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Mother Knows Best: Mechanistic Aspects of Foraging Preference and Differential Arrestment of Gravid Females: A Case Study on Blow Flies (Diptera: Calliphoridae)

By

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Mother Knows Best Mechanistic Aspects of Foraging Preference and Differential Arrestment of Gravid Females: A Case Study on Blow Flies (Diptera: Calliphoridae)

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ABSTRACT

Behavioural plasticity is a concept that describes a change in an organism's behaviour due to their exposure to stimuli that differ from their usual environment. For instance, females of many insect species alter their behavioural responses when exposed to differing substances that they utilize for feeding, oviposition, and/or mating. Behaviours such as the frequency of walking, tasting, grooming exhibited by flies can be associated with the detection and acceptance of a suitable medium. For this thesis, the arrestment and behavioural response of gravid adult females of three species of blow flies (Diptera: Calliphoridae), *Lucilia sericata* Meigen, *Phormia regina* Meigen, and *Calliphora vicina* Robineau-Desvoidy, were tested against six different profiles containing mixtures of three volatile organic compounds (including dimethyl disulfide, indole, phenylacetic acid, and isobutylamine). Each of these organic compounds have been previously found to be attractive to both larval and adult blow flies. Each profile mixture of VOCs corresponds to a diet lacking in one essential amino acid (either phenylalanine, tryptophan, valine, or methionine), each of which are critical to the growth and development of immature flies.

The results of this study indicate that on their own, these volatile cues result in increased arrestment behaviour, but not oviposition. Although demonstrating arrestment, *L. sericata* displayed no preference between the VOC profiles. In contrast, greater arrestment of *P. regina* occurred when the VOC profile lacked isobutylamine. Differing from the other two species, *C. vicina* exhibited a mixed arrestment response whereby no single VOC elicited a consistent response such that a particular cue was always sought or avoided. In terms of the pattern of behavioral switches between arrestment, locomotion, and tasting, overall, across species and VOC profiles, greater arrestment occurred, however, within a few species and within VOC profile differences were present. This was particularly true in *C. vicina*, where only two VOC profile combinations of phenylacetic acid versus indole and isobutylamine versus indole demonstrated greater arrestment than locomotion. Interestingly, this is the opposite for *P. regina* and *L. sericata,* that demonstrated no difference between locomotion and arrestment in the VOC profile combinations of isobutylamine versus indole. This behavioural plasticity demonstrates how different species of fly respond and forage differently despite having the same physiological needs.

DEDICATION

To my wonderfully supportive friends and family, I could not have done this without all your help. To those I lost throughout this journey, you will always be an integral part of my successes and I will always cherish our memories together. To Dakota, Bruiser, and Punkin your lifelong support and comfort has been immeasurable. I will cherish our memories for a lifetime. My heart and soul will always be connected with yours.

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Scientists stand on the shoulders of giants, and we thank them for their contributions.

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Chapter 1. Carrion-Insect Interactions: Volatile Organic Compounds and Microbes

Preference-performance hypothesis, and optimal oviposition theory

The preference-performance hypothesis is a notion that describes how host selection, mostly by phytophagous insects, is made primarily on the detection of a suitable oviposition medium so that offspring development can be optimized (Jaenike, 1978). This hypothesis assumes that the ideal selection of a host resource would maximize an organism's progeny's fitness (Jaenike, 1978). This hypothesis overlaps with the optimal oviposition theory which discusses the specific behaviour in relation to mothers laying clutches of eggs (Jaenike, 1978). A mother would first detect an oviposition resource, then either accept or decline it based on its nutritive qualities (Reisenmen & Scott, 2019). An insect's olfactory system can distinguish scents and these scents lead the insects to media that they can consume or use for other purposes. Insects use their antennae to probe these media based on signals they get from their olfactory system, since these organs are sensitive to scents (Brezolin et al., 2018; Brodie et al., 2015; Lowne, 1870; Sevarika et al., 2020). These qualities are crucial for an insect's survival since they rely on this source throughout their life cycle. Environmental signals such as scents prompt animals to locate resources, and then they can further assess through tasting whether this resource is adequate for what they require (Liu, 2014). This requirement may include the use of medium for oviposition activity, feeding, or mating grounds, as well as others.

Optimal foraging theory

Foraging, or the pursuit of ideal resources, is essential to animals and insects alike. For example, scavengers are organisms that search for natural food sources, and rely on foraging for their survival (Hill et al., 2018). Scavengers can create competition on a single resource with other scavengers, as organisms coexist on a single resource (Hill et al., 2018; Kane & Kendall, 2017). Some organisms are facultative carrion consumers, and others are obligate carrion consumers; this means that some consumers can eat different types of food resources, and others are limited to one specific type of food source, in this case carrion (Dijk et al., 2008; Ruxton & Houston, 2004). Optimal foraging ties into this concept since insects use plant or food resources for larval feeding, but also as adults (Stephens & Krebs, 1986). This theory describes how adults should prefer to feed on resources that give them the highest fecundity and performance as adults

(Stephens & Krebs, 1986). Foraging behaviours can be diverse, and vary from species to species, but behaviours are considered in both optimal foraging and oviposition theories (Thompson 1988; Mayhew, 2001).

Foraging is not limited to insects locating and consuming food sources; it also pertains to searching for resources that address all of their physiological needs. These needs include mating and oviposition sites as well. It is essential for an organism to search for an optimized time in regards to their oviposition/mating resources since their young rely on their mother to make decisions regarding their life. If they choose a suboptimal resource, their progeny may not survive. This risk is especially true in insects that do not practice maternal care as they leave their eggs as soon as they are laid.

Oviposition strategies are driven by a series of complex trade-offs between numerous factors, including resource quality, probability of finding a suitable resource, and clutch size, among others (Janz, 2002). Both abiotic and biotic cues may positively or negatively influence a female to oviposit. Some biotic signals include visual cues and internal cues (e.g., olfactory responses), whereas abiotic stimuli include relative humidity, photoperiod, and temperature (Byrd & Allen, 2001; Hans, 2016). Insect species each practice oviposition strategies in varying ways according to their environment and oviposition resource preferences. Their internal and external signals communicate to the organism that they have hit certain physiological milestones and need to complete certain actions, *i.e.,* oviposition. For example, when a blow fly has consumed enough protein necessary for oogenesis, they then search for an oviposition medium, reproduce, and then lay eggs. If other species have already laid copious amounts of eggs on the carrion, then the fly species may decide to find another resource if she thinks that the competition will be harmful to her young. Alternatively, if she sees another clutch of eggs but senses that the aggregate would create a good environment, she may select to oviposit nearby (Brody et al., 2015). In essence, oviposition strategies depend on physical and chemical cues to assist in the choice of beneficial tactics to employ and ideal locations for oviposition (Riddick et al., 2018). Overall, when a female oviposits, where she chooses to deposit her clutch and the density, she lays correspondingly will affect her offspring.

Resource partitioning and species adaptation

When multiple different organisms coexist on a food source in the wild, they must utilize different strategies to ensure that they acquire what they deem necessary from the source (Torres-Cortés et al., 2019). These interspecific interactions result in organisms partitioning ephemeral resources (Dalu et al., 2017). Resource partitioning involves the division of limited resources by organisms (Xi et al., 2017). If they require different nutritional or reproductive uses for the food source, avoidance may ensue allowing both organisms to extract nutrients from the resource without directly interacting with each other (Cothran et al., 2015; Kyogoku & Kokko, 2020). Competition arises when species conflict with each other to attain resources (Udd et al., 2015). Symbiotic interactions can occur when competition does not, as these interactions allow both organisms to benefit (Itoh et al., 2018). For example, in a carrion-feeding beetle, their microbial symbionts create a biofilm while utilizing carrion that is beneficial to the beetle larvae (Shukla et al., 2018). Different species may exhibit seasonal overlap with others, and the species present may alter the order of colonization (Baumgartner & Greenberg, 1985).

Organisms' dietary choices differ based on what is available to them in their environment, but they can broadly be classified into generalists and specialists. Most insect species are deemed to be specialists, which means that they rely on specific resources to be able to thrive (Futuyma & Moreno, 1988; Shäpers et al., 2015). In contrast to this, generalist species are less selective about their environment and can live in a range of conditions (Schäpers et al., 2015). An example of a generalist would be the lycosid spider (Araneae: Lycosidae). This family of spiders commonly feed on multiple different prey, including fruit flies, aphids, and other qualitatively equivalent prey (Edgar, 2009; Oelbermann & Scheu, 2001). Other generalists may feed on a combination of plants and animals, such as the black soldier fly, *Hermetia illucens* Linnaeus (Diptera: Stratiomyidae), which feeds on both decaying plant and animal matter (Nguyen et al., 2015). A specialist feeder includes the koala, *Phascolarctos cinereus* Goldfuss (Diprotodontia: Phascolarctidae), which feeds exclusively on a diet consisting of eucalyptus leaves due to their metabolic enzyme specificity (Marschner et al., 2017).

The process of adaptation is crucial to the evolution of a species. Adaptations can be of behavioural or morphological origin, and organisms that have more favourable variations of the behaviour/morphological makeup will survive and reproduce (Abdusalam & Li, 2018; Sejian et al., 2018). These traits are heritably passed onto the next generation (Drown & Wade, 2014; Leal et al., 2017). Adaptations occur in response to changing climate or living conditions (Henry et al., 2018; Verde & Di Prisco, 2012). An individual cannot predict the outcome of their changing environment, and thus are not guaranteed to survive into the next generation. Since organisms are not always given a perfect environment to thrive, only individuals in a population of a species who have traits that are favourable for their environment will be able to survive and reproduce. In the wild, diet can be a fleeting resource, with plant and animal sources changing in frequency and composition on a regular basis (Barton et al., 2013; Egert-Berg et al., 2018). Organisms that cannot appropriately adapt to changing environmental conditions will die and thus their bloodline will die out.

Nutritional resources

Ephemeral resources are biological resources that are fleeting in nature and exist for an unpredictable amount of time, but they may also be heterogeneous as far as their composition and nutritive value (Barton et al., 2013; Braack, 1987; Mondor et al., 2012). These resources generally supply food or water for organisms and their composition waxes and wanes over time (Klink et al., 2020; Westerhuis et al., 2020). For species that feed on ephemeral resources, dietary choices will not remain consistent on a year-year basis (Barton et al., 2013). This environmental factor is especially true for carrion-feeding insects, the sarcosaprophages. Factors like climate change, and the consumption of a particular food source by predators may leave these insects with a food source that is less rich in certain nutrients. This circumstance may require choosing a source of food that a competitor would not choose to consume or choosing a source rich in specific essential amino acids over others, in hopes of choosing one that allows her offspring to emerge successfully (Coleman et al., 2014). Plasticity is the ability of a species to change their morphology and/or behaviour to respond to a variety of habitats and their requirements for life (Diggle, 2002). This flexibility in behaviour may be paramount for the survivability of the insect and may aid them in being resilient to various unpredictable lifestyle changes.

Carbohydrates are a necessity for the maintenance of life in blow flies, who can live healthily on a diet mainly consisting of sugar and water (Fraenkel, 1939; Harlow 1956). The most common carbohydrates used to rear blowflies are sugars, such as sucrose or glucose (Fraenkel, 1939). Without these carbohydrates, even with the consumption of protein, the blow flies will die (Strangways- Dixon, 1961). An experiment done by Dethier in 1961 demonstrated that blow flies were able to detect whether or not a source is protein-rich, or if it is carbohydrate-rich. This discrimination of substances may be beneficial to a fly when deciding whether to compete for a protein source, or to wait it out and focus on survival rather than oviposition.

Protein is one component in the insect diets that is especially important for the reproduction of many species (Behmer, 2009; Lee et al., 2002). In blow flies, adults must feed on a food source high in protein for egg development to occur in females, and sperm in males (Stoffolano, 1974). Without it, the flies are not able to reproduce and pass their genetic information to their offspring. Typically, females search for an appropriate carrion source, which contains high levels of protein, and begin to feast on the carrion. Next, they copulate with males, and lay their eggs on an appropriate area for their young to live and grow (Jaenike, 1978). Studies have shown that blow flies can survive on a diet of carbohydrates and water exclusively, but that they will not be able to reproduce since their sex organs never mature without protein (Harlow, 1956).

One nutritional element that organisms consume are amino acids which are crucial to the body. Protein synthesis involves amino acid reactions, which are required for the human body to create energy (Barker, 1906). Amino acids are essential for metabolic processes in the body, and any deficits of amino acids can cause metabolic disease or issues (Scriver, & Rosenberg, 1973). Amino acids are used in the human body for the development and maintenance of various biological reactions (Barker, 1906). Insects are suspected to use amino acids in a similar way, though not much is known about their specific requirements (Harlow, 1956). Insects such as *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) have been analyzed to determine what concentration of amino acids are needed for dietary consumption (Schultz et al., 1946). Metabolism includes all chemical reactions in the body, from creating molecules to breaking down and eliminating waste in the body. It allows the organism to remain alive and continue all its necessary cellular functions. An error in metabolism, or a build-up of waste products in the body (or an error in the creation of a molecule resulting in a mutant molecule) can lead to severe health defects, or death (Willcock, 1906). This nutrition is critical to blow flies, since they feed off human corpses as well as other carrion. Like humans, blow flies also require amino acids, as they cannot produce essential amino acids on their own; they must be consumed through a food source (Matos et al., 2018). The body naturally produces non-essential amino acids whereas essential amino acids must be attained through secondary consumption (Nagao et al., 2015). Insects can survive off of diets lacking certain nutrients, but the diets they consume will affect various factors of growth and oviposition (Piper et al., 2014).

Vitamins are also a crucial part of insect diets in that they provide the insect with compounds that can stimulate growth and help support life (Dadd, 1973). Some vitamins can be synthesized in the body, but the majority must be consumed through dietary means (Douglas, 2017). These nutrients serve in biochemical functions, and a deficit of these may lead to negative outcomes during an insect's life (Dadd, 1973). For example, the vitamin riboflavin has been deemed essential for life in *Phormia regina* (Meigen) (Diptera: Calliphoridae), without it, all larvae died within 2 days of the removal of the vitamin (Brust & Fraenkel, 1955). Other vitamins, such as nicotinic acid, are less important to consume for this species, possibly because these insects may be able to synthesize it in small amounts over time (Brust & Fraenkel, 1955). Insects can reap nutritional benefits from the microbes present around them as they aid in the digestion of carrion (Dadd, 1973; Rhinesmith-Carranza et al., 2018). Overall, the exact concentration and nutritional requirements of vitamin substances will vary depending on the species, what they need to consume, and what they can synthesize on their own. Moreover, some substances will overlap in their nutritional value, and as such, the actual requirements of insects are difficult to define.

Specific nutritive requirements for life in blow fly species fluctuates based on the exact dipteran diet that is used (House, 1962). These necessary nutritional components may be more or less valuable to a blow flies based on their species. Additionally, some constituents of a natural diet may contain trace amounts of others in varying percentages, such as in the case of casein. These components may also not contain purified amounts of amino acids or other dietary substances. For example, it is difficult to eliminate biotin from a synthetic insect diet when casein is supplemented in the diet (Brust & Fraenkel, 1955). Removing even one essential dietary substance can negatively affect the development of the insect (House, 1962; Brust & Fraenkel, 1955).

Decomposition of carrion

One of the ways blow flies assess the nutritional quality of a carrion resource may be through interactions with microbes (Rhinesmith-Carranza et al., 2018). Microbes are minuscule organisms that can live in a huge variety of habitats under a plethora of environmental conditions (Haruta & Kanno, 2015). They constitute the microbiota found on all living organisms (Sears & Garrett, 2014). Microbes are present on an animal both during life, and in death, and are an essential component of the carrion environment (Liu, 2014). They further decomposition processes on a carrion source, and release compounds known as volatile organic compounds (VOCs) (Liu, 2014). These released molecules serve as an indication for the presence of a food source for vertebrate scavengers and insects that feed on carrion, such as blow flies (Chaudhury et al., 2015).

Microbial volatile organic compounds (MVOCs) are airborne compounds that are associated with the microbial breakdown of organic substances. They are produced from carrion as a body decomposes. During the initial stages of carrion decomposition, microbes begin to break down the tissues (Rhinesmith-Carranza et al., 2018). The microbes consume the tissues and, in turn, create VOCs and release amino acids (Barker, 1906). Some MVOCs constitute as fly attractants, which blow flies and other insects use act as chemical cues to locate carrion (Frederickx et al., 2011; Ashworth & Wall, 1994; Liu et al., 2016). These chemicals guide the flies to sources of food that may be rich in essential nutrients. Blow flies use the chemical information to determine the quality of the resource they are living off of (Rhinesmith-Carranza et al., 2018). For example, when the amino acid methionine is broken down, it produces the VOC dimethyl disulfide (DMDS). Thus, if a food source is high in DMDS, it may indicate that there is a high amount of the amino acid methionine present (Frederickx et al., 2011; Liu, 2014). The preferences for these compounds may also be species and dose-specific, and they vary corresponding to their sexual maturity status (Yan et al., 2018; Chaudhury et al., 2015). For example, *Lucilia sericata* Meigen (Diptera: Calliphoridae) is a species that has been tested extensively for its response to a variety of volatiles. A 2014 dissertation by Liu found that both males and females responded differently to doses of DMDS, indole, phenylacetic acid, and isobutylamine. These were demonstrated in differential preferences for VOCs. Moreover, gravid females responded differently than non-gravid ones. For example, females preferred the tested DMDS doses and were more active once they were exposed to it in comparison to males. Female flies also preferred the DMDS concentration of 0.5 mg/ mL when compared to other concentrations.

Blow flies

Blow flies (Diptera: Calliphoridae) are critically important for use in forensic cases (Yang & Shiao, 2012). They are primary colonizers on a corpse, often arriving just minutes after death occurs, and can be used to determine a post-mortem interval (time elapsed since death) (Anderson, 2004; Anderson & VanLaerhoven, 1996; Nuorteva, 1977). This is due to the knowledge surrounding the life cycle of these flies (Anderson, 2004). Once flies or larvae are collected from a crime scene, an expert entomologist can observe them and discern the developmental stage the fly is in. The blow fly life cycle has been studied extensively, with the stage of development of larvae acting as a primary indicator for experts to determine an estimation of time of death of a corpse, as well as additional facts surrounding death (Ody et al., 2017).

Overall, the growth cycle of blow flies is predictable due to the several studies examining their development under specific temperature conditions (Hans, 2016). This time frame is entirely dependent on the selected species, and humidity and temperature conditions of the surrounding environment (Roe, 2014). Generally, as ambient temperatures increase, the rate of development for these insects also increases. Correspondingly, as ambient temperatures decrease, the development rate decreases as well (Byrd & Castner, 2010; Pacheco, 2015). This relationship can be modelled as curvilinear at extreme temperatures, and linear in between (Byrd & Castner, 2010).

