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How attractive are mice carcasses, after being defrosted for 1, 3, 6, and 9 days, to *Calliphora vicina* and *Lucilia sericata*?

An independent research project submitted as part of the requirement of the BSc Forensic Science.

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Abstract

Forensic entomology is the study and application of insects and other arthropods within legal settings (Catts and Goff 1992). The *Calliphoridae* family are involved the estimation of the minPMI, which refers to the first-time insects colonise a body, and can be influence by many factors, such as temperature, insect species, and conditions at the scene. This study aimed to examine *Calliphora vicina* (*C. vicina*) and *Lucilia sericata's* (*L. sericata*) levels of attraction and preference to mice carcasses at different defrosted stages (1-, 3-, 6-, and 9-days).

The mice were thawed for the desired period of time (e.g., 1, 3, 6, and 9 days) in the bottom of a flytrap that has been fashioned out of a 2-litre plastic bottle – 1 mouse per 1 flytrap. Once all the mice had thawed, the flytraps (containing the defrosted mice) were placed into a small insect cage. 30 female and 30 male blowflies were released into the small insect cage and were allocated 6 hours to respond. This experiment was repeated three times, until all 180 flies were used. This procedure was conducted for both *C. vicina* and *L. sericata* on separate days, under the same conditions. Each fly was only used once and, after testing, was killed by freezing at -18° Celsius. Each mouse and flytrap were only used once and were disposed of correctly after the experiment ceased.

Despite the results being inconsistent with the current literature (Johansen et al. 2010; Aak et al. 2010; Byrd and Castner 2009), it was found that *C. vicina* prefer earlier stages of defrosted mouse carcass, whereas *L. sericata* prefer later stages. Additionally, no significant sex behavioural differences were observed for *C. vicina*, while such differences were evident for *L. sericata*. A few limitations were addressed, such as the design of the flytrap, because there were a high proportion of blowflies who did not respond. Despite these limitations, the data obtained from this study adds valuable insights into the behavioural patterns exhibited by *Calliphoridae*.

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1. Introduction

1.1 Forensic Entomology

Forensic entomology is the study and application of insects and other arthropods within a legal settings (Catts and Goff 1992). Lord and Stevenson (1986) define three principal areas of forensic entomology: urban entomology, stored-products entomology, and medicolegal entomology. Urban entomology deals with ligations and civil law actions involving termites, cockroaches, and other insect nuisances that accrue in the human environment (Guitart et al. 1998). Stored-products entomology involves controversy over arthropods and arthropod parts in food and other products; for example, insect debris in food from fast-food restaurants. The final category, and focus of this research project, is medicolegal entomology, which utilises arthropod evidence to help in the investigation of, often, violent crimes, including murder, suicide, and rape (Byrd and Toberlin 2019). Although forensic entomology is a relatively new field of study, for many years medical entomologists have worked alongside physicians and veterinarians in fields such as pathology, dermatology, and infectious diseases.

1.2 Estimating the Post-mortem Interval

Investigators aim to estimate when exactly the death took place and, although there is no scientific way make precise evaluations, a relationship between corpse fauna and patterns of colonisation can aid in determining the minimum period of time since death (minPMI) (Amendt et al. 2010). minPMI refers to the first time insects colonise a body, rather than the actual time of death. The minPMI determination employs two known principles: the time when the body was discovered, and the last time the individual was known to be alive. Correct minPMI estimation is crucial for an investigation of homicide, or other untimely deaths – insurance or inheritance (Henssge et al. 1995) – because such information may help identify both the victim and perpetrator (Catts 1990; Geberth 1996 cited by Wells 2009). The minPMI estimation is a dependent process that relies on many factors, including the conditions at the scene, insect species, and insect succession but can disclose the time of death from one day to more than one month (Smith 1986).

1.3 Brief History of Forensic Entomology

Early documented use of forensic entomology is scarce (Keh 1985). The earliest recording comes from 13th Century China: Sung Tzu's (1235 AD) discussion of a homicide in a book entitled "The Washing Away of Wrongs" (translated by McKnight 1981), where the murder weapon was revealed when flies congregated on the suspect sickle, resulting in the perpetrator's confession. During the 15th and 16th centuries, engravings in woodcuts and ivory – such as "Dances of the Death" and "Skeleton in the Tumba" – document insect-mediated patterns of decay, particularly the reduction of internal organs with large pieces of skin left intact (Benecke and Leclercq 1999). In 1767, biologist, Carl Von Linné, stated that three flies could decimate a horse as quickly as a lion could. In the 18th and 19th centuries, French medical Doctor – Orfila (1831) – observed mass exhumations in France and Germany, and understood and documented, the importance of maggots on decomposing and mummified remains. Orfilas' work was referenced by French hospital physician Bergeret (1855), who expressed cadaver interest, and was asked by the court to examine a child's corpse. The court proposed 5 questions; the first 4 were answered using classical forensic pathology, but the final question proposed to know the time interval between the child's birth and death. Bergeret answered the last question by using blowfly pupae and larval moth succession, and thus became the first to document post-mortem interval (PMI). Despite putative errors, Bergeret's work paved the way for later studies, cited by Nuorteva (1977), Keh (1985), Smith (1986), and Catts and Goff (1992). In 1894, Mégnin was credited for detailing the eight stages of human decomposition and the arthropods associated with them, and continued to develop his theory of predictable, ecological waves of insect life on corpses. This work was later expanded by Leclercq (1969) and Easton and Smith (1970).

During the first half of the 20th century, the foundations for reliable application of forensic entomology were laid by taxonomists Aldrich (1916) and Hall (1948 cited by Wellls and Greenberg (1992), interested in Sarcophagidae (flesh flies) and Calliphoridae (blow flies). Aldrich (1916) was the first to apply concepts by Boettcher regarding the identification of insect genitalia to determine the sex of flesh flies and, following this work, Hall (1948) wrote the 'Blowflies of North America', which laid foundations of modern medicolegal entomology.

With the advancement of technology, recent work – that was once laborious and challenging – by Lui and Greenberg (1989) employed scanning electron microscopy for swift identification of fly species. Moreover, advanced statistical analyses have developed the understanding of relationships between blowfly species (Stevens and Wall 1996). Although expanding research and publications are small in comparison to other scientific fields, publications in the 21st century began with 'The Utility of Arthropods in Legal Investigations' by Byrd and Castner (2000), and since then an average of 14 more research papers have appeared each year. Several more books by Greenberg and Kunish (2002), Wyss and Cheriz (2006), Gennard (2007), Amendt et al (2010 cites by Villet (2010), have expanded the knowledge of forensic entomology.

1.4 Flies (order Diptera)

The most crucial element of forensic entomology is the identification of insects and arthropod species: without correct identification, pupal cases (for example) may be mistaken for rodent droppings at a crime scene. If this forensically important evidence is lost, or otherwise in error, the estimation of minPMI becomes invalid (Byrd and Castner 2009) because few insect species share similar growth and developmental rates. The two major groups of insects who provide most forensic information are flies and beetles (Souza et al. 2008). The family *Calliphoridae* (order Diptera) – otherwise known as blowflies – are a family that provide accurate estimations for minPMI; they are attracted to carrion and excrement, and are the focus of this study.

The adult blowfly size ranges from 6-14mm in length and has evolved to form three regions: the head, thorax, and abdomen. Each segment performs specialised functions to aid the fly in locating sustenance and thus reproduce. The head is a hardened capsule that is the main area of sensory perception and the point of ingestion; it contains the brain, eyes, antennae, and mouthparts. The thorax, a region containing three fused segments, contains the external structures, legs and wings, and is used for locomotion. The final region, the abdomen, contains organs whose functions deal mainly with reproduction. The adult female fly will use the abdomen to lay eggs, oviposit, by extending the telescopic region of their abdomen, ovipositor, and favour oviposition near wounds or natural orifices so that larvae may develop in a moist area (Smith 1986).

