Larval aggregation and competition for resources in populations of Lucilia sericata and Phormia regina

Patricia Obianuju Okpara

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Larval aggregation and competition for resources in populations of *Lucilia sericata* and *Phormia regina* (Diptera: Calliphoridae)

By

Patricia Obianuju Okpara

A Thesis
Submitted to the Faculty of Graduate Studies through the Department of Biological Sciences in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

Windsor, Ontario, Canada

2018

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Larval aggregation and competition for resources in populations of *Lucilia sericata* and *Phormia regina* (Diptera: Calliphoridae)

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May 9, 2018
DECLARATION OF ORIGINALITY

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ABSTRACT

Blow flies (Diptera: Calliphoridae) are commonly used to estimate the post mortem interval of unattended death when this interval is greater than 48 hours. This estimate utilizes the developmental biology and behaviour of these first arriving insects. Female blow flies typically engage in aggregated egg laying, resulting in larval feeding masses once the eggs hatch. These masses often vary in density and species composition and have the potential to impact fitness through different species interactions. This research studies the effects of temperature, density, species interactions and mechanisms of coexistence of two forensically significant blow flies *Lucilia sericata* Meigen and *Phormia regina* Meigen (Diptera: Calliphoridae).

The density and developmental temperatures experienced by the larvae during development was manipulated in the presence of conspecifics (chapter 2) and heterospecifics (chapter 3) in the laboratory. Several life history traits were measured; survival, developmental time and adult body size. The results of this study indicated that the life history traits measured exhibited plasticity with varying temperature, blow flies had decreased survival and adult body size at high developmental temperatures. Blow flies had increased survival and adult body size with density at 15°C decreased adult body size with density at 25°C and decreased survival and adult body size with density at 35°C. *Phormia regina* had higher survival when developing with in the presence of *Lucilia sericata* at 25°C and 35°C. Both blow flies had larger adult body size while developing in the presence of each other at 25°C and 15°C perhaps due to the presence of compounds secreted during larval feeding that aid in digestion and increase nutrient availability.
DEDICATION

To my parents, Patricia Nwakaego Okpara and Simon Ndu Okpara
and my siblings, Nneka, Chioma, Emeka, Amaka, Chimere, and Onyinechi.
moreover, finally, my nieces and nephew.

Thank you for all your unceasing Support, Guidance, and Love.
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Ecological communities are composed of groups of interacting or potentially interacting, species that live together in a defined geographic area (Agrawal et al., 2007; Ricklefs, 2008; Brooker et al., 2009). Gause’s law of competitive exclusion states that two species cannot similarly and simultaneously exploit the same resource without one of the two competitors driving the other to extinction (Gause, 1934). This law suggests that for two or more species to coexist over time, each species must utilize the available resource differently. However, contrary to Gause’s law, carrion communities are often observed with multiple species feeding on a single carrion resource (Fuller, 1934; Hanski, 1987; Prinkkila and Hanski, 1995). It is suggested that within these communities, species coexistence is due to spatial and temporal resource partitioning of limited resources (Prinkkila and Hanski, 1995). These communities are strongly influenced by biotic and abiotic factors, such as ambient temperature, humidity, and species density that influence species interactions and ultimately the fitness of these species at large (VanLaerhoven, 2015).

Colonization of Carrion

Carrion undergoes series of rapid physical, chemical and biological changes, as decomposition is a continuous process that begins at death and ends with the carrion being reduced to a skeleton (Goff 2009). Two critical stages classify the colonization of
carrion by carrion insects such as blow flies (Tomberlin et al., 2011). The first stage is the pre-colonization interval which extends immediately after death until colonization by insects, this stage is characterized by the detection and location of the carrion by arthropods (Tomberlin et al., 2011). The detection of the carrion is controlled by processing external stimuli such as temperature, time of day, precipitation and internal stimuli such as ovarian maturity and mating status (Tomberlin et al., 2011). Detection is aided by insect chemosensory detection of volatile odours produced by the carrion, the microbial community and even visual cues of the carrion (Tomberlin et al., 2011). Once the carrion resource is located, insects use close range cues such as taste and size to evaluate its suitability for oviposition (Tomberlin et al., 2011). The second stage is the post-colonization interval which is marked by colonization and lasts until the dispersal of carrion insects due to complete decomposition of the body or discovery of the body in the case of forensics (Tomberlin et al., 2011). The carrion resource is ephemeral, and communities disassemble as soon as it is consumed (Peschke et al., 1987).

**Carrion communities**

Carrion communities comprise of the carrion flies, detritivores, predators, and parasites that feed on muscle and soft tissue, decaying matter, and other insects respectively. These complex communities are bound together by a network of interactions that species have on one another; competition, predation, facilitation, parasitism, and amensalism (Reis et al., 1999; Faria et al., 2003). The decomposition of carrion is followed by a rapid succession of carrion communities rich in diversity that disassemble upon consumption of resources (Peschke et al., 1987). The limited supply of carrion means, species must utilize this resource efficiently to survive within their
community. It is suggested that coexistence of species within carrion communities, despite high levels of interspecific and intraspecific interactions, is a result of resource partitioning (Atkinson and Shorrocks, 1981; Hanski 1987). Partitioning is achieved temporally, by species arrival time colonizing the resource and altering its environment to support the arrival of latter species (Atkinson and Shorrocks 1981). It is achieved spatially by species ovipositing in more optimal locations and leaving less optimal locations for other species (VanLaerhoven, 2015). Resource partitioning allows for priority effects, which occurs when an early arriving species increases the availability of resources by exposing restricted food sources such as ligaments, internal organs or by altering the microbial community thereby increasing the recruitment of later arriving species (Hobson, 1932). Carrion communities provide an excellent model system to answer profound ecological questions, such as the mechanisms by which similar species coexist simultaneously on a limited resource over a short period, and gives us an opportunity to investigate the structure of macro and micro-communities (VanLaerhoven, 2015).

