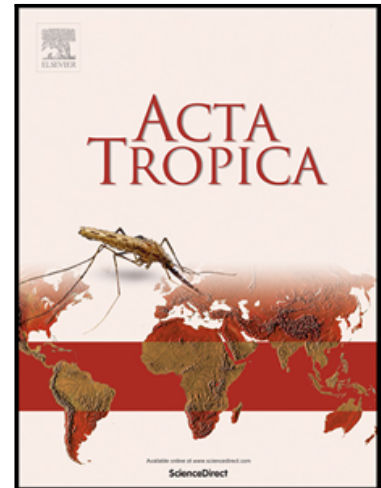


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**Coexistence and intraguild competition of *Chrysomya albiceps* and *Lucilia sericata*
larvae: case reports and experimental studies applied to forensic entomology**

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Short title: Coexistence of *Chrysomya albiceps* and *Lucilia sericata*

Highlights

- Coexistence of the two species affects their mortality, life cycle and adult size
- An adult activity study shows that when the presence of *Ch. albiceps* increases, that of *L. sericata* decreases.
- When they coexist on corpses, *L. sericata* arrives first, allowing PMI_{min} estimation
- One implication of coexistence for the PMI estimation is the increase in their life cycle

Abstract

The larval development of *Chrysomya albiceps* and *Lucilia sericata* is a well-known and valuable tool for estimating the postmortem interval (PMI). The third instar larvae of *Ch. albiceps* are facultative predators of the larvae of other necrophagous dipterans, and this behaviour is undoubtedly an important factor to consider. Both species are found together during human autopsies in Spain and other parts of Europe, where they cohabit, so a precise knowledge of their larval coexistence data is essential. The aim of this study is to investigate their coexistence and the intraguild predation of *Ch. albiceps* on *L. sericata* under experimental conditions and in real case reports. To analyse intra- and interspecific competition, four densities were used under controlled abiotic conditions [25°C, 60-70% RH and 12:12 (D:N)]. The experimental data were compared with data corresponding to their coexistence in natural conditions, so annual activity and forensic case reports for both species were studied. The results indicate that the mortality of immature specimens in both species, the preimaginal developmental time, and adult size were affected by competition. In natural conditions, adult coexistence during the spring-summer period was confirmed. The simultaneity of the two

species colonising human corpses is frequent in the studied area, with *Ch. albiceps* being the dominant species, and *L. sericata* the relevant species for estimating the minimum postmortem interval.

Keywords: *Chrysomya albiceps*, *Lucilia sericata*, forensic entomology, larval competition, adult activity, coexistence.

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1. Introduction

A wide range of species of Diptera usually feed on carcasses, and the most abundant are those of the Calliphoridae, Muscidae and Sarcophagidae families (Byrd and Castner, 2010; Velásquez et al., 2010). The availability of food is limited by the size of the cadaver and this factor can influence the dynamics of populations of many necrophagous and necrophilous species (Denno and Cothran, 1975; Godoy et al., 1993; Ivorra et al., 2019). If the food source is insufficient, there can be various interactions between species, such as competition, cannibalism or predation, but larval competition is the most important phenomenon that takes place on a corpse (Charabidze et al., 2021; Fouche et al., 2021; Ivorra et al., 2019; Polis, 1981; Polis et al., 1989; Ulyett, 1950). The consequences can be observed in different parameters such as larval size and the duration of stages in the life cycle period, but two of the main results are the increased mortality rate and the influence on adult size. One of the tools to analyse the global size is Geometric Morphometrics (Jimenez-Martín et al., 2020), which is based on morphology and statistical analysis, where the discrepancy in size and shape in specific structures is studied (Rohlf and Marcus, 1993). This technique is very useful for distinguishing different species or strains of flies (Grzywacz et al., 2017; Ludoški et al., 2014; Szpila et al., 2019; Vásquez and Liria, 2012), for measuring the level of competition between species (Ivorra et al., 2019), and even for revealing the influence of different larval diets on the adult life-cycle in some Diptera (Gobbi et al., 2013).

Two of the necrophagous species that can usually be found on carcasses are *Chrysomya albiceps* (Wiedemann, 1819) and *Lucilia sericata* (Meigen, 1826) (Diptera: Calliphoridae), and their larval development provides useful information for postmortem interval (PMI) estimation (Bugelli et al., 2014; Grassberger et al., 2003; Martín-Vega et al., 2017; Mehlhorn, 2016; Moffatt et al., 2016; Moore et al., 2013; Okpara and VanLaerhoven, 2019; Shiravi et al., 2011; Smith, 1986; Taleb et al., 2018; Tantawi et al., 1996; Vanin et al., 2008). Both species are

frequently found in Spain in natural conditions and colonising human corpses, so a knowledge of their larval coexistence is important for an accurate estimation of the postmortem interval. *Lucilia sericata* is a necrophagous fly, considered eusynanthropic because adults present a marked preference for human environments and are frequently associated with corpses discovered indoors at urban locations (Anderson, 1995; Pinilla Beltran et al., 2012; Povolný, 1971; Povolný and Rozsypal, 1968). In Spain, it peaks in spring, but is also active in summer and autumn (Martínez-Sánchez et al., 2000; Saloña et al., 2009; Velásquez et al., 2011), and among its natural dipteran predators there are blowflies such as *Ch. albiceps* or muscids such as *Synthesiomyia nudiseta* (van der Wulp, 1883) (Diptera: Muscidae) (Ivorra et al., 2019; Ulyett, 1950). *Chrysomya albiceps* is a necrophilous species that feeds on the dead tissue of insects, but is also a facultative predator of other dipteran larvae during its last larval instar; its predatory behaviour has been widely studied (Barbosa et al., 2021; Faria et al., 2007, 2004, 1999; Faria and Godoy, 2001; Ivorra et al., 2019; Omar, 1995; Reigada and Godoy, 2005; Rosa et al., 2006). It is an exophilous species, but common in urban areas (Baumgartner and Greenberg, 1984), and it is widely distributed over the southwestern Palearctic region (Smith, 1986). In fact, in the last few decades, this species has been recorded toward the North (Grassberger et al., 2003; Kotrba et al., 2012; Lutovinovas and Markevičiūtė, 2017; Makovetskaya and Verves, 2018; Szpila et al., 2008), being the only species of the genus that reaches such northern areas in the world. In Spain, *Ch. albiceps* has a thermophilic character, and is more frequent in the southern half of the country than in the north (Martínez-Sánchez et al., 2001, 2000; Moneo and Saloña, 2009; Velásquez et al., 2015), where it appears in late spring, summer or even autumn, depending on the area studied (Prado E Castro et al., 2012; Saloña et al., 2009).

