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Using an ecological framework to resolve issues in forensic entomology: exploring temperature mediation of species interactions within blow fly (Diptera: Calliphoridae) communities

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By

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ABSTRACT

The blow flies *Lucilia sericata* Meigen, *Phormia regina* Meigen and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) are important decomposers that specifically colonize carrion. Adult flies must make oviposition decisions that impact the survival of their offspring and may be influenced by abiotic and biotic conditions. Although a great deal of research has been conducted regarding their development under different environmental conditions, the influence of species interactions has been scarcely investigated. The objective of my dissertation was to examine the effects of temperature, relative humidity and species interactions on the oviposition behaviour and development of these blow flies.

To accomplish this, I manipulated the temperatures that adult blow fly populations experienced and measured how this affected decisions when ovipositing with conspecifics (Chapter 2) and after heterospecifics (Chapter 3) in the laboratory. These observations were then validated in semi-natural conditions in the field (Chapter 5). The development and eclosion success of blow fly eggs was measured over a range of relative humidities (Chapter 4) and larval development was recorded over multiple temperatures in the presence of conspecifics and heterospecifics (Chapter 6). I predicted that oviposition decisions exhibit plasticity with varying temperature, but shifts in oviposition would be influenced to a greater extent by heterospecifics to either avoid competition or benefit from facilitation. I predicted that species interactions would either facilitate faster development, greater survival and larger adults or lowered survival and smaller adults due to competition. The results indicate that mediation of oviposition decisions by temperature are species-specific, but for *P. regina*, 25°C may be a switching point between facilitation and competition outcomes with heterospecifics. Differential effects of relative humidity on egg eclosion at different temperatures may provide a partial mechanism, as well as developmental impacts on adult size in the presence of heterospecifics.

DEDICATION

To my mother, Catherine

and my sisters,

Chelsea and Angelina.

Thank you for all of your support.

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CHAPTER 1

BLOW FLIES: ENVIRONMENTAL INFLUENCES, SPECIES INTERACTIONS AND REAL WORLD APPLICATIONS

1.1 Summary

The behaviour and development of insects is often influenced by abiotic and biotic conditions, which include temperature, relative humidity and species interactions. The outcome of interactions among species can be mediated by abiotic conditions. The overall objective of this research was to examine the oviposition behaviour of blow flies to investigate how such behaviour could change over a range of temperatures or due to species interactions. These behaviours were then examined under natural conditions to validate the observations made under controlled lab settings. Oviposition decisions made by insects that colonize carrion, such as blow flies, has implications for their offspring's survival and performance, and therefore outcomes in the dynamics of these ephemeral resource-based communities. Additionally, where and when blow flies lay their eggs on a dead body starts a biological clock that is utilized in forensic entomology to estimate how long someone has been dead. Thus, the influences of temperature and species interactions were examined for blow fly development with the goal of exploring potential community outcomes and providing further developmental data useful in estimating time since death.

1.2 Finding a Suitable Oviposition Resource

Carrion represents an ephemeral resource on which a dynamic community of insects arrives, colonizes and develops. Despite the large number of observational studies of species associated with decomposition stages in carrion ecology (Benbow *et al.* 2015a) or the forensic application of these studies (Reed 1958; Payne 1965; Early and Goff 1996; Watson and Carlton 2003, 2005), few ecological concepts and processes have been explicitly tested or incorporated into forensic investigations until recently (VanLaerhoven 2010; Tomberlin *et al*. 2011). Tomberlin *et al.* (2011) proposed a stage-based framework

for describing the behavioural and physiological responses of insects over time as they find and utilize a decomposing resource. The first stage, or the precolonization interval, involves the detection and location of a suitable resource (Tomberlin *et al*. 2011) using external or internal stimuli. External, or allothetic stimuli are often based on abiotic and biotic environmental conditions (Benbow *et al*. 2015b). Abiotic cues may include temperature, relative humidity, and photoperiod whereas biotic cues, either visual or olfactory, may indicate the presence and density of other species that may affect oviposition choice. Idiothetic or internal stimuli are biological and are based on the reproductive status of an insect, such as the egg load or stage of ovarian development (Visser 1988). These behavioural responses can change as the physiological state of the organism changes (Wall and Warnes 1994) indicating a response to both internal and external stimuli. For example, previous studies have observed that in *Lucilia sericata* Meigen (Diptera: Calliphoridae), odours emitted from liver were more attractive to gravid females compared to nongravid females and males (Brodie *et al*. 2014; Wall and Warnes 1994). In parasitoids, females that are experienced in oviposition and have higher egg loads are more receptive to chemical cues than naïve females that have not oviposited (Vinson 1998).

Species that can detect and locate carrion for the purpose of colonization may be influenced by the release of volatile organic compounds (VOC) and changes in the resident microbial community (Ma *et al.* 2012; Tomberlin *et al*. 2012). Blow flies (Diptera: Calliphoridae) probe carrion with their ovipositor to determine an oviposition site which may involve the use of both visual and olfactory cues (Brodie *et al*. 2014). During decomposition, various compounds are released including those containing sulfur, nitrogen, alcohols and acids (Morris *et al*. 1998; Frederickx *et al*. 2012; Paczkowski *et al.* 2012). For insects, the detection of odours is dictated by the quantity of olfactory receptor neurons which are responsible for propagating electrical impulses when stimulated by VOC's (Hansson 2002). A human body can produce over 400 chemical odours during decomposition and these odours change during the decomposition process (Archer and Elgar 2003; Vass *et al*. 2008). These changes in odour profiles may signal different species at different stages of decomposition. For example, female beetles of *Nicrophorus vespilloides* Herbst (Coleoptera: Silphidae) respond only to odours released during

advanced decomposition (Rozen *et al*. 2008) and it could therefore be the attractant quality that regulates the arrival time of this species.

Blow flies may determine the suitability of an oviposition site using close range cues, including chemotactile contact, detected by receptors present on their legs and mouthparts (Chapman 2003). Females must accept the carrion as a suitable resource for oviposition and these choices are critical to the survival of the offspring and the fitness of the ovipositing female (Papaj 2000). The presence of ovipositing females can make the resource visually more attractive to other flies (Barton Browne *et al*. 1969). Semiochemicals released during aggregation, the mass egg laying of multiple females in a single location, may play a role in the acceptance and collective oviposition behaviour of blow flies (Brodie *et al*. 2015). Females can respond to semiochemicals from both conspecifics and heterospecifics (Brodie *et al*. 2015). Flies and larvae release enzymes and microorganisms in their salivary secretions that can facilitate liquefaction of the food source and, as a result, may release volatiles that can attract other fly species (Telford *et al*. 2012; Zheng *et al.* 2013). Brodie *et al*. (2015) found that gravid female blow flies are more attracted to liver that has heterospecifics compared to liver on its own.

Behaviour and acceptance of a resource for egg laying may also depend on a female's perception of fitness consequences for offspring. Determining an optimal site for oviposition is crucial for insects, since the reproductive success of the female depends on the survival of their offspring (Thornhill 1976; Jaenike 1978). Insects such as blow flies do not invest in parental care and their larvae have limited dispersal capabilities. For these organisms, offspring survival is dependent on the resource upon which they are deposited, and this ultimately determines the fitness of the female. Optimal oviposition theory, as outlined by Jaenike (1978) dictates that insects should select oviposition sizes that maximize the development of their offspring. This theory can be interpreted as the preference-performance hypothesis, as female preference for certain oviposition sites should optimize the performance of their offspring (Thompson 1988). Optimal oviposition theory predictions have held true for many insects with a range of life histories, including various dipteran families such as Tephritidae (Joachim-Bravo *et al*. 2001), Drosophilidae (Krebs *et al*. 1992) and Culicidae (Ellis 2008). Ellis (2008) found that for the eastern tree-hole mosquito, *Ochlerotatus triseriatus* Say (Diptera: Culicidae),

oviposition preferences were based on larval density in different breeding sites. Female mosquitoes selected patches with fewer larvae as oviposition sites resulting in greater fitness and reduced development time (Ellis 2008). In the leaf miner, *Liriomyza huidobrensis* Blanchard (Diptera: Agromyzidae), females often preferred to feed on poorer host plants but selected higher quality plants for oviposition when provided with a choice (Videla *et al.* 2012).

Oviposition decisions and arrival of blow flies may vary in terms of the carrion size or type. When comparing carrion of different sizes, Kuusela and Hanski (1982) found that there was no difference in the species attracted to either size carrion, but there were differences in the abundance of the flies present with larger carrion attracting more flies. Denno and Cothran (1975) found that certain species of blow flies preferred different size carrion, where *Lucilia sericata* Meigen and *Phormia regina* Meigen demonstrated a preference for small and large carrion, respectively. The carcass type can also influence the arrival of insects. In a study comparing the insect succession on black bear, deer, alligator and swine, Watson and Carlton (2003, 2005) found that although numerous species arrived to the carrion, only 19 species were collected from all four carcass types. In this study, alligator carrion had the fewest taxa associated with it, most likely due to the lack of suitable oviposition sites due to the morphology of the alligator (closed cloaca, eyes and jaws) (Watson and Carlton 2005).

In addition to carrion size and type, I predict oviposition sites selected across a whole carrion animal resource varies and that the performance of the offspring based on these decisions can vary as well. I expect preference may be divided into a preference for specific oviposition sites by different species, which I will measure by the number of oviposition events by individual females as well as the preference in terms of the number of eggs deposited by these females.

1.3 Abiotic and Biotic Interactions

1.3.1 Abiotic Conditions

Environmental factors often constrain the life history traits of organisms. In particular, conditions such as temperature, relative humidity or photoperiod can change the behaviour and influence the success of an organism. Insects are poikilotherms and rely on ambient heat to maintain body temperature (Harrison *et al*. 2012). Furthermore, their development rate is heavily dependent on temperature (Huey and Kingsolver 1989; Angilletta *et al*. 2004). Insects develop and perform within a thermal range, which contains a critical thermal maximum (CT_{max}) and minimum (CT_{min}) temperature for development of the insect as well as an optimal temperature (T_{opt}) (Huey and Kingsolver 1989; Figure 1.1). These thresholds generally follow a thermal performance curve (TPC), with an optimal temperature as well as a lower threshold temperature, that represents the lowest temperature that insect development can occur (Huey and Kingsolver 1989; Colinet *et al.* 2015). Contrasting this, the highest temperature that maximizes the development rate is the maximum threshold temperature (Huey and Kingsolver 1989). At these thresholds, the rate of development is reduced and insect mortality increases (Wagner *et al*. 1984). Overall, the relationship between temperature and insect development has a wide range of applications in agriculture and forestry with control of pests and disease vectors, and in medicolegal entomology (Arnold 1959; Ames and Turner 2003; Highley and Peterson 1994; Roe 2014).

In addition to temperature, relative humidity can influence development and behaviour of insects. In the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae), mating and oviposition behaviour is greater at higher humidities (Tomberlin and Sheppard 2002) and Holmes *et al*. (2012) found that the successful egg hatch of *H. illucens* also depends on humidity. In addition, the fecundity of the female mosquito, *Aedes aegypti* (L.) (Diptera: Culicidae), is reduced during periods of low humidity and females will delay oviposition due to low humidity stress (Canyon et al. 1999). Periods of low and high humidity can also influence the egg hatching success of various insects, as observed for the bamboo borer beetle, *Dinoderus minutus* (Fabricius)

(Coleoptera: Bostrichidae), with low hatching rates at low (20%) and high (85%) humidity (Norhisham *et al*. 2013). Low relative humidity may have repercussions for eggs as this can lead to desiccation (Evans 1934), loss of lubrication for proper release of larvae from the egg (Guarneri *et al*. 2002) and ultimately increased mortality (Norhisham *et al*. 2013). Blow flies can combat periods of low humidity by clustering eggs during aggregated oviposition (Cruickshak and Wall 2002), thereby reducing exposure to harsh environmental conditions and limiting desiccation of eggs (Stamp 1980).

1.3.2 Biotic Conditions

In addition to these abiotic environmental conditions, blow fly larvae developing on carrion experience varying biotic conditions in the form of intraspecific and interspecific species interactions which can have positive, negative or neutral consequences for individuals involved and range from competition, to mutualism and commensalism. The patchy nature of carrion results in the formation of discrete ephemeral communities, often composed of interacting species that coexist on the transitory resource (Atkinson and Shorrocks 1981; Kneidel 1985; Hanski 1987). For blow flies competing for limited resources, the high density of larvae on carrion often results in scramble competition and can lead to reduced growth rate or increased mortality (Saunders and Bee 1995; Smith and Wall 1997a, 1997b; Davies 1999; Ireland and Turner 2006; VanLaerhoven 2015). Larval crowding on limited resources can produce smaller larvae, pupae and undersized adults (Saunders and Bee 1995; Ireland and Turner 2006). However, faster development may occur at high larval densities due to the higher temperatures generated in larval aggregations (Turner and Howard 1992; Ireland and Turner 2006; Slone and Gruner 2007). Insects using carrion resources face a trade-off between body size and development, where individuals that emerge as adults sooner are often smaller (Nijhout 2003; Davidowitz *et al* 2005; Chown and Gaston 2010). While development of a larger size results in fitness benefits (Kingsolver and Huey 2008), longer development times increase the risk of parasitism or predation (Nijhout 2003).

Aside from competition, insects can experience facilitation, a form of commensalism where one species benefits and the other species is not affected

(Shorrocks and Bingley 1994) and can occur when a resource is modified by the presence of one species, making it easier for another species to utilize. On carrion, early arriving species can modify the resource and make it more suitable for later arriving species (Hanski 1987; Hanski and Kuusela 1977; Kneidel 1983; Brundage *et al.* 2014). Facilitation has been documented for blow fly species that coexist on carrion. In larval stages, *P. regina* has greater survival rates and larger adult body size when feeding on carrion with *L. sericata* (Rosati 2014). The facilitation of feeding in the larval stages may be due to shared salivary enzymes, released by *L. sericata*, which allows for more efficient use of the resource by *P. regina* (Charabidze *et al*. 2011).

Despite these interactions and the ephemeral nature of the resource, numerous species of blow fly manage to coexist on carrion. Species that utilize the same resource can coexist on a resource due to many different mechanisms (Atkinson and Shorrocks 1981; Kneidel 1984; Hanski 1987; Ives 1991) such as partitioning the resource in an attempt to reduce species interactions. The aggregation model of coexistence predicts that competing species can coexist on ephemeral resources if they aggregate in irregular patterns (Atkinson and Shorrocks 1984). It is uncommon for one species to dominate and exclude another species on an ephemeral resource (Shorrocks 1979; Atkinson and Shorrocks 1984). Blow flies also exhibit preferences spatially, in terms of habitat and aggregation on resources (Fiene *et al.* 2014). Baseline studies have examined differences between indoor and outdoor habitats (Anderson 2010), aquatic and terrestrial (Anderson 2010), urban and rural (Hwang and Turner 2005) and sun and shade (Joy *et al*. 2006).

Species can also differ in their temporal availability, which can influence the extent of species interactions that can occur (Hanski and Kuusela 1977; Kneidel 1983; Shorrocks and Bingley 1994). In warmer temperatures experienced in the summer, *L. sericata* arrives to carrion (Smith 1986; Rosati 2014) whereas *C. vicina* is typically active at cooler temperatures and often arrives to carrion during the spring and fall in the temperate zone of North America (Donovan *et al*. 2006; Rosati 2014). Other species, such as *P. regina*, are tolerant of a wider range of temperatures and can be observed on carrion in all three seasons in this region (Hall 1948; Anderson and VanLaerhoven 1996; Byrd and Allen 2001). The arrival time of these species can also change with geographic location. For example, in California and Maine *P. regina* has been reported to arrive later

in decomposition, within 24 - 48 h after death (Denno and Cothran 1976; Lord and Burger 1984). However, in British Columbia, Anderson and VanLaerhoven (1996) collected *P. regina* adults earlier in decomposition, immediately after death.

The dispersal capability of adult blow flies ranges between 4 and 20 km each day (Greenberg 1991; Whitworth 2006). Adult flies feed at pollen and nectar rich flowers to obtain carbohydrates (Karczewski 1967; Grinfel'd 1955), which are required before they feed on protein or engage in mate seeking (Smith and Gadawski 1994; Foster and Takken 2004; Gary and Foster 2006). Large amounts of protein are needed to mature oocytes and flies often acquire protein from pollen, carrion or feces (Evans 1935; Stoffolano *et al.* 1995; Erzinçlioğlu 1996). The egg load and clutch size of female blow flies are dependent on temperature as well. Specifically, ovariole development and the number of eggs that gravid females can carry are influenced by temperature (Harlow 1956; Davies 2006). For *Calliphora vicina* Robineau-Desvoidy females, there is a low threshold at 5°C, and egg maturation does not occur below this temperature (Davies 1998).

1.4 Natural History of Study Species

Among the dipterans commonly associated with carrion, the family Calliphoridae, or blow flies, represent the majority of individuals present (Greenberg 1991; Byrd and Castner 2010). This family is diverse and is comprised of about 1100 species (Smith 1986; Merritt and De Jong 2015). There are five subfamilies found in North America, including the Calliphorinae, Luciliinae and Chrysomyinae (Whitworth 2006) all of which are very closely associated with carrion in both larval and adult stages (Greenberg 1991; Villet 2011; Whitworth 2006). The three species used in this study are locally occurring in southern Canada (Smith 1986; Byrd and Castner 2010) and have been observed colonizing carrion simultaneously (Anderson and VanLaerhoven 1996; VanLaerhoven and Anderson 1999; Sharanowski *et al*. 2008).

The life cycle of blow flies is divided into six stages (Greenberg 1991). Females lay clutches of eggs that hatch into first-instar larvae. The larvae feeds and moults, shedding its exoskeleton, into the second and then a third instar stage. These larval stages are the feeding stages in the lifecycle of the blow fly and other than the change in size the

larvae look very similar. Morphologically, the life stage can be determined by examining the posterior spiracles, where one v-shaped spiracular slit represents a first instar, two separate spiracular slits represent a second instar and three represent a third instar (Erzinçlioğlu 1996). When the larvae acquire enough nutrients to complete their lifecycle and stop feeding, they crawl from the food resource to find a dry place to pupate. During this stage, the cuticle hardens and contracts, forming a puparium, or the outer covering that provides protection for the pupae (Greenberg 1991). When pupal development is complete, an adult fly emerges from the puparium (Greenberg 1991). On carrion resources, blow flies can have multiple generations per year (Erzinçlioğlu 1996). Mean survival for adult Calliphoridae is between three to four weeks, however, adults have been documented to survive for up to 76 days in outdoor and indoor cages (Mackerras 1933; Parman 1945).

The female reproductive system contains two ovaries, composed of ovarioles, which house the developing eggs. Females are anautogenous, requiring protein to mature the eggs and often mate after feeding (Gullen and Cranston 2005). Several days after the first mating, a female will reject further attempts from males due to a peptide transferred to the female during mating from the male accessory glands which reduces the receptivity of the female (Gullen and Cranston 2005). Females that are unreceptive to mating will respond to attempts from males by curling their abdomen or kicking males. Females can deposit between one and four egg masses before remating and this strategy by males ensures paternity of those egg masses (Gullen and Cranston 2005).

In the Luciliinae subfamily, the species *Lucilia sericata* Meigen is cosmopolitan, and is very abundant in the temperate zone of North America. Adult flies are metallic green and range from 6-9 mm in length (Smith 1986; Byrd and Castner 2010). This species is an early carrion colonizer and can arrive and deposit eggs within hours after death (Anderson and VanLaerhoven 1996; Grassberger and Frank 2004; Sharanowski *et al*. 2008). In addition, this species is more common in seasons with higher temperatures above 30°C and prefers open, sunny habitats (Cragg 1955; Smith and Wall 1997b). The lower activity threshold for *L. sericata* is around 14°C (Mellanby 1939), but oviposition typically occurs between 30-40°C (Smith 1986; Zurawski *et al.* 2009). However, *L. sericata* often deposit eggs in more shaded areas of the body, such as inside the mouth,

ears or nostrils (Grassberger and Frank 2004). Adult females commence oviposition between 5-9 days after they emerge from the puparium and can lay approximately 225 eggs at a time (Davies 1998), with a lifetime output of between 2000-3000 eggs (Smith 1986). Development of *L. sericata* is dependent on temperature, with faster development as temperature increases (Kamal 1958; Ash and Greenberg 1975; Greenberg 1991; Wall *et al.* 1992; Davies and Ratcliffe 1994; Greenberg and Reiter 2001; Roe 2014), but the incubation period for eggs is typically between 10-52 hours (Smith 1986). The pre-pupal stage of this species is variable and can last between 3 days to several weeks, depending on the temperature (Smith 1986). The temperature range for development of *L. sericata* is 15°C (Grassberger and Reiter 2001) to 37.5°C (Gosselin *et al.* 2010) and this species overwinters in the third-instar or pre-pupae stage (Erzinçlioğlu 1996).

The species *Calliphora vicina* Robineau-Desvoidy is in the subfamily Calliphorinae and is often referred to as the blue bottle fly due to its dark blue thorax and overall blue appearance (Byrd and Castner 2010). Adult flies of this species are larger than other calliphorids, ranging in length from 10-14 mm (Smith 1986; Byrd and Castner 2010). This species is cosmopolitan and is more abundant in the northern United States and Canada, and is often observed in the spring and fall in temperate areas, when temperatures range between 5-30°C (Smith 1986; Donovan *et al.* 2006; VanLaerhoven, personal observations). This species is an early colonizer to carrion and oviposition often occurs within a few hours. Females can oviposit between 200-300 eggs at a time (Smith 1986; Davies 1998). Egg hatching can occur in temperatures as low as 3.5 °C (Donovan *et al*. 2006) and hatching can take 25 h at 20°C (Ames and Turner 2003). The development threshold for *C. vicina* is between 3.5°C (Myskowiak and Doums 2002; Donovan *et al*. 2006) and 30°C (Smith 1986; Hwang and Turner 2009). At 20°C, development from egg to adult can take up to 22 days (Ames and Turner 2003). This species overwinters in the larval stage (Erzinçlioğlu 1996).

Phormia regina Meigen is in the subfamily Chrysomyinae and is commonly called the black blow fly, although adults are usually olive green in colour. This species is abundant in the United States and southern Canada (Byrd and Castner 2010) and is a medium-sized fly, usually between 7-9 mm in length (Smith 1986). Depending on temperature, the total lifecycle for *P. regina* is reported to range from 10-25 days (James

1947; Kamal 1958; Smith 1986; Greenberg 1991; Byrd and Allen 2001; Roe 2014). Although this species is considered a cooler weather fly in sub-tropical areas of the southern USA (Smith 1986; Byrd and Castner 2010), *P. regina* is abundant in the spring, summer and fall in more temperate regions of North America (Byrd and Castner 2010; Rosati 2014). This species is active between 10-35 °C (Deonier 1940; Byrd and Allen 2001). The arrival of *P. regina* to carrion is variable and this species can arrive later in succession (Denno and Cothran 1975) but has also been observed arriving simultaneously with *L. sericata* early in succession (Anderson and VanLaerhoven 1996; Sharanowski *et al*. 2008; Vanin *et al*. 2013) and can be observed in shaded or sunny areas (Joy *et al*. 2002). Females lay large clusters of approximately 200 eggs (Smith 1986). Development of *P. regina* has been reported to occur in temperatures as low as 15°C and as high as 35°C (Byrd and Allen 2001), with individuals overwintering in the adult stage (Marshall *et al*. 2011).

1.5 Forensic Entomology and its Applications

Forensic entomology is a multidisciplinary field that incorporates aspects of arthropod ecology and forensic investigations. This field is subdivided into three areas (urban, stored-produce and medicolegal), but medicolegal entomology is the primary focus of this thesis and involves utilization of insect evidence in criminal investigations, most of which are of a homicidal and suicidal nature (Byrd and Castner 2001). This information is primarily used to provide answers on the time of death or the place where death occurred (Catts 1992). Life history traits and succession patterns of carrion insects are used in this estimate, which is commonly referred to as the postmortem interval (PMI) or the length of time between death and discovery of the body (Catts 1992; Byrd and Castner 2010). The immediate arrival of insects after death is useful in determining this length of time (Nuorteva 1977; Rodriguez and Bass 1983; Smith 1986; Greenberg 1991; Anderson and VanLaerhoven 1996; Anderson 2001), as the time of colonization (TOC) often closely approximates the PMI (Amendt *et al*. 2007; Tomberlin *et al*. 2011). The TOC can be examined using the various ecological processes that can influence

insects occurring on carrion and may contribute to minimum PMI estimates (Tomberlin *et al.* 2011).

To estimate the PMI, there are two general approaches; one method uses the predictable rates of development of blow flies (Nuorteva 1977; Smith 1986; Catts and Goff 1992) and the other uses the predictable changes in composition in the succession of insects through decomposition (Payne 1965; Schoenly and Reid 1987). Using species-specific growth rates, combined with temperature conditions that the larvae experience during development, the minimum TOC estimate can be calculated. The estimations of the minimum TOC and PMI use calculations and predictions of development based on previously published data. These data, however, are controversial due to different developmental rates reported for identical species (Ash and Greenberg 1975; Anderson 2000; Grassberger and Reiter 2001; Tarone and Foran 2006; Gallagher *et al*. 2010). This issue has been addressed in the forensic entomology community through validation studies that compare estimates of PMI using different developmental data (VanLaerhoven 2008); however, other issues may also impact these estimates.

Most studies examine a range of temperatures that can influence the development of blow flies, but these studies mainly look at growth rate in the absence of competition (Byrd and Butler 1996; Byrd and Butler 1997; Byrd and Allen 2001; Grassberger and Reiter 2002; Nabity *et al*. 2006). Multiple species often colonize a resource simultaneously and their larvae co-develop on one resource (Anderson and VanLaerhoven 1996; Smith and Wall 1997b; Tabor et al. 2004). Species interactions, including competition, occur on these ephemeral resources and can have significant impacts on the development rate, size and mortality of species that are interacting (Hutton and Wasti 1980; Prinkkilä and Hanski 1995; Smith and Wall 1997a; Rosati 2014; Pacheco 2015). Understanding the role of species interactions and temperature on the behaviour and development of blow flies can provide useful information to the forensic entomology community and allow development of confidence intervals for more accurate estimates of TOC and PMI (VanLaerhoven 2010). The incorporation of ecological theory into the precision of these estimates increases the confidence in the interpretation of insect evidence in the judicial system (VanLaerhoven 2010).

1.6 Research Objectives

The overall aim of my research was to examine the effect of temperature, humidity and species interactions on the oviposition behaviour and development of the forensically-relevant blow flies *L. sericata*, *P. regina* and *C. vicina*. The colonization behaviour of adult flies was investigated under different temperature regimes and in the presence of other species to determine if these factors affect the oviposition decisions of female blow flies. The timing and location of oviposition events and the number of eggs were recorded, as these decisions can influence the survival of the offspring (Chapters 2, 3). Based on the temperature ranges in which these species are active, increased temperature should change adult behaviour, resulting in more oviposition events and eggs laid for *L. sericata*, the warm weather species, and *P. regina*, but fewer for the cooler weather species, *C. vicina*. Optimal oviposition theory indicates that blow flies should select sites that benefit their offspring, protecting them from desiccation by placing the eggs in sites such as the natural openings or body folds. Based on the preferences reported in the literature, *L. sericata* and *C. vicina* should oviposit in the mouth and on the face, whereas *P. regina* should oviposit on the body folds, near the legs and all species should avoid open, exposed areas of the carrion resource. When female *P. regina* are in the presence of eggs from other blow fly species, they should increase performance of their offspring by selecting the same sites and aggregating their eggs with those of the other species. The presence of other species eggs should facilitate faster oviposition by *P. regina*. When examined in a field setting, the oviposition behaviour of these three species should reflect those observed in the laboratory, with respect to oviposition timing, site selection and total egg number deposited (Chapter 5).

Egg development was measured under different relative humidities to determine the influence of environmental conditions on hatching time and eclosion success (Chapter 4). Environmental conditions, such as humidity and temperature, should influence the hatching success and development time of blow fly eggs. The developmental temperature thresholds of these blow fly species should determine the egg hatch success and development of larvae. Based on the reported relative humidity thresholds, egg hatching for the warm weather species *L. sericata* should be less successful and should require

more time during periods of low relative humidity. The wide temperature range of *P. regina* suggests that relative humidity would not affect this species, and eclosion time and success would be similar over the range tested. For *C. vicina,* we expected that while low relative humidity would not affect eclosion time or hatching success, high humidity would be challenging for this cool weather species and would result in reduced hatching success.

The development of larvae was measured under different temperature regimes and the interactions of conspecifics and heterospecifics was observed to examine the influences on growth rate, pupal mass, survivorship and adult body size (Chapter 6). The combined effects of temperature and species interactions should result in developmental changes for *L. sericata*, *P. regina* and *C. vicina*. With increasing temperature, *L. sericata* development should increase. Smaller larvae and therefore reduced pupal weight and smaller adults will accompany faster development. Development during interspecific interactions and survival of *L. sericata* will remain largely unchanged over this range of temperatures due to developmental plasticity of this species. The growth rate of *P. regina* will be impacted by temperature and species interactions. As temperature increase and due to facilitation by heterospecifics, the growth of *P. regina* will increase, resulting in larger larvae, heavier pupae and ultimately, larger adults. The survivorship of *P. regina* should remain unchanged as temperature increases, due to the wide temperature range that this species can tolerate. As documented previously, as temperature increases, *C. vicina* growth rate will increase, but at high temperatures, the development of *C. vicina* will be hindered, resulting in high mortality. Based on previous findings that *C. vicina* is more heavily affected by intraspecific interactions, *C. vicina* should experience reduced effects on development, resulting in slower growth and reduced size, due to interspecific interactions.

Through this research, I hope to elucidate interactions among blow fly species on carrion resources that provides some insight into the mechanisms of their coexistence. Furthermore, the conclusions from this research will also highlight the behavioural and developmental differences among blow fly species, providing further information that may be utilized in the interpretation of insect evidence.

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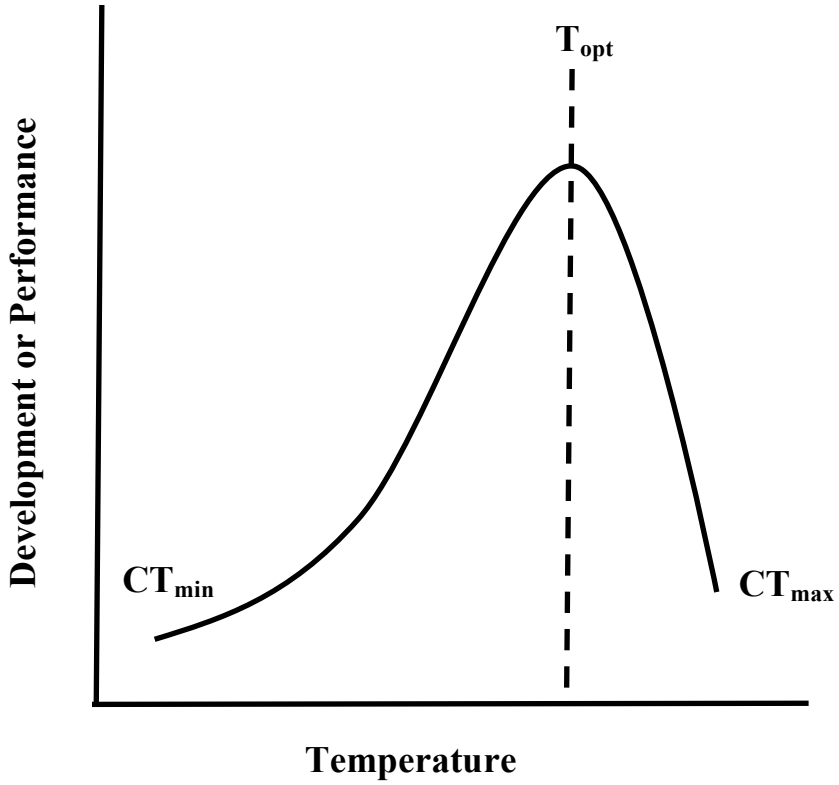

Figure 1.1. Thermal performance curve (TPC) for insects, representing the relationship between temperature and development or performance of an insect. The critical thermal minimum (CT_{min}) and maximum (CT_{max}) represent the thresholds for development or performance. Below the optimal temperature (T_{opt}), there is an increase in development or performance but a decrease in development or performance above the T_{opt}.

CHAPTER 2
THE EFFECT OF TEMPERATURE ON THE OVIPOSITION BEHAVIOUR AND EGG LOAD OF BLOW FLIES (DIPTERA: CALLIPHORIDAE)

2.1 Introduction

Selecting oviposition sites can have consequences that affect the life history traits of a female's developing offspring and therefore her fitness (Janz 2002). Oviposition strategies are a series of complex trade-offs between numerous factors, such as host/resource quality, clutch size, difficulty and probability of finding another suitable host/resource, predation risks, and the mobility of the resultant offspring (Janz 2002). Parental care increases the chances of offspring survival and can result in increased reproductive fitness (Tallamy 1984; Tallamy and Horton 1990). However, parental care can be costly in both energy and risk. Females that do not practice parental care can devote energy to producing more offspring compared to females that do provide parental care (Tallamy and Horton 1990).

Due to the costs of parental care, many insect species ensure their fitness by increasing offspring survival or performance in other ways. For example, some insects use piercing ovipositors to penetrate plant tissues and lay eggs within a leaf; this strategy ensures that the eggs are hidden and protected (Herrera and Pellmyr 2002). Other insects lay large masses of eggs to reduce the number of eggs exposed to predators, parasitoids (Stamp 1980) and the abiotic environment (Clark and Faeth 1998). Egg clustering results in communal feeding which can promote larval survival by deterring predators (Gamberale and Tullberg 1996) or allowing for efficient use of the food source (Goodbrod and Goff 1990; Crowe 1995). Ideally, insects that offer no parental care should position their eggs where a sufficient food supply, or resources of adequate nutritional value are available for offspring development (Jaenike 1978). This theory, referred to as optimal oviposition theory (Jaenike 1978) or the female preference-offspring performance hypothesis (Ellis 2008), has been widely tested as oviposition behaviour by females often determines larval survival for species that have limited larval

mobility and high dependence on the food source selected by the ovipositing female (Joachim-Bravo *et al*. 2001).

On an ephemeral resource, the fitness of individuals developing in clutches can be influenced by clutch size. For ovipositing females, the decision of the number of eggs to lay is often a trade-off. Larger clutch sizes can produce a greater number of offspring, but females may face diminishing returns in fitness due to the limited resource available for their offspring (Skinner 1985; Wilson and Lessels 1994). Competition for ephemeral resources can affect the clutch size decisions made by females, particularly when numerous females are laying clutches simultaneously (Parker and Begon 1986; Ives 1989; Goubault *et al.* 2007). Intraspecific competition should result in fewer eggs laid by each female, since the number of ovipositing females increases (Parker and Courtney 1984; Parker and Begon 1986; Ives 1989). However, some insects demonstrate clutch size decisions based on the presence of conspecifics, and positively respond to the presence of eggs from conspecific females (Wilson 1994).

If avoiding high densities of offspring on ephemeral resources is important (Ellis 2008), then choosing different oviposition sites may be a form of spatial resource partitioning. The preference for oviposition sites may be influenced by a number of factors, such as resource suitability (Jaochim-Bravo *et al*. 2001), resource availability and risk of predation (Giao and Godoy 2007). Some studies outlining the oviposition preferences of blow flies (Diptera: Calliphoridae) have demonstrated that gravid females exhibit preferences for natural orifices (Grassberger and Frank 2004; Smith 1986) with particular species demonstrating strong site preferences on fetal pig carcasses, such as the ears, mouth or legs (Rosati 2014; Pacheco 2015). However, there is little research that explores the impact of temperature on these oviposition decisions.

Insects are poikilotherms and thus are highly responsive to temperature (Harrison *et al.* 2012). Temperature influences seasonal and geographic distribution of blow flies (Anderson 2001) as well as their behaviour and physiology (Grassberger and Reiter 2001; Donovan *et al*. 2006). At low temperatures, performance rates increase with increasing temperature, until they reach a maximum, or T_{opt}, the optimal temperature (Huey and Stevenson 1979; Huey and Kingsolver 1989). After this T_{opt}, these rates rapidly decline, resulting in an asymmetric thermal sensitivity. Numerous processes have similar thermal

sensitivity, such as growth, development and fitness (Huey and Stevenson 1979). The common pattern, called a thermal performance curve (TPC) shows the thermal sensitivity of an organism's performance or fitness (Huey and Kingsolver 1989; Izem and Kingsolver 2005). A shift in this curve vertically, shows variation in the performance of an organism over a temperature range, whereas a horizontal shift indicates a trade-off between temperature and performance (Kingsolver 2009).