Gravid female blow flies begin by laying eggs on a carrion source rich in nutrients. These eggs hatch into first instar larvae and begin to feed. The next stage of growth is when the larvae molt into their second instar, which are a little larger than their predecessors and feed in a mass with other maggots. Later, these larvae molt into third instar larvae. Third instar larvae are known to increase in size and continue to feed vigorously in a maggot aggregate. Once they have surpassed their critical weight to enable them to complete the remainder of their development, they cease feeding on the body (Ribeiro & Zuben, 2018). Next, these post-feeding maggots leave the body and seek a suitable environment to prepare for pupariation (Ahmed, 2015). The timing of pupariation is also variable by species and temperatures. Once development within the puparium has been completed, adult blow flies will emerge from the puparium, or pupal case (Roe, 2014).

Blow flies are poikilothermic, meaning that they rely completely on their environment and its' subsequent temperature to grow to adulthood and to survive. They cannot maintain their own body's homeostasis, and as a result, temperature, humidity, and other conditions must be fulfilled by their environment (Byrd & Castner, 2010; Jaenike, 1978). Female blow flies follow a pattern of oviposition (egg-laying) preferences based on various abiotic factors, such as temperature and humidity (Hans, 2016). A mother chooses a place that allows for immature larvae to intake protein-rich fluids, as first instar larvae are incapable of piercing the skin of a cadaver (Haufe & Nelson, 1957; Pacheco, 2015). Natural orifices (eyes, nose, mouth etc.) are a common spot for oviposition since the areas are comprised of mucosal membranes that are thin and easily punctured by immature larvae. These areas are high in fluid content and would be beneficial for blow fly growth (Pacheco, 2015). Once their eggs are laid, mothers abandon them, and thus they do not practice maternal care. Instead, female blow flies may choose to lay eggs on areas of carrion that will be most beneficial to offspring survival. Afterwards, the mother will depart and allow the young to grow and feed on their own (Jaenike, 1978).

Blow flies are sarcosaprophagous insects whose larvae feed and develop on dead animal flesh (i.e., carrion), while adults use dead animals as a protein-rich meal for development of sperm and eggs, as well as an oviposition medium for eggs (Reid, 2012). These flies assess a carrion resource before deciding to use it as food, or as an oviposition/mating site. If a carrion source appears to be favourable blow flies, then they are more likely to colonize it for their benefit (Rhinesmith-Carranza et al., 2018). Most studies done on blow fly nutrition have focused on the general composition of carrion, and avoids the specific nutrients involved in each food resource. For example, studies have tested blow fly responses to pork liver, bovine liver, pork muscle, bovine muscle, as well as many other tissue types (El-Moaty & Kheirallah, 2013; Thomas et al., 2016).

Previous research done by Rhinesmith-Carranza and colleagues (2018) determined that the blow fly species *Lucilia sericata* seemed to have a preference for diets containing certain amino acids, but not others- indicating that amino acid presence influences behaviour. In their study, maggots in the early third instar stage were given a choice between diets, each lacking one essential amino acid (either valine, tryptophan, phenylalanine, or methionine). The diet lacking methionine was most preferred, followed by the diet lacking valine, then phenylalanine, and the least preferred was the diet lacking tryptophan.

Studies involving sterile blow fly food sources boast different experimental results when compared to non-sterile food sources, as the potential needs of the blow fly change with the presence or absence of microbes (Rhinesmith-Carranza et al., 2018; Thompson & Hinton, 1997). In particular, Rhinesmith-Carranza and colleagues (2018) determined that blow flies (specifically *L. sericata*), show preferences towards diets lacking certain amino acids, but that these preferences shifted once the food medium was sterilized. The difference between a sterile food medium and one that is non-sterile is that microbes are present to aid in the breakdown of carrion tissues. This breakdown not only releases substances that attract necrophagous insects, but also, assists in making an accessible area for immature larvae to feed and live (Liu, 2014). Blow flies secrete salivary enzymes that are able to break down the food further so that it can be consumed and absorbed by the fly (Hobson, 1931). Additionally, these enzymes will allow the female to open the body cavities and lay her clutches near these areas (Matuszewski et al., 2010). Lastly, the gut of blow flies contains intestinal microbiota that aid in digestion through the use of digestive enzymes. The digestion occurs externally first through liquefaction, and then subsequently occurs internally with absorption (Hobson, 1931). Strains of *Proteus mirabilis* Hauser (Enterobacteriales: Enterobacteriaceae) have been found to be present in blow flies *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) and *Lucilia sericata.* In *C. vicina*, this strain was isolated from larvae (Erdmann, 1987), and in *L. sericata* it was isolated from the salivary glands (Ma et al., 2012). *P. mirabilis*, and other microbes may either attract insects or deter insects from a food source; this attraction or repulsion factor may be species-specific, or dependant on whether the bacteria are a wild type or of a mutant phenotype (Liu, 2014).

The aforementioned Rhinesmith-Carranza et al. (2018) research methods were based on paper published by Piper and colleagues in 2013; In this research project, a holidic diet was defined for the fruit fly, *D. melanogaster,* in which the performance of offspring was measured. A holidic diet can be described as a nutritionally complete chemical diet (Piper et al., 2014). This diet was deemed to be effective in supporting the development of subsequent generations of flies across a time span of seven years. This allowed for an investigation into precise amino acid effects on this fly type. This diet was then modified in the Rhinesmith-Carranza et al. study to test blow fly choice in diets deficient in one amino acid. Both diets contained several overlapping chemicals, including amino acids, while some were removed or substituted in the diet used in the Rhinesmith-Carranza et al. (2018) diet to simplify the diet for choice alone. The substances removed were necessary for the development and thus not required for a short-term choice experiment. Others were regarded as indispensable and maintained in both diets. One example is how the removal of cholesterol in the diet shortened life span significantly (Piper et al., 2014).

Blow flies respond to environmental cues such as the presence and concentration of semiochemicals as an indicator of location and quality of resource (Johansen et al., 2013). The physiological state has been statistically proven to affect resource preference of *Lucilia sericata* female flies (Brodie et al., 2014). In addition to this, in the presence of con- and heterospecific flies, *Lucilia sericata* and *Phormia regina* females respond to their respective species' semiochemicals (Brodie et al., 2014). When presented with other species' semiochemicals and liver, both were equally attractive to both species. A preference existed for both species to liver with heterospecific flies than to liver alone (Brodie et al., 2015). This data supports the hypothesis of interspecific semiochemical recognition and attractiveness. Moreover, food sources that had been fed-on were determined to be more attractive to blow flies than non-fed-on food (Dethier, 1955). One study with both field and laboratory data determined that indole, a semiochemicals, is repellant to gravid, oviposition site-seeking *Lucilia sericata* females (Brodie et al., 2015). Indole is present in feces and is associated with the breakdown of the essential amino acid tryptophan (Brodie et al., 2015; Whitley & Thornton, 2012). Indole has been described as a medium for a reliable foraging cue for non-gravid *Lucilia sericata* females due to its' relative abundance in protein and other factors (Brodie et al., 2015).

Study System: *Lucilia sericata*, *Phormia regina*, and *Calliphora vicina*

Species of *Lucilia sericata*, *Phormia regina*, and *Calliphora vicina* (Diptera: Calliphoridae) are geographically distributed in various North American regions as well as across the globe (Hall 1948; Saunders & Hayward, 1998; Sivell, 2018). Although the species overlap in their seasonal distribution, each has a distinctive preference for seasons and temperatures. The species *Lucilia sericata* are tolerant of high-temperature conditions (from 20°C to 33.5°C) and are predominantly active in the spring and summer seasons in various locations globally (Brundage et al., 2011; Tarone et al., 2011). In contrast, *Calliphora vicina* are primarily present during the cooler seasons of fall and spring in temperate regions of the Earth (Lebouvier et al., 2011, Matoba & Terazawa, 2008). Finally, *Phormia regina* is a species that is prevalent year-round and shows no seasonal preference (Brundage et al., 2011). Blow flies overwinter, inducing a state of diapause, which is a halt in their development. This occurs during the cold winter season, so that blow flies can avoid these climate conditions (Coleman et al., 2014). The species *Phormia regina* Meigen, also known as the black blow fly, is classified in the subfamily Chrysomyinae (Hall & Townsend Jr., 1977). They tend to prefer lower temperatures in the southern United States, but warmer temperatures in the northern climates (Brundage et al., 2011; Byrd & Allen, 2001). The lower limit activity temperature of *Lucilia sericata* is roughly 10°C- 12.5°C whereas the same limit for the species *Calliphora vicina* is in the range of 2.5°C to 4°C (Faucherre et al., 1999). The classification of the blue bottle fly, *Calliphora vicina* Robineau-Desvoidy is in the subfamily Calliphorinae (Szpila et al., 2013). Lastly, the species *Lucilia sericata* Meigen can be found in the Luciliinae subfamily (Hall, 1948).

Research Objectives:

This research study aims to establish the mechanistic aspects of individual blow fly oviposition and foraging choice as a response to dietary media which contain a profile of three volatile organic compounds. The approach to this study is two-pronged; first, female blow flies were tracked to view their pre-oviposition behaviour including the metrics of flying, walking, standing, tasting, and grooming behaviour with regards to an agar medium that contains certain VOCs, lacking in one. The order that this behaviour occurs in is also significant to note. Secondly, the choice of females was measured to determine their preference in arrestment for the different profiles varying in volatile organic compound composition. The prediction is that when given a food source that lacks one volatile organic compound but contains others, gravid female blow flies will adjust their actions about oviposition in order to stay near a medium that would create the best outcome for their offspring. It is also expected that with these choices, the offspring will have a variety of survival outcomes. This thesis project aims to ascertain the outcome of gravid blow fly oviposition behaviour on an agar medium rich in three volatile organic compounds but lacking one as well as to view how oviposition choices change based not on a diet, but on VOCs that reflect fluctuating diets.

Choice experiments aim to determine the mechanisms as to why a particular organism makes one choice over others when given multiple options. In this experiment, oviposition choices were tracked through a modification of the choice of medium as well as behaviour. I believe that these alterations would show that blow flies exhibit behavioural plasticity. This experiment involves exposing gravid female blow flies to a nutrient-free agar medium with VOCs corresponding to a diet lacking one amino acid but containing three others. This plasticity may be validated through a marked preference of a particular diet-VOC combination. For example, if a mother can determine what a diet is composed of based on their VOC profile and not the chemical composition of the diet, she can choose the advantageous diet to lay eggs on and would do so sooner than the control. If this is true, this means that offspring whose mothers make poor choices regarding oviposition will be negatively affected in their survival outcomes. Furthermore, if a mother makes a choice that positively affects their offspring, then her offspring are likely to thrive and reproduce. I hypothesized that female gravid blow flies would prefer the agar containing the three VOCs that depict the presence of the amino acids valine, tryptophan, and phenylalanine, but lacking methionine. I hypothesized that the next favoured medium will be that containing the three VOCs that correspond to the presence of the amino acids lacking valine, then the profile with VOCs imitating a diet lacking phenylalanine, and lastly, the profile with VOCs equivalent to the presence of a medium lacking tryptophan.

In respect to oviposition choices, I predicted that the blow flies will demonstrate behavioural flexibility through the behavioural changes brought about through dietary stimulation. This may be done through an increase in oviposition time should the medium be unfavourable to offspring, and a decrease in oviposition time if the medium is more ideal. The lack of one amino acid (and their associated microbial volatile organic compound) will likely indicate to the mothers what the diet is composed of, and she can determine whether or not the diet is preferred for her young. Additionally, behavioural plasticity may be exhibited by an alteration of clutch sizes through exposure to different dietary treatments. If a mother decides that the diet given to her will be beneficial to her offspring, she may decide to lay more eggs in order to give her offspring a better chance at survival. Conversely, if the diet is lacking something the mother deems as critical, then she may hold off and/or lay smaller clutches.

I hypothesized that each blow fly species will prefer VOC profiles matching diets lacking some amino acids while containing others. This partiality may be due to a mother's desire to choose a favourable oviposition source for their young to grow and survive off. A previous study done by Rhinesmith-Carranza and colleagues (2018) demonstrated that the blow fly species *Lucilia sericata* showed a preference in diets missing the amino acid methionine over other dietary treatment options. Their next preferred diet was that lacking valine, then phenylalanine, then tryptophan. The diet treatments in the study each contained a plethora of nutrients and vitamins but with one exception. The four diets lacked one of four essential amino acids, in this case they are methionine, valine, tryptophan, and phenylalanine. I believed that this preference would extend further into plain diets with no nutritive value but with VOCs that represent the presence of dietary components necessary for life. The behavioural modifications they may demonstrate involve not only actual oviposition, but also preoviposition behaviour such as the detection and acceptance of a medium. This may include the frequency and orders of standing, walking, flying, grooming, and tasting behaviours, as well as the location of the arena that these behaviours occur in. Preferences can be demonstrated in marked increases or decreases in behaviours of arrestment, tasting and locomotion.

The Rhinesmith-Carranza et al. study shows that blow flies have a preference towards some amino acids; this may be a result of the interaction between ideal oviposition choices and the performance of the offspring (Rhinesmith-Carranza et al., 2018). I expected that gravid female blow flies would demonstrate more locomotion in their behaviour when exposed to media that are less suitable for their survival, and thus the survival of their young. Conversely, I predicted that blow flies would exhibit more stationary activity when they find a medium that they deem suitable for themselves and/or for their young. Overall, I hypothesized that blow flies display behavioural plasticity through modifications of foraging decisions and that these alterations, based on preference, affect how they colonize a resource. These alterations may be with increased/decreased locomotion in comparison to arrestment, or an increase/decrease in probing behaviours as well as others. Additionally, different species may respond differently due to their seasonal distribution, competition, and/or dietary preferences.

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Chapter 2. Species-specific Arrestment Response to Volatile Organic Compounds in Gravid Adult Blow Flies (Diptera: Calliphoridae)

Organic matter such as animal bodies decompose naturally over time, and detritivorous animals as well as carrion-feeding insects such as blow flies (Diptera: Calliphoridae) take advantage of these corpses for dietary use (Anderson & VanLaerhoven, 1996; Hill et al., 2018). The advantage of consuming decaying flesh for these organisms is that as decomposition occurs, the body components breakdown and become easily available for ingestion (Ma et al., 2012).

Volatile organic compounds (VOCs) are airborne chemicals, or scents, that are released through the breakdown of organic substances (Johansen et al., 2013), primarily by microbes (Barker, 1906). These chemicals are a natural occurrence and have their own unique scent (Adebesin et al., 2017). These scents are attractive or repulsive to various organisms based on what the organism searches for in terms of nutrition (Johansen et al., 2013). Volatiles act as chemical cues for organisms to assess the composition of a food source and whether or not it contains toxins or other contaminants that would make the food source unfavourable (Scheidler et al., 2015).

Blow flies are attracted to the scent of a decaying corpse and can detect a carrion from up to several kilometers away essentially as soon as death occurs (Braack, 1981; MacLeod & Donnelly, 1963; Zajac et al., 2018). Insect antennae are sensitive to odours (Lowne, 1870; Brodie et al., 2014). Blow flies can detect semiochemicals using their olfactory system and use these senses to search for suitable media to feed and oviposit on (Brodie et al., 2015). It is possible that blow flies can detect VOCs and can infer the composition of the amino acids present on a food source due to the presence of their corresponding VOC.

In the decomposition of carrion, microbes consume the tissues, including amino acids, creating VOCs (Barker, 1906). Specific VOCs are associated with the breakdown of corresponding amino acids (Table 2.1) (Kadota & Ishida, 1972; Kim et al., 2007; Richardson, 1966; Wang et al., 2001). The breakdown of the amino acid phenylalanine yields the volatile phenylacetic acid (PAA) (Kim et al., 2007). The consumption of the amino acid methionine produces the VOC dimethyl disulfide (DMDS) (Kadota & Ishida, 1972). The VOC indole is synthesized when tryptophan is broken down whereas isobutylamine is produced through a decarboxylation reaction by valine (Richardson, 1966; Wang et al., 2001). Blow flies use this scent profile to evaluate the quality and composition of the resource they encounter (Frederickx et al., 2011; Ashworth & Wall, 1994; Rhinesmith-Carranza et al., 2018; Scheidler et al., 2015).

Preferences for these volatiles are species and dose-specific, and correspond to the organism's physiological status (Hobson, 1937; Liu, 2014; Yan et al., 2018; Chaudhury et al., 2015). For example, the response to semiochemical baits seems to be more pronounced in female blow flies rather than in males (Vogt et al., l985). Some insects are attracted to sweet scents such as flowers, and other are more interested in aromas that mimic the odours that a decaying corpse releases (Johansen et al., 2013; Wee et al., 2018). Blow flies prefer odours that are emitted from a dead body, rather than sweet ones (Johansen et al., 2013). Scent preference in blow flies is complicated and depends on a variety of factors. Sex and sexual maturity are two crucial factors that affect how a blow fly colonizes and utilizes a resource, as flies of the same species but a different sex respond differently to the same stimulus (Liu, 2014). Additionally, depending on what insects physiologically require, they will alter their behaviour when exposed to stimuli that might be favourable for their use such as food, mates, and oviposition (Lancaster et al., 2020; Leonhardt et al., 2020). Choice volatiles such as dimethyl trisulfide and indole have been used as blow fly attractants in various studies (Wee et al., 2018; Chaudhury et al., 2015). Rates of attraction vary based on the concentration of the compound, and the species in question (Liu, 2014). In addition to these factors, the sexual maturity of insects will create preferences for various volatiles (Hobson, 1937; Liu, 2014). For example, *Lucilia sericata* Meigen males and females responded differently to doses of indole, isobutylamine, phenylacetic acid, and dimethyl disulfide, with gravid females also responding differently than non-gravid ones (Liu, 2014). A previous study done by Rhinesmith-Carranza and colleagues (2018) demonstrated that the blow fly species *Lucilia sericata* showed a preference for diets with tryptophan, then phenylalanine, followed by valine, and lastly methionine in a series of larval choice experiments with each amino acid excluded. Based on VOC cues, this would reflect a preference for indole, phenylacetic acid, isobutylamine and lastly dimethyl disulfide as the least preferred.

Blow flies respond to their environment in various ways. Activities such as initial activation, orientation, and landing behaviour vary based on the stimulus received by the blow fly and result in an acceptance or rejection of the medium (Ashworth & Wall, 1994). To assess the quality of a resource, blow flies probe these sources using their antennae to sense the composition of the resource in question (Brodie et al., 2014). Flies also use visual cues to assess the quality of the resource to see if it is of use by the organism (Brodie et al., 2014). If the medium is fully accepted, then oviposition would occur at that point assuming the blow fly is gravid (Ashworth & Wall, 1994). If an insect detects an unsuitable medium, they depart the resource and search for another, higher quality one (Yang et al., 2008).

