Calliphoridae community composition in sunlit and shaded areas during early colonisation in Metro Vancouver, British Columbia

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ABSTRACT

Necrophagous insects, particularly some species of blow fly (Diptera: Calliphoridae), are extremely useful in medico-legal entomology because they colonise a body very shortly after death, if conditions are appropriate. Their species and development rates can be used to estimate their tenure on the body and so infer minimum time since death. Because many blow flies have specific geographic and seasonal ranges, as well as habitat preferences, their presence on a body can also be used to infer whether a body has been moved from the original death scene. This study focused on the preferences for bait in sunlit or shaded areas. Eighteen beef liver–baited traps were deployed weekly at three sites, equally between sunlit and fully shaded areas. Eight species of blow fly were collected, with the dominant species being *Lucilia illustris* (Meigen), *L. sericata* (Meigen), *Calliphora vicina* Robineau-Desvoidy, and *Calliphora latifrons* (Hough). There was no difference in *Calliphora* spp. between trap placements, but *Lucilia* species were collected more frequently at sunlit sites; statistical differences were seen only with *L. illustris.* Higher numbers of almost all insect species were collected in sunlit traps. This study indicates necrophagous insect preference, or lack thereof, for carrion sites and is important in determining which species may indicate a specific habitat.

Keywords: Forensic entomology; sun and shade, Necrophagous community composition; Calliphoridae; Early colonisation, bait traps

INTRODUCTION

Medico-legal entomology is a branch of forensic entomology used primarily in death investigations to estimate the period of insect colonisation of a body (Tomberlin *et al.* 2011; Rivers and Dahlem 2023) to infer the minimum post-mortem interval (Amendt *et al.* 2011; Rivers and Dahlem 2023). Necrophagous blow flies (Diptera: Calliphoridae) are usually the first colonisers, arriving shortly after death if the conditions are appropriate (Anderson and VanLaerhoven

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1996; Tomberlin *et al.* 2011; Weidner *et al.* 2016). However, although blow flies are usually the first colonisers, the species involved vary with geographic region, habitat, and microhabitat (Anderson 2020). Within a region, extrinsic and intrinsic factors, which include adaptation, predation, temperature, rainfall, light intensity, elevation, and anthropogenic activity, can impact the abundance, species diversity, and oviposition timing of blow flies (Campobasso *et al.* 2001; Sharanowski *et al.* 2008; George *et al.* 2013; Bugajski and Stoller 2017). Some blow fly species have been shown to be attracted preferentially to carrion in urban, peri-urban or rural scenes (Hwang and Turner 2005; Smith *et al.* 2023), others are reported to prefer carrion inside or outside residences or vehicles (Anderson 1995, 2011; Malainey and Anderson 2020), and others are impacted by elevation (Moophayak *et al.* 2014; Gemmellaro 2019). Many species have seasonal (Archer and Elgar 2003; Boudreau *et al.* 2021; Forbes *et al*. 2022; Smith *et al.* 2023) and light intensity preferences (Sharanowski *et al.* 2008; Majola *et al.* 2013; Mashaly *et al.* 2020).

Within-habitat variations, such as that of light intensity, impact blow fly species and diversity. Some species show a preference for shady or sunny areas, with, for example, *Calliphora* species more commonly found in shade and *Lucilia* species preferring direct sunlight (Smith 1986). In addition, some studies have shown higher abundances of blow fly species in cooler, shaded areas, potentially highlighting a protective factor for heat-sensitive species. The inverse has also been recorded, with species known to have sensitivity to cold temperatures shown to seek out oviposition substrates located in warmer, sunlight-exposed areas (Shean *et al.* 1993; Sharanowski *et al.* 2008).The presence of insects on a cadaver that do not fit with the environment in which it was found may suggest that the body has been moved from the death scene to a secondary site, with the insects indicating the scenario of the original site (Madra *et al.* 2015; Weidner *et al.* 2017). For this reason, understanding microhabitat preference is forensically important.

