Blowflies (Diptera: Calliphoridae) activity in sun exposed and shaded carrion in Portugal

Catarina Prado e Castro [1,2], José Paulo Sousa [3], María Isabel Arnaldos [4], João Gaspar [2] & María Dolores García [4]

[1] Centre for Environmental Biology, Department of Animal Biology, Faculty of Sciences, University of Lisbon, Ed. C2, Campo Grande, 1749-016 Lisbon, Portugal

[2] Centre for Forensic Science, National Institute of Legal Medicine, Portugal

[3] Life Sciences Department, Faculty of Sciences and Technology, University of Coimbra, 3001-401 Coimbra, Portugal

[4] Department of Zoology, Faculty of Biology, University of Murcia, 30100 Murcia, Spain

Abstract. The first forensic entomological study performed in Portugal is presented. Two piglet (*Sus scrofa* L.) carcasses were used to determine adult Calliphoridae activity on carrion over a period of 121 days, all along the end of spring and the summer, both in a shaded and a sunny site. Five decomposition stages were observed and a total of 10723 adult Calliphoridae, belonging to 11 species, were collected. *Calliphora vicina, Calliphora vomitoria, Chrysomya albiceps* and *Lucilia caesar* were the dominant species in this study. Decomposition was faster on the carcass exposed to the sun and the number of Calliphoridae specimens was higher there than in the shaded site. It was found a significant effect of the decomposition stage in the number of specimens attracted to the carcass, as well as a significant effect of the interaction between the decomposition stage and insolation regime. *Calliphora* and *Lucilia* species did not show preference for sunny or shaded areas. Important differences in the Calliphoridae community structure were found compared to other regions of the Iberian Peninsula, reinforcing the need of further studies in different environments and regions of this geographical area in order to collect information about the local necrophagous fauna used in forensic practice.

Résumé. Activité des mouches à viande (Diptera: Calliphoridae) sur des charognes exposées à l'ombre ou au soleil au Portugal. La première étude forensique du Portugal 'est présentée ici. Deux carcasses de marcassins (*Sus scrofa* L.) ont été utilisées pour déterminer l'activité des Calliphoridae adultes sur leur charogne durant une période de 121 jours, en fin de printemps et en été, à l'ombre ou au soleil. Cinq stades de décomposition ont été observés et un total de 10723 Calliphoridae adultes, de 11 espèces, ont été collectés. *Calliphora vicina, Calliphora vomitoria, Chrysomya albiceps* et *Lucilia caesar* ont été les espèces dominantes durant cette étude. La décomposition a été plus rapide pour la carcasse exposée au soleil et le nombre de spécimens de Calliphoridae y a été plus élevé que sur la carcasse à l'ombre. On a trouvé un effet significatif du stade d e décomposition sur le nombre de spécimens attirés, de même qu'une interaction significative entre le stade de décomposition et le régime d'insolation. Les espèces de *Calliphora* et *Lucilia* n'ont pas montré de préférence pour les endroits ensoleillés ou ombragés. Des différentes importantes ont été trouvées entre les structures de communauté des Calliphoridae en comparaison avec d'autres régions de la Péninsule Ibérique. Ceci renforce le besoin d'études plus approfondies, dans différents environnements et d'autres régions de cette zone géographique, pour collecter les informations sur les faunes locales de nécrophages utilisées dans la pratique forensique.

Keywords: Necrophagous insects, carrion decomposition, forensic entomology, faunal succession, Portugal.

During the process of decomposition, insects are attracted to the carcass in a predictable successional sequence (Payne 1965; Early & Goff 1986; Tullis & Goff 1987; Catts & Goff 1992; Richards & Goff 1997). Knowledge of the composition and dynamics of the community of entomosarcosaprophagous organisms found in a corpse, together with the knowledge of growth rates, is used to calculate the postmortem interval in forensic cases (Greenberg 1991; Catts & Goff 1992; Goff 1993; Anderson 1995; Anderson & VanLaerhoven 1996; Amendt *et al.* 2007).

Insect species associated with carrion and their times of colonization vary according many factors, one of the most important being the geographic region or biogeoclimatic zone. The biogeoclimatic zone defines the habitat, vegetation, soil type, and meteorological conditions of the area that, obviously, has a major impact on the types and species of insects present, as well as their seasonal availability (Anderson, 2010). Because of that, databases should be developed for

E-mail: cbcastro@fc.ul.pt
Accepté le 10 février 2011

every biogeoclimatic zone in which insects are being used in forensic entomology (Anderson 1995, 2010; Anderson & VanLaerhoven 1996). Season of the year, ambient temperatures and habitat are also important factors that determine the composition and structure of carrion fauna (Smith 1986). Effects of sun versus shaded habitats on decomposition and insect succession were studied and generally report that carcasses exposed to the sun decompose faster than the ones in the shade (Shean *et al.* 1993; Joy *et al.* 2006) and show different patterns of insect succession (Sharanowski *et al.* 2008).

Studies about the Diptera sarcosaprophagous community have been performed in the Iberian Peninsula (Martínez-Sánchez *et al.* 1998, 2000, 2005; Arnaldos *et al.* 2001, 2004; Castillo Miralbes 2002; García-Rojo 2004; Baz *et al.* 2007; Moneo Pellitero & Saloña Bordas 2007), however only few have used animal carcasses (Arnaldos *et al.* 2001, 2004; Castillo Miralbes 2002; García-Rojo 2004), thus being more focused in forensic entomology. Up to date, no such studies were done in Portugal, western Iberian Peninsula, with the exception of Prado e Castro (2005) and related faunistic publications (Prado e Castro *et al.* 2010a, 2010b; Prado e Castro & García 2010).

To study the composition of sarcosaprophagous fauna, inventories are usually done based on direct manual collection of the specimens present on the carcass at the moment when the investigator visits it (Reed 1958; Payne 1965; Early & Goff 1986; Anderson & VanLaerhoven 1996; Tantawi *et al.* 1996; Grassberger & Frank 2004; Matuszewski *et al.* 2008; Sharanowski *et al.* 2008). With this methodology, that most commonly includes the use of insect nets and forceps for sampling, only the fauna present in the moment of the visit is collected and it is dependent on the collector's ability and experience. The manipulation of the carcass, as well as the close contact needed may

Figure 1
The study site, a forested zone within the Botanical Garden of Coimbra city.

be a factor of disturbance to the organisms and the successional process. All these may lead to biased and incomplete inventories that do not accurately represent the sarcosaprophagous community present in a carcass (Ordóñez *et al.* 2008). These limitations can be avoided with the use of specific traps that collect all arthropod fauna that accede, develops and emerges from the carcasses. The efficiency of Schoenly (1981) trap in collecting adult sarcosaprophagous dipterans has recently been proved by Ordóñez *et al.* (2008). With this method, that has been used in few studies (Schoenly 1981; Schoenly *et al.* 1991; Arnaldos *et al.* 2001, 2004; Prado e Castro 2005; Battán Horenstein *et al.* 2007; 2010) the fauna is continuously sampled. Physical contact with the bait during sampling is not needed, diminishing the disturbance in the colonization and the collector bias. While a great variability is found when using the traditional methodologies, with significant differences between replicates, the communities collected by each of the replicates when using the Schoenly trap are very similar (Ordóñez *et al.* 2008). The trap has been confirmed to be a superior methodology for collecting adult dipterans in a decaying corpse, and recommended as the most effective device for characterizing the sarcosaprophagous succession.