Temperature is an important factor that affects decomposition and, for this reason, crime scenes' meteorological information is crucial for an accurate minPMI estimation. Such environmental conditions, including temperature and humidity, will also influence blowfly developmental cycle (Byrd and Castner 2009). The four developmental stages that blowfly will pass through are: egg, larva, pupa, and finally imago. The environmental conditions, such as temperature and humidity, will influence each state, for example, as temperature increases, so does the developmental cycle. It has been assumed that this species-specific influence on temperature and development is linear, within a certain temperature range (Greenber and Kunich 2002), and therefore, physiological age is given in accumulated degree hours (ADH) or accumulated degree days (ADD). ADH and ADD are defined as heat-energy units that represent the combination of chronological time and temperature, which can extend to each developmental stage (egg, larva, pupa etc). Fluctuation of temperature can influence the calculation of ADD and ADH

1.5 Decomposition

A corpse, whether human or animal, will change physically, biologically, and chemically over time and will emit a mixture of volatile organic compounds (VOCs), namely alkanes, alcohols, acids, esters, ketones, aldehydes, cyclic hydrocarbons, aromatic, sulphur- and nitrogen-containing compounds (Brasseur et al. 2012). The decomposition process begins approximately 4 minutes after death has occurred (Vass 2001) and can be categorised into three recognisable stages: autolysis, putrefaction and skeleton bone decomposition (diagenesis) (Gennard 2012). After 1 hour-3 days (Byrd and Tomberlin 2019), autolysis, or self-digestion, begins and the cells of the body are deprived of oxygen; pH decreases, and carbon dioxide and waste accumulate in the blood, which poisons the cells. Simultaneously, enzymes (including lipase, proteases, carbohydrases, etc) digest the cells of the body inside out, causing nutrient-rich fluids to be released (Goff 2010). Vass (2001), found that organs containing high enzyme and water content, such as the brain and liver respectively, begin this process more rapidly than other cells in the body, but it eventually affects all cells. Meanwhile, the corpse will experience stiffness (rigor mortis) followed by the stagnation of blood (livor mortis), and subsequently a reduction of body temperature (algor mortis) (Henge et al. 1995).

Putrefaction is the destruction of fat and muscle tissues by micro-organisms, such as members of anaerobic bacterial genera (*Clostridium* and *Bacteroides*), and consequently the catabolism of tissue into gases, liquids, and simple molecules. After 3-10 days (Byrd and Tomberlin 2019), fermentation gases (hydrogen sulphide, carbon dioxide, methane, ammonia, sulphur dioxide, and hydrogen) are produced, and the body increases in size, otherwise known as bloating (Gennard 2012; eventually the skin, unable to contain such gases, will rupture causing liquid to leak from orifices, including oral and anal cavities.

Shortly after the gases purge due to putrefaction, active decay begins (10-20 days) (Byrd and Tomberlin 2019). Phenolic compounds, such as indole, 3-methylindole, putrescine, cadaverine, and various fatty acids, are yielded by decomposing muscle, composed of protein, and fat (Statheopoulos et al. 2011). Insect activity, such as Calliphoridae larvae, and aerobic and anaerobic bacteria, are present in high numbers. After 20-50 days, advanced decay begins, and the corpse begins to dry. Fragments of flesh, skin and cartilage are present but most notably, there is a decrease in flies (Diptera), and an increase in other necrophagous insects, such as beetles.

Once soft tissue has been broken down, skeletal material remains commence another complex process called diagenesis, after 50-365 days (Byrd and Tomberlin 2019). This occurs when organic (collagen) and inorganic (hydroxy-apatit, calcium, and magnesium) components are altered by environmental conditions, especially moisture (Hedges 2002).

According to Vass (2001), the rate of decomposition to skeletisation can be estimated by the following formula: $Y = 1285/X$, where Y is the number of days to become skeletonised and X is the average temperature (in Centigrade) during decomposition. Although this formula produces only a rough estimation, it may be helpful to crime scene investigators in need of a timeframe to begin their investigation.

1.6 Insect Succession on Carrion

Insects are attracted to carrion immediately after death, frequently within minutes (Smith 1986), and colonise in a predictable sequence. A corpse, whether human or animal, provides a large food resource for many creatures and emanates changing odours as the carcass decomposes. These odours become more attractive to some

species and less attractive to others as time progresses (Nuorteva 1977); for example, it has been found that *Calliphora vicina* prefer fresh carcass when given a choice, whereas *Phormia regina* are often reported arriving later (Erzincliogl u 1996). Johensen et al. (2013) observed similar findings in their study, involving the oriented flight and landings of *Calliphora vicina* on differently aged mice carcass. The mice were defrosted from fresh (1 day) to 33 days old. It was found that attraction to fresh carcasses increased significantly from 36% towards fresh carcasses to 68%, 61%, and 65% towards carcasses ages 3 days, 6 days, and 9 days, respectively.

Some research suggests that *Calliphoridae* are the first to colonise carrion (Byrd and Castner 2009), and that fresher carrion is preferential to most *Calliphoridae* (George et al 2011; Johansen et al; Davies 2002); however, little research has compared *Calliphora vicina* and *Lucilia sericata* preference to defrosted mouse carcass. The aim of this study is to assess the attractiveness of defrosted mouse carcass of *Calliphora vicina* and *Lucilia sericata*. The species will henceforth be referred to as *C. vicina* and *L. sericata.*

2. Aims and Objectives

The main aim of this research is to assess the attractivity of *C. vicina* and *L. sericata* to mouse carcasses that have been defrosted over different periods of time (1-, 3-, 6-, and 9-days). This was achieved by completing the following objectives:

- Assessing the attractivity of carcass *C. vicina* and *L. sericata* to different defrosted mouse carcass.

- Determine the behavioural differences between sexes within a colony when competing for mouse carcasses.

- Compare the choice of *C. vicina* and *L. sericata* to existing publications.

3. Methodology

3.1 Flytrap

An empty, transparent, plastic 2 litre bottle (appendices 1) was used as a vessel for the purpose of trapping the flies. Most commercially available flytraps use chemical agents, herbal materials, soaps, or apple cider vinegar (Hinkle and Hogsette 2021) to attract and ultimately kill flies; however, for this experiment, it was crucial for the flies to remain alive so they could choose a mouse carcass. The flytrap device was similar to that of Gregoire et al. (2022), but modifications were implemented: the head of the bottle was cut 10cm from the top, inverted, and placed into the shaft of the bottle; then duct tape was used to fix the two segments together (appendices 2).

3.2 Carrion Source

This experiment was designed in a manner that would reflect an undisturbed, decomposing human corpse; therefore, unlike Johansen et al. (2014), the mice abdomen was not cut open but left to decompose without trauma. Defrosted mice carcass was used for this experiment because they provide a natural odour source, which is more attractive than a synthetic lure (Aak et al. 2010), to maximise the attraction of *C. vicina* and *L. sericata*. Moreover, they were small enough (average of 10cm x 4cm, weighing 50g) to fit into the bottom of the flytrap vessel. A mammalian body is preferential over a piece of meat, such as liver, for the attraction of flies because their microbial, biochemical, and decomposition activity is closer to that of human carrion (Carter et al. 2007); however, as recommended by Amendt et al. (2010), the ideal carrion source, because of its similarities to human, is a pig, weighing 20-30 kg in weight. Unfortunately, due to the available size, a pig was deemed too large.

The frozen mice were purchased from ‘Reptiles Plus Limited’ (2023) in January 2023, and each weighed an average of 50g. The mice were farm bred in ethical conditions and slaughtered humanely. All frozen mice were stored in a freeze at -18 $°C$ before the start of the decomposition process. A total of 4 mice were used per experiment for each stage of defrosting period. To begin the defrosting process, a mouse was removed from the freezer at 9 am, and was left to thaw in the bottom of their own

flytrap (1 mouse per 1 flytrap). After 3 days, the next mouse was removed and so on. This procedure was repeated until all conditions (1, 3, 6, and 9 days) were met.

3.3 Fly Identification

The flies used in this experiment were *calliphora vicina* and *lucilia sericata* and were purchased online from 'BugzUK' (2023) as puparia in January 2023. Adult *C. vicina* are identifiable by distinct morphological characteristics: they are typically 10-14 mm long; the head is black with red/orange bucca or 'cheeks'; the thorax is grey/black with a dark longitudinal stripe on the dorsal surface between the bases of the wing; the abdomen is a metallic blue with silver markings; and, overall, the body appears bristly (Limsopatham et al. 2018) (appendices 3). Adult *L. sericata* are similar in size and appearance but can be distinguished by their metallic green thorax and abdomen (William and Villet 2014) (appendices 4). Male and female *C. vicina* and *L. sericata* are easily distinguishable by the position of their eyes: in females, there is a large interspace between the eyes (appendices 5), whereas, in males, the eyes are almost touching (appendices 6) (Goff 2016).

3.4 Rearing Blowfly

The rearing of *C. vicina* and *L. sericata* was based on recommendations from numerous sources: Amendt et al. (2010); Byrd and Castner (2009); Byrd (1998); and Gennard (2012). To complete a satisfactory life cycle of a lab reared *Calliphoridae*, a carbohydrate source is required for energy, together with water and protein (Gennard 2012). Some adult *Calliphoridae*, including *C. vicina* and *L. sericata*, are regarded as anautogenous insects, meaning females require two protein thresholds to develop mature oocytes (cells in the ovaries) (Rivers 2014). The first threshold allows the yolk deposition to occur in all available oocytes and the second threshold allows extensive yolk deposition followed by oosorption (resorption of ripe eggs) and maturation of small egg clutches (Wall et al. 2002). Males, however, require protein for the development of their genital glands for insemination (Collatz 2006). The protein source selected is important because, as found by Estrada et al. (2009), artificial diets can negatively affect Calliphoridae development. Pork liver was chosen as a protein source because it is inexpensive, convenient, does not undergo rapid desiccation, and allows full cell development of flies (Byrd 1998). The size of the habitat is also important because if

the cage is too small the insects' wings will become damaged and flight will be affected; a cage ideally around 46 x 36 x 46 cm is recommended (Gennard 2012).

