Variation in Oviposition Behaviour of Blow Flies (Diptera: Calliphoridae) due to Relative Humidity and Intraspecific Interactions

By

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ABSTRACT

Blow flies are usually the first insects to arrive at carrion, using it to oviposit or breed. The behaviour to search for and accept a carrion resource as suitable for oviposition is influenced by abiotic factors, particularly temperature and humidity, as well as by the presence of other insects. My thesis focuses on the influence of relative humidity (RH), combined with intraspecific interactions, on oviposition choices of three blow fly species *Lucilia sericata* (Meigen), *Phormia regina* (Meigen), and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae). Individual fly oviposition behaviour was measured on a temperature gradient (10-40°C) under high (75-85%) and low (25-35%) RH conditions, and in the presence of intraspecific eggs at hotter or cooler non-preferred locations on the temperature gradient under each RH conditions. The choice to oviposit or not, timing of first oviposition event, clutch size, and temperature choice for oviposition location along the temperature gradient were recorded. The results indicated that RH affected whether females would oviposit or not on the gradient, but for those that oviposited, RH did not affect clutch size or timing of oviposition. When in the presence of intraspecific eggs, fewer *L. sericata* females oviposited, with no change in behaviours regardless of RH. In contrast, no *P. regina* females oviposited in low RH but more females oviposted in high RH, ovipositing larger clutches and closer to the non-preferred intraspecific egg location on the gradient.Neither RH, nor presence of conspecific eggs influenced the number of *C. vicina* females that chose to oviposit, however, they laid fewer eggs in the presence of conspecifics. This study demonstrated that behavioural generalizations across insect family, such as blow flies, fail to recognize the species-specific responses to thermal preferences, RH conditions and even to the presence of eggs of their own species. These differences are vital to understanding oviposition behaviours, as it relates to species co-existence and to use of these species in forensic entomology.

DEDICATION

To my parents, Tuan Tran and Hathi Hoang
and my brother, Jonathan.
Additionally, to my partner, Colin, and his family.
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CHAPTER 1

BEHAVIOURAL ECOLOGY & ECOPHYSIOLOGY OF BLOW FLIES

1.1. Abiotic Influences

1.1.1. Temperature

An insect’s life history traits are heavily influenced by their environment. They are poikilothermic, which means that insects will exchange heat with the environment rapidly such that its body temperature will always approximately match ambient temperature (Child 2007; Sanborn 2004). Because of their small size, temperature for insects is both a physical factor and stimulus for their reproduction, movement, and development, as their ideal conditions are within a specific range of temperatures (Abdullah 1961; Rebaudo and Rabhi 2018). To some degree, they are able to regulate their body temperature through physiological and behavioural activity (Heinrich 1974), such as movement of flight muscles to generate internal heat, enabling flight in temperatures that would otherwise be limiting (Heinrich 1995).

Deal (1941) and Uvarov (1931) defined optimum temperature as the average temperature where the greatest number of individuals are reproducing eggs at a given time and the rate of development is at its highest. This means there is a relationship between the number of eggs laid, length of life and mortality. However, within each immature stage, there may be different optimal temperatures required for development (Howe 1967). An insect’s development and fitness can be characterized by a thermal performance curve (TPC), which shows the entire tolerance range of an organism that is nonlinear and highly asymmetric (Martin and Huey 2008) (Figure 1.1). The general curve shape begins with the lower lethal threshold, where performance or fitness is zero since the temperature is too cold, followed by the critical thermal minimum (CT_{min}). There is a gradual increase in performance as temperature increases up to some optimum temperature (T_{opt}) where performance is maximized. This is followed by a steep decline in performance as temperatures exceeds the optimum temperature, reaching a critical thermal maximum (CT_{max}), where an individual experiences thermal stresses within the upper temperature range, where performance or fitness begins to decrease as temperature continues to increase. Temperatures outside the T_{opt} range can result in decreased survival and reduced fitness. Development or fitness can still occur in the upper temperatures before it hits a lethal threshold, where death occurs at this point as temperatures become too hot. (Huey and Berrigan 2001; Huey and Kingsolver 1989; Johansson *et al*. 2020; Phillips *et al*. 2014; Schulte *et al*. 2011; Tuff *et al.* 2016). Temperatures just below or above T_{opt} are suboptimal (Martin and Huey 2008). For example, in the development of *Phormia regina* (Meigen) (Diptera: Calliphoridae), lethal lower temperature for development is 7.5°C and lower, as egg eclosion does not occur (Roe 2014). At 10°C, egg eclosion occurs but no development past first instar (Roe 2014). As temperatures increase, temperatures ranging between 12.5-35°C demonstrates the thermal tolerance breadth, with CT_{min} at 12.5-14°C, where there’s complete development from egg to adult, but longer development (Byrd and Allen 2001; Nabity *et al*. 2006; Roe 2014), and T_{opt} around 25°C, where survival to adult is highest (Okpara 2018). Upper temperatures for high mortality where no adults are able to emerge can be seen between 35-40°C, with 40°C being the lethal limit (Byrd and Allen 2001; Okpara 2018).

There is also an optimal temperature for egg output, which varies among species as it reflects the temperature the species are more active during their reproduction (Engelmann 1970). In the Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), Keena (2006) found that the optimal temperature of 25°C improved the female's reproductive efficiency, based on the observed increase in oviposition sites chewed per week, fecundity, and fertility. For *Lucilia sericata* (Meigen) (Diptera: Callihporidae), oviposition is more likely to be induced within temperatures ranging from 28°C to 33°C, with 30°C observed for *L. sericata* to lay the most eggs (Cragg 1955; Davies 1934; Hans *et al*. 2018; Smith and Wall 1997). Additionally, Ody *et al.* (2017) observed the thermal range of oviposition for *L. sericata*, where females oviposited at temperatures as cold as 17.5°C and as hot as 40°C, with the probability of oviposition occurring as temperature increases. Compared to *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae), females were observed to oviposit within temperatures between 10-35°C, with the probability of oviposition decreasing as temperature increases, with fewest eggs laid at 35°C (Hans 2016, Ody *et al*. 2017).

There will also be unfavourable environmental conditions that will inhibit the survival, growth or reproduction of an insect. One adaptation to avoid these conditions to survive is diapause, either developmental diapause or reproductive diapause (Andrewartha 1952). Diapause for insects within a temperate climate are hormonally controlled to enter a state of dormancy that stops development and decreases the behavioral and metabolic activity when induced to changes in the photoperiod (shorter days and longer nights), or cool temperatures from seasonal changes to overwinter (Allen 2007; Atkinson 1996; Diniz *et al*. 2017; Tougeron 2019). For example, Vinogradova and Reznik (2013) observed that for *C. vicina*, larval diapause in natural settings was induced by both photoperiod and temperature in Russia, where the first indication of larval diapause was in early August with daylength at 16 h and constant temperatures of 17°C, with the highest percentage of larval diapause of 90-100% between late August to October as day length decreased to 12–14-hour days and temperatures decreased to 7°C. For *L. sericata*, the minimum development temperature is between 7.5-10°C, although survival to adulthood is very limited compared to warmer temperatures (Roe and Higley 2015). Even colder temperatures that make the environment unfavourable for development will cause the larvae to enter diapause (Cragg and Cole 1952). Another adaption is quiescence, which is a shorter dormancy period directly caused from unfavourable conditions. Conditions such as low humidity or hot temperatures can trigger quiescence, but when conditions become favourable again, physiological activities resume (Diniz *et al*. 2017). For example, in the eggs of the yellow fever mosquito *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae), they are able to undergo long periods of quiescence by allowing pharate first instar larvae to survive inside the egg from unfavourable environmental conditions, such as a rapid drop in humidity levels or fluctuation in temperature for up to a year (Diniz *et al*. 2017; Oliva *et al*. 2018; Yang 2014). Eggs in contact with water will induce rapid hatching (Diniz *et al*. 2017). In various regions, insects experience different rates of development due to diurnal and seasonal temperature fluctuations (Uvarov 1931). As long as the temperature is within the suboptimal or optimal range, an insect will develop but they take a longer time developing at lower temperature, compared to higher temperatures, where they can develop in shorter times (Janisch 1932).

Similarly, extreme temperatures can also affect the body size of insects, where individuals developing at higher temperatures will develop faster but be smaller as adults compared to individuals developing slower at lower temperatures which will be larger adults (Atkinson 1994; Kingsolver and Huey 2008). In the fruit fly *Drosophila melanogaster* Meigen (Diptera:

Drosophilidae), where adult males and females developed longer mesonotum length, thorax length and larger mean wing area when larvae were reared at lower than optimal temperatures compared to larvae reared at higher than optimal temperature that had a smaller overall body size (French *et al*. 1998).

Body size also affect fecundity. There is a positive correlation between adult body size and fecundity, where larger females have a higher reproductive output (Berger *et al.* 2008; Gotthard *et al.* 2007). For example, large females of zigzag ladybirds (*Cheilomenes sexmaculata* - previously *Menochilus sexmaculatus*) (Fabricius, 1781) (Coleoptera: Coccinellidae) from abundantly fed larvae laid greater number of eggs, laid larger eggs, and had offspring with higher body mass compared to medium and small-sized females (Singh *et al.* 2021). When larvae develop, the size of insects is determined both genetically and by environmental factors, and each of these factors can influence the size of the insect fecundity (Calvo and Molina 2005).

The temperature preference, or preferendum (T_p), is the selection of a certain temperature to which an insect's behavioural regulation of body temperature can occur (Abdullah 1961; Deal 1941). Due to sensitivity to heat, insects will choose thermal conditions that will be optimal for their physiological requirements and tend to avoid lethal temperatures, depending on their given set of conditions (Dillon *et al*. 2009; Haupt *et al*. 2017; Hoffman 2010; Tratter Kinzner *et al*. 2019). Physiologically, a preferred temperature is expected to match the same range as the optimum temperature, but empirically, it may be lower and can differ (Dillion *et al*. 2012; Tratter Kinzner *et al*. 2019). In the alpine fly *Drosophila nigrosparsa* Strobl (Diptera: Drosophilidae), T_p on temperature gradients (14-37°C and -2 to -20°C) within laboratory settings was different than in the field. Flies in the field were found at temperatures at least 5°C and higher than the T_p from the gradient (Tratter Kinzner *et al*. 2019). Similarly, for the caterpillars of the sub-Antarctic moth, *Pringleophaga marioni* Viette (Lepidoptera: Tineidae), T_p on a temperature gradient (0-30°C) was significantly lower than T_{opt} for locomotion, such that the T_p for the treatment groups ranged from 4.2-5.2°C, whereas the T_{opt} for each treatment groups ranged from 20-25°C with temperature differences of 14.8-20.6°C (Haupt *et al*. 2017). The preferred temperature can differ depending on the life stage of an insect (Uvarov 1931). For example, Maclean *et al.* (2019) observed the thermal preference of four adult and larvae *Drosophila* spp (Diptera: Drosophilidae), where the adult behaviours observed were adult thermal preference and egg laying thermal preference. Larval and adult egg laying thermal preferences had the least variation in temperature choice, where the temperature preference range is narrower. However, the adult thermal preference had greater variation in temperature preference and thermal breadth.

For blow flies, they are able modify their behaviour to adapt to their enviornmental optimum within different abiotic factors (Chown *et al.* 2011). Their activity is mediated by temperature, and has been shown to be involved with colonization on bait (George *et al.* 2013) and visitation pattern (Shah and Sakhawat 2004). Each species has their own temperature dependent growth rate, which are affected by the environmental temperature and heat generated from aggregation (Bansode *et al*. 2016), as well as specific thresholds for flight and oviposition behaviour (Berg and Benbow 2013; Hans 2016; Lutz *et al*. 2018). It also affects colonization, as ambient temperature should be around 10-30°C for colonization to occur for many blow fly species (Ody *et al.* 2017).

1.1.2. Humidity

An insect will always be exposed to multiple abiotic factors in natural settings. Along with temperature, relative humidity (RH) interacts with temperature and can affect insect physiology, influencing the development, longevity and oviposition of many insects (Norhisham *et al.* 2013). Insect mortality from desiccation stress can occur if they are in environments of hot or cold temperatures and low RH or could also drown if they are in environments of hot or cold temperatures and high RH (Neven 2000; Punzo and Mutchmor 1980).

The effects of humidity are related to the effects of temperature because changes in temperature are accompanied by the changes in humidity (Engelmann 1970). Relative humidity is the ratio of water vapor in the air to the amount of water vapor air can hold at certain temperatures (Elovitz 1999). Atmospheric humidity affects organisms by modifying its water content (Ludwig 1945). Most organisms lose water when exposed to low RH; in general, the rate of water loss increases the lower the humidity (Ludwig 1945). Each species has a range of humidities considered optimal for certain physiological processes, such as oviposition (Ludwig, 1945). At low humidity, water will be lost due to evaporation and excretion, which will affect egg maturation (Engelmann 1970). Norhisham *et al.* (2013) observed high egg mortality of the bamboo borer *Dinoderus Minutus* (Fabricius, 1775) (Coleoptera: Bostrichidae) at 20% RH, where dehydration at low RH caused both the chorion and the embryo of the egg to shrink.

Humidity also modifies the rate of oviposition and the total egg production of an organism (Ludwig 1945). The number of eggs produced depends on the species and fluctuates depending on the optimal humidity level for those species (Ludwig 1945). The rate of reproduction is increased in higher percentages of RH (Uvarov 1931). Lu and Wu (2011) examined the plant bug

Apolygus lucorum (Meyer-Dur, 1843) (Heteroptera: Miridae) and why their population increased after heavy rainfall. They determined that high humidity from the rain was beneficial for egg and nymph survival, adult longevity, population growth and fecundity. The effects of RH has been observed in several Dipterans, such as egg eclosion and adult emergence in the black soldier fly, *Hermetia illucens* L (Diptera: Stratiomyidae) (Holmes *et al*. 2012), ovarian maturation, and egg production in the olive fruit fly, *Bactrocera (Dacus) oleae* Gmelin (Diptera: Tephritidae) (Broufas *et al*. 2009) and egg mortality in sorghum shootfly, *Atherigona soccata* Rondani (Diptera: Muscidae) (Delobel 1983), to adult mortality in species of mites (Acarina: Macrochelidae) (Singh *et al*. 1967), and even to the invertebrate *Caenorhabditis elegans* (Maupas, 1900) (Rhabditida: Rhabditidae), examining the physiological behaviour within a humidity gradient (Russell and Pierce-Shimomura 2014). Different insects have different sensitivities and adaptations to humidity as there are some that are unaffected by humidity, others that cannot physiologically perform when humidity is overly saturated or too dry, and then there are those that perform optimally within moderate humidity levels, where an insect can evaporate enough water to cool themselves and not cause a fatal loss of water (Buxton 1932). For most species, the optimal RH for egg laying and rates of oviposition is between 80-90% RH (Engelmann 1970).

Temperature, in combination with humidity, plays a complex role in various species' decisions. Using the eggs of *L. sericata*, Evans (1934) noticed that death will occur if temperature, either high or low, are in combination with low saturation deficiency of 10 mm and lower. Eggs were more tolerated between saturation deficiency of 10-20 mm at a median temperature. This could be due to the amount of water forming during metabolism inside the eggshell, as well as the outer chorion layer of the egg governing the loss or gain of water

(Buxton 1932; Davies 1948). If the chorion was damaged or removed upon development, those eggs had a high mortality rate compared to undamaged eggs (Evans 1934). There is a relationship between humidity and water loss; the lower the humidity, the greater the rate of loss of water over the developmental period (Buxton 1932). Lower RH conditions may cause deleterious effects such as decrease in egg production, desiccation of eggs and even egg mortality (Buxton 1932; Guarneri *et al*. 2002). To withstand low humidity, blow flies will oviposit their eggs in a clustering orientation (Hans 2016). Higher RH conditions have proved beneficial, such that Davies (1948) examined that when *L. sericata* are within humidity ranges >50% RH, the rate of development is quicker and eggs hatched more rapidly. Evans (1935) observed that 80-100% RH was more favourable for the growth of ovaries for *L. sericata*, compared to 0-40 RH. Humidity also had no effect on oviposition for *L. sericata* at temperatures of 10-35° C, but at 40° C, 50% RH and lower was unfavourable.

1.2. Biotic Influences

In addition to environmental influences, biotic factors also affect development, reproduction, and survival of an insect (Hamby *et al.* 2016). Individual insects will encounter multiple interactions across a community of diverse species including members of its own species. Their interactions may be positive, negative or neutral and take the form of competition, facilitation, neutralism, aggregation and predation (Godoy 2007; Hodge *et al.* 1999). These various interactions can influence whether there is food available or predators around to prevent or promote foraging and oviposition opportunities (Klomp 1964; Rudolf and Rödel 2004). For insects to select a host or resource to deposit offspring, they depend on a host or resource to be high quality to have hatching success and larval survival (Stamp 1980). Within patchy carrion resources, there is an increase of intraspecific aggregation, defined as a spatial distribution pattern with a high variance in the density of intraspecifics (Fiene *et al.* 2014). Aggregation may be influenced by the behavior of adult females, such as their decision to explore, colonize, and oviposit within a particular resource, along with the local environment (Cruickshank and Wall 2002; Fiene *et al.* 2014). Cruickshank and Wall (2002) observed higher *Lucilia* spp. (Meigen) (Diptera: Calliphoridae) aggregation in field areas that are warmer and more humid in England. Females might oviposit eggs in clutches to save time and energy when locating a mate and suitable oviposition sites if adult population is limited (Stamp 1980). Aggregation of insect eggs has benefits such as reducing desiccation within unfavourable environments and increasing hatching rates by maintaining optimal temperature and RH (Clark and Faeth 1998; Santana and Zucoloto 2016; Stamp 1980). For aggregated larvae, benefits include reduced risk of predation, increase feeding efficiency from a mass release of digestive enzymes to breakdown tissue, and generating temperatures and RH for larvae development (Clark and Faeth 1998; Rivers *et al*. 2011; Rohlfs and Hoffmeister 2004; Santana and Zucoloto 2016; Turner and Howard 1992). Slone and Gruner (2007) determined that within an aggregation volume of 20-50 cm^3, Calliphorid larvae have an internal temperature of 30-35°C, which enables the larvae to be independent of ambient conditions as well as creating protection from drops in temperature.

