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Early colonisation of urban indoor carcasses by blow flies (Diptera: Calliphoridae): an experimental study from central Spain

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Highlights

- Blow flies can colonise indoor carcasses within 24–48 h after death in every season.
- Primary and secondary coloniser species are identified.
- Species composition and contribution to carrion colonisation differ among seasons.
- The mouth was the preferred oviposition site for the first colonisers.

Abstract

Due to their ubiquity and synanthropy, blow flies (Diptera: Calliphoridae) are generally the first colonisers of cadavers and, therefore, frequently used to estimate a minimum post-mortem interval ($_{min}$PMI). Whereas in outdoor situations blow flies are expected to locate and colonise exposed cadavers within hours or even minutes after death, it is usually assumed that the colonisation of a cadaver indoors might be delayed for an uncertain period of time. This uncertainty severely limits the informativity of $_{min}$PMI estimates based on entomological evidence. Moreover, these limitations are emphasised by the lack of experimental data on

insect colonisation of indoor carrion and by the fact that most of the forensic cases involving entomological evidence have been reported to occur indoors. In this study we investigate the early colonisation of pig carcasses placed indoors in a building located in the centre of an urban environment in central Spain. Three carcasses were placed in three equal rooms with a window half opened during five experimental trials: summer 2013, autumn 2013, winter 2014, spring 2014 and summer 2014. The species composition and their contribution to the carrion colonisation differed among seasons. *Calliphora vicina* Robineau-Desvoidy was the sole coloniser of carcasses in winter and colonised the carcasses within the first 24–48 hours in every season, although *Lucilia sericata* (Meigen) was the first coloniser of most summer carcasses. On the other hand, *Calliphora vomitoria* (L.) and *Chrysomya albiceps* (Wiedemann) colonised the carcasses significantly later in spring and in spring and summer, respectively, with a delay of several days. In autumn, however, there were no significant differences in the colonisation times by *C. vicina*, *L. sericata* and *Ch. albiceps*. *C. vicina* and *L. sericata* showed a clear preference for ovipositing in the natural orifices of the carcasses, whereas *Ch. albiceps* oviposited more frequently on the trunk and legs.

Keywords: Forensic entomology; oviposition; minimum post-mortem interval; *Calliphora*; *Lucilia*; *Chrysomya*

1. Introduction

Blow flies (Diptera: Calliphoridae) are considered ideal forensic indicators due to their ubiquity, synanthropy and ability to locate and colonise cadavers promptly after death [1,2]. The colonisation by blow flies starts when the first female lays its eggs on the cadaver. Thus, when a cadaver is found, the time of insect colonisation can be accurately estimated by determining the age of the oldest immature specimens found on or around the body, if developmental data on the pertinent species are available and the environmental temperature

of the forensic scene is modelled [1,3]. The estimated time of insect colonisation is then used to infer a minimum post-mortem interval ($_{min}$PMI) rather than the actual time of death [1], as the latter should also include the pre-colonisation interval [4]. The length of this pre-colonisation interval will thus depend on how promptly the insects located and colonised the cadaver. In outdoor situations, blow flies are expected to locate and colonise exposed cadavers within hours or even minutes after death [1–6], so $_{min}$PMI estimates can be very close to the actual PMI. On the other hand, oviposition might be delayed indoors, as it has been observed that fewer blow fly females are able to locate and access a cadaver under this condition [2,6]. It is therefore of pivotal importance to know which blow fly species are able to colonise indoor cadavers and how promptly, particularly if we take into account that most of the forensic cases involving entomological evidence have been reported to occur indoors [6–9]. However, the vast majority of studies on insect succession on carrion are performed outdoors, whereas available data from indoor locations come mainly from case reports [7–10]. Experimental data from indoor situations are very scarce [2,6], and therefore strongly needed.

The current study aims (i) to determine which blow fly species are able to colonise fresh carcasses placed indoors in an urban environment in central Spain, analysing potential changes in the species composition during the year [11,12]; (ii) to test potential differences in the earliest oviposition times between different blow fly species and seasons; and (iii) to examine if some areas on the cadaver are preferred for oviposition by the earliest colonisers.

2. Material and methods

2.1. Study site and sampling procedure

The experiment was conducted in a two-floor abandoned building (UTM 30T 472091, 4484682; 595 m a.s.l.) located in the centre of Alcalá de Henares, in central Spain (Fig. 1a).

