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An initial study on the succession of sarcosaprophagous Diptera (Insecta) on carrion in the southeastern Iberian peninsula

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Abstract We present the results of the first study concerning Diptera carried out on the sarcosaprophagous fauna of southeastern Spain. This work represents the first attempt to describe dipteran sarcosaprophagous fauna in the Iberian peninsula, the seasonal succession, main features of the population dynamics and the main taxa useful for estimation of the post-mortem interval. The results of this study could be very useful for further forensic case work in the west Mediterranean area.

Key words Sarcosaprophagous Diptera · Iberian Peninsula · Forensic entomology

Introduction

The succession of arthropods has been shown to be very useful for estimating the post-mortem interval and it is therefore interesting to study the order of appearance on a corpse (Anderson 1; Benecke 3; Catts and Goff 5; Goff 7; Nuorteva 9; Smith 11) and this can provide conclusive evidence in forensic case work.

There is, however, a substantial lack of knowledge about the sarcosaprophagous fauna in the west Mediterranean region, and until the study of Tantawi et al. [12] there were no data available for the whole Mediterranean region.

A study of the entomological structure of the sarcosaprophagous community, the succession and seasonal dynamics in the Iberian peninsula could be of great practical importance. This would represent the first valid reference study in forensic entomology for the Iberian peninsula it-

self and for adjacent areas, also largely unexplored in this respect. The locality where the work was carried out is at a latitude of 38°N and a longitude of 1°W, close to the city of Murcia, in the southeast of the Iberian peninsula. The climate of the region is Mediterranean sub-desert, with an annual mean temperature of 17.7 °C and an annual rainfall of 288 mm. The annual mean insolation is close to 3000 h and the area is one of the most arid in the Iberian peninsula. The maximum precipitation occurs in the fall, followed by a secondary maximum in spring. Summer is very dry, with no rain in July and very occasional rain in August.

Materials and methods

Materials

A modified version of the trap designed by Schoenly et al. [10] measuring 120 × 90 × 60 cm and Morrill solution [8] as the preservative solution were used. The trap was baited with chicken carcasses with the flesh partially removed and the viscera present which was considered to be sufficiently representative of a cadaverous ecosystem in which all the stages of faunal succession would occur in the same way as in other corpses, including human.

The design of the trap makes it possible to collect a large variety of arthropods, both those which enter the corpse and those which leave it. The trap does not upset the natural rotting process of the carcass and the faunal succession. Furthermore it allows the composition of the sarcosaprophagous fauna to be recorded and the seasonal dynamics of the populations to be studied using statistical procedures.

Relative humidity and temperature inside the trap were continuously registered with a thermohygrometer (Jules Richard Instruments, Argenteuil, France). Figure 1 shows the variation of temperature throughout the four sampling periods. Micro-environmental conditions of temperature and humidity inside the carcasses were registered by a portable thermohygrometer (MPM 500 Solomat, S.A., 91160, France).

Methods

The trap was positioned in a natural area of the experimental agricultural field in the Espinardo campus of Murcia University, close to a fence and under a tree. It was partially shaded until midday after which it was fully exposed to the sun.