Although there are benefits for insects to aggregate their eggs, there are also consequences for both adults and larvae. For aggregated larvae that are larger and denser, they can face overcrowding, where there are more individuals feeding to food availability, resulting in reduced body size and survival to adult (Okpara 2018; Rivers and Dahlem 2014). Although larval development becomes faster at higher densities, pupal and adult weight decreases with limited food availability, and adult bodies became smaller (Kökdener *et al*. 2019). Additionally, larvae will face thermal stress, as larval aggregation generates a linear increase in internal temperatures. Larvae exposed to species-specific CT_{max} or lethal temperatures will result in incomplete development or death (Okpara 2018; Kökdener *et al*. 2019; Rivers and Dahlem 2014; Slone and Gruner 2007). When *L. sericata* and *P. regina* larvae were developing individually at 35°C on 50 g of liver, larger densities of 400 larvae had the least survival to adult, and 40°C having no survival (Okpara 2018). For females, if they respond negatively to the presence of intraspecific eggs and larvae, they will spend more time searching for oviposition sites, reducing energy for oviposition and food resource (Rohlfs and Hoffmeister 2004; Stamp 1980). In the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), females showed no feeding preference towards grains exposed to either other *S. granaries* individuals or eggs, and strongly avoided feeding on grains containing intraspecific eggs (Woodbury 2008). In contrast, if females decide to aggregate their eggs on the same resource, her offspring will experience high intraspecific competition for nutrients due to overcrowding, resulting in reduced fitness, such as decrease in body size, growth rate, fecundity, or lethal effects on survival (Craig *et al.* 1990; Rivers and Dahlem, 2014; Rohlfs and Hoffmeister, 2004). If there are fewer, smaller adults developing in these clutches, this could affect reproduction, such as ovipositing fewer eggs (Ireland and Turner 2006; Kökdener *et al*. 2019). Furthermore, this could also affect dispersal ability because as body size increases, dispersal ability also improves since increased body size leads to better flight performance, perhaps by being able to store more energy for longer flight times (Dingle and Arora 1973; Juliano 1983). For example, Greenleaf *et al.* (2007) reviewed literature regarding foraging distances for 62 bee species from six families (Andrenidae, Apidae, Colletidae, Halictidae, Megachilidae, and Melittidae), and developed a predictive model, where foraging distance increased with body size.

1.3. Study Species

There are over 1000 species of the family Calliphoridae, and about 50 species belong in the genera *Lucilia*, *Calliphora*, and *Phormia* that all utilize carrion as an oviposition and larval development resource (Smith and Wall 1997). The three species of this study within these genera are native to southern Ontario in Canada (Marshall *et al*. 2011).

Lucilia sericata are commonly called the green bottle flies due to their metallic green appearance. Adult body size ranges from 6-9 mm in length (Byrd and Castner 2002). They are a synanthropic species inhabiting in the Holarctic region, where they are associated with humans and livestock (Grassberger and Reiter 2001; Stevens and Wall 1996). *Lucilia sericata* larvae have been known to cause myiases in sheep throughout the temperate zone of the northern hemisphere, New Zealand, parts of South Africa, and Australia (Wall *et al*. 1992). *Lucilia* species are known to be early arrivers and first colonizers on a carrion in the early stages of decomposition (Anderson and Vanlaerhoven 1996; Bugajski and Stoller 2017). They are seen in Southern Ontario, as they are predominately seen mostly in the summer (Rosati 2014). Wall *et al.* (2001) saw that at 80% RH, 90% of *L. sericata* eggs hatched at around 30°C. Hans (2016) found faster oviposition and more successful egg hatching at RH over 50%. Humidity lower than 50% will result in slower and less successful egg hatching.

The second species in this study is *C. vicina*, known as the blue bottle fly, as they appear to have a greyish blue metallic appearance, is from the genus *Calliphora*. They are widely distributed within the Holarctic region, from temperate and subtropical areas to sub-Artic regions of Europe and Russia (Aak *et al*. 2011; McWatters and Saunders 1996). They are also a very large fly, with adults ranging from 10-14 mm long (Byrd and Castner 2002). They have been known to associate with humans as they are also a group of facultative synanthropic species (Vinogradova 2009), as well as being a pest on fresh food, especially on fish and meats (Aak *et al*. 2011). In southeastern Canada and eastern parts of central Canada, they are found in the cooler months (Leblanc and Strongman 2002; Rosati 2014; Turpin *et al*. 2014), mainly in early spring and fall as they are cool weather species. For *C. vicina*, eggs can hatch about 24h later at 15°C and can reach adulthood in 32 days (Donovan *et al*. 2006; Salimi *et al*. 2018). As temperature increases, the shorter their life cycle becomes and will reach a high mortality at temperatures 28°C and above (Defilippo *et al*. 2013; Hwang and Turner 2009; Salimi *et al.* 2018). For RH, Hans (2016) found that *C. vicina* had successful egg hatching across RH ranges between 30-80% RH, so their eggs are able to survive a wide humidity range.

The third species in this study is *P. regina*, known as the black bottle fly since their body appears black under certain lightings, is from the genus *Phormia*. The body size of *P. regina* adults can range from 7-9 mm in length (Byrd and Castner 2002). This temperate species also has a Holarctic distribution and are commonly found throughout the United States with the exception of southern Florida (Byrd and Castner 2002). They are most prevalent throughout the northern United States in the fall and spring months and during the summer months in the southern United States (Byrd and Allen 2001; MacInnis 2018; Núñez-Vázquez *et al.* 2013). Similarly, in Southern Ontario, they are found in spring, summer, and fall as they have a wider temperature range (Marshall *et al*. 2011; Rosati 2014). Over the winter, they enter diapause during their adult stage (Browne 1974). In laboratory settings, RH increased egg eclosion time and egg eclosion success when conditions reached 50% RH and higher, where the highest hatching success was observed between 70-80% RH (Hans 2016).

1.4. Research Objectives

Relative humidity has been examined during egg hatching success (Hans 2016), but there has been little research examining the effect of RH on blow fly oviposition behaviour. My research evaluated the effects of high (75-85%) and low (25-35%) RH conditions on the choice of individual females of *L. sericata*, *P. regina* and *C. vicina*, as well as documenting the number of females who oviposited or not within each humidity level (Chapter 2). Using individual females from each species, the choice to oviposit or not, and for those that chose to oviposit, the timing to first oviposition, clutch size (defined as set of more than two eggs deposited by one female), and temperature preference for oviposition location was measured under the two different RH conditions. I predicted that females will: 1) be more likely to oviposit under high RH than low RH; 2) oviposit more eggs in the high RH condition as the oviposition medium in this condition will retain moisture, creating a more suitable medium, decreasing the likelihood of egg desiccation and unsuccessful egg eclosion; and 3) oviposit faster under high RH conditions compared to low RH, as stress from low RH could delay oviposition. Hans (2016) observed faster egg eclosion when humidity conditions were at 50% RH and higher. I expect that these differences would be greatest with *L. sericata* and *P. regina* females due to the effect of low RH on eclosion success of these females (Hans, 2016). In contrast, I expect that *C. vicina* females would be least likely to be affected by RH conditions compared to the other two species given the lack of impact of RH on successful egg eclosion (Hans 2016). On the temperature gradient, I predict that temperature choice will be species-specific based on their thermal tolerance breadth within each RH conditions. Humidity will modify each species temperature preference as they have thermo- and hygrosensory neurons that allow them to respond to changes within their environment to avoid unfavourable climates (Enjin 2017; Frank *et al*. 2017; Tochen *et al*. 2015). In both RH conditions, I expect *L. sericata* females to choose temperatures around 30°C as *L. sericata* has an oviposition thermal range between 17.5-40°C, with 30°C to be the optimum temperature observed for *L. sericata* to lay the most egg and avoid lethal temperatures of >35°C and <17.5°C (Cragg 1955; Davies 1934; Hans *et al*. 2018; Ody *et al*. 2017; Smith and Wall 1997). For *P. regina* in both RH conditions, I expect females will choose temperatures over 20°C, as *P. regina* based on development has a thermal range of 12.5-35°C, with optimum temperature for survival to adult at 25°C and avoid lethal temperatures of <14°C and >35°C (Berg and Benbow 2014; Byrd and Allen 2001; Hans 2016; Okpara 2018; Roe 2014). For *C. vicina* in both RH conditions, I expect females will choose more cooler temperatures ranging from 5-25°C. *Calliphora vicina* has an oviposition range of 10-35°C, with the probability of eggs being laid decreasing over 25°C, with 35°C being a lethal temperature (Hans 2016; Ody *et al*. 2017).

Additionally, I tested the oviposition choices, including whether they oviposit or not, of individual females in the presence of intraspecific eggs that were placed at suboptimal temperatures in the same RH condition as above (Chapter 3). I predict that there will be more *P. regina* and *C. vicina* females ovipositing in the arena compared to RH without intraspecifics for both RH conditions. Both species will be attracted to the semiochemical elicited from the intraspecific egg clutches (Giffen-Lemieux *et al.* 2020; Rivers *et al*. 2011), but high RH conditions will have more females ovipositing overall compared to low RH. Both species will oviposit faster, closer to intraspecific eggs, and increase their clutch size in high RH when in the presence of intraspecific eggs. This is due to attraction from semiochemical cues from the eggs along with highly moist medium to retain high RH conditions to increase egg eclosion compared to low RH (Fouche *et al*. 2018; Hans 2016; Heard and Remer 1997; Rohlfs and Hoffmeister 2004). For *P. regina*, I predict females will respond positively to intraspecific eggs suboptimally placed in both cooler and hotter temperatures in each RH condition and will shift their temperature preference to oviposit towards the intraspecific eggs due to the attraction to the semiochemicals of the eggs, as well as fewer thermal limitation as they have a wide temperature range of 12.5-35°C (Berg and Benbow 2014; Brodie *et al*. 2015; Byrd and Allen 2001; Hans 2016; Giffen-Lemieux *et al.* 2020; Rivers *et al*. 2011). For *C. vicina*, I predict females will respond positively towards intraspecific eggs suboptimally placed towards cooler temperatures in both RH conditions and will shift their temperature preferences to be cooler as they will be attracted to the semiochemicals of the eggs, as well as favouring cooler temperatures as they have a minimum oviposition temperature range of 10°C (Giffen-Lemieux *et al.* 2020; Hans 2016; Ody *et al*. 2017; Rivers *et al*. 2011). Compared to intraspecific eggs placed in suboptimally hotter temperatures for both RH conditions, *C. vicina* females will not shift their temperature preference to avoid lethal temperatures (Hans 2016; Ody *et al*. 2017). I also predict that more *L. sericata* females will oviposit in the presence of intraspecific eggs, regardless of RH conditions as they will be attracted to the semiochemical cues of intraspecific eggs (Brodie *et al*. 2015; Giffen-Lemieux *et al.* 2020). Females will also oviposit larger clutches and oviposit faster, as *L. sericata* larvae have shown higher survival to adult emergence when developing on their own depending on temperature and density (Okpara 2018, Smith and Wall 1997). When intraspecific eggs are placed in suboptimally cooler or hotter temperatures, I predict *L. sericata* will avoid ovipositing near them and will not shift thermal preference in both RH conditions as optimal temperatures for oviposition is 30°C, and temperatures less than 17.5°C or greater than

35°C can become lethal (Cragg 1955; Davies 1934; Hans *et al*. 2018; Ody *et al*. 2017; Smith and Wall 1997).

1.5. Significance

The role RH plays in blow fly colonization probability and oviposition behaviour will help determine how abiotic factors, including RH, affect a species' capability to colonize a resource, including the range of habitat in which colonization can take place if RH is at optimal levels. Insect activity can be traced back to the pre-colonization interval (pre-CI) (time period of insect activity where they initially detect carrion until colonization, or oviposition) (Tomberlin *et al*. 2011). This information can be used to reduce reliance on assumptions within forensic science about blow fly colonization, such as generalizations that oviposition occurs immediately, thus affecting the pre-CI. There is variability in the decisions that females make before accepting to colonize on carrion. The response to oviposit on a carrion is shaped by abiotic factors such as temperature, RH, time of day, etc., (Tomberlin *et al.* 2011) and these factors can vary the behaviours differently between blow fly species as each species has its own thermal ranges with different optimal thermal conditions. Likewise, with biotic factors like con- and heterospecific competition, each species behaves differently, where each species either benefit from the presence of con- or heterospecific interactions or performs poorly where females alter the number of eggs she lays and speeds up or delay ovipositing (Hans, 2016). Taking into account the differences in species-specific behaviors during different seasons and interactions can provide more accurate estimates and interpretations of blow fly colonization.

Furthermore, females' oviposition decisions are also mediated by both biotic and abiotic factors, as favourable environments combined with aggregation cues promote the attractiveness of a resource to stimulate oviposition to occur. For females to assess and discriminate among potential oviposition sites, they use visual and olfactory cues to assess temperature, humidity and host quality based on semiochemicals produced by the females at the time of oviposition or microorganisms associated with the eggs (Elsenson *et al.* 2021; Lima and Von Zuben, 2016; Resetarits Jr, 1996). For females to find the ideal oviposition site, they need to consider and balance the time, energy, competition, unfavourable conditions, and availability in resource that are associated with gathering such cues. Such decisions will strongly influence the fitness of her offspring. I hope to provide insight on the interactions of species, their environment, and intraspecific cues in making spatial aggregation decisions and maternal choice.

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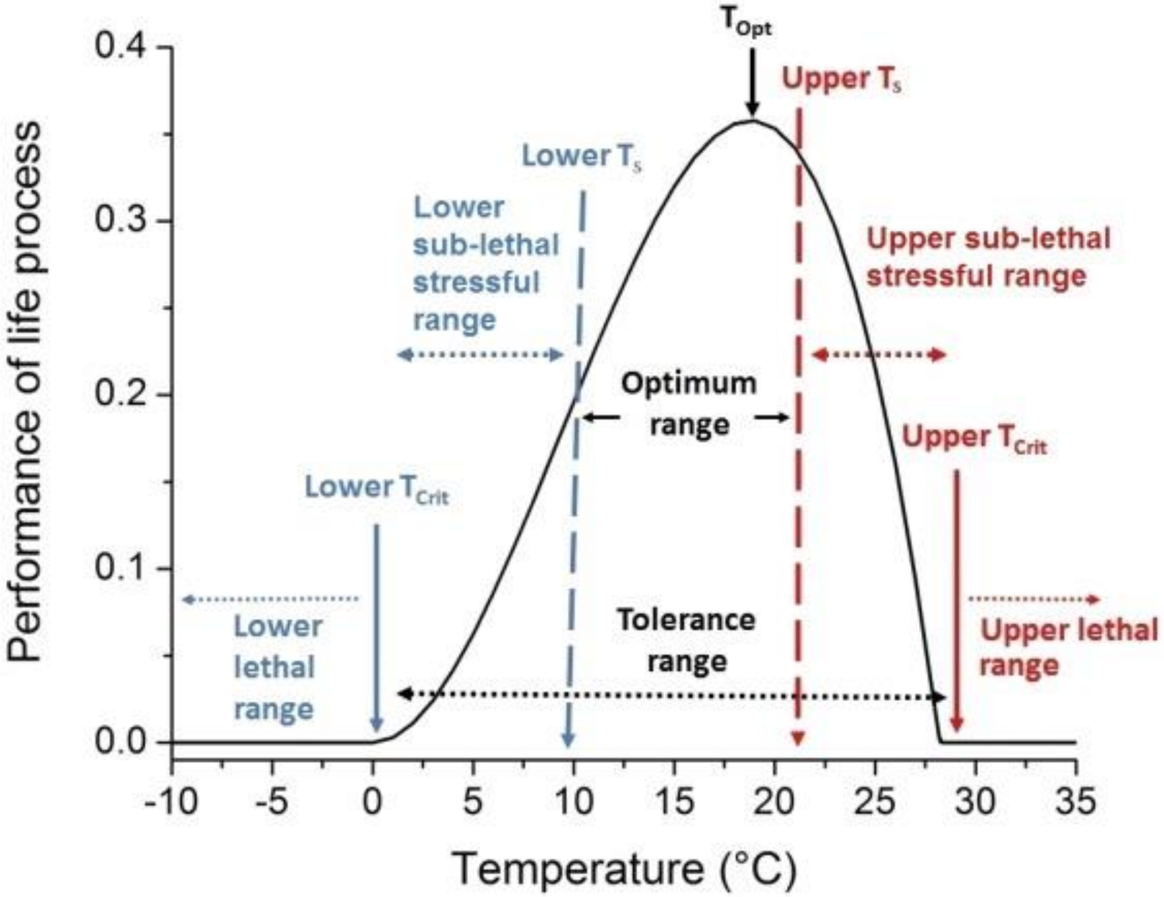

Figure 1.1. Example of a graph depicting the breakdown of the tolerance range within the thermal performance curve (TPC) with performance/ fitness represented on the y-axis and temperature on the x-axis. From B. K. Quinn, 2016, *Threshold temperatures for performance and survival of American lobster larvae: A review of current knowledge and implications to modeling impacts of climate change, 186,* p. 383-396.

CHAPTER 2

BLOW FLY LIKELIHOOD AND LOCATION OF OVIPOSITION ALONG A TEMPERATURE GRADIENT IS INFLUENCED BY RELATIVE HUMIDITY AND SPECIES

2.1 Introduction

Females must make decisions on when and where to oviposit so that they can exploit offspring food resources and maximize their reproductive output (Wall *et al*. 2002). This is also important for adult females that mate and need enough nutrition for vitellogenesis, especially protein ingestion. Wall *et al.* (2002) examined the initiation of yolk deposition and egg maturation of *L. sericata* based on protein meal size and observed that females that fed on suboptimal amounts of protein could not meet the protein threshold, and either no yolk deposition occurred or had arrested oöcyte development. Females could also resorb some oöcytes and mature the remainder due to the slower ovarian development if females have difficulty locating a sufficient protein to initiate ovarian development (Vogt *et al.* 1985).