**Blow flies: A carrion species**

Blow fly (Diptera: Calliphoridae) communities are comprised of four tribes; Chrysomyiini (screwworm flies), Phormiini (black blow flies), Luciliini (green bottle flies) and lastly, Calliphorini (blue bottle flies) which are amongst the first colonizers of decomposing carrion (VanLaerhoven, 2010). The offspring of adult blow flies feed directly on the resource, while other species continue to colonize and make up the rest of the carrion community (Kuusla and Hanski, 1982; Woodcock *et al.*, 2002). The blow fly life cycle consists of six distinct life stages. Eggs are laid by gravid females, and after
accumulating enough degree-days (ADD) which are defined as the measurement of the thermal units required for the growth and development of insects, hatch into the first instar (James 1947; Byrd and Castner, 2010). After obtaining the minimum ADD, the larvae molt into the second instar feeding larvae by shedding off the larval cuticle and mouthparts and finally, after obtaining the minimum ADD, the molts into the third instar feeding and post-feeding stages. After the third instar, larval feeding stage, the larvae enter the post-feeding larval stage and crawls away from the resource in search of a suitable dry place to pupate; this can be the surrounding soil, hair or clothing of the remains (James 1947; Byrd and Castner, 2010). After the pupal stage, the fly emerges from the pupal case and leaves it behind as evidence that the blow fly life cycle has been completed (James 1947; Byrd and Castner, 2010).

**Oviposition choices**

Adult female blow flies are attracted to decomposing flesh to lay their eggs and may lay up to 300 eggs per clutch, three to four times during a single female’s lifespan (Avila and Goff, 1998; Byrd and Castner, 2010; Cragg, 1955; Greenberg and Kunich, 2002; James 1947). Female blow flies do not practice maternal care, so females must choose excellent oviposition sites that provide the highest chance of survival of eggs and adequate resources for the offspring when they hatch (Jaenike, 1978). Females are often attracted to sites on the carrion where the skin is broken through wounding or other means as preferred oviposition locations (Pacheco, 2015). The presence of blood provides an excellent source of protein for newly-hatched blow fly larvae (Avila and Goff, 1998; Byrd and Castner, 2010; Cragg, 1955; Greenberg and Kunich, 2002). Some species of blow fly lay eggs on open wounds of live animals, resulting in myiasis (Byrd
and Castner, 2010; Hanski, 1987). Other than wounds, female blow flies lay eggs near and within natural orifices which are lined with a mucosal layer, which may be easier to penetrate by first instar larvae (Byrd and Castner, 2010; James, 1947). Other sites for oviposition such as between the legs or in skin folds that may increase hatching success by having a higher humidity (Hans, 2016). Recent studies have discovered that female blow flies will preferentially oviposit in dense aggregates on a resource with other female blow flies feeding or ovipositing (Joosse and Verhoef, 1974; Brodie et al., 2015). Aggregation of eggs is thought to reduce the level of desiccation experienced by the eggs, enhancing chances of survival to adulthood (Brodie et al., 2015). It is thought that ovipositing gravid females and feeding flies improve the attractiveness of the resource by semiochemical signals, cues and the release of enzymatic salivary secretions (Connell and Slatyer, 1977; Brodie et al., 2015). Enzymatic secretions by larvae and adult flies may contain antibiotic secretions that facilitate the decomposition of carrion (Greenberg and Kunich, 2002; Rivers et al. 2011).

**Life history traits and abiotic interactions**

Blow flies, like all insects, are poikilotherms; they are unable to regulate their body temperature and depend on ambient temperatures to maintain their body temperatures. Temperature affects growth rate, reproduction, distribution, and abundance; even small changes have a potentially significant effect on fitness and life history characteristics (Angilletta et al., 2002; Denlinger and Yocum 1998; Hoofmann et al., 2003). Studies have reported that temperature affects many biological characteristics such as longevity, survival, sex ratio, fecundity and fertility (Dreyer and Baumgartner, 1996). When insects are exposed to extremely high temperatures, it can result in cell death due
to protein denaturing, or membrane and enzyme structure alteration due to dehydration (Chapman, 1969). The temperature at which thermal injuries occur depends on the threshold temperature of the species, exposure time, and interaction with other factors such as humidity (Chapman, 1969). The lethal temperature range of most insects falls within the range of 40°C to 50°C (Gullan and Cranston, 1994), but this may vary for insects depending on their habitat or other factors.