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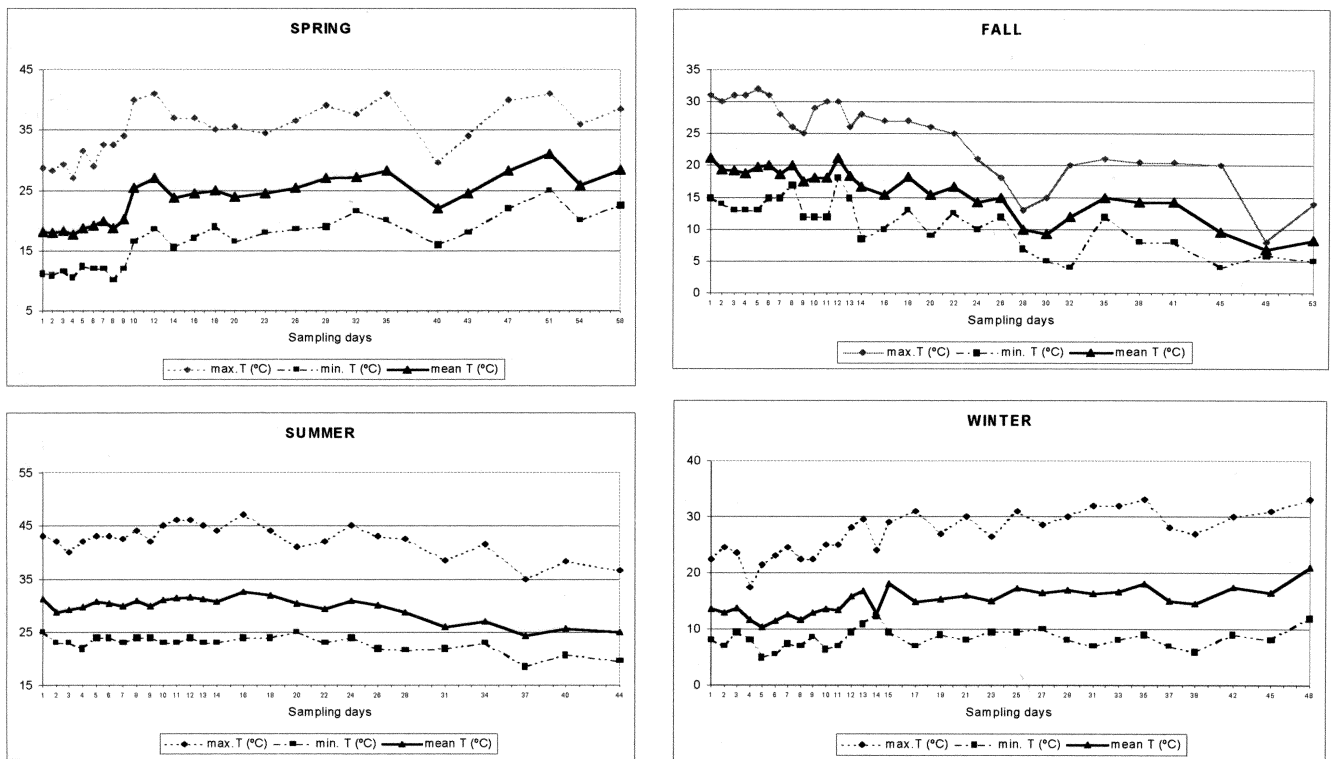


Fig. 1 Temperature during the four seasons studied

Samples were taken in the four seasons during 1 year, in spring from May 16th 1996 to July 12th 1996, in summer from July 27th 1996 to September 9th 1996, in fall from October 19th 1996 to December 10th 1996 and in winter from February 1st 1997 to March 20th 1997. Samples were taken daily during the first 2 weeks in order to collect the main sarcosaprophagous fauna related to post-mortem interval (PMI) determination. After this time, samples were taken every other day or slightly longer until the end of the experiment. Data obtained have been standardized in order to be shown graphically.

We have only taken muscoid Diptera into account because some of the species are the first to arrive on the corpses and appear in a sequential appearance and, as Braack [4] stated, the presence of blowflies is of great importance in the corpse-insects complex and a crucial determinant of the structure of the community. Knowledge of this succession is important in order to estimate the time after death and sometimes the cause of death in medico-legal investigations. The specimens studied are now kept in the entomological collection of the Animal Biology Department of the University of Murcia.

Results

The community of Diptera considered, which represents 41.51% of the fauna collected, is composed of 11 taxa and grouped into the 4 families (Fig. 2) *Calliphoridae* (69.4%), *Muscidae* (16.9%), *Sarcophagidae* (7.3%) and *Fanniidae* (6.3%). All were identified at least to the generic level except for *Sarcophagidae*, which were determined only to the family level because of difficulties in identification, especially of the females, and because the classification is currently under revision in the Iberian peninsula.

During spring (Figs. 3, 4) nine taxa were recorded, of which *Phaenicia sericata* was predominant and arrived in massive numbers on the corpses during the first 4 days. Adults were seen and collected for the first 8 days and then disappeared. *P. sericata* breeds successfully on the corpse as was proven by the emergence of new adults from day 18 onwards from the eggs laid by the first adults. While the first adult population was composed mainly of females (95.7%), the second population showed a male-female ratio of 53.46:46.54.