C. vicina and *L. sericata* were placed in two separated insect cages (measuring 60 x 60 x 60 cm (appendices 7), and subjected to a natural light dark cycle, with an average temperature of 18 $°C$ and humidity of 44.5%; readings were taken from the room's thermometer. After 143 hours, the flies emerged, and were provided granulated sugar (carbohydrate) and water, the former placed on a plastic lid (10cm) and the latter absorbed onto a paper towel, which was placed in a plastic cup (10cm) (appendices 8). Liver was placed in a shallow plastic dish (10cm x 5cm) (appendices 9) in the cage 7 days after emergence and, after 24 hours, was removed. After 3 days, another round of liver was placed in the cage for 3 hours, and removed thereafter to prevent oviposition. It was desirable for females to have fully developed ovaries to increase the attraction towards carrion (Hayes et al. 1999). The flies were starved 24 hours prior to the experiment starting. 180 flies per species were used in total, with an equal number of females and males (90 per sex).

3.5 Problems with Laboratory Rearing

Issues arise when rearing large larval masses in a laboratory environment. It is important to keep the ratio of larvae to food low (ideally 1:1 of maggots to food in grams) because, if the ratio becomes too high, the metabolic heat produced by the larvae will cause the ambient temperature of the environment to rise, thus shortening their developmental duration (Goodbrod and Goff 1990). Such an event is undesirable because, unless maggot mass heat is considered, minPMI estimates derived from their development will also be shortened. Fortunately, this can be monitored with a temperature probe inserted into the larval food source.

Further issues arise when the flies are not field collected because, although there is limited research in this area, decreased genetic homogenisation within a laboratory raised colony may affect their development (Hartmann et al. 2021). Considering this, colonies used to produce growth models for minPMI estimation (Greenberg 1992; Byrd and Allen 2001; Grassberger and Reiter 2001) could be inbred, although some forensic entomologists argue that this does not affect experimental conditions (Wells and Kurahashi 1994; Tarone 2007).

3.6 Final Experiment Design

The experiment was conducted between 10:00 and 16:00 hours (Aak and Knudsen. 2011) because flight activity of *Calliphoridae* is significantly depressed in the dark (Smith 1987) and peak flight activity was found to be around midday (Hedstrom and Nuorteva 1971). The bottle flytraps that contained the mice from different defrosted periods (e.g., 1, 3, 6, and 9 days) were moved into a small insect cage, measuring 30 x 30 x 30 cm (appendices 10). To begin the experiment, 30 male and 30 female blowflies were released into the small insect cage containing the flytraps and were given 6 hours to respond. This was repeated 3 times, until all 180 flies were used.

Each fly was only used once and, after testing, was killed by freezing at -18° Celsius. Each mouse and flytrap were only used once and were disposed of correctly after the experiment ceased. This procedure was conducted for both *C. vicina* and *L. sericata* on separate days, under the same conditions.

3.7 Limitations of Methodology

Although the flytrap design prevents flies from escaping, it is possible for blowflies to leave once inside and, although this was not observed, considering the duration of the experiment, there was a high probability of this transpiring. Such an event can be mitigated when testing a fly individually (Johansen et al. 2013); however, this was deemed undesirable because it would not allow natural interaction between male and female flies. Moreover, this scenario would not replicate an authentic response to carcass.

Moreover, it should be noted that the plastic bottle flytrap may impede the *Calliphoridae* from effectively utilising their visual capabilities for the detection of the mouse carcass.

3.8 Data Analysis

To evaluate the responses of *C. vicina* and *L. sericata*, IBM SPSS Statistic software was used for statical analysis to establish if the choice of defrosted mouse was significant between males and females of both colonies. Using descriptive statistics, the data showed no major deviations from normal distributions and the variances were equal; thus, a one-way analysis of variance (ANOVA) was used to compare the means

of three or more independent groups (e.g., 1, 3 etc) and one variable (sex) (Cassidy 2005).

4. Results

A total of 180 blowflies per species were used (360 total). The flies' responses were determined by counting the number of flies that had entered the flytrap once the experiment ceased. Each flytrap contains a mouse had been defrosted for different periods of time (e.g., 1, 3, 6, 9 days. For ease of explanation, the flytrap will be referred to as the defrosted period of time; for example, the flytrap containing the one-day defrosted mouse carcass = flytrap 1. It was necessary to include flies who were not in any flytrap once the experiment ceased, and flies who had died during the experiment.

The category 'no choice' indicates that the fly was not in any flytrap once the experiment ceased and the category 'dead' indicates that the fly died before choosing a flytrap that contained a defrosted mouse. Both *C. vicina* and *L. sericata* were observed ovipositing on the mouse carrion; however, *C. vicina* began to oviposit immediately, whereas *L. sericata* took approximately 2 hours to begin to oviposit after experiment's commencement.

4.1 *Calliphora vicina* Responses

4.1.1 Female

Out of 90 female *C. vicina* flies, a total of 11 were not in any given flytrap illustrated by 'no choice' in figure 1; this was approximately 3 times more than the number of females who chose flytrap 6, which was the least popular choice, with a total of 4 flies (4.4%). No female flies died during this experiment. Flytrap 1 contained 55 flies in total (61.1%), and was the most popular choice for female *C. vicina*. 3-, 6-, and 9-day-old mouse were less popular. As shown by table 1, there was a significant difference between the choice of defrosted mouse carcass and female *C. vicina* (P=0.017).

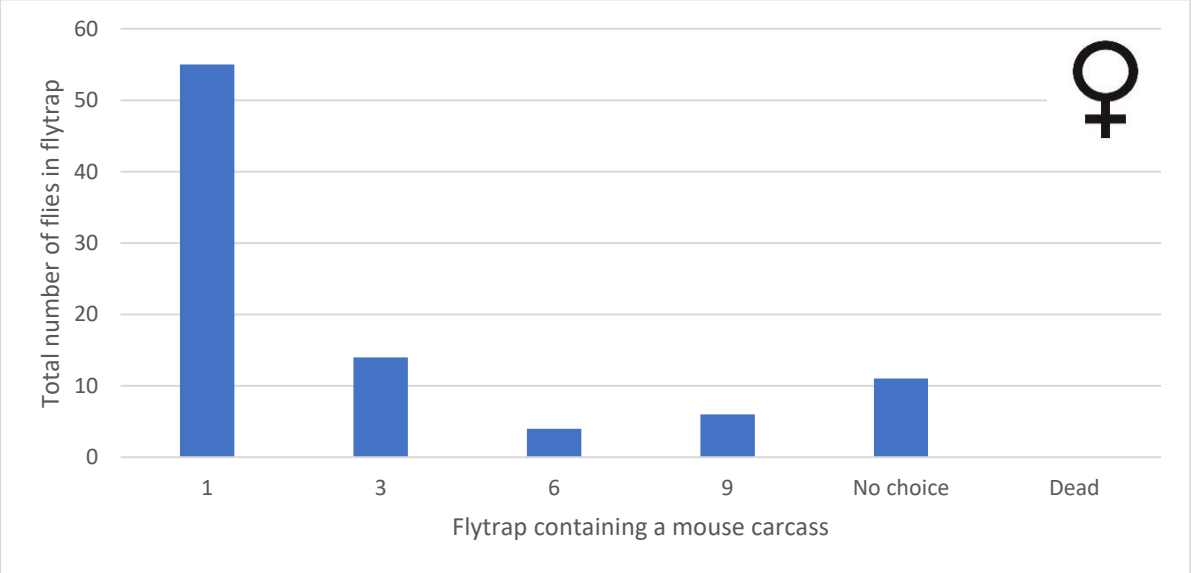

Figure 1: A column graph showing the total number of female *C. vicina* that entered the flytrap containing the defrosted mice, including those who did not make a choice and died. The number on the x axis represents the number of days that the mice were defrosted, e.g., 1 = defrosted for 1 day.

4.1.2 Male

Out of 90 male *C. vicina* flies, 7 made 'no choice' and 1 died. Like female *C. vicina* flies, the most attractive choice for male *C. vinca* was flytrap 1, with a total of 41 in the trap (45.6%) (figure 2). As shown by figure 2, male flies did not show an obvious preference between flytraps 3, 6, and 9. There was a significant interaction between male and female *C. vicina* flies and the choice of flytrap (P=0.017) (table 1).

