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Selma El Batoul Benmira
Laboratoire de Biosystématique et Ecologie des Arthropodes – Constantine, Algérie

Fatima Zohra Guerroudj
Laboratoire de Biosystématique et Ecologie des Arthropodes – Constantine, Algérie

Sélima Berchi
(1). Laboratoire de Biosystématique et Ecologie des Arthropodes – Constantine, Algérie
(2). Ecole Nationale Supérieure de Biotechnologie – Constantine, Algérie

Amel Aouati
Laboratoire de Biosystématique et Ecologie des Arthropodes – Constantine, Algérie

Correspondence
Selma El Batoul Benmira
Laboratoire de Biosystématique et Ecologie des Arthropodes – Constantine, Algérie

Seasonal study of scavenger wildlife of forensic interest in Constantine (Algeria)

Selma El Batoul Benmira, Fatima Zohra Guerroudj, Sélima Berchi and Amel Aouati

Abstract
This study aims atrevealing more information about the seasonal variation of the necrophagous fauna biodiversity. Three entomological experiments were carried out in Algeria, in an open space near the Biosystematic and Arthropod Ecology Laboratory in Constantine at Chaabat Erssas, during three seasons (spring, summer, and autumn) of the year 2012/2013. They allowed us follow the decomposition of three dead-dog corpses. The results showed that the scavenger insects studied in spring were the most diverse one with 21 taxa (Diptera 57.73%, Coleoptera 41.16%, and Hymenoptera 1.1%) as compared to summer (9 taxa) (Diptera 77.77%, Coleoptera 22.22%) and autumn (7 taxa) (Diptera 81.03%, Coleoptera 18.96%). Favorable weather conditions (temperature) partly explain these results.

Keywords: Forensic entomology, adult scavenger insects, corpses, seasons, Constantine

1. Introduction

Forensic entomology is a science that can be applied to all situations in which insects or their actions have a relationship with the legal system [1]. The study of the characteristics of a corpse and its state of decomposition (cadaveric rigidity, presence of lividity or corpse temperature) is only effective for a short period [2]. After this period, the estimation of the post mortem interval (PMI) becomes delicate and imprecise [3]. According to the same author, it is possible to resort to forensic entomology. In fact, necrophagous insects allow, under some conditions, to determine the post mortem interval (PMI) [4], which is often the requisite starting point for the identification of the victim and the circumstances of death [2].

According to Charabidze and Bourel [5], two orders are mainly concerned, Diptera and Coleoptera. Nevertheless, the majority of the works turn around Diptera considers it true bioindicators in the date of death [6, 7, 8]. There are also some Lepidoptera whose larvae are necrophagous (Tineidae), and many Hymenoptera are frequently present on the bodies as predators (ants) or parasites (Chalcididae, Pteromalidae) [9].

Despite all the progress made in forensic entomology, some gaps exist in this discipline. There is, for example, very little data on necrophagous Coleoptera [10] that would be involved in the determination of the Post Mortem Interval. They can also be considered as new bioindicators for forensic entomology in the same way as the Diptera [11, 12, 13, 14, 15].

According to the work of Wyss & Cherix [16] and Charabidze [2], necrophagous species and their sequence of successions may vary according to geographical area, seasons and years. Therefore, the present study aimed at providing more information about the seasonal variation in necrofauna biodiversity.

2. Materials and methods

For this study, three dog corpses recovered from the Hygiene Department have been used as substrate. The first was that of spring (05.04.2012) and weighs about 15 kg. The second was that of summer (07.07.2012) and weighs 13.4 kg and the third was that of autumn (12.12.2012) and weighs 13 kg.

The corpses had been placed immediately after deaths in a cage mounted with a metal frame of 2 m sides and covered with iron netting with 1cm-diameter small meshes to protect them from possible predators. The cage was placed in an open space near the Biosystematic and Arthropod Ecology Laboratory in Constantine at Chaabat Erssas (36°20'16.20"N, 6° 37'33.32"E, altitude 571 m). The investigations were carried out throughout the decomposition with the exception of weekends.

In order to collect the maximum number of species visiting the corpse, the present study adopted a purely qualitative sampling approach. This technique involved coupling an active hunting method with a passive hunting method.