Stage II larvae of *Phaenicia sericata* were detected in the trap on days 3 and 4. A number of post-feeding larvae were collected between days 3 and 12, mainly on day 8 and afterwards. The migrating post-feeding larvae provoked a strong change in the internal conditions of the corpse, and a sharp decrease in the number of Dipteran taxa present in the corpse was observed from this time onwards.

Calliphora vicina was much less abundant than *Phaenicia sericata*, and showed a known preference for cool conditions. It also acted as a primary fly, appearing from days 1 to 7 and post-feeding larvae appeared suddenly on day 6, although they could be collected until day 12. Nevertheless, breeding was not successful and no adult of a new generation was collected.

Only *Muscina stabulans*, which acted as a secondary fly, and *Sarcophagidae* were present throughout the decomposition process.

In summer nine taxa were collected (Figs. 5, 6) including *Phaenicia sericata* which also acted as a primary fly, as did *Calliphora vicina*, the appearance of which was counted as almost accidental due to the sparse specimens

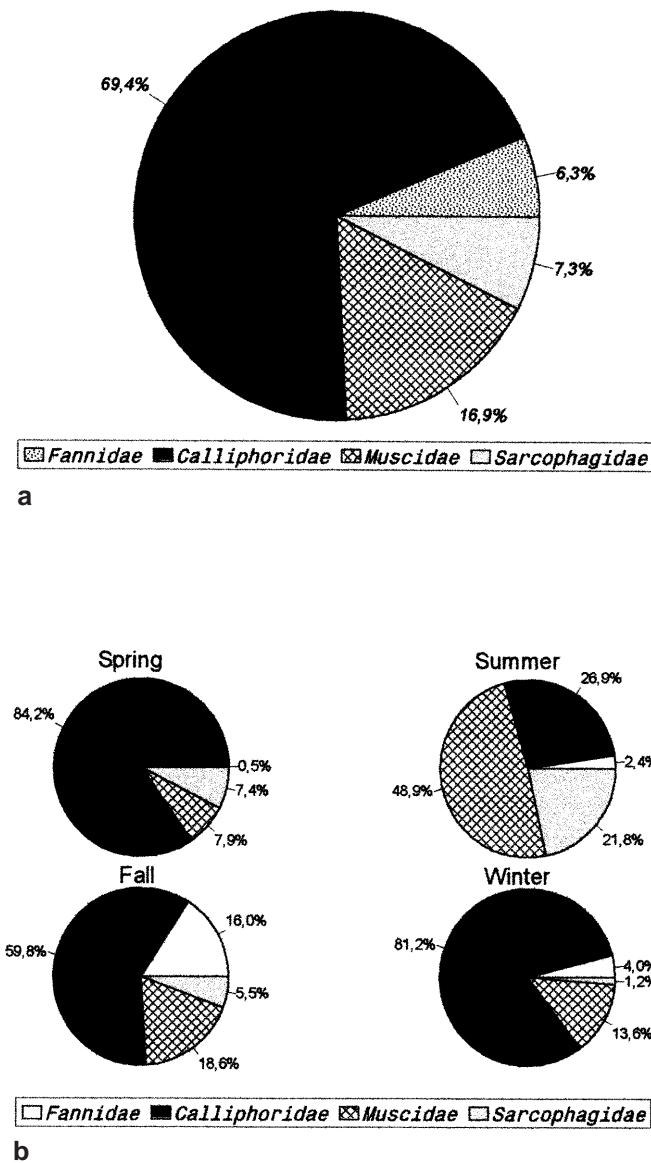
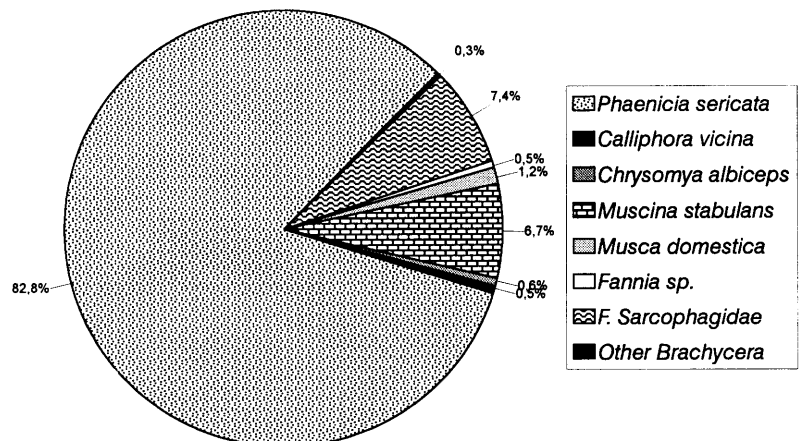