Among the sarcosaprophagous Diptera, the Calliphoridae are the initial and main consumers of carrion. They are considered the most important group of flies to the forensic entomologist, and are used as indicators of the period of time since death of human cadavers (Lane 1975; Smith 1986; Greenberg 1991; Shean *et al.* 1993).

Because, up to date, a study on Calliphoridae sarcosaprophagous community has not been carried out in Portugal, the objective of this paper is the elaboration of a database for blowfly species in this country, which is a preliminary step to start forensic entomology activity in this geographical area, as well as provide other interesting information for forensic purposes.

Material and methods

Site description

Coimbra is the third Portuguese city, one of the most important urban centres of the country, with a municipality population of ≈ 157 000 and covering 319.4 km^2. The climate of the area can be defined as having Mediterranean and Atlantic influences. The experiment was performed in the Botanical Garden of the University of Coimbra, a green area in the centre of the city (Fig. 1). The study site was in an area called "Mata" (40°12'N 8°25'W), which is a forested zone inaccessible to visitors, with shrubby and arboreous vegetation, mainly of *Ailanthus altissima* (Simaroubaceae), *Laurus nobilis* (Lauraceae), *Celtis australis* (Cannabaceae), *Olea europaea* (Oleaceae), *and Eucalyptus spp*. (Myrtaceae). Two sites were chosen, 100 m apart each from other, the first one in a clearing with direct sunlight almost all the day, the other in a shaded area, due to the presence of several trees.

Carcasses and experimental procedures

Two domestic piglets, *Sus scrofa* L., of 5.1 Kg weight each, killed with a stab in the carotid, were selected as animal models. The decomposition of a pig carcass approaches the pattern of human decomposition, providing the most accurate extrapolation of results to human corpses (Catts & Goff 1992; Goff 1993; Anderson & VanLaerhoven 1996; Richards & Goff 1997) and according to Hewadikaran & Goff (1991) the patterns of arthropod succession and development rates are relatively similar for different sized carcasses.

The experiment was carried out using two modified Schoenly (1981) traps, one placed in the sunny area and the other in the shady one. The traps are dodecahedral structures constructed in plywood (Fig. 2). The base of the traps is a large mesh plastic net, so the carcasses were in direct contact with soil; the top of the traps is covered with fine mesh plastic net, which allows good aeration and odour dissipation (Prado e Castro *et al.* 2009). Each trap was baited with one freshly killed piglet. Schoenly`s trap is designed to collect adult entomofauna, theoretically capturing 50% of the arthropods attracted to the carcass, and allowing the other 50% to get inside and access the animal.

Figure 2
Schoenly trap used in the experiment.

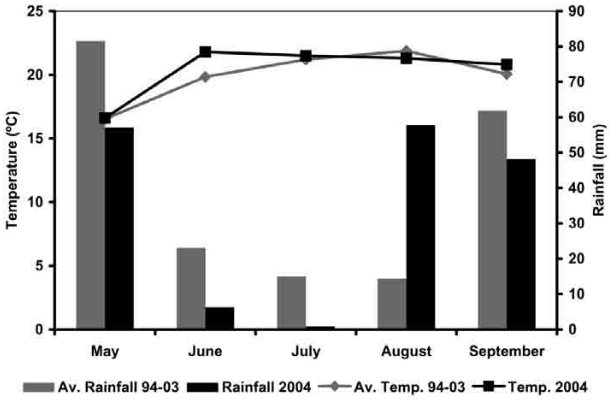


Figure 3
Ambient average monthly temperatures and average monthly rainfall in Coimbra city during the period 1994–2003 and in the year 2004 (data from the weather station).

These uncollected arthropods will eventually leave the corpse, being also captured. Similarly, generations emerging inside the trap are captured. Even though this system is designed for collecting adults, migratory larvae leaving the carcass are also efficiently captured by collector bottles that stand at ground level (Prado e Castro *et al.* 2009). A 40% ethylene glycol solution with formalin and detergent was used in the trap as killing and temporary preservative agent for the arthropods.

This study was conducted during four months, from 26 May to 24 September 2004. Spring was selected as the convenient sampling period since it has been proved to be the most diverse time of the year, at least in other regions of the Iberian Peninsula (Arnaldos *et al.* 2004). Sample collections from each trap consisted in removing the bottles with arthropods (immersed in the preservative solution) and substituting them with others filled with clean solution. This was made always at the same hour, daily in the first 30 days, and afterwards in alternate days until the end of the experiment. The date of death and placement of the pig carcasses in the traps was designated as day 0. The collections started 24 hours after, on day 1.

During each visit to the trap, general weather (e.g. sun, cloudy, rain), physical appearance of the carcass, odours, quantity and quality of species present were recorded, photographs were taken and the arthropods collected from the trap. Ambient temperatures and relative humidity were continuously registered with HOBO Data loggers (24 records/day) placed into the traps, both in sun and shade.

Adult Calliphoridae specimens were identified to species level using taxonomic identification keys (González-Mora & Peris 1988; González-Mora 1989; Peris & González-Mora 1991), except those of genus *Pollenia*. Larvae were collected by the trap mainly from days 7 to 15 and were identified based on Smith (1986) and Reiter & Wollenek (1983). Specimens were all kept in 80% ethanol.

Analysis

To compare the mean temperatures between the sunny and the shaded site, a *z*- test was used, for a 99% confidence level.

To evaluate the effect of the decomposition stage and insolation regime (sun or shade) and their interaction in the number of specimens attracted to the carcass, a two-way multivariate analysis of variance (MANOVA) with Tukey Honest Significant Difference (HSD) *post hoc* test was done. To evaluate the effect of insolation regime in each species attraction to the carcass, a one-way analysis of variance (ANOVA) was done. Statistical analyses were performed using STATISTICA software (ver. 5.0, StatSoft, Inc., Tulsa, OK).