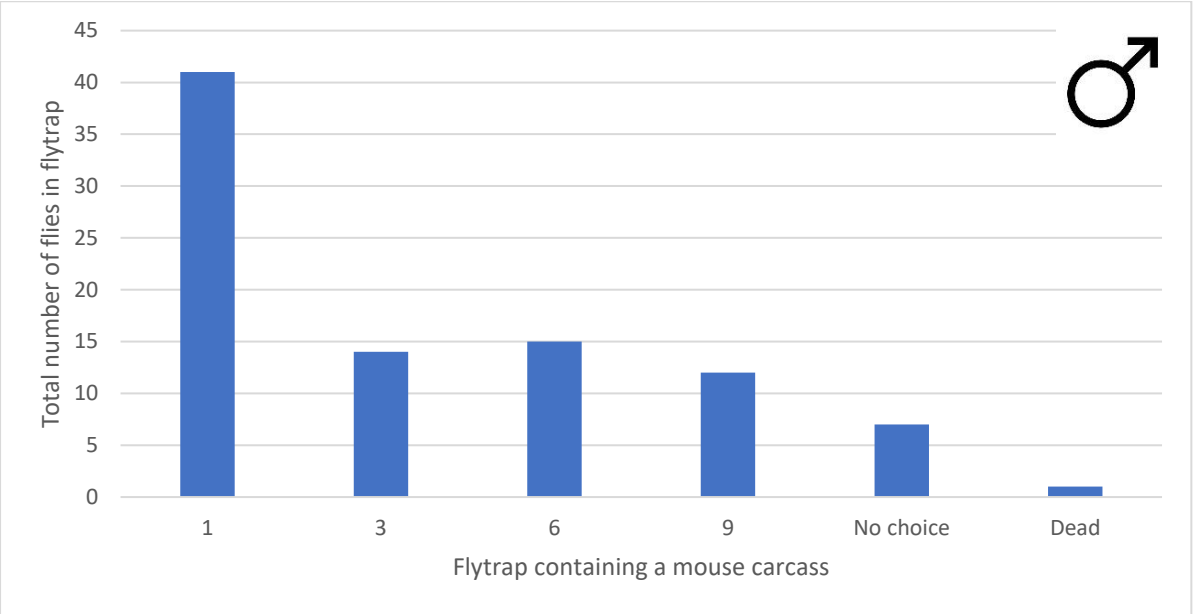

Figure 2: A column graph showing the total number of male *C. vicina* that entered the flytrap containing the defrosted mouse, including those who did not make a choice and died. The number on the x axis represents the number of days that the mice were defrosted, e.g., 1 = defrosted for 1 day.

4.2 *Lucilia sericata* Responses

4.2.1 Female

There were 90 female *L. sericata* in total and out of which 1 died and 12 did not enter any given flytrap as shown in figure 3 by 'no choice'. The flytrap containing the highest number of female flies was flytrap 9, which contained 46 in total (51.1%), and the least popular option was flytrap 6, which only contained 2 flies (2.2%) (figure 3). Flytraps 1 and 3 were equally as popular between the female flies, 16 and 15 in total, respectively (17.8%, 16.7%). There was a significant difference between the choice of defrosted mouse and female *L. sericata*, where P=0.004 (table 1).

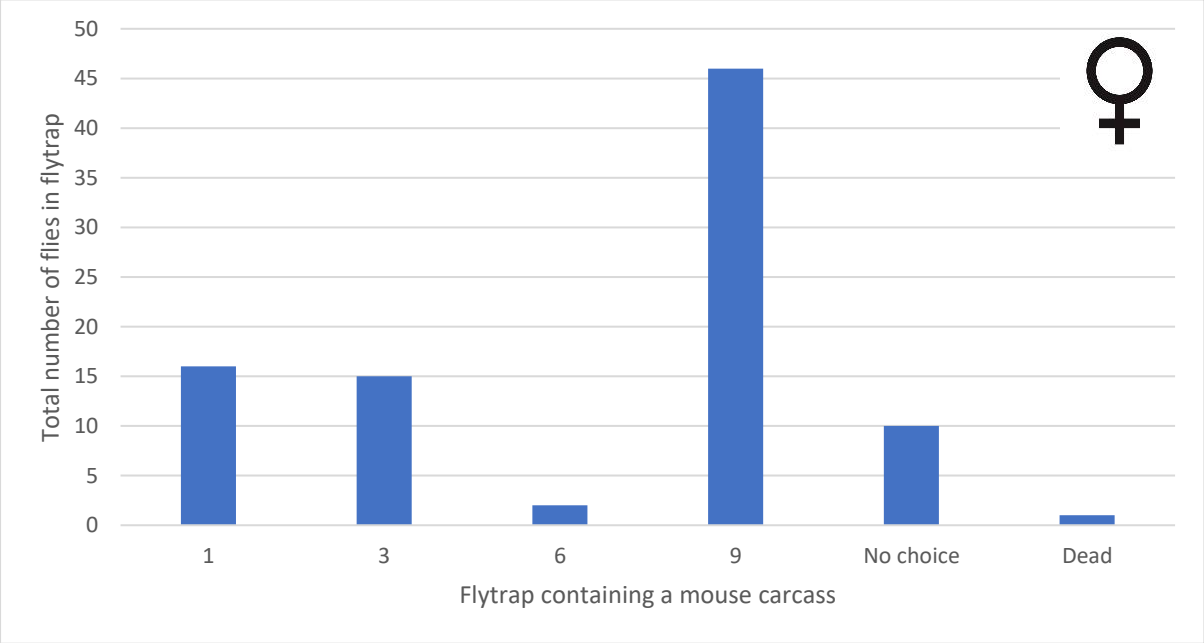

Figure 3: A column graph showing the total number of female *L. sericata* that entered the flytrap containing the defrosted mouse, including those who did not make a choice and died. The number on the x axis represents the number of days that the mice were defrosted, e.g., 1 = defrosted for 1 day.

4.2.2 Male

Out of 90 male *L. sericata* flies, 15 made 'no choice' and 1 died (figure 4). The flytrap containing the highest number of flies was flytrap 1, which contained 26 male flies in total (28.9%). Male *L. sericata* showed no major preference to flytrap 3, 6, and 9, containing 17, 15, and 16 flies, respectively (18.9%, 16.7%, 17.8%). Table 1 shows that were was not a significant difference between male *L. sericata* and the preference of decomposing mouse carcass (P=0.390).

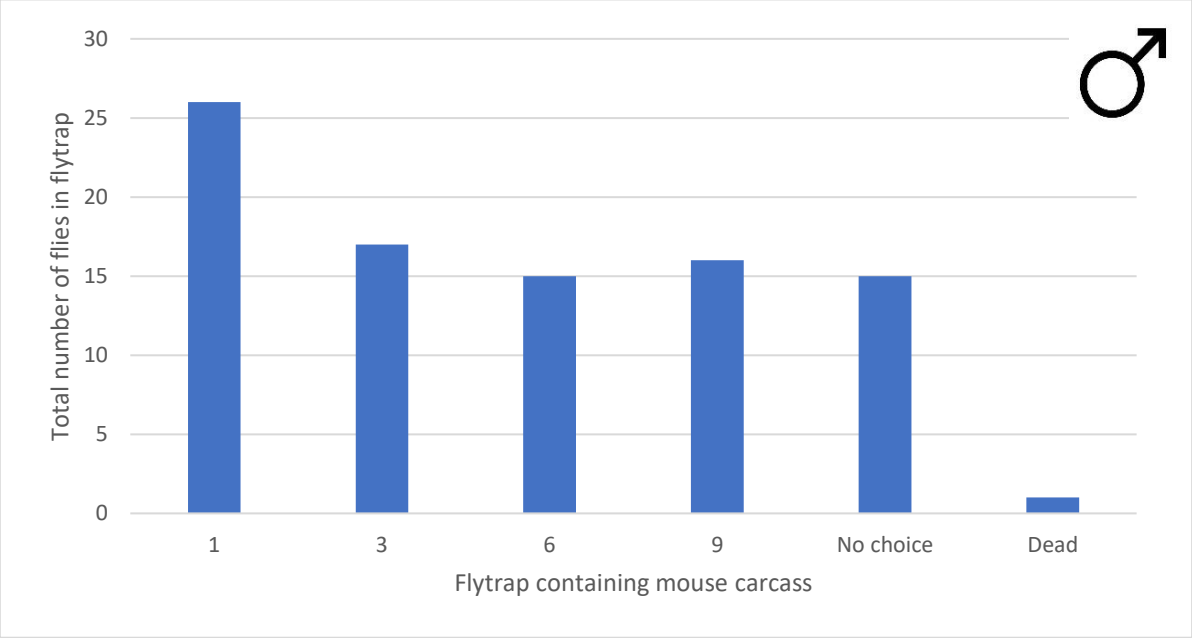

Figure 4: A column graph showing the total number of male *L. sericata* that entered the flytrap containing the defrosted mouse, including those who did not make a choice and died. The number on the x axis represents the number of days that the mice were defrosted, e.g., 1 = defrosted for 1 day.

4.3 ANOVA

The statistical analysis conducted on IMB SPSS Statistics, AVOVA, tested whether there was a significant difference between the choice of decay and sex of the fly, excluding ‘no choice’ and ‘dead’. With a confidence interval of 95%, a p-value less than 0.05 indicates a statistically significant difference between the two variables. Conversely, if the p-value exceeds 0.05, it implies that there is no significant statistical difference.