Arrestment can be described as the process of an organism stopping their locomotion, where an arrestant is defined as a chemical that congregates insects and induces arrestment behaviour (Kennedy, 1978). Contextually, biologists view arrestment as a responsive action to chemical odourants, indicating preference when an insect uses it to remain at a stimulus (Cappadonna et al., 2020). Essentially, when insects find a resource they deem favourable, they will move towards it and remain in that location to utilize it as needed (Ashworth & Wall, 1994).

Blow flies are multivoltine, and as such, can have multiple oviposition events per year (Davies, 2006). In each event, gravid blow flies can lay a clutch of eggs at a size of up to 300 eggs (Davies, 2006). There are several factors that influence blow flies in terms of identifying a fitting source of food and oviposition media. Blow flies first locate a carrion source through chemical cues and will flock to the source (Chaudhury et al., 2015). Once blow flies find a carrion that satisfies their oviposition requirements, they will fly to the resource and lay eggs nearly immediately (Zajac et al., 2018). If they are instead searching for food, or a mate, they will attempt to find this using their senses (Vosshall et al., 2000). Blow flies do not exercise maternal care, so once their progeny hatch, the offspring remain on the food source for the rest of their life cycle (Janz, 2002).

Blow flies demonstrate oviposition preferences dependent on the conditions available to them (Jaenike, 1990). These conditions include temperature, humidity, light intensity, microclimate, and the presence of competition, as well as others. In addition, the composition of the carcass and presence of volatile emissions, as well as the condition of the carrion, affect oviposition behaviour in blow flies. A fresh carrion resource will emit volatiles that attract insects to it, and flies such as blow flies will flock to it and colonize it once they locate it (Archer & Elgar, 2003; Mohr & Tomberlin, 2014). These chemical cues are crucial for insects, since this is what attracts them to a location and determines the suitability of the source for feeding, breeding, and oviposition (Rhinesmith-Carranza et al., 2018). Adult blow flies search for food sources high in protein to achieve success in their growth, and the growth of their progeny (Huntington & Higley, 2010; Jaenike, 1978). Blow flies all function at an optimal level at specific temperatures and humidities, and these values may be species dependent. This is due to their poikilothermic nature (Byrd & Castner, 2010; Jaenike. 1978). Blow fly metabolism rate is temperature compensated as they have both an upper and a lower temperature threshold for survival and development (Faucherre et al., 1999; Ody et al., 2017).

Blow flies rely on a combination of nutritional elements to maintain life and pass on their genetic information to the next generation. In addition to water and carbohydrates (*i.e.,* sugar), they also require a protein source for oogenesis and spermatogenesis (Fraenkel, 1939; Harlow 1956; Huntington, & Higley, 2010). A complete diet contains a combination of these elements but it is not known exactly which concentration of nutrients is optimal for blow flies. Essential amino acids cannot be created in adequate amounts naturally in the body but are required for metabolic processes with deficits of amino acids potentially causing metabolic disease (Barker, 1906; Matos et al., 2018; Scriver, & Rosenberg, 1973). Essential amino acids must be collected through secondary dietary consumption (Nagao et al., 2015).

Flies of *Lucilia sericata*, *Phormia regina* Meigen, and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) are widely distributed geographically in North America and other areas worldwide (Hall 1948; Saunders & Hayward, 1998; Sivell, 2018). Each species has a distinctive preference for seasons and temperatures, with some seasonal overlap. Flies of *L. sericata* prefer conditions ranging from roughly 20 to 33.5°C and exist in the spring and summer seasons wherever the temperature range remains within their limits (Brundage et al., 2011; Tarone et al., 2011). Conversely, *C. vicina* are found during fall and spring in temperate regions of the Earth (Lebouvier et al., 2011, Matoba & Terazawa, 2008). Lastly, *P. regina* shows no distinct seasonal preference and as such can be found in varying seasons (Brundage et al., 2011). Lower limit temperatures range from 10- 12.5°C for *L. sericata*, and 2.5 to 4°C for *C. vicina* (Faucherre et al., 1999).

Based on research of larval preferences to diets lacking in amino acids (Rhinesmith-Carranza et al., 2018) and because of the composition of amino acid breakdown products, and what each VOC represents in terms of nutrition, I hypothesized that blow flies would demonstrate low arrestment when exposed to media containing dimethyl disulfide, thus the greatest arrestment should occur with media lacking dimethyl disulfide. Contrastingly, any media containing indole would be most preferred, so media lacking it would produce the lowest arrestment. Blow fly mothers should choose media that favour the growth and survival of their offspring, though these may or may not correlate with larval preferences, since adults do not practice maternal care and make choices for their young early on. Both larvae and their mothers ideally should choose media that aid in the survival and overall success of the larvae.

In comparing the different blow fly species, I hypothesized that seasonal distribution, combined with species overlap would affect how they respond behaviourally to their environment. Specifically, with *P. regina* demonstrating more specialistic feeding behaviour due to their seasonal preferences, competition performance and species overlap, among other reasons. This species has been previously determined to be slower to oviposit than other blow fly species, possibly because they are searching for specific cues that reflect its preferences due to its choosy nature (Hans et al., 2018; Pacheco, 2015). With these in mind, it is likely that *P. regina* will respond to most profiles similar to the negative control, except in rare cases where they find a medium that is acceptable by their choosy expectations. I expected that *L. sericata* flies would be generalists in their VOC preferences; Previous studies demonstrate that these flies are willing to lay eggs quickly when exposed to oviposition media (Hans et al., 2018; Pacheco, 2015). This should translate into this species of fly responding to all VOC profiles similarly to the positive control, with the exception of the negative control. Flies of the species *C. vicina* should respond similarly to *L. sericata*, with the possibility of some temperature-related preferences due to the volatility of the various cues at lower temperature if temperature-related variation between the cues exists.

Materials and Methods:

Insect Source

Three blow fly species were used for this experiment; they are *Lucilia sericata* Meigen, *Phormia regina* Meigen, and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae). All blow fly colonies required for this experiment were created with adults caught in the wild in the Windsor-Essex area. The blow fly colonies were established and sustained at the University of Windsor, in Windsor, Ontario, Canada. Wild blow flies were caught using plastic King brand wasp traps (King Home and Garden Products, Item ID: 56789) lined with paper towels and liver to attract and trap them. Once trapped, species specific colonies were maintained in aluminum mesh screen cages (46 cm X 46 cm X 46 cm (Bioquip 1450C collapsible cage)) at a 12:12 L:D diel cycle with sugar (carbohydrate), skim milk (protein), and water available *ad libitum*. The room containing the blow fly colonies are kept around 20-30°C and 40-60% RH. To prepare adults for oviposition, roughly 40 g of pork liver was provided as both a protein source and oviposition medium. Once egg masses were obtained (fewer than 200 individuals so as not to crowd the jar), the liver was removed and placed into a 1L Bernardin glass mason jar filled 1/3 of the way with sawdust to provide a pupation substrate. Jars were covered with landscape tarp (provides air flow to flies but does not allow for escape) fed with liver *ad libitum* (at least four times weekly). After adult emergence, flies were released into cages according to species. Colonies were infused with new wild blow flies each year to maintain the genetic diversity of the colonies, limit lab adaptation and inbreeding depression.

Experimental Design

Experimental cages (46 cm^3) of each species were created containing 30 female and 15 male adult flies. Newly emerged adult flies (roughly 24 hours eclosed) were vacuum aspirated into a vial to isolate them and identify their sex. Blow flies are sexually dimorphic, and as such, the space between their eyes can be used to determine their sex (Marshall et al., 2011). Each cage was provided with a carbohydrate source (sugar), a protein source (skim milk), and an Erlenmeyer flask with dental wicks to deliver water but prevent fly drowning. Cages were held at a light: dark cycle of 12:12h, temperature of 23°C to 25°C, and 50% RH. To ensure females were gravid and successfully mated with fertile males prior to behavioural assessment, a five-day high protein pre-treatment was conducted (Hans et al., 2018). Porcine liver (50 g) was placed in the cage for the first two days, then only for an hour a day for the subsequent three days. On day six, females were ready for the experiment. Verification of gravid status was checked post-experimentation by dissection, and only those flies who were gravid were included in the study. A total of 180 flies were used for the experimental treatments, with an additional 24 flies for both positive and negative control (12 each). Each combination (1-6) was tested with 30 flies.

To test the hypothesis that females exhibit differential arrestment and oviposition responses when one of the four critical VOC cues (either indole, isobutylamine, dimethyl disulfide, or phenylacetic acid) is missing, treatments were set up such that all possible combinations of three VOCs with one missing were tested against each other (Table 2.2). In total, this creates six different combinations to test. For each treatment, a mixture of approximately 15 mg of Bacto™

Agar (BD Diagnosis Bacto™ Agar Solidifying Agent) and 20 mL of distilled water was poured in a small petri dish (60 X 15 mm) and three VOCs added in concentrations previously found to be attractive (Table 2.3 (Liu, 2014). Pure solutions of each VOC (>99%) were purchased from Sigma-Aldrich and 10μL of three chosen VOCs were diluted with acetone to achieve the desired concentrations. A positive control combination was conducted using two agar media created using bovine liver, which provides all cues required for oviposition (Bauer et al., 2020; Firoozfar et al., 2011; Parker & Welch, 1991). A negative control combination was conducted using two agar-only petri dishes to test fly arrestment and oviposition in the absence of VOC cues.

A single gravid female was released into a circular arena measuring 5"X 12" (Figures 2.1-2) with two petri dishes of different treatments containing their respective VOC profiles and agar placed on opposite sides of the arena, depending on the treatment combination. In each arena, one treatment was randomly assigned a half in the test arena according to an online randomizer (https://www.random.org/lists/), and the other was assigned to the opposite half. Blow fly location was recorded every 30 minutes for a total of 5 hours. In addition to location, site selection and timing of oviposition was recorded. Each treatment combination was repeated thirty times (n= 30), using a new female for each (n= 90 across all three species). Positive and negative control combinations were repeated twelve times (n= 12) for each species. Ten experimental arenas were run concurrently to reduce the total number of trials needed. Trials were conducted under the same experimental conditions as rearing and pre-treatment, with ca. 23°C to 25°C and 50% RH, as measured by an Elitech temperature and humidity logger.

Statistical Analyses

One sample-proportion tests were applied to data sets of three different blow fly species to determine whether there are significant choice preferences to certain VOC profiles, each lacking one VOC associated with an essential amino acid. In any test, a resulting p-value below 0.05 was deemed significant. Female *L. sericata*, *P. regina*, and *C. vicina* blow fly choice were statistically analysed through one-sample proportion tests with continuity correction. Choice preference was defined as blow flies spending a significantly different (increased/ decreased) proportion of time in one half of the arena. This was done to determine whether there was a difference between the choices that blow flies make in terms of their exposure to differing profiles of volatile organic compounds.

Results:

Lucilia sericata

Females exhibited no arrestment preference for any of the VOC profiles (Figure 2.3), regardless of the treatment combinations as follows: 1) lacking phenylacetic acid or lacking dimethyl disulfide ($X^2_{1,59}$= 1.76, p= 0.18); 2) lacking phenylacetic acid or lacking isobutylamine ($X^2_{1,59}$= 1.47, p= 0.23); 3) lacking phenylacetic acid or lacking indole ($X^2_{1,59}$= 0.03, p= 0.86); 4) lacking dimethyl disulfide or lacking isobutylamine ($X^2_{1,59}$= 2.8, p= 0.09); 5) lacking dimethyl disulfide or lacking indole ($X^2_{1,59}$= 0.08, p= 0.77); and 6) lacking isobutylamine or lacking indole ($X^2_{1,59}$= 2.8, p= 0.09). As expected, the females with the positive controls exhibit no difference in arrestment, varying from a mean arrestment of 47 to 53% between the two sides of the arena (SE= 0.079). In contrast, the females with the negative controls demonstrate no significant difference in arrestment but displays an increased variability in mean arrestment of 37 to 63% between both sides (SE= 0.057).

Phormia regina

Females did exhibit arrestment preferences for some VOC profiles (Figure 2.4). Arrestment behaviour was greater when the media was lacking isobutylamine compared with the media lacking phenylacetic acid ($X^2_{1,59}$= 5.07, p= 0.02), or compared with the media lacking dimethyl disulfide ($X^2_{1,59}$= 14.08, p= 0.0002), or compared with the media lacking indole ($X^2_{1,59}$= 4.08, p= 0.04). None of the other VOC combinations resulted in a preference by females. Arrestment of females did not differ between agar lacking phenylacetic acid and agar lacking dimethyl disulfide (($X^2_{1,59}$= 0.16, p= 0.69), or between media lacking phenylacetic acid and media lacking indole ($X^2_{1,59}$= 3.2, p= 0.07), or between agar lacking dimethyl disulfide and agar lacking indole ($X^2_{1,59}$= 2.08, p= 0.15). The females in positive controls do not exhibit a significant difference in arrestment, varying from a mean (±SE) arrestment of 36 to 63 ± 6% between the two sides of the arena. The females in negative controls in this case also do not exhibit a significant difference in arrestment, with a variation of mean (±SE) arrestment of 37 to 63 ± 9%.

Calliphora vicina

Some significant preferences were found for females when given differing profiles containing VOCs (Figure 2.5). Arrestment behaviour was increased when flies were exposed to media lacking isobutylamine compared to media lacking phenylacetic acid ($X^2_{1,59}$= 12.4, p= 0.00043). Contrastingly, arrestment behaviour was increased for females exposed to media lacking phenylacetic acid when compared to media lacking indole ($X^2_{1,59}$= 5.07, p= 0.024). Lastly, arrestment behaviour of females increased for agar lacking indole when compared to agar lacking dimethyl disulfide ($X^2_{1,59}$= 4.56, p= 0.033). All other tested experimental combinations did not yield significant increases in arrestment of females (media lacking phenylacetic acid compared to media lacking dimethyl disulfide ($X^2_{1,59}$= 1.2, p= 0.27), media lacking dimethyl disulfide compared to isobutylamine ($X^2_{1,59}$= 0.77, p= 0.083), media lacking isobutylamine compared to media lacking indole ($X^2_{1,59}$= 0.16, p= 0.69)). The females in positive controls display a mean (± SE) arrestment varying between 46 to 53 ± 9% between two sides of the arena, with no significant difference in eliciting arrestment. The females in negative controls demonstrate a similar result, with a mean (±SE) arrestment of 37 to 63 ± 9% between sides of the arena.

Discussion:

Although an overall arrestment preference was expected whereby flies would choose any media containing indole, with the indole-missing media least preferred, this was not found to be the case. There are two potential explanations for this. Firstly, a high indole volatile cue alerts blow flies to feces-rich media, which is preferred by non-gravid flies (Brodie et al., 2015) who utilize it as a source of protein to develop eggs and sperm. However, feces is inadequate for blow fly larval growth and not an acceptable oviposition media for gravid flies. Additionally, despite the larval preference exhibited in the work by Rhinesmith-Carranza and colleagues (2018), larval preferences may not be reflective of adult female choices as gravid females may be less choosy as long as the media provides adequate nutrition for successful larval survival to adulthood. In addition, there may be a mismatch between female preference and offspring performance (Dweck et al., 2018).

In addition, an overall arrestment preference was expected whereby flies would preferentially choose a media lacking dimethyl disulfide since the other three more preferred amino acid cues were present. Surprisingly, this was also not found to be the case. Perhaps it is again reflective of the mismatch between larval preference and gravid female choice.

Although the two overall arrestment hypotheses were not upheld, there were some interesting findings within the individual species as the three blow fly species had differential arrestment responses.

Despite *L. sericata* larvae demonstrating clear amino acid preferences (Rhinesmith-Carranza et al., 2018) and the specific concentrations of these VOCs being attractive to females in previous studies (Liu, 2014), there was no arrestment preference exhibited by gravid *L. sericata* females for any of the VOCs in our current study. In addition to the aforementioned possibilities listed above, it is possible this lack of preference is due to a more generalized acceptance of carrion resources by *L. sericata* females. In previous oviposition trials within our laboratory, this species has demonstrated a willingness for rapid, less choosy oviposition on a variety of carrion qualities, temperatures and humidity conditions (Hans et al., 2018; Pacheco, 2015). With utilizing an ephemeral and patchy resource, gravid females of this species may respond to a wider array of cues with less preference exhibited.

Interestingly, *P. regina* females exhibited an arrestment preference, but not for either of the predicted choices. Instead, female *P. regina* preferred media lacking isobutylamine, a VOC associated with the breakdown of the essential amino acid valine (Richardson, 1966). In larval preference studies with *L. sericata*, medium lacking isobutylamine was the second most preferred choice (Rhinesmith-Carranza et al., 2018). This suggests that the other three VOC cues may be more important for *P. regina*. In previous oviposition trials within our laboratory, this species has demonstrated considerable behavioural plasticity, changing the speed at which it is willing to oviposit, and the location, in the presence of other species, quality of the carrion resource, temperature and humidity (Hans et al., 2018; Pacheco, 2015; Tran 2021). This plasticity in oviposition response may reflect the ability of *P. regina* to be a superior competitor when facing interspecific competition against other blow fly species (Prinkkilä & Hanski, 1995; Smith & Wall, 1997) under some circumstances, and facilitated by other species in other circumstances (Hans & VanLaerhoven, 2021). This species has also been found to be more robust than its counterpart *L. sericata*, since they are capable of surviving on unideal carrion, even in cases of higher larval density, and changes in temperature (Pacheco, 2015; Okpara 2018; Hans & VanLaerhoven, 2021).

In contrast with the previous two species that exhibited either no arrestment preference, or a distinct singular arrestment preference, gravid *C. vicina* females exhibited a mixed response.

No single VOC elicited a consistent response such that a particular cue was always sought or avoided. Indeed, the only VOC cue that did not elicit an arrestment to the opposite available choice was dimethyl disulfide, as in no instance did *C. vicina* females choose to preferentially arrest on media lacking dimethyl disulfide. The mixed preference of VOCs demonstrated by *C. vicina* may be due to the temperature range at which this species is active. Volatility of cues should depend on temperature and given the lower temperature threshold of this species down to 3ºC, perhaps its' arrestment response reflects this complexity by utilizing other cues more strongly than simply VOCs (Faucherre et al., 1999).