Temperature may also influence the reproductive potential of blow flies as it can change the ovariole development and number of eggs (egg load) that gravid females carry (Harlow 1956; Davies 2006). Egg maturation is temperature-dependent for blow flies, with a temperature threshold required for the development of eggs (Wall *et a*l. 1992; Wall 1993). The oviposition differences between *Lucilia sericata* Meigen and *Calliphora vicina* Robineau-Desvoidy relating to egg loads oviposited under various conditions have been reported and the variation in egg production may be due to abiotic factors (Hayes 1999; Davies 2006). Davies (1999) reported that in temperatures greater than 11°C, *L. sericata* females lay approximately 225 eggs, whereas *Phormia regina* Meigen and *C. vicina* lay 200 eggs each (Davies unpublished, in Davies 2006). Others have reported varying ranges in egg load for each of these species (Smith 1986; Erzinçlioğlu 1996; Wall 1993) indicating that egg load may be dependent on conditions experienced by the female blow flies.

The flight and oviposition behaviours of insects are also subject to thermal ranges. Although *L. sericata*, *P. regina* and *C. vicina* are common and Holarctic in distribution (Byrd and Castner 2010), they have different temperature thresholds. *Lucilia sericata* prefers to oviposit when temperatures reach 30°C or greater (Smith 1986) and is therefore considered a warm weather, or summer species in Ontario. In northern climates, *P. regina* is a dominant species and displays an activity threshold of 10-12.5°C; below this, activity and oviposition does not occur (Byrd and Allen 2001). However, more northern populations of this species are active below 10°C (VanLaerhoven, personal observations) making it active in spring, summer and fall in southern Ontario. *Calliphora vicina* is considered a dominant species in the early spring and fall in Ontario, and is often active at temperatures below 10°C, and has a lower threshold of 3.5°C (Donovan 2006) and an upper threshold around 30°C (Smith 1986). This species has been observed arriving to

carrion in temperatures up to 32°C in Ontario but is not typically present in the warmer summer months in southern Ontario (VanLaerhoven, personal observation).

The aim of this study was to examine the effect of temperature on the egg load and oviposition behaviour of the blow flies *L. sericata*, *P. regina* and *C. vicina* (Diptera: Calliphoridae). We evaluated oviposition behaviour in terms of site preference, time to the first oviposition event and the number of eggs laid by female blow flies. Due to the relationship between insect activity and temperature, and the temperature tolerances of each species, increased temperature should result in changes in insect behaviour, with more oviposition events and eggs deposited by *L. sericata* and *P. regina*. We expected female blow flies of all three species to prefer the natural orifices (mouth, nostril, ears) and body folds (on legs) for oviposition sites on fetal pig carcasses. We expected different optimum temperatures for each species as they have different temperature tolerances such that *C. vicina* would have the lowest optimum temperature, followed by *P. regina* with a more intermediate optimum temperature and *L. sericata* with the highest optimum temperature demonstrated by more oviposition events, greater egg numbers and greater egg load and the shortest time to oviposition at or near their respective optimum temperatures.

2.2 Materials and Methods

Laboratory colonies maintained at the University of Windsor were used to obtain eggs of *L. sericata*, *P. regina* and *C. vicina*. The colonies originated from wild-caught females in Windsor, Ontario, Canada and were housed in 46 x 46 x 46 cm aluminum cages (Bioquip 1450C collapsible cage). Adult flies were provided with water and sugar *ad libitum*. Fresh pork liver (40 g) was provided for 24 h as an oviposition substrate. When large egg masses (approximately 1000 eggs) were laid, the egg masses were removed from the liver and equally divided among ten 1 L rearing jars containing a new piece of liver (40g) and wood shavings. The wood shavings acted as a pupation medium. Jars were sealed with permeable woven landscape fabric (Quest Brands Inc., Item ID: WBS 50) and a metal ring that permits gas exchange but prevents larvae from leaving the jars. Developing larvae were provided with additional liver as needed. When adult flies

emerged in the rearing jars, they were cold sedated (Ricker *et al*., 1986) and sorted by gender based on spacing between the eyes (Erzinçlioğlu 1996) into treatment cages. Each treatment cage (46 x46 x 46 cm) consisted of 100 females and 50 males of a single species. This density allows for access to the resource, ensuring that each female had an opportunity to oviposit. To minimize any harmful density dependent effects, the density of flies in each cage ensured maximum reproductive rates and survival (Moe *et al*. 2002). In addition, males mate with multiple females and this density is sufficient to ensure that all females in each cage are mated.

Each treatment cage was assigned to one of five temperatures (15°C, 20°C, 25°C, 30°C, 35°C) and placed into a growth chamber (Conviron Adaptis A1000) programmed with a photoperiod of 16:8 (L:D), 50% relative humidity and the appropriate temperature. Each species and temperature treatment was replicated six times. Every 60 minutes, data loggers (HOBO U12-012, Onset, Pocasset, MA) recorded the temperature and relative humidity in the growth chambers. During the first five days of the experiment, 50 g of fresh pork liver was placed into each treatment cage for one hour as a protein source to ensure female ovarian egg development and male spermatogenesis (Erzinçlioğlu 1996; VanLaerhoven and Anderson 2001). On day six, a fetal pig on an aluminum tray was placed into each treatment cage and left for 24 h during which observations were made every hour during daylight hours. The time of the first oviposition event and the location of oviposition events were recorded. Potential oviposition sites included the mouth, ear/nostril, face, neck, legs and abdomen (Rosati 2014). After 24 h the pigs were removed from the cages and the egg masses were photographed with a Nikon D70 camera and AF Micro-Nikkor 60 mm f/2.8D lens with a 15cm ruler for scale. The depth and area of each egg mass was measured following the methods described by Rosati *et al*. (2015). In brief: depth measurements were recorded manually for each section of the egg mass that had a different depth and the surface area of each egg mass was determined using ImageJ software. This information was used to calculate the volume of each egg mass (Rosati *et al.* 2015) and the volumes were used to estimate the number of eggs in each egg mass using species-specific 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*., submitted).

To determine the egg load for each species, three treatment cages were set up for each species in a similar manner as those described above and placed into programmed growth chambers. All cages received fresh pork liver as a protein source daily and females were monitored until they displayed a distended abdomen, indicating that they were gravid (Harlow 1956). Once females were identified as gravid, they were removed from the cages, killed and their ovaries were carefully dissected out from the abdomen. Ovaries were placed into 70% ethanol until egg counts were made using a Meiji EMZ zoom stereomicroscope.

2.2.1 Statistical Analysis

All analyses were performed in R 3.1.1(R Project for Statistical Computing, http://www.R-project.org/). All data presented are back-transformed. The time to first oviposition event data was natural-log transformed and was analyzed using a two-way ANOVA (aov function) to examine the effect of species, temperature and the interaction of species and temperature on time to first oviposition. Significant results were followed with multiple comparisons post-hoc tests. False discovery rate (FDR) was controlled for in order to account for multiple comparisons (Benjamini and Hochberg 1995). The FDR controls for the proportion of hypotheses falsely rejected (Benjamini and Hochberg 1995). We used a maximum false discovery rate of 0.05 on overall p-values.

A two-factor MANOVA (manova function) was used to examine the effects of temperature and species, and the interaction of these two factors on the oviposition sites selected by female blow flies. The data for oviposition site selection was transformed using (ln(site count + 1.5) to meet the normality assumptions of MANOVA. Significant two-way MANOVA results were followed with one-way MANOVA and ANOVA to determine which sites selected were influenced by temperature or species. To determine significance in MANOVA, we compared p values to $\alpha = 0.01$, adjusting for multiple tests.

The effect of species, temperature, and the interaction of species and temperature on the total number of eggs deposited was determined using a two-way ANOVA (aov

function). The data was square root transformed in order to meet parametric assumptions. All significant ANOVA results were followed with multiple comparisons and FDR was controlled for.

The influence of temperature on egg load for all three species was examined using a Generalized Linear Model (glm function, error distribution=Poisson, link=log).

2.3 Results

Temperature and species interacted to affect oviposition time ($F_{8, 75} = 9.97$, $p < 0.001$) such that as temperature increased, the time for first oviposition event decreased for *L. sericata* and *P. regina* (Figure 2.1), but increased for *C. vicina* at the highest temperature tested (Figure 2.1).

Oviposition site selection depended on both temperature and species (temperature x species MANOVA: Wilk's $\lambda = 0.299$, $F_{8,75} = 2.01$, $p < 0.001$; Figure 2.2). The three species differed in their site selection at 15°C (MANOVA: Wilk's $\lambda = 0.033$, $F_{2,15} = 7.52$, $p < 0.001$) and 30°C (MANOVA: Wilk's $\lambda = 0.027$, $F_{2,15} = 8.49$ $p < 0.001$), but not at 20°C (MANOVA: Wilk's $\lambda = 0.299$, $F_{2,15} = 1.38$ $p = 0.25$), 25°C (MANOVA: Wilk's $\lambda = 0.128$, $F_{2,15} = 2.99$ $p = 0.02$) or 35°C (MANOVA: Wilk's $\lambda = 0.194$, $F_{2,15} = 2.11$ $p = 0.07$). Overall, at 15°C, each species differed in site selection of the face (ANOVA $F_{2,15} = 17.06$, $p < 0.001$) whereas at 30°C, there was a difference in selection of the mouth (ANOVA $F_{2,15} = 10.55$, $p = 0.001$) and ears (ANOVA $F_{2,15} = 8.57$, $p = 0.003$). Specifically, temperature affected the oviposition sites selected by *P. regina* (MANOVA: Wilk's $\lambda = 0.124$, $F_{4,25} = 2.43$ $p = 0.002$), such that as temperature increased, *P. regina* used more oviposition sites on the pig carcass such as the neck (ANOVA $F_{4,25} = 6.20$ $p = 0.001$; Table 2.1). The most preferred sites for oviposition overall were the abdomen and the legs, but the legs were preferred at all temperatures included in the study (Figure 2.2). Temperature also affected site selection by *C. vicina* (MANOVA: Wilk's $\lambda = 0.118$, $F_{4,25} = 3.36$, $p < 0.001$), and by *L. sericata* (MANOVA: Wilk's $\lambda = 0.174$, $F_{4,25} = 17.98$, $p = 0.01$). Overall, *L. sericata* demonstrated a preference for oviposition sites on the head, especially the mouth, at all temperatures tested (Figure 2.2). In contrast, female *C. vicina* only chose four oviposition sites and preferred sites on the head, including the face

(ANOVA $F_{4,25} = 5.74$ $p = 0.002$) and also the legs, as temperature increased (Table 2.1; Figure 2.2).

The number of eggs deposited differed for each species, depending on an interaction between species and temperature ($F_{8, 75} = 7.37$, $p < 0.001$) (Figure 2.3). All three species laid the most eggs at 30°C (Figure 2.3), however, at cooler temperatures, both *L. sericata* and *P. regina* laid the fewest eggs (Figure 2.3) whereas for *C. vicina*, the fewest eggs were laid at 35°C (Figure 2.3).

Egg load of females for all three blow fly species depended on temperature (GLM, poisson, $z = 35.57$, $p < 0.001$). Overall, *P. regina* had the highest egg load as temperature increased, with a peak in egg load at approximately 28-30°C. Although *L. sericata* and *C. vicina* had peak egg loads around 20-25 and 20-23°C respectively, *L. sericata* had a higher egg load at this temperature range than *C. vicina* (Figure 2.4).

2.4 Discussion

Overall, we found that temperature influences the oviposition decisions made by female blow flies. We expected that with increasing temperature there would be an increase in egg load and oviposition events, particularly for *L. sericata* and *P. regina*. Each species demonstrated a peak in egg load, with the largest number of eggs overall near 30°C for *P. regina*, followed by *L. sericata* and *C. vicina* between 20-25°C, yet peak egg deposition occurred at 30°C for *L. sericata* and *P. regina* but between 15-30°C for *C. vicina*. The lack of congruence between the peaks in egg load and number of eggs laid for *L. sericata* and *C. vicina* was unexpected, suggesting that clutch size decisions are not predominantly dependent on egg load and that other factors, such as relative humidity or the presence of conspecifics, mediate these decisions.

Given the high temperatures for activity of *L. sericata* (up to 37°C), we expected that egg deposition would peak at the higher temperatures of this study and this finding agrees with our predictions. Additionally, our observation that *L. sericata* females carried fewer eggs and deposited low numbers of eggs at low temperatures of this study agrees with our prediction. Pitts and Wall (2004) found that the oviposition rate of *L. sericata* is lowest below 16°C and is positively correlated with temperature. In this case, the clutch size is behaviourally modified by *L. sericata*, and is dependent on temperature, with

increasing clutch sizes associated with higher temperatures.

For *P. regina*, the number of eggs deposited increased with increasing temperature, with a peak in eggs laid at 30°C that coincides with their peak egg load between 25-30°C. The results for *P. regina* are somewhat surprising, given that in southern Ontario this species is present in a wide range of seasons and temperatures (Byrd and Allen 2001). We expected that *P. regina* would oviposit most frequently at intermediate temperatures of this study. Oviposition decisions by ovipositing females can be influenced by temperature (Forsman 2001). For example, the pygmy grasshopper *Tetrix subulata* L. (Orthoptera: Tetrigidae) oviposits faster in warmer environments, but also takes less time to lay each clutch, compared to those in colder environments (Forsman 2001). Perhaps for *P. regina*, a similar mechanism allows for oviposition to occur at a faster rate with increasing temperature and if given more time, the interval between clutches could be measured to determine if this decreases with temperature as well.

Overall, *C. vicina* deposited fewer eggs than the other two species, across all temperatures tested. There was no difference in egg numbers across treatments, except for 35°C. As expected, at the highest temperature tested, *C. vicina* laid fewer eggs than at any other temperature. It was interesting that there does not appear to be an obvious optimal temperature for oviposition for *C. vicina* within the range of temperatures tested, suggesting that this species utilizes the same clutch size decisions across a larger range of temperatures compared to the other two species. At most temperatures tested, *C. vicina* had a smaller egg load than the other species examined and this was more noticeable at higher temperatures between 25-35°C. *Calliphora vicina* is considered a cool weather species, often active between 3.5° and 30°C (Smith 1986; Donovan *et al*. 2006). Given the temperature threshold of this species, the results for egg load and number are not surprising. Davies (2006) found that *C. vicina* had lower egg production as well as a delay in the production of eggs when compared to *L. sericata*. In addition, after 30 days, Davies (2006) found that only 29% of C. vicina females had oviposited. The lifetime reproductive output of *C. vicina* was observed to be approximately 400 eggs per female, which is much lower than *L. sericata*, with an average of 1400 eggs per female (Davies 2006). According to these results, *C. vicina* may invest more energy into production of larger eggs, which are approximately 1.4 mm in length, 0.40 mm in width, than *L.*

sericata whose average egg length is 1.1 mm in length and 0.33 in width (Greenberg and Singh 1995; Davies 2006).

Temperature affected the time to first oviposition event on fetal pig carcasses for *L. sericata* and *P. regina*, with a decrease in the length of time until oviposition began as temperature increased. Our results for oviposition time were consistent with previous findings. Zurawski *et al*. (2009) found that when temperatures were roughly 30°C, *L. sericata* required approximately three hours for oviposition, whereas *C. vicina* and *P. regina* required five hours (Zurawski *et al*. 2009). Although our results demonstrate that *L. sericata* required only one hour at 30°C, the study by Zurawski *et al*. (2009) was performed in the field and many environmental factors could have influenced their results. Flutctuating temperatures have variable results for fecundity of insects. The reproductive output of some species may be greater in fluctuating temperatures (Terblanche *et al*. 2010) if the temperatures are within an optimal range, whereas insects experiencing stressful temperatures during fluctuating temperatures may have decreased fecundity (Marshall and Sinclair 2010). For *Ceratitis capitata* Wiedemann, (Diptera: Tephritidae), fluctuating temperature resulted in greater egg production (Terblanche *et al*. 2010). For some insects, oocyte development can be impaired by stressful temperatures, which can result in reduced reproductive output (Marshall and Sinclair 2010). Marshall and Sinclair (2010) found that there was a trade-off in survival and future reproduction for flies of *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) where flies that were exposed to cold temperatures multiple times had decreased mortality, but also reduced fecundity, compared to flies that were exposed to cold for the same length of time, but continuously. It would be informative to repeat our experiment in a field setting to determine if our observations hold true in more dynamic and fluctuating abiotic conditions with wild-type females.

Calliphora vicina did not demonstrate a strong dependence on temperature for oviposition in the range of temperatures tested here, where oviposition timing fluctuated between one and three hours. Due to the large body size and higher metabolic rate of *C. vicina*, the tested temperature range may not have had a strong influence on oviposition of this species (Meyer and Schaub 1973). Perhaps we would see a stronger response to temperature by this species if lower temperatures between 0-15°C were tested.

Species that compete for temporally ephemeral resources may partition the resource spatially by ovipositing in different areas of a pig carcass (Ives 1991). It is possible that the site preference exhibited by the species included in this study is the result of their history of interspecific interactions. In southern Ontario, *L. sericata* and *P. regina* are often observed together during the warm summer months, whereas *P. regina* and *C. vicina* are often observed colonizing carcasses in the spring and fall (Rosati 2014). If these species often compete with other blow flies and each other, *L. sericata* should prefer areas that *P. regina* does not commonly colonize. For *L. sericata*, oviposition occurred most frequently in and on orifices of the head. This species often selects sites that contain sufficient moisture, such as the mouth, nose and ears (Grassberger and Reiter 2001; Rosati 2014; Pacheco 2015). In this study, *P. regina* oviposited preferentially on the legs, abdomen and the head, as previously documented (Rosati 2014; Pacheco 2015), but surprisingly preferred the face as an oviposition site at the lowest temperature examined. Finally, *C. vicina* preferred the head as well and the legs, as females of this species oviposited on these sites most frequently.

Based on the oviposition preference-offspring performance hypothesis (Jaenike 1978; Thompson 1988; Ellis 2008) the oviposition preferences demonstrated by each species should be a consequence of differences in offspring performance that arise from selecting different oviposition sites; higher offspring performance at particular sites should result in female preference for those sites. Thus, an important future area of research will be to determine if the site selection preferences by females actually result in increased offspring performance.

Due to intense competition for resources at the larval stages, oviposition behaviour may also be influenced by density of offspring at a site that are the result of individual female clutch size decisions in the presence of conspecifics. The results of our study indicate that decisions females make regarding how many eggs to place in a site can be modified by temperature, and it is expected that these decisions have consequences for offspring. Larval aggregation is beneficial due to the fact that raised temperatures, resulting from the formation of maggot masses, can accelerate development (Baxter and Morrison 1983; Catts 1992; Catts and Goff 1992; Ireland and Turner 2006). In addition, aggregation can increase the quantity of proteolytic enzymes, which aid in resource

decomposition and facilitate feeding (Goodbrod and Goff 1990; dos Reis *et al*. 1999; Ireland and Turner 2006; Kheirallah *et al*. 2007). However, at higher temperatures, high densities of larvae may be detrimental to the offspring, as these aggregations can generate excess heat, which may raise the temperature beyond thermal tolerances for developing larvae. Additionally, at high densities, aggregation can be detrimental as intense competition for resources may result in increased development time and decreased survival rates (Goodbrod and Goff 1990; So and Dudgeon 1990; Saunders and Bee 1995; Smith and Wall 1997) as was also found in the eastern tree-hole mosquito *Ochlerotatus triseriatus* Say (Diptera: Culicidae)(Ellis 2008). Female mosquitoes preferred to oviposit in sites with lower densities of larvae and often avoided the high density sites (Ellis 2008). For blow flies, the optimal density preferred by females has not been determined, but there is most likely an ideal density for each species that may depend on abiotic conditions that interact to influence both site selection and clutch size decisions during oviposition. Our results demonstrate that temperature is an important abiotic factor that influences blow fly oviposition behaviour, yet it is likely that a combination of abiotic factors such as temperature, humidity, photoperiod and wind speed mediate oviposition by blow flies (Zurawski *et al*. 2009) and these factors should all be examined to further quantify the oviposition behaviour of the blow flies studied here.

The intense competition that blow flies face when arriving to a limited resource has driven the evolution of behaviour and decisions made by female blow flies. The carrion resource can be partitioned, both spatially and temporally, by different species arriving to the resource at different times of year, at different times throughout decomposition and by selecting different oviposition sites and modifying clutch sizes. The different optimal temperatures for egg load and differential effect of temperature on the speed of oviposition and clutch size by each of these three species may be community-structuring mechanisms that result in temporal resource partitioning mediating coexistence of these species. Further research measuring oviposition behaviour in the presence of other species and then the outcomes of these oviposition decisions is required to understand the community-level implications of these decisions.

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Table 2.1. Multivariate Analysis of Variance (MANOVA) results to determine the effects of temperature on the oviposition sites selected by *L. sericata P. regina*, and *C. vicina*. Significant effects are indicated in bold font; $\alpha = 0.01$ for all effects.

Oviposition Site	d.f.	F ratio	*p* - value
	Lucilia sericata		
Mouth	4, 25	1.72	0.177
Ears	4, 25	1.63	0.197
Face	**4, 25**	**12.01**	**< 0.001**
Neck	4, 25	0.49	0.741
Legs	4, 25	0.33	0.855
Abdomen	4, 25	1.23	0.323
	Phormia regina		
Mouth	4, 25	3.19	0.030
Ears	4, 25	1.35	0.279
Face	4, 25	3.17	0.031
Neck	**4, 25**	**6.20**	**0.001**
Legs	4, 25	3.35	0.025
Abdomen	4, 25	3.33	0.031
	Calliphora vicina		
Mouth	4, 25	0.40	0.807
Ears	4, 25	3.92	0.013
Face	**4, 25**	**5.74**	**0.002**
Neck	4, 25	1.00	0.426
Legs	**4, 25**	**6.76**	**< 0.001**
Abdomen	4, 25	1.00	0.426

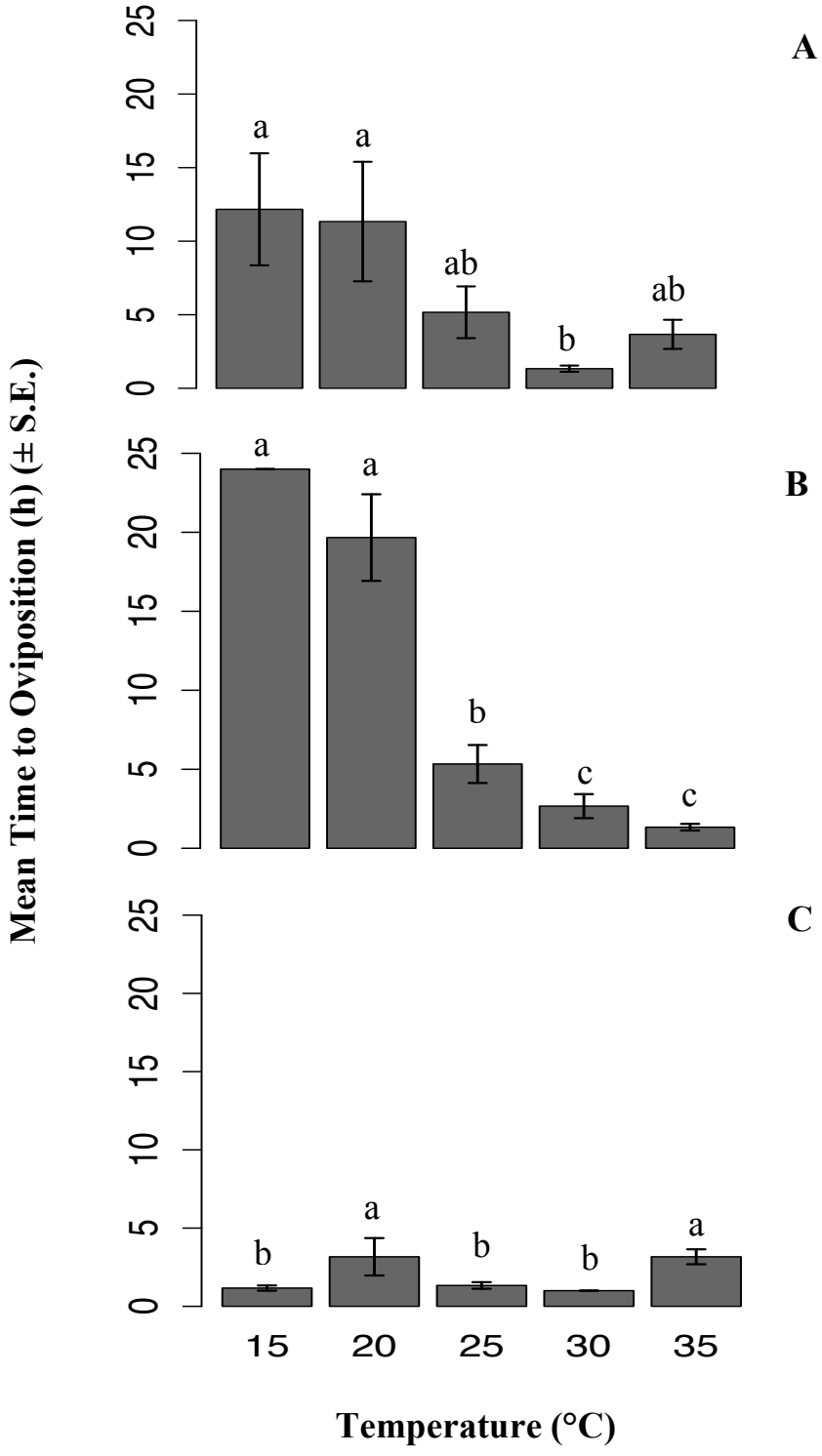

Figure 2.1. The mean time (± S.E.) until the first oviposition event for (A) *L. sericata*, (B) *P. regina* and (C) *C. vicina*. Temperature had an effect on oviposition time for *L. sericata* ($F_{4, 25} = 5.55$, $p = 0.002$), *P. regina* ($F_{4, 25} = 58.49$, $p < 0.001$) and *C. vicina* ($F_{4, 25} = 5.63$, $p = 0.002$). Means with the same letter are not significantly different.

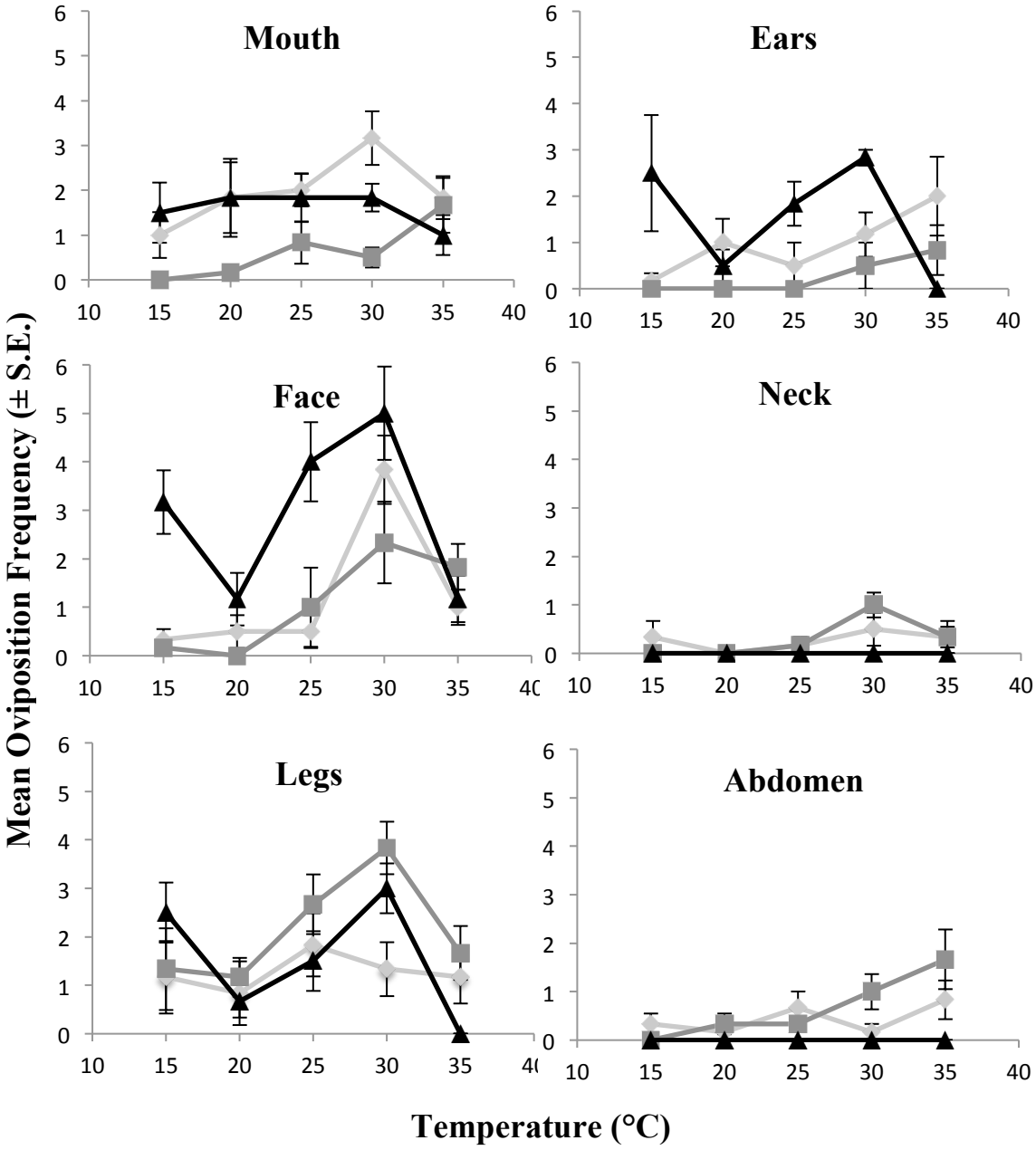

Figure 2.2. Interaction between temperature and species treatment on the mean (± S.E.) oviposition frequency at each site on pig carcasses. ◆ : *L. sericata*, ■ : *P. regina*, ▲: *C. vicina*. Oviposition frequency was calculated as the number of oviposition events that occurred at each site over the observation period. There were no oviposition events by *C. vicina* on the neck and abdomen in any trials over the temperature range tested.

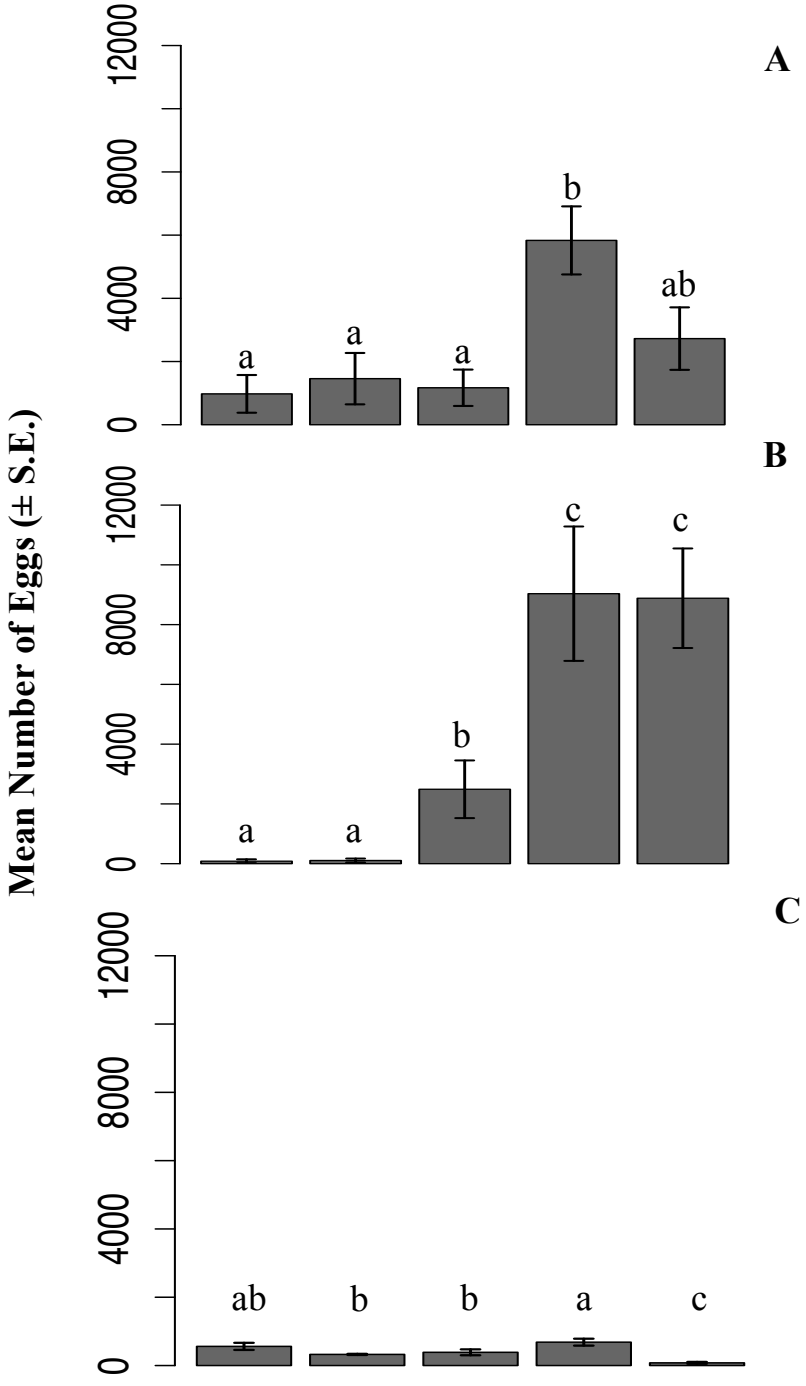

Figure 2.3. The mean number of eggs (± S.E.) deposited by 100 females in each cage of (A) *L. sericata*, (B) *P. regina* and (C) *C. vicina*. Temperature had an effect on egg number for *L. sericata* ($F_{4, 25} = 4.91$, $p = 0.004$), *P. regina* ($F_{4, 25} = 22.98$, $p < 0.001$) and *C. vicina* ($F_{4, 25} = 12.52$, $p < 0.001$). Means with the same letter are not significantly different.

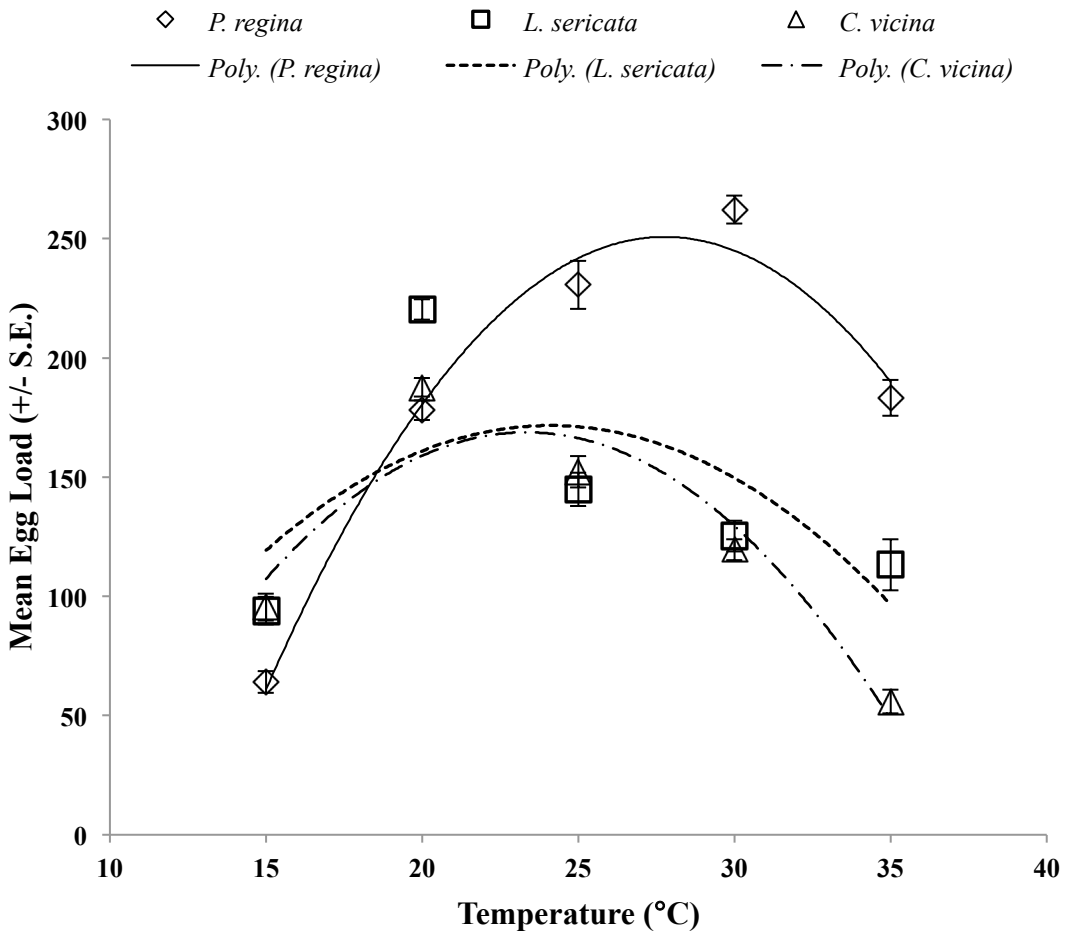

Figure 2.4. Mean (± S.E.) egg load for each species over the temperature ranges tested. Temperature had a significant effect on egg load (GLM, poisson, $z = 35.57$, $p < 0.001$). For *P. regina* ($y = -1.16x^2 + 64.62x - 646.5$), *L. sericata* ($y = -0.63x^2 + 30.45x - 195.3$) and *C. vicina* ($y = -0.88x^2 + 41.26x - 312.6$).