The active method allowed collecting, using plastic tubes, the insects that come to rest on the different parts of the corpse (eye, muzzle, ear, lower abdomen...); it is at the natural orifices that the adults are less agitated and therefore easier to take. The passive method was a Barber-type pitfall trap that relies on the use of containers buried in the soil and containing soapy water. The upper part of these receptacles was flush with the surface and arranged all around the corpse; this type of trap was well adapted for harvesting Coleoptera moving on the ground. In order to obtain more accurate results, we installed a HMI weather station near the substrate deposition site to record the meteorological data essential for the study of cadaveric fauna, such as temperature. The collected samples are placed in a freezer for about 5 minutes or more, at a temperature of 4 °C to 7 °C, in order to get them killed. The insects are then stitched for identification.

The identification of adult necrophagous fauna is carried out under a binocular lens of OPTECH 1.5X type. Recognition techniques used for different families, genera and species are described in various books [17, 18, 19, 20, 21, 22, 23]. Nevertheless, the main used criteria are wing nerves, body shape and color, the color of the respiratory stigma, and the head and bristles at its level.

The present study exploited results using ecological indices of composition such as the distribution of abundance and structure, in particular that of diversity as mentioned below:

2.1 Shannon-Weaver Diversity Index

According to Blondel *et al.* [24], diversity can be defined as the degree of heterogeneity of a stand. Margalef (1958) in Legendre & Legendre [25] proposes the use of the Shannon-Weaver diversity index H' as a measure of diversity, calculated as follows:

H’ = - ∑ni log2 ni

H ': Diversity index expressed in bits

ni: relative abundance of species i relative to individuals in the whole stand

log 2: base-2 logarithme

This index provides information on the diversity of species in each of the seasons taken into consideration. If this value is low, the environment is poor in species, or it is not favorable to necrophagous species. On the other hand, if this index is high, it implies that the environment is very populated in species and that it is favorable to them.

2.2 Maximum diversity

Blondel [26] expresses maximum diversity by the following formula:

H 'max. = Log2 S

H 'max. Is the maximum diversity expressed in bits.

S: is the total wealth of species.

2.3 Equipartition index

According to Blondel [26], equal-distribution is the ratio of observed diversity to maximum diversity. It is given by the following formula:

E = H '/ H' max.

The value of the Equipartition E varies between 0 and 1.

When E tends to 0, it means that the numbers of the collected species are not in balance. In this case one or two species dominate the whole population by their numbers. When E tends to 1 this means that the numbers of the captured species are in equilibrium, their abundances are very similar.

2.4 Statistical analysis

The present study exploited results using centesimal frequencies (FR), Shannon-Weaver diversity index, maximum diversity and equipartition index.

3. Results

3.1 Structure of the necrophagous fauna

Three experiments (autumn, spring and summer) were conducted in an open environment with three dogs. During the experimentation period, we collected 836 samples belonging to 12 families (Table 1). Due to the unavailability of identification keys adapted to all the found families, a part of the samples could not be identified until the species.

Table 1: Families and centesimal frequencies of necrophagous insects collected during the three seasons.

Orders	Families	Number	Frequencies%
Diptera	Calliphoridae	404	48.33
	Sarcophagidae	30	3.59
	Piophilidae	40	4.78
	Muscidae	31	3.71
	Fanniidae	2	0.24
Coleoptera	Dermestidae	81	9.67
	Trogidae	19	2.27
	Silphidae	119	14.23
	Staphylinidae	21	2.51
	Histeridae	64	7.66
	Cleridae	17	2.03
Hymenoptera	Pteromalidae	8	0.96
Total		836	100%

Diptera and Coleoptera represent respectively 60.65% and 38.40% or 99.04% of the total fauna collected. The order of the Hymenoptera is weakly represented with 8 samples (0.96%) belonging to the family Pteromalidae. Among the Diptera, 404 samples belong to the Calliphoridae family, i.e. 48.33%, 30 samples to the Sarcophagidae family (3.59%), 31 to the Muscidae family (3.71%), 2 to the Fanniidae family (0.24%) and 40 to the Piophilidae family (4.78%) (Table 1).

The Coleoptera were divided into 6 families (Table I), Dermestidae with 81 samples (9.67%), Trogidae with 19 samples (2.27%), Silphidae with 119 samples (14.23%), Staphylinidae with 21 samples (2.51%), Histeridae with 64 samples (7.66%) and Cleridae with 17 samples (2.03%). The Hymenoptera collected belong to the family Pteromalidae (Table 1); they were similar to the Fanniidae (Diptera) very poorly represented with 0.96% of the insects caught.