The fitness of a female is determined by the number of viable offspring that she is able to produce. To take advantage of favourable egg-laying opportunities, females must always carry a number of eggs ready for oviposition (Berger *et al*. 2008). Females use many strategies for oviposition, including investing large amounts of time in selecting oviposition sites and also in their clutch sizes, if they cluster their eggs or have limited eggs to lay (Gibbs and Van Dyck 2009; Jervis *et al*. 2005; Rosenheim *et al*. 2008). Based on the optimal oviposition theory, or preference-performance hypothesis, oviposition preference for female blow flies is reflected by maximizing offspring development from choosing to oviposit on high-quality sites to enhance fitness (Jaenike 1978; Scheirs and Bruyn 2002). Any oviposition behaviour-offspring performance relationship is a consequence of initial environmental conditions, as it will complicate the female's choice for oviposition sites (Bonebrake *et al.* 2010).

An environmental factor that is dominant in influencing a blow fly's (Diptera: Calliphoridae) behaviour is temperature. Being poikilothermic, they are unable to thermoregulate using internal physiology, so in order to maintain their body temperature, they exchange heat with their environment (Sanborn 2004; Tatler *et al*. 2000). Temperature also impacts their development, physiology, and behaviour, which influences their seasonal distribution and abundance. Due to fluctuating temperatures in a blow fly's experience, they may need to acclimate at various suboptimal temperatures. Those that are thermal generalists are able to adapt and perform over a broad range of temperatures better than thermal specialists, where they are restricted to a narrow temperature range (Gilchrist 1995; Haaland *et al*. 2020; Hoffman 2010). Although blow flies that are more thermal specialists may not be able to thermoregulate over a larger temperature range, their performance is optimized within the narrow environmental range to which they are adapted (Haaland *et al*. 2020; Hoffman 2010).

Females will make decisions when placed in various thermal environments. For activities such as oviposition, temperature impacts the ability of a female to detect a suitable oviposition site, as well as the successful development of deposited offspring (Dvořák and Gvoždík 2010; Stahlschmidt and Adamo 2013). Temperatures that are too hot or too cold could either delay or cause death to both an adult female and her eggs (Fraenkel and Hopf 1940; Villet *et al*. 2009), thus species want to oviposit within their thermally favourable range (Dillon *et al*. 2009). These decisions may be expressed in terms of temperature preferences. For example, Deal (1941) examined temperatures to which an insect moves if given its choice on a temperature gradient.

Habitat selection for blow flies is influenced by abiotic mediators, predominately time of day and temperature. Their thermal preferences and optimal temperatures often overlap. This depends on species-specific temperature requirements and geographic variation. For example, *L. sericata* chooses to oviposit on carcasses with a surface temperature of 30°C (Cragg 1956; Matuszewski *et al.* 2014; Ody *et al*. 2017). Similarly at 30°C, *L. sericata* eggs in 60-80% RH enabled the most egg hatching (Davies 1948; Wall *et al*. 2001).

Humidity also plays a role in influencing blow fly development, physiology and behaviour as it can modify their body temperature (Ludwig 1945) and affect their internal water balance (Chown *et al*. 2011). Female blow flies use carrion to provide protection and nutrition for their eggs and larvae by searching for openings such as natural orifices or other areas that are thin and hairless (Wardle 1930). These sites offer a humid environment containing enough moisture suitable for larvae development, especially during the early stages of decomposition (Kyerematen *et al*. 2013). Within an optimal humidity range, individuals are able to develop rapidly and survive within a greater range of temperatures (Ludwig 1945). Humidity impacts the rate of oviposition and egg production (Ludwig 1945). High humidity may be a challenge as oversaturation occurs when insects are placed in environments of high temperature and high humidity (Neven 2000). At the opposite end, desiccation stress can occur if humidity is below a species' ideal range. Blow fly eggs are very susceptible to desiccation if there is not enough moisture (Hall *et al*. 2012), causing eggs to lose weight and be unable to complete development (Davies 1948; Evans 1934). Strategies to reduce egg mortality from desiccation include ovipositing eggs in cracks or crevices, or aggregating eggs in densely packed or layered cluster (Clark and Faeth 1998; Tomberlin *et al*. 2015). Prince and Parson (1977) found that when they subjected three *Drosophila* spp. (Diptera: Drosophilidae) in a thermal gradient (16°C - 36°C) at

0% and 100% relative humidity (RH), flies accumulated in the cold end of the gradient at 0% RH, whereas at 100% RH, flies tended to maintain stable distributions centered around 29–32°C. They concluded that flies in dry air gravitate to lower temperatures reduce water loss (Dillon *et al.* 2009).

Humidity also effects the developmental time, and adult longevity. For example, in the azuki bean weevil *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), they developed faster when RH conditions are between 70-75% RH regardless of temperature, and individuals live longer when RH conditions are between 90-95 % RH regardless of temperature (Mainali *et al.* 2015). Additionally, RH has an effect in oviposition responses in stimulating oviposition, site selection and egg aggregation. Relative humidity conditions of >50% elicits a higher oviposition response (Ashworth and Wall 1994; Hans 2016). Furthermore, RH conditions between 60-80% RH promotes the highest success for egg eclosion and adult emergence (Hans 2016; Holmes *et al.* 2012; Matuszewski *et al*. 2010). Although various research has demonstrated that RH influences the physiology of blow flies, there have been limited studies regarding the role RH on the adults and their oviposition behaviours. Blow flies favour sites that are moist as this prevents egg desiccation while promoting higher egg eclosion success (Hans, 2016). Studies from George *et al.* 2013 and Pitt and Wall 2004 observed a negative relationship between RH and colonization occurring, whereas Lutz *et al.* 2022 determined that certain species are influenced by RH to colonize a resource. Although there appears to be differences in the effect of RH, this variation is also due to differences in the experimental setup. George *et al.* 2013 and Lutz *et al.* 2022 focused on field colonization within their country (Australia; Germany) throughout most of the seasons and observed an abundance of species local within their habitat. George *et al.* (2013) observed an abundance of *Calliphora augur* (Fabricius, 1781), *Calliphora dubia* (Macquart 1855), *Calliphora hilli* (Patton, 1925), *C. vicina*, *Calliphora stygia* (Fabricius, 1781), and *Lucillia* spp. Most abundant species for Lutz *et al.* (2022) was *Lucilia ampullcea* Villeneuve, *C. vicina*, *Lucilia caeser* (Linnaeus, 1758), and *L. sericata.* Compared to Pitt and Wall (2004), they focused on using a cage population solely of *L. sericata*, as this species was a primary agent of sheep myiasis at many sheep farms in England and was placed outdoors during spring and summer months to observe oviposition rate. These studies also utilized outdoor environmental parameters, so they examined multiple variables for colonization. For this study presented herein, three co-occuring species from the Windsor-Essex region will be tested under controlled RH settings to eliminate the potential for other variables to affect the outcome. The RH settings used in this study focused mainly on RH extreme levels, and correlate with RH levels experienced within Windsor-Essex seasonally. Compared to the other studies mentioned, they recorded multiple outdoor environmental data and had different ranges for RH (George *et al.* 2013: RH recorded 16-92% RH, Pitt and Wall, 2004: RH recorded 65-88%, Lutz *et al.* 2022: not stated). The main difference is that this study uses a temperature gradient to observe oviposition behaviours. By using a temperature gradient and allowing females to choose the temperature to oviposit, this can validate each species temperature preference for oviposition, as well as comparing temperature preference to optimum temperature without having a fixed temperature influencing their behaviour.

In this study, I measured the influence of high and low relative humidity (RH) on oviposition decisions of individual blow flies of *Lucilia sericata* (Meigen), *Phormia regina* (Meigen), and *Calliphora vicina* Robineau-Desvoidy. Individual females were placed on a temperature gradient (10-40°C) under high (75-85%) or low (25-35%) RH and I measured the choice to oviposit or not, and for those that chose to oviposit, the timing to first oviposition, clutch size (defined as set of more than two eggs deposited by one female), and temperature preference for oviposition location.

Each of these three blow flies differs in their tolerances to temperature and humidity. *Lucilia sericata* are mostly commonly collected during the summer months in southern Ontario (Rosati 2014). They have a relatively narrow thermal range of 17-33°C (Roe and Higley 2015; Okpara 2018; Wall *et al.* 1992), especially for warmer temperatures, which allows them to be present predominately in the summer months in southern Ontario and oviposit on resources within temperatures ranging from 28°C to 33°C (Cragg 1955; Davies 1934; Smith and Wall 1997) and have oviposited at temperatures up to 40°C (Ody *et al*. 2017). Additionally, warmer temperatures are beneficial for their metabolism and growth, as their minimum developmental temperatures between 7.5-10°C will delay their development (Roe and Higley 2015). Thus, I expect that *L. sericata* will choose temperatures ranging from 20-30°C for oviposition on the gradient. Females when in high RH conditions will select optimal temperatures ~25°C, as Okpara (2018) observed highest survival to adult at 25°C and 70% RH, whereas in low RH, *L. sericata* females will select similar temperatures around 25°C as well to avoid thermal stress and water loss if eggs are placed in temperatures too cool or too warm in low RH. Hans (2016) saw that at 50% RH and higher, time to eclosion for *L. sericata* eggs was faster by 20 h and had a higher hatching success of 70 or greater compared to below 50% RH. This suggests more *L. sericata* females will oviposit in high RH, and will oviposit faster, with more eggs laid at high RH. Compared to low RH, *L. sericata*'s mean eclosion success was less than 20% in low humidity (Hans 2016), indicating that *L. sericata* eggs are susceptible to desiccation and will avoid ovipositing in low RH conditions.

For *Phormia regina*, they have a wider temperature range of 12.5-35°C compared to *L. sericata*, with optimal development temperatures between 15-35°C and they typically oviposit at temperatures over 20°C (Berg and Benbow 2014; Byrd and Allen 2001; Roe 2014). This allows them to be active and present across a wider range of seasons, the spring, summer, and fall months in southern Ontario (Marshall *et al*. 2011, Rosati 2014), compared to *L. sericata*. For temperature preferences along the gradient, we predict that *P. regina* will oviposit in temperatures ranging from 15-30°C. In high RH, *P. regina* will select optimal temperatures ~25°C as Okpara (2018) observed highest survival to adult at 25°C and 70% RH. Similarly, in low RH, *P. regina* will select temperatures around 25°C as well to avoid thermal stress and water loss. Hygrosensory neurons will allow them to respond to changes within their environment (Enjin 2017; Frank *et al*. 2017; Tochen *et al*. 2015). For RH preferences, we predict that they will also oviposit more and have a higher oviposition success towards high RH, where they will oviposit larger clusters and oviposit faster, compared to low RH. Hans (2016) observed that the lowest hatching rates were around 50% RH and lower, indicating that *P. regina* eggs are also susceptible to desiccation and will avoid ovipositing in low RH conditions. Compared to high RH, *P. regina's* mean egg eclosion success were within 70-80% RH (Hans 2016)

Lastly, the species *C. vicina* have a wide thermal range towards cooler temperatures observed between 5-30°C (Donovan *et al*. 2006, Hans 2016). Their thermal range allows them to be abundant predominately in cooler seasons (spring/ fall) in southern Ontario and eastern parts of Canada (Leblanc and Strongman 2002; Rosati 2014; Turpin *et al*. 2014). As temperature increases, especially at 28°C and above, their life cycle will be shorter, resulting in high mortality (Defilippo *et al*. 2013; Hwang and Turner 2009; Salimi *et al.* 2018). For temperature choice in both RH conditions, since they are seen in cooler seasons, they will show a preference for cooler temperatures to oviposit, ranging from 5-25°C, specifically choosing optimal temperatures of ~10-20°C as Ody (2017) observed the highest probability of eggs being laid between 10-20°C before the probability declines over 20°C and avoid higher temperatures above 25°C as the temperatures will become lethal. For RH preferences, *C. vicina* will oviposit and produce large clutch sizes at both high and low RH because Hans (2016) observed that they had eclosion successful hatching across all humidities, so they will be unaffected at each RH condition.

2.2 Methods and Materials

2.2.1 Colony Maintenance

Laboratory colonies of *L. sericata*, *P. regina*, and *C. vicina* maintained at the University of Windsor were used in this study. Colonies originated from capturing wild adults around the Windsor, Ontario, Canada area using wasp traps (King Home & Garden) baited with pork liver. New adults are added to the colonies throughout the spring to fall yearly to maintain colony genetic diversity. For *P. regina*, laboratories from Simon Fraser (Burnaby, British Columbia) and Ontario Tech University (Oshawa, Ontario) also provided *P. regina* to maintain colony diversity during the winter of 2019 due to environmental failure of the rearing room to maintain minimum RH rearing conditions. Wild-caught adults were sorted into 46 x 46 x 46 cm aluminum cages (Bioquip 1450C collapsible cage) containing sugar, water in an Erlenmeyer flask with cotton dental wicks to prevent drowning, and skim milk powder as a paste as carbohydrate source ad libitum. Colonies were held under a 12:12 (L:D) diel cycle from 7 AM to 7 PM at 20-30°C and

40-60% RH using full-spectrum artificial sunlight fluorescent grow lights. An oviposition substrate of 40 g of fresh pork liver was provided to adults within a colony cage for 24 hours to collect eggs. Egg masses (~ ca 500 eggs) on liver were placed inside rearing jars (1-L Bernardin mason jar) one-third filled with wood shavings as a pupation medium (Nepco Aspen Shavings). Jars were sealed with landscape tarp (Quest Brands Inc., Item ID: WBS 50) secured with a metal ring to allow for gas exchange while preventing larval escape. Additional liver was provided as needed until pupation. Upon emergence, adults were sorted by gender for placement into experimental cases using a 2–3-minute 4°C chill to slow adult movement for sorting and counting.

Experimental cages were set up in the colony rearing room, consisted of newly emerged 40 females and 20 males that were sorted into an experimental cage to ensure that all females would be mated since males' mate with multiple females. For the first 48 hours, 50 g of liver was placed in the experimental cage to ensure maximum protein uptake for female ovaries and male testes are developed (VanLaerhoven and Anderson 2001), followed by provision of 50 g of liver for 1 h on day 3, 4 and 5. Females were deemed gravid when abdominal stretch was present. On the 6th day of the experiment, individual gravid females were tested for oviposition choices. Experiments were performed between September 2019- November 2020, during which multiple generations were used to reduce generation effect.

2.2.2 Experimental Design

Experiments were performed inside a waterproof environmental box (106.68 cm x 45.72 cm x 45.72 cm) made from 6.35 mm thick plywood, with the door on the front, latched closed, and fully insulated with 2.54 cm thick Styrofoam (Dow Cladmate Extruded Polystyrene Insulation) and foam tape for the pleixglass lid (Figure 2.1). Inside the environmental box, an arena with a temperature gradient ranging from 10 to 15°C at the cold end, and 40 to 47ºC at the hot end. The gradient was created using an aluminum plate (2.54 mm thick; 76.2 cm long x 10.16 cm wide), with 15.24 cm ends bent 90° downward. The hot end was created using a heating wire from a Thermopore heating pad wrapped around the aluminum plate at one bent end. The cold end was created using a Styrofoam container filled with ice water where the other bent end of the aluminum plate was inserted into the ice water bath. Thermocouples (Sper Scientific 800023 4-Channel Thermometer; ±0.2% reading +1°C) were connected underneath the aluminum plate to measure the temperature along the gradient. Manual observations of the thermocouples were made every 30 mins and ice added to the cold end if temperature was at least 1°C above 10°C. At the hot end, the heater was turned off if temperature reached 41°C or higher. At no time, was the cold end below 10°C or the hot end below 40°C. A plexiglass arena (76.2 cm long x 10.16 cm wide x 5.08 cm high) with a lid was placed on top of the aluminum plate. A 4 cm hole cut in the center of the plexiglass arena was the release point for an individual fly, closed using a plug. On the long sides of the arena, ventilation windows were cut and covered with mesh to allow humidity to equalize between the inside and outside of the area. A datalogger (HOBO U12-012, Onset, Pocasset, MA) was placed inside the environmental box to record the relative humidity and overall temperature, with measurements recorded every 30 minutes. The environmental boxes were kept in an experimental laboratory room with standard fluorescent lighting to maintain a constant daylight regime. An oviposition media, made of 500 g of 48 h aged liver in a 35°C growth chamber to speed up decay to mimic 3–4-day old post-death liver (Johansen *et al*. 2014; Von Aesch *et al*. 2003), blended with 500 ml of boiling water with

10 g of agar (BD Diagnosis Bacto™ Agar Solidifying Agent), was placed onto aluminum foil on top of the aluminum plate and enclosed within the plexiglass arena. The aluminum foil allowed for easy cleanup of the plate at the end of each replicate. Cracks were created on the surface using a knife to create crevices, as blow flies tend to oviposit in or around openings, similar to wounds or natural orifices on a corpse (Ames and Turner 2003; Bourel *et al.* 2003). A handheld thermometer (ThermoPro TP-01A Instant Read Digital Meat Thermometer; ±0.9°F (±0.5°C)) and laser thermometer gun (MeasuPro Digital Infrared Thermometer IRT20) was used to measure the temperature on the oviposition medium compared to the temperature of the aluminum gradient.

The two RH treatments were low (25-35% RH), created from room temperature with a dehumidifier (Danby 18.9 L (40 Pint) Dehumidifier; Model: DDR040BFCWDB), and high (75-85% RH), created by using a timed humidifier (Honeywell Ultrasonic Cool Mist Humidifier for Medium Sized Room, 1-Gallon).

An individual female was released into the arena within an environmental box at 10 AM, 3 h after the start of their daylight period, as females laid the most eggs when they were released at 10 AM compared to earlier and later release times based on a preliminary study. Females were left for 8 hours, removed 1 h prior to the end of their daylight period. While in the arena, observations were made hourly as this was the time period used in previous studies (Hans, 2016; Pacheco, 2015). Each experimental trial was considered finished when either the females oviposited on the gradient and were then removed at that hourly check or did not oviposit during the 8-hour experiment. Three test arenas were run at a time. To further eliminate bias of generation effect, each species was alternated between days for each RH trial. For each RH treatment, 30 different individual females of each species were tested for a total of 60 individuals for each species across the two RH treatments. Individual females were only used once.

Oviposition choice was recorded as occurring or not, and if occurring, the timing of oviposition. At the location of egg clutches, the temperature gun and thermometer were used to measure the temperature of the agar. Photographs of egg clutches were taken using a Nikon D70 camera with an AF Micro-Nikkor 60 mm f/2.8D lens, with a 15 cm ruler for scale to estimate how many eggs were oviposited using previously established methods with ImageJ software and species-specific egg number estimation equations (Rosati *et al*. 2015, Hans *et al*. 2018).