Results

Climatic data

Average monthly air temperatures measured by the nearest weather station, 8 km from the study site (40°09'N 8°28'W), were in general similar to the normal values reported for Coimbra for this period

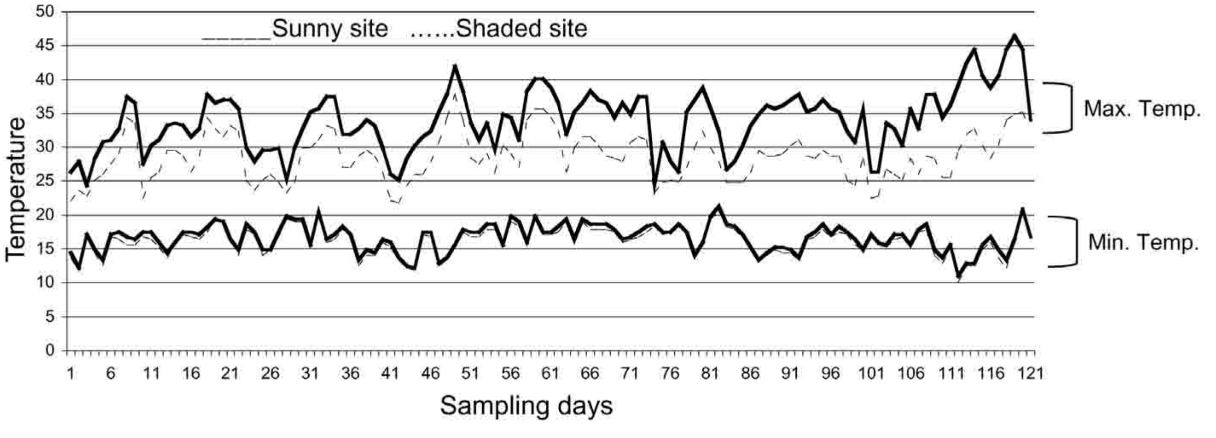


Figure 4
Ambient maximum and minimum daily temperatures in the exposed and shaded sites, measured by data loggers inside the traps.

of the year (Fig. 3). Rainfall values were very atypical for this season, with a high rain in August, resembling springtime.

The monthly mean temperatures measured by the climatic station were approximately 1 °C lower than measured in the shaded trap and 4 °C lower than in the sun.

Daily minimum temperatures were similar in both sites, contrary to maximum temperatures that were higher in the sunny site (Fig. 4). For the duration of the experiment, the mean temperature at the shaded site was 22.4 °C. The mean temperature in the sun exposed site was 25.3 °C, which was significantly different from the shaded site ($F = 1.35$; $df = 120$; $p = 0.01$).

Table 1. Total Diptera specimens collected in sun and shaded sites.

Family	Species	Total number	
		Sun	Shade
Anthomyiidae	*Anthomyia sp.*	379	5243
	Other Anthomyidae	11	14
Calliphoridae	*Calliphora vicina*	1016	1277
	Calliphora vomitoria	1253	1117
	Chrysomya albiceps	2273	920
	Lucilia ampullacea	372	238
	Lucilia caesar	1055	726
	Lucilia illustris	1	7
	Lucilia sericata	278	156
	Lucilia silvarum	1	1
	Pollenia sp.	3	7
	Protophormia terranovae	6	6
	Stomorhina lunata	3	7
Carnidae		248	219
Fanniidae	*Fannia sp.*	564	3941
Heleomyzidae		3	7
Lauxaniidae		15	17
Muscidae	*Hydrotaea capensis*	17	38
	Hydrotaea ignava	2044	8611
	Hydrotaea sp.	673	854
	Musca domestica	19	14
	Muscina sp.	503	1522
	Phaonia subventa	72	71
	Other Muscidae	14	37
Phoridae		75	132
Piophilidae		100	369
Platystomatidae	*Platystoma sp.*	18	76
Rhinophoridae		18	7
Sarcophagidae		419	574
Sphaeroceridae		332	169
Other Brachycera families		37	61
Nematocera families		211	123
Totals		**12033**	**26561**

Decomposition stages

Five decomposition stages were identified as described in Anderson & VanLaerhoven (1996): fresh, bloated, active decay, advanced decay, and dry/ remains.

The fresh stage began at the moment of death and lasted until day 2, both in sunny and shaded sites. On day 3, bloating and odour were evident in the carcass exposed to the sun, while in the one in the shade, odour and only a slight bloat was noticeable. This indicated that putrefaction had begun, so in both sites bloated stage was considered to begin on the 3rd day. The carcass in the sun was very inflated on day 4 and with very strong odour, whereas the carcass in the shade only on day 6 was with equivalent aspect and odour was never so intense as in the sun. Deflation of both carcasses due to Calliphoridae larvae breaking the skin started on day 7, representing the active decay stage. Dipterous larvae began to migrate massively from the carcasses on day 8 in both sites, marking the beginning of advanced decay stage. The migration lasted approximately one week, with the number of larvae collected being higher in the shade. Biomass loss, evaluated visually, was much quicker in the carcass exposed to the sun than in the one in the shade. Dry stage was reached on day 15 in the carcass exposed to the sun, and only on day 42 in the shaded carcass, although the frequent rains re-hydrated the carcasses, attracting more flies.

Species composition and abundance

A total of 38594 adult Diptera were collected during the 121 days of the experiment, including 12033 captured in the sunny site and 26561 collected in the shaded one (Tab. 1). Of these, 10723 belong to the Calliphoridae family, with 6261 individuals collected

Table 2. Total Calliphoridae specimens collected in sun and shaded sites.

Species	SUN			SHADE			Total
	♀	♂	♀♂	♀	♂	♀♂	
Calliphora vicina	600	416	1016	757	520	1277	2293
Calliphora vomitoria	694	559	1253	723	394	1117	2370
Chrysomya albiceps	1555	718	2273	716	204	920	3193
Lucilia ampullacea	291	81	372	207	31	238	610
Lucilia caesar	963	92	1055	689	37	726	1781
Lucilia illustris	1	0	1	7	0	7	8
Lucilia sericata	254	24	278	145	11	156	434
Lucilia silvarum	1	0	1	1	0	1	2
Pollenia sp.	3	0	3	7	0	7	10
Protophormia terraenovae	2	4	6	1	5	6	12
Stomorhina lunata	3	0	3	5	2	7	10
Totals			6261			4462	10723

in the sun and 4462 in the shade (Tab. 2). Information on Sarcophagidae and Piophilidae species present is available in Prado e Castro et al. (2010b) and Prado e Castro & García (2010). Approximately 15000 Calliphoridae larvae were also collected.

Eleven calliphorid species were identified: *Calliphora vicina* Robineau-Desvoidy 1830; *Calliphora vomitoria* (L. 1758); *Chrysomya albiceps* (Wiedemann 1819); *Lucilia ampullacea* Villeneuve 1922; *Lucilia caesar* (L. 1758); *Lucilia illustris* (Meigen 1826); *Lucilia sericata* (Meigen 1826); *Lucilia silvarum* (Meigen 1826); *Pollenia sp.* Robineau-Desvoidy 1830; *Protophormia terraenovae* (Robineau-Desvoidy 1830) and *Stomorhina lunata* (Fabricius 1805).