3.2 Sampling of the necrophagous fauna on the corpse recovered in spring

Sampling began on the first day of the corpse caging on 05.04.2012 and ended when the corpse was completely desiccated on 08.05.2012. These investigations that last 34 days outside the weekends allowed to recover 724 necrophagous insects.

Among the necrophagous fauna captured, 57.73% belong to the Diptera, 41.16% to the Coleoptera and 1.10% to the Hymenoptera. The centesimal frequencies of the identified families are shown in Fig. 1. It was clear that the best represented was that of Calliphoridae (Diptera) with 48.07% followed by that of Silphidae (Coleoptera) with 16.16%. For Sarcophagidae and Piophilidae represented respectively by 2.62% and 5.52%, the species could not be identified.

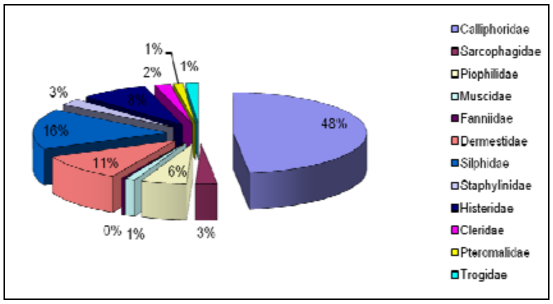

Fig 1: Centesimal frequencies (FR) of families collected on animal substrate during spring.

The Diptera were the first to visit the animal substrate followed by the Coleoptera which appeared from the third day and the first species collected was *Trox hispidus* (Pontoppidan, 1763). The arrival of the Hymenoptera was observed starting from the fifth day, and represented by the family Pteromalidae (1.1%). From the first day of the corpse deposition, the presence of ants in the natural orifices (muzzle, nostril, eyes, and anus) was observed along with the arrival of two species of necrophagous Diptera *Lucilia sericata* (Meigen, 1826) and *Calliphora vicina* (Robineau-Desvoidy, 1830). Both belonged to the Calliphoridae family.

Diptera belong mainly to five families (Fig.1) : Calliphoridae (48.07%) represented by five species [*L. Sericata, Lucilia silvarum* (Meigen, 1826), *C. vicina* (Robineau-Desvoidy, 1830) *Calliphora vomitoria* (Linnaeus, 1758) and *Chrysomia albicep* (Wiedemann, 1819), the Sarcophagidae (2.62%), Piophilidae (5.52%), Muscidae (1.24%) represented by two species: *Muscina stabulans* (Fallén, 1817) and *Hydrotaea dentipes* (Fabricius, 1805)) and finally the Fanniidae (0.28%) was represented by *Fannia scalaris* (Fabricius, 1794).

The Coleoptera belong to six families (Fig.1) : the Dermestidae (10.64%) represented by the species *Dermestes peruvianus* (Laporte de Castelnau, 1840), the Trogidae (1.52%) represented by the species *T. Hispidus*, the Silphidae (16.16%) represented by two species (*Silpha rugosa* (Linnaeus, 1758) and *Silpha obscura* (Linnaeus, 1758)), the Staphylinidae (2.62%) represented by the species *Creophillus maxillosus* (Linnaeus, 1758), the Histeridae (8.29%) represented by four species (*Saprinus semistriatus* (Scriba, 1790), *Saprinus aeneus* (Fabricius, 1775), *Hister unicolor* (Linnaeus, 1758) and *Hister purpurascens* (Herbst), and finally the Cleridae (1.93%) (Fig. 1). The Hymenoptera were represented by the Pteromalidae family (1.1%) (Fig. 1).

3.3 Sampling of necrophagous fauna on the corpse recovered in summer

The investigation began on 07.07.2012 and lasted 11 days corresponding to the complete decomposition of the cadaver. At the end of these investigations, a total of 54 samples were captured, mainly belonging to two categories: the Diptera with 42 samples, ie 77.77%, and the Coleoptera with 12 samples, ie 22.22%.