Alcalá de Henares is a medium sized city of more than 200,000 inhabitants (data from the Spanish National Institute of Statistics), located ~30 km from Madrid, the capital of Spain. The city area is located in the mesomediterranean bioclimatic level, characterised by hot and dry summers and humid and cold winters [12,13]. The abandoned building used for the experiment is part of the "Cuarteles de Lepanto y el Príncipe", an abandoned military complex (~43,000 m^2) built between 1859 and 1864. The complex currently belongs to the University of Alcalá, which is restoring it for academic use. The building used for the experiment was available for the study from June 2013 to July 2014 before being partially demolished for rebuilding. Inside the building, three empty rooms (20 x 20 x 3 m each) of the same characteristics were selected for the experiment. The rooms were contiguous but fully isolated, located in a long corridor, each room with one door facing northwest and one window facing southeast (Fig. 1a). The windows faced a courtyard and consisted of two fixed glass panes and two sliding glass panes (Fig. 1a), and two interior wooden-framed shutters (Fig. 1b). The doors were kept closed during the experiment, whereas the windows were left with one of the sliding panes and the interior shutters opened, leaving an entry area of 120 cm x 50 cm (Fig. 1a).

Domestic pig (*Sus domesticus* Erxleben) carcasses of similar weight (22–27 kg) were used as animal models during this study. Pigs were provided by the Animal Experimentation Centre of the University of Alcalá. In total, 15 pig carcasses were used, three pigs for each trial: summer-1 (from 24/06/2013 to 31/07/2013), autumn (from 23/09/2013 to 19/10/2013), winter (from 13/01/2014 to 30/03/2014), spring (from 22/04/2014 to 27/05/2014), and summer-2 (from 23/06/2014 to 18/07/2014). The end date for each experimental trial was established when no more post-feeding fly larvae were observed dispersing from the carcasses; this and other aspects of the insect succession will be analysed in an upcoming publication. The pigs were killed by sodium phentobarbital overdose in accordance with the

EU Council Directive 86/609/EEC and immediately transferred in plastic bags to the study site building, where they were unwrapped and exposed at 10:00 a.m on the day of death. No further ethical approval was needed in accordance with current regulations on animal experimentation (European Directive 63/2010/EU and Spanish Royal Decree 53/2013).

For every trial, one pig carcass was placed in each one of the three different rooms. The carcasses were always placed in the same position: the head facing west and the belly facing the window (Fig. 1b). Each carcass was placed on an individual mesh platform (100 x 80 cm) in order to enable daily weight measurements (data not included here) and inspection and sampling under the carcass. Each platform was placed on a carpet of the same dimensions, in order to retain part of the decomposing liquids and fluids and facilitate the work around the carcass. Finally, each carcass was surrounded by a wooden strip quadrat (240 x 240 x 14 cm) affixed to the floor with silicone, in order to enable the daily collection of dispersing post-feeding larvae (data not included here). Hourly room temperature data was collected using calibrated data loggers; one logger was used per room.

The carcasses were visited 6, 24, 30, 48, 54, 72, 78, 96, 102, 120, 126 and 144 hours after exposure, and then every 24 hours until the end of each experimental trial (i.e. at 10:00 and 16:00 during the first 6 days and then daily at 10:00 until the end of the experimental trial). During every visit within the first month after carcass exposure, each carcass was visually and carefully inspected by two researchers in the search for egg batches. An egg batch was defined as a cluster of eggs oviposited either by one single female or by several females in aggregation [2]. Newly observed egg batches were recorded by photographs and on paper templates with schematic outlines of the pig bodies. Moreover, new egg batches were marked with nontoxic coloured powder (Fig. 1c), in order to not to record the same egg batch as new more than once. The place of each batch on the carcass was also recorded, distinguishing in this study the following seven oviposition sites: mouth, nostrils, ears, head

(exclusive of mouth, nostrils and ears), trunk (including back, loin, belly and both sides), legs and anus. A small sample (around 10–20 eggs) from each newly observed batch was collected with forceps and placed in small plastic containers (11 x 11 x 7 cm) with chopped ground beef and 2–3 cm of soil as pupariation substrate. The plastic containers were transferred to the laboratory and placed in an incubator under a constant temperature of 25 ºC (± 1ºC) until adult emergence. Fresh chopped ground beef was added when needed, until all the larvae had dispersed to pupariate. Emerged adult blow flies were preserved in 80 % ethanol, identified to the species level and deposited in the collection of the Department of Life Sciences of the University of Alcalá.

2.2. Data analysis

A principal component analysis (PCA) was performed to explore the relationship between the observed blow fly species composition and the different experimental trials. Moreover, similarity percentage (SIMPER) analyses based on the Bray-Curtis measure [14] were performed to identify which blow fly species primarily typified the carrion colonisation within each experimental trial. As some data did not met the assumption of normality according to Shapiro–Wilk tests, non-parametric Kruskal-Wallis analyses with post hoc Bonferroni were performed to test differences in the earliest recorded oviposition times (i) between the observed species within each experimental trial, and (ii) within the same species between different experimental trials, for those species occurring during more than one season. Finally, chi-squared tests followed by pairwise comparisons were performed to assess differences in the frequency of use of the different oviposition sites. For all analyses, differences were considered to be significant at the < 0.05 level. SIMPER analyses were performed using PRIMER v. 5 [14]; the other analyses were performed using Statgraphics Centurion (Statistical Graphics Corp. 1994–2000).