The different preferences or lack of preferences exhibited by each species of blow fly may reflect differences in their seasonality, resource use, and interactions with other species. *Phormia regina* coexists with both *L. sericata* and *C. vicina* in the Windsor-Essex region, whereas *L. sericata* and *C. vicina* have a much more reduced overlap in seasonal distribution with the former primarily summer and early fall, and the later found in early spring and late fall. Decomposition is slower at lower temperatures and airborne cues less volatile at lower temperatures, reflecting the potential lower reliance or mixed response of *C. vicina.* Faster decomposition in the summer such that carrion becomes unsuitable within a few short days may reflect the lack of preference exhibited by *L. sericata* as it must cue in on the resource quickly to take advantage of it in time. The distinct preference exhibited by *P. regina* may reflect its' wide seasonality and ability to vary its' response depending on the circumstances it finds itself in.

Future research in this area could be expanded by providing the actual amino acids in the diet, in addition to the VOC cues. It is likely that blow flies probe their media for the presence of other nutrients, namely amino acids through their presence and not just the odour emitted by the breakdown of their corresponding missing VOC. This means that flies can tell the difference between a medium that has nutritional dietary components and one that does not, even if it has an attractive odour. In addition, in this experiment blow flies exhibited a slight preference for one side of the arena compared to another. Although this was corrected through the use of an online randomizer to vary the orientation of every replicate, other arena designs could be explored to avoid this and to provide more space to allow greater movement of flies. Extending this research through testing in the wild and observing whether there is an obvious choice difference with flies through different distances, or outside compared to an arena is another potential design that could prove fruitful.

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Table 2.1. The Breakdown of Amino Acids Yield VOCs. Studies of four critical amino acids determined that when they are broken down, amino acids emit specific VOCs.

Amino Acid Broken Down	**VOC Released Due to Amino Acid Breakdown**	**Study Citation**
Phenylalanine	Phenylacetic acid	Kim et al., 2007
Methionine	Dimethyl disulfide	Kadota & Ishida, 1972
Valine	Isobutylamine	Richardson, 1966
Tryptophan	Indole	Wang et al., 2001

Table 2.2. Dietary Treatments and their Corresponding Combination Number for this Study. The allocated combination numbers and which dietary treatment (each with their own profiles of VOCs) are assigned to each given number, along with control treatments.

Combination Number	Dietary Treatments Compared
1 (D1 vs. D2)	Medium lacking phenylacetic acid vs. medium lacking dimethyl disulfide
2 (D1 vs. D3)	Medium lacking phenylacetic acid vs. medium lacking isobutylamine
3 (D1 vs. D4)	Medium lacking phenylacetic acid vs. medium lacking indole
4 (D2 vs. D3)	Medium lacking dimethyl disulfide vs. medium lacking isobutylamine
5 (D2 vs. D4)	Medium lacking dimethyl disulfide vs. medium lacking indole
6 (D3 vs. D4)	Medium lacking isobutylamine vs. medium lacking indole
7	Negative Control
8	Positive Control

Table 2.3. Summarized Results of the Liu, 2014 Study. Several VOC concentrations were tested but the results below only include the most attractive concentration that the experiment identified.

VOC	**Most Attractive Concentration Tested**	**Species Tested**	**Significant?**
Indole	5 mg/ mL	*Lucilia sericata*	Yes
Isobutylamine	0.01 mg/ mL	*Lucilia sericata*	Yes
Phenylacetic acid	10 mg/ mL	*Lucilia sericata*	Yes
Dimethyl disulfide	5 mg/ mL	*Lucilia sericata*	Yes

Figure 2.1. Experimental Arena. An image taken above the arena with two petri dishes containing agar, placed in two numbered quadrants. Quadrants are labeled interiorly with numbers 1-4 according to their position. The top of the arena includes a hole plugged by a white cork, located in the centre of the arena. The clear plastic lid contains many small holes to allow air flow within the arena while preventing fly loss.

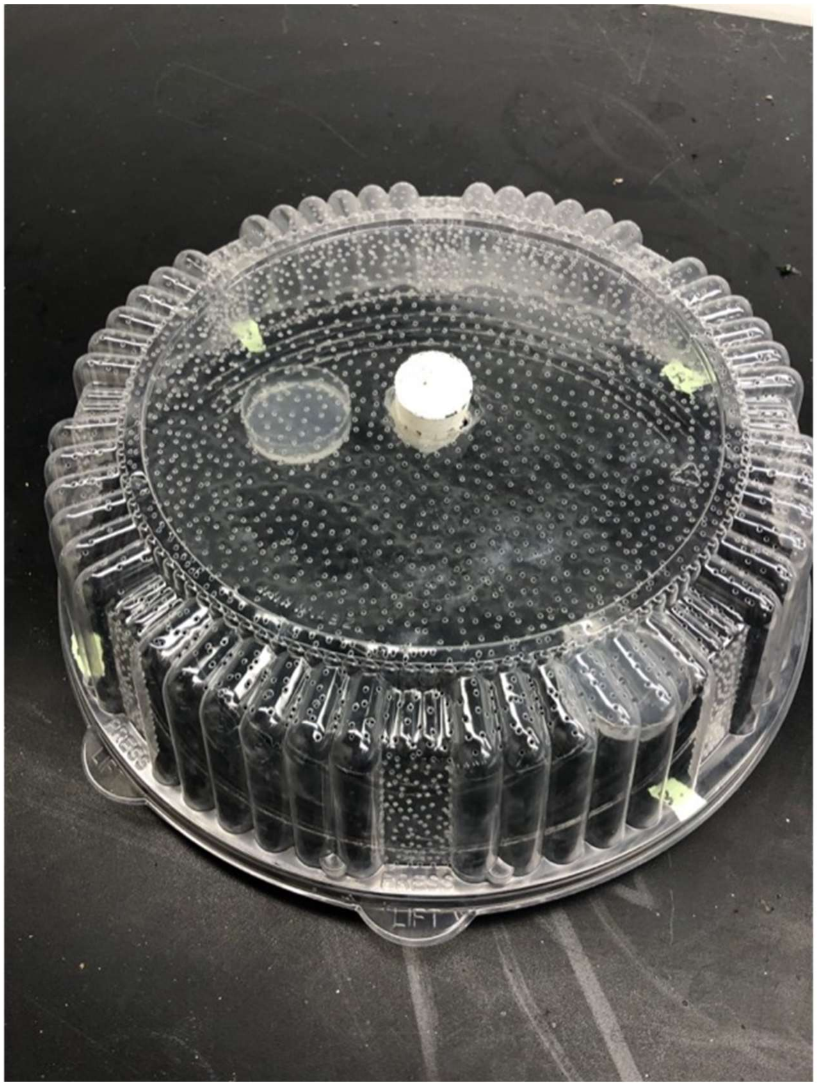


Figure 2.2. Open Experimental Arena. An image taken of the arena with two petri dishes containing agar, placed in randomly assigned sections. Quadrants are labeled with numbers assigned 1-4 according to their position. The lid to this arena is removed but visible at the top of this image.

Figure 2.3. Difference of Mean Proportions of Choice for VOC Profiles in Female *Lucilia sericata* Meigen (Diptera: Calliphoridae). This graph depicts the difference in mean (± SE) proportions of petri dish choice for *L. sericata*, when exposed to different combinations of agar lacking one VOC. Difference was attained through subtracting the mean attained by one VOC combination from the mean of the other combination. Choice was determined through the half of the arena that the fly was located. Bar direction indicates the fly's chosen VOC profile. Differences with asterisks were statistically significant ($P < 0.05$) based on one-sample proportion tests. Abbreviations in red denote which VOC is lacking: PAA= lacking phenylacetic acid; DMDS= lacking dimethyl disulfide; ISO= lacking isobutylamine; and IND = lacking indole.

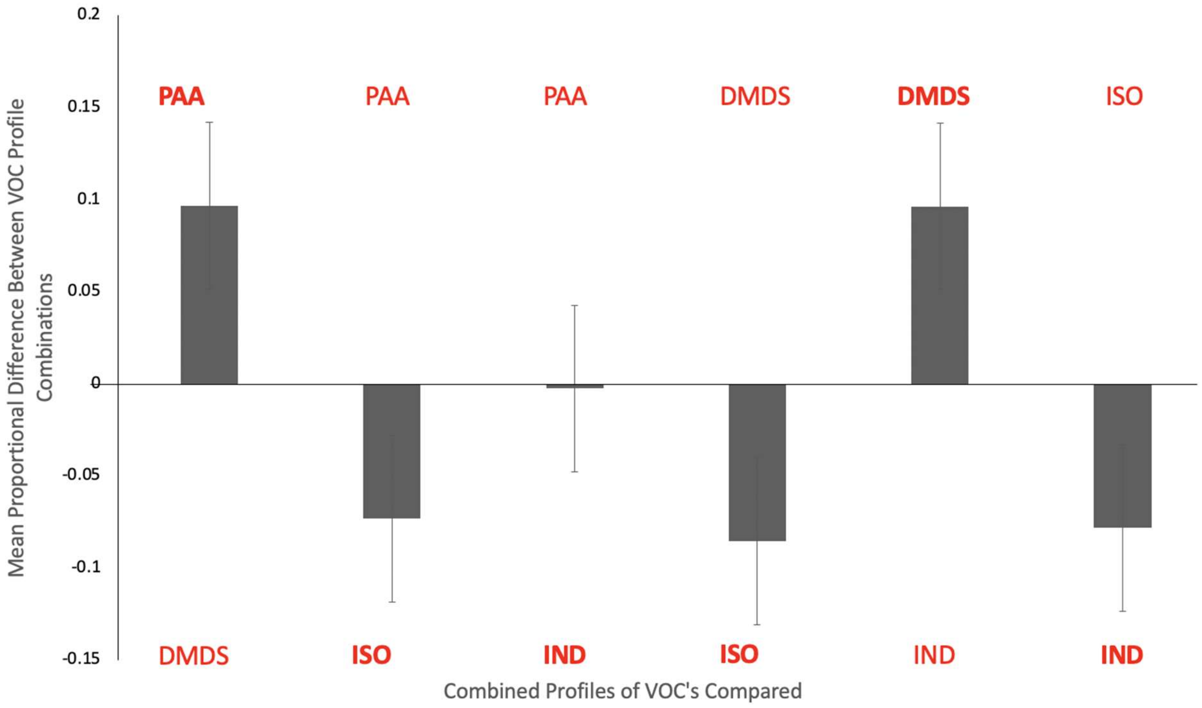


Figure 2.4. Difference of Mean Proportions of Choice for VOC Profiles in Female *Phormia regina* Meigen (Diptera: Calliphoridae). This graph depicts the difference in mean (± SE) proportions of petri dish choice for *P. regina*, when exposed to different combinations of agar lacking one VOC. Difference was attained through subtracting the mean attained by one VOC combination from the mean of the other combination. Choice was determined through the half of the arena that the fly was located. Bar direction indicates the fly's chosen VOC profile. Differences with asterisks were statistically significant ($P< 0.05$) based on one-sample proportion tests. Abbreviations in red denote which VOC is lacking: PAA= lacking phenylacetic acid; DMDS= lacking dimethyl disulfide; ISO= lacking isobutylamine; and IND = lacking indole.

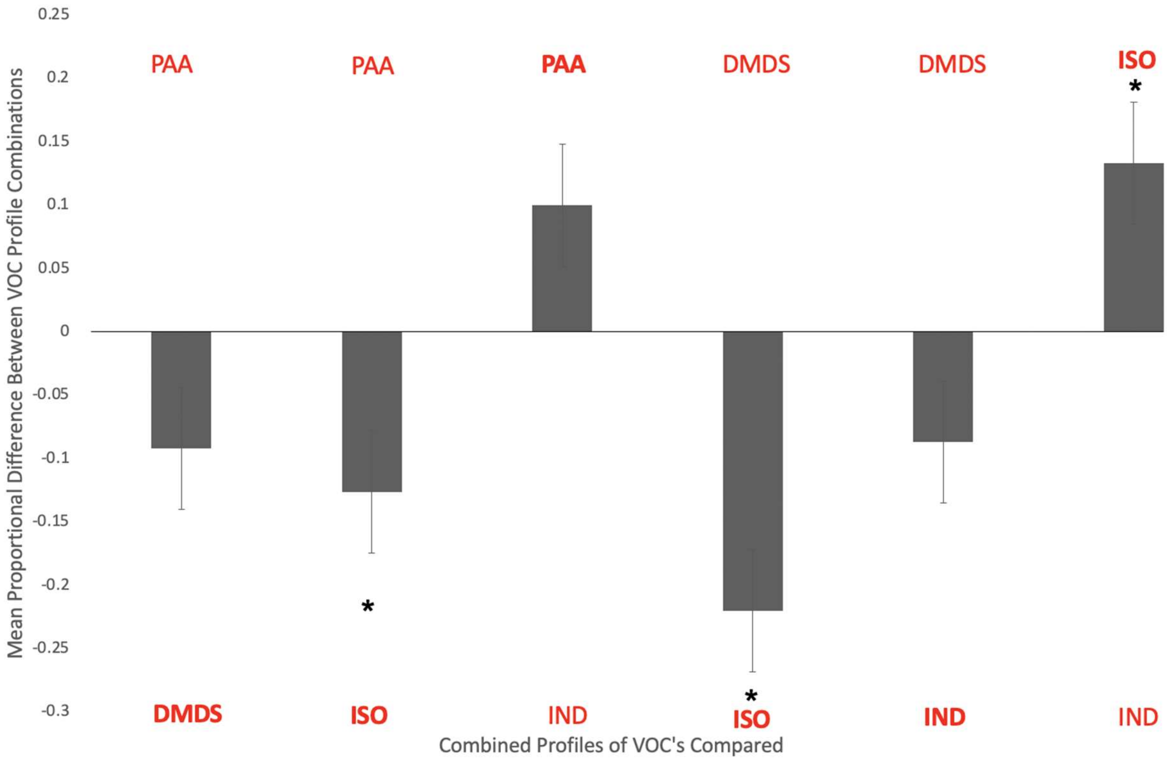
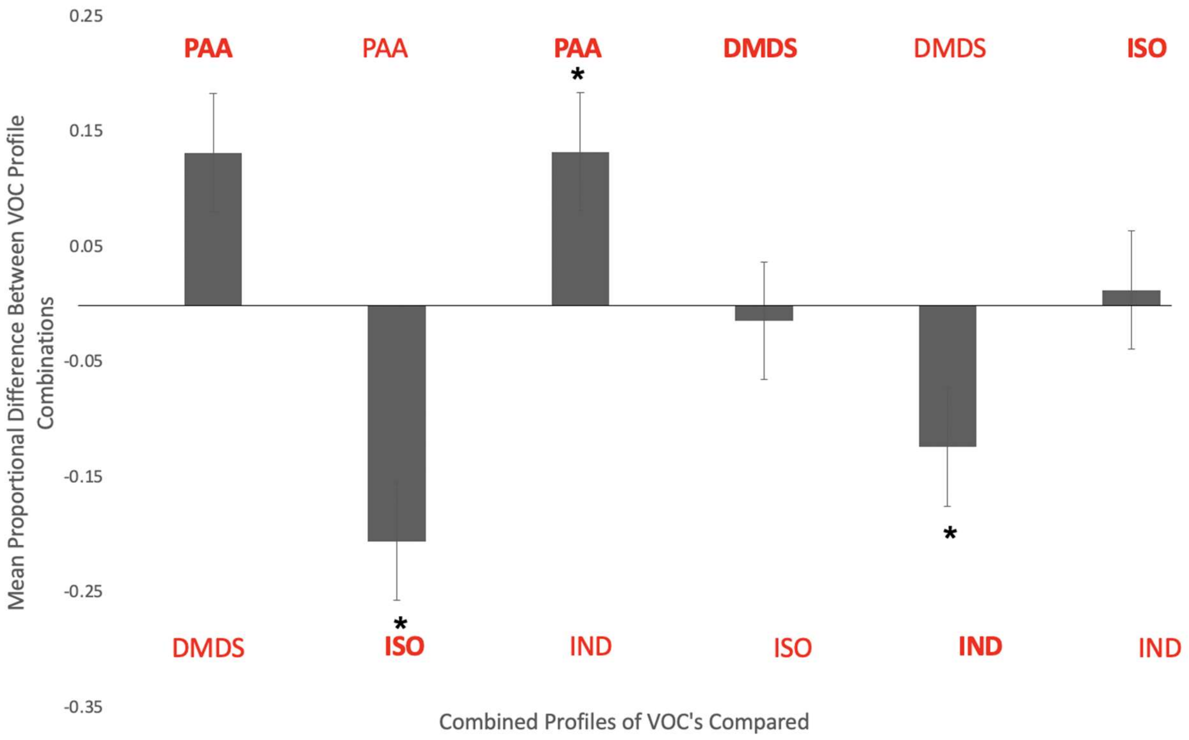


Figure 2.5. Difference of Mean Proportions of Choice for VOC Profiles in Female Calliphora vicina Robineau-Desvoidy (Diptera: Calliphoridae). This graph depicts the difference in mean (± SE) proportions of petri dish choice for *C. vicina*, when exposed to different combinations of agar lacking one VOC. Difference was attained through subtracting the mean attained by one VOC combination from the mean of the other combination. Choice was determined through the half of the arena that the fly was located. Bar direction indicates the fly's chosen VOC profile. Differences with asterisks were statistically significant ($P < 0.05$) based on one-sample proportion tests. Abbreviations in red denote which VOC is lacking: PAA= lacking phenylacetic acid; DMDS= lacking dimethyl disulfide; ISO= lacking isobutylamine; and IND = lacking indole.



Chapter 3. The Influence of Volatile Organic Compounds on the Species-specific Behavioural Response of Blow Flies (Diptera: Calliphoridae)

When an organism actively searches for a food or oviposition source, they are searching for an attractive medium that encompasses all their requirements at that point (Cury et al., 2018). In blow flies (Diptera: Calliphoridae) this is especially true, since they are obligate feeders on carrion and desire a source that contains all their nutrients so they can feed and oviposit on this resource (Byrd & Castner, 2010). To successfully consume and use resources, insects must first detect them (Patt & Pfannenstiel, 2009). Volatile organic compounds (VOCs) are airborne chemicals released through the breakdown of other substances; these materialize as detectable scents (Johansen et al., 2013). Insects often rely on attractive scents to dictate where to move and find both food and oviposition sources (Bish-Knaden et al., 2018; Mansourian & Stensmyr, 2015). These scents travel a distance and essentially lead insects directly to the source that they need (Kandasamy et al., 2019). Insects rely on their sensitive olfactory receptors to detect scents that demonstrate that a resource is available and favourable (Liu, 2014; Voshall et al., 2000), sometimes from a great distance (Braack, 1981; MacLeod & Donnelly, 1963; Popova et al., 2017; Zajac et al., 2018). They also use this same system to detect pheromones (Galizia & Rössler, 2010).