The collected Diptera belong mainly to 3 families (Fig. 2): the Calliphoridae (20.37%) was represented by *C. albiceps*, the Muscidae with 38.88%, the Sarcophagidae (18.51%) represented by the two species *Sarcophaga sp* and *Sarcophaga carnaria* (Linnaeus, 1758). The Coleoptera are divided into four families: the Dermestidae (7.4%) represented by the species *D. peruvianus*, the Staphylinidae (1.85%) represented by *C. maxillosus*, the Histeridae (7.4%) represented by *S. semistriatus* and *S. aeneus*, and the Cleridae (5.55%) (Fig. 2).

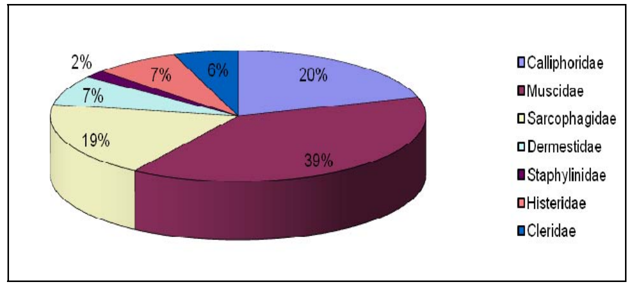

Fig 2: Centesimal frequencies (FR) of families collected in summer.

The first species to settle on the corpse from the first day was *C. albiceps*. Coleoptera intervene only starting from the third day and the first observed family was the Cleridae. On the fourth day, Staphylinidae and Histeridae represented by the species *C. maxillosus* and *S. semistriatus* settle the corpse.

3.4 Sampling of the fauna scavenging on the corpse recovered in autumn

The investigation began on 12.12.2012 and lasted 22 days. To the present study collected a total of 58 samples belonging to the two main categories: Diptera with 47 samples, i.e. 81.03%, and Coleoptera with 11 samples or 18.96%.

Diptera belong to three families; the most abundant is the Calliphoridae with 77.58% of the harvested necrophagous fauna (represented by the two species *C. vicina* and *L. sericata*), followed by the Sarcophagidae (1.72%) and the Muscidae (1.72%) (Fig. 3). Three families of Coleoptera were recorded: Trogidae (13.79%) represented by *T. hispidus*, the Silphidae (3.44%) represented by *S. rugosa*, and the Staphylinidae with 1.72% (Fig. 3).

During autumn season, the first visitors of the corpse were Coleoptera belonging to the species *T. hispidus*. The Diptera didn't intervene until the third day. The first 2 species to came to colonize the corpse were *C. vicina* and *L. sericata*.

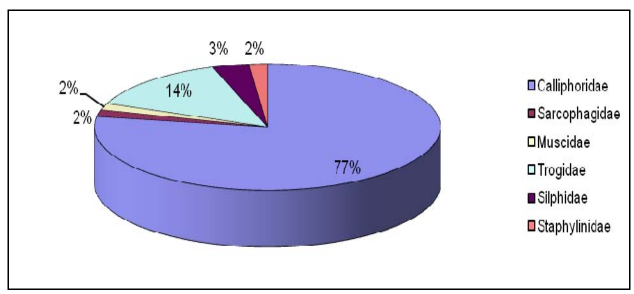

Fig 3: Centesimal frequencies (FR) of families collected in autumn.

3.5 Results analysis by ecological indices of structure

The results presented in Table 2 were studied using the ecological indices of structure showing the quantitative aspect of the necrofauna, namely the Shannon-Weaver diversity index H, the maximum diversity (H 'max) and the equitability index E between species.

Table 2: Ecological indices of structure H ', H'max and E for the three seasons of prospection.

	Spring	Summer	Autumn
H' (in bits)	3.19	2.42	1.67
H' max. (in bits)	4.39	3	2.81
E	0.73	0.81	0.59

H ': Shannon Ŕ Weaver diversity index; H 'max: maximum diversity; E: Equirepartition

The Shannon Weaver index of captured species showed that the H' values record an information of 3.19 bits in spring, 2.42 bits in summer and 1.67 in autumn (Table 2). These values approximate those of H'max maximum diversity; in other words, the population constituting the captured necrofauna is diversified particularly in spring. It was noteworthy that the Equirepartition index values fluctuate between 0.59 and 0.81 (Table 2). These values tend towards 1 for the three seasons reflecting a tendency towards a balance between the numbers of the species present.

3.6 Temperature Influence on the arrival of necrophagous fauna during the three seasons

The influence of temperature on the numbers of collected necrophagous fauna was assessed. To do purpose, the present study calculated the mean temperature of the sampling period for each season. The results are shown in Fig. 4.