There was a significant difference observed between male and female *C. vicina* in terms of their preference for defrosted mice carcass (P=0.017, P=0.017). Additionally, there was a significant difference between female *L. sericata* and the choice of defrosted mice carcass (P=0.004), while there was no significant difference between male *L. sericata* (P=0.390).

Table 1: Statistical analysis to establish whether there was a significant difference between the choice of decay and sex of the fly (excluding ‘no choice’ and ‘dead’), and, if so, how significant. If the p value is <0.05, this means that there is a significant difference between the two variables; if the p value is >0.05, there is not a significant difference (Di Leo and Sardanelli 2020).

Male *C. vicina*	ANOVA, F=6.331, d.f.=3 and 8, P=0.017
Female *C. vicina*	ANOVA, F=6.331, d.f.=3 and 8, P=0.017
Male *L. sericata*	ANOVA, F=1.141, d.f.=3 and 8, P=0.390
Female *L. sericata*	ANOVA, F=10.279, d.f.=3 and 8, P=0.004

5. Discussion

5.1 Overall Attraction

Carcasses provide a rich but ephemeral nutrient source for arthropods, attracting a dynamic and diverse fauna over time (Amendt et al. 2010.) Decomposition is a complex process, where a diverse range of chemical attractants are released from a cadaver as it undergoes decay (Henge et al., 1995), causing some species to become more, or less, attracted as time progresses – this is especially true within the *Calliphoridae* family (Byrd and Castner 2009). This experiment's results suggest, female and male *C. vicina* and *L. sericata* exhibit the ability to discriminate between defrosted mice carcasses of different ages and displayed differential preferences towards them. *C. vicina* females were most attracted to the freshest mouse carcass (1-day-old, 61.1%), whereas female *L. sericata* were most attracted to the oldest mouse carcass (9-day-old, 51.1%).

In accordance with the present results, research suggests *C. vicina* are most attracted to fresher carcasses (Anderson 2010; Shah and Fatima 2007). There is conflicting research on *L. sericata's* level of attraction towards carcasses in the stages of decomposition. An early study conducted by Lane (1975) reported that *L. sericata* were attracted to carcasses only 76 hours after death and described them as 'late primary invaders', which is consistent with the results in the current study. *L. sericata* may colonise carcasses later because they are considered poor competitors, exhibiting negative features, such as reduced body size and reduced survival due to intraspecific competitions (Smith and Wall 1997; Prinkkila and Hanski 1995; Kheiralla et al. 2007). Conversely, it is theorised that *C. vicina* are typically first to colonise carcasses, and prefer fresher decaying carcasses, because their growth rate is much greater than other species, and thus have greater nutrient requirements (Smith and Wall 1997). Moreover, *C. vicina* may not be impacted by interspecific competition, due to their large size, because they have evolutionary advantage compared to smaller species (Rivers 2014).

However, contrary to Lane (1975) and this study's findings, Anderson (2019), The Australian Museum (2009), and Byrd and Castner (2009) found that *L. sericata* are the first insects to attend a corpse, and prefer fresher carcasses. Explanations for the differences in attractivity between *C. vicina* and *L. sericata* might include opportunistic

and adaptation behaviours that the two species exhibited – both demonstrate considerable plasticity in development, growth, and adaptation to their environment (Rivers 2014). Consequently, *C. vicina* and *L. sericata* are distributed worldwide but favour urban habitats, where they frequently infest human remains (Smith 1986). The colonisation of human remains can be influenced by micro-environment conditions, such as the environment, location, and exposure to sunlight (Mann et al. 1990); therefore, necessitates thorough consideration when entomological evidence is employed in a criminal investigation. According to the study conducted by Bonacci et al. (2009), it is imperative to assess forensic evidence on a regional and urban scale rather than relying on general global generalisations; furthermore, the research emphasises the significance of obtaining a greater volume of data to enhance accuracy in medio-legal cases. The findings of this experiment contribute to the improvement of accuracy levels by providing insights into the behavioural differences of *C. vicina* and *L. sericata* to mouse carcasses in the United Kingdom.

5.2 Chemical Communication

Carcass inhabitants can perceive their environments using a variety of chemical signals, otherwise known as semiochemicals, either to detect long-distance carcasses, to release clutches of eggs, or to attract a mate (Tomberlin et al. 2011). Semiochemicals can be categorised into pheromones, chemical signals used to communicate with conspecifics, and allelochemicals, utilised in communication with allospecifics (El-Ghany 2019). The ability to detect semiochemicals via varied receptors is referred to as olfaction, or the sense of smell (Rivers 2014); inhabitants utilise this sensory information to navigate to their preferred carcass.

The results of this study demonstrate that female *C. vicina* were most attracted to the 1-day-old mouse carcass (61.1%) and were less attracted to the 3-, 6-, and 9-day-old mouse carcasses, (15.6%, 4.4%, and 6.7%, respectively). This implies that the 1-day-old mouse carcass released the most attractive VOCs, thus attracting the most female blowflies; however, this discovery is inconsistent with the results of a similar study conducted by Johansen et al. (2013), which explored oriented flight and landings in male and female *C. vicina*. The study revealed that the most attractive mouse carcasses were the 3-, 6-, and 9-day-old defrosted mice, (68%, 61%, and 65%, respectively), whereas the 1-day-old mouse carcass was less attractive (36%). It was

found that, while conducting gas chromatography-mass spectrometry (GC-MS), there was a significant correlation between the amount of chemicals collected and the number of female blowflies responding. For example, female *C. vicina* were most attracted to the 3-day-old defrosted mouse carcass (68%), which was found to contain the highest concentration of three VOCs, namely butylated hydroxyl toluene, 3-hydroxy-2-butanone, and nonanal.

An explanation for the differences between results could be that Johansen et al. (2013) used a different methodology than the current experiment, where blowflies were released and tested individually. The blowflies that were already entrapped may have communicated via conspecific releaser pheromones, produced in the glandular (exocrine) epidermal cells – used to convey alarm, sex attraction, oviposition stimulation, and mate recognition – and beckoned other blowflies. However, Brodie et al. (2014) has challenged *Calliphoridae* conspecific communication, suggesting *L. sericata* and *Phormia regina* seeking a suitable oviposition location do not respond to oviposition pheromones but use semiochemicals associated with feeding flies as resource indicators; this suggests they might chance that resources are appropriate for egg-laying, since there are flies already laying there. The outcome of this study may provide valuable insights into the interactions between male and female *Calliphoridae* when competing for resources, and thus emulates a more pragmatic scenario than the study conducted by Johansen et al. (2013).

This study's mice's decomposition stages range between autolysis (1 hour-3 days after death) and putrefaction (3-10 days after death). Different bacterial activity on decomposing carcasses causes different VOCs to emanate at specific times, informing blowflies that there is high-quality larval medium or limited food (Byrd and Tomberlin 2019). A recent study conducted by Verheggen et al. (2017) revealed that carcasses emit over 800 VOCs during the process of decomposition, and that temperature influences the biological activity of 'catalyst' organisms (e.g.: fungi and bacteria). Warmer conditions cause higher VOC volatility and increased dispersal, whereas cooler temperatures cause odour to linger close to the body (Meyer et al. 2013). The experiment was carried out in an environment with a temperature of 18° Celsius, therefore, VOCs were able to effectively emit and disperse from the mouse carcass. The odour profile of carcasses changes during decomposition; carbohydrates are present during the earlier stages, while acids are prevalent in the later stages (Aak

et al. 2010). According to the results, *C. vicina* females were most attracted to the freshest mouse carcass (1-day-old at 61.1%), whereas female *L. sericata* were most attracted to the latest stage of decomposing mouse carcass (9-day-old at 51.1%). This suggests that female *C. vicina* are more attracted to VOCs emitted during the fresher stage of decay, e.g. carbohydrates and alcohols, whereas *L. sericata* are strongly drawn to VOCs emitted during the putrefaction stage, such as ammonia and sulphur dioxide.

A study conducted by Aak et al. (2010) found that female *C. vicina* are able to distinguish between authentic and synthetic odours; it was found that the odours emitted by dead fish and mice were significantly more attractive than liver, and a synthetic three-compound blend consisting of dimethyl trisulphide, mercaptoethanol and o-cresol. The findings of this study indicate that male *C. vicina* displayed a greater preference for the liver compared to synthetic odours; however, it is worth noting that the attractivity of mouse carcasses was not assessed. This study presents evidence of behavioural differences between male and female *C. vicina*, and states that females with fully developed ovaries (gravid) may respond differently towards authentic and synthetic odours. For example, Singh et al. (2015) found that non-gravid *L. sericata* respond to both faeces and human carcass, whereas gravid females prefer VOC emissions from a carcass.