In the Iberian Peninsula, these two species are the most common thermophilic Calliphoridae found on corpses, and in southwestern Europe they have been recorded in several

forensic cases, but there are no details about their coexistence on corpses (Arnaldos et al., 2005, 2004; Arnaldos and García, 2021; Cainé et al., 2009; Carles-Tolrá, 2002; Domínguez Martínez and Gómez Fernández, 1963, 1957; García-Rojo et al., 2008; García-Rojo and Honorato, 2004; González-Mora et al., 1990; Magaña, 2001; Martín-Vega et al., 2017; Martínez-Sánchez et al., 2006). There are studies of coexistence with other species of Calliphoridae (Barbosa et al., 2021; Flores et al., 2017; Fouche et al., 2021; Komo et al., 2021, 2019; Spindola et al., 2017), but, except for Ulyett (1950) and Kheirallah et al. (2007), there are no studies about the larval coexistence of *L. sericata* and *Ch. albiceps* and how this can affect the estimation of the PMI. For these reasons, the aims of this study are (i) to determine the effect of the coexistence and intraguild predation of *Ch. albiceps* and *L. sericata* in experimental conditions, (ii) to analyse several forensic cases in which both species were detected, and (iii) to learn about their adult activity in the field. To achieve these aims, experiments to analyse intraspecific and interspecific competition were carried out, and the mortality, duration of immature development and adult size were measured; a review of unpublished forensic cases was performed; and baited traps were used to study the annual adult activity of both species. The consequences of the adult and larval coexistence on the estimation of the PMI are discussed.

2. Materials and methods

To establish a colony of *Ch. albiceps* in the laboratory, larvae obtained during autopsy procedures at the Institute of Legal Medicine of Alicante (IMLA) were reared on pig liver *ad libitum* until development was completed; in the case of *L. sericata*, an established colony was already present at the laboratory of the Environmental Sciences and Natural Resources Department (University of Alicante, Spain). All the colonies were maintained in a controlled environment at 23°C, 60-70% RH and with a photoperiod of 12:12 (D:N). To study the coexistence under laboratory conditions [25°C, 60-70% RH and 12:12 (D:N)], intraspecific and

interspecific competition experiments were carried out using groups of first instar larvae neonates (L1, 12±12 h) from different offspring and generations. For this purpose, eggs were previously incubated, and hatching was monitored every 12 hours, until neonate larvae emerged. For intraspecific treatment or pure cultures, four larval densities of 50, 100, 150 and 300 were used and replicated five times; and for interspecific treatment or mixed cultures, the same densities were used but with half of the larvae coming from each of the two species. For each treatment and density, the larvae were placed in a vial containing only 15 g of pig liver to force competition for food (Ivorra et al., 2019; Smith and Wall, 1997), and covered with a fine mesh to prevent the larvae from escaping. After three days, each vial was opened and placed in a bigger container with sawdust at the bottom for pupation. The pupae were collected, and once the adults had emerged and expanded their wings, they were frozen. Then the individuals were sexed and counted, and the wing sizes were measured by Geometric Morphometrics. The total mortality rate was calculated using the number of emerged adults with respect to the initial number of larvae. Regarding the duration of each stage, the mean and SE development time were calculated using the data obtained from each replicate; the larval period was defined by recording when the first pupa of each species was observed in the sawdust, and the pupal period considered the moment at which the first adult of each species emerged; to ensure accuracy with regards to the date, each vial was monitored daily at 9.00 am, until the end of the experiment.

To perform a morphometric analysis of the wings, the centroid size of the right wings was computed using the software: *tpsUtil*, *tpsDig* and *tpsRowl*. On each wing, 17 landmarks (lm) at the connections in different wing veins were used (Jimenez-Martín et al., 2020) (Fig. 1), following previous publications with positive results (Ivorra et al., 2019; Ludoški et al., 2014). All these points are easily distinguishable and homologous in both species. The statistical analysis was carried out with the SigmaStat software, which compared the variables studied,

the average and standard deviation of the rates of mortality, of the sex ratio and of centroid size in relation to treatment and density. The normality of the data was checked using the Kolmogorov-Smirnov test. The parametric data were analysed using the one-way analysis of variance (ANOVA, F) and the t-test (t), followed by a post hoc test (Holm-Sidak method). For the non-parametric independent samples, the Kruskal-Wallis (H) test followed by Dunn's test or the Turkey test were used (U).

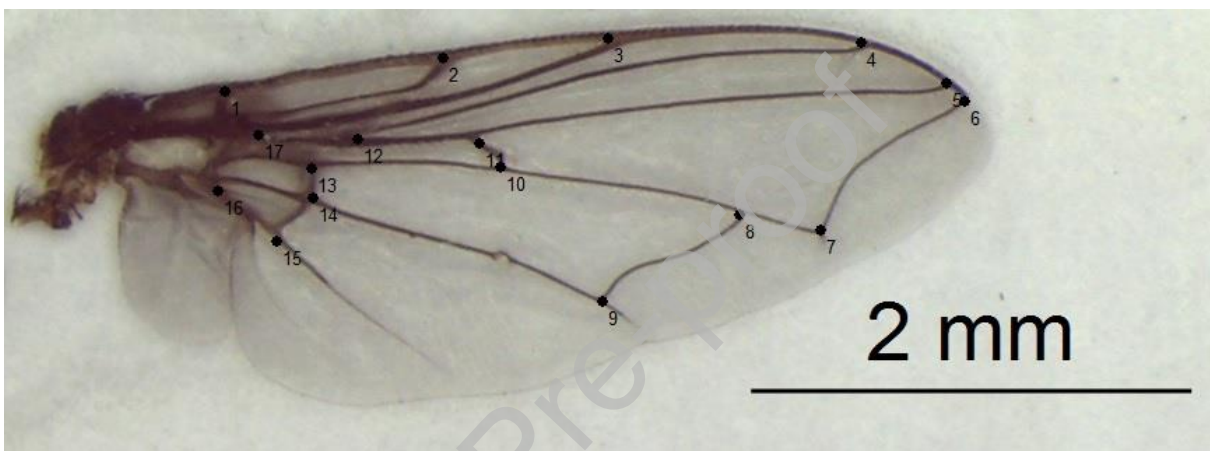


Fig. 1 Wing of *Ch. albiceps* with the 17 landmarks used in this study to carry out the morphometric analysis.