3. Results

The average temperature ± SD in each of the three rooms within each experimental trial were: summer-1: 27.6 ºC ± 2.14, 27.38 ºC ± 2.18 and 27.22 ºC ± 2.14; autumn: 19.73 ºC ± 3.45, 20.22 ºC ± 4.17 and 20.22 ºC ± 4.03; winter: 9.97 ºC ± 3.03, 9.92 ºC ± 3.05 and 10.02 ºC ± 3.03; spring: 19.22 ºC ±2.38, 18.78 ºC ± 2.76 and 18.67 ºC ± 2.66; summer-2: 24.69 ºC ± 2.22, 24.82 ºC ± 2.28, 24.56 ºC ± 2.17. No sudden changes in temperature were recorded in the course of each experimental trial. Recorded temperature values in relation to insect activity and carcass decomposition will be analysed in an upcoming publication.

3.1. Species composition

In total, five blow fly species were observed to colonise indoor carcasses during the current study (Table 1): *Calliphora vicina* Robineau-Desvoidy, *Calliphora vomitoria* (Linnaeus), *Chrysomya albiceps* (Wiedemann), *Lucilia sericata* (Meigen) and *Protophormia terraenovae* (Robineau-Desvoidy). Among them, only *C. vicina* occurred in all four seasons of the year, being the only species colonising the carcasses during the winter trial (Table 1). It must be mentioned, however, that *C. vicina* colonised only two of the three carcass replicates during summer-1 trial and was absent during summer-2 trial (Table 1). On the other hand, *C. vomitoria* and *P. terraenovae* only colonised the carcasses during the spring trial; *C. vomitoria* oviposited on the three carcass replicates whereas *P. terraenovae* oviposited only on two of them (Table 1). Finally, both *Ch. albiceps* and *L. sericata* colonised the three carcass replicates of summer, autumn and spring trials, but not the winter ones (Table 1).

The PCA and SIMPER analysis supported the observed seasonal changes in the composition of the blow fly species colonising carrion during the current study, with the first two principal components accounting for more than 80 % of the variability (Fig. 2). In the PCA plot (Fig. 2), the points represent the scores of the observations (the experimental trials)

and the vectors the weight of the variables (the recorded blow fly species) on the principal components. Points that are close together correspond to experimental trials with similar species composition, whereas vectors pointing in the same direction correspond to species with similar distribution among trials. Within each trial, the species contribution to the colonisation of the different replicate carcasses was very similar (Fig. 2), with average similarities of ~90 % or higher in all cases (Table 2). Between trials, both summer experiments showed similar scores on the principal components (Fig. 2) and very similar species contributions, whereas the highest dissimilarity values were found between winter and both summer trials (Table 3). Both *Ch. albiceps* and *L. sericata* contributed approximately in the same proportion to characterise the carrion colonisation during summer, whereas the colonisation during winter was obviously typified by *C. vicina* alone as it was the only species occurring during that season (Table 2). *Calliphora vicina*, *Ch. albiceps* and *L. sericata* were the three main species characterising carrion colonisation during autumn and spring, although contributing at different levels in each season: in autumn, the three species contributed approximately in the same proportion to the colonisation of carcasses, whereas in spring *C. vicina* was clearly the species dominating the carrion colonisation (Table 2). As mentioned, two additional species colonised the carcasses during spring: *C. vomitoria* and *P. terraenovae* (Table 1). However, their contribution to the carrion colonisation during this season was very low, even negligible in the case of *P. terraenovae* (Table 2).

Overall, the number of observed egg batches was relatively low for every species and experimental trial with the exception of *C. vicina* in both winter and spring trials, when more than 20 egg batches were recorded on every replicate carcass at the end of each experimental trial (Table 1). Even if it is not possible to know how many females oviposited on each carcass, as some egg batches may correspond to several females ovipositing in aggregation, the low recorded numbers (Table 1) suggest that few females were able to locate and colonise

the carrion. It must be highlighted, moreover, that the higher numbers of observed egg batches in winter and spring were due to the fact that carrion colonisation continued during most part of the first month after carcass exposure (Fig. 3) and even beyond: egg batches from *C. vicina* were observed until day 75 after carcass exposure on winter carcasses but not included in the analysis. In contrast, no new egg batches were observed during summer and autumn experiments beyond day 9 after carcass exposure (Fig. 3).