5.3 Oviposition

It was noted that both *C. vicina* and *L. sericata* were witnessed ovipositing on all defrosted mouse carcasses; *C. vicina* commenced oviposition immediately, whilst *L. sericata* took approximately 2 hours before ovipositing. The oviposition site of choice for both *C. vicina* and *L. sericata* were the mice's moist orifices, notably the eyes and anus. This study supports evidence from previous observations (Smith 1986; Smith and Wall 1997; Bourel et al. 2003), who found that female blowfly (*C. vicina*, *L. sericata*, and *C. vomitoria*) frequently oviposition in exposed natural orifices as well as in wounds. Many factors influence *Calliphoridae* oviposition site choice.

One theory – the offspring performance hypothesis – suggests females prioritise the health of their offspring via oviposition site selection (Jaenike 1978) because a dense, secure location for their larvae to feed elevates their survival (Deonier 1940; Norrid and Murray 1964). This evolutionary strategy may be to prevent desiccation, and thus

results in large numbers of maggots in one area, ultimately generating heat and protecting the insects against adverse temperature drops (Byrd and Castner 2009). This strategy was observable in the results of this experiment because female blowflies from *C. vicina* and *L. sericata* congregated in the same flytrap (61.1% and 51.1%, respectively) and oviposited in the same locations.

However, Pacheco et al. (2017) found contradicting evidence, concluding that the anus and mouth were not preferred oviposition sites due to their inability to accommodate a large number of eggs – surface area had a greater impact on determining the location of oviposition. Moreover, Laake et al. (1938) found that female blowflies visit carcasses for reasons other than oviposition; it was observed that *Calliphora hominivorax* feed on the fluids associated with animal wounds, suggesting that they are an important source of nutrition. This study may explain why female *L. sericata* were more attracted to the 9-day-old defrosted mouse carcass, because it was observed that it was the moistest. *L. sericata* have been shown to deviate from typical daytime ovipositing behaviour, and were found to oviposit during cool nocturnal conditions (Catts and Goff 1992); this behaviour is uncommon, and may be dependent on the availability of artificial lights illuminating an area (Baldridge et al. 2006). Numerous factors may cause delayed colonisation, and thus also oviposition, leading to discrepancies when estimating the minPMI (Catts and Goff 1992). Investigating these factors may aid in a suspicious death; for example, identifying oviposition patterns, both the time to oviposition and site selection, can determine whether a suspicious death is considered a homicide rather than natural (Pacheco 2015).

Moreover, gravid females have been analysed using randomly amplified polymorphic DNA (RAPD) that evaluated their genetic relatability; it was found that gravid female blowfly showed a pattern of relatively high relatedness because they oviposit on the same corpse (Picard and Wells 2010). This compelling evidence holds the potential to significantly assist in criminal investigations involving instances of post-mortem relocation of a corpse.

5.4 Sex Behavioural Differences

Blowflies can effectively detect carcass, up to 1 mile away, within minutes by using their olfactory cues coupled with their flying ability (Byrd and Castner 2009). Decomposition tissue changes constantly, and blowflies rely on their olfaction and

vision to source the most desirable material, which can lead to sex-specific responses. For example, female blowflies attend carcasses that will provide a suitable oviposition site, whereas males seek out food and mates (Shorey et al. 1969). Therefore, it is hypothesised that female flies will demonstrate a significant affinity for a particular mouse carcass, while male flies will exhibit no particular attraction. This behavioural response was demonstrated by *L. sericata* but not *C. vicina*; the former demonstrated significant attraction (P=0.004) towards the 9-day-old mouse carcass, whereas males showed no significant attraction to any given mouse carcass (P=0.390). This result differs from *C. vicina* where both females and males showed significant interaction towards the 1-day-old defrosted mouse carcass (P=0.017).

An explanation for sex-behavioural responses is that blowflies possess sexually dimorphic characteristics, such as compound eye structures. Females' eyes are further apart, thus allowing females to rely on vision and olfaction in resource location; conversely males' eyes are closer together, allowing visual cues to identify females at close range (Rognes 2011). This evolutionary adaptation is critical for many *Calliphoridae*, who use their vision for resource acquisition, identifying objects, avoiding collisions, and making controlled landings (Brevault and Quilici 1999; Wall and Fisher 2001). New technology that allows precise chemical analysis has shown that male and female *Calliphoridae* have significant diversities in cuticular hydrocarbonds (CHCs) – organic compounds found on the cuticles of all insects – that act as close-contact pheromones (Butterworth et al. 2020). CHCs were found to be species-specific and sexually dimorphic, and may explain the behavioural differences between male and female *C. vicina* and *L. sericata*.

Sexual dimorphism is scarcely researched within the *Calliphoridae* family, and is underrepresented when estimating the minPMI (Macedo et al. 2018). Isomegalen diagrams are scatter plots that generate a predictable developmental pattern according to size of the larva and temperature, and could facilitate a quick and precise method of estimating the minPMI (Grassberger and Reiter 2001); however, they do not account for the size sexual dimorphisms of blowflies. It was found that female *C. vomitoria*, *C. vicina*, and *L. sericata* are 10% larger than males, which is an important factor to consider when using isomegalen models to estimate the minPMI. While this study does not contribute to the research of the isomegalen model, it does reveal

distinct differences between male and female blowflies, which could be attributed to their sexual dimorphism.

Male blowflies are often less researched, in comparison to females (Frątczak-Łagiewska and Matuszewski 2018), because they are not involved in the minPMI estimation, which constitutes the core objective of forensic entomology (Byrd and Castner 2009). Female blowflies are the ones involved in oviposition, and thus their presence is considered more relevant. Moreover, males exhibit diminished attraction towards decomposing animal or human remains relative to their female counterparts; male blowflies prefer carcasses for the purpose mating opportunities (Rivers 2014). For example, male *C. vicina* blowflies captured in the flytrap containing the 1-day-old mouse carcass (45.6%) exhibited the same response as the female blowfly, suggesting that they synchronised their movements with females to increase their chances of successful mating (Zeil 1986).

5.5 Limitations of Experiment

5.5.1 Experiment Design

The current experiment produced results that differed from previous publications (Johansen et al. 2013; Aak et al. 2010; Byrd and Castner 2009), suggesting the results were unreliable and invalid. If this experiment were to be repeated, it may be beneficial to include later stages of decomposition for a wider comparison; for example, additional 20 and 30-day defrosted mouse carcasses. Later stages of decayed carcasses contain high levels of acids (Cork and Hall 2007), which causes odour profiles to change and signal to blowflies that the food source is of poorer quality. Therefore, the addition of later defrosted mouse carcass may have allowed the *Calliphoridae* a larger comparison range.

This study may have been susceptible to a few confounding variables – a variable that influenced the independent or dependent variable that was unaccounted for (Frank 2000). One variable that can affect the attractivity of a carcass is its wrapping, covering, or concealing. This causes VOCs to remain trapped and, since the rate of diffusion of these compounds is reliant on thermal energy (Brownian motion – the random drifting of particles distributed in a gap depending on thermal energy) (Withers 1992), will slowly emit through small openings. Consequently, the attractive VOCs

might have been released at an exceedingly slow rate, leading to the blowflies in the unable to promptly detect the source of the carcass, demonstrated by the high percentage of flies, 11.9% average of both *C. vicina* and *L. sericata*, who did not choose a flytrap. Moreover, VOCs may have accumulated in the small bug dorm, and therefore it was perhaps difficult for both *C. vicina* and *L. sericata* to distinguish between them – this phenomenon may have occurred due to the small opening of the flytrap. To improve the study, conducting the experiment under a fume cabinet may prevent the accumulation of VOCs.

During this experiment, the blowflies were unable to use their innate flying abilities to detect the defrosted mice carcasses, consequently restricting their natural detection mechanisms (Amendt et al. 2010). If replicated, the inclusion of a wind tunnel bioassay – similar to the methodology employed in studies conducted by Johansen et al. (2013) and Aak et al. (2010) – could attain results that align with an authentic-setting. By implementing this addition, the flight orientation and landing responses of blowflies would be thoroughly examined, leading to the acquisition of supplementary data.

Executing an experimental design where *C. vicina* and *L. sericata* are released together may have produced different results that investigated resource partitioning between the species. Resource partitioning results in reproductive strategies employed by *Calliphoridae* when two species are competing for the same limited resource (Griffin et al. 2011). Such an approach would provide insights into the strategies employed by these species in competitive scenarios.

In future experiment, it would be advantageous to conduct additional chemical evaluations employed by GC-MS, to analyse the VOCs emitted by the defrosted mouse carcasses (Statheropoulos et al. 2005). This approach would eliminate the need for speculation regarding the types of VOCs that attracted the blowflies.