All the species were present both in sunny and shaded sites. In general, a higher number of specimens were collected in the sunny site than in the shaded one, particularly those from *C. albiceps.* The exception was *C. vicina*, which was slightly more abundant in the shade.

Occurrence of the species in the sunny and shaded sites in the first 2 weeks is shown in percentages in fig. 5. In both sites *C. vomitoria* was the species most attracted to the corpses, followed by *L. caesar*, *C. vicina, C. albiceps*, *L. ampullacea* and *L. sericata. Lucilia illustris, L. silvarum, P. terranovae, Pollenia sp.* and *S. lunata* were present in very low numbers, so they were grouped as "other". *Calliphora vomitoria* had a higher relative abundance in the shade, while *L. caesar* and *L. sericata* were more represented in the sun.

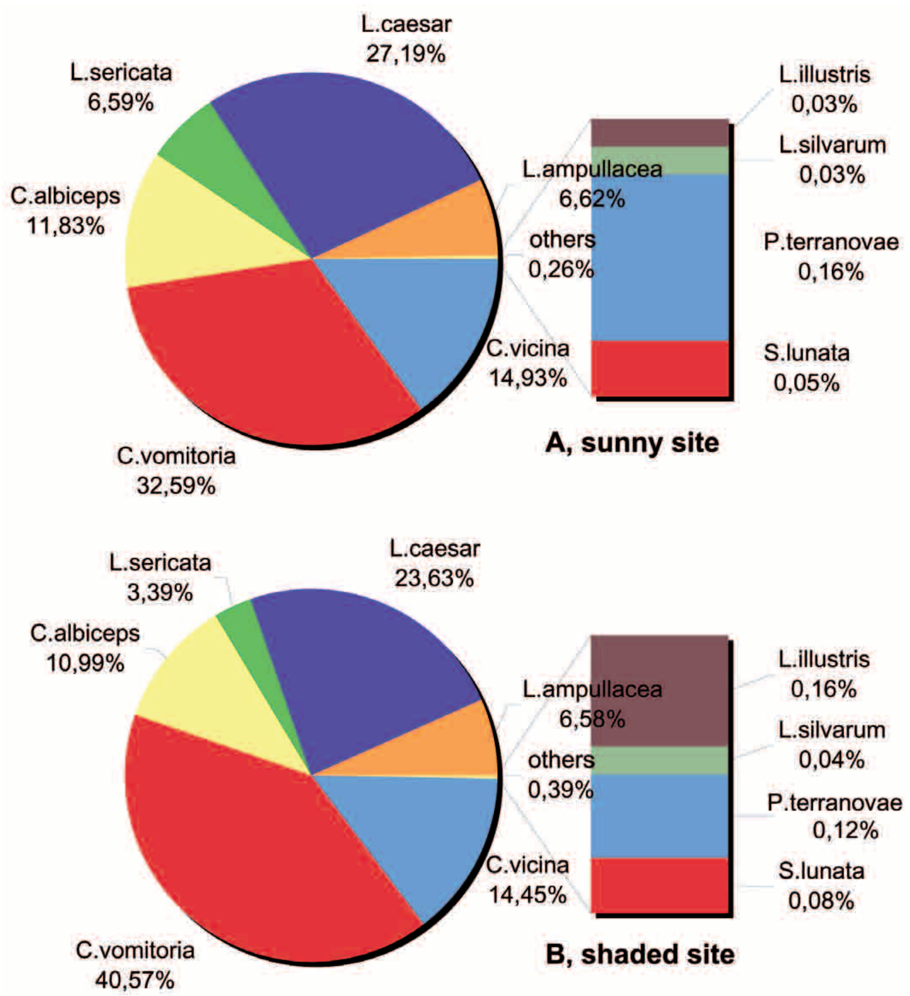


Figure 5
Relative abundance of Calliphoridae species present during the first 2 weeks; **A,** sunny site; **B,** shaded site. The boxes on the right give the detail for the others.

Community structure

The visitation pattern of Calliphoridae throughout the sampling period was different in the exposed and shaded sites as it is shown on Tab. 3 and 4.

As in the study of Goddard & Lago (1985), the carcass in the shaded site attracted fewer flies than the one in the exposed site, in the first days, while in more advanced stages of decomposition the opposite happened, with much more flies being attracted to the carcass in the shade.

Calliphora vicina appeared firstly on day 1 in sunny site and on day 2 on the shaded one. The number of specimens reached its highest peak on day 4 in the exposed site, and day 14 in the shaded one. This species was present during all the sampling period, both in sun and shade.

Calliphora vomitoria arrived to the corpse on the first day in the shaded site and stayed for a longer period of time than in the sunny site, where it was collected on the second day. The visitation pattern in the first couple of days was similar on both sites. After this period, a massive number of individuals appeared in the sunny site, disappearing almost completely after 10 days. In the shaded site the presence was more homogeneous and lasted longer (until day 32).

Chrysomya albiceps showed its typical characteristic of secondary species (Meskin 1986; Tantawi *et al.* 1996; Arnaldos *et al.* 2001), appearing on the third day in the sunny and on the fourth day in the shaded site. At the sunny site, the first generation of this species was confined essentially to the first week, where a conspicuous peak could be observed; in the shaded site, the presence of this species was prolonged, but usually with discrete abundance.

Lucilia ampullacea arrived on the second day to both carcasses and was abundant during the first week, especially in the sun. It was present, although scarcely, at both habitats, almost until the end of the experiment.

Table 3. Adult Calliphoridae species trapped during the sampling period, in the sunny site. Key : 0–1 ind.: · ; 2–10 ind.: • ; 11–50 ind.: ● ; 51–100 ind.: ⬤ ; 101–200 ind.: ⬤ ; 201–500 ind.: ■ ; >500 ind.: ■. Differences in shading represent different decomposition stages. After day 30, the symbols indicate average number of specimens per day.