To compare the data obtained with those corresponding to real coexistence in natural conditions, the annual activity of both species was analysed. Three baited traps (Wind Oriented Traps) with 150 g of fresh fish were used to capture adults in an open area on the campus of the University of Alicante (38°37'98"N, 0°52'69"W), with the distance between each trap being 35 m, thus forming a triangle. For three days every month, the traps were active from March 2012 to December 2012. The temperature and humidity were recorded with a data logger during the whole sampling period. The samples were separated by morphotype and identified using Rognes (1998) for *Lucilia* species and Peris and Gonzalez-Mora (1991) for *Chrysomya* species. Finally, to prove the coexistence of both species in the same niche, autopsies carried out at the

Institute of Legal Medicine of Alicante (Spain), and those where *Ch. albiceps* and *L. sericata* were involved, were analysed. The methodology for recorded samples in autopsies followed that of Velásquez et al. (2013). Samples of larvae of second and third instar were collected, preserved and measured by electronic digital calipers; one part of the larval sample was killed by boiling water and preserved in 70% ethanol, and the rest were reared with pork liver to the adult stage in a chamber under controlled conditions [23-25°C, 60-70% RH, 12:12 (D:N)]. The identification keys of Skidmore (1985), Pont (1991), Fremdt et al. (2012) and Velásquez et al. (2010) were used to identify the larvae, which were later deposited in the Entomological Collection of the University of Alicante (CEUA).

3. Results

3. 1. *Effects of larval competition on mortality*

In intraspecific competition (Table 1), the mortality rates were similar at all densities, increasing slightly in *L. sericata*, and significantly ($H=9.88$, $df=3$, $p=0.020$) in *Ch. albiceps* at a larval density of 300; in this treatment, the necrophagous *L. sericata* showed mortality rates lower than the predator *Ch. albiceps*. However, in interspecific competition (Table 1) mortality increased when densities increased above 100 in *L. sericata*, reaching 100% at densities of 150 and 300; in *Ch. albiceps* the mortality was higher at densities of 50 and 300 than at the intermediate densities of 100 and 150.

When the two treatments were compared (Table 1), the coexistence of both species in the same medium increased the mortality of *L. sericata*, it being significantly different at all the densities (density 50: $t=-4.717$, $df=7$; density 100: $t=-9.774$, $df=8$; density 150: $U=25.00$; density 300: $U=25.00$; $p<0.01$); while in the predator *Ch. albiceps* the mortality dropped significantly at the highest densities (density 150: $t=2.458$, $df=8$; density 300: $t=3.263$, $df=8$; $p<0.01$), but increased at the lowest density of 50 ($p<0.01$) and at 100.

Table 1. Mortality rates (average \pm SE) in intraspecific and interspecific treatments for *Lucilia sericata* and *Chrysomya albiceps*.

Density	Intraspecific mortality (%)		Interspecific mortality (%)	
	<i>L. sericata</i>	<i>Ch. albiceps</i>	<i>L. sericata</i>	<i>Ch. albiceps</i>
50	33.60 \pm 1.72 ^a	65.60 \pm 0.80 ^a	57.60 \pm 12.11 ^{a*}	95.20 \pm 3.88 ^{a*}
100	27.20 \pm 2.52 ^a	52.80 \pm 4.49 ^a	85.60 \pm 5.42 ^{a*}	70.80 \pm 10.19 ^b
150	28.40 \pm 1.63 ^a	74.53 \pm 8.77 ^a	100.00 \pm 0.00 ^{b*}	51.47 \pm 3.34 ^{b*}
300	41.33 \pm 4.78 ^a	96.60 \pm 0.98 ^b	100.00 \pm 0.00 ^{b*}	86.80 \pm 2.84 ^{a*}

^{abc} Different letters between densities (within rows) or ^{*} between intra and interspecific treatment at same density (within columns) indicate significant differences $p < 0.01$.

3.2. Effects of larval competition on adult size

In both species, females were bigger than males and there were significant differences in centroid size between the sexes, so they were studied separately (*Ch. albiceps* $H=147.78$, $df=1$, $p < 0.001$; *L. sericata* $H=393.62$, $df=1$, $p < 0.001$); however, the sex ratio indicated more males than females for both species at all densities, it being 1.32 ± 0.04 for *Ch. albiceps* and 1.14 ± 0.04 for *L. sericata*. Centroid size also showed significant differences between densities, so the data were separated into two groups for their further analysis: low densities (50 and 100 individuals) and high densities (150 and 300 individuals) (*Ch. albiceps*: $H=67.29$, $df=3$, $p < 0.001$; *L. sericata*: $H=817.55$, $df=3$, $p < 0.001$).