At shorter distances, insects rely on visual cues to locate attractive media and once they arrive, use their antennae to assess resource composition (Brodie et al., 2014; Glaser et al., 2013; Klinner et al., 2016). Chemosensory organs on feet allow insects to evaluate resource chemical composition like protein, sugars, and amino acids (Cury et al., 2019). Once contact is made on a substrate, insects can ascertain information, such as taste and texture of substrate, and use this information to choose whether the resource is suitable (Cury et al., 2019; Rockwell & Grossfield, 1978). Flying insects detect sugars and amino acids using chemosensory neurons found on the antennae, mouthparts, and feet (Scott et al., 2001; Amrein and Thorne, 2005). This may lead to an increase in tasting behaviours when insects are exposed to nutrients they deem beneficial, an example of behavioural plasticity.

Insects utilize a plethora of strategies to assess and accept (or reject) a stimulus. The physiological requirements of insects at the searching stage dictate what type of stimulus they will be looking for, in addition to what behavioural choices they make surrounding their exposure to stimuli (Lancaster et al., 2020; Leonhardt et al., 2020). Sex and physiological status (i.e., sexual maturity) play a role in ascertaining attraction or repulsion of volatiles (Liu, 2014). Adult female blow flies that are gravid and have mated search for an appropriate location for egg deposition. Male and female flies of the same blow fly species show differences in their choice of VOCs (Hobson, 1937; Liu, 2014). In addition to factors that males must consider (*e.g.*, mates, nutrition), female insects who oviposit rely on their resources to gain nutrients that are beneficial to their young (Jaenike, 1978; Lancaster et al., 2020). This is especially true in insects such as the blow fly, since their larvae are laid on a dietary medium wherein, they spend a majority of their growth stages (Boulay et al., 2015). If a female chooses wrong and the resource is unsuitable, the patchy and ephemeral nature of carrion resources means the offspring are highly likely to die before being able to acquire a new source of food; larvae cannot move large distances once deposited on a resource. This would also be true if the female had no adequate options and thus could not choose a beneficial one.

Once a baseline is determined for these behavioural actions, an increase or decrease in them may suggest plasticity in response to exposure to varied stimuli (Petzold et al., 2009; Han & Brooks, 2014). Although energetically expensive, the trait may be beneficial if this flexibility allows the organism to successfully respond to rapid environmental changes. For example, in *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), oviposition behaviour was determined to be plastic since female flies adopted alternate oviposition strategies (adjusting clutch size in response to a variation in host density and availability) that directly affected their fitness (Xu et al., 2012).

Insect behaviour is also dependent on both biotic and abiotic conditions (Hans, 2016). Resource composition is commonly heterogeneous, meaning that the insects will not always have single resources with the same composition, or an ideal amount of nutrients according to their specific requirements (Leonhardt et al., 2020). Particularly for insects that utilize decomposing resources, their availability may also be ephemeral, existing for a short period of time and changing in composition over time (Anderson, 2010; Byrd & Castner, 2010).

Insect diet can be variable due to each organism’s differing nutritional requirements (House, 1962). A combination of nutrients such as proteins, carbohydrates, vitamins, and minerals are generally required by a species, but the specific concentrations and ratios of each in relation to each other can fluctuate greatly between species (Cammack & Tomberlin, 2017;

Simpson & Abisgold, 1985). Though not much is known about precise measurements of dietary constituents to maximize a species' survival and reproduction rate, an omission or substitution of certain substances may lead to death or delayed growth (Mainali et al., 2019). For example, in the onion maggot fly, *Hylemya antiqua* Meigen (Diptera: Anthomyiidae), several amino acids are essential, and the omission of even one of these amino acids results in delayed growth and development, and death in most cases (Friend et al., 1957).

Piper and colleagues (2013) produced a study pertaining to insect diet with purified compounds. For this project, a complete holidic diet was produced for *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) wherein generations of offspring were reared on this diet, and subsequently their performance on the diet was measured. A holidic diet is one that is completely chemically defined (Piper, 2017). Overall, the diet was regarded as effective in supporting the development of these flies, though the rate of development was reduced. This study manipulated the use of specific amino acids to complete the dietary requirements of these flies.

Blow flies require a diet composed of various nutritional substances to fulfill their nutritive needs. They do not practice maternal care and as such they abandon their eggs after laying them (Janz, 2002); Because of this fact, the lives of the flies and their young rely on the food they are exposed to throughout their life cycle. Moreover, the choices of medium used by these adult flies (for mating, oviposition and/or feeding) given their environment, are important to maintain life. Flies consume water and carbohydrates (*i.e.,* sugar) to survive but additionally require a protein source for both oogenesis and spermatogenesis (Fraenkel, 1939; Harlow 1956; Huntington, & Higley, 2010). Essential amino acids cannot be created *de novo* and are attained through secondary consumption in the insect's diet (Nagao et al., 2015).

A study by Brust and Fraenkel (1955) was conducted in which the blow fly *Phormia regina* Meigen (Diptera: Calliphoridae) was fed an artificial diet comprised of salts, vitamins, water, protein, and carbohydrate sources. This report discussed the lack of research on the composition of growth media for fly species in their larval form. In this study, larvae were successfully raised on an artificially constructed diet. The base diet determined by this study includes compounds such as casein, cholesterol, water, agar, salt mixture, vitamin solution, and fungicide. These compounds were found to supply the blow flies with the nutrition they require to survive and thrive.

Rhinesmith-Carranza and colleagues (2018) conducted research wherein they allowed *Lucilia sericata* Meigen (Diptera: Calliphoridae) larvae to feed on a selection of four different dietary media. Diets in this study were based off of the diet from Piper and colleagues (2013) and modified for use in blow flies. All diets were identical in composition, with the exception of one essential amino acid missing, either phenylalanine, methionine, valine or tryptophan. Four millilitres of each diet were randomly placed in a petri dish divided in quadrants. The results of this study demonstrated that larvae do, in fact, have a preference for certain diets, and it is presumed that this preference will affect the larvae as they grow to adulthood. In ranked order, the food choice assay determined that the most preferred food for larvae was missing methionine, then lacking valine, then without phenylalanine and, the least preferred diet was one that did not contain tryptophan. It is not known whether or not adult female blow flies would maintain the same selections if offered the same diets.

There are many natural volatiles, and each may have their own unique scent associated with their profile (Adebesin et al., 2017). In addition, the composition of volatiles associated with natural resources vary temporally, spatially, and with both their physical and chemical characteristics (Bell & Cardé, 1984). During the process of decomposition in carrion, microbes consume the body's tissues, and create VOCs. Amino acid breakdown yields VOCs that are specific to the amino acid that is broken down (Barker, 1906) and may indicate what a source is rich in.

Since VOCs can be associated with their corresponding amino acid, the profile of VOCs indicate the quality and composition of the resource (Kadota & Ishida, 1972; Kim et al., 2007; Rhinesmith-Carranza et al., 2018; Richardson, 1966; Scheidler et al., 2015; Wang et al., 2001). Organisms exhibit preferences for these volatiles based on their species and organism's physiological status (Yan et al., 2018; Chaudhury et al., 2015). Additionally, the concentration of VOCs affect the organism's preferential response to the chemical (Liu, 2014). Thus, blow flies are likely to use VOCs to help them find carrion containing nutrients they need to utilize, whether it be dietary, oviposition, or mating purposes (Frederickx et al., 2011; Ashworth & Wall, 1994). Blow fly attraction has been previously studied with substances, such as indole, and others in chemical traps to lure flies towards, or away from a certain location (Chaudhury et al., 2015). Organisms exhibit preferences for these volatiles based on their species and organism's physiological status (Yan et al., 2018; Chaudhury et al., 2015). Additionally, the concentration of

VOCs affects the organism's preferential response to the chemical (Liu, 2014). Thus, blow flies are likely to use VOCs to help them find carrion containing nutrients they need to utilize, whether it be for dietary, oviposition, or mating purposes (Frederickx et al., 2011; Ashworth & Wall, 1994). Blow fly attraction has been previously studied with substances, such as indole, and others in chemical traps to lure flies towards, or away from a certain location (Chaudhury et al., 2015).

Critical amino acids phenylalanine, methionine, valine, and tryptophan are all sought after by blow flies and each amino acid degrades and releases a corresponding VOC. The breakdown of phenylalanine yields phenylacetic acid (PAA), methionine produces dimethyl disulfide (DMDS), indole is produced when tryptophan is broken down, and lastly isobutylamine is created through the breakdown of valine (Kadota & Ishida, 1972; Kim et al., 2007; Richardson, 1966; Wang et al., 2001). Due to this pattern of decomposition and what each VOC represents regarding amino acid nutrition, along with tested larval preferences, I hypothesized that blow flies would demonstrate the lowest locomotion (high arrestment) in media lacking dimethyl disulfide, caused by a preference for media lacking DMDS but containing indole. With preferred media, I also expect tasting will be increased. I additionally hypothesized that blow flies would least prefer the agar medium lacking indole, causing to them to arrest less, locomote more, and taste less often than when exposed to other conditions. This is due to the presence of other VOCs that indicate more vital amino acids are present with media lacking indole, namely tryptophan.

The blow fly species *Lucilia sericata*, *Phormia regina*, and *Calliphora vicina* (Diptera: Calliphoridae) are found in North America and other areas around the world (Hall 1948; Saunders & Hayward, 1998; Sivell, 2018). Blow fly species have distinctive temperature preferences and coexist with other species with similar tolerance ranges. For example, *Lucilia sericata* is considered to be a warmer weather species, preferring conditions ranging roughly from 20 to 33.5°C, compared to *Calliphora vicina* Robineau-Desvoidy with a preference of cooler conditions (found during fall and spring in temperate regions) (Lebouvier et al., 2011, Matoba & Terazawa, 2008). The species *Phormia regina* has a wider temperature range, overlapping with *C. vicina* in the spring and fall, and with *L. sericata* in the summer as long as there is adequate humidity (Brundage et al., 2011; Macleod & Donnelly, 1958). Lower limit temperatures in *Lucilia sericata* range from between 10-12.5°C and 2.5 to 4°C for *Calliphora vicina* (Faucherre et al., 1999).

For the comparison of different blow fly species, I hypothesized that seasonal distribution, in combination with species overlap, affects how flies respond behaviourally to their environment, with an increase or decrease in foraging behaviours depending on their preference. This may include increased locomotion for unfavourable resources (to avoid them) and increased arrestment for more favourable resources (to remain close to the resource), among other strategies. Moreover, I hypothesized that *P. regina* would demonstrate more specialistic feeding behaviour due to their distinct oviposition behaviour and preferences. This may present itself through increased arrestment with preferred profiles, and increased tasting overall. Additionally, this could lead flies to change from being stationary to locomoting more often than generalist species, since they are probing their media to see whether it fits their specific needs, as they are a choosy species when compared to other blow flies. In prior studies, results of interspecific competition were that *L. sericata* competed poorer than *P. regina* (Prinkkilä & Hanski, 1995; Smith & Wall, 1997). It may be that *L. sericata* may operate as generalists in comparison to more specialist *P. regina*, due in part to their negative response to intraspecific and interspecific competition (Smith & Wall, 1997). In contrast, *P. regina*, is better at surviving altered carrion, responding positively to higher larval density and increased competition (Pacheco, 2015). Through prior research, female *L. sericata* flies seem to be generalists that are quick to oviposit on media when given the opportunity (Hans et al., 2018; Pacheco, 2015). This may mean that they are fairly content with media regardless of its amino acid composition and may spend less time tasting or locomoting when exposed to these treatments (when compared to specialist species). Overall, they may accept their media by selecting it and then remaining on it without switching to locomotion or tasting. For flies of *C. vicina*, they are expected to behave similarly to generalist species *L. sericata*, with possible exceptions for preferences of VOCs that remain volatile in temperatures that correspond to this species' lower temperature thresholds. This means that they are likely to respond with little change in behaviour once they select their preferred medium and should respond to treatments similar to the positive control.

Materials and Methods:

Insect Source

Three different blow fly species were used for this experiment: *Phormia regina* Meigen, *Calliphora vicina* Robineau-Desvoidy, and *Lucilia sericata* Meigen (Diptera: Calliphoridae). All fly colonies were established with adults caught in in the wild in the Windsor-Essex area. Using King brand wasp traps (King Home and Garden Products, Item ID: 56789), lined with paper towel, and roughly 30g of porcine liver to attract blow flies. Colonies of each species were maintained at the University of Windsor (Windsor, Ontario Canada) in 46 cm X 46 cm X 46 cm aluminum mesh screen cages (Bioquip 1450C collapsible cage) on a 12:12 L:D diel cycle. Cages were supplied with a source of water, sugar (carbohydrate), and skim milk (protein), *ad libitum*. The colony room is maintained with conditions of ca. 20-30°C and 40-60% RH. Additionally, approximately 40 g of a protein source (pork liver) was placed in the cages *ad libitum* (replaced at a minimum of four times weekly) to create a suitable substance for flies to oviposit onto. Once egg masses were laid on the liver (less than 200 eggs to avoid crowding the jar), the mass was removed and placed inside a 1L glass Bernardin mason jar with sawdust lining approximately 1/3 of the bottom of the jar. This allowed the larvae to move down into the wood shavings to pupate. Jars were covered with a landscape tarp lid which allowed air flow but prevents larval escape. Additional liver was supplied as needed to reach pupation. Upon adult blow fly emergence, they were released back in their respective species cages. Every year, additional wild-caught blow flies were added to colony cages to maintain genetic diversity, avoid inbreeding depression, and limit lab adaptation.

Experimental Design

Experimental cages consisting of 30 adult female and 15 adult male blow flies (for each species) were created using freshly emerged blow flies (< 24 hours eclosed). Individual flies were vacuum aspirated into a vial, then sexed using eye spacing as blow flies are sexually dimorphic (Marshall et al., 2011). Each experimental cage contained a carbohydrate source (sugar), water in Erlenmeyer flasks with dental wicks to prevent drowning, and a protein (skim milk) source. Environmental conditions were a light: dark cycle of 12:12h, ca 23°C to 25°C, and at least 50% RH. To promote gravidity among females and encourage successful mating with fertile males, a five-day high protein pre-treatment was conducted prior to behavioural assessment (Hans et al., 2018). Porcine liver (50 g) was provided in the experimental cage for twenty-four hours a day for the first two days, and then for an hour each day for three days in order to stimulate ovarian growth and spermatogenesis. On day six, females were gravid and ready for the choice experiment. To verify sexual status (gravidity), flies were checked post-experimentation through dissection, and only confirmed gravid flies were included in the experimental study. A total of 180 flies were used for the experimental treatments, with each combination (1-6) were testing with 30 flies. To verify baseline controls, an additional 24 flies were included in both positive and negative controls (12 each).

To test the hypothesis that females demonstrate differential behavioural reactions in response to the removal of one of the four critical VOC cues (either dimethyl disulfide, phenylacetic acid, indole, or isobutylamine) treatments were created where all possible combinations of three critical VOCs, with one missing, were tested against each other (Table 3.2). This results in six total combinations to test. For each created treatment, a mixture of 15 mg of Bacto™ Agar (BD Diagnosis Bacto™ Agar Solidifying Agent) and 20 mL of distilled water were poured in a small petri dish measuring 60 X 15 mm. Next, three essential VOCs were added in concentrations previously found to be attractive (Table 3.1) (Liu, 2014). Pure solutions of each attractant (>99%) were purchased from Sigma-Aldrich, and 10 μL of three VOCs were diluted with acetone to desired concentrations. A combination was created for a positive control using two agar dishes containing bovine liver, which provides all cues necessary for oviposition (Bauer et al., 2020; Firoozfar et al., 2011; Parker & Welch, 1991). Conversely, a negative control combination was generated using two agar-only petri dishes to test fly behavioural response in the absence of VOC cues.

Subsequently, one gravid female was released into a circular testing arena measuring 5”X 12” (Figures 3.1-2) with two petri dishes containing different VOC treatments on agar. For each experimental arena, one treatment was randomly assigned to one half in the arena; Sides were chosen by an online randomizer (https://www.random.org/lists/). Behavioural response of the blow fly was documented every 30 minutes for an overall total of 5 hours. Behaviours were identified as either tasting, grooming, walking, standing, or flying. These behaviours were then further categorized into tasting (tasting/grooming), arrestment (standing), or locomotion (walking/flying). Trials for each treatment combination were repeated thirty times (n= 30), using a new female for each trial (n= 90 across all three species). Both negative and positive control combinations were tested twelve times (n= 12) for each species. At any given time, up to ten experimental arenas were run simultaneously. Experimental conditions remained the same as in rearing and pre-treatment, with ca. 23°C to 25°C and 50% RH, as measured by an Elitech temperature and humidity logger.

Statistical Analyses

Logistic regression analysis and modelling was used to determine whether there were significant behavioural changes within a behavioural category and within a species in response to VOC profile treatment combination, and then predict the magnitude of the change expected. The Akaike information criterion (AIC) is a mathematical estimator that determines the quality of models applied to data sets. Behavioural alteration was defined as blow flies spending a significantly different (increased/ decreased) proportion of time within a behavioural category (arrestment or locomotion).

Non-parametric Kruskal-Wallis ranked sum was used to test overall differences within species in the amount of time spent exhibiting each behaviour compared to other behaviours across pooled treatments, as well as by treatment. Non-parametric Wilcoxon tests for means separation of each pair was conducted to assess specific differences in time spent in each behaviour within treatment and species. Kruskal-Wallis was used to test overall differences within species and within behaviours but between VOC treatment combinations. Dunn's test with control for joint ranks were used for means separation of differences between treatments compared to either the positive or negative controls. Significance was set at $p < 0.05$ for all tests.

Qualitative Analyses

Ethograms are descriptive tables that are commonly used in behavioural studies (Dinesh & Venkatesha, 2012) and include a description of behaviour classifications used in an experiment and other relevant information such as images of described behaviour (Nicholson & O'Carroll, 2021). Identified blow fly behaviours assessed in this experiment have been classified and defined (Table 3.4). Categories were then combined according to likeness. Behaviour A was defined as observing fly standing behaviour. Behaviour B was categorized as flies demonstrating walking or flying behaviour, most locomotive in nature. Lastly, behaviours of grooming or tasting (probing) the medium was catalogued as behaviour C in the analysis.

Once the ethogram has been created, kinetic diagrams are constructed to provide a qualitative analysis of the pattern of behavior. They can be used descriptively to glean insight into how organisms respond over time when exposed to various experimental conditions. Kinetic diagrams demonstrate the order and frequency of the categorized behaviours, including an analysis of transitions from one behaviour to another (Pinzari & Sbordoni, 2013). Kinetic diagrams depict the sequence of behaviours by each species tested in this experiment, which include tallying walking, flying, grooming, tasting, and standing.