The effects of temperature have been studied in different insect species. At very extreme temperatures, death is immediate, but at less extreme temperatures, insects are affected in other ways. Arbogast (1981) exposed the pupae of *Cadra cautella* Walker and *Plodia interpunctella* Hubner (Lepidoptera: Pyralidae) to 40, 45 or 50°C and while all females and males of both species were killed at 50°C, there was a failure to mate, reduced fecundity and complete sterility due to exposure to 40°C and 45°C. The intensity of these effects increased with temperature and length of exposure (Arbogast, 1981). Three-grain beetles, *Trogoderma granarium* Everts (Coleoptera: Dermestidae), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and *Callosobruchus chinensis* Latreille (Coleoptera: Chrysomelidae) were subjected to 30°C, 40°C and 45°C and the percentage of sterility increased with increasing temperature. Extended exposure suppressed adult formation and emergence (Saxena *et al.*, 1992). Developmental abnormalities are observed when the male larvae of *Aedes sierrensis* Meigen (Diptera: Culicidae) were reared at 30-31°C as they became feminized (Horsefall *et al.*, 1964; Anderson and Horsfall 1963). Higher developmental temperatures encourage an increase in fecundity (rate of reproduction of an individual or population) and an increase in oviposition rate (number of eggs laid per day) within a species favourable temperature range (Cunnington
1985; Yadav and Chaudhary 1986; Zulfiqar et al., 2010). This positive relationship is supported by high relative humidity (Cunnington, 1985; Yadav and Chaudhary 1986; Zulfiqar et al., 2010). Although higher temperatures lead to an increase in oviposition rate, fecundity and reduced development time, it also limits the oviposition period and the longevity of the adult fly (Cunnington, 1985; Potting, 1996; Mbapila, 1997; Getu, 2007). The decrease in development time has been shown to be due to an increase in metabolic activity primarily in the larval stage (Srivastava and Omkar, 2003). Development time is inversely proportional to temperature regardless of the effects of relative humidity (Tamiru et al., 2011). Many studies on the effects of temperature on insects’ development support this relationship that higher developmental temperatures cause a decrease in developmental time and an increase in developmental rates (Yadav and Chaudhary, 1986; Muegge and Lambdin, 1989; Levesque et al., 2002; Mbapila et al., 2002).

**Life history traits and biotic interactions**

The influence of density on life history traits has also been investigated. *Lucilia sericata* Meigen (Diptera: Calliphoridae) showed a relationship between survival and density which resembles that of a normal distribution graph with an initial increase in survival with increased density until an optimum threshold was reached, and survival began to decrease as density continued to increase. However, the adult body size of individuals decreased with increasing density (Moe et al., 2002; Smith and Wall, 1997). For *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae), fecundity and adult body size decreased as density increased (Saunier and Bee, 1995; Smith and Wall, 1997). *Chrysomya putoria* Wiedemann and *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) showed that survival was a function of larval densities, such that survival
decreased with increasing larval densities (Reis et al., 1999). The development time of *Chrysomya megacephala* Fabricius and *Chrysomya rufifacies* Macquart (Diptera: Calliphoridae) increased as density increased whereas the larval survivorship decreased as density increased (Shaio and Yeh 2008). As these examples demonstrate, the effects of density on biological parameters such as rate of development, survival, mortality, reproduction, and size, may induce either a one-tailed or a two-tailed response (Peters and Barbosa 1977). A one-tailed response is characterized by the intensity of the effects increasing as density increases, whereas a two-tailed response is characterized by a positive response observed at an optimal density range but with deleterious effects apparent above and below the optimal density (Peters and Barbosa, 1977). Competition is a crucial density-dependent factor that influences the abundance of species population in nature (So and Dudgeon, 1989; Ullyett 1950). Blow flies experience scramble competition which is an indirect form of competitive involving a limiting resource which in the case of blow flies is carrion (Nicholson 1954; Bakker 1961; Beaver 1977; Levot et al., 1979; Baxter and Morrison, 1982). The ability of different blow fly species to withstand interspecific and intraspecific competition is dependent on their competitive ability (Ullyett, 1950). All blow fly colonizers have equal access to the resource but differ in their speed and efficiency for resource exploitation. Blow flies can aggregate themselves in spatially and temporally to coexist on the limited resource (VanLaerhoven 2010; Hattori and Shibuno 2013; Pacheo 2015). Blow flies exhibit both habitat and seasonal/ temporal preferences, and these preferences influence the type and extent of species interactions that occur. Different blow flies species are observed during different seasons. For example, Baumgartner and Greenberg (1985), observed that blue bottle flies
such as *C. vicina* have a preference of cooler temperatures and habitats (fall/spring season), whereas green bottle flies such as *L. sericata* prefer warmer temperatures and open habitats (summer/spring season). However, black bottle flies such as *Phormia regina* Meigen (Diptera: Calliphoridae) have a broad temperature tolerance and can be abundant in both cooler and warmer temperatures (spring/summer/fall season) (Macleod and Donnelly 1958). This temporal variability changes how carrion is utilized and influences the competitive and colonization abilities of blow flies. Blow fly species abundant during specific seasonal conditions exert priority effects on later-arriving species, and this can mediate coexistence (VanLaerhoven, 2015). With positive priority effects, the later arriving species benefits from the presence of the early arriving species. *Lucilia sericata* exerts positive priority effects on *P. regina*, with *P. regina* changing its oviposition choices to more suitable sites (sites with *L. sericata* eggs on them) (Hans, 2016) and having overall increased fitness in the presence of *L. sericata* (Hans, 2016; personal observation). Positive priority effects are seen as facilitation, which occurs when the resource is modified by the presence of one species making it easier to colonize and utilize by other species (Connell and Slatyer, 1977). Facilitation is one of the many species interactions experienced by blow flies aside from competition and has been documented by a few studies (Charabidze et al., 2011; Hans, 2016).