2.2.3 Statistical Analyses

All analyses were performed in RStudio Open-Source Edition (Version 1.3.1073). A generalized linear mixed effect model (GLMER function, family=binomial) was used to test the effects of RH and species on the odds of oviposition occurring for each female between each species. Since there were three replicate box arenas run at a time, box ID was used as a random effect in the model. ANOVA was used to test if box ID influenced the odds of oviposition occurring, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function). In this study, the recorded number of females that oviposited or not was a dichotomous outcome with categories 1 (oviposition occurring) and 0 (no oviposition), and RH and species were treated as categorical variables, where references were assigned.

Clutch size was analyzed using a linear mixed effect model (lmer function) to examine the effect of temperature choice, RH, and the interaction of RH and temperature choice, with species as a covariate and box ID as a random effect in the model. ANOVA was used to test if box ID influenced clutch size, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function). Least squared was used to compare clutch size between species. First timing of oviposition was analyzed using a linear mixed effect model (lmer function) to examine the effect of temperature choice, RH and the interaction of RH and temperature choice, with species as a covariate and box ID as a random effect in the model. ANOVA was used to test if box ID influenced first timing of oviposition, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function). Least squared was used to compare first timing of oviposition between species. Temperature choice was analyzed using a linear mixed effect model (lmer function) to examine the effect of species, RH, and the interaction species and RH, with box ID as a random effect in the model. ANOVA was used to test if box ID was influenced temperature choice, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function). All significant results were separated with a post-hoc test using Tukey’s test (emmeans function, adjust = "tukey").

2.3 Results

The likelihood of oviposition occurring by an individual female did not depend on an interaction between RH and fly species (GLMER: $\chi^2_{2,173} = 0.24$, p = 0.89). However, separately, both factors influence the likelihood of oviposition (RH GLMER: $\chi^2_{1,173} = 5.67$, p = 0.017; Species GLMER: $\chi^2_{2,173} = 10.37$, p = 0.006). The odds of oviposition occurring within low RH are about 45% lower than the odds in high RH conditions (GLMER: $Z_{1,173} = -2.40$, p = 0.017), with more females ovipositing in high RH compared to low RH (Figure 2.2). In terms of species effects, *L. sericata* females were 4 times more likely to oviposit compared to *P. regina* (GLMER: $Z_{2,173}$ = -3.21, p = 0.0013) (Figure 2.2). However, the number of *C. vicina* females ovipositing did not differ from that of *L. sericata* (GLMER: $Z_{2,173}$ = 1.54, p = 0.12), or *P. regina* (GLMER: $Z_{1,173}$ = -0.81, p = 0.42). Likelihood of oviposition occurring did not vary by arena (i.e. AIC, BIC and loglikelihood did not differ by box ID).

For females who did oviposit, clutch size did not depend on RH ($F_{1,87}$ = 0.018, p = 0.90), or an interaction of RH and temperature choice ($F_{1,90}$ = 0.61, p = 0.44). Temperature choice did affect clutch size ($F_{1,88}$ = 3.86, p = 0.05). Species did covary with clutch size ($F_{2,86}$ = 3.10, p = 0.05), where *L. sericata* tended to lay larger clutches compared to *P. regina* (df = 92, p = 0.05) (Figure 2.3). There was no difference between *L. sericata* and *C. vicina* (df =92, p = 0.36) and *C. vicina* and *P. regina* (df = 92, p = 0.69). Arena influenced clutch size (LRT = 7.71, p = 0.0002).

Timing of oviposition was not affected by RH ($F_{1,90}$ = 1.41, p = 0.24) or temperature choice ($F_{1,90}$ = 1.78, p = 0.18) (Figure 2.4). Species at the time of oviposition did not covary with first timing ($F_{2,90}$ = 2.45, p = 0.09). Timing of oviposition did not vary by arena (i.e. AIC, BIC and loglikelihood did not differ by box I.D).

Relative humidity and species interacted to affect temperature choice ($F_{2,90}$ = 11.49, p <0.001). Specifically, *P. regina*'s temperature choice differed between high and low RH (df = 74.8, p = 0.0004), where *P. regina* chose hotter mean temperatures of 30°C ± 1 (mean ± SE), with a range of 25-33°C for oviposition when placed in low RH, compared to high RH where a mean (± SE) of 21°C ± 0.7 with a range of 19-32°C was chosen. At high RH, *L. sericata* females chose higher temperatures for oviposition compared to *C. vicina* and *P. regina*. *Lucilia sericata* chose a mean (± SE) of 28°C ± 1, with a range of 21-41°C for oviposition in high RH. Females in low RH also chose a mean (± SE) temperature of 28°C ± 1, with a slightly smaller range of 21-38°C for oviposition. At both high and low RH, *C. vicina* females chose lower temperatures for oviposition compared to the other two species (Figure 2.5). Females in low RH chose a mean (± SE) temperature of 18°C ± 0.6, with a range of 15-22°C. In high RH, females chose a mean (± SE) temperature of 19°C ± 0.6, with a range of 17-21°C for oviposition. Temperature choice did not vary by arena (i.e. AIC, BIC and loglikelihood did not differ by box I.D).

2.4 Discussion

Previous research has demonstrated that temperature influences the decision by blow flies to oviposit (Hans *et al*. 2018; Ody *et al*. 2017). We hypothesized that relative humidity (RH) would mediate this decision due to the effect of RH on eclosion success of blow fly eggs, and that this effect would be species specific (Hans and VanLaerhoven 2021). Indeed, high RH induced more females to oviposit than at low RH. Our results differ from studies that have found that humidity either had no effect (Shah and Sakhawat 2004) or had a negative influence on colonization, where an increase in RH decreases the probability of bait colonization (George *et al*. 2013). This could be due to differences in experimental set, where both these studies were conducted in the field with multiple abiotic factors present, along with RH fluctuated throughout the day, whereas our study maintained each RH condition, without any external abiotic influences and limited fluctuation from indoor settings.

Although we predicted that the choice to oviposit under different RH conditions would be an interaction with species, it did not, yet the species themselves varied in the choice to oviposit. Abbott (1932) observed that *L. sericata* had a high olfactory response when placed in an environment at 73% RH with 30°C, as well as the lowest olfactory response at 30% RH, regardless of temperature, which indicates that lower RH levels create a less favourable environment for *L. sericata* to locate oviposition resources by olfaction. Although we found that *L. sericata* females chose to oviposit more than *P. regina*, *L. sericata* choice did not vary by RH treatment. Perhaps the higher RH experienced by *L. sericata* adults in the experimental cages prior to being placed in the low RH conditions of the arena cued *L. sericata* adults to oviposit regardless of the low RH arena conditions.

It was not surprising that *C. vicina* did not differ from *L. sericata* females in choosing to oviposit at higher or lower RH, as based on the lack of effect of low RH on *C. vicina* egg eclosion (Hans and VanLaerhoven 2021), we did not predict RH would impact whether or not *C. vicina* chose to oviposit. However, it was surprising that *C. vicina* did not differ from *P. regina* female choice to oviposit or not. Based on Hans (2016), *P. regina* eggs require at least 70% RH to have a hatching success of at least 60%, thus we predicted it would be the most affected by the low RH treatment. Indeed, *P. regina* females chose to oviposit less frequently than that of *L. sericata*, yet *P. regina* response didn't depend on RH treatment, but may depend on other factors that enhance bait attraction for oviposition. Brodie *et al*. (2015), found that gravid *P. regina* had a higher alighting response to liver when gravid and non-gravid intraspecific females, as well liver with gravid heterospecific females were feeding on the liver compared to liver alone.

Surprisingly, RH did not affect clutch size in our study, despite the effect of RH on egg eclosion development and success found by Hans (2016). Clutch size in this study was determined when females cluster more than two of their eggs. For blow flies, laying their eggs in clutches is presumed to be beneficial as it should increase the survival of offspring by preventing egg desiccation for those eggs most surrounded by others as has been found for other species, similar to butterflies clustering their eggs (Clark and Faeth 1998; Godfray *et al*. 1991). This is especially true under low RH conditions as eggs can become dehydrated, shrinking the chorion and embryo (Norhisham *et al*. 2013), as well as reducing eclosion success (Hans and VanLaerhoven 2021; Holmes *et al*. 2012). In contrast, Pitt and Wall (2004) found that as RH increased from 60-90%RH, the oviposition rate (eggs per day) decreased and the mortality rate increased for *L. sericata*, although there is difference between methodology. Hans *et al.* (2018) found that for *L. sericata* and *P. regina*, temperature modified the clutch size such that with increasing temperatures there was an increase in clutch size, thereby concluding that temperature is the main factor in influencing clutch size. In our study, females could choose their location on the temperature gradient to oviposit their eggs, which influenced clutch size. For *L. sericata*, females in our study for both RH conditions chose mean temperature of 28°C to oviposit. The temperature correlates with the findings from Hans *et al.* (2018), where they observed highest mean number of eggs for *L. sericata* at 30°C. In our study for *P. regina*, in high RH, females chose mean temperature of 21°C to oviposit, whereas in low RH, females chose mean temperature of 30°C. Compared to Hans *et al.* (2018), they observed the lowest mean number of eggs for *P. regina* at 20°C and the highest mean number of eggs at 30°C. The temperatures that *L. sericata* and *P. regina* chose in each RH condition correlates with temperatures that were either optimal or suboptimal for clutch size from Hans *et al.* (2018).

Neither RH nor species had an effect on the time to first oviposition. Instead, temperature appears to be more important. Hans *et al.* (2018) found that temperature affected the time to first oviposition for *L. sericata* and *P. regina*, with faster oviposition as temperature increases. In our study, *L. sericata* choosing mean temperature of 28°C in both RH conditions required 3 h for oviposition, which are consistent with the findings from Hans *et al.* (2018) and Zurawski *et al.* (2009). *Phormia regina* choosing mean temperature of 30°C in low RH required 6 h for oviposition. Oviposition time for *P. regina* required longer time compared to Hans *et al.* (2018) and Zurawski *et al.* (2009), where *P. regina* at 30°C required 3-5 h. In high RH, *P. regina* choosing to oviposit at 21°C required 4 h for oviposition. In contrast, Hans *et al.* (2018) observed longer oviposition time of ~20 h at 20°C. The suggests that although RH and species independently play a role in determining if females will choose to oviposit or not, the timing of oviposition event is primarily influenced on temperature.

Interestingly, both RH and species interacted to influence where on the temperature gradient a female chose to lay her eggs. These choices seem to correlate with temperatures that reflect the seasonal distribution of each species. Regardless of RH, *L. sericata* consistently chose a mean of 28°C (21-41°C range), which reflects their higher abundance in the summer in the Windsor-Essex area (Rosati 2014). It was surprising that some females chose to oviposit towards lethal temperatures, as *L. sericata*'s oviposition behaviours and survival rates tend to be low towards 35°C (Hans *et al.* 2018; Okpara 2018; Wall *et al*. 2001), yet some females chose to lay up as far as at 38°C at low RH and up to 41°C at high RH on the gradient. The high moisture atmosphere within the environmental box may have moistened the agar medium, thus decreasing dehydration of the medium to create a more suitable medium towards the hotter end of the gradient.

As expected for the cooler weather species found in spring and fall in the Windsor-Essex area (Rosati 2014), *C. vicina* chose a cooler overall mean of 18°C (14-24°C range) across high and low RH. With different humidity levels, females still chose temperature ranges that are closely similar, within a couple degree differences, which could indicate that *C. vicina* are thermal specialists, less affected by RH, as they preferred to oviposit within in a narrower range of temperatures.

In contrast, temperature choice for oviposition on the gradient was highly dependent on RH for *P. regina.* Under low RH, females chose a mean of 30°C (25-33°C range), whereas under high RH they chose to oviposit at a mean of 21°C (19-32°C range). *Phormia regina* is found throughout spring, summer and fall in the Windsor-Essex area (Rosati 2014), and these results suggest it is a thermal generalist, modifying its preferred temperature range for oviposition depending on local RH conditions. The plasticity of *P. regina* to behaviourally adapt its temperature choice for oviposition under different RH conditions, thereby possibly reducing water loss and thus avoiding desiccation of their eggs (Enjin 2017) may explain how this species is able to persist across all three seasons in the local environment.

We demonstrated that RH influenced the likelihood of oviposition to occur for these three species, as well as influenced their temperature choice on the temperature gradient that was species specific to their thermal tolerance, whereas clutch size, as well as the likelihood of oviposition to occur was dependent on species. The choices that were presented in this study varied by species. High RH was beneficial in this study as this condition increased the probability of oviposition occurring for each species compared to low RH, which could be used as an abiotic indicator to detect a suitable oviposition site based on the moisture content of the substrate (Bauer *et al.* 2020). If more females were to oviposit in low RH, eggs would succumb to desiccation, preventing egg hatching and larval development, thus decreasing reproductive success for these species. Relative humidity did not influence the clutch size or the timing to first oviposition for each species, which indicates that either these species do not rely on RH in determining the number of eggs laid or how fast they oviposit. There might be other factors affecting oviposition decisions that either interacts with RH or on their own, such as semiochemical or visual cues (Brodie *et al*. 2014), or the presence con- and heterospecifics (Brodie *et al*. 2015, Hans *et al*. 2021). Further measurements of the oviposition behavior of these blow flies should be made by analyzing the factors listed above.

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Table 2.1. Summary table of the results for the influence of RH on the likelihood of oviposition occurring and on the oviposition behaviours for each species.

Likelihood of Oviposition Occuring		
Influence	**Yes**	**No**
RH and Species		✓
RH	• Odds of oviposition occurring within low RH are ~45% lower than the odds in high RH • More females ovipositing in high RH compared to low RH	
Species	• When *Lucilia sericata* is the reference species, *L. sericata* is 4x more likely to oviposit compared to *Phormia regina*. • *Calliphora vicina* not different from that of *L. sericata* or *P. regina*	
Oviposition Behaviours		
Clutch Size		✓
First Timing of Oviposition		✓
Temperature Preference	• *P. regina*'s temperature choice differed, chose hotter temp in low RH setting compared to high RH • In low RH: *L. sericata* and *P. regina* chose hotter temperatures than *C. vicina* • In high RH: *C. vicina* and *P. regina* chose cooler temperatures than *L. sericata*	

Figure 2.1. Images of the experimental set up inside the “environmental box” to maintain relative humidity conditions (A). The aluminum temperature gradient with aluminum foil liner and plexiglass arena placed on top (B).

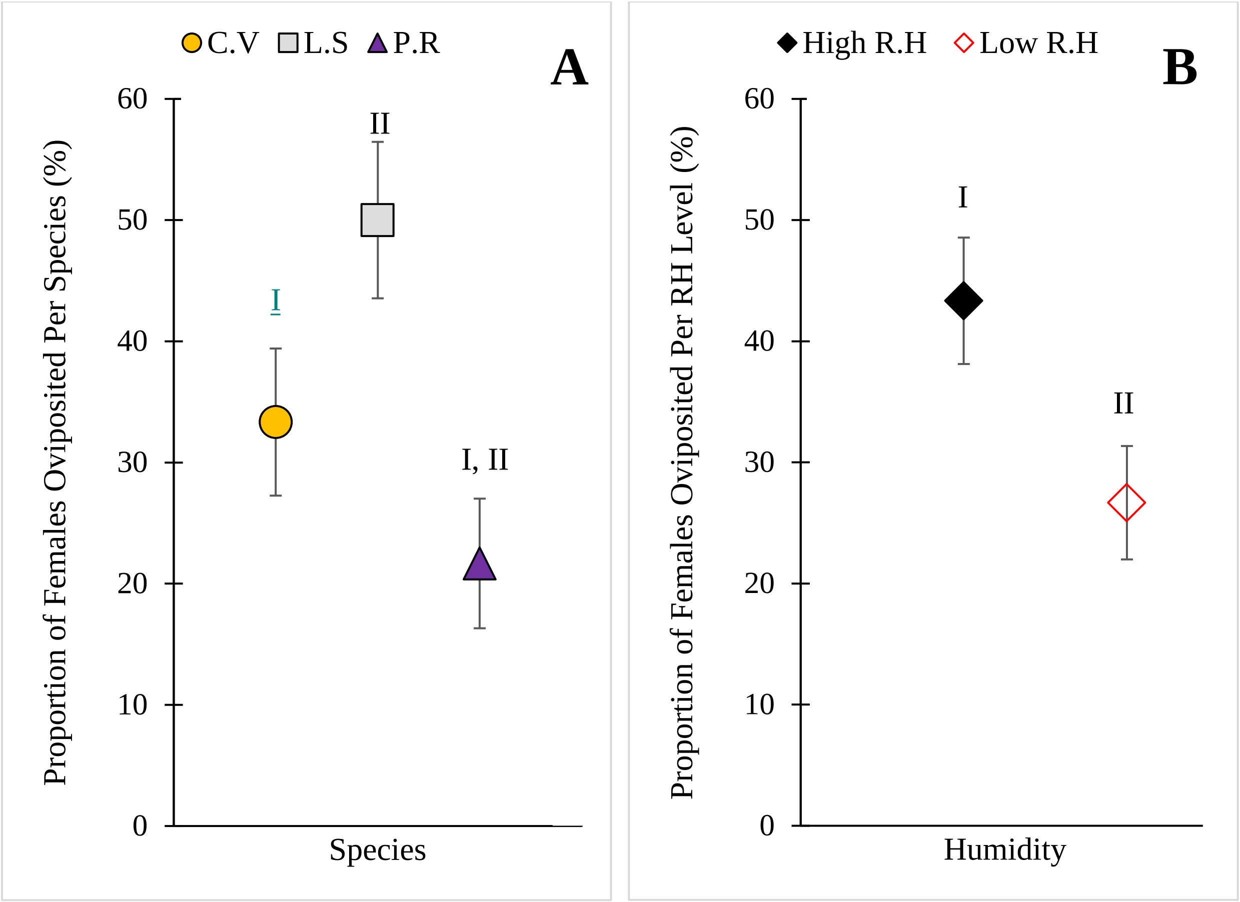

Figure 2.2. Mean (±S.E.) number of females ovipositing by *Calliphora vicina* (n = 20), *Lucilia sericata* (n = 30), and *Phormia regina* (n = 13) (Diptera: Calliphoridae) either (A) pooled across species; or (B) between high relative humidity (75-85%) versus low relative humidity (25-35%). No interaction between relative humidity and species on the likelihood of females choosing the oviposit (p=0.89). Means within the same graph followed by the same roman numeral do not differ ($p > 0.05$).