The goal of this study was to understand the impact of sun and shade on species and abundance of Calliphoridae species in the Metro Vancouver region, British Columbia, Canada, in three different areas. We hypothesised that different environments would have unique compositions of species. Meat-baited traps were used to collect adult blow flies and blow fly eggs. Such traps are very commonly used to assess Calliphoridae populations worldwide because they are easy to deploy and cost effective (Anderson 2000; Hwang and Turner 2005; Brundage *et al.* 2011; Farinha *et al.* 2014; Weidner *et al.* 2017). They have been shown to be excellent predictors of necrophagous insect communities when compared with whole carrion–baited traps (Farinha *et al.* 2014; Weidner *et al.* 2015, 2017); however, it is noted that differences may occur between bait and carrion collections (LeBlanc *et al.* 2021).

MATERIALS AND METHODS

This study was part of a larger survey looking at the effect of season and urbanisation on Calliphoridae diversity in Metro Vancouver (Smith *et al.* 2023). The study location, trap design, experimental design, insect identification, and statistical analyses are described in full in Smith *et al.* (2023) and are summarised below.

Study locations

This study was conducted from 1 July to 5 August 2019, with 18 traps deployed weekly, accumulating a total of six trapping days over a six-week period within Metro Vancouver, British Columbia, Canada, using three locations that differed in geographic area. The Metro Vancouver area lies within the Coastal Western Hemlock zone (Pojar *et al.* 1991). Each site was chosen to be representative of urban, rural, and mixed environments. The Birkdale Place (BP) site was urban, in a residential area of Burnaby (49.2714 N, –122.9423 W) at 103 m above sea level. It includes residences, a wooded landscape, shrubbery, and a stream. The Simon Fraser University (SFU) site was in a mixed urban–rural area (49.2758 N, –122.9148 W) at 330 m above sea level. The trap site was in a locked complex away from flora, although the general area is wooded with shrubbery. The Rural site was in Langley (49.1019 N, –122.5435 W) at 90 m above sea level and consisted of a field and wooded landscape, surrounded by similar properties.

Baited traps

The baited bottle traps were adapted from a design by Hwang and Turner (2005) and constructed from 2-L clear, plastic pop bottles (Smith *et al.* 2023). Each trap had two 2-cm^2 holes cut to allow ingress. Each trap was baited with beef liver (35 ± 2 grams), which was purchased from Surrey Meat Packers, Surrey, British Columbia and was maintained frozen in pre-measured amounts until thawed for placement. Three traps were deployed in full sunshine, and three traps were deployed in shade at each site. The traps were deployed on clear sunny days with no rain forecast to maximise catch and diversity. The traps were hung approximately 1.2 m above the ground and were well spaced (minimum 6 m). Traps were deployed between 7:00 and 8:00 a.m. and collected at 7:00–8:00 p.m., local time. Eighteen traps were deployed weekly from 1 July to 5 August 2019, accumulating a total of six trapping days over a six-week period, and were baited with the same amount and type of beef liver (35 ± 2 grams), as described. Nine traps were placed in shaded areas, and the other nine were placed in areas maximising sunlight. The shaded traps (3 per location; n = 9) were hung approximately 1.2 m above the ground in areas that contained dense foliage or infrastructure to block sunlight. An exception was made for the BP shaded traps, which were hung lower due to limited foliage cover availability.

Liver bait on which eggs had been laid was removed and placed in rearing jars and eggs were raised to adulthood for identification. Adult specimens were frozen for 24 hours, then air dried and identified using a Leica EZ4 dissecting light microscope (Leica Camera AG, Wetzlar, Germany). Calliphoridae were identified to species using Jones *et al.*'s (2019) North American Calliphoridae key. Other specimens were identified to order using information by Millar *et al.* (2000).