CHAPTER 3
SPATIAL AGGREGATION OF *PHORMIA REGINA* (DIPTERA: CALLIPHORIDAE) OVIPOSITION IS MEDIATED BY TEMPERATURE AND THE PRESENCE OF HETEROSPECIFICS

3.1 Introduction

Resources such as carrion, fruit, fungi and dung represent ephemeral resources that many insects select as breeding and oviposition sites. Carrion colonizing flies deposit eggs onto carcasses (Smith 1986; Byrd and Castner 2010) which can be hazardous environments for the developing larvae. Eggs and larvae have a greater chance of desiccation and attack from predators and parasitoids when they are exposed on carrion surfaces. Given these challenges, female blow flies (Diptera: Calliphoridae) should select oviposition sites that contain nutrient rich resources that protect their larvae from natural enemies and desiccation. The sites preferred by female insects for oviposition often determine the performance of their larvae and therefore females should oviposit on sites that maximize the performance of their offspring (Jaenike 1978). It has been previously demonstrated that blow fly females often deposit eggs in natural orifices (Smith 1986; Byrd and Castner 2010) and body folds (Archer and Elger 2003). Due to decomposition and larval feeding, the locations for oviposition may change over time. The arriving flies assess the resource for oviposition sites based on suitability of the sites for their developing offspring, rather than merely having a consistent preference for particular sites (Archer and Elger 2003).

Because females of different blow fly species deposit their eggs on multiple sites on carrion, it can lead to collective oviposition and the spatial aggregation of eggs (Byrd and Castner 2010; Chapter 2) particularly when certain species prefer to deposit their eggs in locations where a large number of eggs or females are located (Barton-Browne 1958; Barton-Browne *et al*. 1969; Brodie *et al.* 2015). Collective oviposition and aggregation may be beneficial for flies as it should indicate the suitability of a resource (Collins and Bell 1996; Jiang *et al*. 2002), enhance the development of larvae by reducing desiccation (Stamp 1980) and enhance digestion of the resource through shared salivary

enzymes (dos Reis *et al*. 1999; Charabidze *et al*. 2011). Additionally, aggregation of larvae can result in accelerated growth due to elevated temperatures resulting from maggot mass formation (Turner and Howard 1992; Ireland and Turner 2006; Kheirallah *et al*. 2007). Yet, there is certainly an optimal density of larvae, above which the negative impacts of competition may result in increased mortality, decreased adult size and longer developmental rates (Ullyett 1950; Goodbrod and Goff 1990; Smith and Wall 1997; Kheirallah *et al*. 2007). Species competing for carrion resources often face exploitative competition, where the consumption of the resource by one species limits the resource for another species (Denno and Cothran 1976). In addition, competing species may engage in interference competition, where access to the limited carrion resource by one species is reduced by another. For ovipositing female blow flies, this can be observed in space available for oviposition. If one species occupies optimal areas, this makes these sites less available for the next arriving species (VanLaerhoven 2015). If high densities of offspring are detrimental (Ellis 2008), then choosing different oviposition sites from other females may be a form of spatial resource partitioning.

Thus, in addition to the oviposition site selected, clutch size decisions made by females can influence offspring survival, particularly on ephemeral resources with numerous females laying clutches simultaneously (Parker and Begon 1986; Ives 1989; Goubault *et al.* 2007). Whereas larger clutch sizes have the potential to produce a greater number of offspring, these offspring may have smaller adult size and reduced fitness due to competition of developing on a limited, ephemeral resource (Goubault *et al*. 2007). Females face many trade-offs where reproduction is costly and the female must determine how much to invest in a reproductive event to maximize the reproductive success over her lifetime (Stearns 1977; Reznick 1985). For organisms that lay multiple clutches in their lifetime, females must also decide how much energy to allot for each breeding cycle in order to maximize her fitness (Forsman 2001). In this case, there may be an additional trade-off between clutch size and clutch interval (Forsman 2001). Forsman (2001) found that female grasshoppers *Tetrix subulata* L. (Orthoptera: Tetrigidae) that were kept in warm conditions not only oviposited faster, but had less time between each clutch laid than females in colder environments. This indicates that

abiotic factors, such as temperature, may also result in differential reproductive performance for female insects (Forsman 2001).

Temperature can affect geographic and seasonal distribution of poikilotherms such as blow flies (Anderson 2001) through physiological impacts on immature development, as well as larval and adult behaviour (Grassberger and Reiter 2001; Donovan 2006; Gomes and Von Zuben 2009). Over the range of 15-35°C, blow fly species differentially change oviposition behaviour by changing their time to oviposition, the location in which they oviposit on carrion and the number of eggs females are depositing (Chapter 2). Physiologically, each blow fly species has different optimal temperatures for egg load (Chapter 2). Since the blow fly *Phormia regina* Meigen is found throughout the year in southern Ontario, together with *Calliphora vicina* Robineau-Desvoidy in the spring/fall and with *Lucilia sericata* Meigen in the summer, we utilized *P. regina* to test the influence of temperature and presence of heterospecific eggs on oviposition behaviour. Here, oviposition behaviour included time to first oviposition event, the sites selected for oviposition as well as the total number of eggs deposited by *P. regina*. These data were utilized to calculate relative strengths of inter and intraspecific spatial aggregation.

Based on previous observations of *P. regina* oviposition behaviour (Chapter 2), we expected that as temperature increased, time to oviposition would decrease, number of eggs deposited would increase and sites selected for oviposition would be the legs and abdomen (Rosati 2014; Pacheco 2015; Chapter 2). This species demonstrated an increased egg load with increasing temperature, which resulted in a greater number of eggs deposited on pig carcasses at higher temperatures (Chapter 2). To test the influence of heterospecific eggs, either *L, sericata* or *C. vicina* were allowed to oviposit for 24 hours prior to introducing *P. regina* to the resource. If *P. regina* is not influenced by the presence of a heterospecific's previous colonization, the oviposition behaviour of *P. regina* should be consistent with what is observed when *P. regina* oviposits in the absence of heterospecifics. If secondary colonization after heterospecifics has a positive effect, *P. regina* will oviposit more quickly and will deposit more eggs when in the presence of heterospecific eggs. If secondary colonization has a negative effect, *P. regina* will exhibit slower oviposition and lower egg deposition in the presence of heterospecific

eggs. Additionally, if *P. regina* colonization is positively affected by the presence of heterospecific eggs, *P. regina* will shift their oviposition site selections to mimic those of the heterospecific colonizers and choose to aggregate their eggs with the other species. If *P. regina* is unaffected, site selection preferences will not differ from those of *P. regina* when colonizing alone. If *P. regina* is negatively affected by the presence of heterospecific eggs, *P. regina* will avoid locations with heterospecific eggs. There are documented examples of *P. regina* facilitation due to the presence of other species (Rosati 2015; Pacheco 2015), indicating that *P. regina* should oviposit faster, deposit more eggs and oviposit closer in the presence of heterospecific eggs.

3.2 Materials and Methods

3.2.1 Colony Maintenance

Blow fly colonies maintained at the University of Windsor were used to acquire eggs of *P. regina*, *L. sericata* and *C. vicina*. All colonies were initiated with wild caught females, collected in Windsor, Ontario, Canada and were stored in 46 x 46 x 46 cm cages (Bioquip 1450C aluminum collapsible cage). Water and sugar were added *ad libitum* and an oviposition substrate of 40g of fresh pork liver was provided to obtain large egg masses (approximately 1000 eggs or larger), which were removed and distributed into 1L rearing jars with fresh liver and wood shavings as a pupation medium. Each jar was then sealed with woven landscape fabric that is permeable to allow gas exchange (Quest Brands Inc., Item ID: WBS 50) and metal rings. Larvae were provided with fresh liver as needed during development. After adult emergence, flies were cold sedated (Ricker *et al*., 1986) and sorted intro treatment cages. All cages received 100 females and 50 males of one species, which allowed for all females to have access to the resource to oviposit. Male blow flies mate with multiple females and the density used ensured that all females would be mated. In addition, this density ensured maximum reproductive rates and survival and minimized density dependent effects (Moe *et al*. 2002). . All species and temperature treatments were repeated six times. Treatment cages were assigned to treatments of: *P.*

regina alone, *L. sericata* as a primary colonizer then *P. regina* as a secondary colonizer or *C. vicina* as a primary colonizer then *P. regina* as a secondary colonizer.

3.2.2 Experimental Design

Treatment cages were assigned to one of five temperatures (15°C, 20°C, 25°C, 30°C, 35°C) and were placed into a programmed growth chamber (Conviron Adaptis A1000) set to the appropriate temperature with a photoperiod of 16:8 (L:D) and 50% relative humidity. During the first five days of the experiment, 50 g of fresh pork liver was provided to flies in each treatment cage to encourage spermatogenesis and ovarian development (Erzinçlioğlu 1996; VanLaerhoven and Anderson 2001). A fetal pig on an aluminum tray was placed into each primary colonizer cage (*L. sericata* or *C. vicina*) as well as cages with *P. regina* colonizing alone, on day six and left for 24h. After this time period, the fetal pig was removed from the primary colonizer cage and transferred into the secondary colonizer (*P. regina*) cages and left for an additional 24 h. Observations were made every hour during daylight hours for the duration of the experiment. Observations included the time of first oviposition event as well as the site selected for oviposition events. Sites included the mouth, ear/nostril, face, neck, legs and abdomen.

3.2.3 Egg Number Estimations

After each 24 h observation period the pigs were removed from the treatment cages and each egg mass was photographed using a Nikon D70 camera and AF Micro-Nikkor 60 mm f/2.8D lens and 15cm ruler for scale. The measurements of each egg mass followed those described by Rosati *et al*. (2015), but will be described briefly here. For each section of an egg mass that had different depth, depth measurements were recorded. Using ImageJ, the surface area of each egg mass was measured and this information was used to calculate volume of each mass (Rosati *et al*. 2015). The volumes were then included in species-specific regression equations to estimate the number of eggs in each egg mass (*L. sericata*: $y = 0.34785 + 0.99974x$; *P. regina*: $y = 0.24706 + 1.02851x$; *C. vicina:* $y = 0.3426 + 0.99603x$) (Hans *et al*., submitted; Rosati *et al*. 2015).

3.2.4 Aggregation Models

The aggregation model of coexistence quantifies intraspecific and interspecific aggregation over patches. The index J_{ab}, quantifies intraspecific aggregation as the increase in the number of conspecifics compared to a random distribution (Ives 1988; Fiene *et al*. 2014); $J_a = \{[\Sigma\, ni\ (ni-1)/(NL)] - N\}/N$, where n represents the eggs in a site i, L is the number of sites, N is the total number of eggs collected from all patches on a pig carcass. Values of J_a indicate intraspecific aggregation, where a more positive value indicates greater intraspecific aggregation and values closer to 0 indicate random distribution.

The aggregation index C_{ab} was used to examine interspecific aggregation, or the extent that aggregation results in an increase in the expected number of species b when it encounters an individual from species a (Ives 1988); $C_{ab} = \{[\,\Sigma\, ni\ mi/(NL)] - M\}/M$, where n_i and m_i represent the number of eggs from species n and m, in each site on a pig carcass, L is the total number of sites, and N and M represent the total number of eggs collected from all sites (L) on a pig carcass. Negative values of C_{ab} indicate a negative association between heterospecifics.

To examine coexistence of heterospecifics, Ives (1988) defines the "relative strength of competitor aggregation" with $A_{ab} = [(Ja+1)(Jb+1)]/(Cab+1)^2$. Interspecific aggregation is greater than intraspecific aggregation if the value of A_{ab} is less than 1.0, where a value greater than 1.0 indicates that intraspecific aggregation is stronger (Ives 1988; Fiene *et al*. 2014). This simply indicates the degree of intra versus interspecific aggregation but does not determine whether the interaction is competitive or facilitative.

3.2.5 Statistical Analyses

All analyses were completed in R 3.1.1(R Project for Statistical Computing http://www.R-project.org/). Data were natural log transformed to meet the assumptions of parametric testing. Analyses were conducted for each species combination (*P. regina* after *L. sericata* and *P. regina* after *C. vicina*) to determine if the presence of

heterospecific eggs influenced time to oviposition, site selection and total egg number of *P. regina* compared to when *P. regina* colonized a pig carcass alone.

The data for time to first oviposition event was analyzed using a two-way ANOVA to examine the effect of temperature, species or the interaction of temperature and species on time to oviposition. The relationship between temperature and time to first oviposition event was determined for each species combination using linear regression analyses.

To examine the effects of temperature, species, and the interaction of temperature and species on the sites selected for oviposition by *P. regina*, a two-factor MANOVA (manova function) was used. The oviposition site data was ln(site count +1.5) transformed in order to meet the assumptions of parametric testing. All significant MANOVA results were followed with a one-way MANOVA and ANOVA in order to determine how site selection was influenced by temperature or species separately. To determine significance, we compared p values to $\alpha = 0.01$, to adjust for multiple tests. The effect of temperature, species and the interaction of temperature and species on the total egg number were determined using a two-way ANOVA. The relationship between temperature and total egg number was determined for each species combination using linear regression analyses.

3.3 Results

The presence of heterospecifics, temperature and the interaction of these factors influenced the amount of time until the first oviposition event by *P. regina* when arriving after *L. sericata* ($F_{4, 50} = 17.57$, $p < 0.001$; Table 3.1). Time to first oviposition event was faster in the presence of *L. sericata* and faster as temperature increased from 15°C until after 25°C, at which point, as temperature increased, *P. regina* oviposition slowed down and was slower in the presence of *L. sericata* than when on its own ($p < 0.001$, $R^2 = 0.69$; Figure 3.1). This same trend was observed in the presence of *C. vicina* eggs as temperature increased ($F_{4, 50} = 7.96$, $p < 0.001$; Table 3.1) such that time to first oviposition event was faster in the presence of *C. vicina* eggs ($p < 0.001$, $R^2 = 0.68$) as

temperature increased from 15°C to 25°C but after 25°C, *P. regina* oviposited slower in the presence of *C. vicina* eggs than when on its own (Figure 3.1).

Temperature and the presence of heterospecific eggs changed the oviposition site selection of *P. regina* (MANOVA: Wilk's $\lambda = 0.304$, $F_{8,75} = 2.68$, $p < 0.001$). Temperature had an effect on *P. regina* oviposition choices when *L. sericata* eggs were present (MANOVA: Wilk's $\lambda = 0.097$, $F_{4,25} = 2.81$, $p < 0.001$), but not when *C. vicina* eggs were present (MANOVA: Wilk's $\lambda = 0.236$, $F_{4,25} = 1.93$, $p = 0.02$). When ovipositing in the presence of *L. sericata* eggs, *P. regina* oviposited more frequently on the legs at temperatures between 20-25°C (ANOVA $F_{4,25} = 5.19$, $p = 0.003$; Figure 3.2; Table 3.2) and as temperature increased, *P. regina* selected the mouth, ears and legs for oviposition, with the mouth and ears being sites previously colonized by *L. sericata*. Across all temperatures, *P. regina* demonstrated a preference for ovipositing on the legs when *C. vicina* eggs were present on this site (Figure 3.2).

The number of eggs deposited by *P. regina* depended on an interaction of temperature and the presence of heterospecific eggs ($F_{4, 50} = 6.84$, $p < 0.001$; Table 3.3). Temperature had a significant influence on the total number of eggs deposited by *P. regina* when colonizing after *L. sericata* ($p < 0.001$) such that as temperature increased, *P. regina* laid more eggs in the presence of *L. sericata* eggs until 25°C, at which point *P. regina* deposited fewer eggs in the presence of *L. sericata* eggs compared on its own (Figure 3.3). There was also an interaction between temperature and presence of heterospecific eggs on the number of eggs deposited in the presence of *C. vicina* eggs ($F_{4, 50} = 10.73$, $p < 0.001$; Table 3.3). As temperature increased, *P. regina* laid more eggs in the presence of *C. vicina* eggs, but after 20°C, *P. regina* laid fewer eggs in the presence of *C. vicina* eggs compared to on its own ($p < 0.001$; Figure 3.3).

Heterospecifics influenced the oviposition site selection and aggregation of eggs of *P. regina*. When *P. regina* was in the presence of *L. sericata* or *C. vicina*, interspecific aggregation was greater than intraspecific aggregation at all sites where oviposition occurred for both species, between 15-25°C (Table 3.4). Above this temperature, intraspecific aggregation was greater than interspecific aggregation of eggs when *P. regina* was in the presence of *L. sericata* or *C. vicina* (Table 3.4).

3.4 Discussion

Coexistence of multiple species on ephemeral resources may be sustained by priority effects (Denno 1975; O'Flynn 1983; Schoenly 1992). The first arriving species may have a competitive advantage due to the ability to select the most beneficial sites and utilize the resource without the effects of interspecific competition (Beaver 1984). This has been demonstrated on many resources, such as mushrooms (Shorrocks and Bingley 1994), fruit (Atkinson and Shorrocks 1977), and carrion (Brundage *et al*. 2014; Kneidel 1983). In *Drosophila*, initial colonizers have an advantage in arriving first, whereas subsequent colonizers often face negative consequences including increased mortality, slower development and smaller size (Shorrocks and Bingley 1994). In carrion systems, initial colonizers may have an advantage if their offspring can consume a large portion of the resource before other species arrive (Hanski and Kuusela 1977).

Although priority effects are often viewed as having negative consequences for the later arriving species, these interactions may have facilitative effects as well. Previous research has demonstrated that for the blow fly *P. regina*, offspring have greater survival rates and larger adult body size when arriving after or at relatively the same time as *L. sericata* (Rosati 2014). Facilitation may be due to egg clustering to reduce desiccation (Stamp 1980) and predation, and in the larval stage may be due to benefits of increased temperatures in a larval mass to increase development rate (Turner and Howard 1992; Ireland and Turner 2006; Kheirallah *et al*. 2007) and sharing of salivary enzymes, as some species may require modification of the resource for more efficient processing (Heard 1994; Hodge *et al.* 1996; Charabidze *et al*. 2011).

The clutch size decisions made by females contribute to the larval density present on the resource. For blow flies, high densities may be beneficial at lower temperatures, where these aggregations can generate heat, which allows for survival and increased rate of development for the larvae present in these masses (Catts 1992; Catts and Goff 1992; Ireland and Turner 2006). However, if females lay large clutches and the resulting larval aggregations are developing in periods of high temperatures, high larval density may be detrimental for the larvae, as the temperatures they experience while in the larval aggregation may extend beyond their thermal tolerances. In order to limit the mortality of

offspring, theories on egg desiccation dictate that the clustering of eggs limits exposure to natural enemies and aridity (Stamp 1980). The position and arrangement of eggs within a layered mass offers protection from desiccation and increases survival (Clark and Faeth 1998). It is possible that abiotic cues of temperature and relative humidity are interacting to determine where female blow flies choose to oviposit.

Attraction of females to particular sites may be due to the presence of heterospecific eggs (Brundage 2012) or microbial communities on the eggs (Lam *et al*. 2007). Brundage (2012) fond that for the blow flies *Chryromya rufifacies* Macquart and *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae), conspecific and heterospecific eggs mediated the oviposition behaviour of gravid adults, with attractant properties of the eggs changing with age and microbial communities. Indeed, previous studies indicate that some species are more attracted to sites where eggs are located rather than sites that are unoccupied (Bryant 1970; Barnard and Geden 1993; Brundage 2012; Brodie *et al*. 2015). These decisions may be affected by cues from aggregation of eggs as if this is beneficial to the offspring and provides protection and promotes survival (Clark and Faeth 1998).

There are two possible ways of interpreting indices of intra versus interspecific aggregation depending on whether facilitation or competition is the predominate outcome of the interaction with heterospecifics. If *P. regina* is facilitated by other species, then it would be expected that it would aggregate its eggs more with heterospecifics. Alternatively, if competition is the predominate outcome, then *P. regina* should avoid heterospecific eggs and aggregate with conspecifics. Based on the results of this study, the outcomes of species interactions may be competitive or facilitative depending on temperature. If there is a trade-off between the impact of competition for resources on the one hand and facilitation through reduction of egg mortality due to desiccation or increased rate of development due to larval mass or more efficient nutrient extraction due to shared salivary enzymes on the other hand, it appears to switch at 25°C.

At and below 25°C, *P. regina* oviposited faster (4-15 h), selected oviposition sites that were previously colonized by other species, such as the face and mouth (after *L. sericata*) and the legs (after *C. vicina*), and laid more eggs that were aggregated with the other species when arriving after *L. sericata* or *C. vicina* compared to when *P. regina* was

alone. These results suggest that at or below 25°C, *P. regina* is facilitated by *L. sericata* or *C. vicina*. However, it will be important to determine if aggregation with heterospecific eggs at and below 25°C actually results in an increase in the resulting offspring size, lower mortality during development or faster development for *P. regina* to conclude that this is facilitation.

At 25°C, there appears to be a switch in the outcome of the interaction to competition, indicated by slower oviposition by *P. regina* in the presence of other species compared to on its own, and aggregation of its eggs more with conspecifics instead of heterospecifics in terms of site selection and number of eggs laid. It will be important to determine if aggregation with heterospecific eggs above 25°C results in increased mortality during development, smaller adult size or longer development times for *P. regina.*

The results of oviposition behaviour in this study demonstrate that both abiotic and biotic conditions influence the oviposition behaviour of the blow fly *P. regina*, providing some much needed insight into potential mechanisms of coexistence within this diverse community of decomposers. Temperature appears to mediate the outcomes of species interactions with *P. regina*, suggesting a switch from facilitation to competition around 25°C, resulting in changes in time to first oviposition and degree of aggregation with heterospecifics or conspecifics in both site selection and clutch size decisions. This strategy may ensure that *P. regina* offspring have increased survival at lower temperatures than they would on their own, yet at higher temperatures, when offspring survival is more likely, *P. regina* chooses to aggregate its eggs with conspecifics and lessen competitive interactions with other species. This change in resource partitioning and spatial aggregation based on temperature may contribute to the coexistence of *P. regina* with other members of this community. It is likely other community members employ different strategies as we observed in Chapter 2. Exploring the oviposition behaviour of other blow flies in the presence of heterospecifics at different abiotic conditions and under natural conditions is an important next step to understanding the structure of this community.

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Table 3.1. Analysis of Variance (ANOVA) results to determine the effects of species, temperature and the interaction of these effects, on the time to first oviposition event for *P. regina* when arriving secondary to *L. sericata* or *C. vicina*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects.

Effect	d.f.	F ratio	*p* - value
Phormia regina after *Lucilia sericata*			
Species	1, 50	12.42	**< 0.001**
Temperature	4, 50	57.50	**< 0.001**
Species * Temperature	4, 50	17.57	**< 0.001**
Phormia regina after *Calliphora vicina*			
Species	1, 50	5.82	**0.029**
Temperature	4, 50	30.72	**< 0.001**
Species * Temperature	4, 50	7.96	**< 0.001**

Table 3.2. Multivariate Analysis of Variance (MANOVA) results to determine the effect of temperature on the oviposition sites selected by *P. regina* when ovipositing after *L. sericata or C. vicina*. Significant effects are indicated in bold font; $\alpha = 0.01$ for all effects.

Oviposition Site	d.f.	F ratio	*p* - value
Phormia regina after *Lucilia sericata*			
Mouth	4, 25	0.94	0.453
Ears	4, 25	0.67	0.665
Face	4, 25	2.19	0.099
Neck	4, 25	1.54	0.222
Legs	**4, 25**	**5.19**	**0.003**
Abdomen	4, 25	3.50	0.021
Phormia regina after *Calliphora vicina*			
Mouth	4, 25	0.75	0.567
Ears	4, 25	3.40	0.024
Face	4, 25	2.04	0.119
Neck	4, 25	2.22	0.096
Legs	4, 25	1.37	0.271
Abdomen	4, 25	0.78	0.55

Table 3.3. Analysis of Variance (ANOVA) results to determine the effects of species, temperature and the interaction of these effects on the total number of eggs deposited by *P. regina* when arriving secondary to *L. sericata* or *C. vicina*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects.

Effect	d.f.	F ratio	*p* - value
Phormia regina after *Lucilia sericata*			
Species	1, 50	9.29	**0.004**
Temperature	4, 50	7.99	**< 0.001**
Species * Temperature	4, 50	6.84	**< 0.001**
Phormia regina after Calliphora vicina			
Species	1, 50	34.45	**< 0.001**
Temperature	4, 50	11.62	**< 0.001**
Species * Temperature	4, 50	10.73	**< 0.001**

Table 3.4. The strength of intra versus interspecific aggregation (A_{ab}) across all temperatures for *P. regina* interacting with heterospecifics, *L. sericata* and *C. vicina.* Dashes indicate sites in which oviposition did not occur for both species. Interspecific aggregation is greater than intraspecific aggregation if the value of A_{ab} is less than 1.0, where a value greater than 1.0 indicates that intraspecific aggregation is stronger.

Temp (°C)	**Oviposition Site**	***P. regina* after *L. sericata***	***P. regina* after *C. vicina***
Measures of Aggregation		**A_{ab}**	**A_{ab}**
15	Mouth	0.013	-
	Ears	-	-
	Face	0.03	-
	Neck	-	-
	Legs	-	0.010
	Abdomen	-	-
20	Mouth	-	-
	Ears	-	0.019
	Face	0.002	0.004
	Neck	-	-
	Legs	0.001	0.03
	Abdomen	-	-
25	Mouth	0.002	0.004
	Ears	0.0008	0.004
	Face	0.001	0.002
	Neck	-	-
	Legs	0.01	0.02
	Abdomen	-	-
30	Mouth	0.98	-
	Ears	0.99	-
	Face	0.99	0.99
	Neck	-	-
	Legs	0.99	0.99
	Abdomen	0.99	-
35	Mouth	0.99	-
	Ears	0.99	-
	Face	0.99	0.99
	Neck	-	-
	Legs	0.99	0.99
	Abdomen	-	-

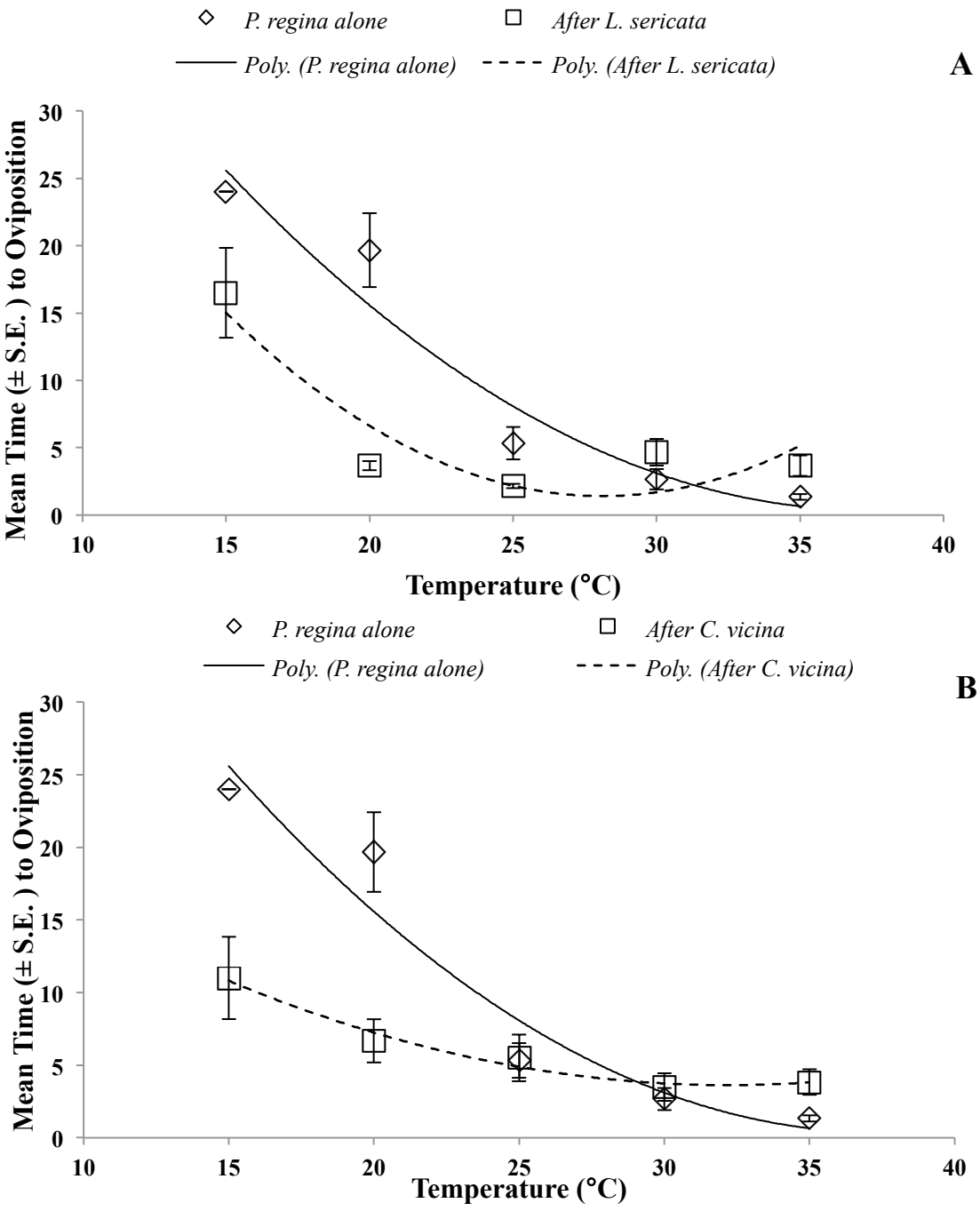

Figure 3.1. Mean time (± S.E.) to first oviposition event across all temperatures for *P. regina* colonizing alone ($y = 0.05x^2 – 3.77x + 70.79$) compared to *P. regina* arriving after *L. sericata* colonization (A) and after *C. vicina* colonization (B). Temperature had a significant influence on time to first oviposition event by *P. regina* when colonizing after *L. sericata* ($p < 0.0001$, $y = 0.08x^2 – 4.45 + 63.9$, $R^2 = 0.64$) and after *C. vicina* ($p < 0.0001$, $y = 0.02x^2 – 1.56 + 28.81$, $R^2 = 0.68$).

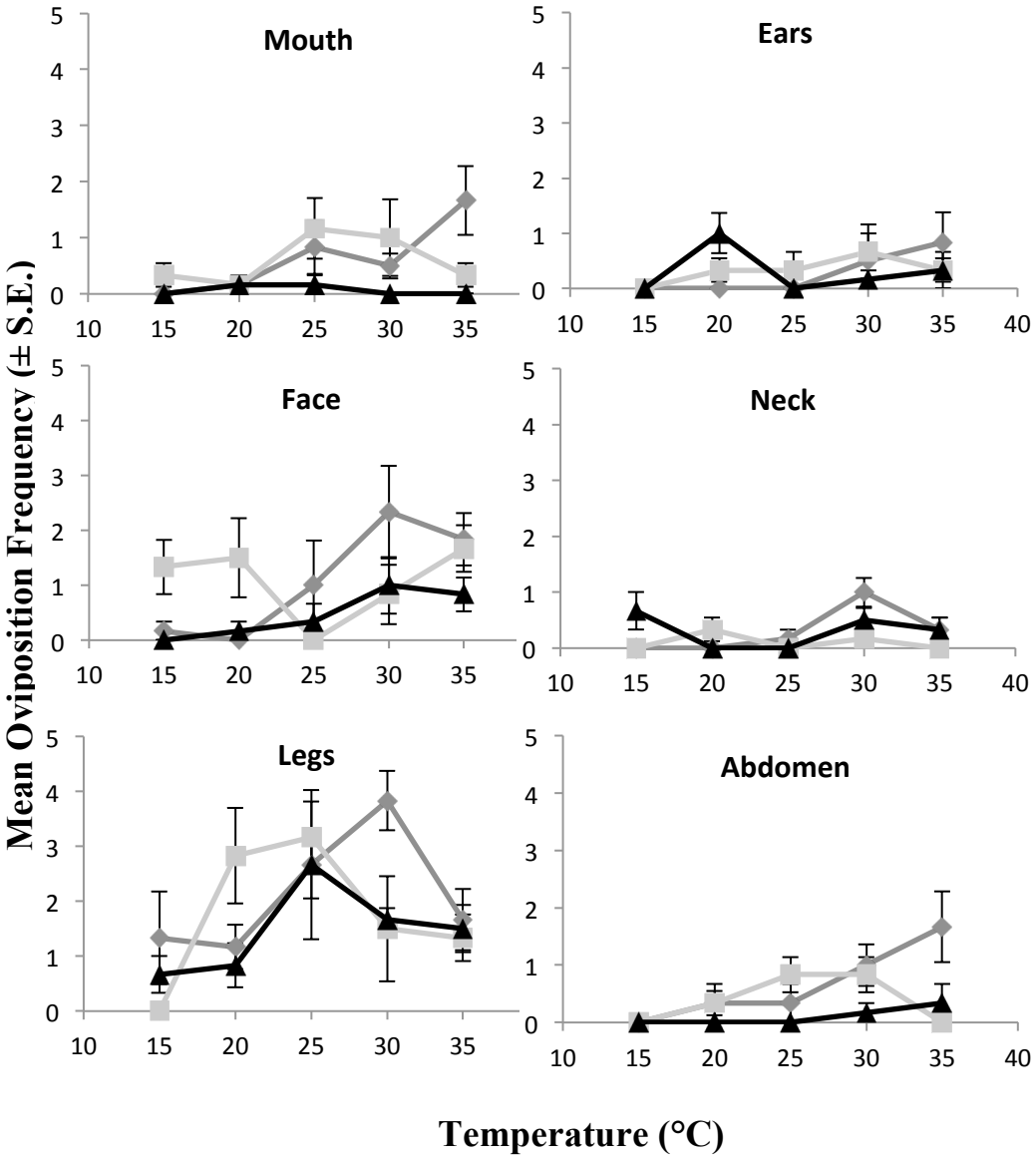

Figure 3.2. Interaction between temperature and species on the mean (± S.E.) oviposition frequency at each site on pig carcasses. ■ : *P. regina* alone, ◆ : after *L. sericata*, ▲ : after *C. vicina*. Oviposition frequency was calculated as the number of oviposition events that occurred at each site over the observation period.

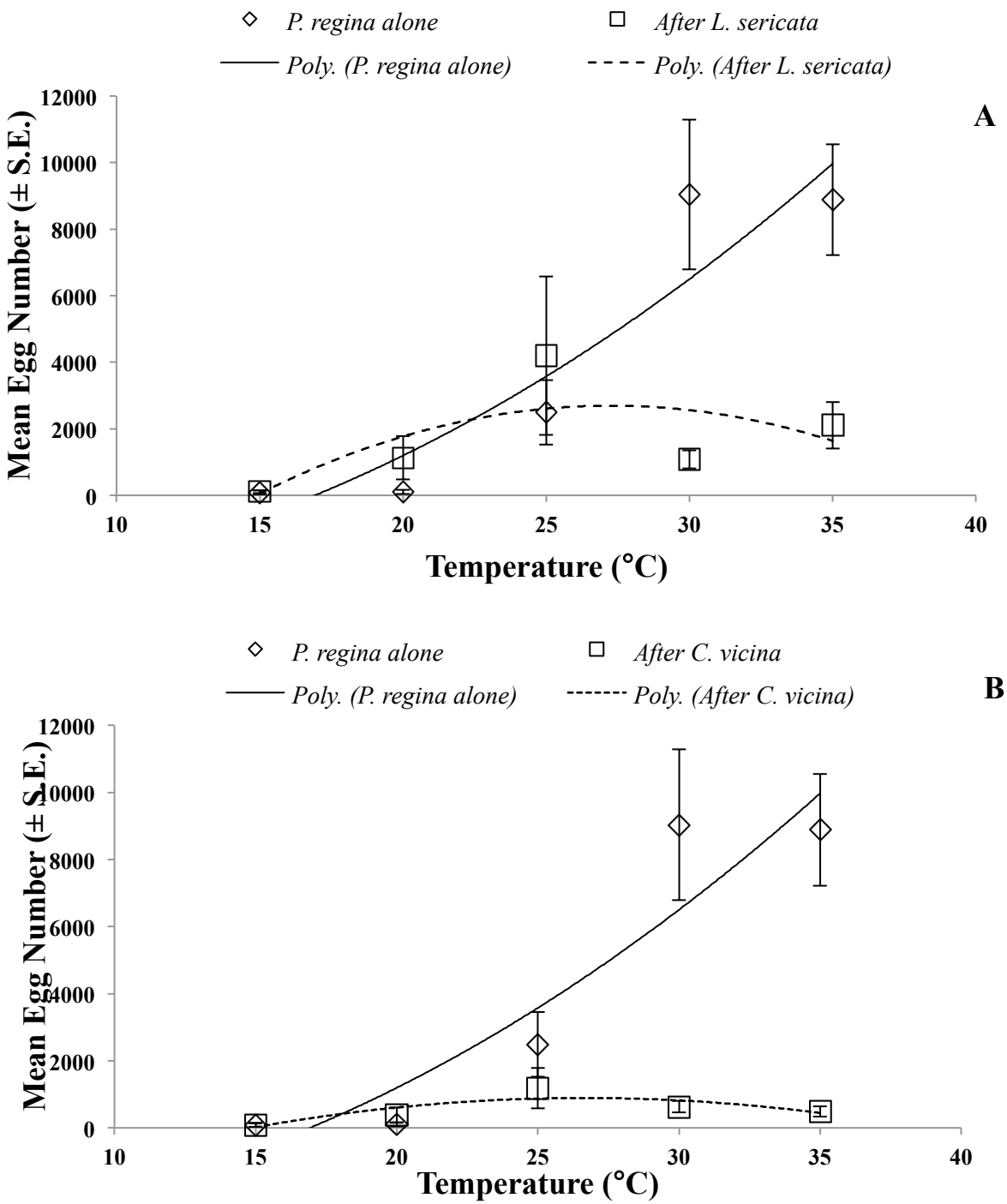

Figure 3.3. Mean (± S.E.) number of eggs deposited by 100 females in each cage across all temperatures for *P. regina* colonizing alone ($y = 10.89x^2 – 13.84x – 2883.7$) compared to *P. regina* arriving after *L. sericata* colonization (A) and after *C. vicina* colonization (B). Temperature had a significant influence on the number of eggs deposited by *P. regina* when colonizing after *L. sericata* ($p < 0.0001$, $y = -17.63x^2 + 960.38x – 10388$, $R^2 = 0.44$) and after *C. vicina* ($p < 0.0001$, $y = -6.42x^2 + 341.9x – 3665.8$, $R^2 = 0.72$).