**Study species**

*Phormia regina* is a part of the subfamily Chrysomyinae, abundant in North America with adult ranging from 7-9mm (Bryd and Castner 2010; Marshall et al., 2011; Smith 1986). *Phormia regina* overwinters in the adult form, and its larval stages are implicated in secondary myiasis in animals, and it is a pest in livestock (Marshall et al.,
The seasonal distribution of *P. regina* is broad as it is found during all seasons of the year but more prevalent during the fall months, depending on the geographical location (Weidner *et al.*, 2015). It dominates the southern regions of the United States during the winter months and the northern climates of the United States during the summer months (Byrd and Allen 2001). The development time of *P. regina* can be as short as 8.8 days depending on the developmental temperature (Bishop 1915; Byrd and Allen 2001; James 1947; Kamal 1958). The thermal activity limits of *P. regina* is currently noted as between 10°C/12.5°C up to 40°C, with no adult emergence at this upper temperature (Deonier 1940; Haskell 1993).

*Lucilia sericata* is a cosmopolitan species in the subfamily Luciliinae. It is most abundant in temperate regions due to its preference for warmer and open sunny habitats (Smith and Wall 1997b). It has been implicated in blow fly strike, which can be described as a parasitic infection of maggots on live animal tissues and has been reported in the Netherlands (Baudet and Nieschultz 1938), Germany (Liebish *et al.*, 1983), South Korea (Jang *et al.*, 2013) and many more countries. The life cycle of this species can range from 13 days to several weeks depending on the developmental temperature (Kamal, 1958; Wall *et al.*, 1992; Hans, 2016).

*Lucilia sericata* and *P. regina* are two forensically important blow fly species commonly found in southern Ontario, Canada (Smith, 1986; Byrd and Castner, 2010). They have been reported to inhabit and feed on the same carrion (Anderson and VanLaerhoven, 1996; VanLaerhoven and Anderson, 1999). *Lucilia sericata* and *P. regina* have been reported to show different preferences in their oviposition behaviour on carrion. Gravid *L. sericata* females preferentially oviposit in moist locations such as the
mouth, eyes, and nostrils when the colonize the carrion before or at the same time as *P. regina* (Hans, 2016; Pacheco, 2015). However, gravid *P. regina* females have been shown to prefer ovipositing on dryer locations on the carrion such as the abdomen, between the legs and on the head (Hans, 2016; Pacheco, 2015). When *P. regina* colonize the carrion after *L. sericata*, they switch their oviposition preferences and lay their eggs on or close to *L. sericata* eggs (Hans, 2016). High interspecific and intraspecific competition in *L. sericata* maggot masses can result in increased larval mortality and a decrease in adult size (Cragg, 1995; Hutton and Wasti, 1980; Kheirallah *et al.*, 2007; Prinkkila and Hanski, 1995; Smith and Wall, 1997a; Ullyett, 1950). *Lucilia sericata* has been reported to experience high interspecific competition in the presence of other blow fly species which result in decreased larval survival, longevity, and fecundity in adults (Prinkkila and Hanski, 1995; Smith and Wall, 1997a). Interspecific competition between *P. regina* and *L. sericata* resulted in no adverse effects on larval survival and adult size in *L. sericata* (Hutton and Wasti, 1980; Hans, 2016). The opposite was observed for *P. regina* when developing in the presence of *L. sericata*; there was an increase in survival (Hans 2016). Contrary to this observation Hutton and Wasti (1980) observed 100% larval mortality of *P. regina* in the presence of *L. sericata*. Large larval densities of *P. regina* indicate that high intraspecific competition results in a reduction in successful adult emergence (Hutton and Wasti, 1980). Pacheo (2015) studied the effects of species interaction on survivorship rates, body size, and development using burnt carrion. Increasing burn levels on the carrion led to an increase in survivorship rates for both *L. sericata* and *P. regina*. However, there were no significant effects of species interactions on survival (Pacheo 2015). Larger posterior cross vein length was observed for flies in
mixed species showing a positive effect of species interactions in adult body size of *L. sericata* and *P. regina* (Pacheo 2015). More recent work by Hans (2016) investigating the influence of temperature and species interactions on the development of *L. sericata* and *P. regina* found that there were positive effects of species interaction on *P. regina*. This positive interaction was a function of temperature, and at temperatures approaching the upper thermal limits for *P. regina*, the interaction turned negative with survival decreasing in the presence of *L. sericata*. Peak survivorship of *L. sericata* was at 30 °C in the presence of conspecifics. However, this was reduced to 25°C in the presence of heterospecifics (Hans, 2016).

*Applications of carrion species in forensic entomology*

Forensic entomology is the use of arthropod ecology in criminal investigations, primarily utilized to determine post-mortem interval (PMI) (time of death) of deaths which are homicidal or suicidal (Byrd and Castner, 2001; Catts 1992). The successional patterns and developmental time of carrion insects are used in this estimate as blow flies arrive within hours after death (Greenberg, 1991; Anderson and VanLaerhoven, 1996). After remains are discovered, insect samples of the oldest developmental stages are collected from and around the body and compared to the known development times of blow flies based on the temperature(Catts 1992).