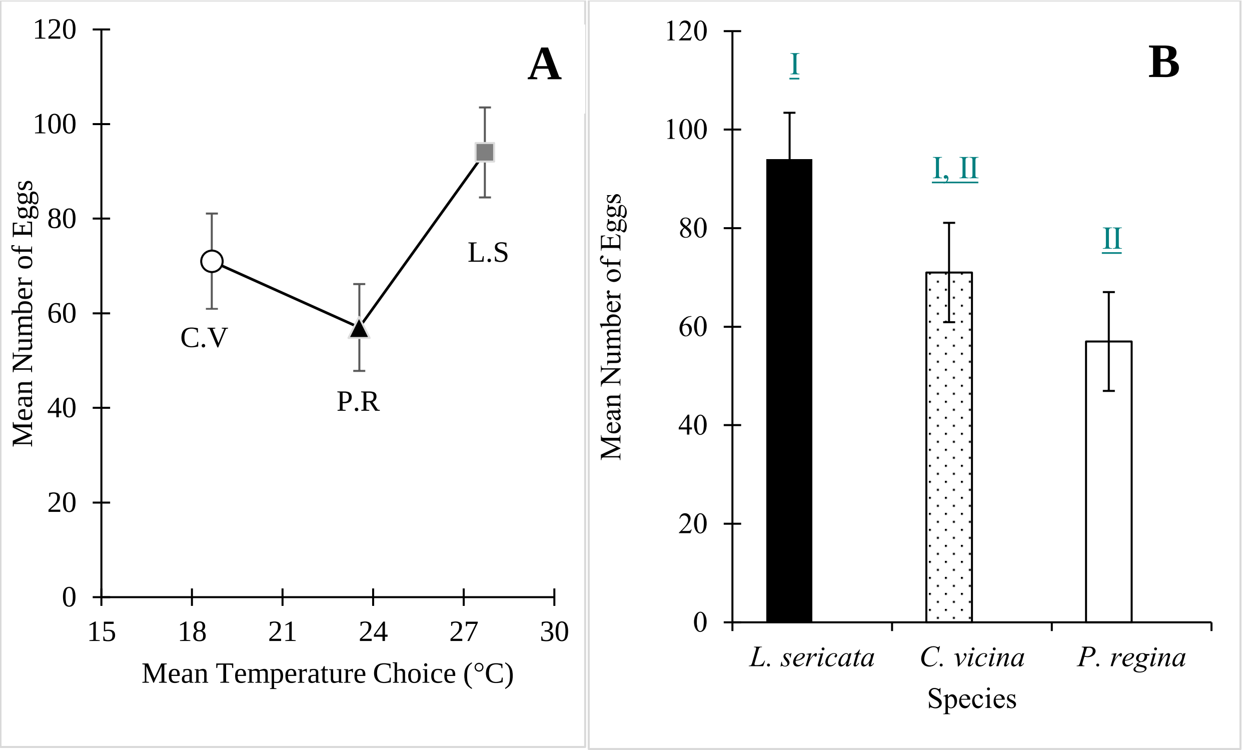

Figure 2.3. Mean (±S.E.) number of eggs oviposted by individual females of *Calliphora vicina* (n = 20), *Lucilia sericata* (n = 30), and *Phormia regina* (n = 13) (Diptera: Calliphoridae) pooled across humidity levels. RH did not influence clutch size ($F_{1,87} = 0.018$, p = 0.90). Temperature choice did affect clutch size ($F_{1,88} = 3.86$, p = 0.05) (A), as well as species ($F_{2,86} = 3.10$, p = 0.05) (B). Means with the same roman numerals are not significantly different.

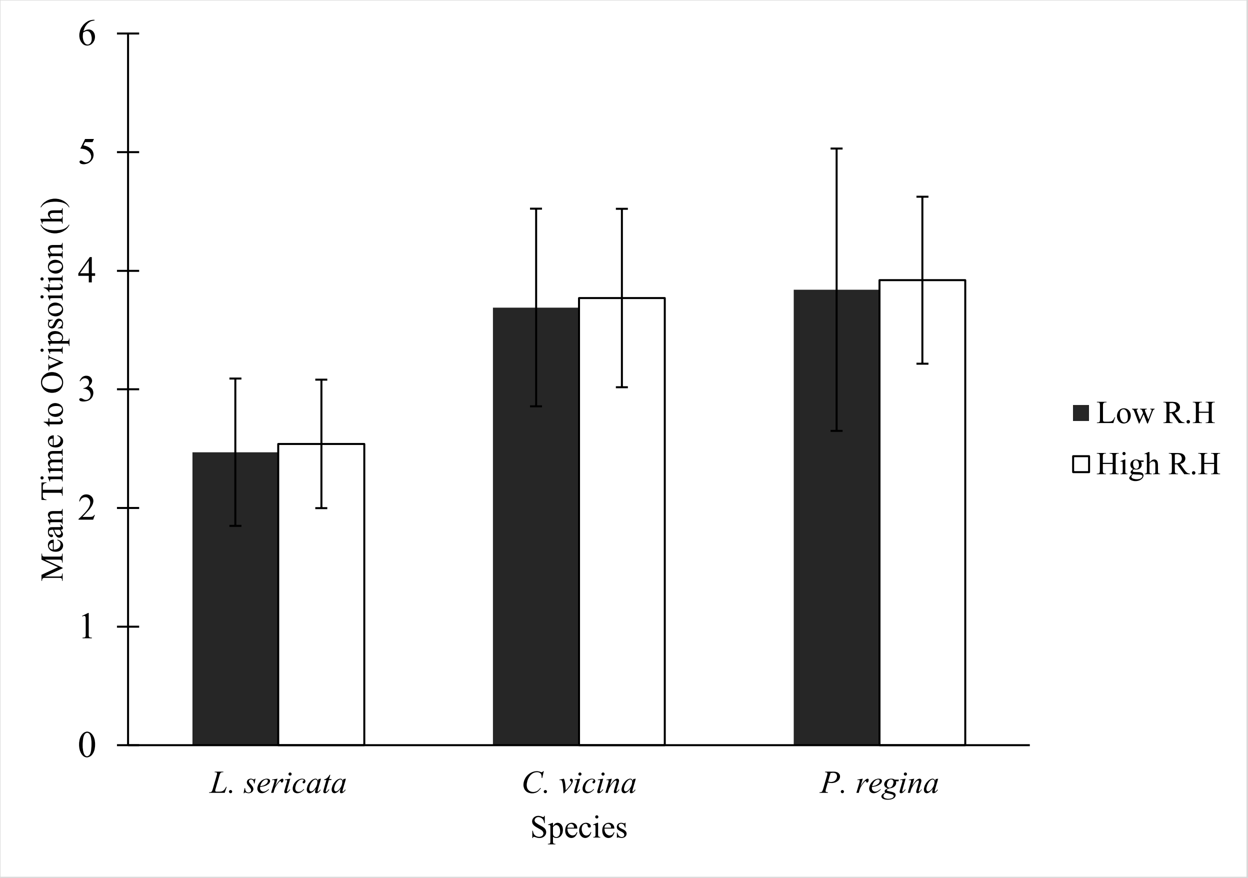

Figure 2.4. Least-Squared mean (±S.E.) timing of first oviposition by individual females of *Calliphora vicina* (n = 20), *Lucilia sericata* (n = 30), and *Phormia regina* (n = 13) (Diptera: Calliphoridae) at high (75-85%) and low (25-35%) RH. There was no difference between high and low RH ($F_{1,90} = 1.41$, p = 0.24), temperature choice ($F_{1,90} = 1.78$, p = 0.18), or between species ($F_{2,90} = 2.45$, p = 0.09).

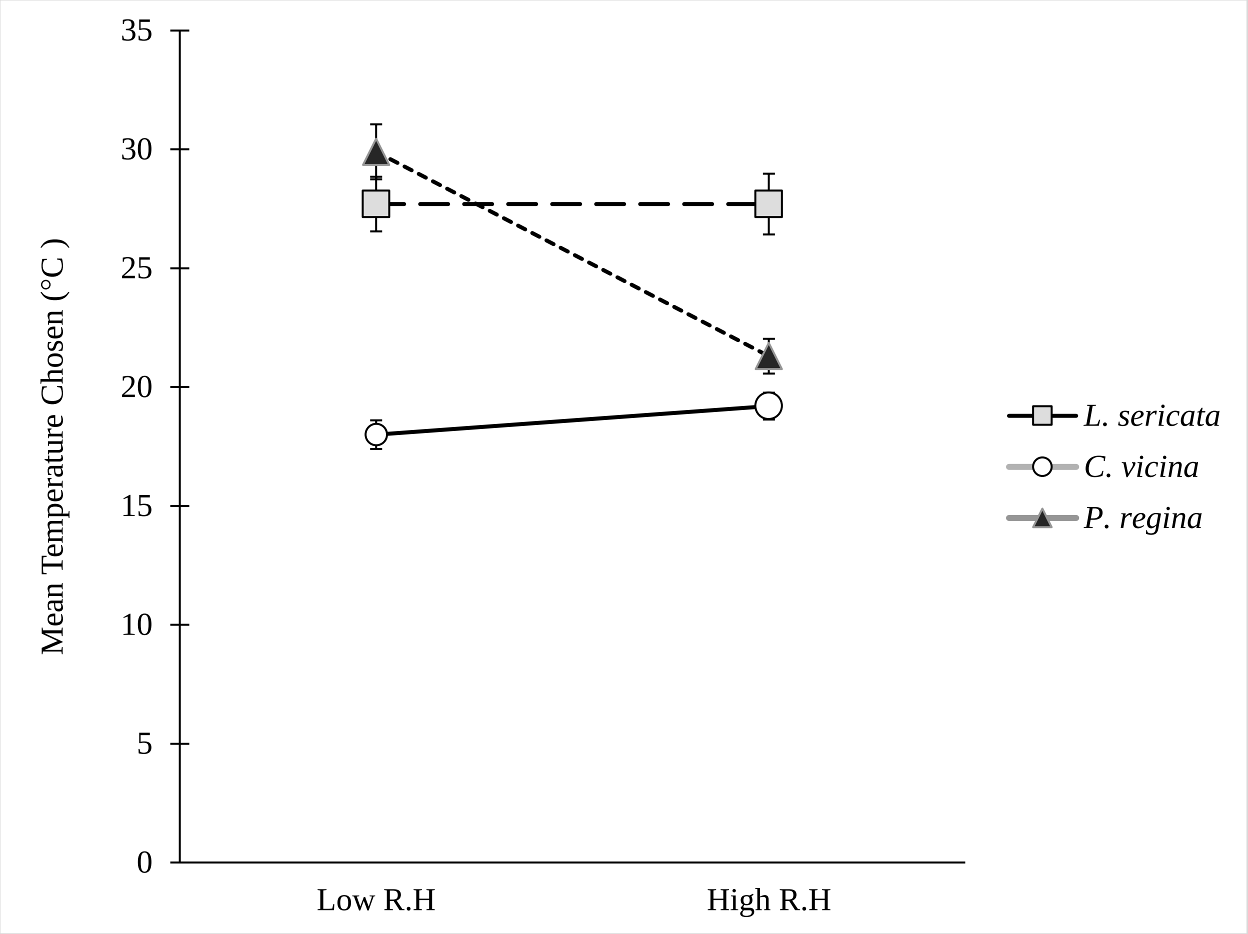

Figure 2.5. Least-squared mean (±S.E.) temperature choice along the gradient between individual females of *Calliphora vicina* (n = 20), *Lucilia sericata* (n = 30), and *Phormia regina* (n = 13) (Diptera: Calliphoridae) between high (75-85%) and low (25-35%) relative humidity. The interaction between species and humidity ($F_{2,90} = 11.49$, $p < 0.001$) influenced temperature choice.

CHAPTER 3

INTRASPECIFIC EGGS INTERACT WITH RELATIVE HUMIDITY TO AFFECT SPECIES-SPECIFIC BLOW FLY LIKELIHOOD AND LOCATION OF OVIPOSITION ALONG A TEMPERATURE GRADIENT

3.1 Introduction

When a blow fly (Diptera: Calliphoridae) lays eggs on a resource, it becomes more attractive to other females who then oviposit in the same area (Anderson 2002; Brodie *et al*. 2014; Lima and von Zuben 2016; Prokopy *et al*. 1999). This is possibly due to pheromones produced by gravid females (Jiang *et al.* 2002; Judd and Borden 1992), semiochemicals produced by the salivary gland of females feeding on the substrate (Brodie *et al.* 2014), and the eggs themselves (Giffen-Lemieux *et al.* 2020; Lima and von Zuben 2016; Judd and Borden 1992). If the abiotic environmental conditions are favourable, high intraspecific aggregation is often produced due to shared preference for similar environmental and resource conditions (Fiene *et al*. 2014). Individual female experiences, age of the female, egg load status and perceptions of the value of an oviposition substrate will influence their choice to oviposit on a resource (Minkenberg *et al*. 1992; Rohlfs and Hoffmeister 2004). Aluja *et al.* (2001) observed that diet, female age, and density accounted for the variation in egg load in the Mexican fruit fly, *Anastrepha ludens* Loew (Diptera: Tephritidae) and the West Indian fruit fly, *Anastrepha obliqua* Macquart (Diptera: Tephritidae), where *A. obliqua* had higher oocyte counts at 30- or 45-days post emergence on a well-fed diet and an increase in density, compared to *A. ludens* females with higher oocyte counts at 15 days post emergence on a well-fed diet. If a female lays a few large clutches, her offspring might experience a higher cost of intraspecific competition among her offspring, but if she decides to lay many small clutches, there is a higher cost of searching and travelling to different oviposition sites (Heard and Remer 1997).

For blow flies aggregating their eggs, this can be beneficial for the adults, as this strategy can reduce predation, conserve energy in locating a resource, and reduce risk of ovipositing on a low-quality resource (Faraji *et al*. 2002; Rivers *et al*. 2011; Stamp 1980). Additional benefits include increased survival of larvae. Laying eggs in locations where previous eggs have been laid will result in larval masses, as females typically lay large number of eggs (Charabidze *et al.* 2011). Larval aggregation can be beneficial for feeding larvae because they secrete proteolytic enzymes and ammonia to macerate the food, leading to more efficient feeding (dos Reis *et al.* 1999; Ireland and Turner 2006; Kökdener *et al*. 2019; Scanvion *et al*. 2018). However, the carrion resource is limited, ephemeral food resource so higher densities of individuals result in competition for food. Depending on whether the aggregation is beneficial or detrimental, trade-offs for larvae are differences in survival, growth rate, and body size (dos Reis *et al.* 1999; Okpara 2018; Smith and Wall 1997). Dos Reis *et al.* (1999) found that the rate of *Chrysomya putoria* (Wied) and *Cochliomyia macellaria* (F) (Diptera: Calliphoridae) survival to adulthood decreased as larvae densities increased when reared with intraspecifics. Additionally, higher density population can reduce individual fitness, with some components of fitness such as body size, growth rate, fecundity, or survivorship (Craig *et al.* 1990; Rohlfs and Hoffmeister 2004). Kökdener *et al.* (2019) observed a decrease in pupal and adult weight, as there is limited food available for developing larvae, leading to smaller adult bodies in *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae).

Considering that blow fly larvae are poikilotherms as well, larval development depends on temperature; faster development as temperature increases to optimal development temperature. The temperatures they experience are either from ambient temperature, or heat emissions produced from larval crowding during development (Aubernon *et al.* 2019; Slone and Gruner 2007). Larvae also release digestive enzymes as they develop. Together with high temperatures within larval crowding and constant movement, these factors liquefy the resource for easy consumption (Rivers *et al*. 2011; Thompson *et al*. 2013). Aggregation for larvae can be beneficial because it can protect against environmental conditions, reduce water loss, and increase survival by reducing development time due to increase in temperature (Broly *et al*. 2013; Fouche *et al.* 2018). Slone and Gruner (2007), found that when aggregations have a volume of 20-50cm^3, they produce an internal temperature of about 30-35°C, which is an optimal temperature range for larval growth and resistance to diapause. Larval masses can generate temperatures up to over 20°C above ambient temperature (Fouche *et al*. 2018). Larger larval masses can result in temperatures that can become too lethal, killing individual larvae and reducing overall larval survival (Kökdener *et al*. 2019; Okpara 2018; Rivers *et al*. 2011; Slone and Gruner 2007). Okpara (2018) observed smaller adult bodies of *Phormia regina* (Meigen) (Diptera: Calliphoridae) and *Lucilia seriata* (Meigen) (Diptera: Calliphoridae) at larger densities, as well as a decrease in survival, especially when temperatures are at 35°C.

High intraspecific larval competition on limited resources and at high temperatures results in smaller bodied adults which can affect various life history traits. For females, body size can affect fecundity, longevity, locating oviposition sites and mating (Honěk 1993; So and Dudgeon 1989; Tomberlin *et al*. 2009). In emerged *Lucilia eximia* (Wiedemann, 1819) (Diptera: Calliphoridae), the survival and fecundity of resultant adults decreased as larvae density increased; as larvae density increased over 100 larvae, mean number of eggs per females decreased from 7 eggs to 4 eggs per female while survival to adult emergence decreased from 91% to 36% (Silva *et al*. 2003). For fecundity, by ovipositing fewer eggs, this could lead to lower reproductive success as there is less energy allocated towards reproductive success (Ireland and Turner 2006; Kökdener *et al*. 2019). For males, body size can also affect mating. Stoffolano Jr *et al.* (2000) observed mating of *Phormia regina* (Meigen) (Diptera: Calliphoridae) where individuals from both sexes were either small (pupae weighing 20 mg or less) or large (pupae weighing 40 mg and more). Large females mating with small males had the lowest successful insemination, whereas large females mating with large males had the highest insemination success. Similarly, with *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), smaller males were less competitive for mating compared to larger males (Pitti *et al*. 2011).