CHAPTER 4
EFFECTS OF RELATIVE HUMIDITY ON EGG DEVELOPMENT AND HATCHING SUCCESS OF BLOW FLIES (DIPTERA: CALLIPHORIDAE)

4.1 Introduction

The life history of insects is often constrained by environmental factors. Temperature, photoperiod and relative humidity can all impact the physiology, development, behaviour and success of an organism. As mostly terrestrial organisms, insects face challenges in conserving water (Harrison *et al*. 2012). Water loss and gain is due to changes in the water content of an insect; to gain water, an insect can ingest food, drink or absorb water (Harrison *et al*. 2012). Excretion from the respiratory system and cuticle results in water loss (Harrison *et al.* 2012). Insects can choose environments that are suitable, in terms of humidity, as a way to control water loss (Harrison *et al*. 2012).

The ability to regulate water content during fluctuations in ambient conditions is influenced by relative humidity (Romoser and Stoffolano 1998). Insects living in habitats with high humidity have longer lifespans and greater fecundity than those in habitats with low humidity (Ouedraogoa *et al*. 1996). Tomberlin and Sheppard (2002) found that environmental cues, such as temperature and humidity, correlated positively with oviposition in the black soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae). In mosquitoes, oviposition is delayed by periods of low humidity and results in reduced egg numbers and decreased survival of *Aedes aegypti* (L.) (Diptera: Culicidae) (Canyon 1999). For the bamboo borer, *Dinoderus minutus* (Fabricius) (Coleoptera: Bostrichidae), a decrease in egg eclosion, or hatching, occurred at the lowest and highest relative humidity levels examined (20-85%) (Norhisham *et al*. 2013). Low relative humidity can prevent the development of an embryo or make it difficult for larval release from an egg due to a loss of lubrication (Guarneri *et al*. 2002), resulting in high mortality (Norhisham *et al*. 2013).

For blow flies (Diptera: Calliphoridae), environmental cues have important impacts on physiology and development. For example, blow fly development rates accelerate with increasing temperatures (Ames and Turner 2003). Blow flies rely on patchy resources, such as carrion, and often engage in egg clustering and aggregation,

both of which are advantageous for the survival of offspring in heterogeneous environments (Cruikshak and Wall 2002). When facing periods of extreme temperature or humidity, egg clustering limits the number of eggs that are exposed to environmental conditions and can act to limit egg mortality (Stamp 1980). Low relative humidity leads to dehydration of eggs, which can make it difficult for larvae to be released from the chorion of the egg (Norhisham *et al*. 2013). Water loss can extend development of the egg stage, inducing dormancy for some species (Zrubek and Woods 2006). On the other hand, excessive moisture due to high humidity can result in greater mortality for insect eggs (Guarneri *et al*. 2002; Norhisham *et al*. 2013). Species that can tolerate a wider range of humidity should demonstrate more successful egg hatching than species that have a more limited range. For species that have higher optimal temperatures, high levels of humidity should reflect greater egg hatching ability, whereas species that have lower optimal temperatures should have greater egg hatching at lower humidities as well.

The amount of water in the air depends on temperature, where increased temperature results in greater capacity of air to hold water (Anderson 1936). The vapor pressur deficit (vpd) is the difference between saturation vapor pressure (e_s) and actual vapor pressure (e_a) (Anderson 1936). To calculate saturation vapor pressure: $e_s = 0.611 \times 10(\frac{17.5 \times T}{T+237.3})$, where T is temperature in degrees Celsius (Melesse and Abtew 2013). The actual vapor pressure is calculated for a given relative humidity as: $e_a = es(1 - (\frac{RH}{100})$ and vapor pressure deficit (vpd) $= e_s - e_a$ (Monteith and Unsworth 2013). Vapor pressure deficit is a sensitive indicator for atmospheric water vapor conditions compared to relative humidity (Anderson 1936).

The aim of this study was to examine the influence of relative humidity on the egg eclosion time and hatching success of three forensically important blow flies that are common in Southern Ontario: *Lucilia sericata* Meigen, *Phormia regina* Meigen and *Calliphora vicina* Robineau-Desvoidy. *Lucilia sericata* is a ubiquitous species, abundant in the US and Canada, that demonstrates a preference for sunny, open habitats (Byrd and Castner 2010). This species is commonly observed at temperatures above 30°C (Smith 1986) and is typically collected in the summer months in Windsor, Ontario. *Phormia regina* is often observed in southern Canada in the spring, summer and fall months (Byrd

and Castner 2010) when temperatures are above 10°C (Byrd and Allen 2001). In contrast, *C. vicina* is a cool weather species, abundant in the northern US and Canada and can be found in the spring and fall months in this area (Byrd and Castner 2010) when temperatures range between 3.5°C (Donovan *et al*. 2006) and 30°C (Smith 1986). Due to the temporal distribution of these three species, we predicted that eggs of the warm weather species *L. sericata* would require more time to first eclosion and have reduced hatching success at low humidity. Based on the wide temperature range that *P. regina* can tolerate, we expected that this species would remain largely unaffected by changes in relative humidity and should have similar eclosion time and success over the humidity range tested. We predicted that lower humidity levels would not significantly affect the time to first eclosion or hatching success of *C. vicina*.

4.2 Materials and Methods

Eggs of *L. sericata, P. regina* and *C. vicina* were collected from laboratory colonies maintained at the University of Windsor, Ontario, Canada. The colonies were maintained at 23 ± 1°C, 60 ± 5 % RH and 12L:12D photoperiod, in aluminum cages (Bioquip 1450C collapsible cage, 46 x 46 x 46 cm) and originated from wild type females collected using liver-baited traps in Windsor. Colonies were provided with water and sugar *ad libitum*. Fresh pork liver (40g) was used as an oviposition substrate to collect eggs from the colony cages. Egg masses (5-7 mm in diameter) were carefully removed from the liver to maintain the structure of the egg mass and prevent damage to the eggs. The surface area and depth of the egg masses was measured to estimate the number of eggs per egg mass as detailed in Rosati *et al*. (2015). A one-way analysis of variance (ANOVA) was used to determine if there was a difference in the number of eggs in each egg mass for each species. Despite the egg masses being within the same overall size range across all three species, the number of eggs in each cluster was significantly different by species composition ($F_{2,216} = 213.37$, $p < 0.001$) potentially due to differences in egg size and orientation in the mass. Due to these differences, the percent of successful eclosion was used in this study.

Egg masses were then distributed into 266 mL glass containers, lined with moistened filter paper and sealed with paper towel and an elastic band. The containers were arranged into a clear plastic tray and assigned one of six relative humidity treatments: 30, 40, 50, 60, 70 and 80%. The relative humidity treatments were selected based on previous work done by Clark and Faeth (1998) and Holmes *et al.* (2012). Growth chambers (Conviron Adaptis A1000) were programmed to 25°C with a photoperiod of 16:8 (L:D) and the appropriate relative humidity level. Ten replicates of each species treatment at each humidity level were used in this study. Data loggers (HOBO U12-012, Onset, Pocasset, MA) recorded the relative humidity and temperature every 30 minutes and the mean (±SE) relative humidity and temperature in each treatment is provided in Table 4.1. Vapor pressure deficit was calculated for each temperature and relative humidity (Table 4.1) by subtracting actual vapor pressure from saturation vapor pressure, as described above.

Following the initial emergence of larvae from the eggs, observations continued every six hours for two days, as the majority of eggs that would hatch did so in this time frame (personal observation). The egg masses and neonatant larvae were submerged in 70% ethanol and stored until manual counts of larvae could be made. The time until egg eclosion and the percentage of successful eclosion were recorded for each replicate within each relative humidity treatment.

Logistic regression was used to examine the effect of relative humidity and species identity on the time to eclosion and mean percentage of successful eclosion, using the glm function in R 3.1.1(R Project for Statistical Computing, http://www.R-project.org/).

4.3 Results

Relative humidity had a significant influence on the time to eclosion for all blow fly species (GLM, poisson, $z = -16.8$, $p < 0.001$) such that as relative humidity increased, eclosion time was faster for all three species (Figure 4.1). Compared to *P. regina*, the time to eclosion was slightly slower for *L. sericata* (GLM: $z = 3.99$, $p < 0.001$) and *C. vicina* (GLM: $z = 2.27$, $p = 0.02$) as relative humidity increased (Figure 4.1).

There were differences in the hatching success of all three species due to relative humidity (GLM, binomial, $z = 40.29$, $p < 0.001$) such that *Phormia regina* had the lowest rate of hatching success compared to *L. sericata* and *C. vicina*, which had the highest rate overall (Figure 4.2). *Calliphora vicina* had relatively high hatching success (65-80%) across all relative humidities, whereas *P. regina* had hatching rates of less than 50% at low humidities, but had the highest hatching success at 70-80% (Figure 4.2). As relative humidity increased, the rate of hatching success also increased, with significant differences for *L. sericata* (GLM, binomial, $z = -2.63$, $p = 0.009$) and *C. vicina* (GLM, binomial, $z = -19.02$, $p < 0.001$) (Figure 4.2). Under the lower humidities, *L. sericata* had the lowest hatching success, but this appeared to change at 50% relative humidity, where this species experienced 70% hatching success or greater at all higher relative humidities (Figure 4.2).

4.4 Discussion

Survivorship of insects at the egg stage can be dependent on abiotic factors, with water loss becoming a critical factor for successful development and eclosion. The harmful effects of low relative humidity in this study are apparent in the low survival of eggs for *L. sericata and P. regina* eggs, which is most likely the result of desiccation. At relative humidity treatments below 50%, *P. regina* demonstrated a greater survival rate compared to *L. sericata*. Due to the array of temperatures that *P. regina* experiences in Windsor from the spring through the fall, it was expected that this species can withstand lower humidity than a summer species such as *L. sericata*, which performed better than *P. regina* at higher humidity treatments (50% and above). *Lucilia sericata* exhibited slower and less successful egg hatching at lower humidities, which is in agreement with a previously described minimum development threshold of 50% relative humidity (Davies 1947). At lower humidities, egg hatching occurs in the middle of the egg mass and eggs along the surface are often desiccated (Davies 1947). As expected, the low relative humidity treatments were not detrimental to *C. vicina* eggs, as this species often experiences lower relative humidity in Southern Ontario during the spring and fall months in Windsor.

Egg hatching rate and survival changes with humidity, not just for the blow fly species examined in this study, but for other insects as well. Black soldier flies, *Hermetia illucens* L. (Diptera: Stratiomyidae) had greater eclosion success as eggs and adults, as well as a decrease in development time, with increasing humidity. For pine caterpillars *Dendrolimus tabulaeformis* Tsai et Liu (Lepidoptera: Lasiocampidae), extreme low and high relative humidity resulted in prolonged development time and reduced hatching success (Han *et al*. 2008). The area of origin may play an important role in tolerance to desiccation (Juliano *et al.* 2002). For the mosquito *Aedes albopictus* Skuse (Diptera: Culicidae), egg mortality was strongly dependent on both relative humidity and temperature, with high egg mortality except at the highest relative humidity for this species, whereas for *Aedes aegypti* L., low mortality was observed across the range of temperatures and humidities tested (Juliano *et al*. 2002).

Differences in egg size between these three blow fly species could account, in part, for the differences in time to eclosion. *Lucilia sericata* has the smallest eggs of the three species in this study, with a mean size of 1.1 ± 0.2 x 0.33 ± 0.05 mm, whereas the largest eggs were those of *C. vicina*, with a mean size of 1.4 ± 0.2 x 0.4 ± 0.05 mm (Greenberg and Singh 1995). According to Greenberg and Singh (1995) *P. regina* eggs are of 'intermediate' size, with a mean size of 1.2 ± 0.1 x 0.3 ± 0.03mm. The variation in the egg sizes as well as the orientation of the eggs within a mass can affect the number of eggs that are present within masses as well as offer protection from desiccation (Clark and Faeth 1998).

Our results indicate that relative humidity may be an abiotic cue that influences the timing and success of egg hatching for blow flies. Eggs cannot regulate water loss, although the chorion, or eggshell provides protection (Woods 2010). However, by laying eggs in large masses, female blow flies can account for potential water losses (Ireland and Turner 2006). Egg clustering is advantageous and egg aggregation can reduce egg predation and parasitism in addition to reducing egg exposure to environmental factors such as desiccation due to low humidity (Stamp 1980). In harsh ambient conditions, an egg mass experiences desiccation of the outer layer of eggs, which then protects the eggs inside the mass and ensures the survival of the majority of the offspring (Clark and Faeth 1997). Based on our results, abiotic cues such as humidity may be driving the aggregation

of eggs by female blow flies, resulting in mixed species egg masses which may enhance the survival of the offspring. For *C. vicina*, high egg hatching success was maintained across the relative humidity ranges tested here, indicating that this species may not require large aggregations of eggs to ensure survival. For *P. regina*, however, eclosion success was more heavily influenced by humidity and this species only exhibited higher success at 70 and 80% relative humidity. Because *P. regina* may have higher egg mortality at lower humidities, females may prefer to oviposit in areas where eggs are already located to assemble aggregations of eggs and facilitate more successful egg hatching.

Studies examining the oviposition behaviour of female flies would benefit greatly from examining the influence of relative humidity and vapor pressure deficit on their choices. Based on our results, decisions regarding oviposition site selection and egg aggregation may be determined in part, by relative humidity. The relative importance of competition due to density of offspring compared to facilitation due to protection from desiccation is likely to be mediated by these abiotic conditions and partly explain clutch size decisions and the likelihood of mixed species egg masses. Furthermore, field validation studies to examine the influence of natural weather conditions with fluctuating temperatures and humidity would contribute to the knowledge of hatching success of blow flies. Due to the constraints of the equipment, the lowest humidity allowed in this study was 30%. It would be valuable to explore a lower range of humidity levels and determine the effect of such extreme conditions. In the moth, *Manduca sexta* (Lepidoptera: Sphingidae), eggs are protected by wax, which offers resistance to water loss (Woods *et al*. 2005). Additionally, around the eggs is a boundary layer of air, and the thickness of this boundary layer depends on the wind speed and the substrate used for oviposition (Woods *et al*. 2005). For insects facing fluctuating temperatures and periods of low humidity, either the wax layer or the boundary layer of air may provide additional protection.

Based on this information, the influence of relative humidity on the life history of blow flies, in terms of egg development and hatching success, can have implications for the behavioural ecology of blow flies. The oviposition behaviour of *P. regina* in the presence of heterospecifics indicated a transition from facilitation to competition at 25°C

(Chapter 3). This transition may be driven by other changing environmental conditions, such as fluctuations in relative humidity. The hatching success of offspring over a range of relative humidity indicates that for some species, low relative humidity may present a challenge for egg hatching or may delay development of the egg. For females ovipositing in low relative humidities, these environmental conditions may facilitate the aggregation of eggs to ensure greater offspring survival. For *P. regina*, the aggregation of this species eggs with heterospecifics at low temperatures and low relative humidites could result in greater success for the offspring. Not only does this information advance the field of insect ecology, but also provides information pertaining to the potential mechanisms for aggregation and coexistence of multiple species within a community. It is vital to understand the role of environmental factors that modify egg development and oviposition decisions for coexisting species.

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Table 4.1. Mean temperature and relative humidity recorded for each treatment and calculated vapor pressure deficit.

RH (%)	Mean (±SE) Temperature °C	Mean (±SE) RH (%)	Vapor Pressure deficit (kPa)
30	24.9 ± 0.06	29 ± 0.34	0.99
40	24.8 ± 0.1	41 ± 0.95	1.27
50	24.8 ± 0.01	51 ± 0.75	1.58
60	25.2 ± 0.03	60 ± 0.37	1.90
70	24.7 ± 0.05	70 ± 0.91	2.22
80	24.6 ± 0.15	81 ± 0.07	2.54

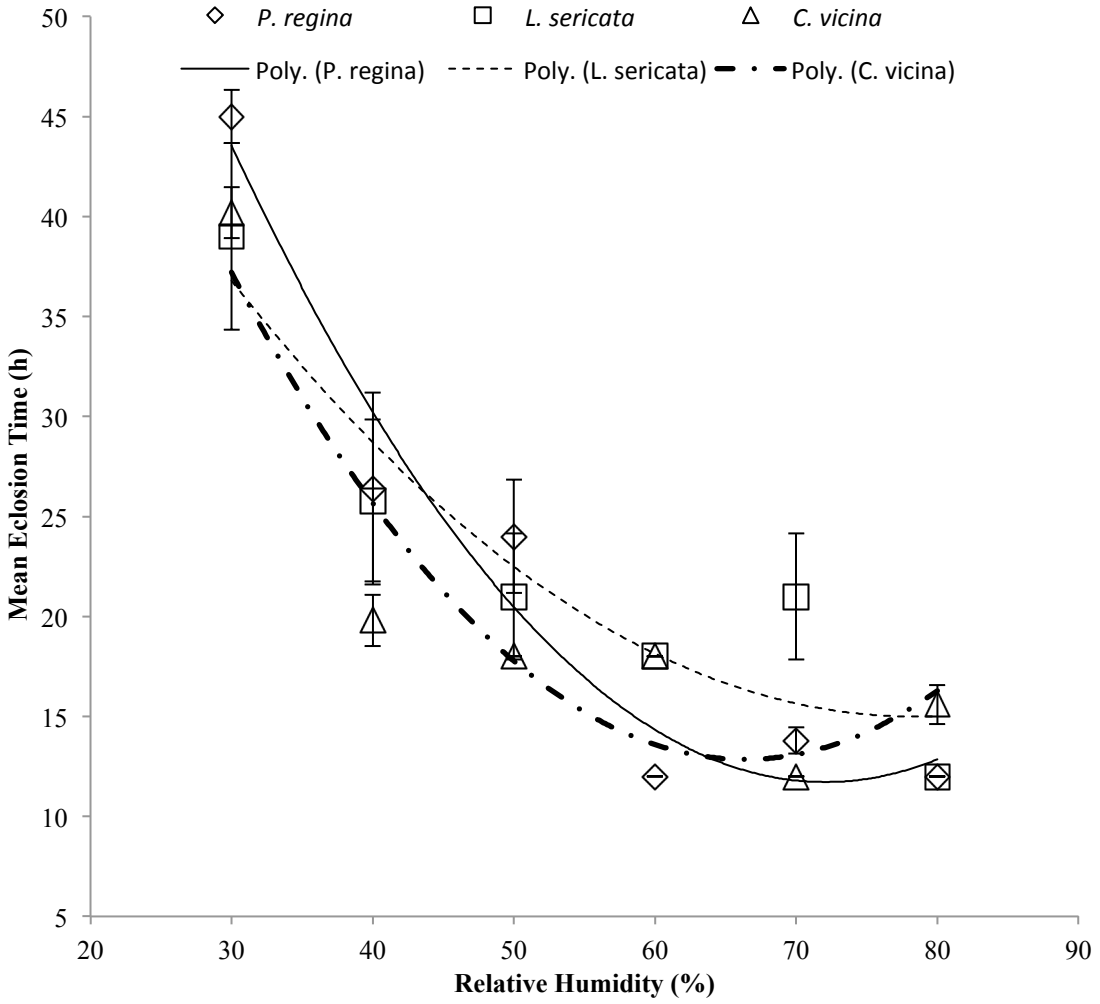

Figure 4.1. Mean (+/- S.E.) eclosion time for each species treatment over the range of relative humidities tested (30-80%). Relative humidity had a significant influence on the time to eclosion for all blow fly species (GLM, poisson, z = -16.8, $p < 0.001$). For *P. regina* ($y = 0.02x^2 - 2.59x + 105.2$, $R^2 = 0.75$), *L. sericata* ($y = 0.009x^2 – 1.46x + 72.23$, $R^2 = 0.51$) and *C. vicina* ($y = 0.018x^2 – 2.45x + 93.97$, $R^2 = 0.57$).

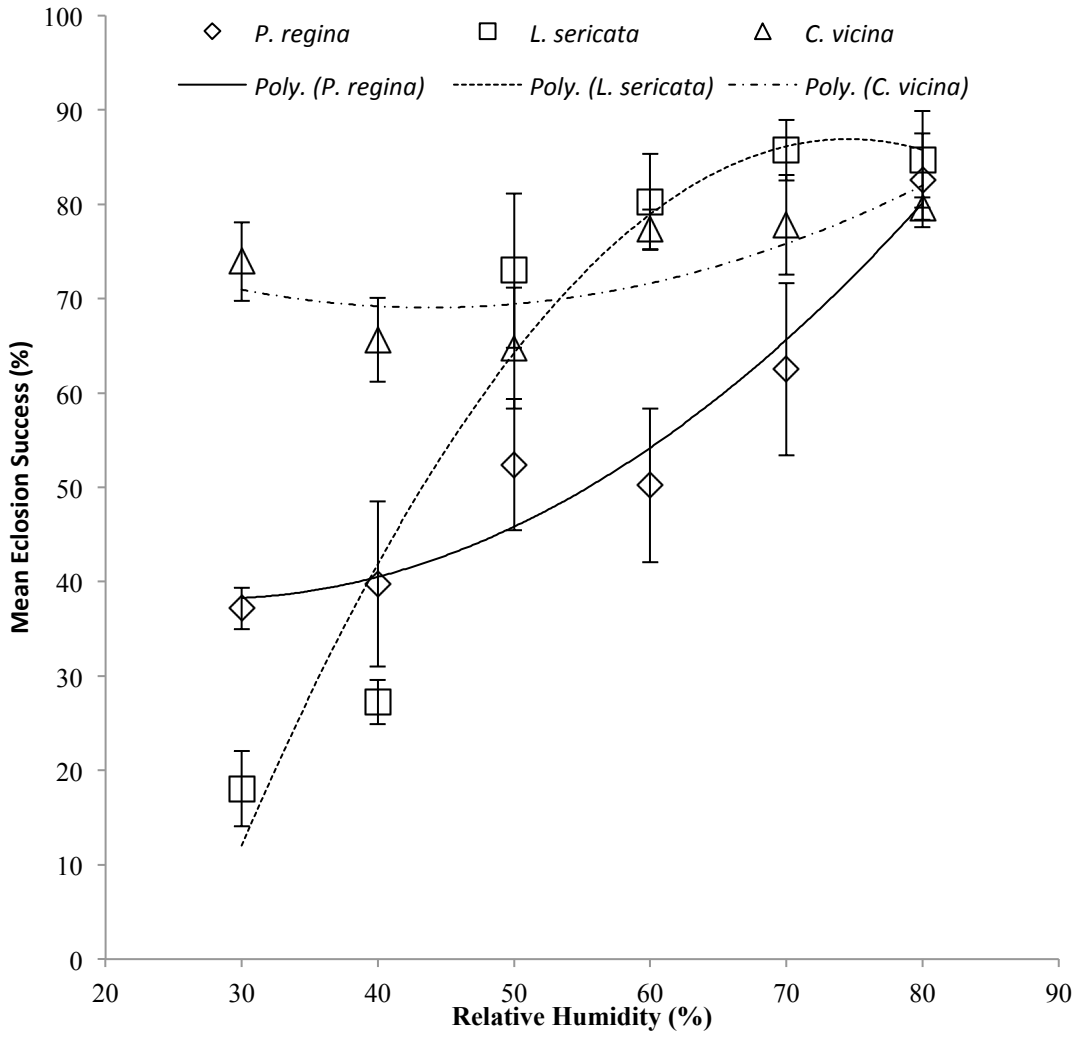

Figure 4.2. Mean percentage (+/- S.E.) of eclosion success for each species treatment over the range of relative humidities tested (30-80%). Relative humidity had a significant influence on the hatching success of all three species (GLM, binomial, $z = 40.29$, $p < 0.001$). For *P. regina* ($y = 0.015x^2 – 0.85x + 49.93$, $R^2 = 0.54$), *L. sericata* ($y = -0.047x^2 + 5.63x - 122.89$, $R^2 = 0.63$) and *C. vicina* ($y = 0.010x^2 – 0.87x + 88.08$, $R^2 = 0.58$).

CHAPTER 5
DOES OVIPOSITION BEHAVIOUR OF BLOW FLIES IN THE LABORATORY REFLECT BEHAVIOUR IN THE FIELD?

5.1 Introduction

The measurement of life history traits is crucial to understanding the population dynamics of insect species. For small, highly mobile insects, quantifying rates of activity and oviposition is difficult in a field setting. In order to evaluate these traits, controlled laboratory conditions are used to measure characteristics such as the rate of development or oviposition; however, these traits are infrequently assessed in semi-natural field conditions (Bezemer and Mills 2003; Casas *et al.* 2004). Controlled conditions and laboratory colonies may provide different results from field trials due to differences in the lab and the variable climatic conditions in the field.

Blow flies (Diptera: Calliphoridae) are used in forensic entomology to estimate the postmortem interval (PMI), or time between death and discovery of the body based on their arrival time, behaviour and subsequent immature development (Catts 1992; Tomberlin *et al.* 2011). Female blow flies are attracted to animal tissues after death and can arrive within minutes (Smith 1986; Byrd and Castner 2010). Blow flies prefer natural orifices as well as skin folds on the legs and abdomen as oviposition sites (Smith 1986; Byrd and Castner 2010; Rosati 2014; Pacheco 2015). Often, PMI estimates are based on insect behavior and development data collected under controlled laboratory conditions (Kamal 1958; Greenberg 1991; Anderson 2000), yet these data are not sufficient alone; they should be compared to data collected under field conditions in order to be applied in forensic investigations (Byrd and Castner 2001).

Under controlled abiotic conditions in laboratory settings, oviposition behaviour may be influenced by various factors, such as the presence of conspecifics or heterospecifics (Chapter 3; Yang and Shiao 2012), the presence of predators (Giao and Godoy 2007) or the structural integrity of the oviposition medium (Pacheco 2015). However, in the field, climatic conditions vary and flight activity and oviposition behavior of wild flies may also be influenced by additional factors such as wind speed

and precipitation (Catts 1992; Fink and Volkl 1995; Erzinçlioğlu 1996), solar radiation (Von Aesch *et al*. 2003) or light levels (Catts 1992; Erzinçlioğlu 1996), relative humidity (Holmes *et al*. 2012) and temperature (Byrd and Allen 2001; Zurawski *et al.* 2009). In particular, temperature affects the activity and development of blow flies (Grassberger and Reiter 2001; Ames and Turner 2003; Donovan 2006) with reports suggesting that blow fly activity ceases below 10°C (Williams and Richardson 1984), yet, solar radiation may also be an important factor during colder seasons (Von Aesch *et al*. 2003). Although blow fly activity, arrival times and oviposition site selection have been described in laboratory settings (Chapter 2; Chapter 3;Yang and Shiao 2012; Rosati 2014; Pacheco 2015) and field settings (Hanski and Kuusela 1980; Thomas and Mangan 1989; Tessmer *et al.* 1995), few studies have examined these simultaneously in order to compare observations.

The aim of this study was to compare the oviposition behaviour of three local, forensically relevant blow flies *Lucilia sericata* Meigen *Phormia regina* Meigen and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) in terms of timing of the first oviposition event, oviposition site preference and the number of eggs deposited, between controlled laboratory conditions and in semi-natural field cages. *Calliphora vicina* is active during cooler temperatures (Donovan *et al*. 2006) and arrives to carrion in the spring and fall in southern Ontario, when temperatures range between 5-30°C (Rosati 2014); *Lucilia sericata* is often active between temperatures of 20-37°C (Smith and Wall 1997; Zurawski *et al*. 2009) and arrives in the summer and early fall in Ontario (Rosati 2014). *Phormia regina* is often dominant in northern climates during the summer months, but is also tolerant of cooler temperatures with a temperature range between 10-35°C. (Hall 1948; Byrd and Allen 2001) and is present during all three seasons, fall, spring and summer in southern Ontario (Rosati 2014). Average seasonal temperatures in Southern Ontario are 2.6-14.5, 14.4-34.8 and 10.3-25.8°C, for the spring, summer and fall seasons, respectively (Environment Canada National Historical Database).

Thus, we tested the oviposition behaviour of laboratory-raised *C. vicina* and *P. regina* at 15°C in spring, of *P. regina* and *L. sericata* at 35°C in the summer and of all three species at 25°C in the fall under semi-natural conditions and with wild flies under natural conditions, comparing to the oviposition behaviour of laboratory-raised *C. vicina*,

P. regina and *L. sericata* under laboratory conditions of 15, 25 and 35°C. Based on the assumption that mean temperature is the predominate factor determining these oviposition behaviours in the absence of heterospecifics, we predicted that time of first oviposition event, oviposition site preference and the number of eggs deposited should be similar between blow flies in natural conditions and controlled laboratory conditions, testing how reliable these measures are under natural conditions (Nunez-Vasquez *et al.* 2013). In controlled laboratory conditions, oviposition occurred faster with increasing temperature for *L. sericata* and *P. regina* and more eggs were deposited by these species (Chapter 2), thus we predict oviposition rate (number of eggs laid/hour) to be faster with increasing temperature for *L. sericata* and *P. regina* in the field. For *C. vicina*, oviposition remained relatively unaffected by increasing temperature 15 or 25°C (Chapter 2), thus we predict no change in oviposition rate with temperature in the field. In order to test these predictions, we measured oviposition behaviour and calculated species-specific oviposition rates comparing between semi-natural field caged flies and lab caged flies.

5.2 Materials and Methods

Eggs were obtained from laboratory colonies of *L. sericata*, *P. regina* and *C. vicina* maintained at the University of Windsor in aluminum cages (Bioquip 1450C collapsible cage, 46 x 46 x 46 cm). All colonies originated from wild type females collected in Windsor, Ontario and were provided with water and sugar *ad libitum*. All egg masses were collected using fresh pork liver (40 g) as an oviposition substrate. When large egg masses (approximately 1000 eggs) were present, they were removed from the substrate and divided among 10 rearing containers, which were composed of a 1L mason jar with wood shavings, sealed with landscape tarp (Quest Brands Inc., Item ID: WBS 50) and a metal ring. Fresh liver was provided to the developing larvae as needed. Once adult flies emerged, they were cold sedated (Ricker *et al*., 1986) and sorted by gender by examining eye morphology (Erzinçlioğlu 1996) for placement into treatment cages. Each treatment cage received 100 females and 50 males for all species examined. The density of flies in each treatment cages ensured that there would be no damage to the adult flies due to high density of individuals. In addition, this density ensured that all females would be mated,

since males mate with multiple females and that each female would have access to the resource and an opportunity to oviposit. Density dependent effects were minimized with the density of individuals in each cae, ensuring maximum survival and reproductive rates (Moe *et al*. 2002). All species and temperature treatments were replicated three times. Fresh pork liver (50 g) was provided for each treatment cage during the first five days of the experiment to ensure ovarian development and spermatogenesis (Erzinçlioğlu 1996; VanLaerhoven and Anderson 2001); this process ensured that the female flies within the treatment cages were gravid on experimentation days.

For the laboratory observations, the treatment cages were assigned to one of three temperature treatments (15°C, 25°C, 35°C) and placed into a growth chamber programmed with a photoperiod of 16:8 (L:D), 50 ± 0.09-0.95 % relative humidity and the appropriate temperature. Each species and temperature treatment was replicated six times. Every 60 minutes data loggers (HOBO U12-012, Onset, Pocasset, MA) recorded the temperature and relative humidity. The data set provided for the laboratory trials was collected as a subset of oviposition behavior data (Chapter 2), however, the current analyses test different hypotheses.

5.2.1 Semi-Natural Field Study Site

On day six of experimentation, three treatment cages of each species were transported to the field site. These treatment cages represented semi-natural field cages, as the flies were observed in treatment cages, but were held in natural conditions at a field site. In the field experiments, the recorded temperatures and relative humidities for each set of field trials can be found in Table 5.1. Based on the seasonal preferences and temperature thresholds of the species to be studied, the semi-natural field cages were constructed to contain the species expected to arrive during each season. Trials conducted in the spring had mean temperatures close to 15°C, when it was predicted that *C. vicina* and *P. regina* would arrive, and cages containing these two species were brought to the field site. In the summer, when mean temperatures were close to 35°C, cages of *P. regina* and *L. sericata* were brought to the field site. In the fall, mean temperatures were close to 25°C, and cages of all three species were brought to the field site.

The field site was located in Ojibway Park (42.2578° N, 83.0691° W) within the

Ojibway Prairie Complex in Windsor, Ontario. This nature reserve is approximately 160 acres and is composed of various habitats, including pin oak forest, tallgrass prairie, savanna and aquatic areas. The treatment cages were placed 50 m apart (Amendt *et al*. 2010) in the transition zone between the pin oak forest and the tall grass prairie. In addition to the treatment cages, three fetal pigs (1-2 kg) were placed throughout the field site 50 m apart to attract and observe the oviposition behaviour of wild flies, and to verify the arrival of the species of interest. The wild colonized pig carcasses were secured with hexagonal wire and rebar to protect from scavenging activity. For observational studies on wild colonizing blow flies, pig carcasses were observed every 1 h during daylight hours 24 h and then at 3 h intervals for 48 h. Observations were made as to species arrival and oviposition sites selected, which included the mouth, ears, face, neck, legs and abdomen. Samples of approximately 100 eggs and larvae were collected from each oviposition site that contained eggs to identify species that colonized the carcasses. Total egg numbers present on the pig carcasses colonized by wild flies were not calculated due to the large number of flies that arrived and the uncertainty of the species identity of the egg masses making absolute quantification of each species' contribution to individual egg masses not feasible.

5.2.2 Oviposition in Controlled Settings

For both laboratory and field trials, a deceased fetal pig was removed from the freezer and thawed overnight before being placed on an aluminum tray and positioned inside each treatment cage on the sixth day of experimentation. Observations were made hourly during daylight hours for 24 h to measure the amount of time until oviposition occurred, the site selected for egg deposition and the number of eggs oviposited.

After the observation period was complete, the fetal pigs were removed and all egg masses were photographed using a Nikon D70 camera and AF Micro-Nikkor 60 mm f/2.8D lens and a 15cm ruler for scale. Measurements of depth were recorded for each egg mass at all areas of the mass with different depths. The procedure used to calculate the number of eggs is outlined in Rosati *et al.* (2015), but will be briefly outlined here. Using the photographic analysis software, ImageJ, the surface area of each egg mass was

measured. With the STRAIGHT line tool, a 10mm line was drawn and superimposed onto the ruler in each photograph. The scale in each photograph was calibrated using the ANALYZE > SET SCALE function. All egg masses were outlined at each depth using the polygon selection tool. Measurements of surface area were obtained using the ANALYZE > MEASURE function. To calculate the volume of eggs, depth measurements were multiplied by their corresponding surface area measurements and this was repeated for all egg masses (Rosati *et al*. 2015). The volumes calculated were put into species-specific regression equations (*L. sericata*: $y = 0.34785 + 0.99974x$; *P. regina*: $y = 0.24706 + 1.02851x$; *C. vicina:* $y = 0.3426 + 0.99603x$), which provided an accurate estimate of the total number of eggs within a given egg mass (Rosati *et al*. 2015; Hans *et al*. submitted).

5.2.3 Statistical Analyses

All analyses were performed in R 3.1.1(R Project for Statistical Computing, http://www.R-project.org/). Analyses were conducted separately for all three blow fly species using the mean temperature of the field trials and corresponding laboratory trial temperatures.

The data for time to first oviposition event were log transformed to meet the assumptions of normality and homogeneity of variance for parametric testing. A two-way ANOVA (aov function) was conducted for each species to examine the effect of temperature and location (laboratory, field, wild) on time to first oviposition event. Regression analyses were performed for each species to examine the relationship between temperature and oviposition time.