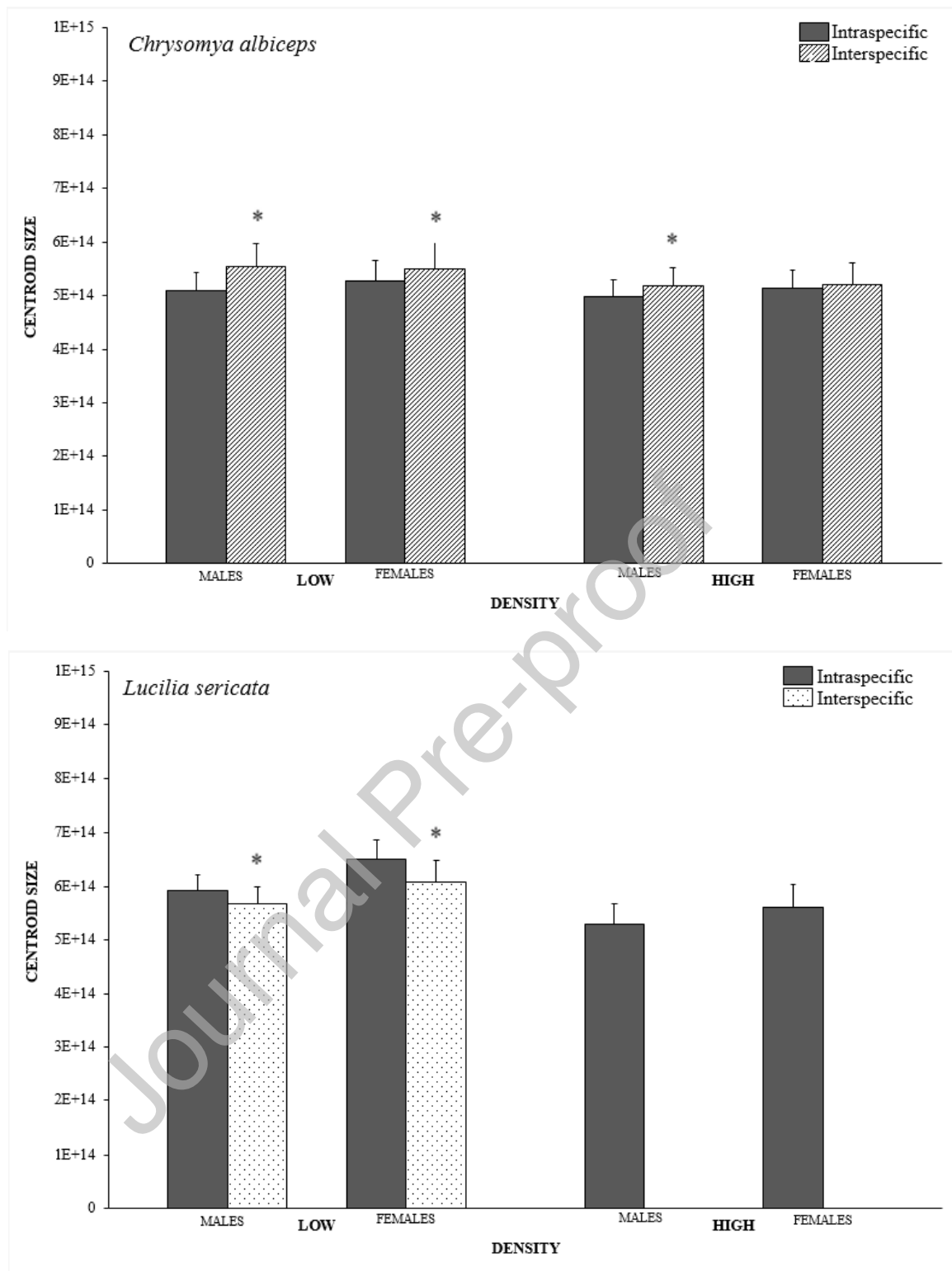


Fig. 2 Centroid size of females and males of *Chrysomya albiceps* (upper chart) and *Lucilia sericata* (lower chart) under different treatments and densities (Low: 50 and 100; High: 150 and 300 larvae/15 g liver). An asterisk indicates significant differences between intraspecific and interspecific competition ($p < 0.05$).

The centroid size in *L. sericata* was bigger than that of *Ch. albiceps* under the same rearing conditions ($H=952.21$, $df=7$, $p<0.001$). In the case of *Ch. albiceps* centroid size decreases as density increases in both sexes for intra and interspecific treatments ($H=63.324$, $df=7$, $p=0.001$). Its wing sizes in interspecific competition are larger than in intraspecific competition, but not in females obtained at high larval densities (150 and 300; $p>0.05$) (Fig. 2). With regards to *L. sericata*, its wing size in interspecific competition is smaller than in intraspecific competition, with differences in both sexes at low densities (males: $U=6812.00$, $p<0.001$; females: $U=6981.00$, $p<0.001$); but it was not possible to study adult size in interspecific competition at high densities for *L. sericata* due to the total mortality of these samples. At low densities, the centroid size for this species in intraspecific competition is greater than at high densities ($H=792.667$, $df=3$, $p<0.001$) (Fig. 2).

3. 3. Effect of larval competition on development time

The development time of both species is shown in Table 2. The larval, pupal and total preimaginal periods (including egg period) in *Ch. albiceps* were generally slightly shorter than in *L. sericata*. In intraspecific competition the larval period in each species was shorter when densities increased, but there were only significant differences at a density of 50. The larval period in *Ch. albiceps* was from 4.00 to 4.60 days, it being longest at a density of 50 (5.60 ± 0.55 days). The same tendency is observed in the case of *L. sericata*, for which the larval period was between 4.60 and 5.60 days, and the longest period was at a density of 50 (6.00 ± 0.71 days). The pupal period was similar at all densities in each species ($p>0.05$), with the range being 4.80-5.00 days in *Ch. albiceps*, and 6.00-6.20 days in *L. sericata*. Finally, the total immature cycle in pure cultures was affected by density in *Ch. albiceps* ($H=12.277$, $df=3$, $p=0.006$) and *L. sericata* ($H=13.660$, $df=3$, $p=0.003$), and a reduction in the number of days

was observed with an increment in density, but only at a density of 50 there were significant differences. This is probably a consequence of the variation in the larval stage and not of a modification in the pupal stage period, which was similar at all densities (Table 2).

Table 2. Duration of larval, pupal, and total preimaginal (from egg to adult) development (average \pm SE) in *Ch. albiceps*, and *L. sericata* under both treatments: intraspecific and interspecific competition at different densities.

		Duration (days)				
Period	Treatment/Density	50	100	150	300	
<i>Chrysomya albiceps</i>	LARVA	Intraspecific	5.60 \pm 0.55 ^a	4.60 \pm 0.55 ^b	4.00 \pm 0.00 ^b	4.20 \pm 0.45 ^b
		Interspecific	6.00 \pm 0.00 ^a	5.20 \pm 0.45 ^a	4.40 \pm 0.55 ^a	4.60 \pm 0.55 ^a
	PUPA	Intraspecific	4.80 \pm 0.45 ^a	5.00 \pm 0.00 ^a	5.00 \pm 0.00 ^a	5.00 \pm 0.00 ^a
		Interspecific	6.50 \pm 0.71 ^a	5.20 \pm 0.45 ^a	4.60 \pm 0.55 ^a	4.40 \pm 0.55 ^a
	TOTAL PREIMAGINAL	Intraspecific	11.40 \pm 0.55 ^a	10.60 \pm 0.55 ^b	10.00 \pm 0.00 ^b	10.20 \pm 0.45 ^b
		Interspecific	13.50 \pm 0.71 ^a	11.40 \pm 0.89 ^a	10.00 \pm 0.00 ^a	10.00 \pm 0.00 ^a
<i>Lucilia sericata</i>	LARVA	Intraspecific	6.00 \pm 0.71 ^a	5.60 \pm 0.55 ^b	4.60 \pm 0.55 ^b	4.60 \pm 0.55 ^b
		Interspecific	6.80 \pm 0.84 ^a	6.80 \pm 0.84 ^{a*}	-	-
	PUPA	Intraspecific	6.20 \pm 0.45 ^a	6.00 \pm 0.00 ^a	6.00 \pm 0.00 ^a	6.20 \pm 0.45 ^a
		Interspecific	6.80 \pm 0.45 ^a	5.20 \pm 0.45 ^{b*}	-	-
	TOTAL PREIMAGINAL	Intraspecific	13.20 \pm 0.45 ^a	12.60 \pm 0.55 ^b	11.60 \pm 0.55 ^b	11.80 \pm 0.45 ^b
		Interspecific	14.60 \pm 1.14 ^{a*}	13.00 \pm 1.22 ^a	-	-