For females locating and choosing an oviposition site, there are various factors that influences the attractiveness of the site, as well as the female's oviposition behaviour. One factor includes favourable environmental conditions such as temperature (Hans *et al.* 2019; George *et al*. 2013; Ody *et al*. 2017). Additionally, relative humidity (RH) influences their oviposition behaviour, where we observed more females of *Lucilia sericata* (Meigen), *Phormia regina* (Meigen), and *Calliphora vicina* Robineau-Desvoidy ovipositing in high RH (75-85%) compared to low RH (25-35%) conditions (Chapter 2), which could be due to the high moisture level maintained on the oviposition medium to promote higher egg eclosion success. Another factor influencing the attractiveness of the site and female's oviposition behaviour is the presence of intraspecifics. The presence of either eggs or larvae provides social information as visual, tactile or semiochemical cues. These cues can either be interpreted as avoiding the site to minimize intraspecific competition since the site is already colonized or can be attracted to the site, which leads to aggregation of eggs to increase offspring survival (Buxton *et al*. 2020; Fiene *et al*. 2014; Raitanen *et al*. 2013). It is possible that there is a complex interaction between environmental factors and intraspecific interactions that influence females' oviposition behaviours. In Chapter 2, RH alone influenced whether females would oviposit or not, as well as temperature choice along the gradient. Favourable environmental conditions may promote colonization, but other factors such as semichemical cues and the presence of conspecifics may also promote colonization. For example, Brodie *et al.* (2015) observed greater fly landing of *L. sericata* and *P. regina* on livers that had gravid and nongravid conspecific females. Lima and von Zuben (2016) observed greater colonization of *Chrysomya megacephala* (Fabricius) in livers that contained larger conspecific egg masses compared to liver alone. These studies primarily focused on semiochemical cues as attraction as these studies were performed under controlled conditions (Brodie *et al.* 2015: 30–40% RH, and 23–25°C, Lima and von Zuben: 27°C and 60% RH). To date, there have been no studies measuring the influence of RH, combined with the presence of intraspecific eggs on blow fly oviposition behaviours on a temperature gradient. By using a temperature gradient, we are able to create a semi-natural condition with varying RH conditions and temperature to mimic environmental changes. Along with females being able to choose where to oviposit on the temperature gradient in different RH conditions, this study will also observe whether females will aggregate their eggs or oviposit away from conspecific egg masses. Observing these species-specific responses in the presence of conspecifics and in varying RH conditions allows us to understand more about their decision process to accept carrion, and this information can be used to predict the probability of these species occurring while validating their temperature preferences.

The aim of this experiment was to measure the oviposition choices of individual female blow flies of *Lucilia sericata* (Meigen), *Phormia regina* (Meigen), and *Calliphora vicina* Robineau-Desvoidy interacting with both intraspecific eggs and RH within a temperature gradient. The information can provide a better understanding of why and when colonization occurs, which can be useful in estimating the pre-colonization interval (pre-CI) (time period of insect activity where they initially detect carrion until colonization, or oviposition) (Tomberlin *et al.* 2011), as well as factors influencing aggregation decisions. Based on previous observations on the number of females ovipositing (Chapter 2), we expect more females to oviposit in high RH when in the presence of intraspecific eggs, as high RH will reduce desiccation and increase attractiveness of the resource by retaining moisture, as well as semiochemical cues elicited from the intraspecific egg clutches (Giffen-Lemieux *et al*. 2020; Rivers *et al.* 2011). For *C. vicina* and *P. regina*, we predict they will oviposit faster, closer to intraspecific eggs, and increase their clutch size when in the presence of eggs in high RH because they can recognize the eggs based on aggregation vectors (visual, auditory, tactile or chemical stimuli efficient) (Fouche *et al*. 2018), along with a more favourable environment as high RH will retain moisture on the medium and on the intraspecific eggs. For *P. regina,* females will respond positively to intraspecific eggs, regardless of egg temperature location, in each RH condition and will shift their temperature preference to oviposit towards the intraspecific eggs due to the attraction to the semiochemicals of the eggs, as well as fewer thermal limitation as they have a wide temperature range of 12.5-35°C (Berg and Benbow 2014; Brodie *et al.* 2015; Byrd and Allen 2001; Hans 2016; Giffen-Lemieux *et al*. 2020; Rivers *et al.* 2011). For *C. vicina*, females will respond positively towards intraspecific eggs suboptimally placed towards cooler temperatures in both RH conditions and will shift their temperature preferences to be cooler as they will be attracted to the semiochemicals of the eggs, as well as favouring cooler temperatures as they have a minimum oviposition temperature range of 10°C (Giffen-Lemieux *et al*. 2020; Hans 2016; Ody *et al.* 2017; Rivers *et al.* 2011). Compared to intraspecific eggs placed in suboptimally hotter temperatures for both RH conditions, *C. vicina* females will not shift their temperature preference to avoid lethal temperatures (Hans 2016; Ody *et al*. 2017). For *L. sericata*, we hypothesize that there will be more females ovipositing in the presence of intraspecific eggs for both RH conditions as they will be attracted to the semiochemical cues of intraspecific eggs (Brodie *et al*. 2015; Giffen-Lemieux *et al*. 2020). Females will also oviposit larger clutches and oviposit faster, as *L. sericata* larvae have shown higher survival to adult emergence when developing on their own depending on temperature and density (Okpara 2018, Smith and Wall; 1997). When intraspecific eggs are placed in suboptimally cooler or hotter temperatures, I predict *L. sericata* will avoid ovipositing near them and will not shift thermal preference in both RH conditions as optimal temperatures for oviposition is 30°C, and temperatures less than 17.5°C or greater than 35°C can become lethal (Cragg 1955; Davies, 1934; Hans *et al*. 2018; Ody *et al.* 2017; Smith and Wall 1997).

3.2 Materials and Methods

3.2.1 Colony Maintenance & Experimental Cage Set-Up

Laboratory colonies of *L. sericata*, *P. re*gina, and *C. vicina* maintained at the University of Windsor and originating from wild adults collected yearly around the Windsor, Ontario, Canada area were used for this study. New adults are added to the colonies throughout the spring to fall yearly to maintain colony genetic diversity. For *P. regina*, laboratories from Simon Fraser (Burnaby, British Columbia) and Ontario Tech University (Oshawa, Ontario) also provided *P. regina* to maintain colony diversity during the winter of 2019 due to environmental failure of the rearing room to maintain minimum RH rearing conditions. Colonies are maintained in 46 x 46 x 46 cm aluminum cages (Bioquip 1450C collapsible cage) containing sugar, water in an Erlenmeyer flask with cotton dental wicks, and skim milk powder paste *ad libitum* under a 12:12 (L:D) diel cycle from 7 AM to 7 PM at 20-30°C and 40-60% RH using full-spectrum artificial sunlight fluorescent grow lights.

Eggs are collected by providing adult cages with 40 g of pork liver for 24 h as an oviposition substrate. Masses of ca. 500 eggs with 40 g of liver are placed into individual larval rearing jars (1-L Bernardin mason jar) filled with 1/3 wood shavings as a pupation medium (Nepco Aspen Shavings Bulk 8 CuFt) and sealed with landscape tarp (Quest Brands Inc., Item ID: WBS 50) and metal ring to prevent larval escape. Upon adult emergence, adults are sorted by gender using a short (ca. 3 min) 4°C chill to slow down movement enough to allow handling.

Experimental cages were comprised of 40 female and 20 male freshly-eclosed adults, provided sugar, water and milk paste as previously described. For the first 48 hours, 50 g of liver were placed in experimental cages to ensure maximum protein uptake for female ovaries and male testes are developed (VanLaerhoven and Anderson 2001), followed by provision of 50 g of liver for 1 h on day 3, 4 and 5. Females were deemed gravid when abdominal stretch was present. On the 6th day of the experiment, individual gravid females were removed from the experimental cage for use in the testing arenas that took place between August 2020- February 2021.

Experimental intraspecific eggs were collected from each species colony cages from 40 g of liver placed in the colony cages the day prior to each experiment. Eggs were placed in a refrigerator at 4°C for 12-18 hours (overnight) to slow development and prevent egg hatching during each experimental trial.

3.2.2 Experimental Design

Experiments were performed inside a waterproof environmental box (106.68 cm x 45.72 cm x 45.72 cm) made from 6.35 mm thick plywood with a door opening from the front that closes using latches, and fully insulated with Styrofoam (Dow Cladmate Extruded Polystyrene Insulation) and foam tape for the plexiglass lid (Figure 2.1). Inside the environmental box, a thermal gradient ranging from 10 to 15°C at the cold end, and 40 to 47°C at the hot end was used. The gradient was created by an aluminum plate with a thickness of 2.54 mm that is 76.2 cm long with 15.24 cm sides bent 90° downward, with heating wire from a heating pad (Thermopore) wrapped around the aluminum to create the hot end, and the cold end was created by inserting the aluminum into a styrofoam container (20.10 cm x 15.24 cm x 18.29 cm) filled with an ice water bath. Thermocouples (Sper Scientific 800023 4-Channel Thermometer; ±0.2% reading +1°C) were connected underneath the aluminum plate to measure the temperature along the gradient. Manual observations of the thermocouples were made every 30 mins and additional ice was added to the cold end if temperature was at least 1°C above 10°C. At the hot end, the heater was turned off if temperature reached 41°C or higher. At no time, was the cold end below 10°C or the hot end below 40°C. A plexiglass arena with lid to enclose the flies inside is 5.08 cm high and the same length and width as the gradient. At the center of the arena, a 4 cm hole with plug provides a release point for the flies. The front and back sides of the arena have windows covered with mesh to allow humidity to equalize between the inside and outside of the arena. A datalogger (HOBO U12-012, Onset, Pocasset, MA) was placed inside the environmental box to measure relative humidity. The environmental boxes were kept in an experimental laboratory room with standardfluorescent lighting to maintain a constant daylight regime during the 8 h experimental period.

An oviposition media, made from 500 g of 2-day old liver that was aged in a 35°C growth chamber to speed up decay to mimic 3–4-day decomposed liver (Johansen *et al*. 2014; Von Aesch *et al*. 2003), blended with 500 ml of boiling water with 10 g of agar (BD Diagnosis Bacto™ Agar Solidifying Agent), was placed onto aluminum foil on top of the aluminum plate and enclosed within the plexiglass arena. The aluminum foil allowed for easy cleanup of the plate at the end of each replicate. The media also had cracks on the surface cut with a knife to promote oviposition in or around openings, similar to wounds or natural orifices on a corpse (Ames and Turner 2003; Bourel *et al.* 2003). A handheld thermometer (ThermoPro TP-01A Instant Read Digital Meat Thermometer; ±0.9°F (±0.5°C)) and laser thermometer gun (MeasuPro Digital Infrared Thermometer IRT20) were used to measure the average temperature on the oviposition medium compared to the temperature of the gradient.

Intraspecific eggs were placed in sub-optimal temperatures that were either 5-10°C hotter or 5-10°C colder from the mean temperatures chosen from Chapter 2 (Table 3.1) and were acclimated to the temperature on the gradient for 30 mins before females were released in the arena. An individual female was released into the arena within the environmental box that was either high RH (75-85%), created by the use of a timed humidifier (Honeywell Ultrasonic Cool Mist Humidifier for Medium Sized Room, 1-Gallon), or low RH (25-35%), created from room temperature and a dehumidifier (Danby 18.9 L (40 Pint) Dehumidifier; Model: DDR040BFCWDB). Three experimental boxes and arenas were conducted at a time, for a total of 30 replicates of each humidity level for each of the 3 species. Intraspecific egg placement was either hotter (15 replicates) or cooler (15 replicates) for each of the 30 replicates per humidity level per species. New females were used for every replicate. Individual females were released into the arena at 10 AM, 3 h after the start of their daylight period, as females laid the most eggs when they were released at 10 AM compared to earlier and later release times based on a preliminary study. Females were left for 8 hours and removed 1 h prior to the end of their daylight period. While in the arena, observations were made hourly as this was the time period used in previous studies (Hans, 2016; Pacheco, 2015)

Females were left for 8 hours, during which observations were made hourly to observe their time spent along the gradient. Dataloggers were placed in the environmental boxes and recorded the temperature and RH of the environment every 30 minutes, while the thermocouples measured the temperature of the temperature gradient. Experimental trials were finished when females either oviposited on the gradient or did not oviposit during the 8-hour experiment.

At the end of the experimental trial, whether or not the female oviposited was recorded, and for females who oviposited, the time of the first oviposition event was noted. A temperature gun and thermometer were used to measure the temperature location of the intraspecific clutch. Egg mass photos with a 15 cm ruler for scale were taken using a Nikon D70 camera with an AF Micro-Nikkor 60 mm f/2.8D lens. The depth of the egg clutch was measured using a 15 cm ruler and the area was measured following ImageJ methods described by Rosati *et al*. (2015) to calculate volume of each egg mass. Estimates of the number of eggs in each egg mass was calculated using species-specific volumetric regression equations (L. sericata: $y = 0.34785 +$

0.99974x; P. regina: y = 0.24706 + 1.02851x; C. vicina: y = 0.3426 + 0.99603x) (Rosati *et al.* 2015; Hans *et al*. 2018).

3.2.3 Statistical Analyses

All analyses were performed in RStudio Open-Source Edition (Version 1.3.1073). All differences were compared between the absence of intraspecific eggs with intraspecific eggs placed in sub-optimal temperatures (hotter or colder) in either high or low RH. To compare the difference in the number of females who oviposited in the presence of intraspecific eggs versus none between each humidity level, a generalized linear mixed effect model (GLMER function, family=binomial) was used to calculate the difference of oviposition occurring for each female between each species. Since there were three replicate box arenas run at a time, box ID was used as a random effect in the model. An analysis of variance (ANOVA) was used to test if box ID influenced the odds of oviposition occurring, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function).

The difference in clutch size was analyzed using a linear mixed effect model (lmer function) to examine the effect of temperature choice, RH, and the interaction of RH and temperature choice, with species as a covariate and box ID as a random effect in the model. An ANOVA was used to determine whether box ID was significant or not for clutch size, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function). Least squared was used to compare clutch size in the presence verses absence of intraspecifics. The difference in first timing of oviposition was analyzed using a linear mixed effect model (lmer function) to examine the effect of temperature choice, RH and the interaction of RH and temperature choice, with species as a covariate and box ID as a random effect in the model. An ANOVA was used to determine whether box ID was significant or not for first timing of oviposition, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function). Least squared was used to compare first timing in the presence verses absence of intraspecifics. The difference in temperature choice was analyzed using a linear mixed effect model (lmer function) to examine the effect of species, RH, and the interaction species and RH, with box ID as a random effect in the model. An ANOVA was used to determine whether box ID was significant or not for temperature choice, along with Exact (Restricted) Likelihood Ratio Tests for Mixed and Additive Models (RLRSim package, exactLRT function). Least squared was used to compare first timing in the presence verses absence of intraspecifics. All significant results were separated with a post-hoc test using Tukey's test (emmeans function, adjust = "tukey").

3.3 Results

There was a difference in the overall number of *L. sericata* females ovipositing in the presence of intraspecific eggs versus absence, such that fewer *L. sericata* individuals oviposited when in the presence of intraspecific eggs (GLMER: $\chi_1 = 4.46$, $p = 0.03$). This was particularly true when different RH conditions were considered, where females were less likely to oviposit under low RH compared to high RH (GLMER: $\chi_1 = 9.92$, $p = 0.002$) (Figure 3.1). For *C. vicina*, when in the presence of intraspecific eggs overall did not affect the chance of females ovipositing (GLMER: $\chi_1 = 0.45$, $p = 0.5$), nor did specific RH conditions (GLMER: $\chi_1 = 1.29$, $p = 0.26$) (Figure 3.1). There was no overall difference in the number of *P. regina* females ovipositing in the presence of intraspecific eggs (GLMER: $\chi_1 = 0.06$, p = 0.81). However, there was a difference under different RH conditions, where no females oviposited in low RH when in the presence of intraspecific eggs (GLMER: $\chi_1 = 11.85$, p = 0.0006) (Figure 3.1). Likelihood of oviposition occurring did not vary by arena (i.e. AIC, BIC and loglikelihood did not differ by box ID).

For females who did oviposit, overall, there was no difference in clutch size when in the presence of intraspecific eggs versus absence of intraspecific eggs ($F_{1,188} = 2.46$, p=0.12), nor was there an effect of RH ($F_{1,189} = 0.12$, p=0.73), or interaction of RH and temperature choice ($F_{1,188} = 1.26$, p = 0.26). Temperature choice influenced clutch size ($F_{1,188} = 4.20$, p=0.04) (Figure 3.2 B), as well as species ($F_{1,186} = 5.76$, p=0.004), where *C. vicina* laid fewer eggs in the presence of intraspecific eggs compared to *L. sericata* (df = 192, p = 0.004) and *L. sericata* laid more eggs than *P. regina* (df = 192, p = 0.049) (Figure 3.2 A). *Calliphora vicina* and *P. regina* also did not differ in clutch size (df = 192, p = 0.54). Likelihood of oviposition occurring did not vary by arena (i.e. AIC, BIC and loglikelihood did not differ by box ID).

Overall, there was a difference in timing of oviposition when in the presence of intraspecific eggs versus absence, such that females oviposited faster when in the presence of intraspecific eggs ($F_{1,189} = 5.28$, p = 0.023). *Lucilia sericata* and *P. regina* oviposited 1 hour faster in high RH, while C. vicina oviposited 3 hours faster in high RH. In low RH, *L. sericata* and *C. vicina* oviposited slower by 1 hour. First timing of oviposition was not affected RH ($F_{1,189} = 1.12$, p = 0.29) or temperature choice ($F_{1,189} = 0.24$, p = 0.62). Species at the time of oviposition did covary with first timing ($F_{2,189} = 3.48$, p = 0.03), where *P. regina* oviposited faster compared to *C. vicina* (df = 189, p= 0.02) and *L. sericata* (df = 189, p = 0.030) (Figure

3.3). Timing of oviposition did not vary by arena (i.e. AIC, BIC and loglikelihood did not differ by box ID).