To examine the effects of temperature and location, and the interaction of temperature and location on the sites selected for oviposition by *P. regina*, *L. sericata* and *C. vicina*, a two-factor MANOVA (manova function) was used. To meet the assumptions of parametric testing, the oviposition site data was ln (site count +1.5) transformed. To determine how site selection for each species was influenced by temperature or location independently, all significant MANOVA results were followed

with one-way MANOVA and ANOVA. P values were compared to $\alpha = 0.01$ for MANOVA.

To compare the rate of oviposition in the lab and in the field, oviposition rate was calculated by dividing the total number of eggs on each pig carcass by the number of hours female blow flies laid eggs. These values were square root transformed to meet the assumptions of normality and homogeneity of variance for parametric testing. A two-way ANOVA (aov function) was performed to examine the difference in oviposition rate due to temperature and location for each species.

A one-way ANOVA (aov function) was used to examine the relative change in the total amount of eggs oviposited between the laboratory and caged field trials over the temperature range tested.

5.3 Results

As expected, the wild fly populations that arrived to pig carcasses in the spring consisted of *P. regina* and *C. vicina*, in the summer of *P. regina* and *L. sericata* and in the fall, of all three species.

Time to first oviposition event depended on the interaction of temperature and location for both *P. regina* ($F_{2, 30} = 27.05$, $p < 0.001$) (Figure 5.1) and *C. vicina* ($F_{2, 18} = 14.36$, $p < 0.001$) (Figure 5.2). For *P. regina*, there were significant differences between all temperatures (Figure 5.1). As temperature increased, *P. regina* required less time for oviposition in all locations (Figure 5.1), but demonstrated delayed oviposition in the laboratory at 15°C compared to in the field (Figure 5.1). For *C. vicina*, there was a significant difference in oviposition time between the laboratory and the field trials (Figure 5.2). For this species, delayed oviposition occurred at 15°C in the field, but time to oviposition was between 1-2 hours when tested at 25°C (Figure 5.2). There was no effect of temperature ($F_{1, 18} = 3.32$, $p = 0.09$) or location ($F_{2, 18} = 2.83$, $p = 0.08$) on the time to first oviposition event for *L. sericata* (Figure 5.3).

There was an effect of location (MANOVA: Wilk's $\lambda = 0.277$, $F_{2,27} = 4.35$, $p = 0.002$; Table 5.2) on *P. regina* oviposition site selection, indicating that *P. regina* selected different sites depending on the location in which it was ovipositing. For *P. regina*, the

preferred oviposition sites in the lab were the legs and areas of the head (Figure 5.4). In the field, however, *P. regina* primarily selected the legs, mouth and face (Figure 5.4). Wild colonizing *P. regina* preferred the legs and face at temperatures of 15 and 25°C, but demonstrated a preference for the mouth, face and legs at 35°C (Figure 5.4).

For *C. vicina*, there was a significant effect of location on site selection (MANOVA: Wilk's $\lambda = 0.09$, $F_{2,18} = 5.86$, $p < 0.001$; Table 5.2), indicating that *C. vicina* selected different sites when ovipositing in different locations. In both the lab and the field, *C. vicina* demonstrated a similar preference for sites, choosing the face, mouth, legs and ears (Figure 5.5). The oviposition sites selected by wild colonizing *C. vicina* were the mouth and face, at both 15 and 25°C (Figure 5.5).

Location had a significant effect on oviposition site selection of *L. sericata* (MANOVA: Wilk's $\lambda = 0.092$, $F_{2,27} = 4.97$, $p < 0.001$; Table 5.2) where *L. sericata* selected different oviposition sites more frequently depending on the location it was ovipositing. Wild *L. sericata* selected the mouth more frequently, whereas *L. sericata* in the field selected the mouth more frequently at 25°C, but less frequently at 35°C, compared to the wild and lab flies (Figure 5.6).

The oviposition rate of *C. vicina* was different due to location ($F_{1,14} = 6.42$, $p = 0.02$, Table 5.3) and the rate of *C. vicina* oviposition in the field was greater than in the lab. Oviposition rate of *P. regina* was influenced by the interaction of temperature and location ($F_{2,14} = 12.89$, $p < 0.001$, Table 5.3), and the rate was greater in the field at 15°C, but greater than in the lab at 25 and 35°C. There was no effect of location ($F_{1,14} = 1.86$, $p = 0.194$, or temperature ($F_{1,14} = 1.26$, $p = 0.283$), on the oviposition rate of *L. sericata*.

Fewer eggs were deposited by *L. sericata* in the field than in the lab at 35°C ($F_{3,8} = 9.08$, $p = 0.04$, Figure 5.7). *Calliphora vicina* demonstrated no difference in the number of eggs deposited on pig carcasses in the lab and the field at any of the temperatures tested (*C. vicina*: $F_{3,8} = 2.94$, $p = 0.16$). The number of eggs laid by *P. regina* differed in the lab and in the field ($F_{5,12} = 9.62$, $p = 0.02$) such that at 15°C, more eggs were deposited in the field than in the lab; however, at 25 and 35°C, more eggs were oviposited in the lab than in the field (Figure 5.7).

5.4 Discussion

Based on our results, there are noticeable differences between the observations in the field setting compared to the lab setting. The differences in the time to the first oviposition event between the lab and field at 15°C for *P. regina* and *C. vicina* may be due to fluctuating temperatures in the field compared to constant temperatures maintained in a laboratory setting (Byrd and Allen 2001), as temperature has been shown to influence the time to first oviposition (Chapter 2). Whereas previous studies have examined the influence of fluctuating temperature on development of eggs and larvae (Byrd and Allen 2001; Niederegger *et al.* 2010), little information is available for the influence of fluctuating temperature on the behavior of the adult blow flies. It is also possible that different levels of humidity between the lab and field environment result in different speeds of first oviposition as humidity has been shown to influence egg eclosion success (Chapter 4) and therefore, may impact adult oviposition behaviour.

Differences in humidity thresholds among species for egg hatching success may play a role in the number of eggs a female chooses to deposit in a given location. When female *P. regina* are ovipositing in the presence of heterospecific eggs, the flies deposit more eggs at lower temperatures, however, at higher temperatures, *P. regina* deposits fewer eggs in the presence of heterospecific eggs (Chapter 3). The switch in behaviour may be due to *P. regina* females choosing to aggregate their eggs with heterospecific eggs under low temperature/low humidity conditions when their eggs are most susceptible to reduced hatching success due to desiccation, whereas at higher temperatures and higher humidities, *P. regina* has greater hatching success and may not rely on aggregating eggs with those of heterospecifics to ensure offspring survival.

Although the rate of oviposition for *L. sericata* was no different between the lab and the field locations at either temperature tested, there was an increase in the number of eggs laid per hour for this species. As expected, *P. regina* oviposition rate increased with increasing temperature, but only in the lab. In the field, the greatest rate of oviposition occurred for *P. regina* at the lowest temperature tested, and this resulted in a greater total number of eggs deposited by *P. regina* in the field at this temperature. The rate of oviposition by *C. vicina* was greater in the field than in the lab. Although natural

conditions may have resulted in greater time to first oviposition event, *C. vicina* females deposited a greater number of eggs per hour in the field, at both temperatures tested.

In both the laboratory and field, *L. sericata* selected orifices such as the mouth and ears, as was expected for this species based on previous work (Rosati 2014; Pacheco 2015). This species often selects areas that can provide moisture and protection for their offspring, from factors such as desiccation, predators and parasitoids (Kamal 1958; Grassberger and Reiter 2001). Site selection by *P. regina* females demonstrated that the legs, face and mouth were the preferred sites when tested in the lab as well as the field. This behaviour has been documented previously (Rosati 2014; Pacheco 2015) in which *P. regina* selects the larger areas on a carcass. Lastly, *C. vicina* preferred the face as an oviposition site when tested in the laboratory, but demonstrated a preference for the face, legs and mouth when examined in the field. In this study, *P. regina* and *C. vicina* females preferred to oviposit on larger areas of the pig carcass, such as the legs and the face, in both the laboratory and field setting. This preference for oviposition sites may be due, in part, to the surface area that is available for egg deposition (Pacheco, Hans and VanLaerhoven, in prep). Smaller orifices, such as the nostrils or ears, could limit the number of eggs that can be deposited into these areas, whereas larger areas allow for more eggs to be laid and the formation of larger egg masses. Selecting larger areas for oviposition, and therefore, the ability to form larger aggregated egg masses, may be beneficial for the developing offspring. The large egg masses can increase development temperatures (Catts and Goff 1992; Ireland and Turner 2006), facilitate resource consumption by increasing the amount of digestive enzymes present (Goodbrod and Goff 1990; Reis *et al*. 1999; Ireland and Turner 2006) and dilute predatory effects (Stamp 1980), although there is likely a density at which larger egg masses are detrimental.

This study demonstrated the importance of verifying conclusions drawn on lab-based behaviour with field-based studies as the response by each species differed in different ways between the lab and field, yet with good correspondence between field-caged and wild flies. The differences in the behaviour of *P. regina,* in particular, emphasize the caution that is required when applying various parameters measured in a lab-based setting to a more natural setting. Still to be explored is the added factor of species interactions on the oviposition behaviour of these flies in a more naturalized

setting and much more investigation is required to understand the mechanisms driving their oviposition behaviour. Few studies combine observations of behaviour and development made in a controlled setting to that of insects under natural conditions (Pitts and Wall 2004). Observing the natural history of these organisms in field settings provides valuable information, particularly when these observations can be compared to those made in controlled conditions. Caution must be applied when taking measurements collected under controlled settings and applying them our understanding of these organisms in the wild. Because the behaviour of organisms in these settings can differ, it provides the means to test the robustness of hypotheses developed in controlled mechanistic experiments to real world environments.

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Table 5.1. Mean temperature and relative humidity for the controlled laboratory and field trials.

Treatment	Mean (±SE) Temperature °C	Mean (±SE) % RH
Lab 15	15.4 ± 0.27	52 ± 0.09
Lab 25	24.8 ± 0.01	51 ± 0.75
Lab 35	34.8 ± 0.06	51 ± 0.95
Field 15	14.7 ± 1.12	50 ± 0.6
Field 25	24.7 ± 0.5	62 ± 1.4
Field 35	35.3 ± 2.2	71 ± 0.7

Table 5.2. Multivariate Analysis of Variance (MANOVA) results to determine the effect of location (lab, field, wild) on the oviposition sites selected by *L. sericata P. regina*, and *C. vicina*. Significant effects are indicated in bold font; α = 0.01 for all effects.

Oviposition Site	d.f.	F ratio	*p* - value
	Lucilia sericata		
Mouth	**2, 18**	**15.21**	**< 0.001**
Ears	2, 18	4.74	0.022
Face	**2, 18**	**5.86**	**0.010**
Neck	2, 18	0.47	0.633
Legs	2, 18	0.24	0.778
Abdomen	2, 18	2.89	0.082
	Phormia regina		
Mouth	2, 27	2.03	0.151
Ears	2, 27	0.14	0.867
Face	**2, 27**	**8.99**	**0.001**
Neck	2, 27	0.08	0.924
Legs	**2, 27**	**7.34**	**0.003**
Abdomen	2, 27	1.50	0.241
	Calliphora vicina		
Mouth	2, 18	1.94	0.173
Ears	2, 18	3.75	0.044
Face	2, 18	4.81	0.021
Neck	2, 18	1.13	0.346
Legs	**2, 18**	**7.32**	**0.004**
Abdomen	2, 18	3.31	0.061

Table 5.3 Mean oviposition rate (number of eggs per hour) for each species at each temperature and location. For these analyses, *L. sericata* was not tested at 15°C and *C. vicina* was not tested at 35°C.

		Mean Oviposition Rate (#eggs/h)		
Location	**Temperature**	***L. sericata***	***P. regina***	***C. vicina***
Lab	**15**	-	2.8 ± 2.8	51.5 ± 9.3
	25	135.4 ± 52.1	298.6 ± 104.4	35.8 ± 7.6
	35	287.3 ± 78.2	841.5 ± 162.9	-
Field	**15**	-	313.5 ± 49.3	68.1 ± 12.8
	25	145.1 ± 35.9	118.1 ± 91.2	110.4 ± 45.2
	35	214.9 ± 36.9	204.6 ± 116.1	-

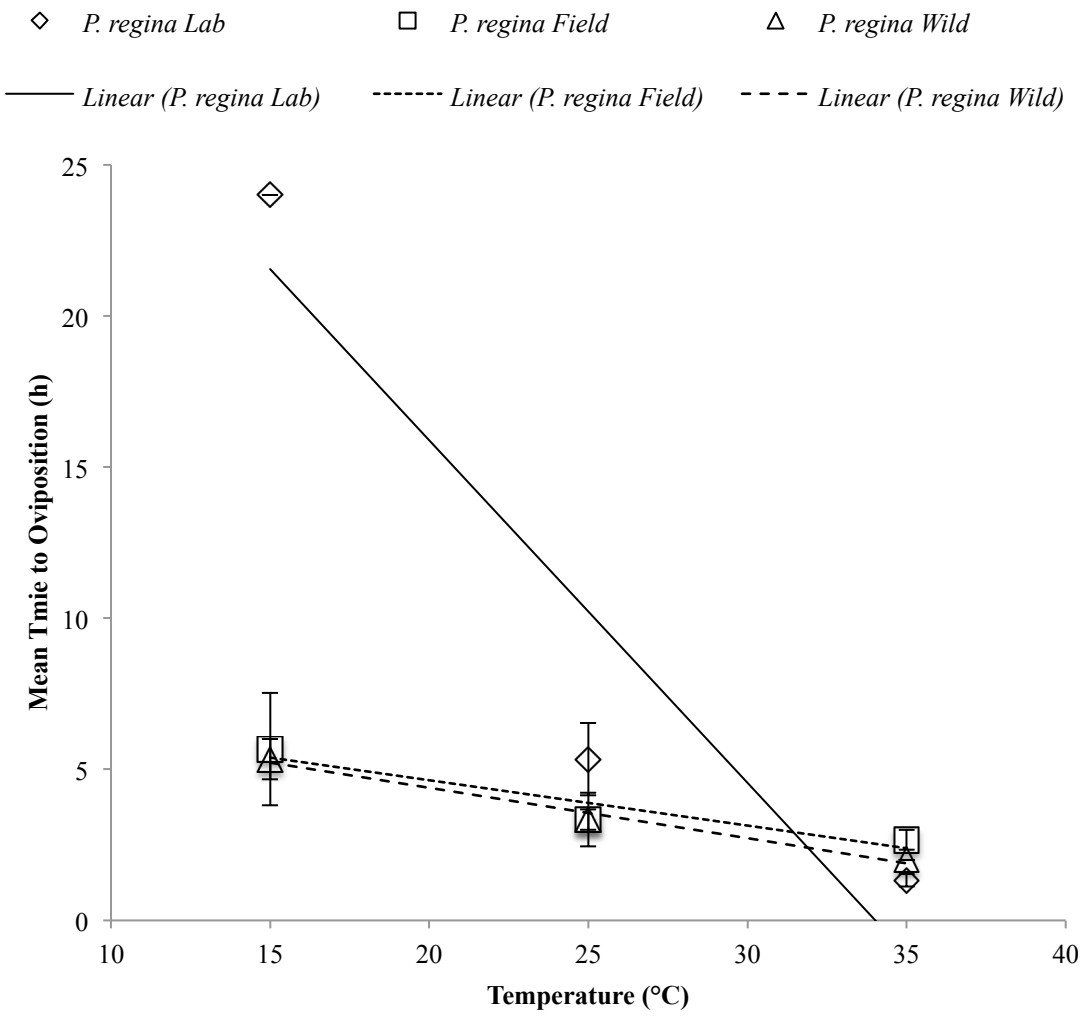

Figure 5.1. Mean time (± S.E.) to first oviposition event across all temperatures for *P. regina* ovipositing in the laboratory, field or colonization by wild *P. regina*. There was a significant interaction between temperature and location on *P. regina* oviposition time ($F_{2, 30} = 27.05$, $p < 0.0001$). For *P. regina* Lab ($y = -1.13x + 38.56$, $R^2 = 0.87$), *P. regina* Field ($y = -0.15x + 7.64$, $R^2 = 0.69$), and *P. regina* Wild ($y = -0.17x + 7.72$, $R^2 = 0.82$).

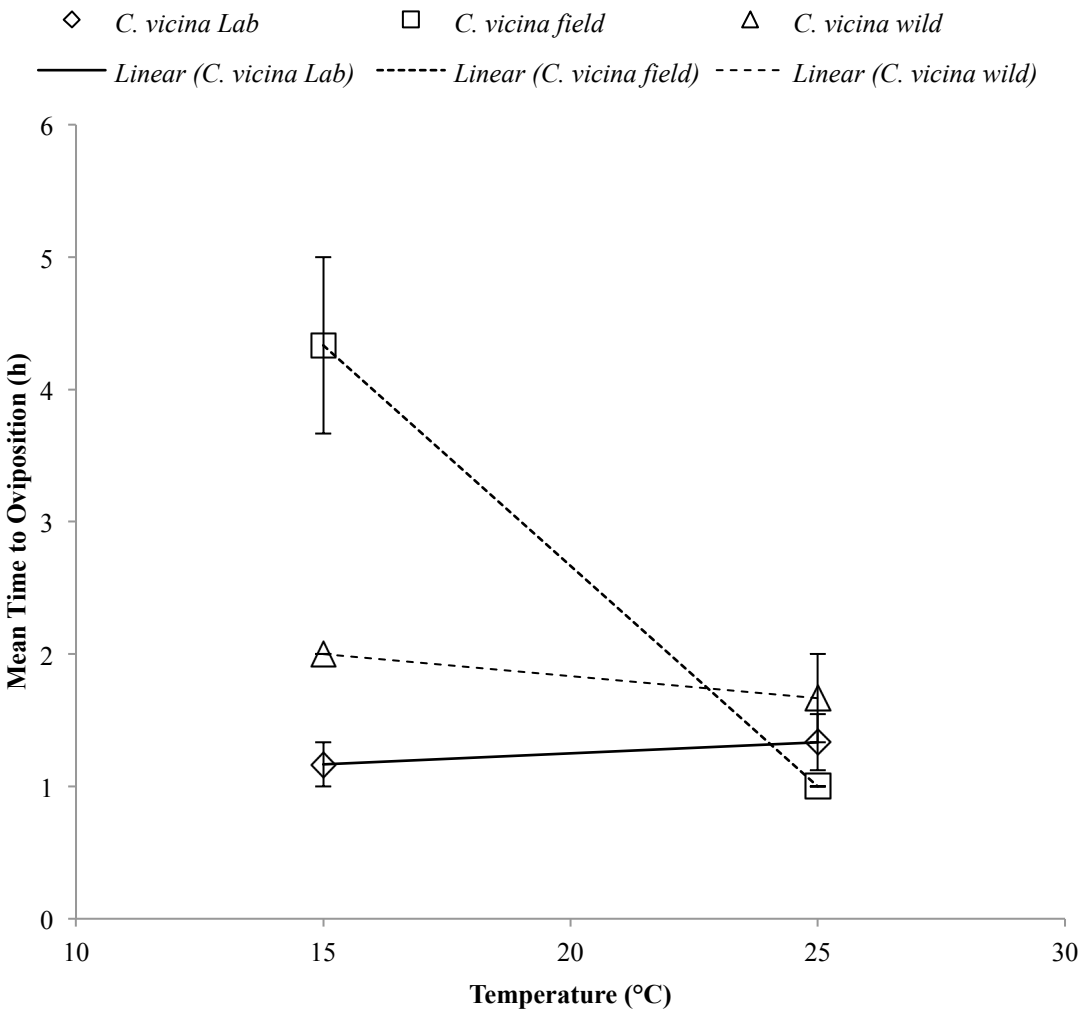

Figure 5.2. Mean time (± S.E.) to first oviposition event across all temperatures for *C. vicina* ovipositing in the laboratory, field or colonization by wild *C. vicina*. There was a significant interaction between temperature and location on *C. vicina* oviposition time ($F_{2, 18} = 14.36$, $p = 0.0002$). For *C. vicina* Lab ($y = 0.02x + 0.92$, $R^2 = 0.40$), *C. vicina* Field ($y = -0.33x + 9.33$, $R^2 = 0.86$), and *C. vicina* Wild ($y = -0.03x + 2.50$, $R^2 = 0.20$). For these analyses *C. vicina* was not tested at 35°C.

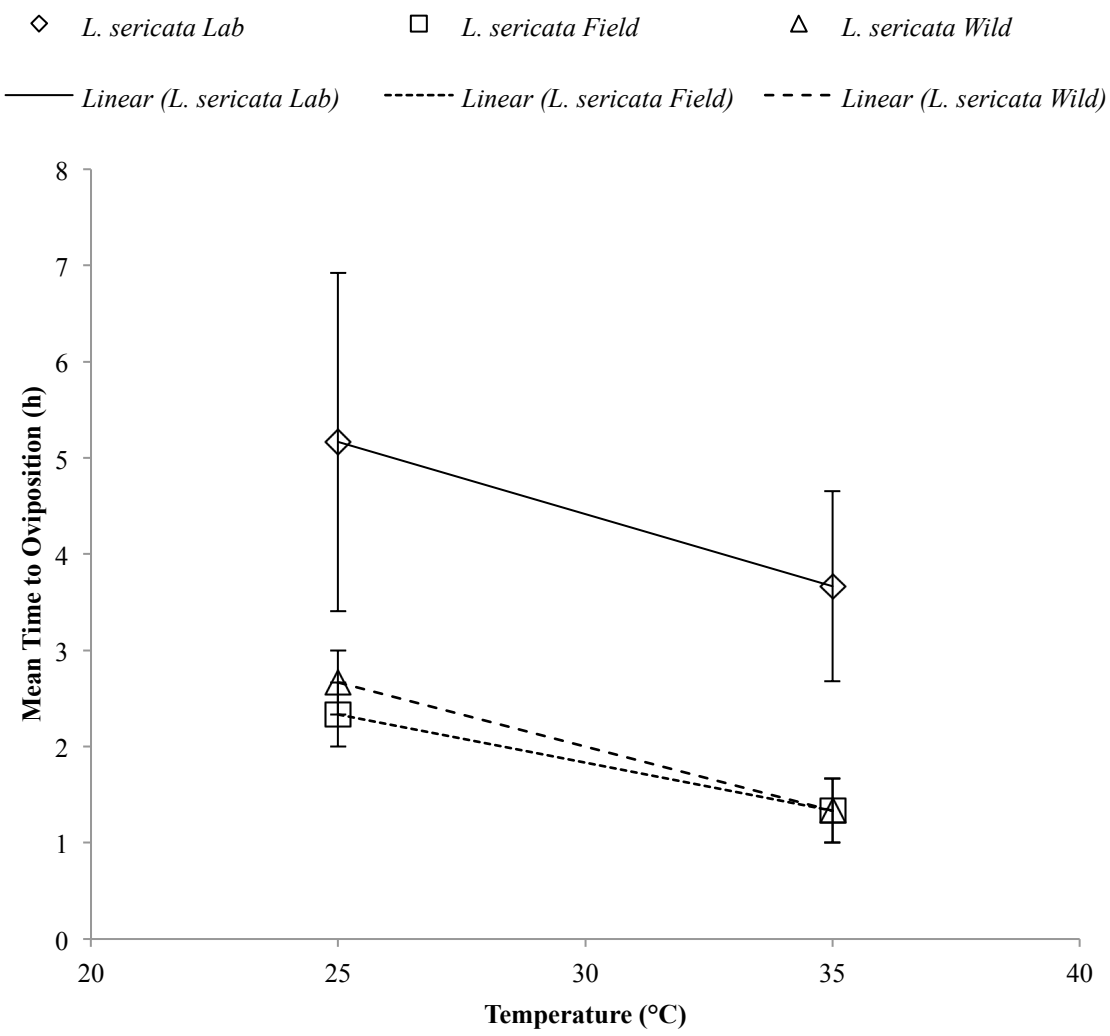

Figure 5.3. Mean time (± S.E.) to first oviposition event across all temperatures for *L. sericata* ovipositing in the laboratory, field or colonization by wild *L. sericata*. There was no significant effect of temperature ($F_{1, 18} = 3.32$, $p = 0.09$) or location ($F_{2, 18} = 2.83$, $p = 0.08$) on *L. sericata* oviposition time. For *L. sericata* Lab ($y = -0.15x + 8.92$, $R^2 = 0.05$), *L. sericata* Field ($y = -0.10x + 4.83$, $R^2 = 0.53$), and *L. sericata* Wild ($y = -0.13x + 4.08$, $R^2 = 0.67$). For these analyses *L. sericata* was not tested at 15°C.

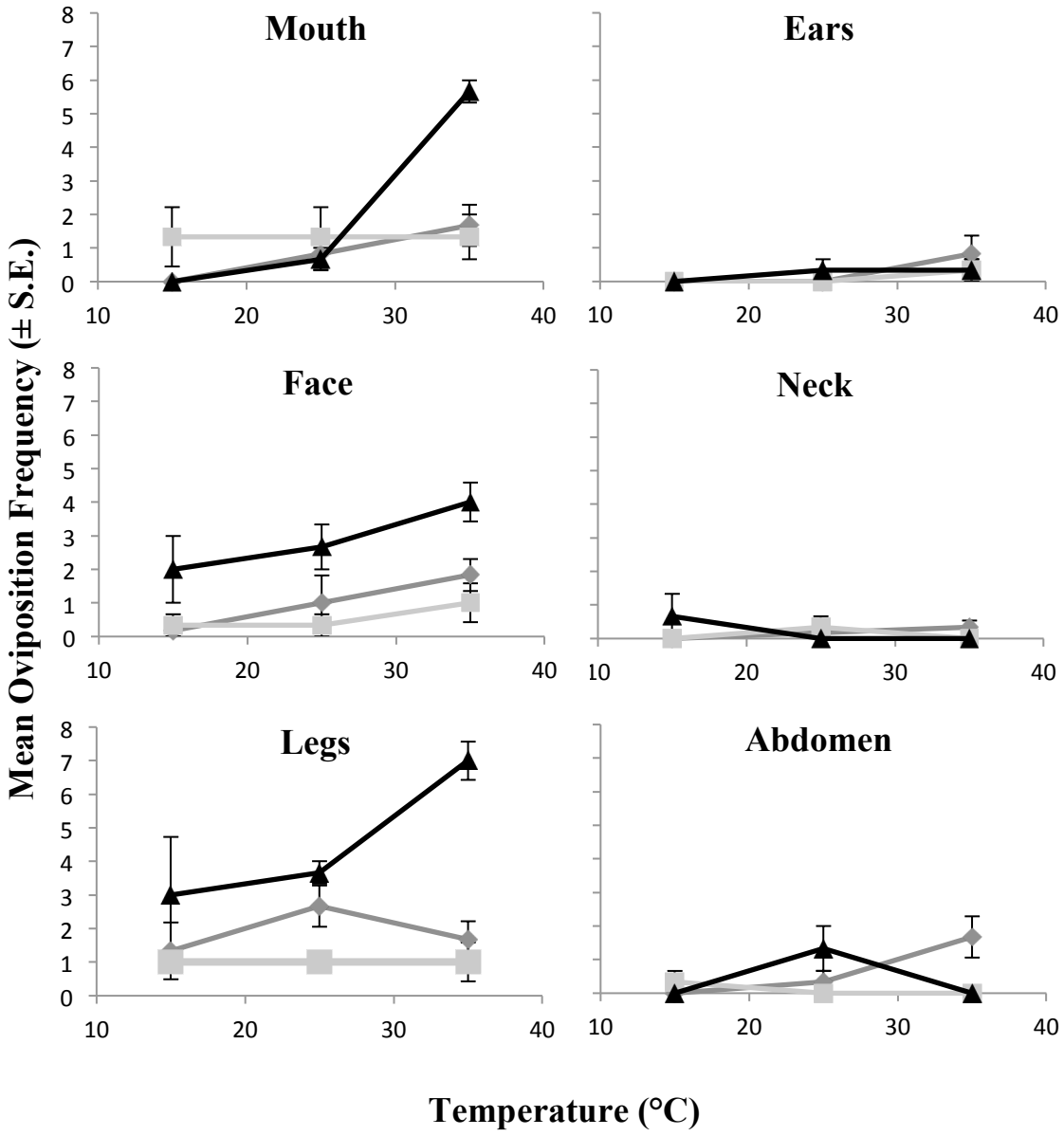

Figure 5.4. Interaction (MANOVA: Wilk's $\lambda = 0.277$, $F_{2,27} = 4.35$, $p = 0.002$) between temperature and location on the mean (± S.E.) oviposition frequency by *P. regina* at each site on pig carcasses. ▩ : *P. regina Lab*, ◆ :*P. regina* Field, ▲ : *P. regina* Wild. Oviposition frequency was calculated as the number of oviposition events that occurred at each site over the observation period.

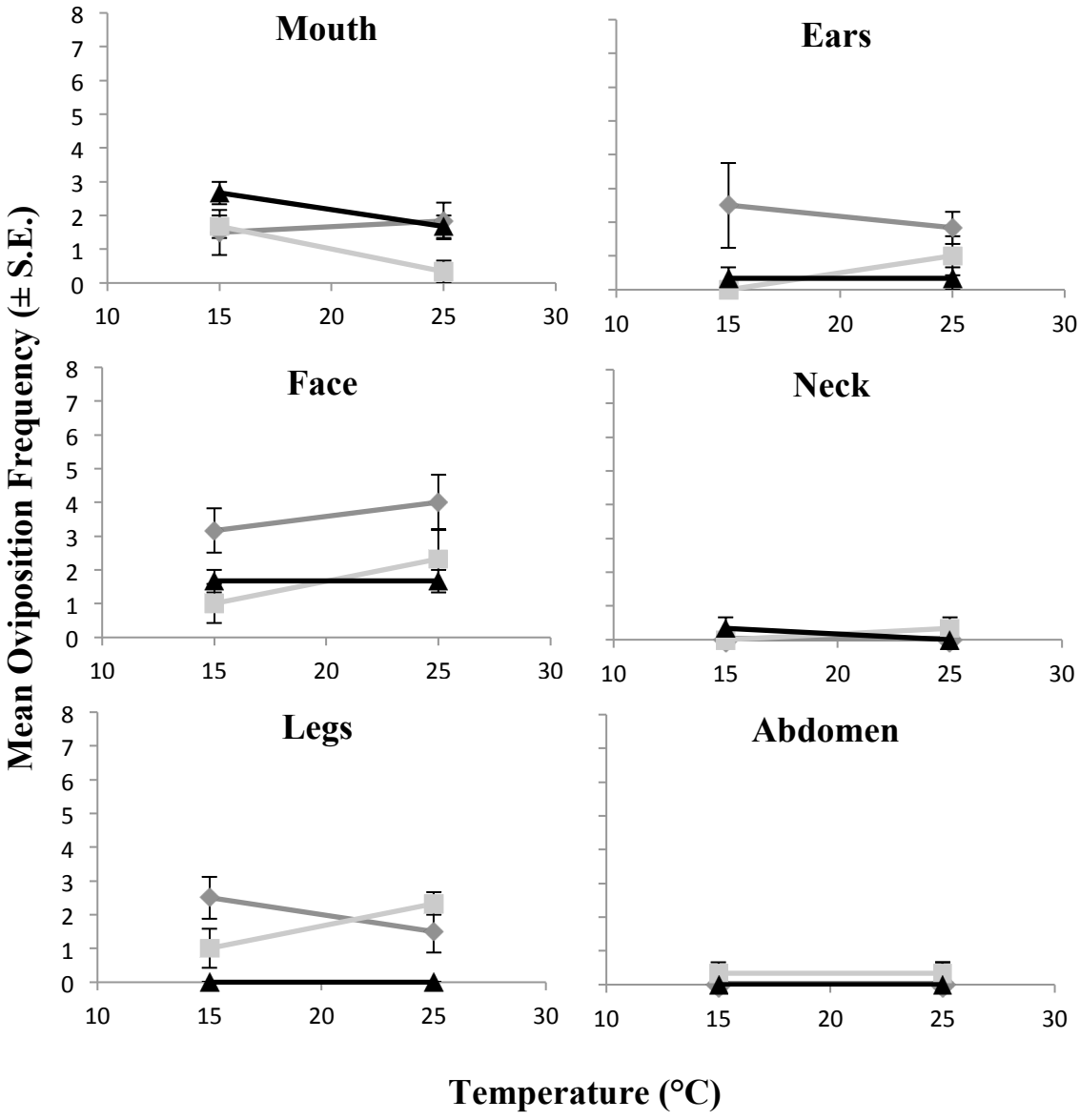

Figure 5.5. Interaction (MANOVA: Wilk's $\lambda = 0.09$, $F_{2,18} = 5.86$, $p = 0.0003$) between temperature and location on the mean (± S.E.) oviposition frequency by *C. vicina* at each site on pig carcasses. ■ : *C. vicina Lab*, ◆ :*C. vicina* Field, ▲: *C. vicina* Wild. Oviposition frequency was calculated as the number of oviposition events that occurred at each site over the observation period.

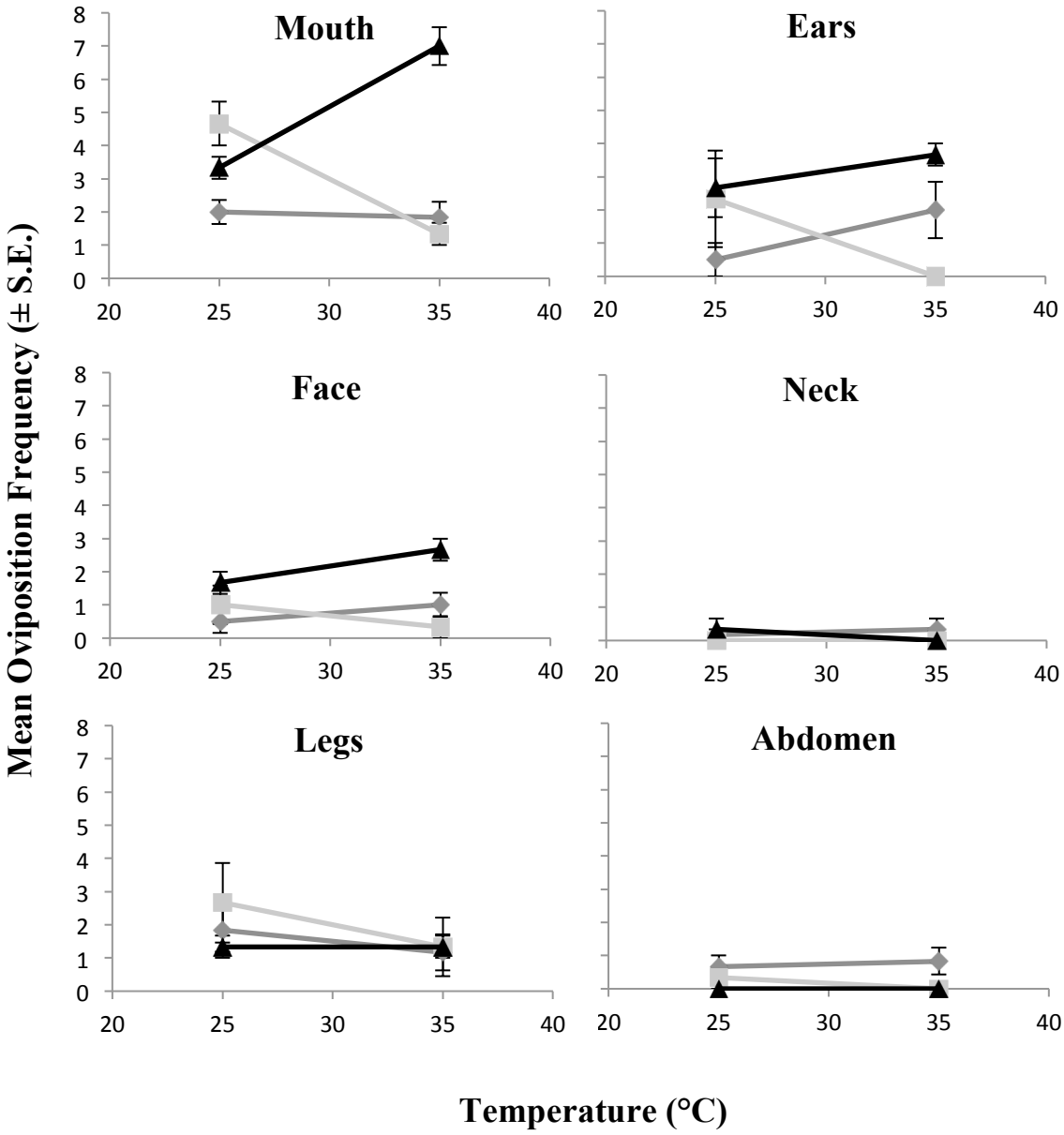

Figure 5.6. Interaction ((MANOVA: Wilk's $\lambda = 0.092$, $F_{2,27} = 4.97$, $p = 0.0003$) between temperature and location on the mean (± S.E.) oviposition frequency by *L. sericata* at each site on pig carcasses. ■ : *L. sericata Lab*, ◆:*L. sericata* Field, ▲: *L. sericata* Wild. Oviposition frequency was calculated as the number of oviposition events that occurred at each site over the observation period.

