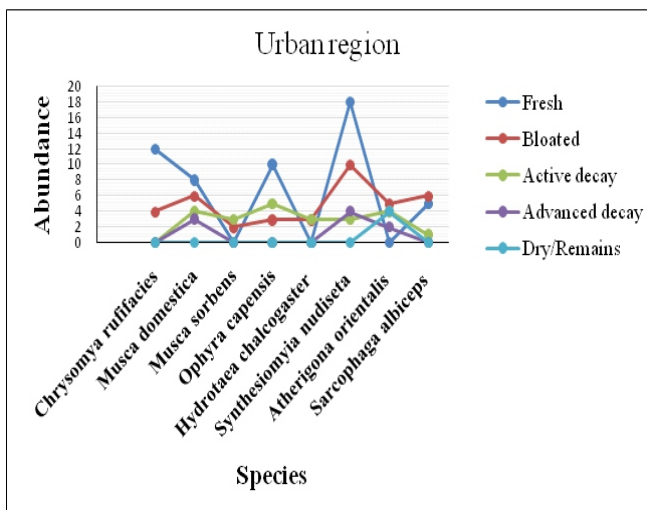


Figure 4: Fly species abundance in different stages of decomposition of Indian mole-rat carcasses at the urban locality.

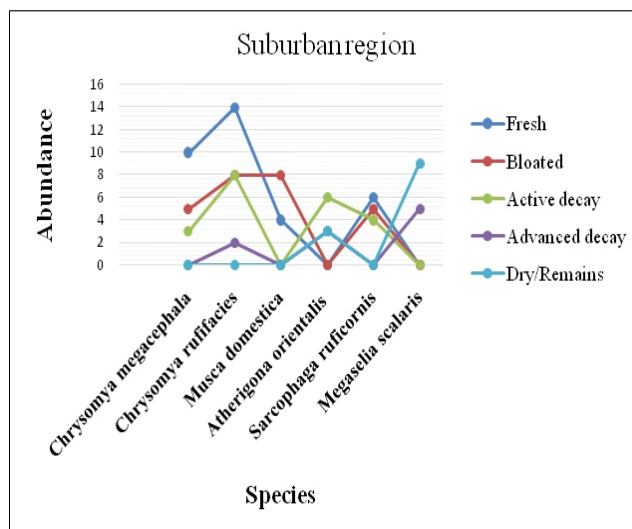


Figure 5: Fly species abundance in different stages of decomposition of Indian mole-rat carcasses at the suburban locality.

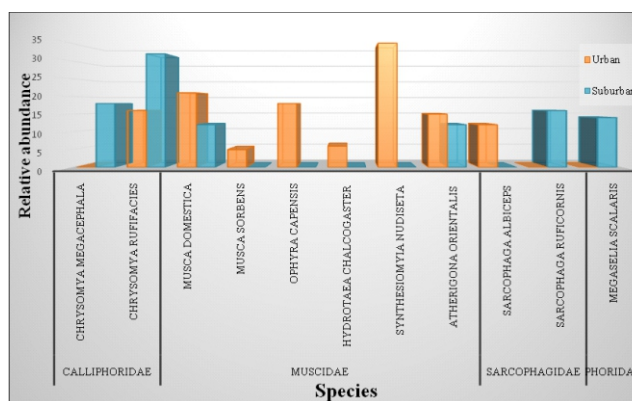


Figure 6: Comparative analysis of relative abundance among the fly species on Indian mole-rat carcasses in two different localities.

DISCUSSION AND CONCLUSION

The present study comprising of detailed observations, descriptions and documentations of the different decompositional stages and dipterofaunal activity on a mammalian forensic model, Indian mole-rat carcass in two different habitats viz. an urban and a suburban locality studied simultaneously, is the first of its kind studied in Indian perspective. It was observed that, at environmental conditions with insignificant differences between the two study sites, substantial amount of dissimilarity was noticed not only in terms of

carrion dipterofauna species composition and abundance but also in the successional patterns of carcass colonization as well.

The number and types of decompositional stages observed from the present study is similar to that observed in previous studies (Tullis and Goff 1987; Anderson and Van Laerhoven 1996; Wolff *et al.*, 2001; Grassberger and Frank 2004; Martinez, Duque & Wolff, 2006). No difference was observed regarding the number and types of decompositional stages of the carcasses placed in two different habitats. Dissimilarity was noted regarding the rate of decomposition of the Indian mole-rat carcasses in the two localities, with the decomposition process being faster in the urban region in comparison to the suburban region. The difference was observed in the number of advanced decay and dry remains stages in the two habitats. As the rate of decomposition of the carcass depends upon quite a number of factors like biotic and abiotic factors of the habitat like environmental variables, carcass biochemistry, carcass biomass (Campobasso *et al.*, 2001; Gunn 2006), it is often difficult to interpret the results as the study is in its initial phase. But assumptions can be made, that the differential species composition and abundance of the dipterofauna recorded can play a contributing factor for changes in rate of decomposition in our present study. In our study, it is observed that in the urban context, the overall abundance of dipteran species was higher than the suburban region and moreover, the diversity and frequency of muscid species was more in the urban area than as recorded in the suburban region which could contribute to the changes in rate of decomposition observed.

The differential species composition in the two habitats in the present study can also attribute to the fact that calliphorid and muscid flies are anthropocentric as well as synanthropic. Thus, more abundant in habitats close to anthropogenic activities, like in urban areas with high human population density, in contrast to sarcophagids which are more abundant in distant, open places rather far away from human settlements as proposed in previous studies conducted on filth-inhabiting flies from West Bengal (Joseph and Parui, 1980; Majumdar *et al.*, 2008). This hypothesis can also

explain the prevalence of a diverse group of Muscidae (six muscid species) in the urban locality with congested human settlements, in contrast to only two muscid species in the suburban region.

In forensic entomological studies, the entomological approach to cases of corpse relocation is based on observed differences between different habitats in carrion insect communities. Therefore, the ecological separation of species across habitats forms the foundation based on which entomological approach to cases of corpse relocation can be studied. This method rests on a hypothesis that particular species of carrion insects have different environmental demands, and as a result they colonise carcasses in different habitats. Once the sequence of colonizing dipterofauna on the carrion is known, an analysis of the dipterofauna on a carcass can be used to determine time since death in legal investigations (Anderson and Van Laerhoven, 1996).

Recent forensic entomological studies carried out on different habitats like rural open to rural forest habitats of Central Europe and urban settlements provide entomological evidence of certain specific indicator dipteran species that can act as forensic indicators for corpse relocation (Grassberger and Frank 2004; Matuszewski *et al.*, 2008; Matuszewski, Szafałowicz. & Jarmusz, 2013).

If we put the same approach in our present study, that the data obtained from the study the difference in species abundance and succession patterns in the two different habitats could act as database or potential candidates that can be regarded as entomological indicator species for these two types of habitats. Thus this may be extremely useful in future forensic entomological studies regarding corpse relocation in homicide cases.

Furthermore, our study also has its relevance in future research on community dynamics of carrion flies, their ecological roles in nutrient recycling and resource partitioning in carrion ecological studies from this region.

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