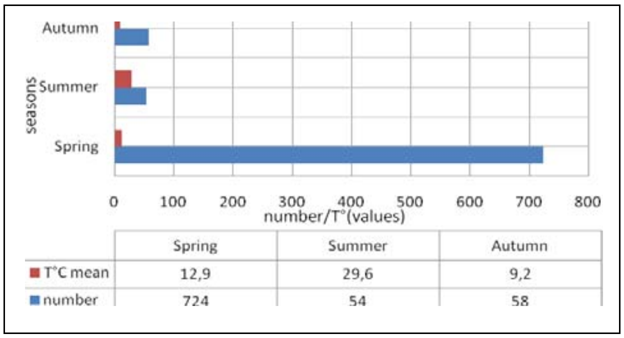

Fig 4: Temperature influence on the number of necrophagous fauna collected during the three investigation seasons.

According to Fig. 4, the warmest season, summer (T ° Cmoy = 29.6 ° C) was noted to be the least rich in numbers with only 54 samples collected. During autumn (T ° Cmoy = 9.18 °C), the number collected was similar to that of summer with 58 samples. The most abundant season was spring (12.92 °C) with a total of 724 samples, 12.48 times higher than autumn, and 13.4 times higher than summer.

4. Discussion

A decomposing body is a particularly resource-rich environment. A large number of insect species use this energy and proliferate quite rapidly on the corpse. Decomposition attracts early some insects on the body, and others later. This is the principle of the squads defined by Mégnin [27]. The best represented fauna on a decomposing substrate is that of necrophagous insects [16, 14]. It is this order of insects in the adult state taken from the three decaying dog substrates during the three seasons of spring, summer and autumn.

Contrary to the work of Bensaada *et al.* [28] who carried out their investigations on cats cadavers dead for some time, the present study began just after the dogs were slaughtered by the hygiene department, which allowed regular monitoring of the necrophagous fauna immediately after their death.

The number of necrophagous insects collected in spring (724 samples) represents 86.60% of the total number collected during the three seasons, which was much higher than in summer (54 samples) or 6.45% or in autumn 58 samples) or 6.93%. The obtained results are similar to those of Charabidze *et al.* [29] who captured a significant number of samples during the spring-summer period, unlike the autumn and winter seasons. These authors explain this by the typical seasonal variations that indicate that populations of necrophagous insects remain at a very low level during autumn/winter unlike the warm (spring/summer) period.

One of the essential characteristics of any community is its organization degree; generally, in a given environment, there is a high index of diversity when living conditions are favorable. Otherwise, the recorded values are low. Bigot and Bodot [30] report that when living conditions in an ecosystem are favorable to the whole, many species exist and each of which is represented by a number of individuals such that the index of diversity is then high. On another hand, when conditions are unfavorable, only a small number of species are found, and the value of the index is low. According to the obtained results, the Shannon diversity index observed during spring (H '= 3.19 bits) approaches the maximum diversity (H'max = 4.39 bits), which means that the population of the collected necrofauna was diversified during this season. If we refer to equitability E = 0.73 for this season, we can deduce that the collected population is in equilibrium. This balance was observed in spite of the domination of the species *L. sericata*. Indeed, all the other species present in the population are poorly represented. According to Daget [31], when the value of the equitability tends to 1, this reflects a balance between the numbers of the different present populations. This is the case of the results we obtained in spring.

In summer, the fauna was less diversified than spring (H '= 2.42 bits) but well balanced with an equitability E = 0.81. These results are in consistent with Charabidze *et al.*[29] who explained that by what they called the typical seasonal variations.

The least diversified season was autumn as the Shannon index calculated for this season has the lowest value comparing to the other two seasons (H = 1.67 bits). Nevertheless, a certain balance exists in the population (E = 0.59).

Among the necrophagous fauna collected during the three seasons, the two main categories are Diptera and Coleoptera. Hymenoptera appeared only in spring; this order was represented by the Pteromalidae family, unlike the results interpreted by Bensaada *et al.*[28] in the region of Gouraya where they mentioned that the Hymenopteran order is represented by three different families (Braconidae, Chalcidae and Formicidae).