Fig.2 Relative abundance of Dipteran families considered a throughout the sampling period and b in every season

Fig.3 Relative abundance of different taxa during spring expressed as percentages



collected, appearing only on days 1 and 2, and not breeding. *Chrysomya albiceps* appeared from the first day but acted as a secondary fly, with a maximum appearance at day 3 similar to *Musca domestica*, which was the predominant species of this season.

It is worth mentioning the earlier presence of post-feeding larvae, which were collected in very small numbers on days 6 and 8 as well as the general shortening of the different decomposition stages.

Summer was the only season in which skeletonization was accomplished within the 7 weeks of sampling.

The shorter period of time during which flies were evident could be due to the environmental dryness and heat. *Sarcophagidae* seem to be indifferent to the ambient changes, and appear throughout the process.

None of the species seemed to be able to breed successfully in summer, at least no adults from a new generation were collected. This fact may be due both to the climatic features and to the predation of larvae of second and third instars of *Chrysomya albiceps* on larvae of *Phaenicia sericata*, potentially the most active breeder of the season, with which it is synchronous.

In fall ten taxa were collected (Figs. 7, 8). *Chrysomya albiceps* was the predominant species, appearing from day 3 to day 18 with a maximum on day 5.

Phaenicia sericata and *Calliphora vicina* acted as primary flies but the densities were much lower than that of *Chrysomya albiceps*, which predominated.

Sarcophagidae also acted as primary flies in this season, but with a customary low density. The capture of larvae of third instar of this family in days 9–13 revealed its breeding capacity.

Post-feeding larvae of *Phaenicia sericata* were collected mainly on day 30 but some were also collected on day 11. This fact demonstrates a conspicuous lengthening of the initial decomposition stages and of the whole decomposition process. As occurred in summer, there was little or no successful breeding. The few adults of *Calliphora vicina* collected from day 22 onwards showed a male:female ratio of 15.38:84.62, far from the 50:50 characteristic of the second generations.

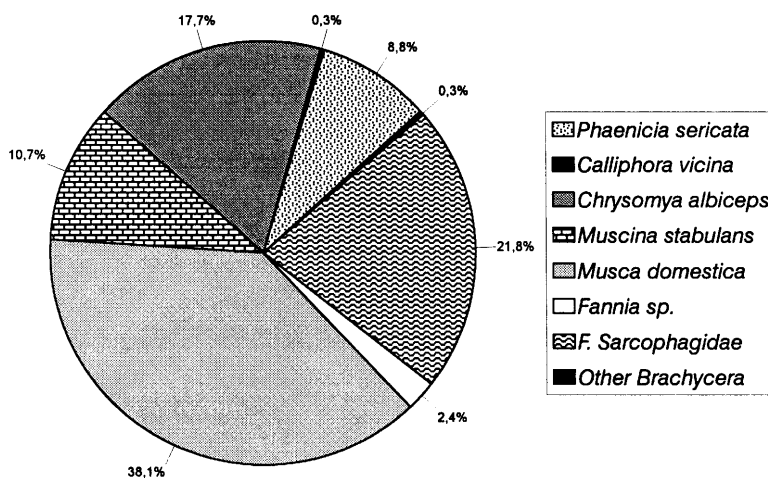
SPRING	Collect 1:1 days										Collect 1:2 days					Collect 1:3 days					Collect 1:4 days						
	Taxa	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	23	26	29	32	35	40	43	47	51	54	58
<i>Phaenicia sericata</i>	●	●	●	●	●	●	●	●	●	●					●	●	●	●	●	●	●	●	●	●	●	●	●
<i>Calliphora vicina</i>	●	●	●	●	●	●	●	●	●	●																	
<i>Chrysomya albiceps</i>																											
<i>Pollenia sp.</i>																											
<i>Muscina stabulans</i>	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●												
<i>Muscina assimilis</i>		●	●																								
<i>Musca domestica</i>	●	●	●	●	●	●	●	●	●	●																	
<i>Fannia sp.</i>	●	●	●	●	●	●	●	●	●	●																	
<i>F. Sarcophagidae</i>	●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	