SUN	Collect 1:1 days																													
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
C.vicina	·	●	●	⬤	⬤	●	●	•	•	·	·	·	·	·	·	·	·	·	•	·	•	•	•	•	•	•	•	·	·	·
C.vomitoria		•	●	■	■	■	■	●	·	·								·												
C.albiceps			·	●	●	●	⬤	•	•	·	·				·	·	•	●	⬤	■	■	·			·	·		·	·	·
L.ampullacea		⬤	⬤	•	·	·	·									·	·	·	·	·	·	·	·	·	·	·	·	·		·
L.caesar		⬤	■	■	■	⬤	●	•								·					·			·					·	
L.illustris				·																										
L.sericata	·	·	•	•	●	●	•	•	·	·			·				·							·	·		·	·	·	·
L.silvarum						·																								
Pollenia sp.																														
P.terranovae						·	·	·		·																				
S.lunata										·					·															·

SUN	Collect 1:2 days												Coll. 1:3 days			Collect 1:4 days				Collect 1:5 days				Coll. 1:7 days		
Taxa	32	34	36	38	40	42	44	46	48	50	52	54	57	60	63	67	71	75	79	84	89	94	99	106	113	121
C.vicina	·	•	·	•	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·		·	·	·	·	·	
C.vomitoria				·																						
C.albiceps	·	·	·	·		·	·	·					·						·							
L.ampullacea		·	·	·	·	·	·	·	·	·	·	·	·	·		·								·		·
L.caesar	·	·					·		·				·													
L.illustris																										
L.sericata	·	·	·	·			·											·						·		
L.silvarum																										
Pollenia sp.																			·		·					
P.terranovae																										
S.lunata																										

Lucilia caesar arrived on day 2 in both sites, but in much higher numbers in the sun, from where it practically disappeared on day 9. The largest number of specimens in the shade occurred on day 4, the species being present until day 36.

Lucilia sericata had a regular appearance in the first days in the sunny site, being present in large numbers on days 5 and 6; in the shaded site it was present throughout the experiment.

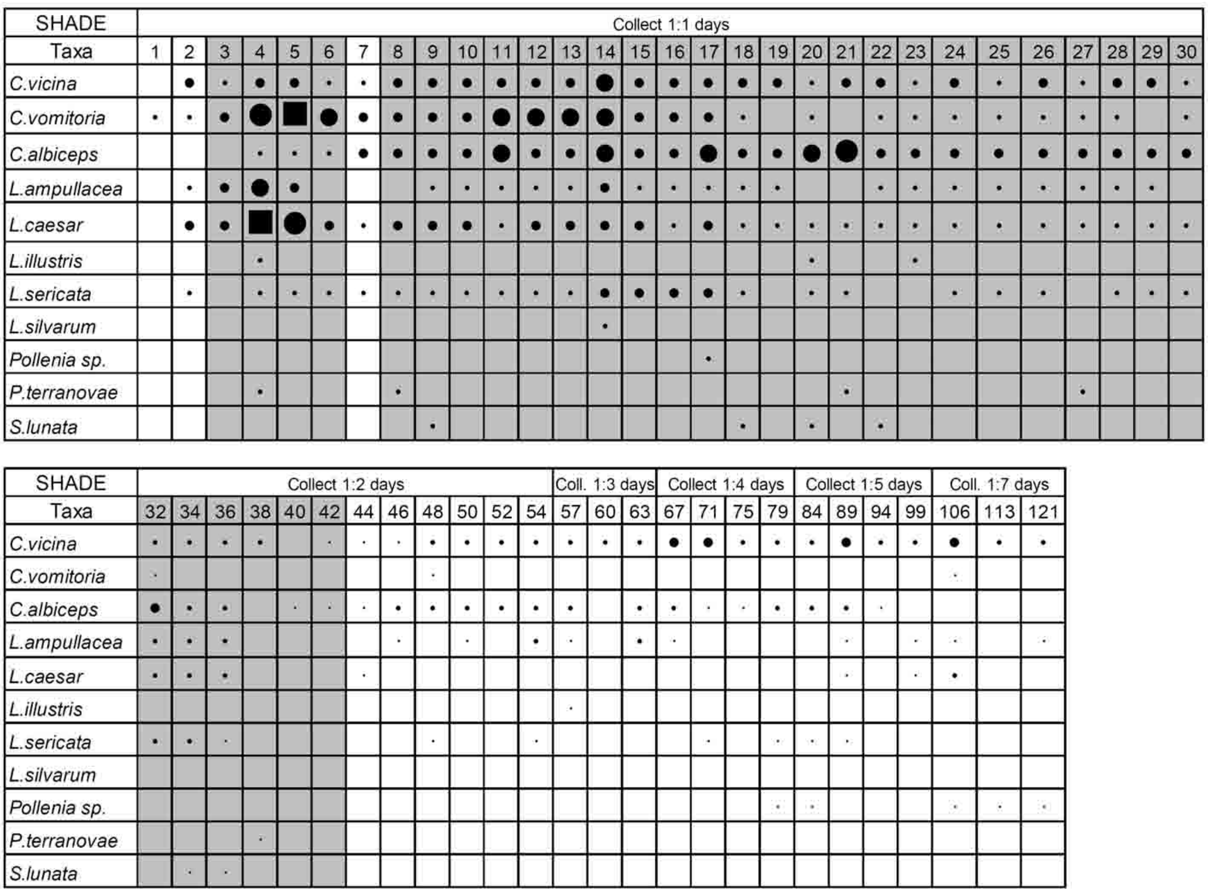
The presence of the other species was irregular and scarce on the carcasses.

All species took more time to reach large numbers in the shade, compared to the sun. The carcass in the shaded site remained attractive to the species in the advanced decay stage, which evidences a slower decomposition process. In the sunny site we can clearly define a particular moment in which the majority of the post-feeding larvae migrated, observed on day 8 and 9 of the experience, allied to a change in the aspect of the carcass. In the shaded site the larval migration was more prolonged, observed mainly between day 6 and 11. In this case the carcass remained slowly decomposing, with some larval activity and a considerable number of adult flies present.

Even though the primary objective was to study adult community, 3rd instar post-feeding larvae were also collected, of which nearly 10% were identified, revealing the presence of: *C. vomitoria* (day 7- shade; day 8- sun), *C. albiceps* (day 7- shade; day 8 to 15- sun), *L. ampullacea* (days 7 and 9- shade), *L. caesar* (days 7, 8, 9 and 13- shade; day 8- sun) and *L. sericata* (day 8- sun).

In the sunny site, for *C. vicina*, *C. albiceps*, *L. ampullacea* and *L. sericata*, two peaks of abundance were observed (Tab. 3). The first correspond mainly to flies that were attracted to the carcass to oviposit, in the initial stages of decomposition. The second peak is the emergence of flies that developed from the eggs laid.

Table 4. Adult Calliphoridae species trapped during the sampling period, in the shaded site. Key : 0–1 ind.: • ; 2–10 ind.: ● ; 11–50 ind.: ● ; 51–100 ind.: ● ; 101–200 ind.: ● ; 201–500 ind.: ■ ; >500 ind.: ■. Differences in shading represent different decomposition stages. After day 30, the symbols indicate average number of specimens per day.



These second generations reared on the carcass showed a close to 1:1 (male: female) sex-ratio, differently to that of the specimens attracted initially, that were mainly females. From day 15 onwards the second generation of the six more abundant species started to emerge. This was particularly obvious for *C. albiceps* that were captured, from day 15 to 22, by the trap exposed to the sun. In the shade the same has happened for this species, but it was not so clear, probably because of the lower number of specimens and constant presence.