Data from each of the three traps in the same habitat were respectively pooled for each date and location, providing for 18 samples each for sunlit and shaded traps (6 dates × 3 locations). Data pooling might have produced an exaggerated effect when analysing differences between trapping locations due to microsite conditions. Descriptive statistics assessed the distribution and normality of all variables. Chi-square tests were performed to assess significant changes ($P < 0.05$) in *species ID* against *shade* versus *sunlit* traps within and between all trapping locations. Statistical analyses were performed using IBM SPSS Statistics, version 24, software (IBM, Armonk, New York, United States of America; https://www.ibm.com/products/spss-statistics).

RESULTS

A total of 678 (n = 678) specimens, identified to 13 groups, were collected from all traps, with statistically significant differences ($X^2 = 41.111$, $df = 12$, $P = 0.000$) observed in species composition between shaded and sunlit traps. Tests were then performed for individual taxa and showed that only *Lucilia illustris* (Meigen) had significant ($P < 0.002$) differences in abundance, with a higher proportion observed in sunlit traps. Three insect orders (Dermaptera, Diptera, and Hymenoptera) were identified, with Diptera representing the majority, followed by Hymenoptera (Table 1). Dermaptera were caught rarely. Four dominant species of Calliphoridae were identified: *Calliphora vicina* Robineau-Desvoidy and *Lucilia sericata* (Meigen) were represented equally, followed in abundance by *Calliphora latifrons* Hough and *L. illustris*. All other Calliphoridae represented only 10.4% of the captured blow fly population.

Table 1. Frequency (n) of adult specimens in sunlit and shaded traps at all trapping locations, July–August 2019, in Metro Vancouver, British Columbia, Canada

	Rural		BP		SFU		Total	Total	Total
Species ID	Sun	Shade	Sun	Shade	Sun	Shade	Sun	Shade	Catch (%)
Calliphora latifrons	6	8	11	11	11	13	28	32	60 (8.8)
Calliphora montana	1	0	0	0	0	0	1	0	1 (0.15)
Calliphora vicina	11	8	9	21	16	11	36	40	76 (11.2)
Calliphora spp.	1	1	1	0	0	1	2	2	4 (0.59)
Cynomya cadaverina	0	0	0	0	1	0	1	0	1 (0.15)
Lucilia illustris	25	6	12	1	1	1	38	8	46 (6.78)
Lucilia sericata	37	22	13	1	3	0	53	23	76 (11.2)
Lucilia spp.	10	7	3	0	1	0	14	7	21 (3.1)
Phormia regina	1	0	0	0	0	0	1	0	1 (0.15)
Non-Calliphoridae Diptera	54	59	13	18	14	13	81	90	171 (25.2)
Angioneura spp.	1	0	1	0	0	0	2	0	2 (0.29)
Dermaptera	0	0	0	5	0	1	0	6	6 (0.88)
Hymenoptera	54	44	35	15	31	34	120	93	213 (31.4)
Total	201	155	98	72	78	74	377	301	678

Species composition in sunlit areas

Three hundred and seventy-seven specimens from 12 identified groups were obtained from traps placed in sunlit areas (Table 1). Hymenoptera had the highest collected abundance, with *L. sericata* being the dominant Calliphoridae species. Most dominant groups including *C. vicina*, *C. latifrons*, and non-Calliphoridae Diptera, did not present significant differences ($P > 0.05$) in abundance between sunlit and shaded traps. However, Hymenoptera and *Lucilia* (*L. illustris* and *L. sericata*) species were collected more frequently in sunlit traps. Examination of sunlit and shaded traps within each trapping location revealed similar trends, with *Lucilia* species observed in equal or higher frequencies in sunlit traps compared with in shaded traps at all locations, whereas Hymenoptera were collected in higher frequencies in sunlit traps at the Rural and BP locations and in lower frequencies at the SFU location compared with in shaded traps.