Key:

≤1: (0-0,999); 1 – 10: ● (1-9,999); 10-50: ● (10-49,999); 50-100: ● (50-99,999); 100-200: ● (100-199,999); >200: ■

Fig.4 Appearance of considered taxa during spring (1:1 collected every day, 1:2 collected every other day, etc.)

Fig.5 Relative abundance of different taxa during summer expressed as percentages



SUMMER	Collect 1:1 days														Collect 1:2 days								Collect 1:3 days				1:4
	Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20	22	24	26	28	31	34	37	40	44
<i>Phaenicia sericata</i>	●	●	●	●	●																						
<i>Calliphora vicina</i>	●	●																									
<i>Chrysomya albiceps</i>	●	●	●	●	●																						
<i>Pollenia sp.</i>												●															
<i>Muscina stabulans</i>	●	●	●	●	●	●					●		●								●		●	●	●	●	●
<i>Alloeostylus sp.</i>		●																									
<i>Musca domestica</i>	●	●	●	●	●	●					●										●						
<i>Fannia sp.</i>		●	●	●	●	●																					
<i>F. Sarcophagidae</i>	●	●	●	●	●	●	●			●	●		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●

Key:

≤1: (0-0,999); 1 – 10: ● (1-9,999); 10-50: ● (10-49,999); 50-100: ● (50-99,999); 100-200: ● (100-199,999); >200: ■

Fig.6 Appearance of considered taxa during summer (1:1 collected every day, 1:2 collected every other day, etc.)

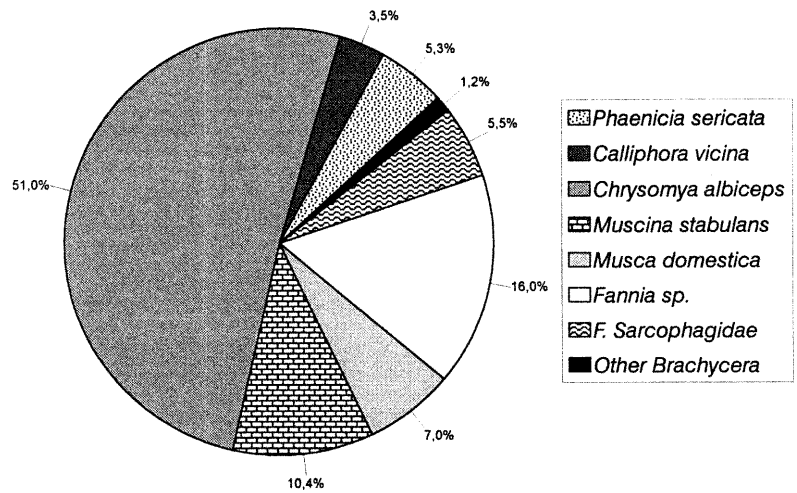
In general, the fall species were observed as adults for a long period of time, emphasizing the lengthening of the decomposition process and, as a consequence, their own capacity to feed and breed in the corpse.

In winter 11 taxa were collected (Figs.9, 10) and the dominant species was *Calliphora vicina*, which appeared in important densities from day 2 onwards. In this

season it is able to breed successfully on the corpse, and a number of post-feeding larvae were collected between days 12 and 25, with a maximum on day 21. Adults emerging from eggs laid by the first invading adults (26.26 males:73.74 females) were recorded from day 33. This generation showed a male-female ratio of 48.74: 51.26.