^{abc} Different letters between densities (within columns) or * between intra and interspecific treatment at same density (within rows) indicate significant differences $p < 0.01$.

Regarding interspecific competition (Table 2), the larval, pupal and total preimaginal periods were not affected by density, except in the pupae of *L. sericata*. In *Ch. albiceps*, the larval, pupal and total immature periods were longer than in intraspecific competition, but statistical differences were not found ($p > 0.05$). In addition, the total preimaginal period was similar or shorter in intraspecific competition, at high densities, and for the pupal period too ($p > 0.05$). In *Lucilia sericata*, the larval and total preimaginal periods were also longer in interspecific competition than in intraspecific competition, but significant differences were only observed in the total preimaginal period at a density of 50 ($t = -2.556$, $df = 8$, $p = 0.034$), and in the larval period at a density of 100 ($t = -2.683$, $df = 8$, $p = 0.028$). However, the pupal period at density

100 was shorter ($U=2.500$, $df=5$, $p=0.032$) than under the intraspecific treatment. It should be noted that in the case of *L. sericata* at high densities (densities of 150 and 300 individuals) it was impossible to analyse the life cycle due to the total mortality in interspecific competition.

3.4. Annual activity

The relative abundance for each month was calculated on the basis of the total number of individuals collected by the trap over the whole sampling period. Our results indicate that *Ch. albiceps* ($n=1,931$) was more abundant in the samples than *L. sericata* ($n=859$). Figure 3 shows the annual activity of both species from March to December, with the first peak of *L. sericata* being in the middle of spring (May - 48.19% of individuals), while *Ch. albiceps* peaks later, at the end of summer (September - 43.75% of individuals), and the maximum of activity in both species was at 23°C. Adult activity in the two species was inversely proportional, and the presence of *L. sericata* decreased when *Ch. albiceps* appeared. The abundance of *Lucilia sericata* increased as temperatures increased during the spring, up to 23.73°C. *Chrysomya albiceps* appeared when *L. sericata* peaked (May at 23.73°C) and increased in number as temperatures rose throughout the summer and also at the end of the summer, when temperatures began to decrease, down to 23.36°C, when it peaked in September. Both species were absent below 14.58°C (Fig. 3).

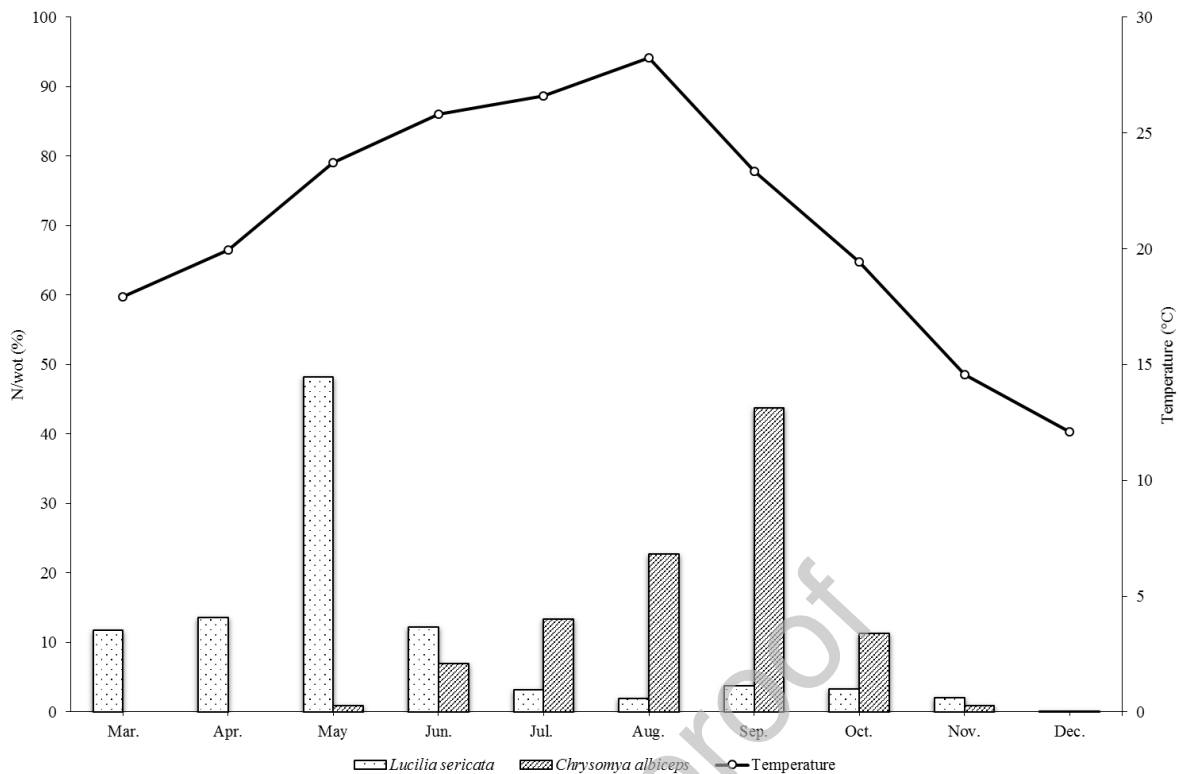


Fig. 3 Annual adult activity of *Ch. albiceps* and *L. sericata* during the year 2012 in Alicante (Spain), based on relative monthly abundance of individuals. The line shows the average temperature in each month.