5.5.2 Temperature Tolerance

Calliphoridae are poikilothermic ectotherms, which means they cannot regulate internal body temperature through production of internal heat (Rivers 2014). Subsequently, the varying temperature of their environment affects their ability to efficiently search for carcasses, mate, and oviposit. If exposed to extreme temperatures, irreversible injury, such as cold shock (temperature decline) or heat

shock (temperature increase), can lead to death (Henssge et al. 2000). This means that insects peak in abundance during the warm summer months, but lower temperatures can lead to cold avoidance where several families (including *Calliphoridae*) refuse to oviposit (Campobasso et al. 2001). It has been reported the optimal temperature for *Calliphoridae* to perform at their optimum efficiency is 20-30° Celsius (Byrd and Butler 1996), and unfortunately, the ambient room temperature was 18° Celsius, which may have caused both *C. vicina* and *L. sericata* to underperform. This is another limitation of the experiment that may have caused unreliable and invalid results, as shown by the significant number of flies that did not choose a flytrap (11.9% average across species), as well as the few that died (1 male *C. vicina*; 1 male and 1 female *L. sericata*).

5.5.3 Carcass Attraction

Carcass size, colour, and shape may also impact its attractiveness to *Calliphoridae* (Erzinclioglu 1996). For example, *Calliphora vomitoria* blowfly, another forensically important species, show a significant preference for black cardboard, while coloured carboards attracted a limited number of flies (Benelli et al. 2018); moreover, *L. sericata* females have demonstrated their preference for blue cues (Wall and Fisher 2001). Research suggests that the aid of visual cues may become increasingly important when searching for a carcass at close range (Wall et al. 1992); although semiochemicals cues locate the desired carcass, other components of their respective mammalian host provide visual landing cues (Rakusin 1970) (e.g., eyes, nostrils, etc). This may explain why a high percentage, averaging 11.9% for both female and male *C. vicina* and *L.* sericata did not locate a flytrap containing a mouse carcass, because their visual cues were impaired by the plastic bottle flytrap.

The size of the carcass may also affect its attractiveness to blowflies; for example, *Calliphora vomitoria* reportedly prefer larger carcasses (Davies 1990), whereas *Lucilia richardsi* prefer small rodent carcasses (Nuorteva 1959). However, in general, larger carcasses tend to be more attractive to blowflies because it increases the surface area for egg-laying (Reibe and Madea 2010). This evidence could provide an explanation for the relatively high proportion (11.9% on average) of blowflies that did not select a defrosted mouse carcass, as the size of the carcass might have influenced their perception of its suitability as a resource.

Catts and Goff (1992) recommend using a domesticated pig, weighing approximately 23 kg, because it shares similarities to that of human remains, and thus has applications to real-world scenarios. Research involved in blowfly attraction to human remains is relatively sparse – in some cases under conditions not naturally encountered (Rodriguez 1997). The data acquired through this experiment provides valuable insights into the attraction patterns of *C. vicina* and *L. sericata*; however, it is important to acknowledge that these findings may not be directly applicable to real-world scenarios.

Another compounding variable that was unaccounted for was the amount of time that the mice carcass was frozen. Several studies used carcasses that were frozen and thawed prior to exposure, but Miscozzi has demonstrated difference in the character of decomposition between fresh and thawed carcasses. Frozen-thawed carcasses showed predominantly aerobic decomposition in the field whereas fresh carcasses showed anaerobic putrefaction. Also, decomposition of thawed corpses in northern latitudes is accelerated following prolonged freezing exposure.

Another contributing factor that was not taken into consideration was the duration of freezing for the mice carcasses. Various studies utilized carcasses that underwent the process of freezing and subsequent thawing before being exposed. However, Miscozzi's research has revealed differences in the decomposition characteristics between fresh and thawed carcasses. Specifically, frozen-thawed carcasses exhibited predominantly aerobic decomposition in natural environments, while fresh carcasses underwent anaerobic putrefaction. Furthermore, in northern latitudes, the decomposition of thawed corpses was observed to accelerate following prolonged exposure to freezing temperatures.

Another confounding variable that may have affected mouse carcass, was the amount of time the carcass was frozen before it was used in the experiment. Various studies utilise carcasses that were frozen and thawed prior to their exposure to blowfly (Johansen et al. 2010; Aak et al. 2010). However, Micozzi’s (1986) research has revealed differences in decomposition characteristics between fresh and thawed carcasses; frozen-thawed carcasses exhibited aerobic decomposition in natural environments, while fresh carcasses underwent anaerobic putrefaction. Furthermore, in northern latitudes, the decomposition of thawed corpses was accelerated following

prolonged exposure to freezing temperatures (Catts 1990). Unfortunately, the amount of time that the mice were frozen is undetermined, and thus this may have impacted the decomposition, and subsequently the attractivity.

6. Conclusion

Blowflies are an extremely important insect that can aid in legal investigations because they are typically the first to colonise carrion (Byrd and Castner 2009), thus provide beneficial entomological evidence. The blowfly development and ambient temperature is linear, and therefore is used to determine minPMI estimation. Although this study does not contribute to the minPMI estimation, it does highlight the complexity of blowfly behaviour, who can distinguish between the various VOCs of defrosted mice carcass. The results demonstrated that female and male *C. vicina* were most attracted to the 1-day-old mice carcass, while female *L. sericata* were highly attracted to the 9-day-old mice carcass, while male *L. sericata* did not show any preference towards a particular carcass. The findings of this study demonstrate that blowflies possess the ability to use their olfaction abilities to distinguish between the various VOCs emitted by defrosted mice carcasses (Tomberlin et al. 2011).

Another significant finding pertained to the difference between sex and choice of defrosted mice carcass. While female *L. sericata* exhibited a preference for the 9-day-old defrosted mice carcass, male blowflies did not demonstrate any significant attraction. This observation showcases the differences in resource-seeking behaviours of females and males: females exhibit a preference for carcasses as sites for oviposition, whereas males prioritise remains for mating and acquiring nourishment (Shorey et al., 1969). Moreover, there are sexual dimorphic characteristics, such as eye width and size, may also explain the differences between male and female flies. However, some publications disregard the sexual dysmorphic aspects of blowfly (Macedo et al. 2018), which may influence the minPMI estimation.

It was found that female and male *C. vicina* showed similar preference towards the freshest mice carcass (1-day-old), which was not in concurrence with prior literature (Johansen et al. 2013; Aak et al. 2010; Byrd and Castner 2009). There was a slight variation in methodology, as this experiment released the flies collectively instead of individually, which could account for these differences; it is plausible that female blowflies enticed males into the same flytrap using conspecific pheromones.

The methodology employed in the study had several limitations, which could have resulted in a significant number of blowflies not selecting a flytrap. One potential limitation was associated with the design of the bottle flytrap, as its small opening

restricted the exposure of attractive compounds – this is evident from the high percentage of flies that did not choose a flytrap. Moreover, the design of the flytrap prohibited blowfly from using their visual cues to access carrion and, many publications (Brevault and Quilici 1999; Wall and Fisher 2001) have shown that visual cues are equally important as olfactory cues. If this experiment were to be repeated, the inclusion of a wind tunnel assay would enable the blowflies to utilise their flying abilities for the detection of carcasses. Furthermore, GC-MS would facilitate precise identification of the VOCs that attract the blowflies, and thus would eliminate the need for speculation.

This study exemplifies the intricate behavioural responses of *Calliphoridae* towards defrosted mice carcasses, and highlights the multitude of variables that influence these decisions. Further research would be beneficial to expand the knowledge on forensically important insects who aid in legal investigations.

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8. Appendices

8.1 Learning Contract

The learning contract is an agreement between student and supervisor: it should clearly indicate what is expected from both sides. The text in Sections 2 and 3 provides guidance and can be modified to give more details reflecting what has been agreed, such as deadlines for submission of drafts and provision of feedback, word count limits/exclusions and number/timing of meetings.

Importantly, the document checklist helps students to follow the required procedures (e.g. ethical approval and risk assessment) and communicate what has been done to the supervisor.

The student should submit a draft of the completed form to the supervisor and request a meeting to discuss and finalise the content. Both the student and the supervisor are responsible for keeping a signed copy of this document and following what has been mutually agreed.

1. YOUR DETAILS

Student name: Rose-Ellen Toon

Degree Programme: Forensic Science

Proposed IRP Title or Set Project: Forensic Entomology

Supervisor name: Luca Manelli

2. As the student undertaking the above project I agree to:

- E-mail my supervisor on a fortnightly basis with a progress report
- Meet with my supervisor at least once a month to discuss progress and I understand that it is my responsibility to organise these meetings
- Comply with the terms of this learning contract and the guidance set out in the Guide to Independent Research Projects
- I understand that this is an *independent* project and that I am solely responsible for its completion
- I agree to comply with all ethical, laboratory and fieldwork protocols established by the Faculty.

3. As the supervisor of this project I agree to:

- Meet with the student undertaking this project on at least a monthly basis and to respond to the progress e-mails as appropriate
- To meet formally with the student during the first week in November to undertake the interim interview
- To provide guidance and support to the student undertaking this project bearing in mind that it is an *independent* research project. This is inclusive of commenting on drafts of the final report in a timely fashion.