Results:

When pooled across experimental VOC profile combinations, and regardless of species (*L. sericata*: $X^2_{2,203} = 324.70$, $p<0.0001$; *P. regina*: $X^2_{2,204} = 395.66$, $p<0.0001$; *C. vicina*: $X^2_{2,204} = 416.50$, $p<0.0001$), flies consistently spent more time in arrestment, followed by locomotion, and the least amount of time in tasting. However, within individual VOC profile combinations, this overall ranked difference between each behaviour did not hold true in every instance (Figure 3.3). *Lucilia sericata* females spent the same amount of time in arrestment as in locomotion, both longer than time spent tasting when given the choice between media lacking phenylacetic acid or media lacking dimethyl disulfide ($X^2_{2,30} = 52.08$, $p<0.0001$). *Calliphora vicina* females also spent the same amount of time in arrestment as in locomotion, both longer than time spent tasting when in the absence of VOCs (negative control) ($X^2_{2,12} = 16.47$, $p= 0.0003$). Females of all three species spent the most time in arrestment, with no difference between locomotion and tasting when in the presence of liver media (positive control) (*L. sericata:* $X^2_{2,12} = 22.31$, $p<0.0001$; *P. regina:* $X^2_{2,12} = 24.91$, $p<0.0001$; *C. vicina:* $X^2_{2,12} = 25.12$, $p<0.0001$).

For *L. sericata* females, time spent in arrestment differed between liver media (positive control) and blank agar media (negative control) ($X^2_{7,30} = 19.35$; $p= 0.007$; Figure 3.3). There was no difference between the presence of liver media (positive control) and any VOC treatment combination in terms of time spent in arrestment, however when media lacking phenylacetic acid and dimethyl disulfide, or media lacking isobutylamine and indole were offered, time spent in arrestment did not differ from that of the negative control. Time spent in arrestment also differed between liver media (positive control) and blank agar media (negative control) for *C. vicina* females ($X^2_{7,30} = 17.28$; $p= 0.016$; Figure 3.3) and there was no difference between the positive control and any VOC treatment combination. However, it was only when media lacking phenylacetic acid and indole, or media lacking isobutylamine and indole combinations were offered that arrestment also differed from the negative control. For *P. regina* females, arrestment did not differ between positive or negative controls, or any treatment combinations ($X^2_{7,30}$ = 8.27; p= 0.3; Figure 3.3).

For *L. sericata* females, time spent in locomotion differed between liver media (positive control) and blank agar media (negative control) ($X^2_{7,30}$ = 31.05; p<0.0001; Figure 3.3). There was no difference between the presence of liver media (positive control) and any VOC treatment combination, except for the media combination lacking phenylacetic acid and dimethyl disulfide, in terms of time spent in locomotion. Only the media combination lacking phenylacetic acid and dimethyl disulfide did not differ in time spent in locomotion from that of the negative control. This same pattern in locomotion was observed for *C. vicina* females ($X^2_{7,30}$ = 22.88; p= 0.0018; Figure 3.3). For *P. regina* females, locomotion did not differ between positive or negative controls, or any treatment combinations ($X^2_{7,30}$ = 8.80; p= 0.3; Figure 3.3).

Time spent tasting did not differ between treatments compared to controls, nor between controls for any of the three species (*L. sericata:* $X^2_{2,30}$ = 12.50, p= 0.08; *P. regina:* $X^2_{2,30}$ = 10.66, p= 0.2; *C. vicina:* $X^2_{2,30}$ = 9.88, p= 0.2; Figure 3.3).

Kinetic diagrams demonstrate that the most frequent behavioural sequence for gravid females all three species is switching between standing (arrestment) and walking/flying, or vice versa (Figures 3.4-6). Interestingly, in the presence of any VOC media treatment and across all three fly species, the number of switches between standing and walking/flying increased, often more than double, compared to either of the controls.

In the presence of the liver agar (positive control) compared to the blank agar (negative control), *L. sericata* females increase the number of switches between grooming/ tasting, and standing (arrestment), particularly from grooming to standing, to be either equal to or more than the number of switches between walking/flying (locomotion) and grooming/tasting (Figure 3.4). Using this pattern as a baseline, flies exposed to media lacking phenylacetic acid and indole, or media lacking dimethyl disulfide and isobutylamine, or media lacking isobutylamine and indole all exhibit the positive control behavioural pattern. In contrast, flies exposed to media lacking phenylacetic acid and dimethyl disulfide exhibit the negative control pattern, whereas the last two VOC combinations exhibit an intermediate behavioural pattern with approximately equal switching between standing and grooming/tasting, and between flying/walking and grooming/tasting.

In the presence of the liver agar (positive control) compared to the blank agar (negative control), *P. regina* females increase the number of switches from grooming/ tasting to standing (arrestment) and have an equal or reduced number of switches between walking/flying and grooming/tasting, particularly from grooming/tasting to walking/flying (Figure 3.5). Using this pattern as a baseline, flies exposed to media lacking phenylacetic acid and dimethyl disulfide, or media lacking phenylacetic acid and indole, or media lacking dimethyl disulfide and isobutylamine, or media lacking dimethyl disulfide and indole all exhibit the positive control behavioural pattern. In contrast, flies exposed to media lacking phenylacetic acid and isobutylamine, or media lacking isobutylamine and indole exhibit the negative control pattern.

In the presence of the liver agar (positive control) compared to the blank agar (negative control), *C. vicina* females increase the number of switches from grooming/ tasting to standing (arrestment) and vice versa and have a lower number of switches between walking/flying and grooming/tasting compared to the number of switches between grooming/tasting and standing (Figure 3.6). Using this pattern as a baseline, flies exposed to all VOC treatments exhibited a positive control behavioural pattern.

Percentage of increase or decrease in behaviours of locomotion and arrestment were summarized according to treatment conditions (lacking VOCs) when compared to others (Table 3.4). Overall, the treatments elicited more than 50% decrease in arrestment when compared to negative control and more than 80% increase in locomotion. Treatment conditions elicited more than 50% increase in arrestment with roughly 50% decrease in locomotion when compared against positive control.

The percentage of increase or decrease in locomotion and arrestment were summarized according to treatment conditions (lacking VOCs) when compared to other treatments (Table 3.5). Overall, many of the treatment conditions did not show a significant difference when compared to controls.

Percentage of increase or decrease in behaviours of arrestment and locomotion were tabled according to treatment conditions (lacking VOCs) in comparison to others (Table 3.6). To summarize, treatment conditions showed over 60% decrease in arrestment and more than 150% increase in locomotion when compared to negative control. Except for lacking phenylacetic acid vs. lacking indole, and lacking isobutylamine vs. lacking indole, all other treatments compared to positive control yielded roughly 100% increased arrestment and roughly 80% decrease in locomotion.

Discussion:

The three blow fly species demonstrated the expected overall pattern of arrestment being the most prevalent behaviour in experimental treatments, followed by locomotion, and lastly by tasting in the presence of VOCs that represent essential amino acids required for their offspring.

Despite an expected increase in arrestment and decrease in other behaviours for preferred VOC combinations containing indole compared to the VOC profile combination lacking indole, this was not observed. Indeed, for *L. sericata* when presented the choice of media lacking isobutylamine or lacking indole, arrestment was intermediate and not different from either control, suggesting the lack of indole isn't a critical cue for *L. sericata* when other essential amino acid cues are present. For *C. vicina*, the greatest arrestment, although not different from the other VOC combinations) was when media lacking indole was paired with two VOC combinations that contained indole but lacked another VOC (isobutylamine or phenylacetic acid), although interestingly, it was not the predicted combination of media missing dimethyl disulfide (but containing indole) compared with media missing indole. It is possible that this is because larval preferences and adult preferences may not align perfectly since females may be less choosy so long as the media provides sufficient nutrition for larvae to survive (Dweck et al., 2018).

Although there was the prediction that media missing dimethyl disulfide but containing indole would result in higher arrestment, this was not the case. Interestingly, the combination of media lacking dimethyl disulfide compared with media lacking phenylacetic acid resulted in greater locomotion compared to other VOC combinations, and equivalent to the negative control, for both *L. sericata* and *C. vicina*, suggesting the flies found this less acceptable than the other VOC combinations.

Despite the prediction that *P. regina* would be the most choosy of the three blow fly species, the percentage of time spent in the individual behaviours didn't vary between any of the VOC media combinations. However, in the presence of the phenylacetic acid lacking with isobutylamine lacking media, or the isobutylamine lacking with indole lacking media, this species was almost twice as likely to switch to locomotion from tasting, then to switch from locomotion to tasting. It is unclear what this suggests, although it could reflect a movement away from the alternative choice presented and towards the isobutylamine lacking media, as observed in the previous chapter.

The different results exhibited by each species of blow fly may be a result of their seasonality, resource use, and coexistence with other species. For example, *P. regina* coexists with both *L. sericata* and *C. vicina* in the Windsor-Essex region. In contrast to this, *L. sericata* and *C. vicina* overlap much less seasonally. Species *L. sericata* exists primarily during summer and early fall seasons, with *C. vicina* found in early spring and late fall. Processes of decomposition occur at a slower rate at lower temperatures, and *C. vicina*'s corresponding low temperature threshold led to a mixed response in this species. Warmer weather (*I.e.*, summer) increases the rate of decomposition meaning *L. sericata* must colonize the resource quickly to consume it before it disappears. The species *P. regina* has a wider seasonality than other species, and its' response varies due to its' choosy nature.

The agar media with VOCs are not suitable for oviposition but is attractive to these flies. Compared to the negative control lacking VOCs and the positive liver control that is suitable for oviposition and provides all of the cues, all three species approximately doubled the number of behavioural switches between arrestment, locomotion, and tasting, suggesting increased foraging behaviour by these flies. In addition, the time spent within various behaviours differed and flies may exhibit different behavioural patterns based upon the time spent assessing attractive media.

Overall, blow flies forage differently to the preferences of their larval counterparts, though parental choice directly affects their offspring. In addition to this, blow flies of differing species behaviourally respond differently with profiles of volatile scents. Preferences may be subtle or obvious (depending on the species) but are caused by a combination of complex factors such as coexistence, seasonality, sterility, sexual status, and more. Behavioural changes can be demonstrated through increases or decreases in arrestment, tasting, or locomotion. Each of these behaviours serve a different function but exist to allow the fly to probe the media and move towards, or away from a stimulus.

This research could be extended through adding the corresponding amino acids to experimental VOC cues and flies subsequently tested similar to this experiment. Behaviours tracked here can indicate whether it is the scent or the actual presence of the amino acid that attracts the flies. Additionally, testing in the natural environment of the flies would provide further whether the fly behaviour is altered significantly when they are able to travel large distances. Lastly, comparing each treatment profile created to only the positive and negative controls would garner information about how relatively attractive the profiles are, and would confirm exactly how different flies respond in predictable cases.

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Table 3.1. Most Attractive VOC Concentrations for *Lucilia sericata* Meigen (Diptera: Calliphoridae) (Liu 2014). These concentrations were used in the current study.

VOC	**Most Attractive Concentration Tested**	**Species Tested**	**Statistically Significant?**
Indole	5 mg/ mL	*Lucilia sericata*	Yes
Isobutylamine	0.01 mg/ mL	*Lucilia sericata*	Yes
Phenylacetic acid	10 mg/ mL	*Lucilia sericata*	Yes
Dimethyl disulfide	5 mg/ mL	*Lucilia sericata*	Yes

Table 3.2. Dietary Treatments and their Corresponding Combination Number for this Study. Dietary treatment (each with their own profiles of VOCs) are assigned to each given number, along with control treatments.

Combination Number	**Dietary Treatments Compared**
1 (D1 vs. D2)	Medium lacking phenylacetic acid vs. medium lacking dimethyl disulfide
2 (D1 vs. D3)	Medium lacking phenylacetic acid vs. medium lacking isobutylamine
3 (D1 vs. D4)	Medium lacking phenylacetic acid vs. medium lacking indole
4 (D2 vs. D3)	Medium lacking dimethyl disulfide vs. medium lacking isobutylamine
5 (D2 vs. D4)	Medium lacking dimethyl disulfide vs. medium lacking indole
6 (D3 vs. D4)	Medium lacking isobutylamine vs. medium lacking indole
7	Negative Control
8	Positive Control

Table 3.3. Ethogram for Determining Blow Fly Behaviour. Five typical blow fly behaviours are represented in this ethogram. The behaviours were recorded in order from the beginning of the experiment to the end of the experiment. One hundred and twenty blow flies of three different species were tested for behaviour every half hour during a trial including ten checkpoints (roughly five hours in total length).

Name of Behavior	Description	Picture	Function
Grooming	A behaviour that depicts the fly rubbing their legs together and may include them rubbing their legs onto their mouthparts, abdomen, or wings.		This behaviour is thought to be associated with blow fly cleaning.
Tasting of medium	When the blow fly uses their mouthparts to probe their media and taste a food source.		To taste and sense the validity of a food source.

Flying	Flying occurs when the blow fly uses its wings to lift off of the ground and become suspended in midair.		Locomotion from one location to another (towards or away from some stimulus)
Walking	This is when the blow fly uses its legs to locomote from one location to another.		Locomotion from one location to another (towards or away from some stimulus)
Standing on one spot	When a fly is standing on one spot they are immobile but standing on their legs.		Resting or remaining at one location (possibly due to a positive stimulus).

Table 3.4. A Summary Chart of Increased/ Decreased Percentage of Behaviours Obtained through Logistic Regression Modelling for the Species *Lucilia sericata* Meigen (Diptera: Calliphoridae). These values are demonstrated for all experimental combinations and control treatments. Tabulated AIC is included within the table. Abbreviated VOCs demonstrate what VOC is lacking for each treatment. PAA refers to medium lacking phenylacetic acid. DMDS refers to medium lacking dimethyl disulfide. IND refers to medium lacking indole. ISO refers to medium lacking isobutylamine.

		Behaviours	
Combination	**Compared against**	**Arrestment**	**Locomotion**
PAA vs. DMDS	Negative Control	51%↓ AIC= 727.9	82%↑ AIC= 740.7
PAA vs. DMDS	Positive Control	140%↑ AIC= 705.79	75%↓ AIC= 623.12
PAA vs. ISO	Negative Control	67%↓ AIC= 712.56	175%↑ AIC= 711.8
PAA vs. ISO	Positive Control	59%↑ AIC= 690.46	62%↓ AIC= 594.22
PAA vs. IND	Negative Control	67%↓ AIC= 927.11	297%↑ AIC= 860.13
PAA vs. IND	Positive Control	61%↑ AIC= 905	47%↓ AIC= 743.87
DMDS vs. ISO	Negative Control	60%↓ AIC= 1133.3	272%↑ AIC= 1027.2
DMDS vs. ISO	Positive Control	93%↑ AIC= 1111.2	48%↓ AIC= 909.61
DMDS vs. IND	Negative Control	62%↓ AIC= 1129.8	206%↑ AIC= 1071
DMDS vs. IND	Positive Control	86%↑ AIC= 1107.7	57%↓ AIC= 953.4
ISO vs. IND	Negative Control	58%↓ AIC= 1137.2	163%↑ AIC= 1100.5
ISO vs. IND	Positive Control	104%↑ AIC= 1115.1	63%↓ AIC= 982.95
Negative Control	Positive Control	387%↑ AIC= 598.34	86%↓ AIC= 541
PAA vs. DMDS	PAA vs. ISO	149%↑ AIC= 820.02	65%↓ AIC= 793.92
PAA vs. DMDS	PAA vs. IND	148%↑ AIC= 1034.6	47%↓ AIC= 943.53
PAA vs. DMDS	DMDS vs. ISO	0%↓↑ AIC= 1240.8	48%↓ AIC= 1109.3
PAA vs. DMDS	DMDS vs. IND	0%↓↑ AIC= 1237.3	58%↓ AIC= 1153.1
PAA vs. DMDS	ISO vs. IND	0%↓↑ AIC= 1244.6	68%↓ AIC= 1182.7
PAA vs. ISO	PAA vs. IND	0%↓↑ AIC= 1019.2	71%↓ AIC= 914.63
PAA vs. ISO	DMDS vs. ISO	0%↓↑ AIC= 1225.4	73%↓ AIC= 1080.4
PAA vs. ISO	DMDS vs. IND	0%↓↑ AIC= 1222	0%↓↑ AIC= 1124.2
PAA vs. ISO	ISO vs. IND	0%↓↑ AIC= 1229.3	0%↓↑ AIC= 1153.7
PAA vs. IND	DMDS vs. ISO	0%↓↑ AIC= 1440	0%↓↑ AIC= 1230
PAA vs. IND	DMDS vs. IND	0%↓↑ AIC= 1436.5	0%↓↑ AIC= 1273.8
PAA vs. IND	ISO vs. IND	0%↓↑ AIC= 1443.8	143%↑ AIC= 1303.4
DMDS vs. ISO	DMDS vs. IND	0%↓↑ AIC= 1642.7	0%↓↑ AIC= 1439.6
DMDS vs. ISO	ISO vs. IND	0%↓↑ AIC= 1650	141%↑ AIC= 1469.1
DMDS vs. IND	ISO vs. IND	0%↓↑ AIC= 1646.6	0%↓↑ AIC= 1512.9
N= 2040			

Table 3.5. A summary chart of increased/ decreased percentage of behaviours obtained through logistic regression modelling for the species *Phormia regina* Meigen (Diptera: Calliphoridae). These values are demonstrated for all experimental combinations and control treatments. Tabulated AIC is included within the table. Abbreviated VOCs demonstrate what VOC is lacking for each treatment. PAA refers to medium lacking phenylacetic acid. DMDS refers to medium lacking dimethyl disulfide. IND refers to medium lacking indole. ISO refers to medium lacking isobutylamine.