3.5. Analysis of forensic case reports

Two case reports (out of 11 in total) involving the coexistence of these two species were analysed. One of the cases took place outdoors in summer, and the other one was indoors in spring. Both *Ch. albiceps* and *L. sericata* were found developing on the corpses.

IMLA Case: 556/2009

On 30 July 2009, at 23:30 pm, the corpse of a man was found in the bloated stage in a clearing in the locality of Callosa del Segura (Alicante, Spain) ($38^{\circ}07'21''\text{N}$, $0^{\circ}52'47''\text{W}$) (Fig. 4). The body was naked below the waist and showed a *perimortem* contusion in the left occipital region, but the cause of death was undetermined. The man's description matched that of a chronic drug addict known to the police and who was last seen alive earlier that week. The maximum

postmortem interval was estimated by the pathologist to be 5 days (meaning death occurred on 25 July). The average temperature in the area, seven days prior to the discovery of the body, was 28.4°C. The entomological samples were larvae of second (L2) and third (L3) instars of *L. sericata* and of second instar of *Ch. albiceps*. The maximum length of L3 of *L. sericata*, which was located on the back, was 11.56 mm, and for L2 of *Ch. albiceps*, which was located on the head, the maximum length was 8.67 mm.

For the analysis, developmental data for *L. sericata* and *C. albiceps* existing in our research group at the University of Alicante and the bibliography were used. According to Martínez-Sánchez et al. (*in prep.*), at a constant temperature of 28°C, the larvae of *L. sericata* reach stage III and a length of 11.56 mm in 3 days. In the case of *Ch. albiceps*, a larva of 8.67 mm requires approximately 2.5 days (Al-Misned et al., 2003; Grassberger et al., 2003). On the other hand, the Accumulated Degree-Days (ADD) method was used and calculated for 3 days (at 28.4°C and $D_0=9.78$) in *L. sericata*, resulting in 55.86 ADD. In order to obtain a total of 55.86 ADD, with the temperature recorded the days before discovery, 38.34, 56.56 and 75.98 ADD are reached for two, three and four days, respectively. Thus, a minimum postmortem interval of around 3 days (27 July), based on *L. sericata*, was estimated.

IMLA Case: 385/2010

The corpse of a man was found indoors in the bloated stage (with loss of epidermis) in the locality of San Vicente del Raspeig (Alicante, Spain) (38°23'47"N, 0°31'31"W) on 1 June 2010. The body was fully clothed (with shirt, jeans, jacket, socks, and shoes) and was located on the couch, face down, resting on his knees and with his head on the armrest. Near the corpse, capsules of anxiolytic (benzodiazepine) were found. The house remained closed until the discovery of the body, and the man was last seen alive between 7 and 10 days before the body was discovered (22-25 June). No signs of violence were established by the pathologist.

Dipteran larvae were collected from the head, back, genitals and legs, and an important larval mass was found on both feet. The specimens were identified as *L. sericata*, *Ch. albiceps*, *S. nudiseta* and *Sarcophaga tibialis* Macquart, 1851 (Diptera: Sarcophagidae). The average temperature was interpolated, using the nearest meteorological station to the scene of death, on the basis of the ten days prior to the discovery of the body, giving a figure of 20.9°C. Third instar larvae of *L. sericata* constituted the predominant species found (>200 individuals). The higher number of *L. sericata* last instar larvae and their displacement towards the lower body, specifically towards the feet, suggests that these larvae were close to leaving the body to pupate. Around 15 larvae of *Chrysomya albiceps* (maximum length 15 mm) were collected from the corpse, and with *L. sericata* were the first species to finish their life cycle in the laboratory. For the estimation of the PMI, larval length, life cycle data and isomorphen graphs of *L. sericata* (Martínez-Sánchez et al., *in prep.*) and *Ch. albiceps* (Al-Misned et al., 2003; Grassberger et al., 2003) were used. It was established that *L. sericata* reach the prepupal stage in 5 days at 23°C; and *Ch. albiceps* larvae need between 6 to 8 days to reach 15 mm. *Lucilia sericata* was again the species with which a minimum postmortem interval, of around 5 days, was estimated.

4. Discussion

Interaction between species and population aggregation on carrion are important factors to consider when estimating the postmortem interval in real cases (Fiene et al., 2014; Ivorra et al., 2019). The coexistence of *Ch. albiceps* and *L. sericata* in forensic cases is not frequently cited (Arnaldos and García, 2021; Tantawi et al., 1996; Ulliyett, 1950), although the effect of their coexistence has been confirmed in this study.

Among other studies on the development time in *L. sericata* (Anderson, 2000; Grassberger and Reiter, 2001; Greenberg, 1991; Kheirallah et al., 2007; Marchenko, 2001),

Martínez-Sánchez et al. (2007), using Spanish specimens, established a preimaginal development time of 16.84 ± 0.88 days at 25°C , while the preimaginal development times found in our study in intraspecific (13.20-11.80 days) or interspecific competition (14.60-13 days) were shorter. The result reported by Velásquez (2011) for the preimaginal development time is similar (13.60 ± 0.50 days) to our results (13.20 ± 0.45 days), but Velásquez (2011) reported it at 28°C , and in our study the temperature was 25°C and at the lowest density, i.e. 50 individuals. However, our times are shorter than those in Velásquez's study at 23°C (16.80 ± 0.8 days). On the other hand, Kheirallah et al. (2007) reported a preimaginal development time of 331.36 ± 0.29 hours (~ 13.80 days) with 40 individuals in pure cultures of *L. sericata* at 23°C , a bit longer than in our results in pure cultures with 50 individuals at 25°C . The contrary is true if the results for interspecific competition are compared: in mixed cultures, Kheirallah et al. (2007) reported a development time of 327.36 ± 0.35 hours (~ 13.64 days) with 40 individuals, which is shorter compared with our results (14.60-13 days). Martínez-Sánchez et al. (2007) showed that larval and pupal development times were not significantly different and did not change with density, as in this study in which only the larval period was significantly higher at density 50; but actually, in both studies, when density increased the larval periods shortened, but not significantly. Scanvion et al. (2018) demonstrated that the larval density significantly influences the development period. In the case of interspecific cultures, when other species are feeding together with *L. sericata*, both effects can be found: (i) the larval periods increase with respect to controls or pure cultures when sharing food with *Ch. albiceps*, as happened in this study; or (ii) they can decrease when *L. sericata* is reared with other species such as *Ch. albiceps* (Kheirallah et al., 2007) or *Calliphora vicina* Robineau-Desvoidy, 1830 (Diptera: Calliphoridae) (Komo et al., 2021). With regards to mortality, the rates obtained in pure cultures in this study were between 27% and 41%, without significant differences between densities. These results partially coincided with those of Martínez-Sánchez et al. (2007), in