3.2. Earliest ovipostion times

Regarding the earliest oviposition times, *C. vicina* was the first coloniser in autumn, spring and on one carcass in summer-1, and the only coloniser in winter, laying eggs within the first 24 hours in most cases. The exceptions were the spring carcasses, where the first egg batches were observed 30 hours after carcass exposure in the three replicates, and one replicate carcass in winter, where the first egg batch was observed 168 hours after carcass exposure (Fig. 4). Nevertheless, no significant differences were found in the earliest oviposition times of *C. vicina* between trials ($H = 4.37$; $p > 0.05$). *C. vicina* was also the first coloniser in all the carcasses where it occurred with one exception: one replicate carcass in summer-1 trial, where it was observed 48 hours after carcass exposure, after the colonisation of *L. sericata* within the first 6 hours after exposure (Fig. 4). *L. sericata* was, besides *C. vicina*, the only other species able to colonise carcasses within the first 24 hours, although this was only observed in two of the replicate carcasses of summer-1 trial (Fig. 4). In general, *L. sericata* colonised the carcasses within 48–72 hours after carcass exposure in summer and autumn, whereas its arrival was delayed until 126–192 hours after carcass exposure in spring (Fig. 4). There were, however, no significant differences in the earliest oviposition times of *L. sericata* between trials ($H = 7.09$; $p > 0.05$).

On the other hand, *Ch. albiceps* was clearly the last coloniser of carcasses in spring and summer, ovipositing 384–552 and 78–102 hours after carcass exposure, respectively, but it colonised the autumn carcasses significantly earlier ($H = 9.51$; $p < 0.05$), showing similar earliest oviposition times to *L. sericata* (Fig. 4). In accordance with all these observations, significant differences in the oviposition times by the different species were found within spring ($H = 9.79$; $p < 0.05$), summer-1 ($H = 3.85$; $p < 0.05$) and summer-2 ($H = 3.97$; $p < 0.05$) trials, with *Ch. albiceps* colonising carcasses significantly later and *C. vicina* and *L. sericata* being the primary colonisers in spring and summer, respectively (Fig. 4). In contrast, no significant differences between species were found within autumn trial ($H = 5.77$; spring ($H = 9.79$; $p > 0.05$). Finally, in the spring trial, *C. vomitoria* appeared to act as a secondary coloniser of carcasses, arriving 168–216 hours after carcass exposure, whereas *P. terraenovae* showed an irregular colonisation pattern, with only two egg batches recorded 78 and 432 hours after carcass exposure, respectively (Fig. 4).

3.3. Oviposition sites

The mouth was the oviposition site where the first observed egg batch was most frequently found ($\chi^2 = 70.65$; $p < 0.05$). Indeed, the mouth was preferred as the first site for colonisation in every experimental carcass with the exception of one of the replicate carcasses of winter trial, where the first egg batch was observed inside one of the nostrils. It must be emphasised once again that *C. vicina* was the first coloniser in virtually all the experimental carcasses where this species was present (i.e. all the experimental carcasses except one of summer-1 trial and the three of summer-2). *Lucilia sericata* was the first coloniser in those summer carcasses where *C. vicina* was absent, as well as in one of the summer-1 carcasses which *C. vicina* colonised later (Fig. 4). Taking into account the total number of egg batches at the end of the experiment, the three most frequent species (*C. vicina*, *L. sericata* and *Ch. albiceps*) used every body part as oviposition sites. However, *C.*

vicina ($\chi^2 = 14.81$; $p < 0.05$) and *L. sericata* ($\chi^2 = 15.15$; $p < 0.05$) oviposited more frequently in natural orifices (mouth, nostrils, ears and anus), whereas *Ch. albiceps* laid its eggs more frequently on the trunk and legs ($\chi^2 = 61.6$; $p < 0.05$).

4. Discussion

The five blow fly species recorded in the present study have been previously reported colonising human cadavers not only in Spain [10,15,16] but also across Europe and North America, including indoor situations [6–10,17]. Among them, *C. vicina* justifies its role as arguably the insect most widely used as a forensic indicator: it is commonly found across wild, rural and urban habitats [11,12,18], and the present results show that it might be able to colonise cadavers located indoors within the first 24–48 hours after death in every season (Fig. 4), therefore potentially being the first species colonising a corpse under most situations. Primary colonisation of indoor carcasses by *C. vicina* within the first 24–48 hours had also been reported by Reibe and Madea [2] using piglets during spring and summer in central Germany. In central Spain, *C. vicina* peaks in abundance during autumn and spring, whereas the number of active adults decrease drastically during summer at low elevations [11,12,18]. This explains why summer was the only season when this species did not colonise all the experimental carcasses (Fig. 4). On the other hand, *C. vicina* can be the only active blow fly species during winter [5,11,12,18], potentially monopolising the colonisation of carcasses during this season (Figs. 3 and 4). Domínguez Martínez and Gómez Fernández [15] reported how the colonisation of human cadavers by *C. vicina* alone was observed "over and over again" during winter in Spain, suggesting that the adults of this species may overwinter inside buildings and dwellings, thus enabling the rapid colonisation of carrion indoors even during the coldest periods of the year. A previous study conducted using carrion-baited traps in a nearby building [12] showed that *C. vicina* was also able to access carrion indoors during every season, even when doors and windows were closed, although it was absent in the

hottest months of the year. Nonetheless, indoor carrion colonisation times under restricted access environments (i.e. no open doors or windows or other entry areas) still need to be investigated, as they may indicate a delayed access to the cadaver [9].