Species composition in shaded areas

In total, 301 specimens from nine identifiable groups were recovered from traps placed in shaded areas, with the majority collected in the Rural location (Table 1). The highest abundance was recorded for Hymenoptera, with the dominant Calliphoridae species being *C. vicina*. Although accumulated totals of *C. latifrons*, *C. vicina*, and non-Calliphoridae Diptera were higher in shaded traps, these differences were minimal (± 10 specimens).

Captured versus *reared specimens: differences in species composition*

A combined total of 6187 blow flies were reared from eggs laid in the sunlit and shaded traps (n = 1995 and n = 4192 respectively), consisting of four identifiable species (*C. latifrons, C. vicina, L. illustris*, and *L. sericata*), with results demonstrating a higher likelihood of *C. latifrons* (n = 2797; 45.2)) and *C. vicina* (n = 1715; 27.7%) oviposition. A higher number of both *Calliphora* species were reared from the shaded traps (*C. vicina,* n = 1213; *C. latifrons*, n = 2427) compared with the sunlit traps (*C. vicina*, n = 502; *C. latifrons*, n = 370), with opposite results observed in both *Lucilia* species (shaded traps: *L. illustris*, n = 283; *L. sericata*, n = 229; sunlit traps: *L. illustris*, n = 343, *L. sericata*, n = 768). Analysis of eggs reared *versus* females captured during a collection period revealed distinct differences. High escape rates were observed in both shaded and sunlit traps (50.0% and 32.6% respectively), with higher escape rates observed in shaded traps (*C. vicina*: 52.4%; *L. illustris*: 50.0%; *L.* sericata: 66.7%) compared with in sunlit traps (*C. vicina*: 41.7%; *L. illustris*: 14.3%; *L. sericata*: 9.1%) for all species, except for *C. latifrons* (shaded and sunlit traps, both 61.5%). These numbers are probably much higher because the only way to prove that escape had occurred was by rearing species from eggs that were not captured as adults in that trap on that date. It is likely that some species that were trapped as adults may also have escaped. In addition, there is no way to determine whether male adults escaped.

DISCUSSION

Necrophagous insect species may be influenced by light intensity differences in sunlit *versus* shaded bait traps. The present findings showed a higher abundance and diversity of insects overall in sunlit traps compared with in shaded traps. These finding match results found in Saskatchewan, Canada on carrion (Sharanowski *et al.* 2008) and in other regions, including Saudi Arabia (Mashaly *et al.* 2020) and South Africa (Majola *et al.* 2013), indicating that certain insect species may prefer to colonise resources in sunlit environments. This finding likely is influenced by the increased decomposition rate of sun-exposed cadavers because these studies relate to collections from carrion, allowing insect succession to proceed faster with sun-exposed cadavers compared with shaded cadavers (Shean *et al.* 1993; Sharanowski *et al.* 2008). However, in the present study, the baits were always fresh and were not exposed long enough to have been impacted by increased temperatures: these data therefore indicate preference for sunlit areas rather than decomposition stage. In Portugal, however, Prado e Castro *et al.* (2011) found that more than twice as many Diptera were collected in shade rather than sun, although calliphorids were much more common in sun.

Of the collected Calliphoridae, both *Lucilia* species (*L. sericata* and *L. illustris*) were found more frequently in sunlit traps compared with in shaded traps, but only *L. illustris* had a statistically significant difference. This result indicated that light intensity might influence resource choice. Sharanowski *et al.* (2008) found that *L. sericata* colonised in greater abundance on sun-exposed carrion; however, they observed more *L. illustris* on shaded carrion. In Washington state, United States of America, *L. illustris* was more commonly attracted to carrion in a sunlit site than in shade (Shean *et al.* 1993). However, these studies were based on carrion exposure rather than bait traps.