which the mortality rate in a Spanish population was around 36-40% at densities of 50 and 150, while at density 300 mortality increased up to approximately 50%; also the study of Kheirallah et al. (2007) showed similar mortality results (20.83-39.67% at densities of 40 and 200, respectively) to those of the current study. Mortality was not affected by intraspecific competition at the larval densities studied, but adult size did decrease as the density increased, and the same tendency was reported in previous studies with the same species (Kheirallah et al., 2007; Martínez-Sánchez et al., 2007).

As mentioned above, *Ch. albiceps* is a well-known facultative predator of other dipteran larvae. The mortality rate obtained by us is higher than in Queiroz (1991), in which the authors reported a mortality of 8% with a larval density of 150 at 27°C. Apart from the temperature, they used more rearing medium (1 g of food per larva) than in this study. The pre-adult development time obtained was 10.86 ± 0.03 days, which is similar to our results (10.00 ± 0.00 days) with the same initial number of larvae (150 larvae). Al-Misned et al. (2003) reported a total development time of 14.10 ± 0.49 days at the same temperature as in our study, which is longer than in our results under the same conditions (11.40 ± 0.55 days), and with regards to mortality they reported a rate of only 1%, but they provided more food for the larvae (100 g for 50 larvae). In the case of Kheirallah et al. (2007), the same tendency occurs, as their results indicated a development time of 382.06 ± 0.14 hours (~15.91 days) with 40 individuals in pure cultures at 23°C and a mortality rate of 21%, reporting a longer period compared with our results (11.40 ± 0.55 days) and a lower mortality rate than in this study (65.60%) in pure cultures with 50 individuals at 25°C. In the above studies, the quantity of food provided was greater than in our study, as we only used 15 g of pig liver, following the methodology of Smith & Wall (1997) and Ivorra et al. (2019), so *Ch. albiceps* could also have played a cannibalistic role, explaining in this way its high mortality under both treatments because its mortality in intraspecific competition was higher than that of the necrophagous species *L. sericata*, and moreover the

mortality rate of *Ch. albiceps* was higher at high densities than at low densities in interspecific competition. However, this hypothesis should be tested in specific studies about the cannibalistic role of this species.

If we look at the coexistence of these species that display necrophagous and necrophilous behaviour, the mortality rate, the development time and the size of the adults were affected by the presence of the other species. The mortality of *L. sericata* increased with the presence of the predator *Ch. albiceps* and as density increased, even reaching 100% at the highest densities, as Ulyet (1950) and Kheirallah et al. (2007) showed. The contrary happened with the mortality of *Ch. albiceps*, which decreased when the density increased in the presence of the other blowfly, compared with the pure cultures. These results, if the K value (\log_{10} initial larvae - \log_{10} adults emerged) is calculated and compared with the log of initial density (Begon et al., 1996), could indicate that mortality is independent of density in *Ch. albiceps* and dependent on *L. sericata* in a culture with high densities, as was observed in Martínez-Sánchez (2003). Ulyett (1950) found that the presence of *L. sericata* benefits *Ch. albiceps* because it increases the size of individuals in the surviving population, and this has been confirmed in this study because the adult size of *Ch. albiceps* in mixed cultures was greater than in pure cultures; but in our study, in which food is limited, this was possibly due to the high mortality rate. Unlike the results of Ulyett (1950), the adult size of *L. sericata* in mixed cultures was smaller than in pure cultures. However, the same occurred in the study of Brundage et al. (2014), which found a reduction in the adult size of *Cochliomyia macellaria* (Fabricius, 1775) in the presence of the predatory larvae of *Chrysomya rufifacies* (Macquart, 1842) (Diptera: Calliphoridae). With regard to the development time, the effect of the presence of predatory larvae has been studied before (Flores et al., 2014; Ivorra et al., 2019; Reid, 2012). These studies indicate that the effect on the prey was a faster development in order to leave the carrion resource promptly in order to pupate and escape from predation. However, in this study the behaviour in the prey *L.*

sericata was different, as the total preimaginal period and the larval stage were longer, and the pupal stage was shorter in mixed cultures than in pure cultures, though not always significantly. For the predator *Ch. albiceps* the larval, pupal and total development time generally increased, although at the highest densities the pupal and the total development time decreased, but not significantly, possibly due to cannibalism, as the larval food was a limiting factor in this experiment. Perhaps, contrary to other studies, the food limitation in this study prevents *L. sericata* from moving to the substrate to pupate, and then the pupation happened quickly to avoid the predation by *Ch. albiceps*. These results are not conclusive and more studies with different densities and proportions within species could help us to learn more about this prey-predator behaviour.

Environmental factors such as temperature, relative humidity and photoperiod can influence the annual activity of a species. However, Bauer et al. (2020) recently suggested that the photoperiod does not affect the development of *Ch. rufifacies*. In our study, the peak of both species was at a similar temperature (about 23°C), but *Ch. albiceps* appeared in a wetter month (September with RH 75.31%) than *Lucilia sericata* (May with RH 64.90%), so both of them have a markedly thermophilic character and their presence can be affected by the relative humidity in the field. The species of the genus *Chrysomya* originate from the Old World, and their original distribution is in tropical areas (Azevedo and Krüger, 2013), and *Lucilia* originates from the Palearctic region and is distributed in temperate areas (Richards et al., 2009). Another factor is that *L. sericata* can be the prey of *Ch. albiceps*, but it always peaks earlier (Saloña et al., 2009; Velásquez et al., 2015). In addition, the seasonal coexistence of the two species in summer and autumn has previously been confirmed (Martínez-Sánchez et al., 2000; Tantawi et al., 1996; Ulyett, 1950). It is important to take this into account when a forensic case is analysed. In both cases (one outdoor and one indoor) presented in this study, the same tendency was observed. In the outdoor case (IMLA case: 556/2009) in July, *Ch. albiceps* was more