The history of forensic entomology dates back to 13th century China when a murderer confessed based on the arrival and presence of insects on the murder weapon (Tz’u, translated by Mckinght 1981). In 1855, insect evidence was used to develop a timeline for the death of a mummified infant child (Bergeret, 1855). The life history traits
of blow flies and flesh flies were extensively documented by Aldrich (1916), Knipling (1936), Hall (1948), Kamal (1958). However, forensic entomology was not applied in the courtroom for forensic investigations until 1970’s in the US and 1990’s in Canada (Catts, 1992; Anderson and VanLaerhoven, 1996)

**Significance**

During PMI estimations, forensic entomologists use known predictable development times of blow flies. A problem with this approach is that most studies examine the effects of temperature on development time without incorporating the possible effects of species interactions (intra-specific and interspecific)(Bryd and Allen, 2001; Grassberger and Reiter, 2002). This study aims to identify implications blow flies in forensic entomology and their role in the carrion community.

There are two primary approaches used to determine post-mortem interval; one uses the known development times of blow flies, and the other uses the changes in successional patterns of carrion insects during decomposition (Catts and Goff, 1992). These successional patterns are affected by the size of carrion, state of decomposition climate and habitat (Anderson and VanLaerhoven, 1996; VanLaerhoven and Anderson, 1996). The second aspect relates to the fact the carrion community, especially blow fly species are essential in the process of decomposition and recycling nutrients back into the ecosystem while providing nutrients for higher trophic levels. It also serves a model system for the study of interactions between species within and between trophic levels (VanLaerhoven, 2015) as well as the study of community assembly patterns and mechanisms of coexistence (VanLaerhoven, 2010). This research aims to understand the
role of species interactions, temperature, and density on the development of blow flies, as incorporating these factors might provide relevant information to forensic entomologist and help for more accurate PMI estimations.

**Research Objectives**

The first aim of this project was to investigate the effects of temperature, density and intra-specific interactions on the life history traits of forensically significant blow flies *L. sericata* and *P. regina* (Chapter 2). It is expected that the life history traits of survival, adult size and development time will be affected by larval population density and different developmental temperatures. Based on previous literature, development time, adult size and survival should be a function of temperature and should decrease with increasing developmental temperatures. Density should be negatively correlated with survival, development time and adult body size. Given that the temperature range for *L. sericata* is 10.6°C – 32.5°C (Roe and Higley 2015) and *P. regina* are 10°C- 40 °C (Gosselin *et al.*, 2010), *P. regina* should have a shorter development time and higher survival at higher developmental temperatures.

In chapter 3, the effects of larval population density at different temperatures in mixed species cultures of *P. regina*, and *L. sericata* was examined. Different species ratios were investigated for mixed cultures, to determine if there are priority effects of one species over the other due to a higher ratio of the first species in comparison to the second species. Species ratio should influence development by giving a competitive advantage to the species in a higher ratio, such as increased survival and body size for *P. regina* in the ratio 75% *P. regina* to 25% *L. sericata* or vice versa for *L. sericata* in 25% *P. regina* to 75% *L. sericata*. 
Development time should decrease with increasing developmental temperatures for both species, *L. sericata* and *P. regina* regardless of species ratio or larval density. Given that the developmental temperature range of both species; *L. sericata* should have decreased survival and smaller adult body size at higher temperatures whereas *P. regina* should have increased survival at higher temperatures and adults in comparison to *L. sericata*. Also, given the recent results from Hans (2016), *P. regina* should demonstrate increased adult size and survival in the presence of *L. sericata*. Due to *P. regina* having a wider temperature range for development, *P. regina* should have a shorter development time in comparison to *L. sericata*. Lastly, a negative linear relationship is expected between larval density and survival, development time and adult body size such that these life history traits decrease with increasing larval density due to increased levels of interspecific competition.

This research strives to understand the patterns of blow fly coexistence within the complex community structure of carrion communities and provides some in-depth understanding of developmental changes in blow fly biology due to temperature, species interactions, and density. This information can then be applied to forensic entomology.
REFERENCES


Fuller, M.E., 1934. The insect inhabitants of carrion: a study in animal ecology. *Australia Council for Scientific and Industrial Research* 82: 4-63


Hans, K.R., 2016. Using an ecological framework to resolve issues in forensic entomology: Exploring temperature mediation of species interactions within blow
fly (Diptera: Calliphoridae) communities (Doctoral dissertation, University of Windsor (Canada)).