Although it shows a spatial and seasonal distribution similar to *C. vicina* [11,18], *C. vomitoria* only colonised carcasses during spring in the current study, and significantly later than *C. vicina* in every carcass replicate (Fig. 4). Spring is, indeed, the season of maximum abundance for *C. vomitoria* in central Spain [11,18]. This species shows, besides, a less synanthropic character than *C. vicina* [19], which has been suggested as one of the reasons why *C. vomitoria* is less frequently observed colonising human cadavers [15]. Comparing the colonisation of indoor and outdoor piglet carcasses, Reibe and Madea [2] found *C. vomitoria* colonising only the carcasses placed outdoors. However, it must be taken into account that their study [2] was limited to the first 48 hours after carcass exposure, so later colonisations of indoor carcasses by *C. vomitoria* (Fig. 4) cannot be discarded. Velásquez et al. [20] found *C. vomitoria* colonising pig carcasses placed outdoors in a periurban environment in the south east of Spain during both autumn and winter, although in very low numbers and with a delay of 8–9 days after carcass exposure. Evidence therefore suggests that *C. vomitoria* is a secondary coloniser of carrion, under both indoor and outdoor conditions, so its presence on a human cadaver might be indicative of several days of exposure [20].

In their review of the insects collected in several indoor cases from Italy, Bugelli et al. [9] highlighted that *Ch. albiceps* and *L. sericata* were frequently found together colonising human cadavers. In our study, both species colonised every carcass replicate during both summers, autumn and spring trials (Fig. 4). Our results suggest, however, that *Ch. albiceps* tends to colonise indoor carcasses later than *L. sericata*; this was particularly clear during the spring trial (Fig. 4). Indeed, *L. sericata* and *Ch. albiceps* have been regarded as primary and secondary colonisers of carrion, respectively [5]. Nevertheless, the significant delay in

carcass colonisation by *Ch. albiceps* recorded during the spring trial in the current study (Fig. 4) is more likely related to the scarcity of active adults of this species during early spring in the study area [12]. Although both species are only active during the warm months in the Iberian Peninsula, *L. sericata* adults show a longer period of annual activity, from early spring —"as soon as the warm weather can be felt" [15]— to late autumn, whereas *Ch. albiceps* only peaks in abundance during the warmest months from mid-summer to early autumn [11,12,18]. It has been suggested that these different seasonal patterns might also represent an adaptive response by *L. sericata* to the well-known facultative predacious behaviour of *Ch. albiceps* larvae on the larvae of other blow fly species [11,20,21]. Although *L. sericata* may be able to colonise indoor carcasses within hours after death, it seems to locate them generally less promptly than *C. vicina* overall (Fig. 4). This agrees with the observations of Reibe and Madea [2] who observed *L. sericata* colonising indoor carcasses only after 48 hours of exposure, in a situation where *C. vicina* colonised them within the first 24 hours.

Regarding the occurrence of *P. terraenovae*, our results suggest that it might be just an exceptional coloniser of indoor cadavers in central Spain, given the low number of recorded egg batches (Table 1) and the irregular pattern of colonisation observed (Fig. 4). As a cold-climate species, *P. terraenovae* can be frequently found in central and northern Europe and North America, where it can certainly be one of the first colonisers of indoor cadavers [6,8]. However, the records of this species in the Mediterranean region are very scarce and it is usually absent in the carrion succession studies conducted in this area [22]. Interestingly, *P. terraenovae* had only been recently reported colonising human cadavers in Spain: specifically, a single puparium was recovered from an indoor case in Madrid (central Spain) in spring, where the species was found together with *C. vicina*, *L. sericata* and *Ch. albiceps* immatures [22]. The occurrence of *P. terraenovae* in warm mesomediterranean locations

might be related to the fact that its larvae are widely used as live bait for angling in Spain [22]; nonetheless, this needs further investigation.