For temperature choice on the gradient, overall, there was a difference when in the presence of intraspecific eggs versus absence ($F_{1,189}$ = 4.61, p = 0.03), due to an interaction of relative humidity and species that affected temperature choice ($F_{2,185}$ = 8.96, p = 0.0002). Eggs were only laid under high RH conditions by *P. regina* females and oviposition location temperature choice depended on the placement of intraspecific eggs such that females were choosing hotter temperatures (df = 185, p = 0.0002) compared to their preferred temperature choice in the absence of intraspecific eggs (Chapter 2) (Figure 3.4). For *L. sericata*, there was no difference in temperature choice, regardless of RH (df = 196, p = 0.9923), as well as for C. vicina (df = 195, p = 0.56). The random effect of box ID also influenced temperature choice (LRT = 4.77, p = 0.003).

3.4 Discussion

When multiple individuals of the same species exploiting the same limited resource, they interact and influence each other by emitting semiochemicals (Brodie *et al.* 2014; Giffen-Lemieux *et al.* 2020; Jiang *et al.* 2002; Judd and Borden 1992; Lima and von Zuben 2016). As a result, this affects the community assembly within an ecological community, such as a necrobiome (community of species associated with decomposing remains (e.g., animal carrion or human corpses) (Benbow *et al*. 2013; VanLaerhoven 2019).

When in the presence of intraspecific eggs, there was lower overall number of females who oviposited for *L. sericata* in low RH conditions in comparison to no eggs present (Chapter

2). This suggests that *L. sericata* might examine the resource as unfavourable and reject ovipositing to avoid intraspecific eggs. It is also possible that other factors mediate *L. sericata* to oviposit. *Lucilia sericata* heavily relies on cues, predominately olfactory cues, along with temperature for oviposition (Brodie *et al*. 2014; Chaudhury *et al*. 2015; Hans *et al*. 2018). Compound mixtures that include indole, dimethyl disulfide, dimethyl trisulfide, or ammonia-rich compounds elicits an oviposition response, whereas compound mixtures that include sulfur-rich compounds elicits attraction and landing responses of *L. sericata* (Ashworth and Wall 1994; Hobson 1936; Chaudhury *et al*. 2015). Overall, the oviposition behaviours for *L. sericata* did not vary when in the presence of intraspecific eggs. For clutch size, *L. sericata* did not oviposit more or less eggs in comparison to no eggs present (Chapter 2), regardless of RH condition. For timing to first oviposition, *L. sericata* again did not deviate, regardless of RH condition. Lastly, for temperature choice, *L. sericata* did not digress from their preferred average temperature chosen from Chapter 2. The decisions made by *L. sericata* demonstrate that females did not alter their behaviours since they appeared to not be influenced by the eggs present on the gradient, regardless of the temperature placement.

For *C. vicina* when in the presence of intraspecific eggs, there was no difference in the number of females ovipositing compared to absence of intraspecific eggs, regardless of RH. As well, there was no difference in timing to oviposition at either RH conditions when in the presence intraspecific eggs, and females did not deviate from their preferred temperatures. There was a difference in clutch size, where females laid fewer eggs when in the presence of intraspecific eggs. This suggests that there is a trade-off between clutch size and preference to aggregate with intraspecifics. It would be important to measure the impact of these choices on offspring size and survival to further explore this potential trade-off.

For *P regina* in low RH, surprisingly, no females oviposited, regardless of intraspecific egg temperature. This could be due to the unfavourable environment, where intraspecific eggs placed in low RH loss water during the duration of each trial (Evans 1934), which could shift resource preferences to avoid intraspecific eggs or reject the resource to oviposit. In high RH, although the same number of females chose to oviposit, those females oviposited more eggs faster in the presence of intraspecific eggs but laid the fewest eggs overall between species. Females were also choosing to oviposit towards hotter temperatures. This suggests that the presence of intraspecific eggs, together with suitable RH, may provide an indication of a suitable oviposition resource for *P. regina* but that individual females do not choose to aggregate their eggs necessarily at the same location or temperature as intraspecifics.

Additional research for oviposition behaviour includes the presence of heterospecific interactions to further understand the coexistence of these species. There are a lot of compounds within different species eggs, described by Giffen-Lemieux *et al.* (2020), that act as chemical cues to promote oviposition for both con- and heterospecific interactions. If volatiles play a bigger role in combination with favourable abiotic factors (Fiene *et al*. 2014), different strategies to promote olfactory cues within the gradient should be considered. Different strategies to increase olfactory cues could allow adults to feed on the oviposition medium before each trial (Uriel *et al*. 2018), or aging eggs, as Brundage *et al.* (2017), found that the age of eggs influenced the attraction of both *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae) *and Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae), specifically eggs aged between 3-9 hours. Attraction of *Ch. rufifacies* may also be impacted by the later instar predation behaviour on other blow fly larvae so the presence of older eggs lets females know another food resource is available for their offspring. As this predation behaviour is restricted to the *Chrysomya* genus, this would not necessarily explain the effect of egg age on *Co. macellaria*.

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Table 3.1. Temperature location for placement of intraspecific eggs in suboptimal temperatures (5-10°C warmer or cooler) away from mean temperature chosen (Chapter 2) by each species within each RH treatment.

Low Relative Humidity (25-35%)			
Species	**Cold Intraspecific Egg Placement Temperatures**	**Mean Temperature Chosen (Chapter 2)**	**Hot Intraspecific Egg Placement Temperatures**
Lucilia sericata	18-22°C	28°C	33-38°C
Calliphora vicina	8-13°C	18°C	23-28°C
Phormia regina	20-25°C	30°C	35-40°C
High Relative Humidity (75-85%)			
Species	**Cold Intraspecific Egg Placement Temperatures**	**Mean Temperature Chosen (Chapter 2)**	**Hot Intraspecific Egg Placement Temperatures**
Lucilia sericata	18-22°C	28°C	33-38°C
Calliphora vicina	9-14°C	19°C	24-29°C
Phormia regina	11-16°C	21°C	26-31°C

Table 3.2. Summary table of the results for the influence of RH and the presence of intraspecific eggs on the likelihood of oviposition occurring and on the oviposition behaviour for each species.

Likelihood of Oviposition Occurring			
	Species		
	Lucilia sericata	*Calliphora vicina*	*Phormia regina*
Yes	• Low RH: fewer females oviposited when in the presence of intraspecific eggs	/	• Low RH: no females oviposited when in the presence of intraspecific eggs
No	• High RH: no difference	• No difference for both RH conditions	• High RH: no difference
Oviposition Behaviours			
Clutch size	• No difference between RH	• Smaller clutches in presence intra eggs, regardless of RH	• No eggs laid in low RH
First Timing of Oviposition	• No difference between RH	• No difference between RH	• Females oviposited faster
Temperature Preference	• No change in temperature preferences	• No change in temperature preferences	• Chose hotter temperatures in the presence of intra eggs

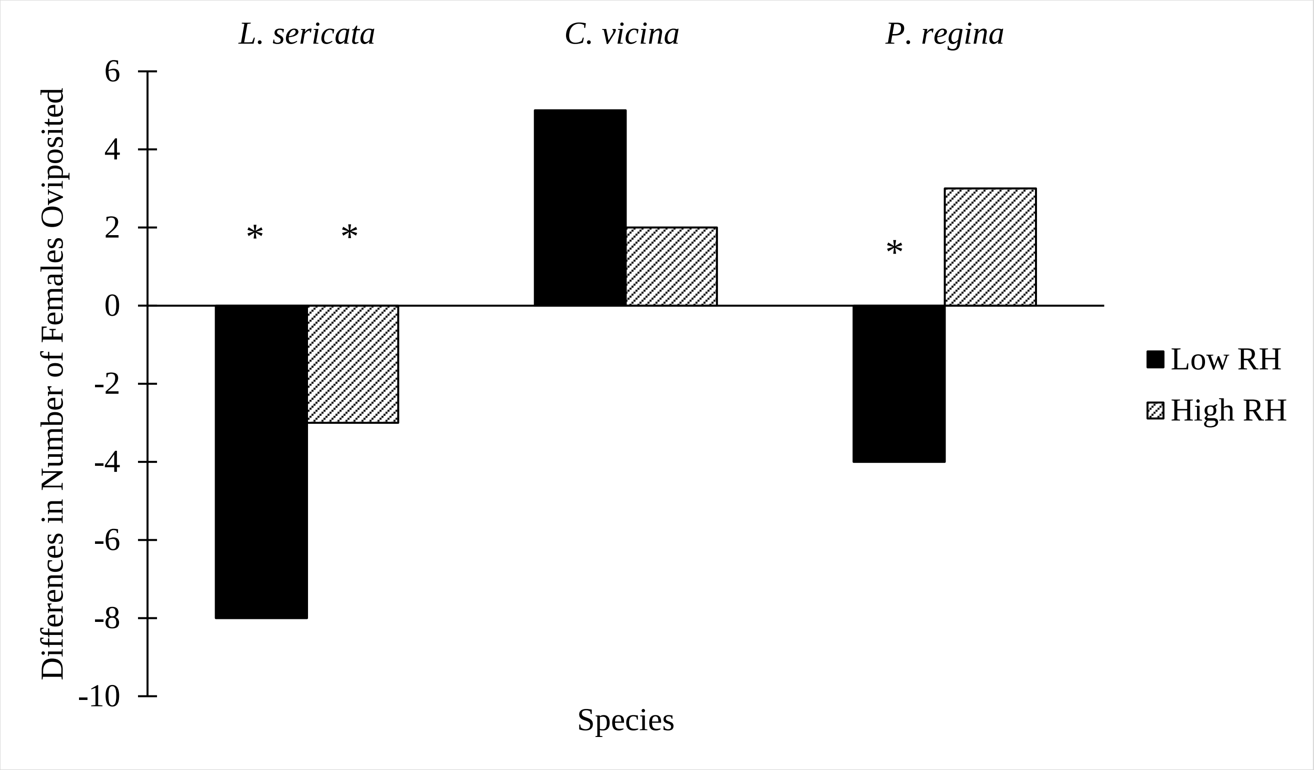

Figure 3.1. Difference in the number of females of *Calliphora vicina* (n = 27), *Lucilia sericata* (n = 19), and *Phormia regina* (n = 12) (Diptera: Calliphoridae) that oviposited in the presence of intraspecific eggs compared to a baseline of no intraspecific eggs between high humidity (75-85%) verses low humidity (25-35%). Negative differences indicate fewer females ovipositing in the presence of intraspecific eggs. The proportion in the number of females that oviposited are different from the baseline for *L. sericata* in both RH (p= 0.002) and *P. regina* in low RH (p=0.0006), indicated with asterisks.

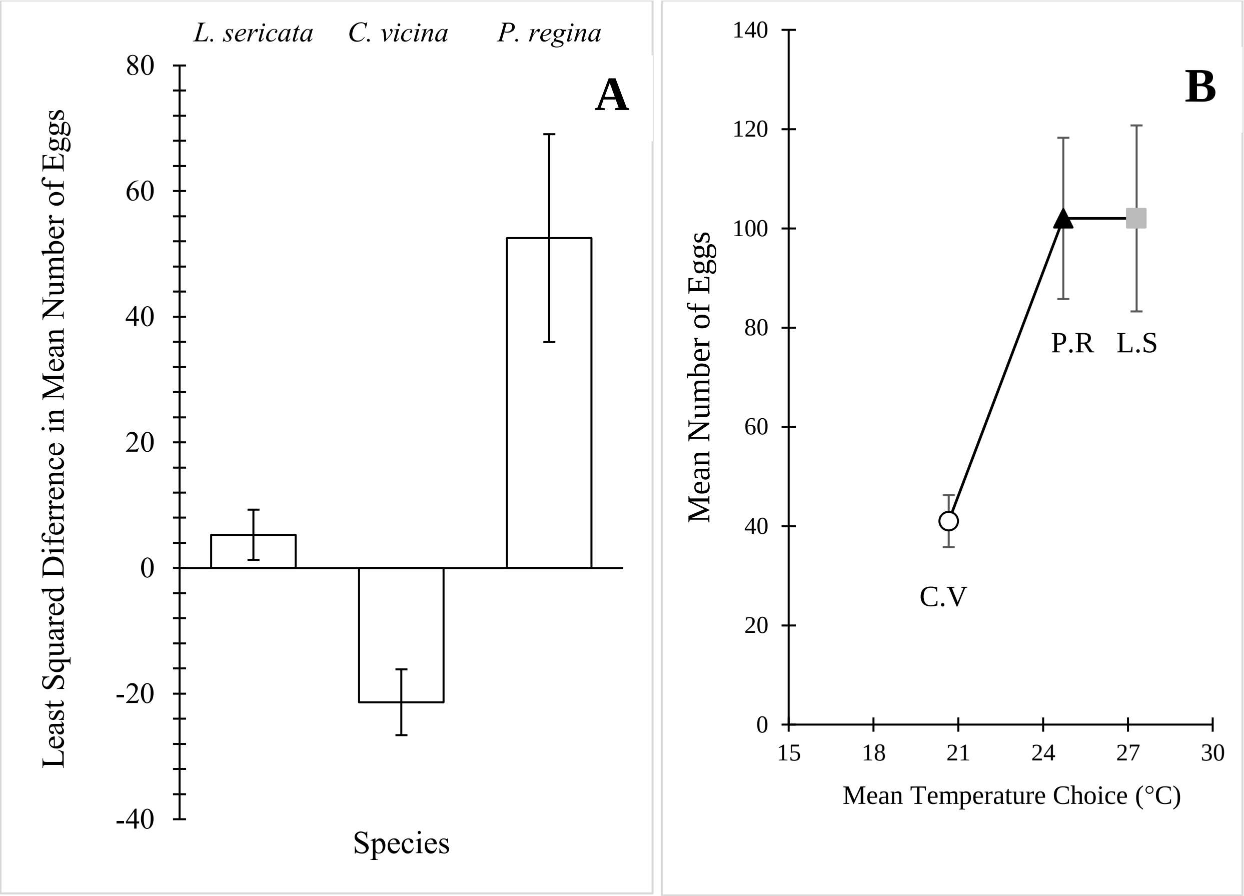

Figure 3.2. Difference in mean (± S.E.) number of eggs oviposited by *Calliphora vicina* (n = 27), *Lucilia sericata* (n = 19), and *Phormia regina* (n = 12) (Diptera: Calliphoridae) in the presence of intraspecific eggs compared to a baseline of no intraspecific eggs, pooled across humidity treatments. Intraspecific egg placement was either hotter or cooler than mean temperature choice in the absence of intraspecific eggs. Negative differences indicate fewer eggs oviposited in the presence of intraspecific eggs. Temperature choice also affected clutch size ($F_{1,188} = 4.20$, p=0.04) (B).

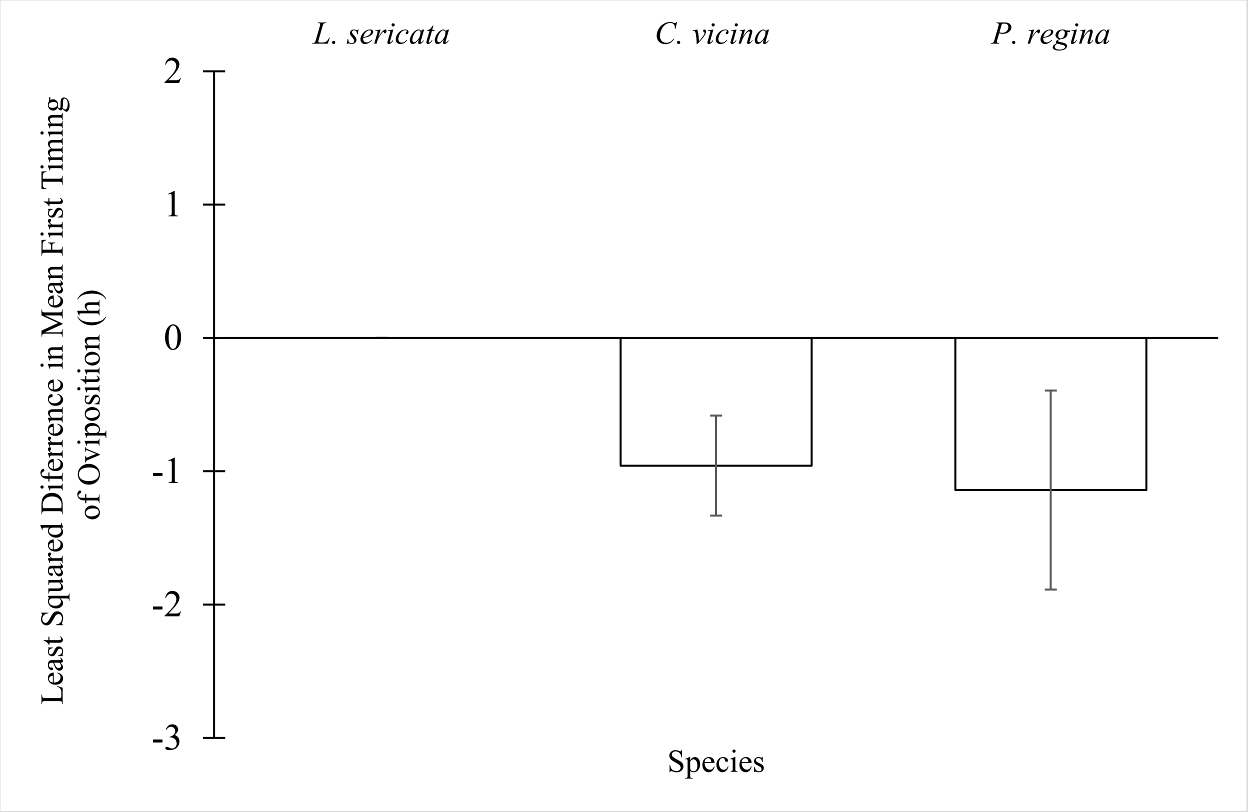

Figure 3.3. Difference in mean (± S.E.) number of eggs oviposited by *Calliphora vicina* (n = 27), *Lucilia sericata* (n = 19), and *Phormia regina* (n = 12) (Diptera: Calliphoridae) in the presence of intraspecific eggs compared to a baseline of no intraspecific eggs, pooled across humidity treatments. Intraspecific egg placement was either hotter or cooler than mean temperature choice in the absence of intraspecific eggs. Negative differences indicate faster oviposition in the presence of intraspecific eggs.