Statistical analysis

The six more abundant species (*C. vomitoria*, *L. caesar*, *C. vicina, C. albiceps*, *L. ampullacea* and *L. sericata*), correspond to 99.77% and 99.58% of the total number of individuals captured in the first 2 weeks, respectively in the sun and in the shade. They were analysed for this period of time that comprises the fresh, bloated, active and advanced decay stages.

Considering the overall number of specimens, a significant effect of the decomposition stage was found ($F = 8.4$, $p \leq 0.001$), whereas the insolation regime induced no significant differences in this parameter ($F = 1.7$, $p \geq 0.05$). However, a significant interaction between decomposition stages and insolation regime was observed ($F = 3.0$, $p \leq 0.05$).

The highest number of specimens was collected in the bloated stage, in the carcass exposed to the sun (Fig. 6). This number was significantly higher than the number collected in the other decomposition stages in the same carcass ($p < 0.01$ for all). Specimens were collected in lower number in the shade, compared to the sun, with the exception of advanced decay stage, where the average number of individuals collected in the shade surpasses the number collected in the sun.

No significant differences were found, in none of the species, in the number of individuals attracted to the sunny or to the shaded site ($p \geq 0.3$ for all).

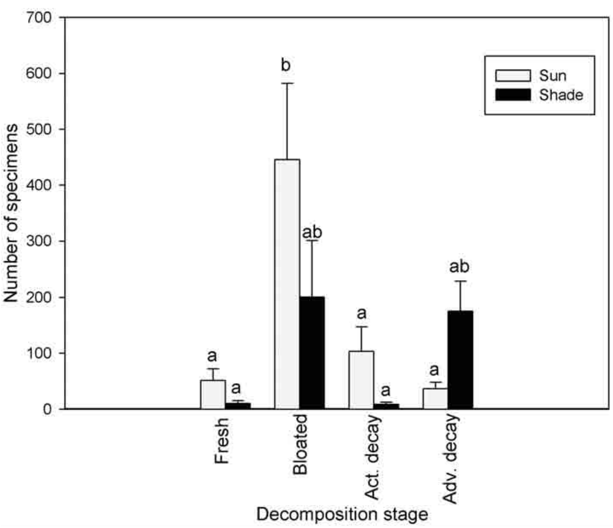


Figure 6
Number of Calliphoridae specimens collected in the first 2 weeks, in the different decomposition stages (fresh, bloated, active decay and advanced decay) in the carcasses exposed to different insolation regimes (sun and shade). Values presented are daily means and standard error. Different letter represent significant differences.

Discussion

As in previous studies by Shean *et al.* (1993), Joy *et al.* (2006), Sharanowski *et al.* (2008), where the effect of sun vs. shade was analysed, differences in decomposition rates were observed between the two carcasses. Daily observation in the field suggested that the decomposition stages were progressing at different rates, much slower in the shady site; as an example of this, bloating was more advanced in the sunlit carcass, similarly to what was observed by Shean *et al.* (1993) and Joy *et al.* (2006). In the shaded carcass the swelling occurred slowly, with about 2 days delay relatively to the carcass placed in the sun. A closer analysis of the characteristics and key-moments that marked the progression from one stage of decomposition to another (odours, bloating, perforation of skin by larvae, migration and dryness) showed that they were simultaneous in the initial stages, in sunny and shaded site, but with differences occurring in later stages. The advanced decay stage lasted longer in the shaded site, dry stage starting later than in the sunny site. The higher temperatures in the exposed site, due to direct sunlight, intensified the odour, attracting a large number of flies to oviposit on the corpse. The exposure of the growing larvae to the sun may have increased their metabolic rate, which accelerated the decomposition process and the apparently observed loss of biomass.

Consistent with Shean *et al.* (1993), the arrival of flies and oviposition started essentially at the same time in both piglets, but continued for a longer period of time on the shaded carcass, due to the prolonged availability of suitable carrion.

Considerable differences between Calliphoridae succession dynamics in both sites were noticed. In the sunny site, Calliphoridae were the main and quicker flesh consumers, arriving in massive numbers in the first week, breeding on the cadaver and voraciously consuming it. In the shade, the same species were present in the first week, but in lower numbers compared to the sun. As decomposition progressed, the opposite happened, with large number of individuals present in the shaded site. The slower decomposition process in the shade and the continuous availability of carrion allowed other families, as Muscidae, Fanniidae and Anthomyiidae, among other Diptera, to appear in very large numbers. In the sun, most of the other dipterans were less abundant, as dry stage was reached earlier and less carrion was left to consume.

Relatively to the number of Calliphoridae specimens attracted to the carcasses, despite the clear differences observed between sun and shade conditions, there were no significant differences between them. In the first three decomposition stages, the carcass exposed to the sun had a higher number of specimens than in the shade. But an inverse trend occurred in the last decomposition stage, leading to a significant interaction effect between decomposition stage and insolation regime.

From a faunistic point of view, it is worth of mention that 9 species previously unrecorded to continental Portugal (Martínez-Sánchez *et al.* 2002) were collected: *C. vicina, C. vomitoria, C. albiceps, L. ampullacea, L. caesar, L. illustris, L. sericata, L. silvarum* and *P. terraenovae* (Prado e Castro 2005, Prado e Castro *et al.* 2010). These references enlarge the knowledge on Diptera in the country and constitute a solid basis for the establishment of a database for forensic purposes.

From a forensic entomological point of view, during this study, *C. vicina, C. vomitoria, C. albiceps, L. ampullacea, L. caesar* and *L. sericata* were clearly predominant in relation to the other species present on the corpses, both in sun and shade. Post-feeding larvae and second generations collected reveal that these six species are able to breed on corpses in this time of the year, in Portugal. The presence of the same species was recently reported from larvae collected in human corpses in the north of Portugal (Cainé *et al.* 2009).

The high numbers of *C. albiceps* adults caught in the sun exposed trap is explained by a second generation that emerged inside the trap. It was observed that this species mainly pupated over the carcass and near it; consequently, the trap captured almost all the adults emerged. However, the other Calliphoridae species showed an active migratory behaviour, their post feeding larvae moving away from the corpse. Thus, the trap could collect part of these larvae, and another part could get outside the trap to pupate. For this reason, the adults that emerged inside the trap were very low numbered compared to *C. albiceps*. Even though 3rd instar larvae of *C. albiceps* are predatory upon other larvae (Zumpt 1965), we believe that in this case, the absence of abundant second generations of the other species is mainly due to larval migration, since migrating larvae were captured in very large quantities.

Calliphora vomitoria is considered more rural in its distribution than *C. vicina*, and when the two species are together, usually is less abundant (Greenberg 1971; Smith 1986). This was not observed in this experiment, where *C. vomitoria* was more abundant than *C. vicina*. The large green area in which the experiment was performed, creating an island in the middle of the urban center, may explain its marked presence and agrees with previous observations from Grassberger & Frank (2004).