As expected, the natural orifices of the cadaver, and particularly the mouth, were the first areas to be colonised by blow flies [1,20,23]. This is due to the fact that natural orifices offer both protection and soft tissues as mucous membranes for first-instar larvae to feed [23]. Natural orifices should therefore be carefully examined when searching for entomological evidence in fresh cadavers. As putrefaction advances and the epidermis detaches from the dermis [24], the oviposition on other body parts may be facilitated for later colonisers. The presence of gravid females and/or feeding larvae in a given area, moreover, can stimulate further oviposition [23,25,26]. During the feeding process, *C. vicina* and *L. sericata* larval masses move to the thoracic and abdominal regions of the body [20], so the observed preference of the secondary coloniser *Ch. albiceps* for those areas in the current study may respond to its aforementioned facultative predacious behaviour [20,21]. However, previous studies using carrion baits in Brazil did not found any clear preference of *Ch. albiceps* for those baits already containing other blow fly larvae [27]. Further research on the interactions between *Ch. albiceps* and other coexisting blow flies, mainly *L. sericata* in Spain, is needed.

Finally, it is important to emphasise once again that it is not possible to know the exact number of egg-lays (i.e. oviposited by a single female) from our data. Blow fly females can oviposit in aggregations on carrion [26] and therefore each egg batch counted in this experiment (Table 1) might well have contained eggs from an undetermined number of females. Nevertheless, Reibe and Madea [2] reported significantly far higher numbers of egg batches on outdoor carcasses than on indoor carcasses for all tested exposure times. Anderson [6] also noted much fewer insects attending indoor carcasses than outdoor carcasses. Cadaveric volatile organic compounds [28,29] released by outdoor cadavers are likely more

easily detected, and perhaps females of some blow fly species are more efficient detecting them than others. Whatever the case may be, the low numbers of blow fly females attending indoor carcasses results in low larval densities feeding on carrion, and this results in turn in a slow decomposition process [2,6]. As noted by Anderson [6], carcasses placed indoors may thus provide suitable oviposition sites for much longer than carcasses placed outdoors. This was particularly clear during winter and spring trials in the current experiment (Fig. 3). Nonetheless, a slow rate of decomposition can also be expected in carcasses placed outdoors during cool seasons [30,31].

5. Conclusion

Although delayed colonisation may occur, blow flies are able to colonise indoor carcasses within 24–48 hours in every season if the environment is not completely sealed (e.g. with windows left open). *Calliphora vicina* is expected to be the first coloniser in every season in central Spain with the exception of the warmest months, when *L. sericata* might be the first species laying its eggs on the cadaver. Both species would therefore be the most potentially useful ones for estimating a $_{min}$PMI. On the other hand, the presence of secondary colonisers like *C. vomitoria* or *Ch. albiceps* on an indoor carcass may be an indicator of several days of exposure in certain seasons.

There are obvious limitations to the placement of carcasses indoors that explains the paucity of studies on insect colonisation and succession on indoor carrion in urban environments [6]. Ideally, the present study would have included replicate buildings, an outdoor control pig and the completion of, at least, a second year of surveys, thus replicating every season. Regrettably, this was not possible due to the limited availability of the building. Nevertheless, the current study should contribute to fill the existing gap in experimental data on insect colonisation of indoor carrion, particularly in the Mediterranean region.

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Figure legends

Fig. 1. (a) Building used for the experiment, showing the half-opened windows (arrows) of the three rooms. (b) Interior view of one of the rooms with the pig carcass placed on a mesh platform and surrounded by a wooden strip quadrat. (c) Egg batch marked with coloured powder.

Fig. 2. Biplot for the blow fly species and the five experimental trials showing two axes in the plane defined by the two first principal components. Complete species names are given in Table 1.