No statistical differences were seen between sun and shade sites in any of the *Calliphora* spp. in the present study. This was unexpected because *Calliphora* spp. are traditionally considered to prefer shaded, cooler areas. However, this has particularly been noted for *Calliphora vomitoria* (Linnaeus) (Shean *et al.* 1993; Smith 1986), which was not collected in the current study. In earlier carrion studies in the Metro Vancouver area, *C. vomitoria* was regularly collected in forested areas (Malainey and Anderson 2020), but no *Calliphora* species were collected in sunlit pastureland (Anderson and VanLaerhoven 1996).

In Alberta, carrion studies indicated similar species in both sun and shade during early decomposition, although only one blow fly species, *Protophormia terraenovae* (Robineau-Desvoidy) was collected (Hobischak *et al.* 2006). In other studies, no differences were observed between blow fly species collected from carrion in South Africa (Majola *et al.* 2013), and in Portugal, studies using two piglet carcasses found no differences in *Calliphora* or *Lucilia* spp. in sun or shade, although overall numbers of Calliphoridae were much higher in sun (Prado e Castro *et al.* 2011).

Relatively similar numbers of adult blow flies were collected in sunlit and shaded traps in the present study. However, other studies suggest a higher abundance of blow flies in sunlit areas, such as in Mississippi, United States of America (Goddard and Lago 1985) and in Portugal (Prado e Castro *et al.* 2011), although these studies, again, were carried out on carcasses rather than with baited traps.

Bait traps have been used worldwide for more than a century to document fly species in various region and habitats (*e.g.,* Bishopp 1925; MacLeod and Donnelly 1956; Vogt 1974) and remain popular because they are inexpensive and easy to use and they collect insects in a relatively undamaged manner, thereby facilitating identification (Sanford 2017; Weidner *et al.* 2017). However, their value in directly representing species that colonise carrion and, thus, human cadavers, has been questioned (LeBlanc *et al.* 2021). In a New Brunswick study comparing flies collected with pork liver–baited traps *versus* pig carcasses, although bait traps were found to be valuable in documenting necrophagous fly communities, differences between the two bait types were found in the presence and assemblage of flies (LeBlanc *et al.* 2021). In contrast, a study in New Jersey, United States of America, comparing blow flies collected from carcasses *versus* beef liver–baited traps, found that all species that represented at least 1% of the overall adult and larval collection of blow flies were the same for both carrion and traps (Weidner *et al.* 2017). Similarly, a study in Portugal found blow fly species collected in baited traps were similar to those collected from carrion on the Iberian Peninsula (Farinha *et al.* 2014). These studies suggest that, although differences may occur, in general, baited traps are an excellent tool for surveying local necrophagous communities.

It was clear from this study and the larger survey (Smith *et al.* 2023) that many blow flies entered the traps, oviposited on the bait, then escaped. In the larger study, an escape rate of more than one-third was recorded across species and of up to two-thirds in some species (Smith *et al.* 2023). This measurement is an underestimate because escape rates can be determined only by the presence of eggs of a species of which no female adults are collected; there is no way to determine whether males of a species escaped or if female individuals of a captured species escaped. In addition, large numbers of predatory Hymenoptera, primarily yellowjackets and hornets, were attracted to the traps and were observed killing and eating both adult flies and eggs. This confounding factor, which was also observed in carrion studies in Australia (Archer 2003) and Brazil (Somavilla *et al.* 2019), impacts both species abundance and diversity results. The above observations indicate that, although such traps are useful in studies, improvements in trap design are needed to prevent escape and to discourage Hymenoptera.

Although significant differences between sunlit and shaded traps were observed only for *L. illustris*, preferences may still exist, and further study is needed to identify patterns of species composition between different light intensities. This study shows an overall preference for sunlit sites in necrophagous insects and also indicates that, in this study area, data on preferences for shade sites in *Calliphora* species, from other regions, may not be valid. Developing baseline data on carrion insects is vital to determine which species are present in an area and their location preferences in order to further inform forensic entomology casework and to encourage further research in this area.

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