Only *Muscina stabulans* and *Fannia spp.* were present more or less continuously throughout the process, with the numbers falling after the migration of post-feeding

Fig. 7 Relative abundance of different taxa during fall expressed as percentages



AUTUMN	Collect 1:1 days														Collect 1:2 days								Collect 1:3 days			Collect 1:4 days		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16	18	20	22	24	26	28	30	32	35	38	41	45	49
<i>Phaenicia sericata</i>	•	•	•	•	•	•	•	•	•	•																		
<i>Calliphora vicina</i>	•	•	•	•	•	•	•			•	•						•						•	•	•			•
<i>Chrysomya albiceps</i>			•	•	•	•	•	•	•	•	•	•	•	•	•	•												
<i>Muscina stabulans</i>		•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•										
<i>Muscina assimilis</i>					•																							
<i>Muscina pabulorum</i>					•																							
<i>Musca domestica</i>			•	•	•	•	•	•		•	•	•	•	•	•	•	•						•					
<i>Alloeostylus</i>		•	•				•	•		•	•	•	•				•											
<i>Fannia sp.</i>			•	•	•	•	•	•	•	•	•	•	•	•		•	•	•	•									•
<i>F. Sarcophagidae</i>	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•									

Key:

≤1: . (0-0,999); 1-10: • (1-9,999); 10-50: ● (10-49,999); 50-100: ● (50-99,999); 100-200: ● (100-199,999); >200: ■

Fig. 8 Appearance of considered taxa during fall (1:1 collected every day, 1:2 collected every other day, etc.)

larvae. Both acted at least as secondary species, the lengthening of the decomposition process permitted a delay in the maximum of appearance.

The almost complete absence of *Sarcophagidae* in this season was noteworthy.

Discussion and conclusions

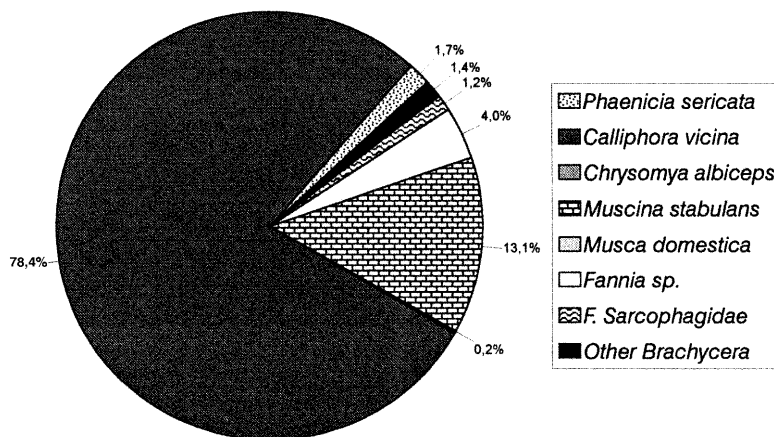
The results obtained in this study can be compared with those of Tantawi et al. [12] because of the geographical vicinity and a year long study. As in Tantawi et al. [12], the decomposition process in southeastern Spain varied with the seasons and was more rapid in spring and summer than in fall and winter. According to the Dipteran populations recorded in our experiments, corpses supported a richer community in cooler seasons but on the other hand, seasonal communities were characterised by a single fly species.

We found a close relationship between Dipteran population patterns and the seasonal period. Thus *Calliphoridae* were the dominant flies in all seasons except summer,

when they are replaced by *Muscidae*. At the species level, it is possible to characterize the different seasons. Spring was related with *Phaenicia sericata*, summer with *Musca domestica*, fall with *Chrysomya albiceps* and winter with *Calliphora vicina*. Our results showed some differences from the results of Tantawi et al. [12], as for example, in our case *Chrysomya albiceps* did not appear in spring, *Phaenicia sericata* reached its highest prevalence in spring not summer and only bred in this former season and *Sarcophagidae* did not appear as primary invaders except in summer, when the decomposition process was shortened. The differences are not surprising and can be attributed to the geographical, climatic and zoogeographical differences between the Iberian peninsula and north eastern Africa. Tantawi et al. [12], themselves, recognized that Egypt shares characteristics of both the Palaearctic and Afrotropical regions, while the Iberian peninsula, despite its particular situation, belongs only to the Palaearctic region, showing a mixture of Mediterranean and European biotic elements.