		Behaviours	
Combination	**Compared against**	**Arrestment**	**Locomotion**
PAA vs. DMDS	Negative Control	0%↓↑ AIC= 542.67	0%↓↑ AIC= 490.58
PAA vs. DMDS	Positive Control	0%↓↑ AIC= 526.46	0%↓↑ AIC= 477.96
PAA vs. ISO	Negative Control	0%↓↑ AIC= 567.56	0%↓↑ AIC= 514.33
PAA vs. ISO	Positive Control	99%↑ AIC= 551.35	48%↓ AIC= 501.71
PAA vs. IND	Negative Control	0%↓↑ AIC= 517.03	0%↓↑ AIC= 422.56
PAA vs. IND	Positive Control	0%↓↑ AIC= 500.82	0%↓↑ AIC= 409.946
DMDS vs. ISO	Negative Control	0%↓↑ AIC= 522.45	0%↓↑ AIC= 474.12
DMDS vs. ISO	Positive Control	0%↓↑ AIC= 506.25	0%↓↑ AIC= 461.5
DMDS vs. IND	Negative Control	0%↓↑ AIC= 547.86	0%↓↑ AIC= 496.2
DMDS vs. IND	Positive Control	0%↓↑ AIC= 531.65	0%↓↑ AIC= 483.58
ISO vs. IND	Negative Control	0%↓↑ AIC= 547.86	0%↓↑ AIC= 498.01
ISO vs. IND	Positive Control	0%↓↑ AIC= 531.65	0%↓↑ AIC= 485.39
Negative Control	Positive Control	0%↓↑ AIC= 298.57	0%↓↑ AIC= 256.82
PAA vs. DMDS	PAA vs. ISO	69%↓ AIC= 795.46	0%↓↑ AIC= 735.48
PAA vs. DMDS	PAA vs. IND	0%↓↑ AIC= 744.92	58%↓ AIC= 643.71
PAA vs. DMDS	DMDS vs. ISO	0%↓↑ AIC= 750.35	0%↓↑ AIC= 695.27
PAA vs. DMDS	DMDS vs. IND	0%↓↑ AIC= 775.75	0%↓↑ AIC= 717.35
PAA vs. DMDS	ISO vs. IND	0%↓↑ AIC= 775.75	0%↓↑ AIC= 719.16
PAA vs. ISO	PAA vs. IND	182%↑ AIC= 769.82	46%↓ AIC= 667.46
PAA vs. ISO	DMDS vs. ISO	174%↑ AIC= 775.24	69%↓ AIC= 719.02
PAA vs. ISO	DMDS vs. IND	0%↓↑ AIC= 800.64	0%↓↑ AIC= 741.1
PAA vs. ISO	ISO vs. IND	0%↓↑ AIC= 800.64	0%↓↑ AIC= 742.9
PAA vs. IND	DMDS vs. ISO	0%↓↑ AIC= 724.71	147%↑ AIC= 627.25
PAA vs. IND	DMDS vs. IND	0%↓↑ AIC= 750.11	178%↑ AIC= 649.33
PAA vs. IND	ISO vs. IND	0%↓↑ AIC= 750.11	181%↑ AIC= 651.14
DMDS vs. ISO	DMDS vs. IND	0%↓↑ AIC= 755.53	0%↓↑ AIC= 700.89
DMDS vs. ISO	ISO vs. IND	0%↓↑ AIC= 755.53	0%↓↑ AIC= 702.69
DMDS vs. IND	ISO vs. IND	0%↓↑ AIC= 780.94	0%↓↑ AIC= 724.78
N= 2040			

Table 3.6. A summary chart of increased/ decreased percentage of behaviours obtained through logistic regression modelling for the species *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae). These values are demonstrated for all experimental combinations and control treatments. Tabulated AIC is included within the table. Abbreviated VOCs demonstrate what VOC is lacking for each treatment. PAA refers to medium lacking phenylacetic acid. DMDS refers to medium lacking dimethyl disulfide. IND refers to medium lacking indole. ISO refers to medium lacking isobutylamine.

		Behaviours	
Combination	**Compared against**	**Arrestment**	**Locomotion**
PAA vs. DMDS	Negative Control	66%↓ AIC= 509.94	174%↑ AIC= 472.71
PAA vs. DMDS	Positive Control	106%↑ AIC= 441.5	80%↓ AIC= 357.35
PAA vs. ISO	Negative Control	67%↓ AIC= 507.76	205%↑ AIC= 458.74
PAA vs. ISO	Positive Control	102%↑ AIC= 439.32	78%↓ AIC= 343.38
PAA vs. IND	Negative Control	80%↓ AIC= 440.60	392%↑ AIC= 395.01
PAA vs. IND	Positive Control	0%↓↑ AIC= 372.25	64%↓ AIC= 279.64
DMDS vs. ISO	Negative Control	68%↓ AIC= 501	205%↑ AIC= 458.74
DMDS vs. ISO	Positive Control	91%↑ AIC= 432.56	78%↓ AIC= 343.38
DMDS vs. IND	Negative Control	64%↓ AIC= 518.3	174%↑ AIC= 472.71
DMDS vs. IND	Positive Control	120%↑ AIC= 449.86	80%↓ AIC= 357.35
ISO vs. IND	Negative Control	74%↓ AIC= 476.06	218%↑ AIC= 452.85
ISO vs. IND	Positive Control	0%↓↑ AIC= 407.62	77%↓ AIC= 337.49
Negative Control	Positive Control	506%↑ AIC= 268.27	93%↓ AIC= 214.65
PAA vs. DMDS	PAA vs. ISO	0%↓↑ AIC= 680.98	0%↓↑ AIC= 601.44
PAA vs. DMDS	PAA vs. IND	70%↑ AIC= 613.92	55%↓ AIC= 537.7
PAA vs. DMDS	DMDS vs. ISO	0%↓↑ AIC= 674.23	0%↓↑ AIC= 601.44
PAA vs. DMDS	DMDS vs. IND	0%↓↑ AIC= 691.53	0%↓↑ AIC= 615.41
PAA vs. DMDS	ISO vs. IND	0%↓↑ AIC= 649.28	0%↓↑ AIC= 595.55
PAA vs. ISO	PAA vs. IND	167%↑ AIC= 611.74	62%↓ AIC= 523.73
PAA vs. ISO	DMDS vs. ISO	0%↓↑ AIC= 672.05	0%↓↑ AIC= 587.47
PAA vs. ISO	DMDS vs. IND	0%↓↑ AIC= 689.35	0%↓↑ AIC= 601.44
PAA vs. ISO	ISO vs. IND	0%↓↑ AIC= 647.11	0%↓↑ AIC= 581.58
PAA vs. IND	DMDS vs. ISO	62%↓ AIC= 604.98	161%↑ AIC= 535.73
PAA vs. IND	DMDS vs. IND	54%↓ AIC= 622.29	179%↑ AIC= 537.7
PAA vs. IND	ISO vs. IND	0%↓↑ AIC= 580.04	0%↓↑ AIC= 517.85
DMDS vs. ISO	DMDS vs. IND	0%↓↑ AIC= 682.6	0%↓↑ AIC= 601.44
DMDS vs. ISO	ISO vs. IND	0%↓↑ AIC= 640.35	141%↑ AIC= 581.58
DMDS vs. IND	ISO vs. IND	0%↓↑ AIC= 657.65	0%↓↑ AIC= 595.55
N= 2040			

Figure 3.1. Experimental Arena. An image taken above the arena with two petri dishes containing agar, placed in two numbered quadrants. Quadrants are labeled interiorly with numbers 1-4 according to their position. The top of the arena includes a hole plugged by a white cork, located in the centre of the arena. The clear plastic lid contains many small holes to allow air flow within the arena while preventing fly loss.

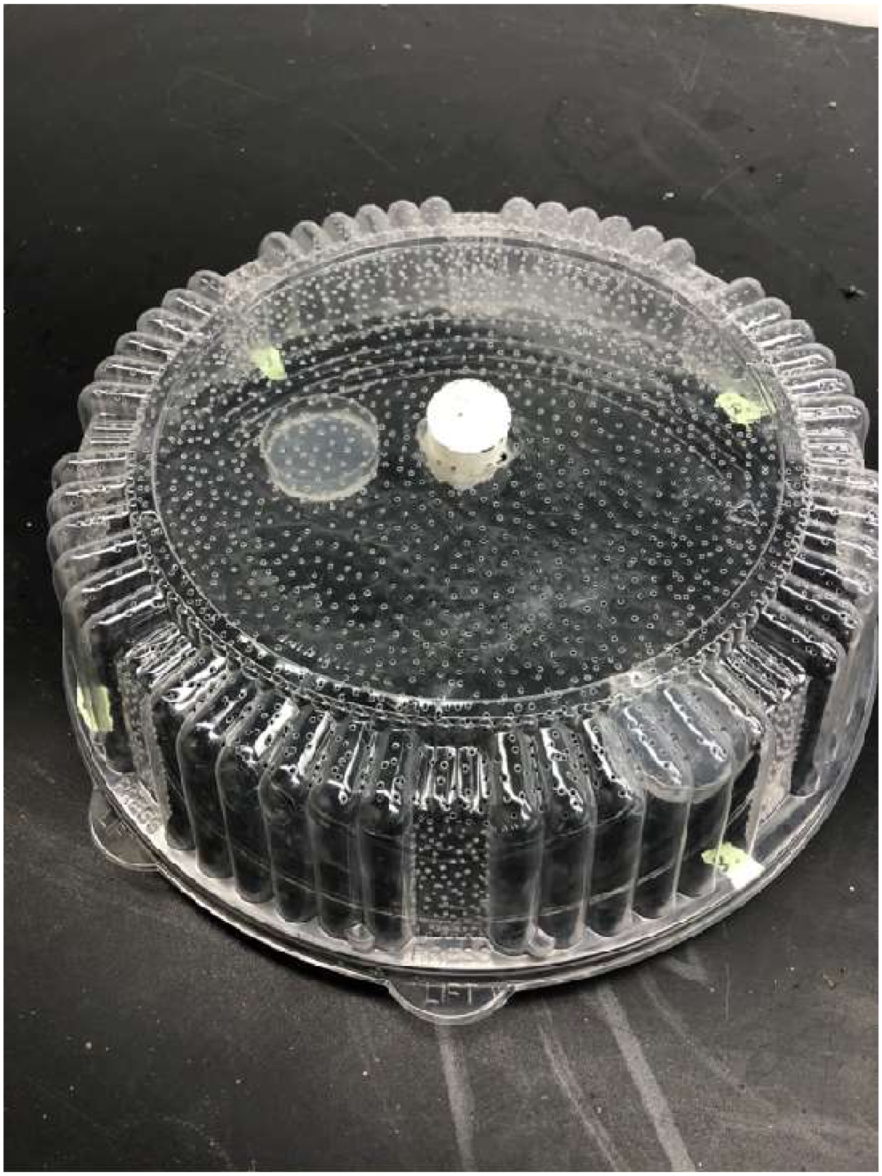


Figure 3.2. Open Experimental Arena. An image taken of the arena with two petri dishes containing agar, placed in randomly assigned sections. Quadrants are labeled with numbers assigned 1-4 according to their position. The lid to this arena is removed but visible at the top of this image.

Figure 3.3. Mean (+SE) Percentage of Time Spent Exhibiting Arrestment, Locomotion, or Tasting Behaviour by *Lucilia sericata* Meigen, *Phormia regina* Meigen, and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) in response to VOC treatment profiles. Behaviours were assessed across ten checkpoints (N= 5400). Means followed by the same letter within treatment are not different (P>0.05). Means followed by the same roman numeral across treatments are not different (P>0.05). Media profile abbreviations tested: PAA = lacking phenylacetic acid; DMDS = lacking dimethyl disulfide; ISO = lacking isobutylamine; and IND = lacking indole.

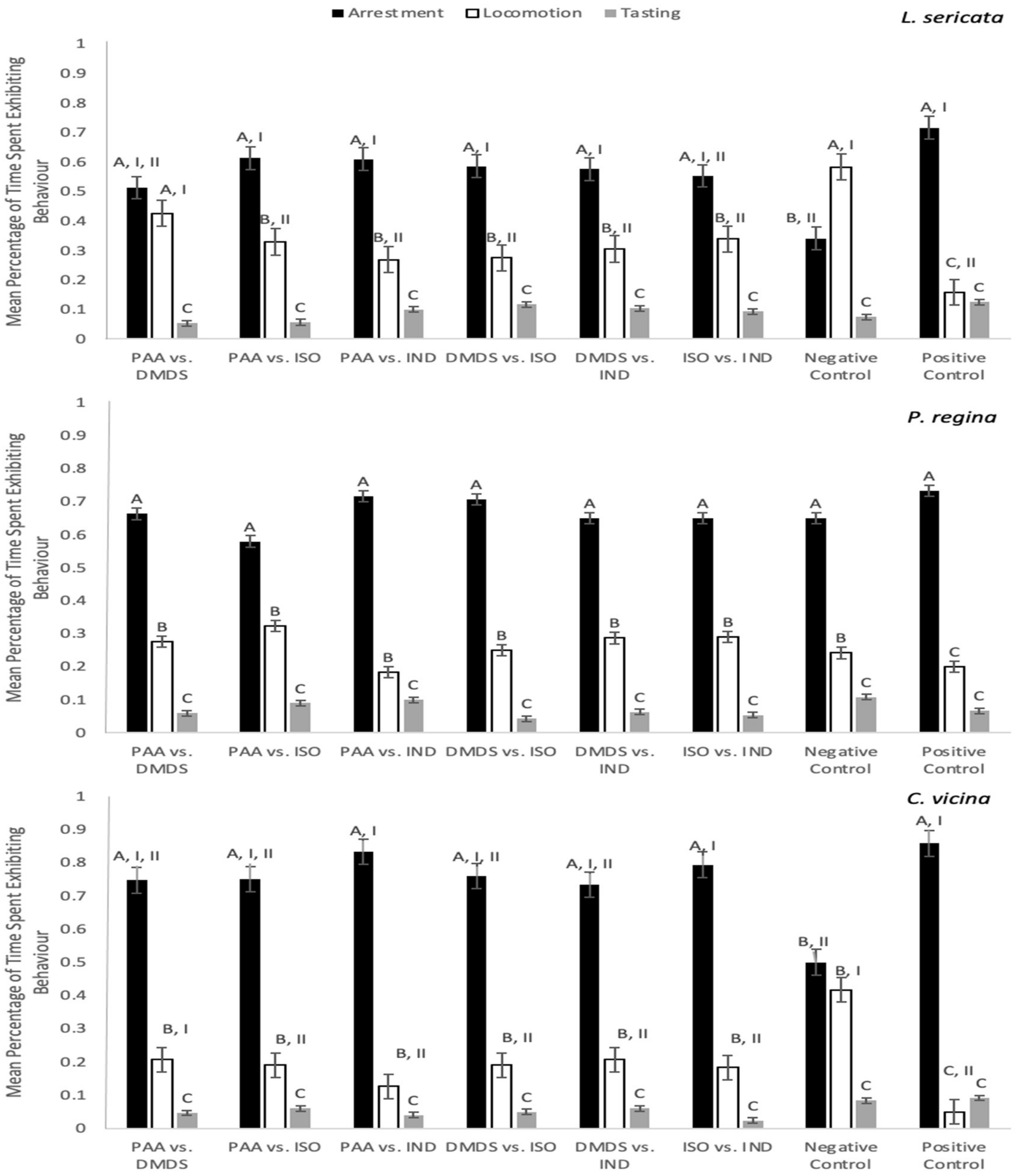


Figure 3.4. Blow fly Order and Frequency of Demonstrated Behaviour for Treatment Conditions (VOCs lacking listed in upper right corner) in *Lucilia sericata* Meigen (Diptera: Calliphoridae). Arrows indicate what behaviour occurred from a given position towards another throughout experimentation. Numbers indicate how many times a blow fly changed from one behaviour to another. Media profile abbreviations tested: PAA = lacking phenylacetic acid; DMDS = lacking dimethyl disulfide; ISO = lacking isobutylamine; and IND = lacking indole.

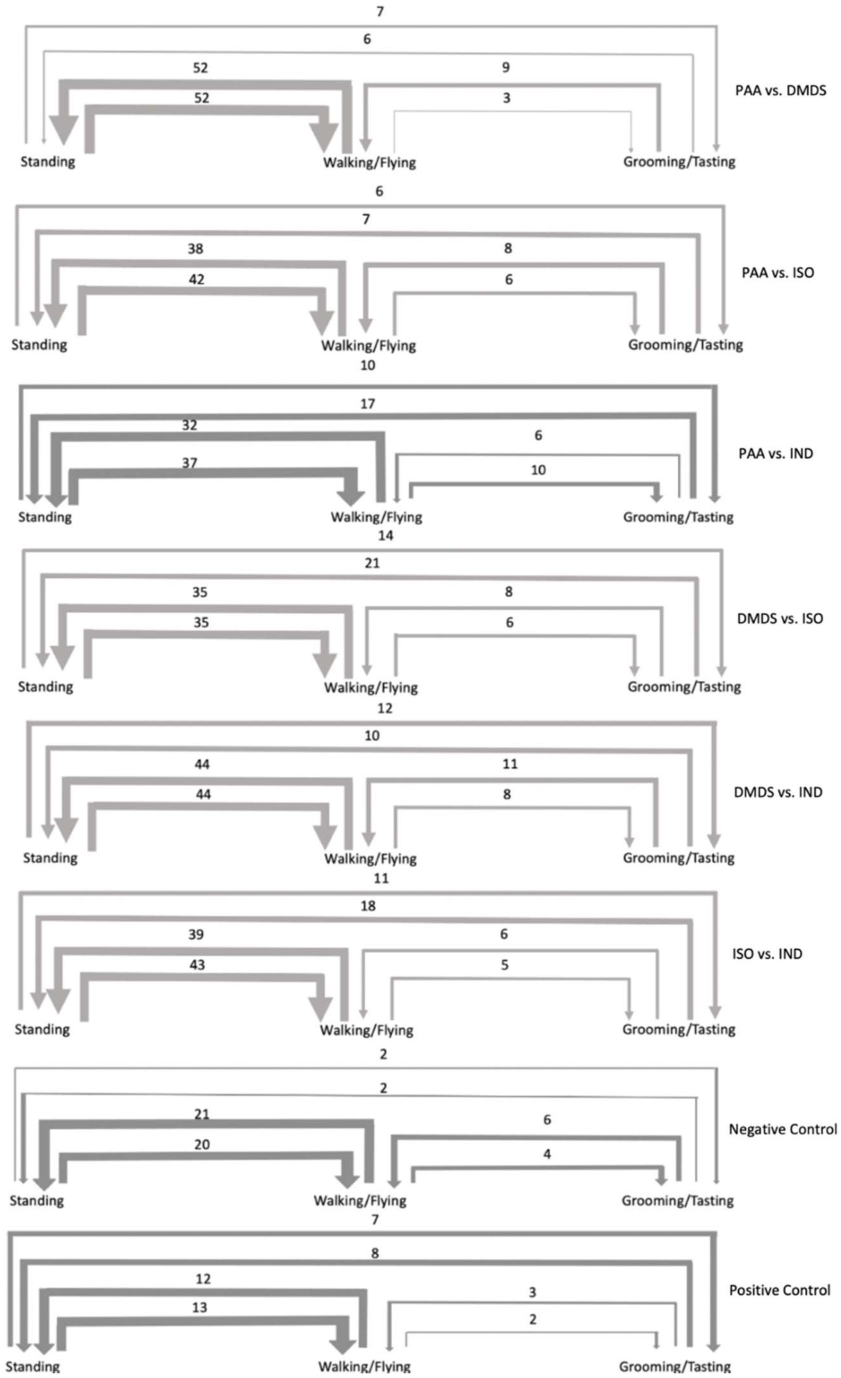


Figure 3.5. Blow fly Order and Frequency of Demonstrated Behaviour for Treatment Conditions (VOCs lacking listed in upper right corner) in *Phormia regina* Meigen (Diptera: Calliphoridae). Arrows indicate what behaviour occurred from a given position towards another throughout experimentation. Numbers indicate how many times a blow fly changed from one behaviour to another. Media profile abbreviations tested: PAA = lacking phenylacetic acid; DMDS = lacking dimethyl disulfide; ISO = lacking isobutylamine; and IND = lacking indole.