During the decomposition of a corpse, the odors emitted are made up of a mixture of chemical molecules [32] which necrophagous insects are able to perceive rather quickly whereas they are not perceived by humans. In spring and autumn, the most abundant family was Calliphoridae with 48.07% and 77.58%, respectively; they are the first to visit the corpses. According to Wyss & Cherix [16], it is probably the most important family in forensic entomology. Moreover, when the climatic conditions are favorable, this family comes to colonize a corpse very quickly earlier than any odors thanks to their particularly developed olfactory system [2]. This observation is in perfect agreement with our results, which show that the *L. sericata* species appears on the first day in both spring and autumn.

In summer, the dominant family was Muscidae with 38.88% followed by Calliphoridae with 20.37%. However, the representatives of this last family remain the first to colonize the corpse. The first species that comes to visit the corpse was *C. albiceps*.

The present experiments results are similar to those conducted by Wyss on a series of pig corpses [16], which reveal that the diversity of necrophagous Diptera varies according to the seasons. Indeed, most of the species that we have inventoried are reported by these authors.

In spring, the dominant species was *L. sericata* with a total of 276 samples, it is a species well adapted to hot climate [29]. In autumn, where the temperature tends to decline, the best represented species was *C. vicina* with a total of 37 samples. According to Charabidze *et al.* [29], this species is well adapted to the cold. For Wyss *et al.* [4], Diptera of the *Calliphora* genus are active starting from 4.5 °C. Bouleknefet *et al.* [33] also reported the presence of this species in winter with 71.82% of collected Diptera. *C. vicina* appears to be well adapted to the autumn temperature because it can accumulate thermal energy by exposure to the sun or by placing it on a warm substrate [34].

Concerning the species *L. sericata* and *C. vicina*, the present study results showed their first appearance and their respective abundance during spring and autumn. These results are in perfect agreement with the studies carried out by Bensaada *et al.* [28] and Wolff *et al.* [35].

Previous works [36-43] are consistent with the results obtained in the current study, including the arrival and seasonal preferences of these species.

Coleoptera intervene later than Diptera; during the degradation of the fats, volatile fatty acids like butyric acid is released [2]. They appear on the second day in spring and autumn seasons and represented by the Trogidae families for the two in addition to the Silphidae in spring. In summer, they intervene starting from the third day and the first family that colonizes the corpse is the Cleridae.

The temperature, especially when in high degrees, plays an important role in insect activity, but it does not seem to be the only physical parameter determining their arrival [29]. Indeed, the presence of Calliphoridae bunches on a corpse placed under the snow would have been reported by Wyss *et al.* [4].

In spring, the present study experienced large variations in temperature during the 34 days of prospection (6 °C to 19.6 °C). Based on the number of collected insects which was 86.6% of the total fauna of the three seasons, conditions appear favorable for the arrival of the necrophagous fauna during this season. Autumn was weak in numbers because temperature decreases; this indicates unfavorable conditions for the arrival and activity of necrophagous insects [29].

In summer, temperatures are higher (Average T °C = 29.6 °C); this encourages the activity of insects given their flying speed. They become faster and therefore more difficult to catch mainly by the active method than we have adopted. According to Hall (1995) in Charabidze *et al.* [29], there is no ideal solution that would allow an optimal capture of all the species.

It is commonly known that when climatic conditions are favorable, colonization occurs within few hours of death, but the present study didn't able to estimate whether the captured insects came to the body to lay immediately after the deposition of the substrate or after some time. However, if the study of climate data provides information on the activity of necrophagous insects, it doesn't allow determining the populations' status [44]. Hence, the lack of a species can be explained by climatic conditions unfavorable for insect inactivity, but also by a small number of the population at the study site. Reciprocally, a local temperature favorable to insects does not necessarily suppose the presence of a necrofauna [45].

5. Conclusion

Our contribution to the seasonal study of fauna necrophagous is one of the first at the region of Constantine. Furtherthorough studies are needed to better understand the biology of the larval stages associated with the climatic preferences of this fauna. The proposed methodology consists first of all in estimating, for the species of interest, the numbers present in the different environments of the territory and determining according to the local climatic data the activity periods of the insects. The results obtained from this study allowed us to note that the necrophagous insects are primarily responsible for the decomposition of the corpses. Their role consists of the mechanical dislocation and digestion of cadaveric tissues. The first representatives of the necrophagous fauna which colonize the corpse immediately after his death belong to the order of the Diptera. The Coleoptera and Hymenoptera come just after. Climatic conditions play a very important role in the diversity of necrophagous species.

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