In order to estimate post-mortem intervals (PMI), the most useful species vary with the seasons where, regardless of the dominant taxa, *Calliphoridae* primary flies are the most important. A very important point to determine the PMI is knowing the moment at which the post-feeding larvae migrate. This moment varies from day 8 in spring

Fig. 9 Relative abundance of different taxa during winter expressed as percentages



WINTER	Collect 1:1 days															Collect 1:2 days										Collect 1:3 days				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	17	19	21	23	25	27	29	31	33	35	37	39	42	45	48
<i>Phaenicia sericata</i>					●	●		●				●																		
<i>Calliphora vicina</i>		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
<i>Calliphora vomitoria</i>						●		●				●																		
<i>Chrysomya albiceps</i>			●			●							●	●	●															
<i>Pollenia sp.</i>											●	●	●	●	●															
<i>Muscina stabulans</i>		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
<i>Muscina assimilis</i>													●	●	●															
<i>Muscina pabulorum</i>												●	●	●	●															
<i>Alloeostylus sp.</i>													●	●	●															
<i>Fannia sp.</i>				●	●	●	●	●				●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	
<i>F. Sarcophagidae</i>							●																							

Key:

≤1: . (0-0,999); 1 – 10: ● (1-9,999); 10-50: ● (10-49,999); 50-100: ● (50-99,999); 100-200: ● (100-199,999); >200: ■

Fig. 10 Appearance of considered taxa during winter (1:1 collected every day, 1:2 collected every other day, etc.)

to day 6 in summer, day 22 in fall and day 25 in winter, the species involved belonging to *Calliphoridae* in all cases. This fact should be taken in account in any investigation. Another very important point is the first appearance of species that cannot be detected by traps, at least not on the level of eggs or 1st instar larvae.

It may be asked whether our results can be extrapolated to human remains. In general, data obtained from

non-human corpses are used to estimate the PMI and to apply the results to investigations where human remains are involved. Previous forensic investigations support the possible extrapolation from our results to human remains because the results agree with some of the data. In this respect, the results from several actual cases from routine case work of our laboratory are shown in Table 1. In these cases, the post-mortem interval could be determined precisely by other data from the judicial inquiry. Nevertheless, some differences could be detected in the details, for example, differences in corpse size could influence the

Table 1 Data from forensic case work of our laboratory in which the date of death was estimated on the basis of entomological evidence

Case	Sex	Arthropods collected	Collected	Estimated date
1/95	Male	Puparium <i>Calliphoridae</i> died Larvae III <i>Calliphoridae</i> mummified Puparium <i>Phiophilidae</i> Larvae III <i>Phiophila foveolata</i> Larvae <i>Dermestidae</i> <i>Dermestes frischii</i> Larvae <i>Nitidulidae</i>	4-13 February 1995	1 month
2/95	Male	Eggs, larvae I, II <i>Phaenicia sericata</i> and <i>Calliphora vicina</i>	24 November 1995	3-4 days
1/96	Male	Larvae <i>Phaenicia sericata</i> and <i>Calliphora vicina</i>	28 February 1996	4 days
1/98	Female	Larvae III <i>Calliphora vicina</i>	12 January 1998	15 days
2/98	Male	Eggs and larvae <i>Calliphora vicina</i>	23 November 1998	2 days

availability of useful resources. Denno and Cothran [6] found that carcass size had a direct relationship to fly populations but, according to Anderson and VanLaerhoven [2] this only applied to extremely small carcasses, such as small rodents, lizards or toads. We have found that the general pattern of appearance of different Dipteran taxa reflected the expected pattern and matched the data available for the other comparable region studied in depth.

Insect succession in carrion, when known, can provide useful information for determining time of death. The information provided here is of interest because it gives valuable information on Dipteran carrion fauna, and the times of appearance in corpses during seasonal periods, in the Iberian peninsula.

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