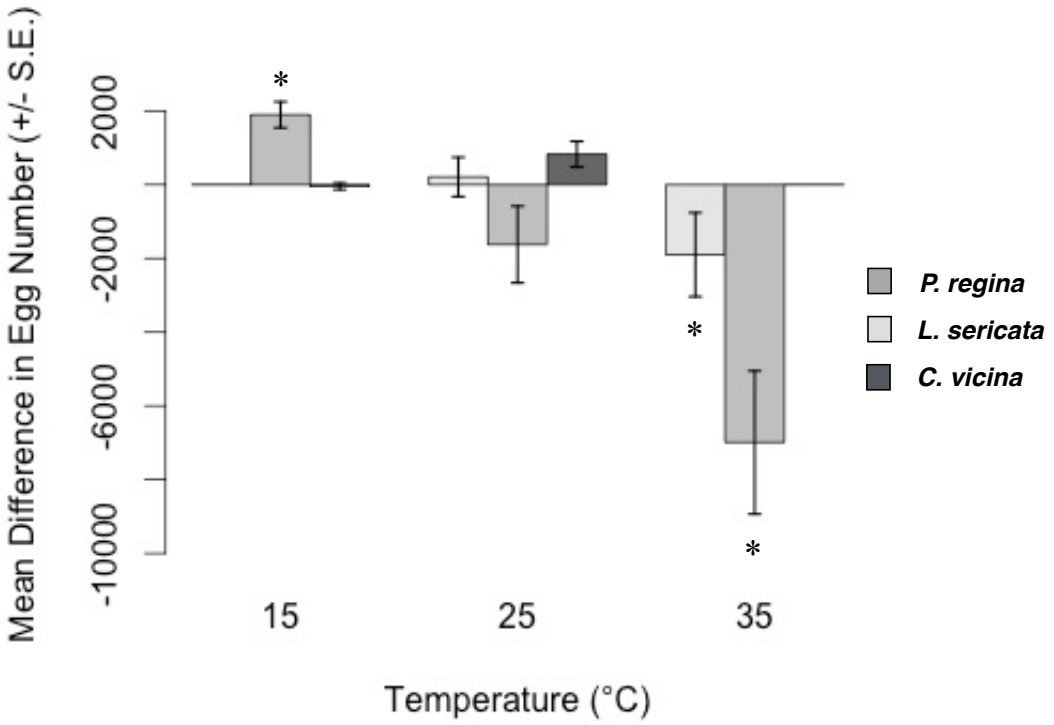

Figure 5.7. Mean difference (±S.E.) in the number of eggs oviposited by 100 females in each cage in the semi-natural field trials compared to a baseline of the eggs oviposited under controlled laboratory conditions for *P. regina*, *L. sericata* and *C. vicina*. There were significant differences in number of eggs deposited by *P. regina* ($F_{5, 12} = 9.617$, $p = 0.02$) and *L. sericata* ($F_{3, 8} = 9.08$, $p = 0.04$), but not *C. vicina* ($F_{3, 8} = 2.941$, $p = 0.16$). For these analyses, *L. sericata* was not tested at 15°C and *C. vicina* was not tested at 35°C. Asterisks indicate a difference between the baseline number of eggs oviposited under controlled laboratory conditions and the semi-natural field trial for that species that that temperature.

CHAPTER 6
INFLUENCE OF TEMPERATURE AND SPECIES INTERACTIONS ON DEVELOPMENT ON LARVAL DEVELOPMENT AND ADULT SIZE OF BLOW FLIES (DIPTERA: CALLIPHORIDAE)

6.1 Introduction

Insects are poikilotherms and therefore rely on ambient conditions to maintain their body temperatures (Harrison *et al.* 2012). The association between temperature and insect development has been thoroughly documented (Huey and Kingsolver 1989; Angilletta *et al*. 2004). Insect development occurs within a thermal range, having an optimal temperature (T_{opt}) for development, which lies between a critical thermal minimum (CT_{min}) and maximum (CT_{max}) (Huey and Kingsolver 1989). This represents the lowest and highest temperatures at which an insect can develop (Huey and Kingsolver 1989). This relationship between temperature and development can be demonstrated as a thermal performance curve (TPC), that shows thermal sensitivity of an organism over a range of temperature (Huey and Kingsolver 1989; Izem and Kingsolver 2005). Shifts in a TPC can reflect changes in development (Kingsolver 2009). A vertical shift in the height of a TPC indicates variation in the development of the organism over a temperature range and a shift horizontally demonstrates a trade-off between development and temperature, at low or high temperatures (Kingsolver 2009).

These trade-offs have been examined in evolutionary biology, as reaction norms, which describe the relationship between a phenotype and an environmental variable, and involve a series of trade-offs relating to allocation, acquisition and specialist-generalists (Angilletta *et al.* 2003). Allocation trade-offs are described as allocating the available resources to one function over another, whereas acquisition trade-offs focus on minimizing mortality and maximize the foraging and consumption of resources (Angilletta *et al*. 2003). Specialist-generalist trade-offs describe the specialization of an organism in a particular environment, resulting in greater performance over a range of environmental conditions (Angilletta *et al.* 2003). For insects, thermal reaction norms examine the trade-offs associated with physiological and behavioural processes in a given environment (Angilletta *et al.* 2003).

The thermal environment can affect ectotherms, including insects, by altering the rates of growth and development or affecting the final adult size of the organism (Atkinson 1993; Angilletta *et al.* 2003). Typically, high temperatures result in faster development, resulting in smaller organisms (Atkinson 1994; Davidowitz and Nijhout 2004; Kingsolver 2009). Kingsolver *et al*. (2004) explain that plasticity can occur in two ways. In a TPC, performance or development of an individual is affected by temperature (Kingsolver *et al*. 2004). This can be compared to a thermal development reaction norm where a trait in a later stage is influenced by temperature during development and growth. Thermal development reaction norms and TPCs are both considered forms of phenotypic plasticity, where there is considerable variation in the traits of organisms in response to the thermal environment (Kingsolver *et al.* 2004).

For carrion feeding insects, development of offspring occurs on decomposing tissue. Adult blow flies (Diptera: Calliphoridae) are attracted to carrion where they feed on blood and decomposition fluids and reproduce while their larvae feed on body tissues (Greenberg 1991). Gravid females use decomposing material as an oviposition substrate to deposit their eggs. The hatching larvae develop through three (L1, L2 and L2) larval instars. Once individual larvae have met their nutritional requirements in the third larval stage, larvae crawl from the food source to pupate (Hutton and Wasti 1980). During pupation, larvae shorten in length and the outer membrane hardens to form a puparium; following metamorphosis, an adult fly emerges from the puparium (Greenberg 1991).

Blow fly species have different growth and developmental rates, which have been measured for numerous species including: *Lucilia sericata* Meigen (Kamal 1958; Ash and Greenberg 1975; Greenberg 1991; Wall *et al.* 1992; Davies and Ratcliffe 1994; Grassberger and Reiter 2001), *Calliphora vicina* Robineau-Desvoidy (Kamal 1958; Reiter 1984; Greenberg 1991; Davies and Ratcliffe 1994; Donovan 2006); and *Phormia regina* Meigen (Kamal 1958; Greenberg 1991; Byrd and Allen 2001; Nabity *et al*. 2006). Numerous studies provide examples of variable development within species of blow flies. This may be a result of constant versus fluctuating temperatures, or different geographic populations. Growth rate, larval weight and adult size may all be influenced by temperature (Kingsolver and Huey 2008).

Generally, there are three principles associated with temperature, size and fitness (Kingsolver and Huey 2008). These ideas utilize the term 'better' to denote greater fitness, which can be associated with population increases and reproductive rates. However, due to the difficulty associated with quantifying these metrics, components of fitness, such as fecundity or survival, are often measured (Kingsolver and Huey 2008). The first rule, bigger is better, suggests that a larger body size leads to increased fitness compared to individuals with a smaller body size (Kingsolver and Huey 2008). However, attaining a large size involves trade-offs where increased energy demands often require more time, leaving the individual more susceptible to predation (Kingsolver and Huey 2008). The second principle is the temperature-size rule, which proposes that higher temperatures result in the development of smaller adults, due to faster growth rates which shorten development time (Atkinson 1994; Atkinson and Sibly 1997; Angilletta and Dunham 2003; Kingsolver and Huey 2008). The third rule, hotter is better, indicates that species with a higher optimal temperature have greater fitness or development (Frazier *et al.* 2006). Studies of insect development have taken these principles into consideration, however, many of these studies are not looking at all aspects that can influence insect development.

Although many studies examine the influence of temperature, many of these studies examine only one species developing, with species interactions noticeably absent (Byrd and Butler 1996; Byrd and Butler 1997; Byrd and Allen 2001; Grassberger and Reiter 2002; Nabity *et al*. 2006) and it is likely that species interactions together with environmental effects result in variable blow fly development. Group oviposition sets up a potential scenario for different species interactions among larvae, such as intraspecific and interspecific competition (Saunders and Bee 1995; Smith and Wall 1997; Von Zuben *et al*. 2001). Species interactions, including competition, can alter body size and fecundity of blow flies (Ives 1988; Catts 1992; Von Zuben *et al.* 1996; Faria *et al.* 1999; Reis *et al*. 1999; Ireland and Turner 2006). Overall, there is a lack of information regarding the influence of species interactions on the development of coexisting blow flies.

The blow flies *L. sericata*, *C. vicina*, and *P. regina* are three of the most ubiquitous species that are frequently encountered on decomposing remains (Haskell and

Williams 2008). These blow fly species often arrive to carrion and oviposit large aggregations of eggs. *Lucilia sericata* and *P. regina* often colonize the same carrion resource (Anderson and VanLaerhoven 1996; VanLaerhoven and Anderson 1999; Sharanowski *et al.* 2008; Vanin *et al*. 2013). The developmental temperature range for *L. sericata* is between 10-35°C (Gosselin *et al*. 2010; Roe and Higley 2015), however, this species demonstrates increased mortality between 10-17.5°C (Roe and Higley 2015). Adult *L. sericata* are smaller than the other species in this study, with a size between 6-9 mm (Byrd and Castner 2010). It is generally believed that *L. sericata* is a poor competitor, exhibiting negative effects such as reduced body size and reduced survival due to intraspecific competition (Prinkkilä and Hanski 1995; Smith and Wall 1997; Kheirallah *et al*. 2007). However, when competing with *P. regina*, *L. sericata* development and survival were not reduced, indicating a potential benefit due to interspecific interactions (Hutton and Wasti 1980; Rosati 2014).

The temperature range of development for *P. regina* is between 10-35°C (Deonier 1940; Byrd and Allen 2001) and adults are usually between 7-9 mm (Byrd and Castner 2010). When *P. regina* develop with heterospecifics, differing responses have been documented. For example, Hutton and Wasti (1980) found that *P. regina* larvae were completely eliminated from resources when competing with *L. sericata*. However, others found that *P. regina* survival and adult fitness increased when developing with *L. sericata* (Reid 2012; Rosati 2014).

*Calliphora vicin*a has the lowest temperature tolerance of the three species, with a development range between 3.5-30°C (Donovan 2006; Hwang and Turner 2009). At temperatures above 30, Reiter (1984) reported that *C. vicina* larvae exhibit inhibited growth, with high mortality and few surviving to pupation. The growth rate of *C. vicina* is often greater than that of other species, due to the larger size of this species (Smith and Wall 1997), with an overall greater pupal mass and adult body size (10-14 mm) (Byrd and Castner 2010). This species experiences increased mortality and reduced adult size due to intraspecific competition during development, indicating that the growth of *C. vicina* should be reduced during intraspecific interactions (Saunders and Bee 1995; Smith and Wall 1997). Due to their large size, *C. vicina* may not be as heavily impacted by interspecific competition when competing with smaller larvae and may have the ability to

outcompete and exclude a smaller species. Conversely, the larger size of *C. vicina* may result in increased competition with other species due to their greater resource requirements.

The objective of this study was to determine the effects of temperature and intraspecific and interspecific species interactions on the growth rate, pupal mass, survivorship and resulting adult size of the blow flies *L. sericata*, *C. vicina* and *P. regina*. We were specifically interested in development of *L. sericata* when developing with *P. regina* as these two species co-occur in the summer in southern Ontario, *C. vicina* when developing in the presence of *P. regina* as these two species co-occur in the spring/fall in southern Ontario, and *P. regina* when developing in the presence of each of these species separately as it is found throughout the spring, summer and fall. Given its affinity for warmer temperatures (Smith 1986), *L. sericata* should have a faster growth rate at higher temperatures, resulting in a lower pupal mass, increased survivorship and smaller adult body size. With *C. vicina* being a cold-adapted species (Smith 1986; Donovan *et al*. 2006), high temperatures should have a negative impact on the growth rate, pupal mass and survivorship of larvae, with smaller adults emerging at higher temperature. Due to the wide temperature range for development of *P. regina*, we expect that development will be faster at lower temperatures compared to *L. sericata*, but slower at lower temperatures than *C. vicina*. In addition, we expect *P. regina* will have faster growth rate, lower pupal mass, greater survivorship and smaller adult body size as temperature increases.

Furthermore, we predicted that the growth rate of *L. sericata* would not change due to both intra- and interspecific interactions. However, we believe that interspecific interactions would result in smaller adult size and therefore decreased pupal mass as documented in previous studies (Fuller 1934; Smith and Wall 1997). The larger blow fly, *C. vicina*, should exhibit stronger intraspecific interaction effects when compared to interspecific effects, as demonstrated by reduced pupal weight and smaller adults. Based on previous work on interspecific interactions among blow flies (Rosati 2014; Pacheco 2015), which indicates the facilitation of *P. regina* larval growth, we expected larger adult flies directly resulting from increased growth rates and greater pupal mass. Although *P. regina* oviposition is facilitated by the presence of *L. sericata* and *C. vicina*

at low temperatures, there is a switch at 25°C, where rather than facilitation, *P. regina* faces competition from these species (Chapter 3). Based on these results, we expect similar outcomes for faster development when *P. regina* larvae are developing in the presence of *L. sericata* and *C. vicina* below 25°C and potentially slower development in the presence of heterospecifics above 25°C.

6.2 Materials and Methods

6.2.1 Colony Maintenance

All adult flies were maintained in colonies at the University of Windsor and were housed in 46 x 46 x 46 cm aluminum cages (Bioquip 1450C aluminum collapsible cage). Colony cages were provided with sugar and water *ad libitum*. Pork liver was used as an oviposition substrate for gravid females within the colony cages. Egg masses (approximately 1000 eggs) were removed from the liver and placed into 1L rearing jars with wood shavings as a pupation medium and pork liver as a food source. The openings of all jars were secured with landscape tarp (Quest Brands Inc., Item ID: WBS 50) and metal rings to ensure the larvae remained within the jars. Larvae were given fresh liver throughout development and were monitored until emergence of adults. After emergence, flies were transferred to clean colony cages.

6.2.2 Experimental Design

The species treatments for this study were as follows: (1) *P. regina* only, (2) *L. sericata* only, (3) *C. vicina* only, (4) *P. regina* and *L. sericata,* (5) *P. regina* and *C. vicina*. For treatments 1-3, the species developed with conspecifics and therefore experienced intraspecific interactions. Treatments 4 and 5 represent mixed species treatments, with the species experiencing both intra and interspecific interactions. All species and temperature treatments were replicated five times at every sample point. For each temperature and species treatment, 40 cups were prepared and were composed of 20

first stage larvae were transferred to 59 mL polystyrene cups using a dampened paintbrush. Density of larvae was maintained at 20 individuals regardless of treatment, thus in mixed species treatments, 10 individuals of each species were placed into the cup. Each cup contained 20 g of pork liver to ensure that excess liver would be present, as each larvae requires between 0.5- 1 g liver (Ives 1991; Reid 2012) and 1.5 cm of sawdust, to act as a pupation medium (Hutton and Wasti 1980). All cups were covered with landscape tarp and secured with a plastic lid. Cups were placed into growth chambers (Conviron Adaptis A1000) that were programmed to a constant temperature, with 50% (± 0.41- 2.59) RH and a 12:12 L:D cycle. Temperatures within the growth chambers were programmed to be one of five temperatures (15°C, 20°C, 25°C, 30°C, 35°C (±0.66)). Dataloggers (HOBO U-12 data loggers, Onset, Pocasset, MA) were placed into the growth chambers to record both temperature and relative humidity, hourly.

6.2.3 Sampling

At each time point, one cup from each species and temperature treatment was removed from the growth chambers. All larvae present in one cup, representing each species and temperature treatment were removed from their respective polystyrene cups at 12 h intervals. Larvae were immediately placed into boiling water for 30 s to prevent shrinkage and were then preserved in 70% ethanol. This continued until pupation of all larvae in the polystyrene cups was observed. Following pupation, pupae were sampled in 24 h increments. The wet mass of larvae and pupae were measured to the nearest 0.1 mg using an analytical scale (Denver Instruments M-120). After emergence, adult flies were counted in each cup to determine percentage of survival. Size of adults was measured to the nearest 0.01 mm using the length of the cm-du cross vein (Smith and Wall 1997).

6.2.4 Statistical Analyses

All analyses were completed in R 3.1.1(R Project for Statistical Computing, http://www.R-project.org/). Analyses were conducted for each mixed species combination (*L. sericata* with *P. regina*, *C. vicina* with *P. regina*, *P. regina* with *L. sericata* and *P. regina* with *C. vicina*) to determine if species composition influenced growth rate, pupal mass, wing vein length and survivorship. To satisfy the assumptions of normality and homogeneity of variance for ANOVA, variables were square-root (larval weight, wing vein length), log (pupal mass) or arc sine (survivorship) transformed.

Growth rate was examined using the weight of larvae at each sampling interval. A two-factor ANOVA (aov function) was performed to determine the effect of temperature, species composition and the interaction of temperature and species composition on growth rate. This was performed for all species combinations used in this study. Polynomial contrasts (lsmeans package, Lenth 2013) were used to determine the differences in the growth rates between heterospecific and conspecific treatments at each temperature.

For each species composition, a two-factor ANOVA was performed to examine the effect of temperature, species composition and the interaction of temperature and species composition on pupal mass. Multiple comparisons post hoc tests were used to examine differences among treatment groups, with a Tukey adjustment to account for multiple comparisons (lsmeans package). Regression analyses were performed to examine the relationship between temperature and pupal mass for each species combination.

For all species treatments, two-factor ANOVA was performed to examine the effect of temperature, species composition and the interaction of temperature and species composition on survivorship. Survivorship was calculated as number of emerged adults divided by the number of initial larvae. To examine the relationship between temperature and survivorship, regression analyses were performed for each species combination.

A two-factor ANOVA was performed to examine the effect of temperature, species composition and the interaction of temperature and species composition on wing vein length. This was done for all species combinations. The relationship between

temperature and wing vein length was examined for each species combination using regression analyses.

6.3 Results

6.3.1 Growth Rate

Temperature significantly affected the growth rate of all species in this study (Table 6.1, Figures 6.1-6.3). For *L. sericata*, growth rate was faster when developing with heterospecifics, but larvae weighed less at 15°C (Figure 6.1). The growth rate of *C. vicina* was influenced by temperature with the slowest growth at 35°C (Table 6.1, Figure 6.2), but not by interactions with *P. regina* (Figure 6.2). The growth rate of *P. regina* was influenced by temperature and the presence of other species (Table 6.1) such that as temperature increased, the growth rate of *P. regina* increased in all species treatments (Figure 6.3).

6.3.2 Pupal Mass

When *L. sericata* developed with *P. regina*, there was a significant interaction of temperature and the presence of *P. regina* on the pupal mass of *L. sericata* ($p = 0.04$) such that the pupal mass of *L. sericata* when developing alone was lower than when developing with *P. regina* at 15°C, yet the pupal mass of *L. sericata* was greater at 35°C when in the presence of *P. regina* (Table 6.2, Figure 6.4).

The pupal mass of *C. vicina* was influenced by an interaction between temperature and the presence of *P. regina* ($p < 0.001$) (Table 6.2). When developing alone, *C. vicina* pupal mass increased with increasing temperature; however, no *C. vicina* pupated at temperatures above 30°C. When developing with *P. regina*, *C. vicina* pupal mass was greatest at 15 and 30°C (Figure 6.5).

Pupal mass of *P. regina* was influenced by the interaction of species composition and temperature when developing with *L. sericata* ($p = 0.002$) however was only marginally significant when *P. regina* developed with *C. vicina* ($p = 0.05$) (Table 6.2).

When developing alone, *P. regina* pupal mass was greatest at 15°C. When developing with *L. sericata*, *P. regina* had a greater pupal mass at 20, 25 and 35°C (Figure 6.6). In contrast, when developing with *C. vicina*, *P. regina* pupal mass was lowest at temperatures between 15-20°C, but greater at temperatures above 20°C (Figure 6.6).

6.3.3 Survivorship

Temperature and species composition interacted to influence the survivorship of *L. sericata* ($p < 0.001$) (Table 6.3). When *L. sericata* developed with conspecifics, as temperature increased, survivorship increased to a peak at 30°C (Figure 6.7). In contrast, when developing in the presence of *P. regina*, *L. sericata* survivorship was reduced at 25-30°C (Figure 6.7).

Survivorship of *C. vicina* also depended on an interaction of species composition and temperature ($p < 0.001$) (Table 6.3). The survivorship of *C. vicina* increased with increasing temperature to 20°C, yet there were no survivors at higher temperatures of 30 and 35°C (Figure 6.8). In the presence of *P. regina*, *C. vicina* survivorship was greatest at 20°C and *C. vicina* survived at 30°C, albeit at a low rate (Figure 6.8).

Temperature and species composition interacted to influence the survivorship of *P. regina*, when *P. regina* developed with *L. sericata* and *C .vicina* ($p < 0.001$) (Table 6.3). When developing with conspecifics, *P. regina* survivorship increased with temperature, peaking between 30-35°C (Figure 6.9). In the presence of *L. sericata* larvae or *C. vicina* larvae, *P. regina* survivorship displayed similar results peaking around 30°C, but had lower survival at 15°C with heterospecifics than when on its own (Figure 6.9).

6.3.4 Adult Body Size (Wing Vein Length)

The wing vein length of *L. sericata* was influenced by temperature ($p < 0.001$) (Table 6.4). As temperature increased, the wing vein length of *L. sericata* increased, with a peak at 20°C, then decreased at higher temperatures (Figure 6.10). The wing vein length of *C. vicina* was influenced by species composition ($p < 0.001$) as well as

temperature ($p < 0.001$) (Table 6.4). The wing vein length of *C. vicina* decreased with increasing temperatures when developing alone and with *P. regina* (Figure 6.11).

The difference in *P. regina* wing vein length was based on the interaction of temperature and species composition when developing with *L. sericata* ($p < 0.001$) and *C. vicina* ($p = 0.001$) (Table 6.4). The wing vein length of *P. regina* increased with increasing temperature, with the largest adults occurring at 25°C when developing alone (Figure 6.12). When developing with *L. sericata*, *P. regina* wing vein length increased, with a peak between 20 and 25°C (Figure 6.12). When *P. regina* developed with *C. vicina*, *P. regina* wing vein length increased with increasing temperature, with a peak between 30-35°C (Figure 6.12).

6.4 Discussion

Ecological conditions can influence development time and adult body size in many organisms. For insects that face competition on limited resources, a trade-off exists between faster development times, which result in increased chance of survival, or the production of larger and more fit adults (Prinkkilä and Hanski 1995; Smith and Wall 1997). It has been hypothesized that the minimum requirements for the occurrence of life history events, or the development thresholds, influence physiological processes (Day and Rowe 2002). Adaptations to combat adverse conditions, either environmental or due to species interactions, consist of rapid growth during larval stages, having a lower critical weight to reach before pupation or an overall reduction of adult body size in order to promote greater survivorship (Ullyett 1950; Saunders and Bee 1995). There are many interactions, both positive and negative, that can influence individuals that are feeding and developing on an ephemeral resource. The nature of these interactions experienced by the developing larvae will impact the adult size, survivorship and fitness of these individuals. In the present study, interactions, both intraspecific and interspecific, influenced larval development of *L. sericata*, *C. vicina* and *P. regina*.

6.4.1 Lucilia sericata

The survival and success of larvae developing on ephemeral resources, such as carrion, depends on the rate of development and the time they require to reach a minimum weight for pupation (Ullyett 1950; Levot *et al*. 1979). The growth rate for *L. sericata* was similar during both intra and interspecific interactions. Previous studies have found that *L. sericata* is a poor competitor, by displaying negative effects on survival and adult size due to interspecific competition (Hutton and Wasti 1980; Smith and Wall 1997; Kheirallah *et al.* 2007). Other studies have indicated that *L. sericata* may be facilitated by the presence of other species, such as *P. regina* (Rosati 2014; Pacheco 2015). Our study, however, demonstrates that the outcomes of these interactions among different species may be dependent on temperature. When developing in the presence of conspecifics, *L. sericata* had the smallest pupal mass at 15°C and the largest mass at 20°C indicating that this species has delayed development between these two temperatures. This is corroborated by previous data, as Higley *et al*. (2014) reported delays in development of *L. sericata* and *P. regina* at temperatures below 17.5°C.

The survivorship of blow flies can be dependent on temperature and species interactions. For *L. sericata*, decreases in survivorship were more apparent at higher temperatures during interspecific interactions with *P. regina*. This agrees with findings of Smith and Wall (1997) in which *L. sericata* was negatively influenced by the presence of other species.

Lucilia sericata demonstrates plasticity in growth and development and are often more successful in reaching the adult stage compared to other blow fly species when developing under adverse conditions (Ullyett 1950; Tarone and Foran 2006). The adult size of *L. sericata* in this study indicates that temperature had a strong influence, yet species interactions did not. Although others have documented a reduction in size when *L. sericata* interacts with heterospecifics (Hutton and Wasti 1980; Prinkkilä and Hanski 1995; Smith and Wall 1997; Kheirallah *et al*. 2007), the results of this study demonstrate that there are no differences in adult size due to species interactions. This may be due to

the low density of larvae used in this study, as we did not allow them to naturally colonize or manipulate density of individuals. It would be of great interest to add the factor of density to outcomes of species interactions at different temperatures as our previous results demonstrate that females change the number of eggs that they lay in the presence of heterospecifics and at different temperatures (Chapters 2-3).

6.4.2 Calliphora vicina

For *C. vicina*, temperature had an influence on development, as expected. Growth rates of *C. vicina* were similar in both conspecific and heterospecific treatments and, overall, growth rate increased with temperature. Although the differences were not significant when developing in the presence of conspecifics, there was an overall increase in pupal mass with increasing temperature. At temperatures between 30-35°C, *C. vicina* did not successfully pupate. This is not surprising given that *C. vicina* is considered a cold weather species (Smith 1986; Donovan *et al*. 2006). However, when developing with *P. regina*, *C. vicina* larvae successfully pupated at 30°C, a possible indication that *P. regina* facilitated the development of *C. vicina* at this temperature.

Critical weights required for pupation have been studied in many insect systems (Kingsolver 2000; Davidowitz 2003; Mirth and Riddiford 2007). When examining the influence of temperature and diet quality on critical weight of *Manduca sexta* L. (Lepidoptera: Sphingidae), Davidowitz *et al*. (2003) found that while temperature had no influence, critical weight decreased with the decreasing quality of resources. Saunders and Bee (1995) found that the minimum pupal weight for *C. vicina* was 30 mg when developing in low densities of less than 50 larvae, but this critical weight was reduced to 15-20 mg when *C. vicina* was developing at higher densities of 150 larvae or more. These results indicate that critical weight may fluctuate due to the influence of species interactions and competition. In our study, the mean pupal mass of *C. vicina* was greater than 40 mg in all species and temperature treatments, but could have been influenced by the low density used in this study.

Species interactions had mixed effects for *C. vicina*. Survivorship was negatively and positively affected, varying with temperature. Although survivorship was low when developing with *P. regina* at 30°C, there were no *C. vicina* survivors when developing with conspecifics at the same temperature. The presence of *P. regina* larvae positively impacted *C. vicina* development at higher temperatures. This may be due to the smaller size of *P. regina* larvae, compared to *C. vicina*, resulting in less competition for food during interspecific interactions at the same density of larvae.

For *C. vicina*, increasing temperature resulted in smaller adults. Adult *C. vicina* were larger when interacting with *P. regina* compared to intraspecific interactions, although these differences were not significant. Similar to the survivorship results, the body size of *C. vicina* may be greater when developing with *P. regina* due to the smaller size of these larvae, and the lower nutritional requirements of *P. regina*.

6.4.3 Phormia regina

When developing with *L. sericata*, *P. regina* pupal mass was greater at most temperatures tested, indicating possible facilitation. Others have suggested that the presence of proteolytic enzymes released by *L. sericata* may facilitate more efficient feeding by other blow fly species (Baxter and Morrison 1983; Reis *et al*. 1999; Ireland and Turner 2006; Kheirallah *et al*. 2007). The release of these enzymes may be a mechanism that facilitates feeding by *P. regina*, resulting in larger larvae, with greater mass at pupation. A similar trend was observed when *P. regina* developed with *C. vicina*, but was more noticeable at higher temperatures. This increase in *P. regina* pupal mass may be the result of the low *C. vicina* survivorship at higher temperatures, allowing the food resource to be more accessible for *P. regina*.

The reduction in pupal mass at the highest temperatures might be due to the tradeoffs associated with metabolism and food acquisition costs, which can affect development (Higley and Haskell 2010; Ribiero and Von Zuben 2010; Tarone *et al*. 2011). Temperature can also have an effect, as there is a direct relationship between temperature and feeding rates; as temperatures increase, feeding rates increase and larvae may not be able to metabolize as quickly, leading to smaller adults (Atkinson 1996;

Tarone and Foran 2006). Competition for food, especially between larvae that differ in size such as *P. regina* and *C. vicina*, can result in reduced larval weight for the smaller species. The larger size and higher metabolic rate of *C. vicina* (Meyer and Schaub 1973) may have influenced the availability of food for *P. regina,* leading to a reduction in pupal mass at lower temperatures.

In regards to survivorship of *P. regina*, this species demonstrated both positive and negative effects due to species interactions. When developing with *L. sericata*, *P. regina* had reduced survivorship at 15, 25 and 35°C and enhanced survivorship only at 20 and 30°C. There was an increase in survivorship between 20-25°C, when *P. regina* was with conspecifics and heterospecifics, indicating that 25°C may represent an optimal temperature for *P. regina* survivorship. Survivorship of *P. regina* was greatly increased in the presence of *C. vicina* at low temperatures between 15-20°C, indicating that *P. regina* is facilitated by *C. vicina* at these temperatures.

The wing vein length and therefore adult size of *P. regina* increased with temperature. When interacting with *L. sericata*, *P. regina* displayed greater adult size, but this difference was only prominent at 20°C. When *P. regina* developed in the presence of *C. vicina*, adult size increased with increasing temperatures and was greatest at 30 and 35°C, but again, this was most likely due to the diminished survivorship of *C. vicina* at these temperatures, which made the food resource more available.

6.4.4 Future Areas of Consideration

Although the present study examined species interactions in various combinations of blow flies, it would be worthwhile to examine these interactions at varying densities. It is likely that initial densities of each species' population affect the outcome of the interaction, where the more abundant species has the greatest probability of dominating a resource (Mittelbach 2012; VanLaerhoven 2015). Known as founder control, at each carrion resource, a different species could arrive to and colonize the resource at a greater abundance ultimately resulting in different potential outcomes on each of these carrion patches (Mittelbach 2012; VanLaerhoven 2015). The density of 20 larvae utilized in this study limits the amount of competition that the larvae can experience while developing.

On carrion, much higher densities of larvae occur, which can result in the formation of a maggot mass. The large number of larvae associated with maggot masses produce heat due to metabolic activity, resulting in elevated temperatures and accelerated development (Catts and Goff 1992; Turner and Howard 1992). This may be an advantage when temperatures are below the optimum temperature for a species but may be detrimental if it results in temperature over the thermal tolerances of a species. As we did not manipulate density, it remains to be seen if the outcomes observed here would differ.

Associated with density, the nutritional requirement for each species, as well as the growth rate and size may impact the interactions. For example, *C. vicina* is a larger fly and may have the ability to exclude smaller species by dominating a food source. However, *C. vicina* generally has a longer developmental period, which can be a disadvantage when developing on an ephemeral resource if they run out of food before achieving their critical weight. In addition, development on different food types can result in variable growth rates (Kaneshrajah and Turner 2004; Clark et al. 2006; Tarone and Foran 2006; Reid 2012). Tarone and Foran (2006) found that *L. sericata* developed faster on whole rat carcasses when compared to liver alone. Others documented faster development of *C. vicina* when reared on kidney, brain and heart tissues (Kaneshrajah and Turner 2004). Therefore, the development times and interaction outcomes reported in this study may change if a different food source is utilized, depending on the nutritional requirements of each species.

Temporal variability can also impact the interactions among species and the outcomes of such interactions. Chesson and Warner (1981) describe the lottery model, in which the outcome of species interactions depends on fluctuating population dynamics as well as resource availability. Competitive exclusion can be prevented due to fluctuation in the environmental conditions such as temperature that changes throughout the year; however, the species that has the greatest number of offspring when a resource is made available at the same time temperatures are also favourable for flight and oviposition is more likely to establish a population on that resource. Because carrion resources occur randomly and the population size of species at any given time is unpredictable, the changes of locating and establishing a population on this resource can be equated to winning a lottery (Chesson and Warner 1981; VanLaerhoven 2015). It is possible that

temporal and spatial fluctuation, prevent any one species from always being favoured, thereby allowing the coexistence of multiple species that utilize this ephemeral resource.

At a smaller temporal scale, the development of blow flies under constant temperatures, as presented here, may also differ from those developing under fluctuating temperatures (Greenberg 1991; Davies and Ratcliffe 1994; Byrd and Allen 2001). Fluctuating temperatures exemplify temperatures blow flies experience in natural conditions, but the results of fluctuating temperatures on larval development are mixed. Some species develop faster under fluctuating temperatures, such as *Calliphora vomitoria* L. (Hagstrum and Hagstrum 1970), whereas development of *L. sericata*, *P. regina* and *C. vicina* are delayed under periods of fluctuating temperatures (Greenberg 1991; Niederegger et al. 2010). Since temperature interacts with development to mediate species interactions, fluctuating temperatures have the potential to change outcomes of species interactions. For *C. vicina*, constant temperatures of 30°C and above proved lethal in the third instar stage when developing with conspecifics, yet some *C. vicina* survived these temperatures when developing with heterospecifics. It would be important to determine if fluctuating temperature changed the outcome of this interaction.

6.4.5 Conclusion

The information obtained here demonstrates that there are differences in blow fly development due to abiotic and biotic factors, which may change outcomes of community dynamics as these factors, such as temperature and the presence of other species, fluctuate over a spatial and temporal scale. Species coexistence can occur for carrion communities by spatial and temporal heterogeneity (VanLaerhoven 2015). Spatially, coexistence can occur due to the partitioning of species by habitat or season, or by aggregation of individuals in different sites on the resource (Atkinson and Shorrocks 1984; Fiene *et al*. 2014). This assumes that the primary outcome of species interactions is competition, where greater intraspecific compared to interspecific competition results in coexistence of multiple species on a limited resource (Fiene *et al*. 2014; VanLaerhoven 2015). Yet, oviposition behaviour of *P. regina* suggested that it is facilitated by the presence of other species at temperatures at or below 25°C (Chapter 3) and indeed, in the

presence of *L. sericata*, *P. regina* adults are larger and have a larger egg load as a result (Reid 2012) and in the presence of *C. vicina*, has a higher survival than on its own at some temperatures below 25°C, thereby providing some evidence of facilitation at the density tested here.

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Table 6.1. Analysis of Variance (ANOVA) results to determine the effects of species composition, temperature and the interaction of these effects, on the growth rate (mg/sampling) for *L. sericata*, *C. vicina* and *P. regina*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects.

Effect	d.f.	F ratio	*p* - value
	Lucilia sericata* with *Phormia regina		
Species Composition	1, 176	0.97	0.327
Temperature	**4, 176**	**2.65**	**0.035**
Species Composition * Temperature	4, 176	1.68	0.157
	Calliphora vicina* with *Phormia regina		
Species Composition	1, 212	0.46	0.497
Temperature	**4, 212**	**2.69**	**0.032**
Species Composition * Temperature	4, 212	0.31	0.874
	Phormia regina* with *Lucilia sericata		
Species Composition	1, 142	2.61	0.109
Temperature	**4, 142**	**4.95**	**< 0.001**
Species Composition * Temperature	4, 142	1.33	0.263
	Phormia regina* with *Calliphora vicina		
Species Composition	**1, 152**	**8.19**	**0.005**
Temperature	**4, 152**	**4.73**	**0.001**
Species Composition * Temperature	4, 152	0.48	0.752

Table 6.2. Analysis of Variance (ANOVA) results to determine the effects of species composition, temperature and the interaction of these effects on the pupal mass (mg) for *L. sericata*, *C. vicina* and *P. regina*. Significant effects are indicated in bold font; α = 0.05 for all effects.