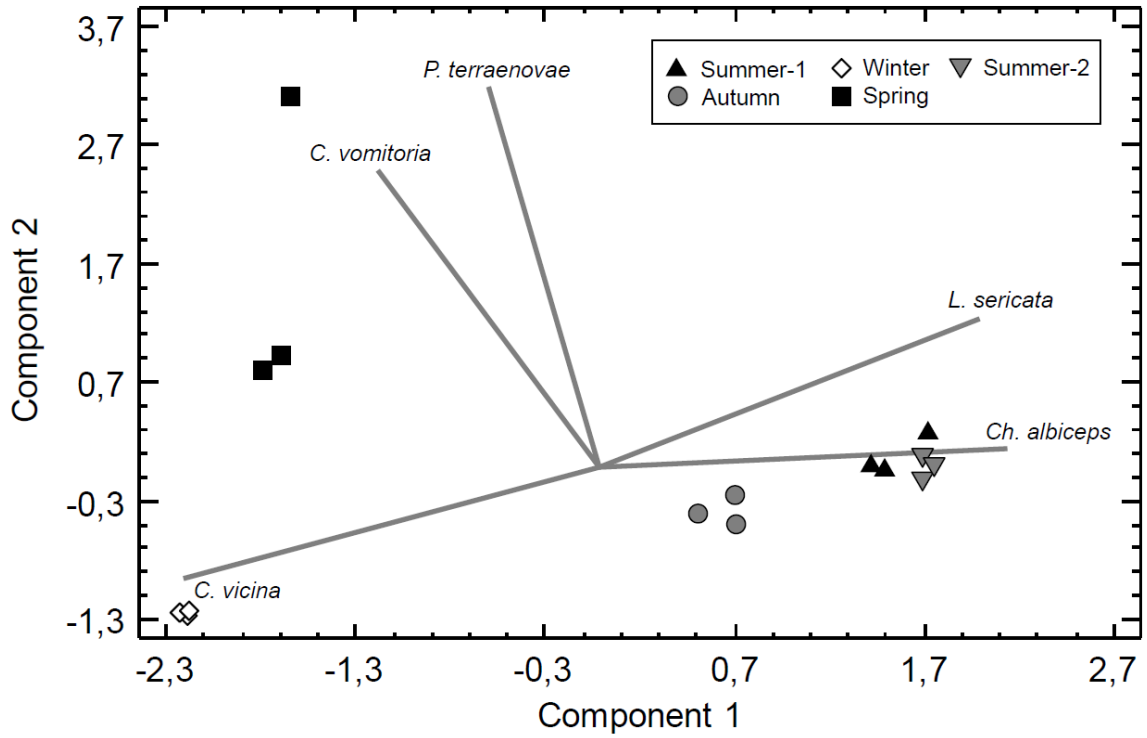

Fig. 3. Days with newly observed egg batches for each blow fly species within each trial. Only the first 30 days after carcass exposure are shown, but new egg batches of *Calliphora vicina* were observed until day 75 during winter trial (*).

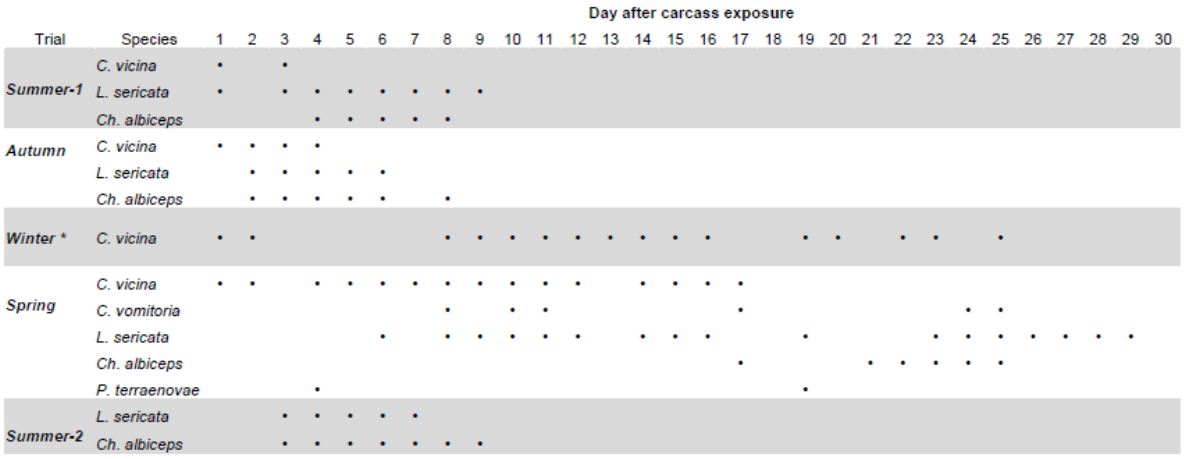

Fig. 4. Earliest oviposition times in hours after carcass exposure, for each blow fly species within each season. The size of each dot denotes the number of carcass replicates where oviposition was observed.