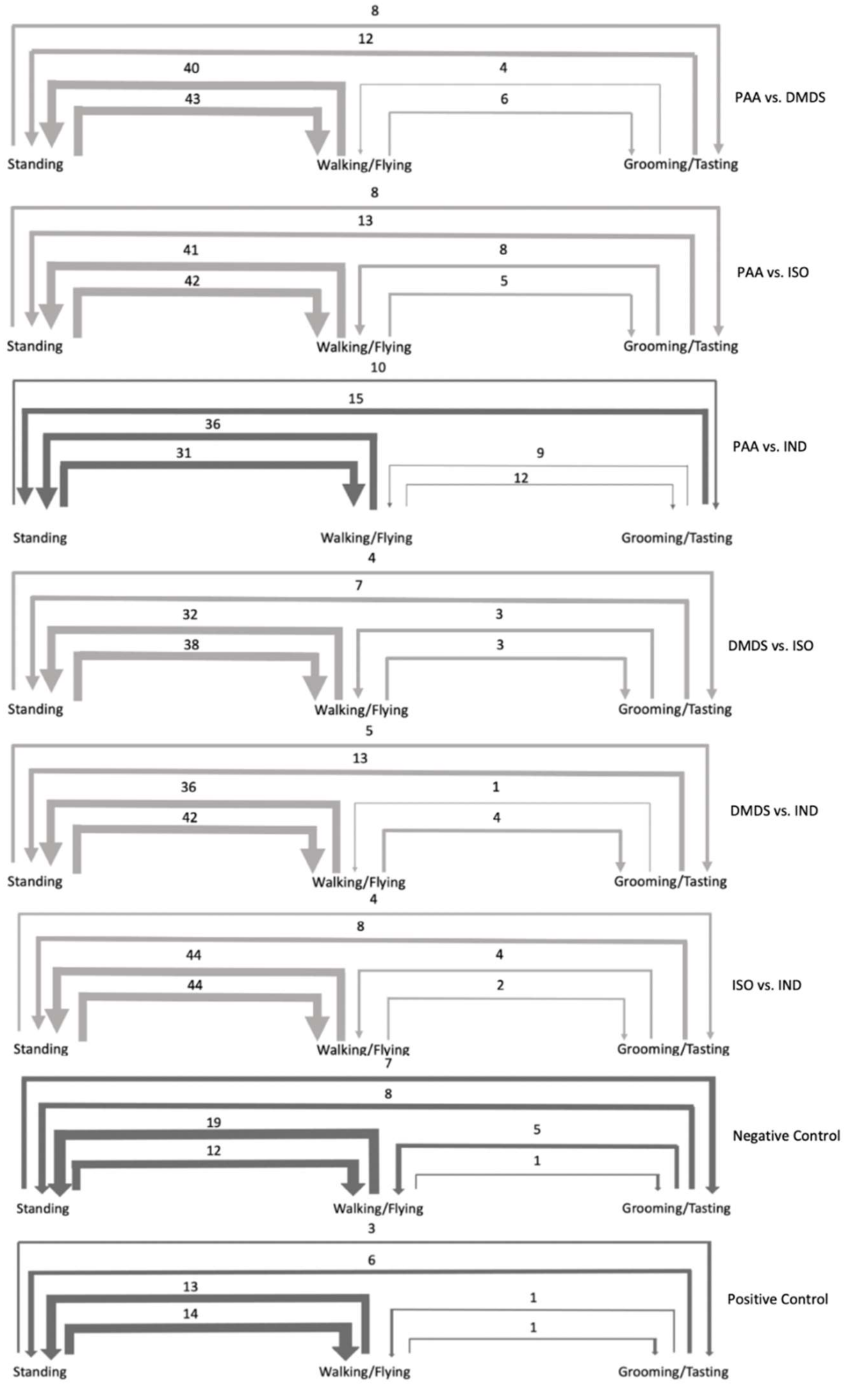
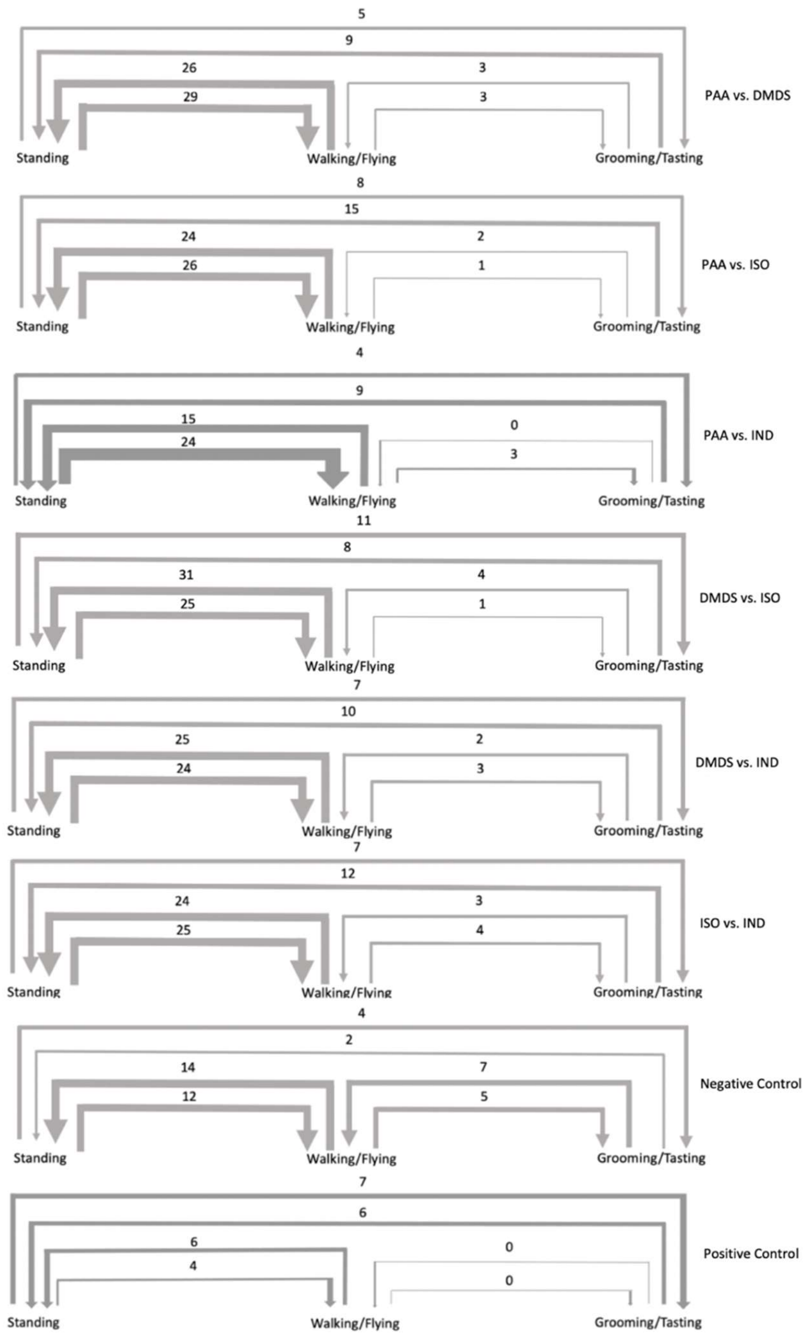


Figure 3.6. Blow fly Order and Frequency of Demonstrated Behaviour for Demonstrated Behaviour for Treatment Conditions (VOCs lacking listed in upper right corner) in *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae). Arrows indicate what behaviour occurred from a given position towards another throughout experimentation. Numbers indicate how many times a blow fly changed from one behaviour to another. Media profile abbreviations tested: PAA = lacking phenylacetic acid; DMDS = lacking dimethyl disulfide; ISO = lacking isobutylamine; and IND = lacking indole.



Chapter 4. Understanding Species Adaptability and Plasticity

Selection pressures

Charles Darwin theorized about the concept of evolution during his expedition of the Galapagos Island. This theory revolutionized the field of ecology and discussed the succession of animals from their ancestors. He proposed the concept of descent with modification, which explains that organisms that survive and reproduce pass on their genes to their offspring, but that these genes also evolve through time. The complexity of organisms increases as more simplistic organisms have selection pressures placed on them before reproduction (Darwin, 1809-1882; Winkler, 2012). Random genetic mutations occur as errors in replication arise in cells. These mutations are preserved when they aid the organism in their survival and are subsequently passed on to their offspring. This mechanism is known as natural selection; it preserves any mutations that can be useful for the environment of the organism and can allow for the loss of any mutations that are not favourable. It is important to note that although mutations that cause phenotypes favourable to the reproduction and survival of progeny will be maintained in the population, other unfavourable mutations can be maintained if they do not cause death before reproduction occurs. If, however, the mutation leads to a failure in reproductive processes, then it is easily phased out of the population as it is not passed onto other generations. As this process continues, organisms become increasingly more complex, and accumulate beneficial mutations (Ayala, 2010). Due to selection pressures, speciation can occur in which the changes in genetic code can genetically isolate one organism from another in the form of two different species (Hendry, 2009).

Organisms can tolerate stress and competition for resources in their environment but also undergo other physiological and biological disturbances during their lifetime (Haruta & Kanno, 2015). Environmental conditions are constantly changing and can flux and flow between changes (e.g., years of drought and then no drought). Due to this ephemerality, traits can be favourable one year, and then unfavourable the next. Selection pressures are specific forces that act on organisms, which drive the evolution of a species (Haasdijk & Heinerman, 2018). This process of elimination is crucial in living systems since it weeds out maladaptive traits, behaviours, and genetic material (Haasdijk & Heinerman, 2018). The mechanism of how selective pressures operate correlates with the environmental conditions present at the time of selection. These pressures also are influenced by climate change, as the environments of species change, and species are expected to adapt to shifting conditions or die trying (Henry et al., 2018; Valtonen, 2010; Merilä & Hendry, 2014). An organism that is rigid in the toleration of its habitat's fluctuating conditions will likely not survive long enough to pass on their genetic material to the next generation. If they do survive, then their offspring will not be adapted to the conditions and may die. Conversely, an organism that is flexible in its traits will be able to endure varying environmental conditions, even if the conditions do not stay constant (Jaenike, 1978). If an organism has a phenotype that is not favourable to its environment, then they are less likely to reproduce; if they do reproduce, their offspring may not survive unless the conditions change to be more favourable to their traits.

Selection, plasticity, and evolution

Selection, plasticity, and evolution work together dynamically in ecosystems to change an organism through time. These changes may better adapt the organism to its environment or may lead to the demise of the organism. Although an organism cannot predict what behaviours will assist their young in survival, mothers employ the tactics they believe will satisfy the requirements of life for their offspring (Royle, 2016). For example, females who don't practice maternal care must choose the environment to lay their eggs and allow their progeny to grow. If they choose a poor environment, their genetic line dies out. The goal of every organism is essentially to pass on their genetic information to continue their lineage, and some organisms do this by expending a great amount of energy on a small clutch to ensure their survival and provide parental care whereas others produce many young so that some will inevitably survive (Fischer & O'Connell, 2018). In a study by Huang, & Pike (2011), long-tailed skink mothers directly affect the fitness of their offspring by controlling the temperature of incubation through the selection of a nesting site. It has been suggested that climate change would change the benefits of this activity, and the mothers would have to modify and adapt their behaviours (Henry et al., 2018; Huang & Pike, 2011). This is because climate change causes a change in the organism's environment, whether it be an increase/decrease in temperature, increased rain levels, decreased land mass, or through other ways. In this case, the choices of the mother are either selected for or against, which affects the fitness of her offspring. The ability and flexibility of the adaptation of the organism depicts their plasticity. Evolution is the driving force behind plasticity and selection as it follows the varying environmental conditions and shapes what behaviours will be ideal for survival.

Behavioural Plasticity

Behavioural plasticity represents a change in an organism's behaviour because of their exposure to stimuli that vary from the environment they are accustomed to (Petzold et al., 2009; Han & Brooks, 2014). This mechanism requires an energetic expense but encourages the organism to become flexible in their behaviours. This expense may pay off if the trait is adaptive and creates positive outcomes, but on the other hand, the trait may also create negative outcomes for the organism. Many organisms exhibit behavioural plasticity. For example, in *Bactrocera dorsalis* Hendel (Diptera: Tephritidae), oviposition behaviour was determined to be plastic (Xu et al., 2012). In this study, female flies adopted alternate oviposition strategies that directly affected their fitness. They adjusted their egg clutch size in response to the variation in the host density and availability.

Resource partitioning on carrion

Carrion is an ephemeral resource that is utilized by both specialists and generalists alike (Anderson, 2010; Byrd & Castner, 2010). Insects, in particular, coexist on this resource and use it to their benefit to get nutritional value for growth and development (Byrd & Castner, 2010; Weidner et al., 2016). Insects such as blow flies rely primarily on carrion as their food source for themselves and additionally for their young to grow and thrive (Jaenike, 1978). Blow flies may have different seasonal preferences and specific dietary requirements; however, they can be found with other insects, and even with other blow flies on a single carrion source (Anderson, 2010). This coexistence may be due to dietary preferences, or other factors that influence an organism's choice of how to utilize a resource amongst other organisms. Competition, as well as other types of interactions (*e.g.*, predation etc.) can exist on a single resource, but positive and negative interactions likely exist in situations such as these (Okpara, 2018). Coexistence can occur between many species of insects, while positive and negative interactions are occurring on the same resource.

Relevance

Forensic entomology involves the collection and analysis of insects in criminal investigations, predominantly utilized in the determination of the post-mortem interval of the deceased, commonly referred to as time of death (Byrd and Castner, 2010). Blow flies are known as primary colonizers of corpses, which are useful indicators of a predictable interval of time of death in forensic investigations, as their development follows a predictable pattern (Ody et al., 2017). These successional patterns, combined with other information regarding insect ecology are used in this estimate, as blow flies arrive within hours or minutes after death (Anderson, 2004; Anderson & VanLaerhoven, 1996; Nuorteva, 1977).

It is not known exactly how different species of blow flies coexist in the wild and the mechanisms they use to survive despite sharing the same ephemeral resource. The fly's highly sensitive sensillary system on their mouthparts allow them to taste a substance, and their ability to detect a substance from a far distance aid in their foraging decisions. It is evident that they rely on visual, scent, and gustatory cues to assess their environment. It is possible that they have different dietary preferences in hopes of avoiding other species. This may be the case in *L. sericata* according to results attained in chapter two. *P. regina* on the other hand, may be more of a specialist, and may prefer to remain near one medium they deem favourable. *C. vicina* seems to have some preference, but nothing distinctive; they may go to one medium over another if it is more desirable to them and their needs. These preferences may be due to a tissue preference *i.e.,* different tissues, or tissues decomposing at different times may differ in VOC and nutrient composition (El-Moaty & Kheirallah, 2013; Thomas et al., 2016). The difference in preferences on a species basis may indicate that different species of blow flies forage and utilize a resource differently. This resource partitioning could allow blow flies to avoid direct competition. In the area of Windsor-Essex, where the experimentation took place, *P. regina* naturally coexists with both *L. sericata* and *C. vicina*. In this area, *L. sericata* are typically abundant during summer and spring seasons, whereas *C. vicina* are present during fall and winter (Baumgartner & Greenberg, 1985). Conversely, *P. regina* are found nearly year-round, with exception of the period of diapause that all blow flies engage in (Macleod & Donnelly, 1958). Additionally, *L. sericata* does not have a distinct preference for the medium they visit so long as it is somewhat attractive, possibly because they are active during warmer weather, which encourages the decomposition of carrion, allowing for better access to nutrients. Two species of blow fly (*C. vicina* and *P. regina*) seem to have distinct choice preferences for media lacking isobutylamine, and thus possibly valine. Flies of *C. vicina*, who are present in the fall, demonstrated an incomplete hierarchy in their preferences for media lacking specific VOCs. This is possibly a mechanism of avoidance since they interact with *P. regina* on the carrion resource at the same time. *P. regina* only demonstrated a preference for media lacking isobutylamine. Isobutylamine is associated with the breakdown of the amino acid valine (Richardson, 1966; Wang et al., 2001); which has been previously demonstrated to be critical for the development and growth of insects (House, 1962). However, the need for this amino acid could be substituted with the presence of other dietary constituents (House, 1962). Results of kinetic diagrams suggest there is no distinct difference in the frequency of the changing of behaviours overall. Lastly, compared proportions of walking to flying and tasting were as expected, with the overall pattern of arrestment being the most commonly exhibited in treatment conditions, followed by locomotion and finally by tasting.

Summary

This thesis primarily revolves around two main theories; these theories are the optimal oviposition theory and the oviposition preference- offspring performance hypothesis. The optimal oviposition theory concerns the deposition of egg clutches made by females in response to what she believes will improve her offspring's chances of survival. In this theory, mothers choose areas for oviposition that they think are of a good quality for their offspring (Jaenike, 1978). If the mother can read environmental cues about the conditions of their environment, they can observe whether the site is likely to remain beneficial for their offspring. They may be able to sense whether her young's food source has been previously colonized and consumed, and the quality of the food source, whether the source is high in essential nutrients. In blow flies the females do not practice maternal care. This means that the oviposition medium chosen by the mother would accompany and nourish their young throughout most of their life cycle (Ody et al., 2017). It is in the mother's best interest to select the environment that will support the living conditions of immature blow flies, since an organism's ultimate goal in life is to reproduce and pass on their genes to the next generation. The second theory, the oviposition preference-offspring performance hypothesis, deals with how offspring fitness is related to maternal choice when it comes to oviposition (Thompson, 1988); essentially, this is an extension of the aforementioned theory (Pacheco, 2015). This is because an optimal oviposition choice will positively affect the outcome of their progeny. The selection of oviposition strategies varies depending on the type of organism, as they each function best at differing preferences. Blow flies resort to ovipositing their clutches in densities that support the survival of that clutch. Eggs are thought to be laid in aggregates to avoid desiccation (Brody et al., 2015).

Future studies could further extend the knowledge gained from this study to determine exactly what compounds influence blow fly choice and how mechanistically these behavioural choices break down. Applications for this not only aid in determining how a blow fly assesses and accepts or rejects a resource, but how they demonstrate behavioural choices and partition a resource during its colonization. Once information is gathered about the substances blow flies need, an artificial chemical blow fly diet can be created to simplify and modify experimental rearing processes, among other uses.

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