Effect	d.f.	F ratio	*p* - value
	Lucilia sericata* with *Phormia regina		
Species Composition	1, 117	2.18	0.143
Temperature	**4, 117**	**13.68**	**< 0.001**
Species Composition * Temperature	**4, 117**	**2.89**	**0.039**
	Calliphora vicina* with *Phormia regina		
Species Composition	**1, 41**	**8.85**	**0.005**
Temperature	**4, 41**	**26.75**	**< 0.001**
Species Composition * Temperature	**4, 41**	**9.85**	**< 0.001**
	Phormia regina* with *Lucilia sericata		
Species Composition	1, 109	1.00	0.319
Temperature	4, 109	1.79	0.136
Species Composition * Temperature	**4, 109**	**4.51**	**0.002**
	Phormia regina* with *Calliphora vicina		
Species Composition	1, 103	1.54	0.218
Temperature	4, 103	2.09	0.089
Species Composition * Temperature	**4, 103**	**2.98**	**0.049**

Table 6.3. Analysis of Variance (ANOVA) results to determine the effects of species composition, temperature and the interaction of these effects on the survivorship of *L. sericata*, *C. vicina* and *P. regina*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects.

Effect	d.f.	F ratio	*p* - value
	Lucilia sericata* with *Phormia regina		
Species Composition	**1, 131**	**62.80**	**<0.001**
Temperature	**4, 131**	**7.25**	**<0.001**
Species Composition * Temperature	**4, 131**	**18.33**	**<0.001**
	Calliphora vicina* with *Phormia regina		
Species Composition	**1, 87**	**21.66**	**<0.001**
Temperature	**4, 87**	**131.84**	**<0.001**
Species Composition * Temperature	**4, 87**	**24.67**	**<0.001**
	Phormia regina* with *Lucilia sericata		
Species Composition	1, 137	1.422	0.234
Temperature	**4, 137**	**98.16**	**<0.001**
Species Composition * Temperature	**4, 137**	**8.36**	**<0.001**
	Phormia regina* with *Calliphora vicina		
Species Composition	**1, 141**	**3.65**	**<0.001**
Temperature	4, 141	34.46	0.056
Species Composition * Temperature	**4, 141**	**10.83**	**<0.001**

Table 6.4. Analysis of Variance (ANOVA) results to determine the effects of species composition, temperature and the interaction of these effects on the wing vein length (mm) for *L. sericata*, *C. vicina* and *P. regina* mixed with *L. sericata* and *C. vicina*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects.

Effect	d.f.	F ratio	*p* - value
	Lucilia sericata* with *Phormia regina		
Species Composition	1, 131	0.20	0.652
Temperature	**4, 131**	**118.9**	**< 0.001**
Species Composition * Temperature	4, 131	2.35	0.053
	Calliphora vicina* with *Phormia regina		
Species Composition	**1, 87**	**12.94**	**< 0.001**
Temperature	**4, 87**	**81.68**	**< 0.001**
Species Composition * Temperature	4, 87	0.36	0.699
	Phormia regina* with *Lucilia sericata		
Species Composition	**1, 137**	**22.52**	**< 0.001**
Temperature	**4, 137**	**81.32**	**< 0.001**
Species Composition * Temperature	**4, 137**	**12.23**	**< 0.001**
	Phormia regina* with *Calliphora vicina		
Species Composition	1, 141	0.362	0.547
Temperature	**4, 141**	**129.93**	**< 0.001**
Species Composition * Temperature	**4, 141**	**5.85**	**0.001**

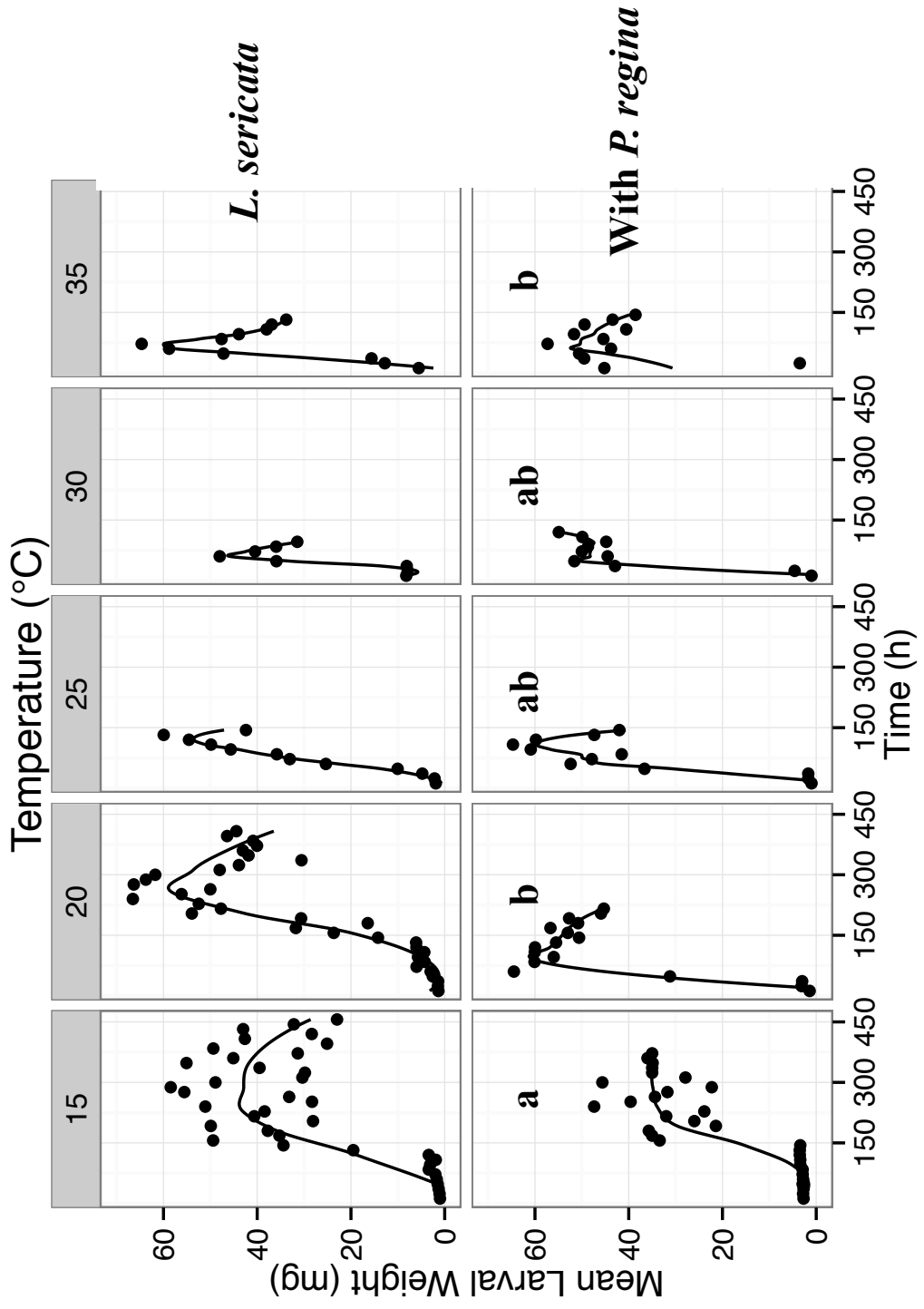

Figure 6.1. Mean larval (first instar through third instar) growth rate across all temperatures for *L. sericata* when developing alone and mixed with *P. regina*. There was a significant effect of temperature on growth rate of *L. sericata* ($F_{4, 176} = 2.65$, $p = 0.035$) but no effect of species. Letters indicate significant differences between temperatures.

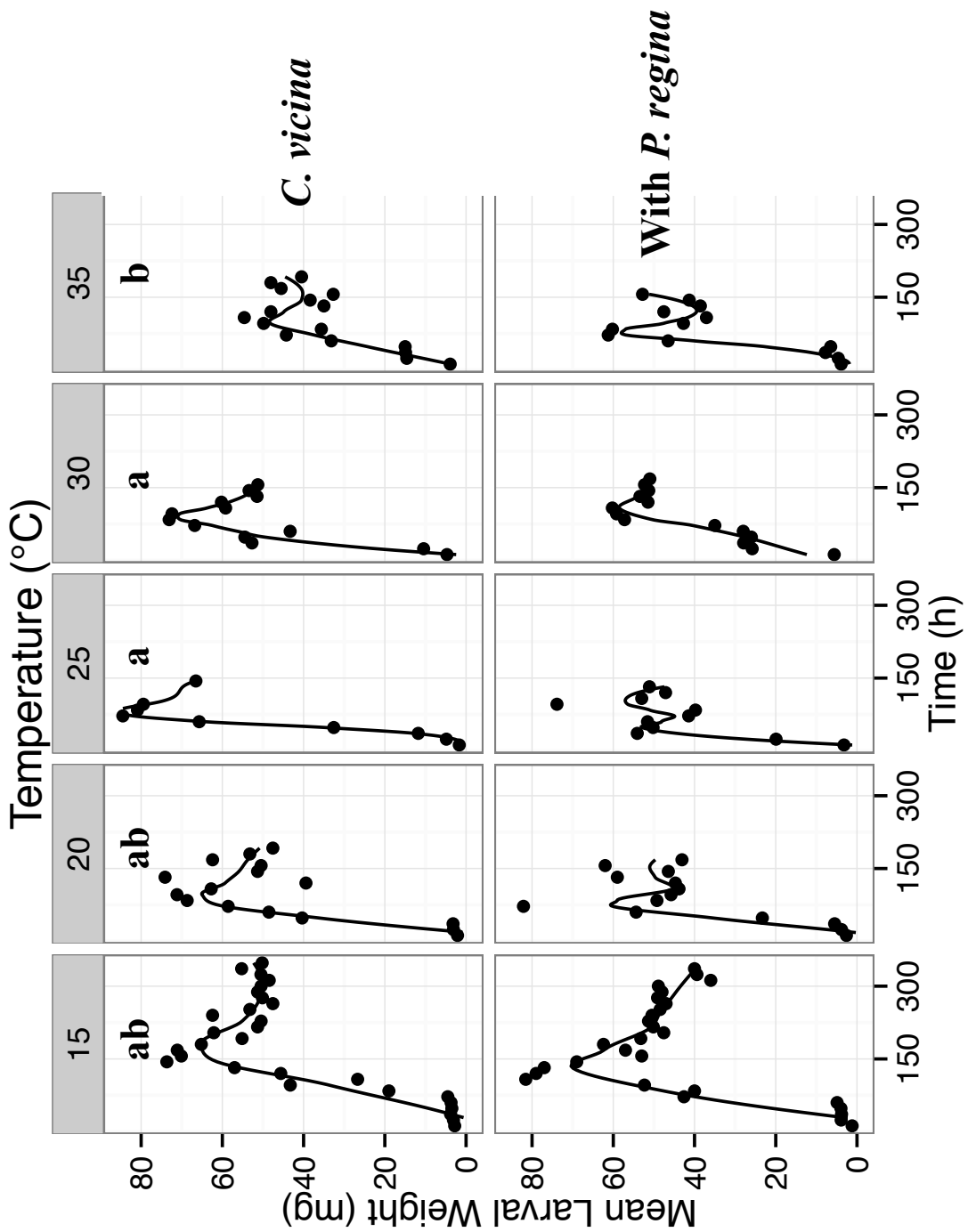

Figure 6.2. Mean larval (first instar through third instar) growth rate across all temperatures for *C. vicina* when developing alone and mixed with *P. regina*. There was a significant effect of temperature on growth rate ($F_{4, 212} = 2.69$, $p = 0.032$) but no effect of species. Letters indicate significant differences between temperatures.

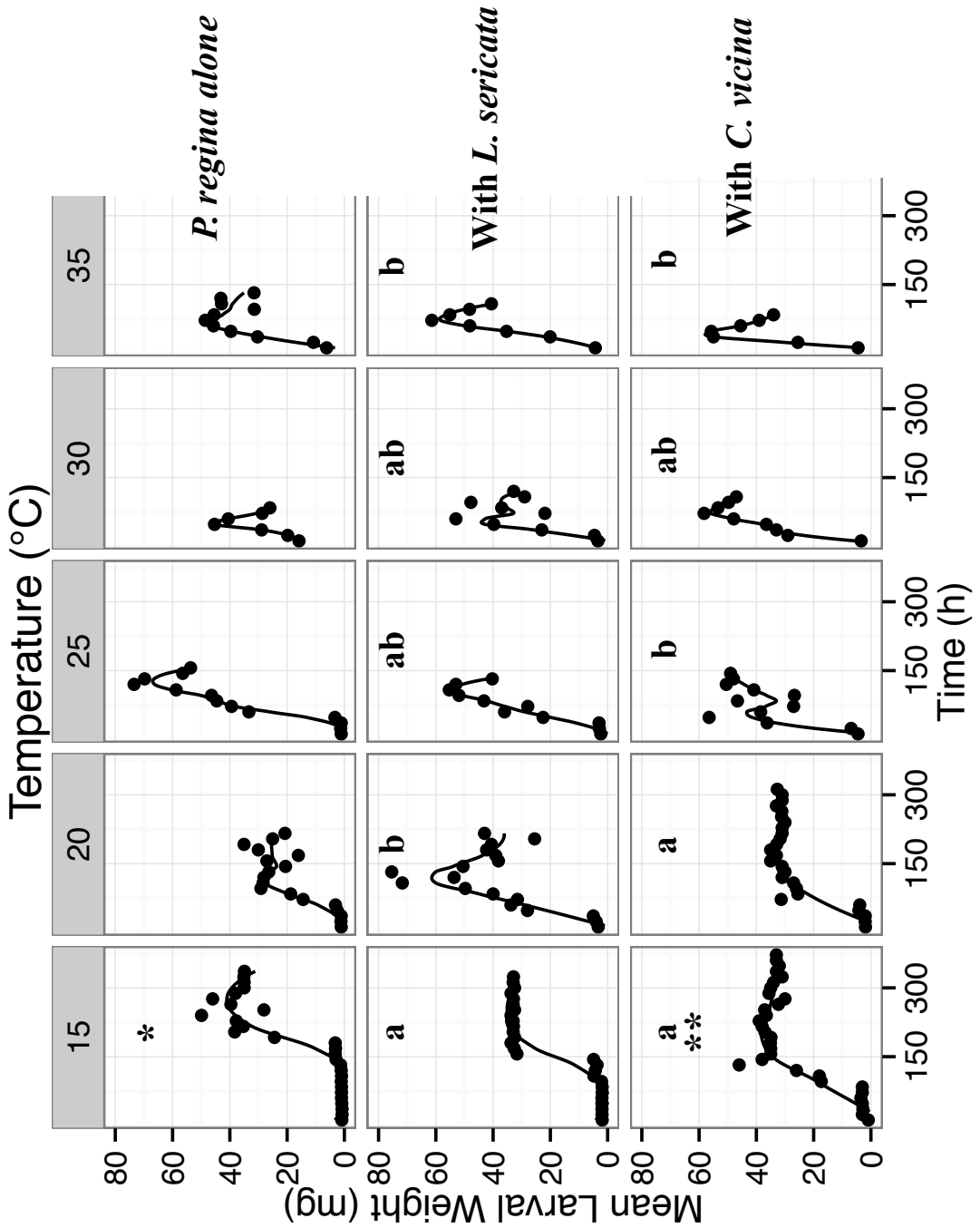

Figure 6.3. Mean larval (first instar through third instar) growth rate across all temperatures for *P. regina* when developing alone and mixed with *L. sericata* and *C. vicina*. There was a significant effect of temperature on growth rate of *P. regina* in mixed treatments with *L. sericata* ($F_{4, 142} = 4.95$, $p < 0.001$). There was a significant effect of temperature ($F_{4, 152} = 4.73$, $p = 0.001$) and species composition ($F_{4, 152} = 8.19$, $p = 0.005$) on *P. regina* growth rate in mixed treatments with *C. vicina.* Asterisks indicate the effect of species within temperatures among species combinations.

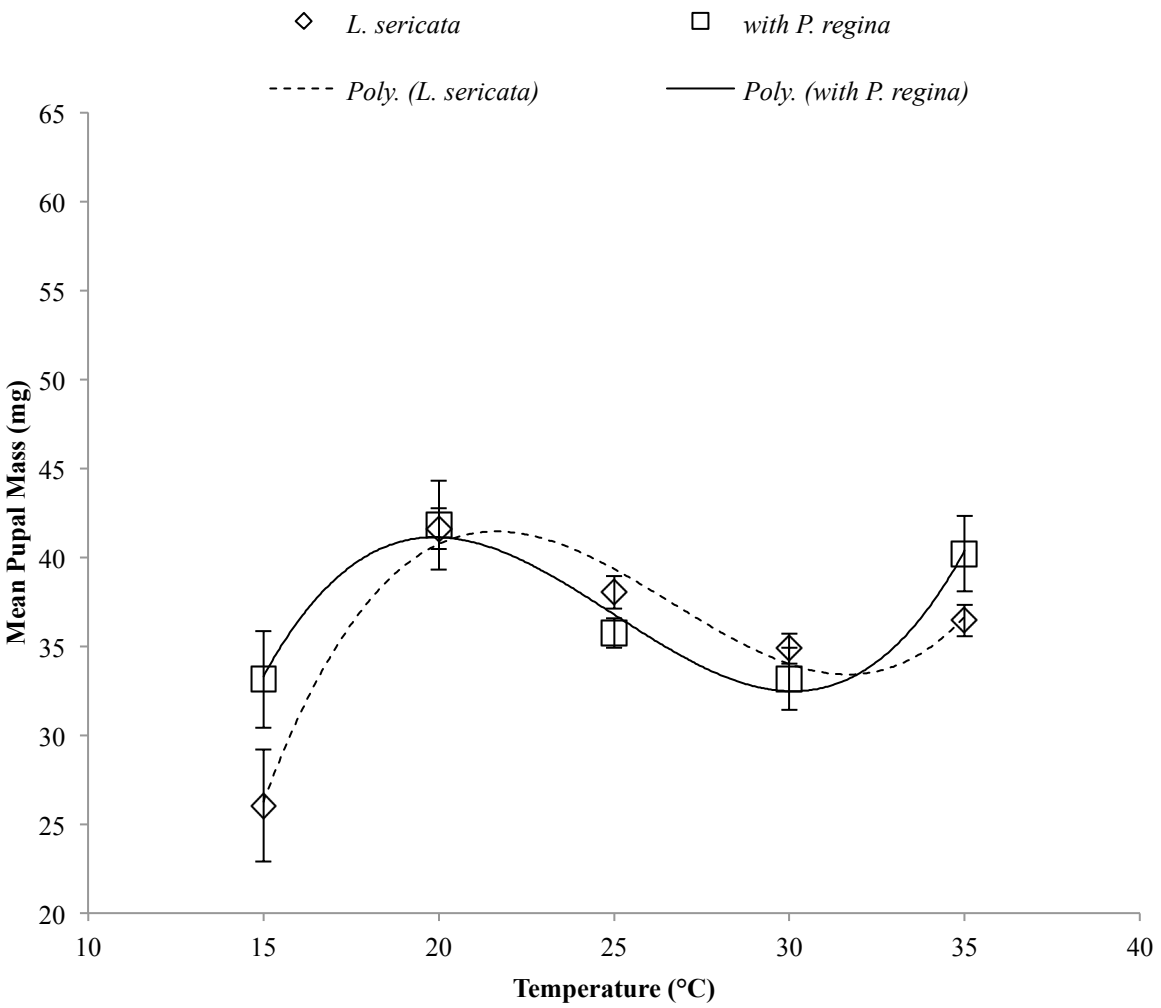

Figure 6.4. Mean pupal mass (±S.E.) across all temperatures for *L. sericata* when developing alone and mixed with *P. regina*. The interaction between temperature and species composition was significant for *L. sericata* pupal mass ($F_{4,117} = 2.89$, $p = 0.039$). For *L. sericata* alone ($y = 0.016x^3 – 1.27x^2 + 32.72x – 231.86$, $R^2 = 0.64$) and for *L. sericata* mixed with *P. regina* ($y = 0.017x^3 – 1.22x^2 + 29.16x – 184.91$, $R^2 = 0.66$).

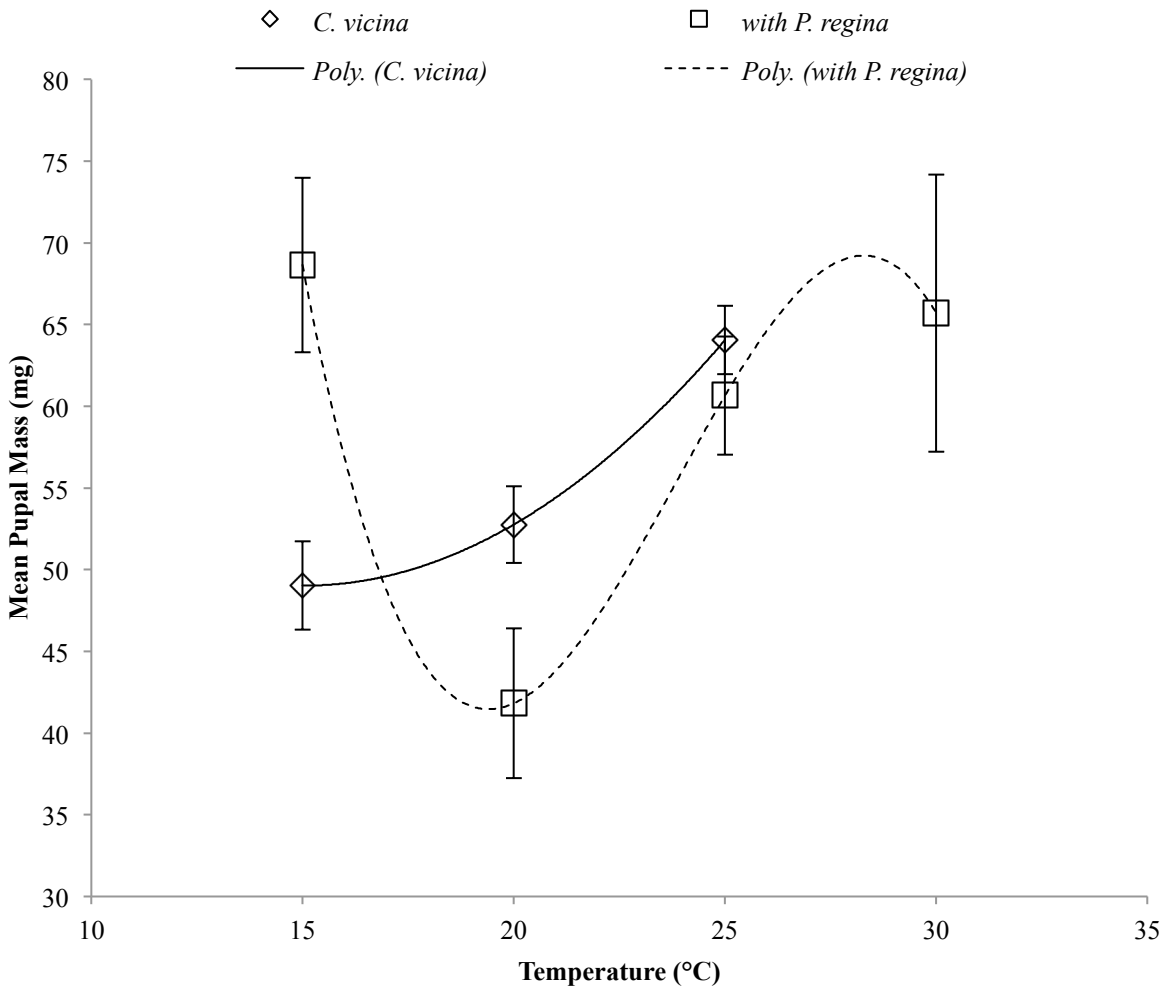

Figure 6.5. Mean pupal mass (±S.E.) across all temperatures for *C. vicina* when developing alone and mixed with *P. regina*. The interaction between temperature and species composition was significant for *C. vicina* pupal mass ($F_{4,41} = 9.85$, $p < 0.001$). No *C. vicina* pupated at 30°C when alone or at 35°C for either treatment. For *C. vicina* alone ($y = 0.151x^2 – 4.54x + 83.15$, $R^2 = 0.82$) and for *C. vicina* mixed with *P. regina* ($y = -0.079x^3 + 5.66x^2 -130.41x + 1017.2$, $R^2 = 0.89$).

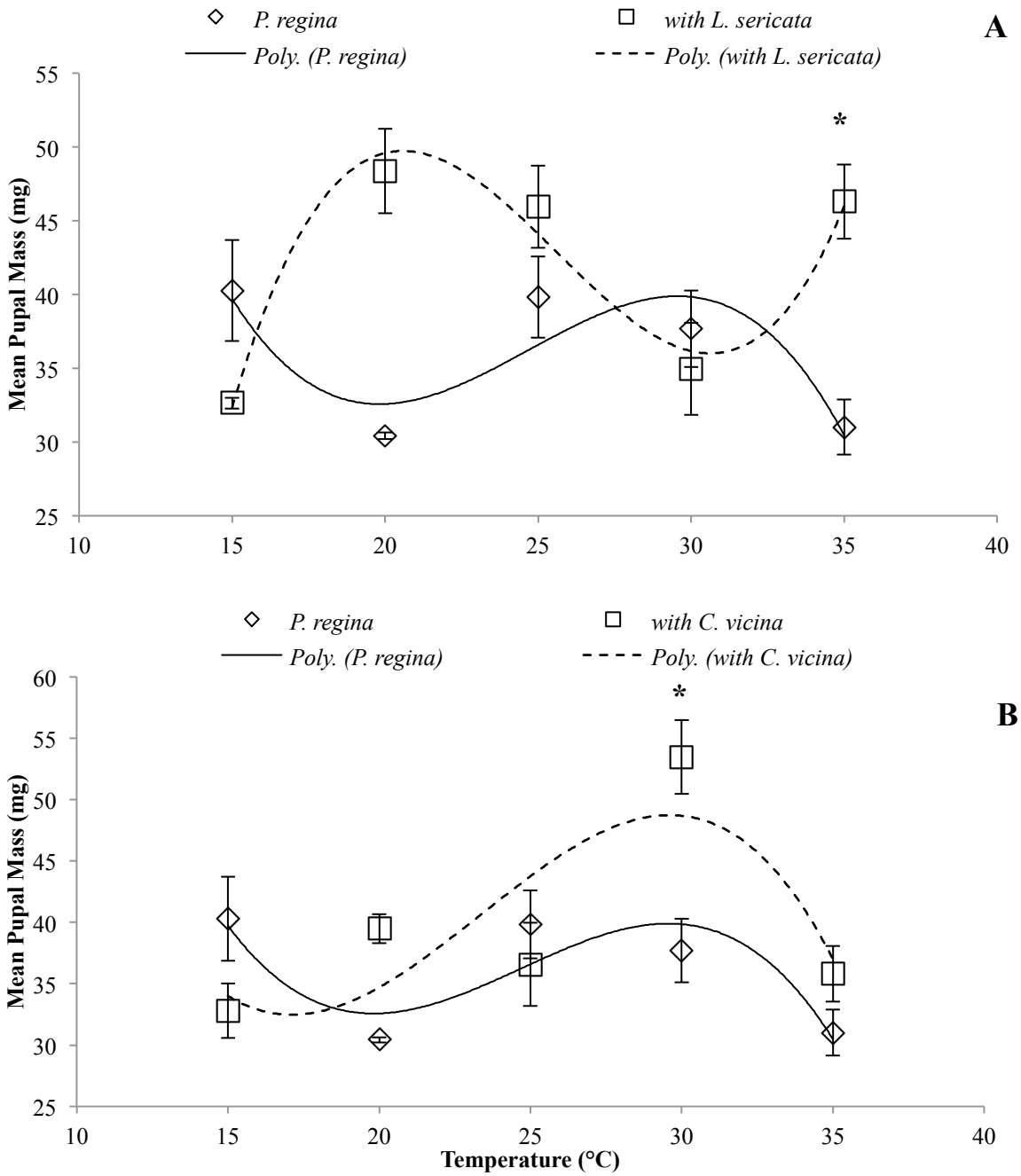

Figure 6.6. Mean pupal mass (±S.E.) across all temperatures for *P. regina* when developing alone and mixed with *L. sericata* (A) or *C. vicina* (B). The interaction between temperature and species composition was significant for *P. regina* pupal mass when developing with *L. sericata* ($F_{4,109} = 4.51$, $p = 0.002$), but only marginally significant when developing with *C. vicina* ($F_{4,103} = 2.48$, $p = 0.05$). For *P. regina* alone ($y = -0.016x^3 + 1.16x^2 - 27.88x + 247.13$, $R^2 = 0.78$), mixed with *L. sericata* ($y = 0.027x^3 - 2.07x^2 + 51.12x - 358.45$, $R^2 = 0.76$) and mixed with *C. vicina* ($y = -0.017x^3 + 1.16x^2 - 25.27x + 206.89$, $R^2 = 0.62$). Means with an asterisk indicate a difference between *P. regina* alone or with *L. sericata* (A) or *C. vicina* (B) at that temperature.

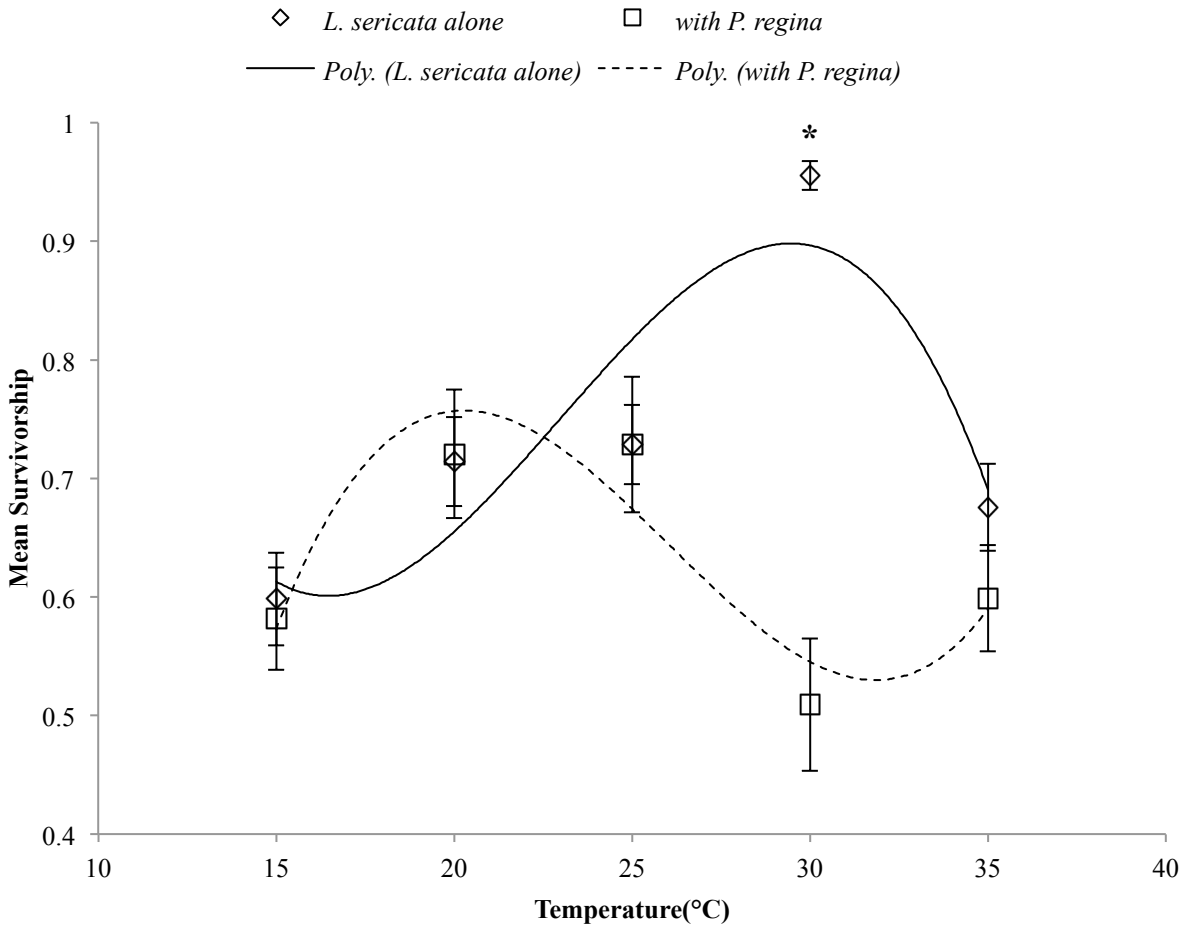

Figure 6.7. Mean survivorship (±S.E.) across all temperatures for *L. sericata* when developing alone and mixed with *P. regina*. The interaction between temperature and species composition was significant for *L. sericata* survivorship ($F_{4,131} = 18.33$, $p < 0.001$). For *L. sericata* alone ($y = -0.0003x^3 + 0.019x^2 – 0.39x + 3.35$, $R^2 = 0.78$) and for *L. sericata* mixed with *P. regina* ($y = 0.0003x^3 – 0.023x^2 + 0.57x – 3.78$, $R^2 = 0.64$). Means with an asterisk indicate a difference between *L. sericata* alone or with *P. regina* at that temperature.

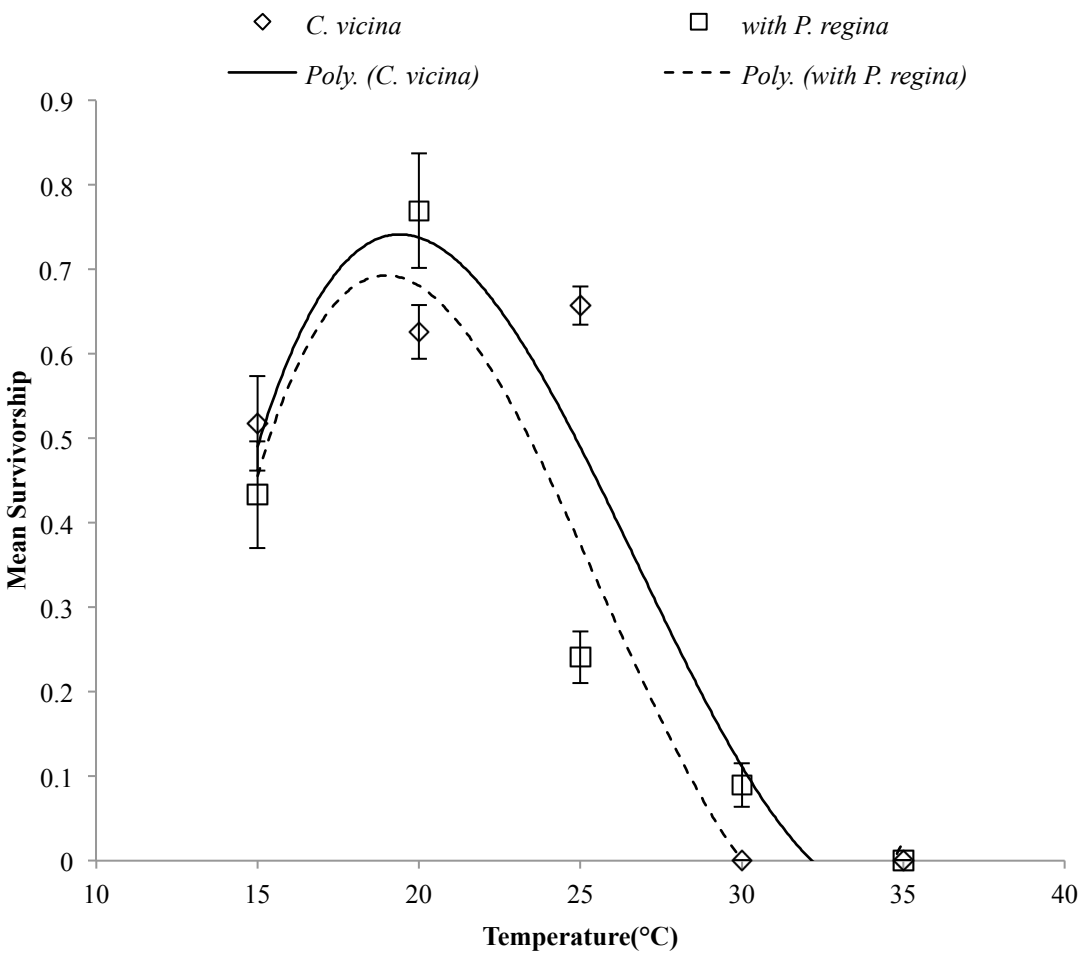

Figure 6.8. Mean survivorship (±S.E.) across all temperatures for *C. vicina* when developing alone and mixed with *P. regina*. The interaction between temperature and species composition was significant for survivorship of *C. vicina* when developing with *P. regina* ($F_{4,87} = 24.67$, $p < 0.001$). For *C. vicina* alone ($y = 0.0005x^3 + 0.039x^2 + 0.97x + 6.90$, $R^2 = 0.67$) and for *C. vicina* mixed with *P. regina* ($y = 0.0006x^3 – 0.05x^2 + 1.14x – 8.04$, $R^2 = 0.70$). Means with an asterisk indicate a difference between *C. vicina* alone or with *P. regina* at that temperature.