Calliphora species are generally described as preferring shade habitats, while *Lucilia* prefers to be in the sunlight (Smith 1986). In the present study, all the species of the two genera were more numerous in the sun, except *C. vicina* that was slightly more represented in the shade. However, even this species was extremely abundant in the sun. During the first weeks of exposure, *C. vomitoria* was more represented in the shade, however it was simultaneously the most abundant species in the sun. *Calliphora vicina* and *L. ampullacea* had similar relative abundances in both habitats. *Lucilia caesar* and *L. sericata* were slightly more represented in the sun. These calliphorids did not show evident preferences for sunny or shaded areas, as it was demonstrated in our results, with no significant differences in the number of individuals, from each species, attracted to each carcass.

In most of the studies performed in the Iberian Peninsula, with variable methodologies, for the same time of the year, *L. sericata* is a very dominant species (Martínez-Sánchez *et al.* 2000, 2005; Arnaldos *et al.* 2001; Castillo Miralbes 2002; García-Rojo 2004). In the present study, in central Portugal, from the group of six species considered dominant in the carcass, *L. sericata* was the least abundant. *Lucilia caesar*, a very dominant species in this study, has been reported to be present abundantly in the north of Spain (Moneo Pellitero & Saloña Bordas 2007), but in low numbers in the west and centre (Martínez-Sánchez *et al.* 1998, 2000; García-Rojo 2004; Baz *et al.* 2007) and absent in northeast and southeastern Spain (Arnaldos *et al.* 2001; Castillo Miralbes 2002). *Lucilia ampullacea*, that is a well represented calliphorid in Portugal, has not been reported in any of the previously referred studies in the Iberian Peninsula, with the exception of Moneo Pellitero & Saloña Bordas (2007). *Calliphora vomitoria*, similarly to this experiment, has been found very abundantly in central Spain (Baz *et al.* 2007), even though very different methodologies were used. *Calliphora vicina*, not having been collected in southeastern Spain at this time of the year (Arnaldos *et al.* 2001) is a common species throughout the Iberian Peninsula (Martínez-Sánchez *et al.* 2000; García-Rojo

2004; Baz *et al.* 2007), as *C. albiceps*, which is generally very abundant in this geographical region.

These data reveal important differences on sarcosaprophagous community structure and dynamics depending on local bioclimatic characteristics in the Iberian Peninsula. For forensic purposes, the availability of regional data is extremely important for the correct interpretation of casework. All these results reinforce the need of further studies on sarcosaprophagous fauna in different environments and regions of this geographical area, as well as in other seasons of the year, to understand the sarcosaprophagous community under different environmental conditions. The information on blowfly community obtained in this work, even though only covering spring and summer periods, is valuable for future applications, in the evaluation of evidence obtained in forensic cases, similarly to Arnaldos *et al.* (2005) that have used baseline data collected with the same methodologies as described in this study. Moreover, the data presented are the first in the country, precluding the use of foreign studies in favour of the regional data, now available.

Acknowledgements. The authors would like to thank the following entities: University of Coimbra Botanical Garden, for allowing the realization of the field work and Ministerio de Asuntos Exteriores, Agencia Española de Cooperación Internacional of the Spanish Government for the MAE-AECI grant provided to CPC, which supported part of this work.

References

Amendt J., Campobasso C.P., Gaudry E., Reiter C., LeBlanc H.N., Hall M.J.R. 2007. Best practice in forensic entomology - standards and guidelines. *International Journal of Legal Medicine* **121**: 90-104.

Anderson G.S. 1995. The use of insects in death investigations: an analysis of cases in British Columbia over a five year period. *Canadian Society of Forensic Science Journal* **28**: 277-292.

Anderson G.S. 2010. Factors that influence insect succession in carrion, p. 201-250 *in*: **Byrd J.H., Castner J.L. (eds.)**, *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. 2nd edition. CRC Press, Taylor & Francis Group, Boca Raton.

Anderson G.S., VanLaerhoven S.L. 1996. Initial studies on insect succession on carrion in southwestern British Columbia. *Journal of Forensic Sciences* **41**: 617-625.

Arnaldos I., Romera E., García M.D., Luna A. 2001. An initial study on the succession of sarcosaprophagous Diptera (Insecta) on carrion in the southeastern Iberian Peninsula. *International Journal of Legal Medicine* **114**: 156-162.

Arnaldos M.I., Romera E., Presa J.J., Luna A., García M.D. 2004. Studies on seasonal arthropod succession on carrion in the southeastern Iberian Peninsula. *International Journal of Legal Medicine* **118**: 197-205.

Arnaldos M.I., García M.D., Romera E., Presa J.J., Luna A. 2005. Estimation of postmortem interval in real cases based on experimentally obtained entomological evidence. *Forensic Science International* **149**: 57-65.

Battán Horenstein M., Linhares A.X., Rosso B., García M.D. 2007. Species composition and seasonal succession of saprophagous calliphorids in a rural area of Córdoba, Argentina. *Biological Research* **40**: 163-171.

Battán Horenstein M., Linhares A.X., Rosso B., García M.D. 2010. Decomposition and dipteran succession in pig carrion in central Argentina: ecological aspects and their importance in forensic science. *Medical and Veterinary Entomology* **24:** 16-25.

Baz A., Cifrián B., Díaz-Aranda L.M., Martín-Vega D. 2007. The distribution of adult blow-flies (Diptera: Calliphoridae) along an altitudinal gradient in Central Spain. *Annales de la Société entomologique de France* (n.s.) **43**: 289-296.

Cainé L.M., Corte Real F., Saloña-Bordas M.I., Martínez de Pancorbo M., Lima G., Magalhães T., Pinheiro F. 2009. DNA typing of Diptera collected from human corpses in Portugal. *Forensic Science International* **184**: e21-e23.

Castillo Miralbes M. 2002. Estudio de la entomofauna asociada a cadáveres en el Alto Aragón (España). *Monografías SEA* **6**: 1-94.

Catts E.P., Goff M.L. 1992. Forensic entomology in criminal investigations. *Annual Review of Entomology* **37**: 253-272.

Early M., Goff M.L. 1986. Arthropod succession patterns in exposed carrion on the island of Oahu, Hawaiian Islands, USA. *Journal of Medical Entomology* **23**: 520-531.

García-Rojo A.M. 2004. Estudio de la sucesión de insectos en cadáveres en Alcalá de Henares (Comunidad Autónoma de Madrid) utilizando cerdos domésticos como modelos animales. *Boletín Sociedad Entomologica Aragonesa* **34**: 263-269.

Goddard J., Lago P.K. 1985. Notes on blow fly (Diptera, Calliphoridae) succession on carrion in Northern Mississippi. *Journal of Entomological Science* **20**: 312-317.