CHAPTER 2

THE INFLUENCE OF TEMPERATURE AND DENSITY ON THE DEVELOPMENT AND SURVIVAL OF BLOW FLIES (DIPTERA: CALLIPHORIDAE)

Introduction

Temperature affects distribution, abundance and several life history characteristics of insects (Angiletta et al., 2002; Bale et al., 2002; Denlinger and Yocum, 1998; Hoofmann et al., 2003; Zheng et al., 2008). The rate of insect development changes with changing environmental temperature, because insects are poikilotherms (Taylor, 1981). Temperature influences the physiology of insects because few can survive at temperatures below 0°C and above 45°C. Below 0°C, insects can experience cold shock injuries due to improper muscle development caused by oxidative stress that ultimately leads to adverse effects on development (Lee, 1991; Yocum et al., 1994). Above an insect’s tolerance temperature range, enzyme degradation, protein denaturation (Chown and Nicholson, 2004) and membrane disruption (Hochachka and Somero, 2002) occur which also lead to adverse effects on development (Zheng et al., 2008). Huey and Kingsolver (1989) documented that insects have a temperature tolerance zone with minimum and maximum limits with optimum developmental temperatures lying somewhere in between. Shifts within this tolerance zone can indicate developmental variations or trade-offs between development and temperature (Kingslover, 2009). Insects are widely distributed over areas with varying environmental temperature ranges; this often results in insects exhibiting different changes in development with most insects
exhibiting smaller body size in hotter environments and larger adult body in colder temperatures (Angilletta et al., 2004). This variation is body size with changes in temperatures is termed the “Temperature-Size rule.” There are three general rules associated with temperature and life history traits of insects; firstly, bigger is better, hotter is smaller, and lastly, hotter is better (Kingsolver and Huey, 2008). These rules measure how temperature affects fitness (rate of population increase and rate of reproductive rates) of an individual/population. The bigger is better rule states that individuals with larger body size will have increased fitness compared to smaller individuals, however growing to a larger size also involves costs such as decreased rate of development, increased risk of predation due to delayed development, and increased energy demands (Kingslover and Huey, 2008). Hotter is smaller, suggests that insects developing at higher temperatures will have smaller adult sizes when compared to those at colder temperatures (Atkinson, 1994; Angilletta and Dunham, 2003; Kingslover and Huey, 2008). This rule represents the phenotypic plasticity of body size which is affected by environmental conditions (Kingsolver and Huey, 2008). The relationship between size and the developmental temperature is called the thermal reaction norm (Angilletta et al., 2003). Lastly, the hotter is better proposes that individuals/population with higher optimal developmental temperatures will also have higher fitness (Frazier et al., 2006; Kingsolver and Huey, 2008). Due to the difficulty associated with quantifying fitness, components of fitness such as fecundity, survival, adult body size, and rate of development are measured (Kingsolver and Huey, 2008).

The effect of temperature on the rate of development was investigated for the water chestnut beetle, Galerucella birmanica Jacoby (Coleoptera: Chrysomelidae), using seven
different temperature regimes (Zheng et al., 2008). In this study, development time decreased with increase in developmental temperatures (Zheng et al., 2008). The developmental time of the cotton pest, *Aphis gossypii* Glover (Hemiptera: Aphididae) also decreased with increasing temperature from 4.5 days at 30°C to 12 days at 15°C (Kersting et al., 1999). Development occurs within a thermal range with an optimum developmental temperature within this range (Huey and Kingsolver, 2008). Studies have shown that development outside the optimum temperature can result in adverse effects. This is seen in *A. gossypii*; larvae reared at higher than optimum temperatures developed slower than larvae reared at more favourable temperatures (Kersting et al., 1999). The relationship between development time and the temperature is linear until it exceeds the developmental temperature range (minimum and maximum). Below or above this, development time increases or halts until the optimum temperature for development is reached (Briere et al., 1999).

The influence of temperature on survival of arthropods has been investigated in different insect species. Tun-Lin et al. (2000) observed the survival to adulthood of *Aedes aegypti* Linnaeus (Diptera: Culicidae) was poorest when developing in temperatures that were below and above *A. aegypti*’s optimum thermal range (88-93 % at 20°C-30°C, but 23.5% at 15°C and 67% at 35°C). Mortality of *Ephestia cautella* Walker and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) increased when pupae were exposed to maximum thermal temperatures (40°C, 45°C, 50°C) (Arbogast 1981). *Ephestia cautella* was eight times more tolerant to these maximum thermal temperatures (40°C, 45°C), suggesting that *E. cautella* might have a broader thermal range than *P. interpunctella*. The spider mite, *Tetranychus medanieli* McGregor (Acarina: Tetranychidae) and its
predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) were investigated at 12 constant temperatures ranging 10°C - 38°C and their development modeled as a function of temperature. The optimum temperature for survival ranged from 14°C to 36°C for both species with low survival at both ends of the temperature thresholds.

The temperature-size rule is a well-established phenomenon that describes the relationship between temperature and growth by which individuals that are developing at low temperatures exhibit larger body size (Atkinson, 1994; Angilletta et al., 2003). Adaptive and nonadaptive theories have been offered to explain the driving force for this thermal plasticity of adult body size (Angilletta et al., 2003). Nonadaptive theories suggest that temperature affects enzymes and molecular processes which in turn affects the rate of development. In contrast, adaptive theory suggests a cost-benefit relationship for particular life history traits. For example for individuals to achieve a significant body size in cold environments, they must prolong development and postpone reproduction in comparison to individuals in warmer environments. Fischer and Fiedler (2002) compared four populations of the butterfly *Lycaena hippothoe* Linnaeus (Lepidoptera: Lycaenidae) and observed smaller adults at higher developmental temperatures.