abundant than *L. sericata*, while the contrary was true in the indoor case (IMLA case: 385/2010) in June; moreover, *L. sericata* is eusynanthropic, which is not always the case of *Ch. albiceps* (Baumgartner and Greenberg, 1984; Schnack et al., 1989). If we compare these data with the annual adult activity (Fig. 3), the necrophagous *L. sericata* was more abundant in June, whilst *Ch. albiceps* was in July. Both corpses were in the bloated stage and the postmortem interval estimated by the pathologist was similar, and it should be borne in mind that *Ch. albiceps* is a necrophilous species, so this species generally colonizes carcasses after *L. sericata* does. The ovipositional behaviour of predators and prey in blowflies was studied by Gião & Godoy (2007), and they reported that *Ch. albiceps* and *Lucilia eximia* (Wiedemann, 1819) (Diptera: Calliphoridae) have a preference for laying egg in places where any species has laid before, with the number of eggs laid being lower in such cases. Recently, Barbosa et al. (2021) confirmed that *L. eximia* uses escape and avoidance behaviour when sharing the same niche with *Ch. albiceps* once the larvae are together. Furthermore, Richards et al. (2009) also reported that *Ch. albiceps* larvae were collected from separate masses on the periphery of carcasses. Therefore, the case reports presented in this study corroborate that *L. sericata* and *Ch. albiceps* can coexist in the same niche too.

As mentioned above, the confirmation of their coexistence has consequences for the estimation of the postmortem interval in an entomological report, especially due to the predatory behaviour of *Ch. albiceps*, since the duration of the life cycle and the mortality of both species are affected by density and interspecific competition: (i) the mortality is higher in *L. sericata* when coexisting with *Ch. albiceps*, with lower mortality of the latter, but *L. sericata* reaches the corpse sooner; and (ii) the prey reduces its development time to avoid be predated (Flores et al., 2014; Ivorra et al., 2019; Reid, 2012), leaving the body sooner to complete the life cycle, but not in our study. As Wilson (2000) stated, predators and prey are closely associated on carrion. This has been confirmed by recent studies in which the aggregation of

different necrophagous species can be mutually beneficial (Charabidze et al., 2021; Fouche et al., 2021; Hans and Vanlaerhoven, 2021; Komo et al., 2021, 2019), increasing or decreasing the size, the mortality or even the developmental time of the species in the same niche. The last of these parameters should be taken into account more than the others because it can be influenced by the co-occurrence of both species, and therefore the minimum postmortem interval in a forensic case can be estimated inaccurately if it is not considered. In our results, the development time of *L. sericata* is similar or slightly longer in interspecific competition than intraspecific, so the estimation based on *L. sericata* at constant temperatures and in pure cultures appears to be correct. On the other hand, our results for *Ch. albiceps* show a longer development time in interspecific competition than in intraspecific competition, so the use of the habitual data for *Ch. albiceps* for the estimation of the PMI will only be correct if this species is captured alone on corpses, but when other species are present, the estimation of the PMI could be affected and significantly underestimated. However, two species do not necessarily interact when they occupy the same body because they can be located on different parts of the corpse (Charabidze et al., 2021), as has been observed previously on South African rhinos' corpses, on which a larval mass of *Ch. albiceps* was observed on the periphery with respect to the larval mass of other blowflies (Richards et al., 2009). So this could happen when a dead body is found, different species can lay eggs in different parts of the body to avoid interspecific competition, as Gião & Godoy (2007) and Richards et al. (2009) have established, or the prey can avoid and escape the predator, as Barbosa et al. (2021) have stated, because the niche is bigger, thus allowing a fast development that could lead to an inaccurate estimation of the postmortem interval in an entomological report.

5. Conclusions

In conclusion, as the coexistence of species is a factor to take into account in a real forensic case, it is important to carry out more studies of the coexistence of common species on a corpse in different ratios (e.g. 20:80 or 40:60) or in different larval instars (e.g. spp1 in 1st instar and spp2 in 2nd instar) to check different times of oviposition and apply the results correctly in the estimation of the minimum postmortem interval.

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Author's contribution

Tania Ivorra: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing - original draft, Writing - review & editing. **Anabel Martínez-Sánchez:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - review & editing. **Santos Rojo:** Project administration, Resources, Supervision, Visualization, Writing - review & editing.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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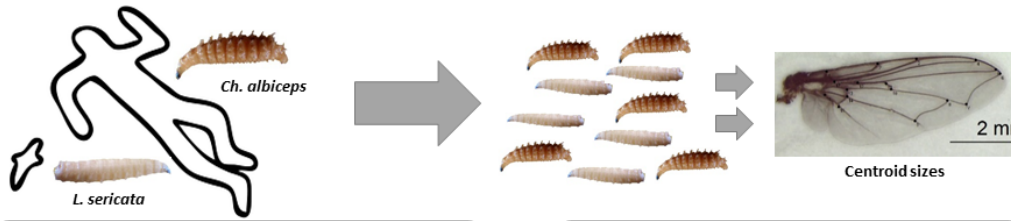
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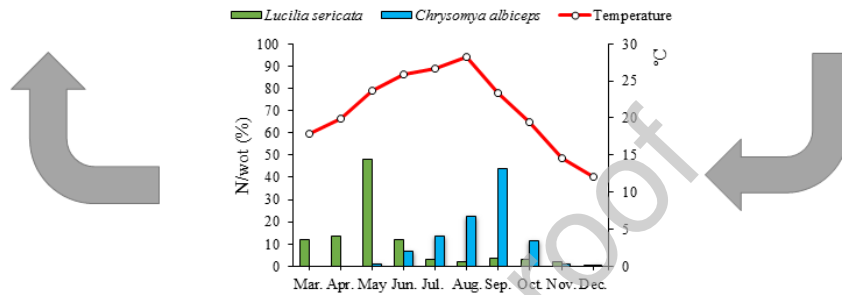
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Chrysomya albiceps and *Lucilia sericata* are a well-known and valuable tool for estimating the postmortem interval (PMI)



In forensic cases where both species were detected, the PMI_{min} was estimated with *L. sericata*

Coexistence of these species affects their mortality, life cycle span and adult size



In the field, an adult activity study shows that when the presence of *Ch. albiceps* increases, that of *L. sericata* decreases

Graphical abstract

Journal Pre-proof