Goff M.L. 1993. Estimation of postmortem interval using arthropod development and sucessional patterns. *Forensic Science Review* **5**: 81-94.

González-Mora D. 1989. Los Calliphoridae de España, II: Calliphorini (Diptera). *Eos* **65**: 39-59.

González-Mora D., Peris S. V. 1988. Los Calliphoridae de España: 1: Rhiniinae y Chrysomynae (Diptera). *Eos* **64**: 91-139.

Grassberger M., Frank C. 2004. Initial study of arthropod succession on pig carrion in a central European urban habitat. *Journal of Medical Entomology* **41**: 511-523.

Greenberg B. 1971. *Flies and Disease, Volume I: Ecology, Classification and Biotic Associations*. Princeton University Press, New Jersey, 856 p.

Greenberg B. 1991. Flies as forensic indicators. *Journal of Medical Entomology* **28**: 565-577.

Hewadikaram K.A., Goff M.L. 1991. Effect of carcass size on rate of decomposition and arthropod succession patterns. American Journal of Forensic Medicine and Pathology **12**: 235-240.

Joy J.E., Liette N.L., Harrah H.L. 2006. Carrion fly (Diptera: Calliphoridae) larval colonization of sunlit and shaded pig carcasses in West Virginia, USA. *Forensic Science International* **164**: 183-192.

Lane R.P. 1975. Investigation into blowfly (Diptera-Calliphoridae) succession on corpses. *Journal of Natural History* **9**: 581-588.

Martínez-Sánchez A.I., Rojo S., Rognes K., Marcos-García M.A. 1998. Califóridos con interés faunístico en agroecosistemas de dehesa y catálogo de las especies ibéricas de Polleniinae (Diptera: Calliphoridae). *Boletín Asociación de española de Entomología* **22**: 171-183.

Martínez-Sánchez A., Rojo S., Marcos-García M.A. 2000. Annual and spatial activity of dung flies and carrion in a Mediterranean holm-oak pasture ecosystem. *Medical And Veterinary Entomology* **14**: 56-63.

Martínez Sánchez A., Rognes K., Báez M. 2002. Calliphoridae, p. 204-205 *in* **Hjorth-Andersen M.C.T. (ed.)**, *Catálogo de los Diptera de España, Portugal y Andorra (Insecta)*. Monografías SEA 8, Zaragoza.

Martínez-Sánchez A., Marcos-García M.A., Rojo S. 2005. Biodiversidad de la comunidad de dípteros sarcosaprófagos en ambientes insulares del sudeste Ibérico (Diptera: Calliphoridae, Muscidae, Sarcophagidae). *Nouvelle Revue d'Entomologie* **22**: 251-265.

Matuszewski S., Bajerlein D., Konwerski S., Szpila K. 2008. An initial study of insect succession and carrion decomposition in various forest habitats of Central Europe. *Forensic Science International* **180**: 61-69.

Meskin I. 1986. Factors affecting the coexistence of blowflies (Diptera: Calliphoridae) on the Transvaal Highveld, South Africa. *South African Journal of Science* **82**: 244-250.

Moneo Pellitero J., Saloña-Bordas M.I. 2007. Califóridos (Diptera, Calliphoridae) de interés forense recogidos en el entorno universitario del campus de Leioa (Vizcaya, España). *Boletín de la Sociedad Entomológica Aragonesa* **40:** 479-483.

Ordóñez A., García M.D., Fagua G. 2008. Evaluation of efficiency of Schoenly trap for collecting adult sarcosaprophagous dipterans. *Journal of Medical Entomology* **45**: 522-532.

Payne J.A. 1965. A summer carrion study of the baby pig *Sus scrofa* Linnaeus. *Ecology* **46**: 592-602.

Peris S.V., González-Mora D. 1991. Los Calliphoridae de España, III: Luciliini (Diptera). *Boletín de la Real Sociedad Española de Historia Natural* (Sección Biológica) **87**: 187-207.

Prado e Castro C.B. 2005. *Studies on Sarcosaprophagous Diptera (Insecta) in Central Portugal: Application to Forensic Entomology*. M.Sc. thesis, Universidade de Coimbra, Coimbra, 97 p.

Prado e Castro C., Arnaldos M.I. , García M.D. 2010a. Additions to the Calliphoridae (Diptera) fauna from Portugal, with description of new records. *Boletín Asociación española de Entomología* **33** (3-4) (2009): 425-437.

Prado e Castro C., Chichorro D., Serrano A., García M.D. 2009. A modified version of Schoenly trap for collecting sarcosaprophagous arthropods. Detailed plans and construction. *Anales de Biología* **31**: 1-6.

Prado e Castro C., García M.D. 2010. Additions to the Piophilidae (Diptera) fauna from Portugal, with new records. *Graellsia* **66:** 101-105.

Prado e Castro C., García M.D., Arnaldos M.I., González-Mora D. 2010b. Sarcophagidae (Diptera) attracted to piglet carcasses including new records for Portuguese fauna. *Graellsia* **66:** 285-294.

Reed H.B. 1958. A study of dog carcass communities in Tennessee, with special reference to the insects. *The American Midland Naturalist* **59**: 213-245.

Reiter C., Wollenek G. 1983. Zur Artbestimmung der Maden forensisch bedeutsamer Schmeissfliegen. *Zeitschrift fur Rechtsmedizin* **90**: 309-316.

Richards E.N., Goff M.L. 1997. Arthropod succession on exposed carrion in three contrasting tropical habitats on Hawaii Island, Hawaii. *Journal of Medical Entomology* **34**: 328-339.

Schoenly K. 1981. Demographic bait trap. *Environmental Entomology* **10**: 615-617.

Schoenly K., Griest K., Rhine S. 1991. An Experimental Field Protocol For Investigating The Postmortem Interval Using Multidisciplinary Indicators. *Journal of Forensic Sciences* **36**: 1395-1415.

Sharanowski B.J., Walker E.G., Anderson G.S. 2008. Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons. *Forensic Science International* **179**: 219-240.

Shean B.S., Messinger L., Papworth M. 1993. Observations of differential decomposition on sun exposed v. shaded pig carrion in coastal Washington-State. *Journal of Forensic Sciences* **38**: 938-949.

Smith K.G.V. 1986. *A Manual of Forensic Entomology*. The Trustees of the British Museum (Natural History), London, 205 p.

Tantawi T.I., Elkady E.M., Greenberg B., ElGhaffar H.A. 1996. Arthropod succession on exposed rabbit carrion in Alexandria, Egypt. *Journal of Medical Entomology* **33**: 566-580.

Tullis K., Goff M.L. 1987. Arthropod succession in exposed carrion in a tropical rain-forest on Oahu island, Hawaii. *Journal of Medical Entomology* **24**: 332-339.

Zumpt F. 1965. *Myiasis in man and animals in the old world*. Butterworths, London, 267 p.