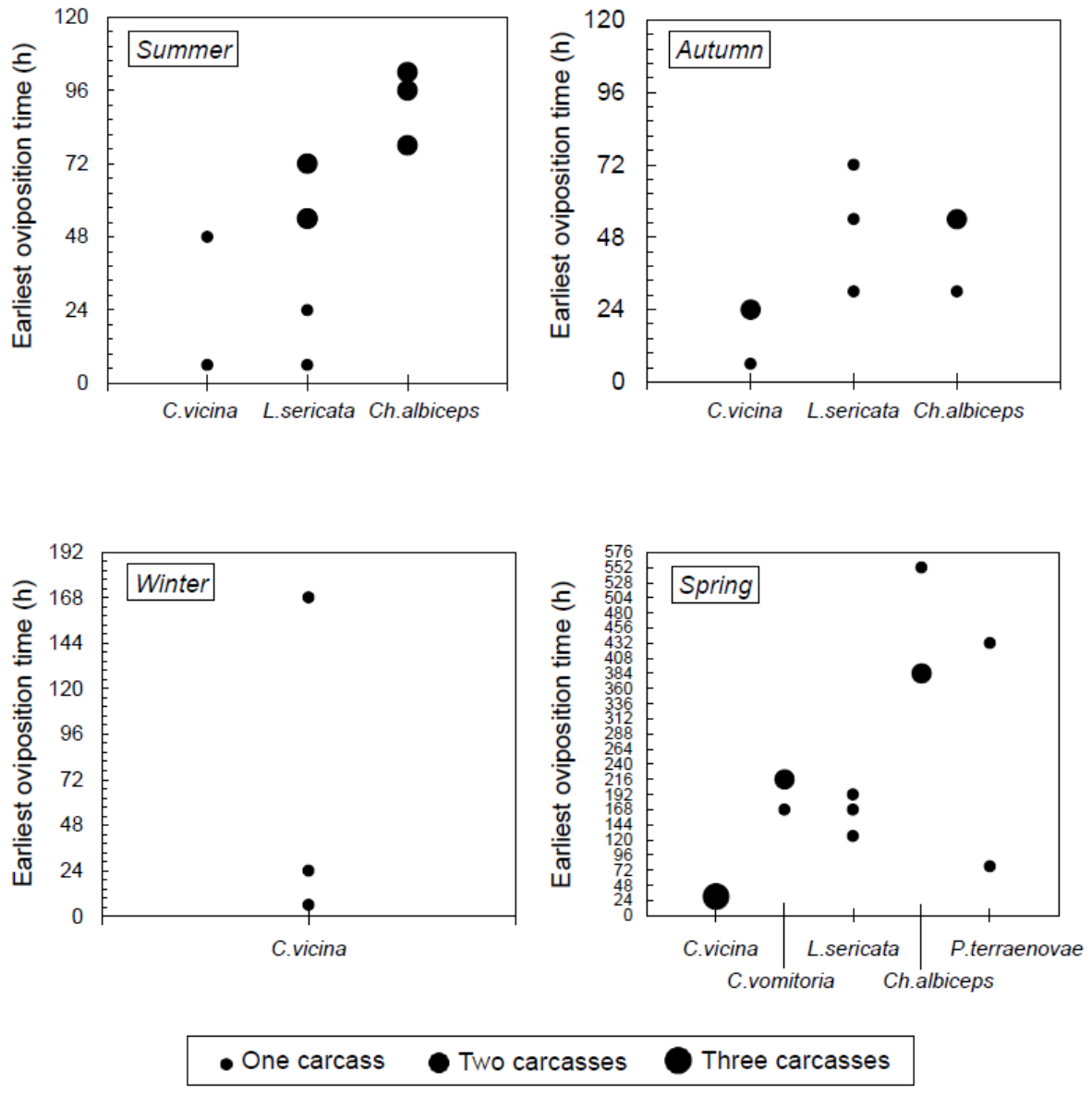

Table 1. Number of observed egg batches for each blow fly species, per trial and carcass replicate.

Trial	Carcass replicate	Number of observed egg batches				
		C. vicina	*C. vomitoria*	*Ch. albiceps*	*L. sericata*	*P. terraenovae*
Summer-1	1	1	0	8	8	0
	2	1	0	7	8	0
	3	0	0	4	7	0
Autumn	1	7	0	8	9	0
	2	3	0	9	3	0
	3	4	0	6	4	0
Winter	1	36	0	0	0	0
	2	25	0	0	0	0
	3	29	0	0	0	0
Spring	1	24	2	1	11	1
	2	29	2	5	15	1
	3	27	2	4	8	0
Summer-2	1	0	0	12	9	0
	2	0	0	9	10	0
	3	0	0	9	16	0

Table 2. Percentage of contribution of each blow fly species to the carrion colonisation within each trial, with average similarity between carcass replicates.

Trial	Average similarity (%)	Species	Contribution (%)	Cumulative (%)
Summer-1	87.9	*L. sericata*	50.1	50.1
		Ch. albiceps	44.4	94.5
		C. vicina	5.5	100
Autumn	92.1	*Ch. albiceps*	38.1	38.1
		C. vicina	31.9	70
		L. sericata	30	100
Winter	100	*C. vicina*	100	100
Spring	88.2	*C. vicina*	47.3	47.3
		L. sericata	28.3	75.6
		Ch. albiceps	12.8	88.4
		C. vomitoria	11.6	100
Summer-2	94.4	*Ch. albiceps*	52.2	52.2
		L. sericata	47.8	100

Table 3. Matrix of percent dissimilarities in blow fly species composition between trials.

	Summer-1	Autumn	Winter	Spring	Summer-2
Summer-1	-	19.3	87.2	44.1	11.4
Autumn		-	61.5	27.5	24.4
Winter			-	44.3	100
Spring				-	51.9
Summer-2					-