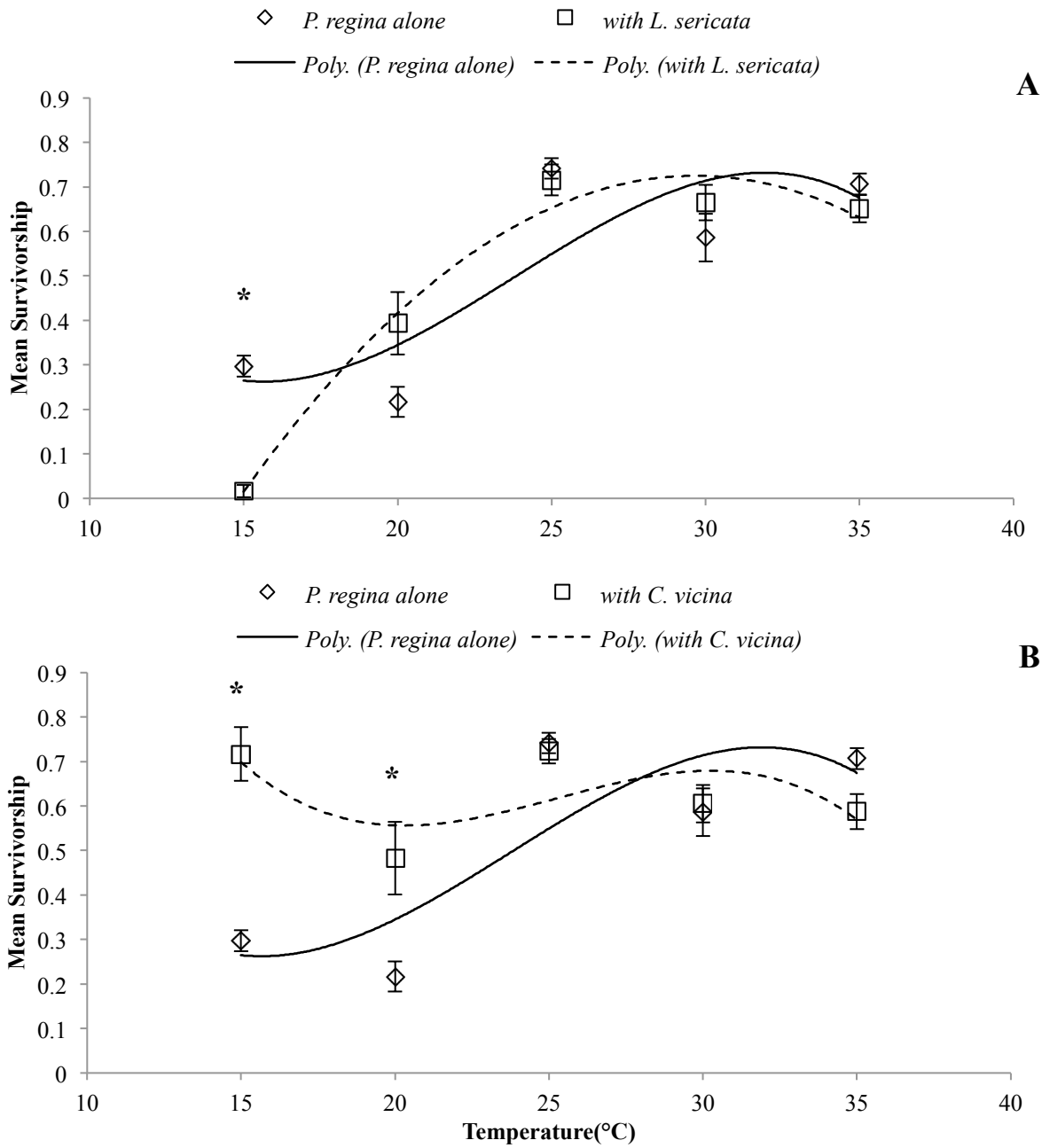

Figure 6.9. Mean survivorship (±S.E.) across all temperatures for *P. regina* when developing alone and mixed with *L. sericata* or *C. vicina*. The interaction between temperature and species composition was significant for *P. regina* survivorship when developing with *L. sericata* ($F_{4,137} = 8.36$, $p < 0.001$) and *C. vicina* ($F_{4,141} = 10.83$, $p < 0.0001$). For *P. regina* alone ($y = -0.002x^3 + 0.02x^2 - 0.33x + 2.4$, $R^2 = 0.68$), mixed with *L. sericata* ($y = -0.003x^2 + 0.19x - 2.18$, $R^2 = 0.67$) and mixed with *C. vicina* ($y = 0.0002x^3 + 0.02x^2 - 0.46x + 4.19$, $R^2 = 0.40$). Means with an asterisk indicate a difference between *P. regina* alone or with *L. sericata* (A) or *C. vicina* (B) at that temperature.

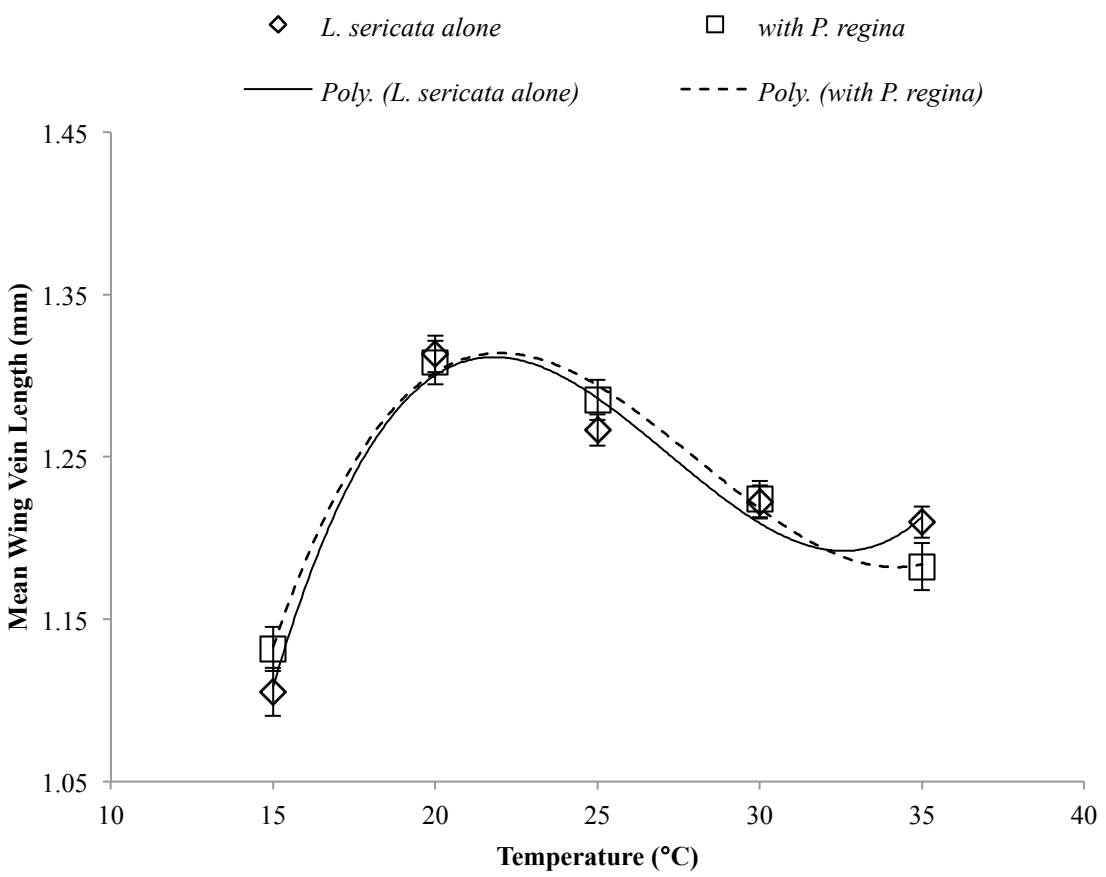

Figure 6.10. Mean wing vein length (±S.E.) across all temperatures for *L. sericata* when developing alone and mixed with *P. regina*. There was a significant effect of temperature on wing vein length ($F_{4,131} = 118.9$, $p < 0.001$). For *L. sericata* alone ($y = 0.0002x^3 - 0.016x^2 – 0.41x – 2.14$, $R^2 = 0.76$) and for *L. sericata* mixed with *P. regina* ($y = 0.0001x^3 – 0.01x^2 + 0.34x – 1.53$, $R^2 = 0.79$).

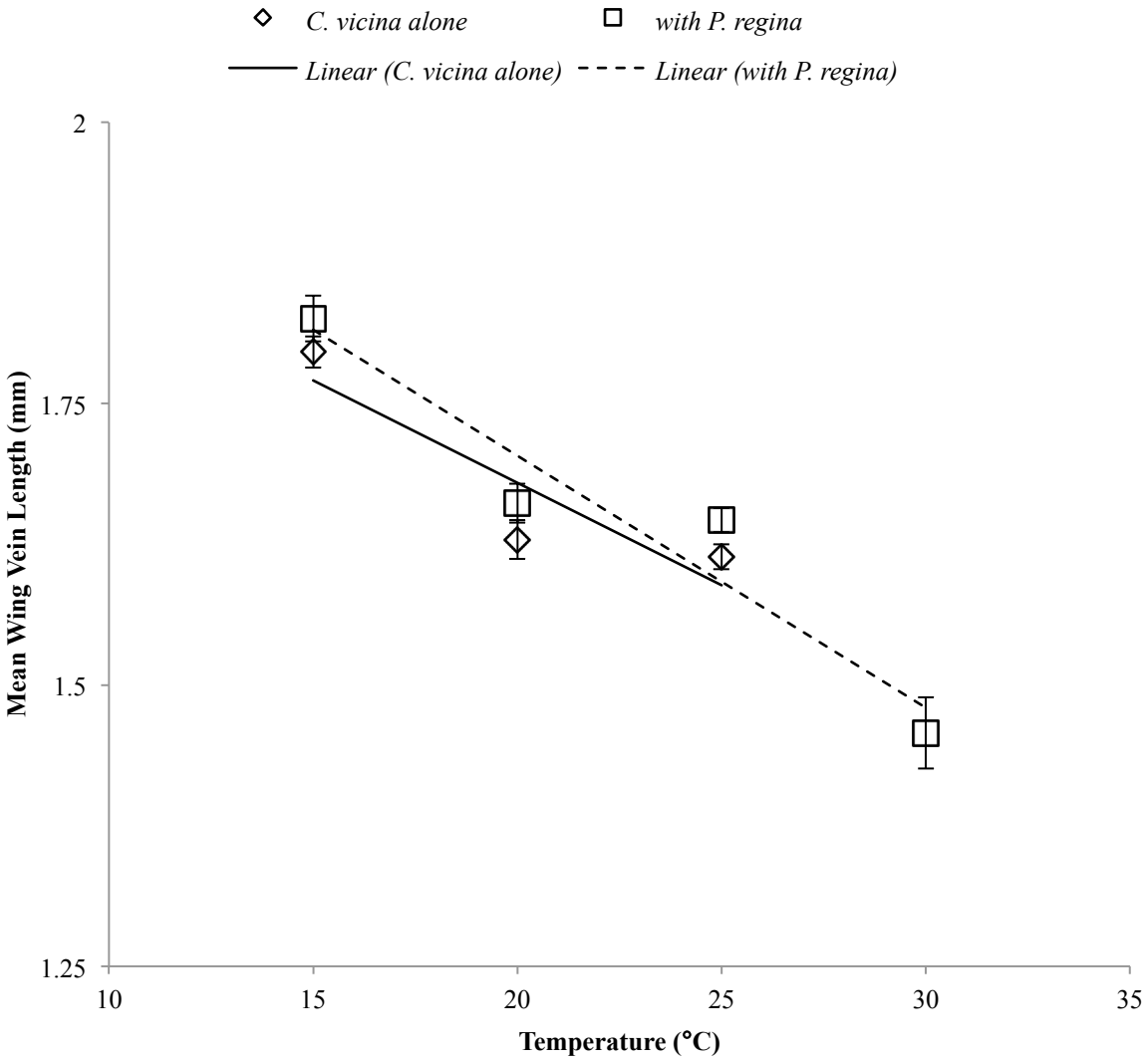

Figure 6.11. Mean wing vein length (±S.E.) across all temperatures for *C. vicina* when developing alone and mixed with *P. regina*. There was a significant effect of temperature ($F_{4, 87} = 81.68$, $p < 0.001$) and species composition ($F_{1, 87} = 12.94$, $p < 0.001$) on wing vein length. No *C. vicina* survived at 30°C for *C. vicina* mixed with P. regina or at 35°C for either treatment. For *C. vicina* alone ($y = -0.018x + 2.04$, $R^2 = 0.93$) and for *C. vicina* mixed with *P. regina* ($y = -0.022x + 2.15$, $R^2 = 0.89$).

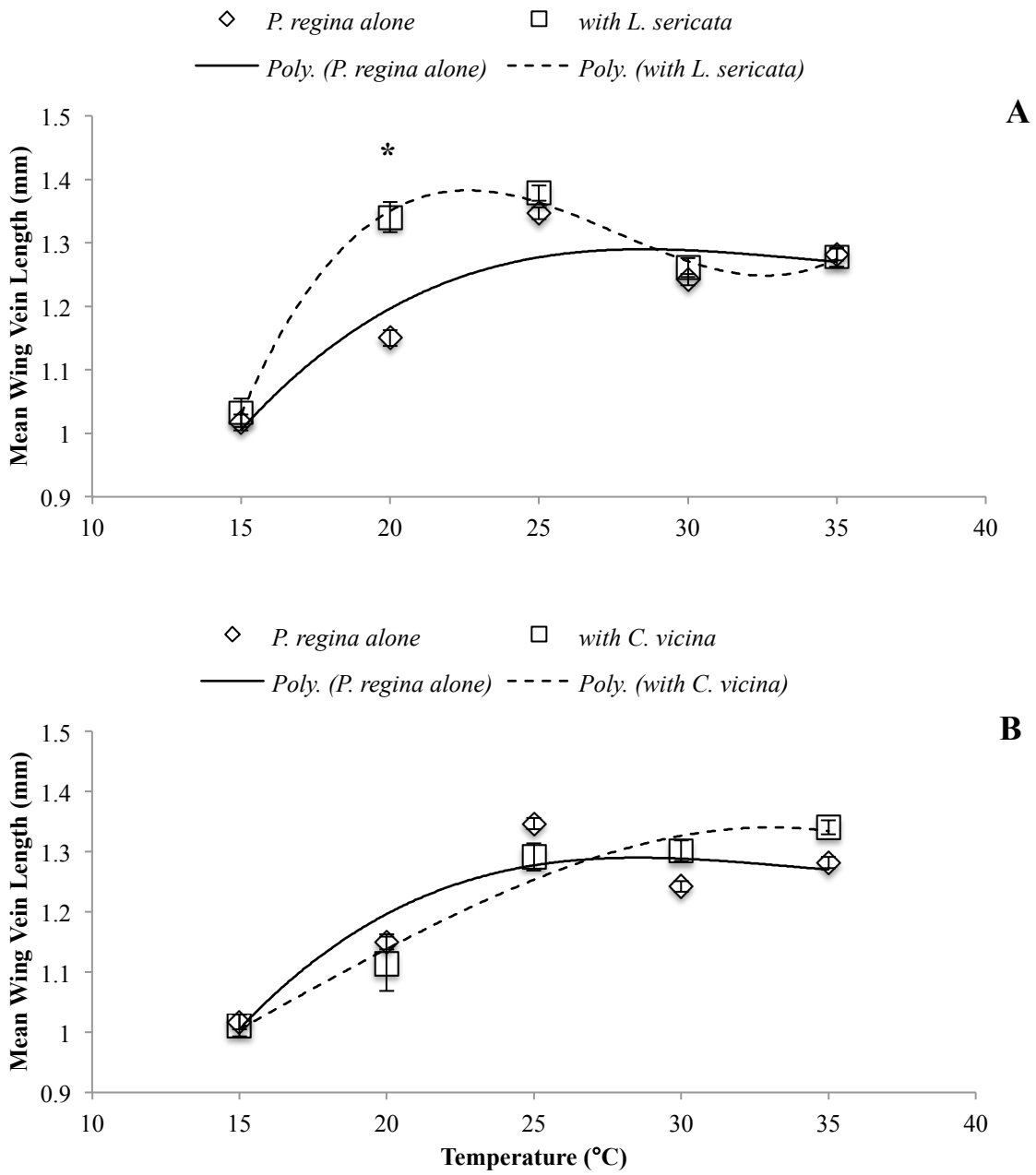

Figure 6.12. Mean wing vein length (±S.E.) across all temperatures for *P. regina* when developing alone and in mixed treatments with *L. sericata* (A) or *C. vicina* (B). The interaction between temperature and species composition was significant for *P. regina* when developing with *L. sericata* ($F_{4,137} = 12.23$, $p < 0.001$) and *C. vicina* ($F_{4,141} = 5.85$, $p = 0.001$). For *P. regina* alone ($y = 0.0005x^3 - 0.005x^2 + 0.18x - 0.64$, $R^2 = 0.65$), mixed with *L. sericata* ($y = 0.0003x^3 - 0.02x^2 + 0.59x - 3.79$, $R^2 = 0.79$) and mixed with *C. vicina* ($y = 0.0005x^3 + 0.002x^2 + 0.003x + 0.73$, $R^2 = 0.66$). Means with an asterisk indicate a difference between *P. regina* alone or with *L. sericata* (A) at that temperature.

CHAPTER 7
SPECIES INTERACTIONS AND TEMPERATURE INFLUENCE THE BEHAVIOUR AND DEVELOPMENT OF BLOW FLIES. IMPLICATIONS FOR FORENSIC ENTOMOLOGY

7.1 Overview

Environmental conditions can influence the behaviour and development of insects. Although many studies have documented the arrival times of forensically relevant species during different seasons and at various temperatures, few studies have investigated how these temperatures affect the behaviour of blow flies when arriving to and ovipositing on carrion. The goal of this research was to determine if the oviposition behaviour of blow flies changed over a range of temperatures, or due to species interactions and to determine if these factors interacted to affect development over this temperature range. The role of species interactions and temperature and their effect on blow fly development and behaviour provides information on insect behaviour as well as has applications for forensic entomologists that can assist in more accurate estimates of the time of colonization (TOC). Understanding the ecological concepts associated with blow flies allows for incorporation of this information into making more precise estimates and interpretation of the insect evidence. To complete this study, three species of blow fly, locally occurring in southern Canada, were used: *Lucilia sericata* Meigen, *Phormia regina* Meigen, and *Calliphora vicina* Robineau-Desvoidy.

7.2 Behavioural, Evolutionary and Community Ecology

Natural selection shapes the behaviour of animals, assuming there is genetic variability associated with variable behaviour, and the adaptive significance of certain behaviours may be determined by the impact of their behaviour on reproduction and survival, or fitness (Mousseau and Roff 1987). Reproductive behaviour involves all behaviours associated with locating and selecting mates, as well as allocating energy and

time to rear offspring. The reproductive strategy utilized by an animal consists of the behaviours that maximize their reproductive success and have evolved due to the energetic costs of reproduction under the environmental conditions they experience (Krebs and Davies 1993). In carrion flies, the energetic costs involve locating a suitable resource and site to lay eggs as well as the number of eggs laid per clutch. The female flies in my study differed in their oviposition behaviour, depending on the temperature they experienced, as well as the conspecifics and heterospecifics present on the resource. When ovipositing in the presence of heterospecifics, *Phormia regina* Meigen selected oviposition sites that were occupied by heterospecific eggs, in sites such as the mouth and ears. In the presence of heterospecifics, *P. regina* females laid more eggs at a faster rate, but only at temperatures below 25°C. Above this temperature, *P. regina* oviposition time was greater and fewer eggs were deposited.

Determining the factors that contribute to fitness, or the evolutionary ecology of these flies, involves looking at traits that are linked to fitness. For these flies, egg production and body size correspond to reproduction and fitness, where larger bodied females carry larger egg loads. Temperature influenced survival and resulting adult body size of these blow flies developing under those conditions, thus impacting their fitness through egg loads and potential reproductive success. For *Calliphora vicina* Robineau-Desvoidy, smaller adults resulted at higher temperatures, whereas, for *P. regina*, adult size was greater at higher temperatures than at lower temperatures. Temperature can also influence the number of eggs a female carries while she is an adult, despite her body size, where lower temperatures result in diminished egg loads, resulting in reduced fitness for these individuals. These traits determine the ability of individuals to successfully reproduce and to leave viable offspring. The carrion system provides a means to study reproductive behavioural strategies of species on ephemeral resources and the outcomes of these strategies on the success of individuals that may drive the evolutionary ecology of these species.

In community ecology, the overall objective is to explain distribution patterns, abundance of species and the interactions between species. There are many types of species interactions that can occur, with six theoretical categories defined, which can be positive, negative or neutral for the species interacting (Haskell 1947). Interactions can be

intraspecific, between members of the same species, or interspecific, between members of different species. It is assumed that one of the strongest interactions in carrion communities is competition due to the ephemeral and unpredictable nature of the resource. For blow flies utilizing carrion resources, there are often high densities of individuals on the resource, which generates different outcomes of interactions and individuals can trade-off adult body size for faster development or female flies can choose different locations to deposit offspring (VanLaerhoven 2015). Competition between these individuals can influence the survival and development of these offspring. Competition can occur in different ways, as exploitative competition, where the consumption of the resource by one species makes it less available for another species or through contest competition, where access to the resource for one species is inhibited by another species (Mittelbach 2012). For blow flies depositing eggs on carrion, space for oviposition sites is limited in areas such as the eyes, mouth or nose. The selection of these sites for oviposition by one species restricts other species from depositing eggs in these suitable locations.

Another interaction that can occur between species is mutualism, where both species have positive outcomes due to the interaction. For example, interactions between blow flies and microbes on carrion can be mutualistic if blow flies use cues emitted from microbes to detect suitable resources, and transport microbes to other resources (Tomberlin *et al.* 2012). A more common interaction among species is facilitation, where one species can benefit from the interaction while the other is unaffected. When one species colonizes a resource and alters the environment, making it more suitable for later arriving species, this can be considered facilitation (Connell and Slatyer 1977).

The current study highlighted potential mechanisms that may promote coexistence of blow flies on ephemeral carrion resources. By exploring the decisions made by ovipositing female blow flies and the development of their offspring under variable environmental conditions and in the presence of other carrion fly community members, this work demonstrated the potential for differential outcomes of species interactions at different temperatures and humidities, switching between facilitation and competition. Unlike previous work that suggested competition was the predominate interaction between these species, such that temporal and spatial resource partitioning

with fugitive species were the sole structuring mechanisms for coexistence (Denno and Cothran 1976; Hanski 1987), the current study suggest that fluctuating abiotic conditions over time may mediate the outcomes of these interactions such that no one species consistently has a competitive advantage, and may in fact, benefit from the presence of other species under some abiotic conditions.

Species interactions influence the structure and diversity of communities that assemble on ephemeral resources. Although species interactions have been explored in various communities, there is a need for examining such interactions in communities that utilize ephemeral resources, as these interactions can have greater consequences for these species due to the limited availability of the resources and outcomes at the individual community patch level have implications for the wider meta-community, suggesting variable outcomes amongst patches may stabilize the meta-community over time (VanLaerhoven 2015). This work can be scaled up by looking at the additional interactions among not only blow flies, but carrion beetles and even vertebrate scavengers.

7.3 Conclusions

7.3.1 Oviposition Behaviour

Incorporating theories of optimal oviposition behaviour and offspring performance (Jaenike 1978; Thompson 1988), I examined the influence of temperature and species interactions on female oviposition behaviour (Chapters 2,3). I also performed field validation studies, expecting that blow flies would demonstrate similar behaviours under natural conditions as they do in controlled laboratory settings (Chapter 5).

Oviposition varied due to temperature, and this relationship was stronger for *L. sericata* and *P. regina* compared to *C. vicina* (Chapter 2). With increasing temperature, the time to the first oviposition event decreased and the number of eggs deposited increased for *L. sericata* and *P. regina*. This trend was not observed for *C. vicina*, as temperature did not affect oviposition timing and the number of eggs oviposited was only

reduced at the highest temperature tested. As *L. sericata* is active at warmer temperatures up to 30-40°C, it was expected that it has a higher optimal temperature for oviposition than *C. vicina*, which is active at cooler temperatures between 5-30°C (Smith and Wall 1997; Donovan *et al*. 2006; Zurawski *et al*. 2009). However, it is surprising that there was no apparent optimum temperature for oviposition by *C. vicina* within the range tested here. Although *P. regina* is often considered tolerant of a wide temperature range, between 10-35°C (Hall 1948; Byrd and Allen 2001), it was expected that it would have an optimum temperature for oviposition that was intermediate between the other two species. Perhaps in southern Ontario, local *P. regina* populations are more of a warm weather species. These differences in oviposition timing due to temperature challenge previous assumptions regarding the oviposition behaviour of these blow flies, including the belief that oviposition occurs immediately after arrival to the resource.

Optimal oviposition predicts that females should select oviposition sites that will maximize the performance of their offspring, optimizing her fitness (Jaenike 1978). Stronger preferences for particular oviposition sites should reflect suitability of a site for offspring survival. The species examined in this study demonstrate clear preferences in oviposition site. Whereas *L. sericata* and *C. vicina* often selected natural orifices (mouth, nose) and areas on the face, *P. regina* deposited eggs more often on the legs. This can be explained by the protection that these sites offer from predators and parasitoids as well as the space available for large egg masses to form, limiting chance of desiccation for these eggs.

When interacting with heterospecifics, the oviposition behaviour of *P. regina* changed (Chapter 3). The oviposition timing of this species was significantly faster when arriving after *L. sericata* and *C. vicina* at lower temperatures, but slower at higher temperatures. Additionally, *P. regina* laid more eggs when arriving secondary to the other species at lower temperatures, but fewer eggs at higher temperatures. *Phormia regina* selected similar sites as these species for oviposition. The shift in behaviour of *P. regina* among the low and high temperature treatments indicate that the mechanisms driving species interactions can change from facilitation to competition.

Field validation of oviposition behaviour was examined for these three species, observing egg laying behaviours under natural conditions (Chapter 5). *Phormia regina*

exhibited differences in oviposition behaviour between the controlled laboratory and natural field conditions, where *P. regina* deposited eggs faster in the field at temperatures between 15-25°C, compared to in the lab. The acceleration in oviposition may be due to fluctuating temperatures in the field, as well as other abiotic factors, such as light levels or humidity. The results of this field validation study highlight the caution that should be applied when using generalizations about insect behaviour obtained from controlled laboratory settings in practical applications under fluctuating natural conditions.

Although understanding behaviours of individual species is valuable for an ecological perspective, on carrion, multiple species often arrive simultaneously. Our results indicate that the oviposition behaviour of *P. regina* is dependent on the presence of heterospecific eggs, with *P. regina* ovipositing faster, at temperatures below 25°C, when interacting with heterospecifics. The plasticity in the oviposition behaviour of *P. regina* suggests that this response may also occur in other species and future studies should expand this work to examine the influence of *P. regina* on other blow fly species.

7.3.2 Development

Egg development and hatching success of blow fly eggs can be influenced by abiotic conditions. Due to the short duration of the egg, this stage can provide valuable information for a forensic entomologist (Byrd and Tomberlin 2010). Understanding the influence of abiotic factors, such as temperature or relative humidity, can provide information pertaining to the mechanisms driving female oviposition behaviour. The results of this study indicate that for some species, periods of low relative humidity can delay egg hatching for up to 48 h, which can have implications for the survival of the developing offspring.

Based on the preferences demonstrated by female blow flies in their oviposition behaviour, I expected that larval development would reflect this, where development would occur faster at higher temperatures for some species, resulting in smaller adults (Chapter 6). Faster development can provide a competitive advantage for larvae feeding on an ephemeral resource, allowing them to disperse from the source quickly and avoid predation or parasitism from later arriving species.

Many aspects of blow fly development can be assessed to determine the influence of external factors. In addition to temperature, the influence of relative humidity is important for blow fly eggs, as they are susceptible to desiccation (Evans 1934). Blow flies often engage in aggregative oviposition and deposit large egg masses to combat the risk of desiccation and dilute predatory effects for their offspring (Stamp 1980). Few studies have examined the effect of relative humidity on blow fly egg hatching timing or success (Davies 1947; VanLaerhoven and Anderson 2001). Overall, I observed that hatching time decreased with increasing relative humidity for all three species, but hatching success differed (Chapter 4). At low relative humidities, *L. sericata* was the least successful and this was expected due to the proclivity of this species for warmer temperatures (Smith 1986). A relatively high hatching success was observed for *C. vicina* over the range of humidity tested and *P. regina* demonstrated increasing hatching success with increasing humidity.

To investigate this, I looked at changes in development over a range of temperatures, within both intraspecific and interspecific interactions (Chapter 6). Overall, species demonstrated an increased growth rate with increasing temperature, which was expected based on previous research on the relationship between insect development and temperature. There were differences in larval and pupal size due to interspecific interactions and this was noticeable for *C. vicina* and *P. regina*, indicating potential facilitative and competitive mechanisms influence the development of these species. I also examined survival rates of these blow flies and found that interspecific interactions resulted in increased survivorship for *C. vicina*. This species typically has very low survivorship when developing at constant temperatures of 30˚C and above (Smith 1986), but when in the presence of *P. regina*, mean survivorship was around 15%. Additionally, due to the influence of temperature on development, it would interesting to examine the presence and change in heat shock proteins during development of these blow flies.

7.4 Suggestions for Future Studies

The oviposition decisions made by female blow flies have consequences for their developing offspring and can provide useful information in a forensic entomology investigation. While previous studies have addressed insect oviposition behaviour, few

have examined the combined effects of abiotic and biotic interactions. The behaviour and development of the blow flies *L. sericata*, *P. regina* and *C. vicina* are influenced by temperature and species interactions. Oviposition behaviour may depend on many factors relating to the physiological state of the female or previous experience. It would be worthwhile to examine differences in oviposition behaviour of naïve and experienced females. In this study, all females used were considered naïve due to their inexperience with oviposition until fetal pig carcasses were introduced. Experienced females, having had the opportunity to oviposit, may behave differently under the temperatures tested here. Differences between naïve and experienced female insects have been observed in their egg loads and response to chemical cues (Vinson 1998) and this idea would be interesting to explore in blow flies. Additionally, the age of the insect may change their behaviour, as older females may be more likely to deposit eggs in less suitable oviposition sites or may deposit fewer eggs overall. Although temperature was a primary focus of this study, other abiotic factors can change blow fly behaviour and should be investigated. For example, relative humidity can change oviposition behaviour in the soldier fly *Hermetia illucens* L. (Diptera: Stratiomyidae), with more oviposition events occurring at higher periods of relative humidity (Tomberlin and Sheppard 2002). For other species, periods of relative humidity can result in a delay in oviposition (Canyon *et al.* 1999). Understanding how abiotic conditions influence the oviposition decisions made by female blow flies can provide valuable information relating to the community of insects colonizing a carrion resource and can have applications in forensic investigations in which the oviposition timing and sites selected can provide evidence pertaining to the crime scene.

This research can be expanded to not only look at adult behaviour, but development of larvae based on the oviposition decisions of the females. The differential success of larvae based on the sites selected and the clutch sizes of females may be dependent on temperature or species interactions. In studies of these species developing under different temperatures and in mixed species compositions, differences were observed in the growth rate and body size of the larvae and adults (Chapter 6). These differences may be more pronounced if larvae are developing in larger densities and in specific oviposition sites selected by female blow flies. It would be of interest to

understand how the female's oviposition decisions impact her offspring by examining the behaviour of the offspring and future generations.

Future work on the development of blow flies should expand to examine other forensically relevant species that coexist on carrion. Although only three species were selected for this study and are commonly occurring in this area, studying other species could provide valuable information regarding species interactions and their influences on behaviour and development on an ephemeral resource. Blow flies on carrion represent a system that can be used to examine coexistence patterns and mechanisms that has applications in forensic entomology as well as community ecology.

7.5 Forensic Entomology Implications

Historically, studies in forensic entomology have involved examining decomposition and the insects associated with each stage. Although the earliest recorded use of forensic entomology dates back to 13th century China where a homicide was investigated and a confession was derived based on the arrival of insects to the suspects weapon (Tz'u, translated by McKnight 1981), it was not until the mid 1800's that medicolegal entomology was applied in a more formal sense. In 1855, the mummified remains of an infant were discovered and the insects present on the body were used to develop a timeline of death (Bergeret 1855). Human decomposition was thoroughly described by work of Mégnin (1894) and many have followed in his footsteps to describe the insects associated with each stage of decomposition (Review in Anderson 2010).

Foundational work completed by Payne in the 1960's contributed to the idea of investigating insect succession on carrion within an ecological framework, where the organisms interact with the food source, changing the resource in a predictable sequence, thereby making it attractive to different types of insects during the stages of decomposition (Payne 1965). Payne (1965) demonstrated the importance of insects during decomposition by comparing pig carcasses exposed to and protected from insects, showing that decomposition can take as long as 100 days when insects are denied access compared to only 6 days when carrion is exposed to insects.

Life history traits and taxonomy of the families of flesh flies and blow flies began to be documented during the first half of the 20th century (Aldrich 1916; Knipling 1936;

Hall 1948). The development of forensically important species was examined initially by Kamal (1958) and many others have expanded upon this work but this information was not utilized to estimate the postmortem interval (PMI), or time between death and discovery of the body, until the 1990's (Catts 1992; Wallace *et al*. 2015). Forensic entomology was not brought into the courtroom until the 1970's in the US and this triggered a dramatic increase in empirical research since this time (Wallace *et al*. 2015). Research in the last 40 years has examined patterns of insect succession, decomposition and insect arrival in various terrestrial and aquatic habitats, anthropological influences, entomotoxicology as well as DNA techniques (Byrd and Castner 2010). Genetic sequencing has been performed in blow flies to determine species identity (Sperling and Anderson 1994) as well as the degree of relatedness among individual flies collected in one area (Picard and Wells 2009; Picard and Wells 2010). Tarone and Foran (2011) examined gene expression of blow flies during development to allow for greater precision in blow fly aging in relation to PMI estimations. Recently, it was suggested that genetic sequencing should be incorporated into behavioural studies to better understand blow fly behaviours and the genes that may be expressed during different behaviours (Sanford *et al*. 2015).

Although forensic entomology has made great strides, incorporating more ecological processes to test some of the long-standing assumptions in this field can strengthen the credibility of forensic entomology. For example, the assumption that blow flies arrive and oviposit immediately after death is not true in all circumstances. Although insects arrive and colonize carrion in a fairly predictable fashion, the behaviour and development of these insects is shaped by the biotic and abiotic conditions they experience. In order to better understand these behaviours, the mechanisms driving them must be established and thoroughly tested.

My study aimed to address some of these issues and to incorporate ecological theory in explaining blow fly behaviour and development, which can then be applied in forensic entomological investigations. The effects of temperature, humidity and species interactions on the oviposition behaviour and development of three ubiquitous and forensically relevant species were examined. The influence of abiotic conditions on oviposition behaviour is important in an applied sense, given that forensic entomologists

often incorporate temperature data into the estimation of PMI. We predicted that given that some species are considered warm weather species (*L. sericata*), some are cool weather species (*C. vicina*) and others have a wide temperature tolerance (*P. regina*), these species should change their oviposition behaviour in accordance with their temperature tolerances. Although oviposition timing is thought to occur almost immediately, our study examines how the timing can be delayed or accelerated due to temperature or species interactions. Information regarding the oviposition decisions of blow flies can provide valuable evidence for forensic entomologists, as changes in oviposition timing can alter the estimates of the TOC and PMI.

We also examined the influence of temperature, relative humidity and species interactions on blow fly development. We expected that given the thresholds for egg hatching of these species, low humidity would result in water loss and would delay egg hatching timing and success, compared to high relative humidity, for *L. sericata* and *P. regina*, but not for *C. vicina*. The timing of egg hatch is also a crucial stage in development that can provide information in forensic entomological investigations. The duration of this stage is relatively short, compared to other life stages in blow flies and understanding what mechanisms can influence the development of eggs is useful in accurate estimates of the TOC.

In terms of larval development, we expected that increasing temperature would result in faster development for these species, but high temperatures should result in higher mortality for cool weather species and lower temperature would result in higher mortality for warm weather species. The presence of heterospecifics was expected to influence the development of *P. regina*, as previous studies indicate that *P. regina* development is facilitated when in the presence of heterospecifics. The results of our study on larval development indicate that the presence of heterospecifics in conjunction with temperature act to influence the development of blow flies. This data provides useful information for forensic entomologists and can used to provide more accurate estimations of the PMI.

The results of this study highlight the need for further examination of the natural history of blow flies in an ecological context. Within the field of forensic entomology, various aspects of the behaviour and development of these organisms are often assumed

to occur in similar manners across species. This is not the case, as the results of my study indicate. The choices made by female blow flies are susceptible to variation due to factors such as temperature and humidity, and these choices have consequences for the developing offspring. For a forensic entomologist to utilize information obtained from these organisms, the full spectrum of the adult and larval behaviour and development must be understood. These assumptions have been overlooked and with more information, the field of forensic entomology can advance ecologically, which will improve the validity of this field within forensic science.

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