High temperature encourages an increase in fecundity, which is described as the actual reproductive rate of an organism or population, in oviposition rate and the number of eggs laid per day. This positive behaviour is also supported by high relative humidity (Cunnington, 1985; Yadav and Chaudhary, 1986; Zulfiqar et al., 2010; Mabapila, 1997). Whereas higher temperatures lead to an increase in oviposition rate, fecundity and reduced development time, it also limits the oviposition period and the longevity of the adult life (Cunnington, 1985; Potting, 1996; Mabapila, 1997; Getu, 2007). Fecundity rate
of *A. gossypii* increased with increasing temperature (Kersting *et al.*, 1999). The fecundity of the water chestnut beetle also increased initially with increasing temperature, peaks at an optimum thermal range and then decreases once this range is exceeded (Zheng *et al.*, 2008). Exposure of the pupae of *Cadra cautella* Walker (Lepidoptera: Pyralidae) to high temperatures of 40, 45 and 50°C resulted in failure to mate, reduced fecundity, and sterility (Arbogast, 1981). Temperature limits fecundity in two ways, egg maturation and oviposition opportunities, both dependent on temperature. Temperature strongly influenced the rate of egg maturation which increased as developmental temperature increases (Berger *et al.*, 2008).

The fitness of female insects is often determined by her reproductive rate (fecundity), a factor that is strongly dependent on her body size (Honek 1993). Within a female’s favourable thermal range, the reproductive output should be directly affected by the number of resources she can convert to eggs and hence, larger females should have larger reproductive output in comparison to smaller females. Females do not have unlimited oviposition events, as females can oviposit only during favourable thermal conditions that support insect activity and under these conditions, the selection for larger female body size is weak. Females experience a cost of reproduction due to the allocation of resources to egg maturation. High egg maturation rates are associated with high metabolic activities due to higher developmental temperatures. However, this allocation could leave fewer resources for other process and could lead to decreased longevity, decreasing adult body size and reduced performance (Jervis *et al.*, 2005). Research on the temperate butterfly, *Pararge aegeria* Linnaeus (Lepidoptera: Nymphalidae) showed that fecundity was limited by temperature dependent egg-maturation and oviposition
under favourable thermal conditions and temperature dependent adult body size (Berger et al., 2008). The relationship between these two-temperature dependent life history traits is often complicated. A comparative study by Honek (1993) determined that adult body size is determined genetically and modified by environmental conditions such as temperature and resource availability during development and each factor can influence fecundity in different ways. Fecundity varies with female body size, which varies with developmental temperature (Berger et al., 2008). Under constant environmental conditions, fecundity is positively correlated with female adult body size (Rosenheim and Rosen, 1992; Kapranas and Robert, 2008; Aung et al., 2009).

In addition to environmental factors that can influence the variation in insect development, density has also been implicated (Southwood et al., 1972; Dijkstra, 1986; Merritt et al., 1992; Arnvist and Johansson, 1998; Agnew et al., 2002; Gimnig et al., 2002; Stav et al., 2005; Legros et al., 2009; Roberts and Kokkinn, 2010; Couret et al., 2014). The relationship between temperature and density is often positively correlated. Blow fly larvae (Diptera: Calliphoridae) form dense larval feeding aggregates; these aggregates can generate internal heat due to exothermic digestive process and have been recorded at >50°C when ambient temperatures were below 30°C (Anderson and VanLaerhoven, 1996; Campobasso et al., 2001; Joy et al., 2002). Turner and Howard (1992) noted that size of the aggregation (density) influenced its temperature. Slone and Gruner (2007) observed a strong relationship between aggregation volume and internal temperature, aggregations volumes at 20-50cm³ had internal temperatures of approx. 30-35. In contrast, aggregation volumes larger than 50cm³ had internal temperatures higher than optimum thermal range and resulted in increased larval mortality. Interestingly,
Slone and Gruner (2007) observed larvae deliberately moving in and out of the larval aggregates to the cooler periphery to escape the adverse effects the high internal maggot mass temperatures. The effects of density on insect development have been widely investigated. Increase in density-dependent competition for a limiting resource adversely affected adult size, fecundity, and survival (Credland et al., 1986; Dijkstra, 1986; Reeve et al., 1998). There was delayed pupation, reduced survival and adult body size in the mosquito *A. aegypti* and the fruit fly *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) (Agnew et al., 2002). At high densities, the spider mite, *T. mcdanieli* showed reduced developmental rate and reduced survivorship (Wrensch 1978). Density can have indirect effects on development times by mediating resource availability. Survivorship and adult body size decreased at higher larval densities of *Anopheles gambiae* Giles (Diptera: Culicidae) during development. Another example of the influence of density on size is seen in the cabbage looper *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae) with decreasing pupal weights as well as adult size as larval densities increased (Henneberry and Kishaba, 1966).