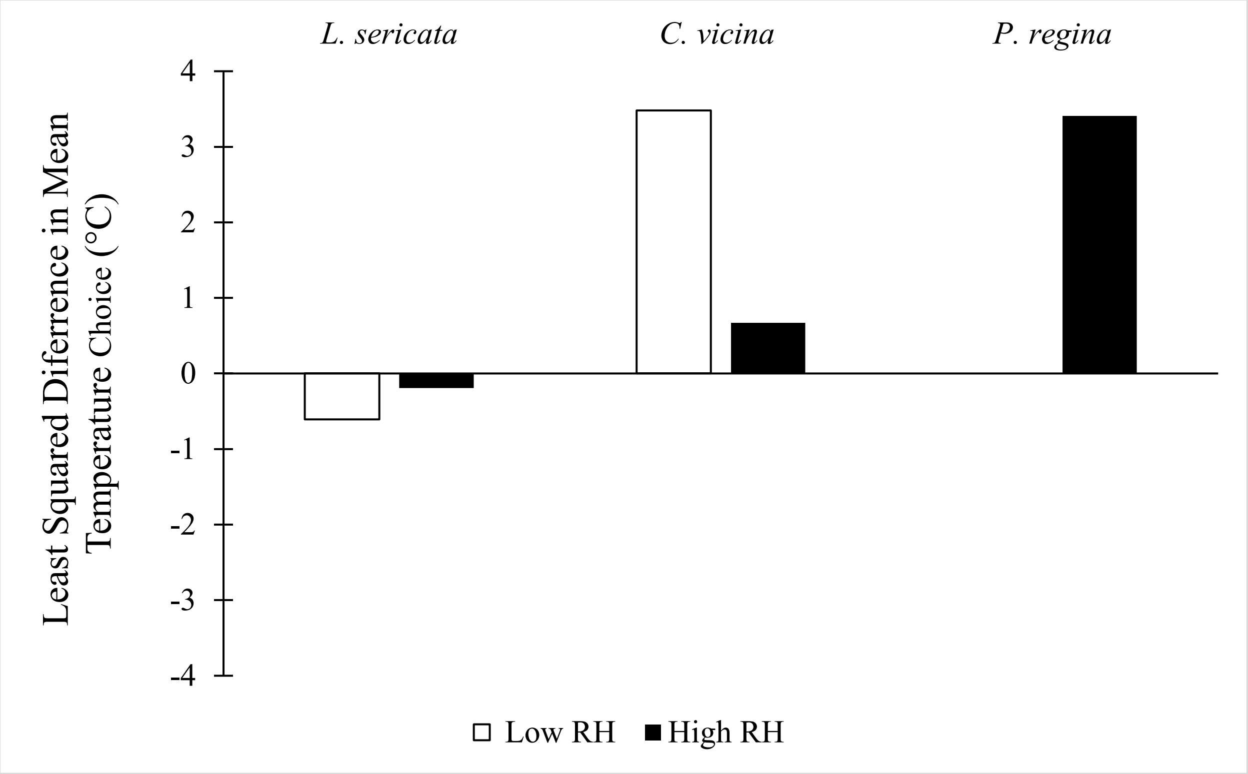

Figure 3.4. Difference in mean (± S.E.) temperature choice for oviposition location by *Calliphora vicina* (n = 27), *Lucilia sericata* (n = 19), and *Phormia regina* (n = 12) (Diptera: Calliphoridae) in the presence of intraspecific eggs compared to the baseline of no intraspecific eggs within high (75-85%) and low (25-35%) relative humidity treatments. Negative differences indicate colder temperatures chosen for oviposition in the presence of intraspecific eggs.

CHAPTER 4

INFLUENCE OF HUMIDITY AND INTRASPECIFIC INTERACTIONS IN THE OVIPOSITION BEHAVIOUR OF BLOW FLIES; IMPLICATIONS WITHIN FORENSIC ENTOMOLOGY AND ECOLOGICAL PERSPECTIVES

4.1 Forensic entomology implications

Blow fly species are not only important for medical and veterinary purposes as they are able to carry pathogens and cause diseases to humans and animals (Galindo 2016; Horenstein and Salvo 2012), they also play a critical role as indicators in criminal investigations as insect evidence. Forensic entomology is a branch of forensic science, where the use of insects and their life cycle and behaviour is used to interpret evidence in a legal context relating to both humans and wildlife (Gennard and Szpila 2012). There are three main applications used within forensic entomology: urban entomology (interactions between insects and human environments), stored products entomology (insects found in products), and medico-legal entomology (criminal proceedings involving animal cruelty or humans in cases of violent crime or unexpected death) (Brundage and Byrd 2016; Hall *et al*. 2012). Focusing on the medico-legal entomology application, the use of insects is significant in estimating the time since death. After 72 hours, when medical information is no longer accurate (Anderson 2004), either the succession of insects that are present or absent on the body, or life cycle stage that are present on the body is used as evidence to provide an estimated minimum post-mortem interval (PMI_{min}) (Gennard and Szpila 2012). They are collected as either preserved, to identity larvae stage or species found on the body or live to rear under laboratory conditions. When rearing larvae found on the scene, temperatures taken from the body, maggot masses, ambient temperature in the environment, soil and humidity are taken into consideration (Hall *et al*. 2012). Crime scene temperature can also be obtained from local weather stations.

There are caveats that must be made to estimate time of death for any one case using insect evidence, such as blow flies arriving immediately on a body, or that the period of insect activity corresponds to the PMI_{min} (Amendt *et al*. 2007; Hans *et al.* 2018). Since blow flies tend to be the first insects to arrive at the scene, their physiology and behaviour can be used to estimate the pre-colonization interval (pre-CI) (time period of insect activity where they initially detect carrion until colonization, or oviposition), which is part of the estimate of how long an individual has been deceased, i.e., the full estimate post-mortem interval (Tomberlin *et al*. 2011). The behaviour and physiology of these adults responds to cues, whether it's environmental, or semiochemicals from the resource (Charabidze and Hedouin 2019; Mohr and Tomberlin 2015). Environmental factors have a stronger influence in colonization because unfavourable environments outside a blow fly's optimal thermal range prevents fly activity as it creates unfavourable ranges for oviposition (Campobasso *et al*. 2001; George *et al*. 2013). Temperature is one of the most significant factors in colonization, as it has shown to affect blow fly colonization (George *et al*. 2013; Matuszewski *et al*. 2014; Mohr and Tomberlin 2014; Ody *et al*. 2017; Wall *et al*. 1992) . George *et al.* (2013), determined that ambient temperature was a positive predictor variable, where an increase in temperature increased the probability for colonization. In contrast, Matuszewski *et al.* (2014), observed an exponential decrease relationship in adult preappearance interval (interval from the moment of death until the arrival of first adult of a given species) and oviposition preappearance interval (interval from the moment of death until the appearance of the first eggs of a given species) as temperature increases.

This study was conducted to determine if relative humidity (RH) is also an abiotic factor that influences colonization. We showed that RH does influence the probability for oviposition to occur, where the high RH range of 75-85% RH increases the probability in the number of females to oviposit for *Lucilia sericata* (Meigen), *Phormia regina* (Meigen) *and Calliphora vicina* Robineau-Desvoidy. This contradicts the findings that higher RH decreases colonization (George *et al*. 2013; Pitt and Wall 2004), although there are experimental differences to take into consideration, as well as individual variation. Higher RH resulted in more oviposition by females of each species compared with low RH. This could be that the oviposition medium in this study remained moistened, which increased the attractiveness for oviposition to occur (Campobasso *et al*. 2001). Moisture content in a food source has shown to affect development, where a decrease in moisture content prevents egg hatching and reduces larval devleopment (Bauer *et al*. 2020, Campobasso *et al*. 2001). For the three species tested here, it is beneficial for females to oviposit their eggs where the environment is favourable to promote a higher succcess of egg hatching and larval development. Although oviposition did occur within the low RH range of 25-35%, this may cause the eggs to slow or stop embryogenesis, as well as diapause during larval development in conditions of RH below 40%, as well as cold temperatures of 6-8°C (Campobasso *et al*. 2001; Tauber *et al*. 1998). Additional experiments at different humidity levels in between 20 and 80% can create a species-specific performance curve for humidity and fitness to compare limits varied by each species.

Additionally, RH in this research has shown that it changes the thermal preferences of these species. For *L. sericata*, regardless of RH conditions and presence of intraspecific eggs, females chose to oviposit at a mean of 28°C. Around this temperature, *L. sericata* oviposits the highest number of eggs (Hans *et al*. 2018; Ody *et al*. 2017). With *C. vicina*, females in high RH chose to oviposit at a mean of 19°C, whereas females in low RH chose to oviposit at a mean of 18°C. Difference in temperature choice only differed by 1°C between both RH conditions. When in the presence of intraspecific eggs, *C. vicina* females either oviposited at the same, or warmer temperatures than in the absence of intraspecific eggs. For *P. regina*, temperature choice depended on RH. In low RH females chose to oviposit at warmer temperatures and at cooler temperatures in high RH. The presence of intraspecific eggs placed in cooler than preferred locations under high RH resulted in females choosing warmer locations instead of aggregating eggs with intraspecific eggs. If females are choosing to lay eggs at different temperatures, this could affect hatching time, thus affecting the total development time of these species. Temperature and development time are the most critical factors for calculating PMI as they have implications for calculating PMI using insect development. Hans *et al.* (2016) observed the slowest eclostion time for *L. sericata*, *P. regina* and *C. vicina* at 30% RH, and as RH increased, the fastest and most successful eclosion success was at 80% RH at 25°C. Additionally, larvae developing at temperatures hotter than their species-specific optimal temperature can develop faster, but emerge as smaller bodied adults (Atkinson 1994; Hans and Vanlaerhoven 2021; Kingsolver and Huey 2008; Rivers *et al.* 2011).

Relative humidity within entomological protocol plays a role in understanding what abiotic conditions adult blow flies are able to arrive at a carcass. This study demonstrated that RH influences blow fly activity, where the likelihood of whether females will oviposit increases as RH increases. It is important to recognize that in low RH conditions, females may chose to not lay eggs, so the assumption of lack of oviposition as part of the entomological time of death timeline in cases where the body is in low RH conditions needs to be carefully considered. Clearly, immediate (i.e) within a few hours of death, is not a resonable assumption under these conditions. More research is needed testing individual blow fly species.

Relative humidity could also be a cue used to assess a resource before colonization during the acceptance phase, which can add information to further understand the pre-CI, and the switch between the pre-CI and the post colonization interval (Mohr and Tomberlin 2014; Tomberlin *et al.* 2011). Additionally, this can be useful in predicting the distribution of these species based on climatic factors and habitat types as their thermal ranges allows different species to be abundant between seasons and geographical areas (Azevedo and Krüger 2013; Klong-klaew *et al*. 2018; Zabala *et al.* 2014).

4.2 Behavioural ecology

Habitat selection during colonization shapes abundance and composition of taxa of community assembly (Kraus and Vonesh 2010; VanLaerhoven 2019). Community assembly is a process where species from a regional pool colonizes and interacts to form a local community with two main process affecting community composition: environmental filter (abitotic factors creating constraints) and limiting similarity (biotic forces such as competition keeping coexisting species from being too similar (HilleRisLambers *et al*. 2012; Pavione *et al*. 2010). Selecting a habitat is important as it help protects against predators and unfavourable environments, as well as increases reproductive success (Sempo *et al*. 2013). One indication of habitat suitability is the presence of intraspecifics, as this provides an auditory, tactile or chemical cues that attracts females (Buxton *et al.* 2020; Raitanen *et al.* 2013). Various studies have looked into chemical cues, or attraction to bacteria, from either eggs or gravid females as a signal that induces oviposition (Brodie *et al.* 2014; Brodie *et al*. 2015; Lam *et al.* 2007; Wertheim 2005; Wertheim *et al*. 2005; Tomberlin *et al*. 2016). In this research, the presence of intraspecific eggs in each RH treatment illicited different behaviours that was species-specific. *Phormia regina* in high RH oviposited more eggs but egg placement was not located closer to intraspecific eggs. In contrast, *P. regina* in low RH had no females ovipositing. Females typically show a strong increase in response when semiochemical cues from either the eggs or associated with feeding flies are accompanied with a coattractant (Brodie *et al.* 2015; Wertheim *et al*. 2005; Wall and Fisher 2001). In the case of *P. regina* in low RH, the unfavourable environment indicated that the resource was not suitable for oviposition, which may have caused females to reject to oviposit. For *P. regina*, the presence of intraspecific, along with high RH act as cues to mediate the decision for females to oviposit as these factors together indicate a suitable oviposition resource. *Lucilia sericata* in both RH conditions made choices independantly when in the presence of intraspecific eggs. In low RH, *C. vicina* experienced a trade-off between optimal temperatures and preference to aggregate when intraspecific eggs were placed in suboptimally warmer temperatures as females ovipoisted faster and closer towards intraspecific eggs in suboptimally warmer temperatures. It is apparent from this research that individual species vary in their assessment of suitability based on decisions of intraspecific females' willingness to lay eggs. This, in turn, effects the aggregation of intraspecifics within a local resource patch, and the distribution of a species across a habitat.

Additionally, environmental factors can have an indirect effect on biological diversity since a large community exists on a carrion, there could be an affect on the overall species composition (Hoermann *et al*. 2018). Temperature is a predominate factor for colonization to occur that influences the spatial distribution, with RH next as another main factor (George *et al.*

2013; Ody *et al.* 2017; Zarkos 2019). Zabala *et al.* (2014) analyzed multiple blow fly species seasonal distribution in Western Europe. There was a strong relationship between species abundance and environmental conditions, such as temperature and RH, where high abundance of *Lucilia* spp. and *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) were apparent only in the summer, whereas *Calliphora* spp. were abundant throughout the year.

For different species, their thermal tolerance ranges allow them to be active within specific seasons geographically. For thermal generalists, individuals will be active over a broader range of temperatures and are more likely to find a suitable habitat after dispersal, while being less sensitive to environmental changes (Gilchrist 1995; Jacob *et al*. 2018; Seebacher *et al*. 2015). Compared to thermal specialists, individuals are more active within a specific narrow range of temperatures and would be more sensitive to environmental changes outside an individual's range (Gilchrist 1995; Jacob *et al*. 2018; Seebacher *et al*. 2015). For the species tested here, each species has different thermal tolerance range that drives their seasonal and temporal distribution in Windsor-Essex. In my study, *L. sericata* appeared to be more of a thermal generalist towards hotter temperatures, as females oviposited a wide temperature range of 21-38°C in low RH and 21-41°C. This correlates with their seasonal distribution and high abundance mainly in the summer (Babcock *et al*. 2020; Langer *et al*. 2019; Schroeder *et al.* 2003). For *C. vicina*, females oviposited within a narrow temperature range of 15-22°C for low RH and 17-21°C in high RH, correlating with their seasonal distribution prominently in spring and fall, and winter depending on geographical location (Babcock *et al*. 2020; Langer *et al*. 2019; Schroeder *et al.* 2003; Weidner *et al.* 2016). When *P. regina* are in low RH, females have a wider temperature range of 19-32°C, while in high RH, females have a narrow temperature range of 25-33°C. This shift in thermal ranges due to RH could play a role in habitat selection and abundance for *P. regina* within different seasons. This species can be found throughout each season, and winter depending on geographical location (Babcock *et al*. 2020; Brundage *et al*. 2011; Langer *et al*. 2019; Weidner *et al.* 2016). All three species have temperatures that overlap with one another that allows them to be present on a resource together but may utilize different areas. For example, Hans *et al.* (2018) observed that temperature influenced site selection on a pig carcass for *L. sericata*, *P. regina* and *C. vicina*, such that all three species chose different sites at different temperatures. Each site on the carcass may provide different microclimates that each species prefers to oviposit within.

Species thermal preferences can be used to predict distribution within difference microhabitats. For example, differences in temperature ranges can infer habitat preferences in either sunny or shady habitats, several research have found that species abundance and diversity is greater when carrion are sun-exposed compared to shaded depending on the season (e Castro *et al.* 2011; Hobischak *et al*. 2006; Sharanowski *et al*. 2008). This could be due to exposure of sun and increase in ambient temperatures increasing the rate of decomposition of a carrion, releasing different volatile compositions in each stage to attract a diversity of species. This can also infer habitat preference within urban or rural habitats. Abundance and diversity can vary as urban areas could have less solar radiation, higher air temperatures, lower wind speed, lower RH, more cloud and more precipitation (Hwang and Turner 2009), compared to rural areas, which can be more open fields with more vegetation can have direct sunlight (Bugajski and Stoller 2017). Babcock *et al*. (2020) observed high abundance of *P. regina* within rural habitats, and *L. sericata* within urban habitats of Mid-Michigan. This is similar with Langer *et al*. (2019), where high abundance of *P. regina* and *C. vicina* were caught in rural habitats, but *L. sericata* was also abundant within rural habitats across six Canadian provinces. Habitat choice should promote the selection of optimal habitats and in turn reduce movements to suboptimal habitats. Taking into consideration of various species thermal preferences can be used to predict their distribution seasonally and geographically and can be used to create a predictive distribution map to assess the biodiversity of blow flies within a particular season and geographical area. This may also drive coexistence and reduce competition, where they are partitioned on different resources over space and time due to environmental preferences.

4.3 Future Direction

This research provided each species an opportunity to respond to each humidity level by choosing where they prefer to oviposit and how they respond when given the choice. By allowing females from each species to choose their preferred temperature, the temperatures chosen can be used to compare optimal temperatures found in various studies regarding optimal development or oviposition temperatures. Validation studies to compare preferred and optimal temperatures for each species can be performed by setting up bait colonization experiments for each preferred temperature to observe the likelihood for oviposition to occur.

Relative humidity, along with the presence of intraspecific eggs, influenced the probability of oviposition to occur, as well as temperature selection. Specifically, high RH, along with intraspecific eggs, may act as cues for females to determine when to oviposit. These factors indicate a suitable oviposition resource, especially for *P. regina*. By including other species within the geographical area to determine the probability of oviposition to occur, the information can be used to develop models to predict species occurrence on a resource. Other incorporations would be to include heterospecific eggs in the arena to compare oviposition decisions between species to further understand the interactions when adults arrive on a resource. Would females aggregate their eggs with other species eggs or avoid ovipositing near them?

Any effects on the carrion or on the influence of the adults can change the behaviours within and between blow fly dynamics. Understanding these physiological constraints provides additional information on how these study species behave under unfavorable environments to understand their thermal and desiccation stress before and after arriving on a resource, and what adaptation strategies are needed to prevent these stresses.

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