3. DOCUMENT CHECKLIST

Research Proposal or Plan Attached? /☒ **YES** ☐ **NO**

☒ /☐ **YES NO** **Risk Assessment for fieldwork and evidence of COSSH assessment for all laboratory procedures (online risk assessment completed)**

8.2 Interim Comments

☒ YES / ☐ NO	**Completed booking for all field equipment**
☒ YES / ☐ NO	**Letters of permission where appropriate providing evidence of access to such things as field sites and/or museum archives**
☒ YES / ☐ NO	**Completed Ethics Checklist**

4. INTERIM INTERVIEW – Progress evaluation

Revised the original experiment; initially used VOCs on filter paper but we have decided that mice carcass is more suitable. I need to obtain more equipment (e.g. 14 more plastic bottles for the fly traps). Discussed the need for a control to run at the same time as well as more replicates and another condition. The next steps are to fill out another risk assessment, COSSH, and an ethics form.

Interim Review Date: 05/12/2022

5. Variance from the Independent Research Project Guide

The IRP assessment is normally governed by the guidance provided in the Independent Research Project Guide. Any variance in terms of format (e.g. technical report, scientific paper) and word limit should be agreed and specified here. Submission date cannot be changed unless evidence of mitigating circumstances is provided in accordance with the standard BU Guidelines.

Any changes? ☐ **YES** ☒ **NO** If YES please provide details below:

Both of the undersigned parties agree to be bound by this learning contract:	
Student Signature:	Rose-Ellen Toon
PRINT NAME:	Rose-Ellen Toon
Date:	22/12/2022

Supervisor Signature:	Recoverable Signature X  Luca Manelli Signed by: eb9506e7-271c-47e6-8f3c-812d63a71460
PRINT NAME:	Luca Manelli
Date:	26/12/2022

8.3 Risk Assessment

<table>
<tr><td colspan="2">1. Assessor:
2. Assessment Date:</td><td colspan="3">3. Assessment Review:</td></tr>
<tr><td>Rose-Ellen Toon</td><td>26/10/2022</td><td>Next Review Date:
26/10/2023</td><td>Reviewed on: Date:</td><td>Reviewed By:</td></tr>
<tr><td colspan="2">4. Summary of process or method (or make specific reference to written protocol to be used) :</td><td colspan="3" rowspan="2"></td></tr>
<tr><td colspan="2">Frozen mice carcass, 3 days thawed, and 9 days thawed.</td></tr>
<tr><td colspan="2">5. Key Activity/Task (in relation to exposure potential e.g. mixing, filling, spraying, etc.):</td><td colspan="3">6. People who <u>could</u> come to harm (number & roles e.g. students)</td></tr>
<tr><td colspan="2">Skin contact may occur when placing mice into fly traps</td><td colspan="3">One student, Rose-Ellen Toon.
One staff member, Luca Manelli.</td></tr>
<tr><td colspan="2">7. Duration of Exposure (minutes, hours and how often):</td><td colspan="3">8. Location and Conditions of Use (e.g. lab, room, temp etc.)</td></tr>
<tr><td colspan="2"></td><td colspan="3">C139 lab, in a fume cabinet.</td></tr>
</table>

1 student, 2 hours, once a day until experiment is complete. Estimate time: 1-4 weeks.						
9. Hazardous ingredients: (copy form/add more rows as req'd)		**10. Quantities Used**	**11. Workplace Exposure Limit (WEL)**	**12. Hazard and Precaution statements**	**13. Actual Potential Route of Exposure** (E.g. by inhalation)	**14.Datasheet Attached? Y/N**
A	Mice carcass	15 in total	N/A	H315: Causes skin irritation. H335: May cause respiratory irritation. P280: Wear eye protection/face protection.	• Inhalation • Skin contact • Eye contact • Ingestion	N
D					•	
E					•	
F					•	
G						
H						

15. Control Measures

1) Less hazardous alternatives

N/A.

2) Engineering controls

Use in fume hood to avoid inhalation.

3) Personal Protective Equipment

Lab coats, safety glasses, and gloves when working with insects and mice.

4) Storage

In freezer until needed. When thawing, they can be stored in the fly traps

Now mark in the letters from the list of 'Hazardous Ingredients' above to indicate potential danger:

16. Indication of Danger				**17. Route of Exposure**		**18. Chemical State**		**19. Flammability**		**20. Volatility**		**21. Dust rating**	
Very Toxic		Irritant	A	Inhalation	All	Solid	A	Flammable		Low	A	Low	A

Toxic		Sensitiser		Skin Contact	All	liquid		Highly flammable		Medi um		Mediu m	
Corrosive		Carcinogen		Eye Contact	All	Gas/v apour		Extremely flammable		High		High	
Harmful		Mutagenic		Swallo wing	All			Oxidising					
Biological Agent		Toxic to reproduction		Injectio n	All			Explosive					

22. First Aid Procedures (as advised from Material Safety Data Sheet)

If inhaled	**If skin contact**	**If eye contact**	**If swallowed**	**If injected**
Move to fresh air If breathing is difficult, give oxygen. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; induce artificial respiration with a respiratory medical device (use chest compressions alone if one is not available & heart stops). Immediate medical attention is required.	Wash off immediately with plenty of water for at least 15 minutes Obtain medical attention	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes Obtain medical attention	Do not induce vomiting Call a physician or Poison Control Centre immediately	Obtain medical attention

23. Spillage Procedures:	~~Obs~~erving the control measures outlined in section 15:			
24. Disposal Arrangements	Dispose mice in labelled waste, to be collected and disposed elsewhere. Dispose of PPE in normal waste, such as gloves, after each experiment is complete.			
Collection	**Swill down sink**	**Evaporation**	**In normal waste**	**Other**
			X	
25. Are the risks adequately controlled? (Write 'Yes' or 'No'):		Yes		

8.4 Data

Table 2: Numerical figures showing the amount of *C. vicina* caught in the flytrap once the experiment ceased.

Sex of fly	1	3	6	9	No choice	Dead
Male	10	6	6	3	4	1
Female	22	5	1	0	2	0
	1	3	6	9	No choice	Dead
Male	13	8	6	3	0	0
Female	12	3	3	6	6	0
	1	3	6	9	No choice	Dead
Male	18	0	3	6	3	0
Female	21	6	0	0	3	0
Average	1	3	6	9	No choice	Dead
Male	13.7	4.7	5.0	4.0	2.3	0.3
Female	18.3	4.7	1.3	2.0	3.7	0.0

Table 3: Numerical figures showing the amount of *L. sericata* caught in the flytrap once the experiment ceased.

Sex of fly	1	3	6	9	No choice	Dead
Male	9	9	5	2	4	1
Female	5	8	0	12	4	1
	1	3	6	9	No choice	Dead
Male	8	6	2	7	7	0
Female	6	0	0	21	3	0
	1	3	6	9	No choice	Dead
Male	9	2	8	7	4	0
Female	5	7	2	13	3	0
Average	1	3	6	9	No choice	Dead
Male	8.7	5.7	5.0	5.3	5.0	0.33
Female	5.3	5.0	0.7	15.3	3.3	0.33

8.5 Supplementing Pictures

All pictures were taken using the iPhone 13 pro max.

Appendices 1: A picture of the plastic bottle used for the flytrap.

Appendices 2: A picture of the final bottle flytrap that includes the defrosted mouse carcass.

Appendices 3: Picture of *Calliphora vicina.*

Appendices 4: Picture of *Lucilia sericata.*

Appendices 5 (left) and 6 (right): A female *C. vicina* (left) whose eyes are further apart.

A male *C. vicina* (right) whose eyes are narrower.

Appendices 7: An example of the bug dorm (60 x 60 x 60 cm) that separately housed *C. vicina* and *L. sericata.*

Appendices 8: The dish on the left contains the carbohydrate source (sugar). The dish on the right contains the paper towel that has been moistened with water.

Appendices 9: The plastic container that contains the liver protein source.

Appendices 10: The smaller bug dorm (30 x 30 x 30 cm) that contained the 4 bottle flytraps.

8.6 Evaluation Supplement

Overall, there were significant challenges throughout this project, primarily attributable to two main factors. Firstly, the rearing of Calliphoridae proved exceedingly arduous, as it involved an unfamiliar process. Secondly, both the initial and subsequent experiments encountered failures: the first was unsuccessful because all male blowflies had died before the experiment commenced, which a new colony needed to be reared; the subsequent experiment yielded unsatisfactory results because most blowflies escaped through a small opening in the insect cage. Consequently, the duration of the experiment extended far beyond the anticipated timeframe, resulting in considerable frustration.

I hold great admiration for individuals proficient in working with flies, as it has revealed my own limitations in terms of patience. Through this independent research project, I have acquired valuable skills, including statistical analysis, and designing and conducting experiments. I am very grateful for the opportunity to research forensic entomology because, although it was stressful at times, I found it fascinating.