**Blow Flies**

Blow flies are among the first colonizers of carrion; gravid females locate carrion by chemosensory detection and volatile odours produced by the remains (Tomberline et al., 2011). Adults are attracted to the remains where they feed on blood and decomposition fluids (to help with ovarian development) and reproduce, resulting offspring feed directly on the resource (Kuusla and Hanski 1982). Blow fly larvae grow and molt through three instar larval stages, after feeding the larvae crawls away from the resource in search of a suitable pupation site and finally emerges as a fully formed adult.
Gravid female blow flies will oviposit in natural body orifices (mouth, nose, ears, anus) or some cases open wounds and lesions (Rivers et al., 2011). Female blow flies lay their eggs in aggregates, and these aggregates can vary in location, density and species composition (Rivers et al., 2011). Greenberg and Kunich (2002) suggested that larvae benefit from larval aggregations as multiple individuals penetrate tissues and release enzymatic secretions that aid digestion. The effects of density have been studied in numerous blow fly species; for example, in *Chrysoma megacephala* Fabricius (Diptera: Calliphoridae), there is an inverse relationship between density and development time (Goodbrod and Goff, 1990). Also, survival increased initially with increasing density until the optimum density was reached and then decreased with increasing larval density (Goodbrod and Goff, 1990). Smith and Wall (1997) studied the effects of increasing densities on pure cultures of *Calliphora vicina* Robineau-Desvoidy and *Lucilia sericata* Meigen (Diptera: Calliphoridae) and observed that survival and adult body size decreased with increasing larval density.

Like all insects, blow flies are poikilotherms, which means their distribution, survival, and colonization are all greatly influenced by environmental factors such as temperature. Grassberger and Reiter (2002) studied the development of *Protophormia terraenovae* Robineau-Desvoidy (Diptera: Calliphoridae) at different constant temperatures and showed that development time was inversely related to temperature. Similarly, the influence of temperature on activity was investigated on four forensically important blow fly species; *L. sericata*, *Lucilia cuprina* Wiedemann, *Chrysomya rufifacies* Macquart and *Calliphora stygia* Fabricus (Diptera: Calliphoridae) and the results indicated that higher temperature encourages an increased burst of activity in the
sheep-blow flies (Nicholson, 1934). The growth curves for the egg, larva, and pupa of the secondary screwworm, *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) was studied by Byrd and Butler (1996) and, the development time for the different developmental stages decreased with increasing temperature. High environmental temperature influenced the hatching success of *L. sericata* eggs; eclosion success was negatively impacted by increasing temperatures (Evans, 2009). The development time and survival of *L. sericata* were adversely affected by high developmental temperatures (Tarone *et al.*, 2011). Goodbrod and Goff (1990) studied the effects of larval density on the developmental rates of *Ch. megacephala* and *Chysomya rufifacies* Macquart (Diptera: Calliphoridae) and observed an inverse relationship between density and duration of the larval stage. Contrary to other insect species, the effect of temperature on adult body size of blow flies is often dependent on the thermal range of a species. Adult body size of *L. sericata* and *Phormia regina* Meigen (Diptera: Calliphoridae) increased with increasing developmental temperatures until it reached a maximum and exceeded its thermal range, and then the opposite is observed with decreasing body size with continuous increase in temperature (Hans, 2016). *Lucilia sericata* and *P. regina* are two forensically important species because they frequently oviposit on decomposing remains (Anderson and VanLaerhoven 1996; VanLaerhoven and Anderson 1999). *Lucilia sericata* has a thermal range of 10.6℃-32.5℃ per Gosselin *et al.* (2010), Roe and Higley (2015) and is an inferior competitor, exhibiting decreased survival and adult body size with intraspecific and interspecific competition (Smith and Wall 1997). *Phormia regina* has a thermal range of 10℃-40℃ (Byrd and Allen 2001). Hutton and Wasti (1980) observed 100% mortality of *P. regina* in the presence of *L. sericata*. However, this observation is
controversial and contrary to other research that found facilitation of *P. regina* in the presence of *L. sericata* (Reid 2012; Hans 2016). However, Hans (2016) determined that this facilitation is dependent on temperature, that is, at different temperatures facilitative effects become competition. Although there has been some research conducted examining the effects of temperature on the development of *P. regina* and *L. sericata*, all of the aspects that might influence insect development such as intra-specific interactions have not been investigated to date.

The primary focus of this research is to determine the relative importance of temperature and density on the life history traits of *L. sericata*, and *P. regina* reared in single species cultures at a range of densities. We assessed development time by calculating accumulated degree days, percent survival to adult and fecundity, using adult body size as a proxy. We investigated how these life history traits are affected by three different constant temperature regimes for *L. sericata* and four different constant temperature regimes of *P. regina*, both at five densities. For both species, we predicted a negative linear relationship between accumulated degree days and temperature, such that as temperature increases, fewer ADD are required to complete development. Survival should be affected by temperature and density for both *L. sericata* and *P. regina*, such that increasing densities and temperatures should increase the intensity of species interactions. We expect survival to differ for *L. sericata* and *P. regina* based on their different critical thermal ranges. Survival should be highest at the optimum developmental temperature and lower at the minimum and maximum critical temperatures for both species. We expect the resulting adult body size for all species to follow the temperature-size rule by decreasing with increasing developmental
temperatures (Kingslover and Huey 2008). The “hotter is better” rule by Kingslover and Huey (2008), states species with higher optimal temperatures have relatively higher fitness. The developmental temperature range of *L. sericata* is between 10.6 -32.5°C, with peak survival between 25- 30°C (Roe & Higley 2015; Hans 2016) and *P. regina* is between 10°C - 40°C with peak survival between 25- 35°C (Byrd and Allen 2001). We expect *P. regina* to have shorter development time (lower ADD) and increased survival at higher temperatures in comparison to *L. sericata*. Finally, we predict that development time (ADD), adult body size and survival should decrease with increasing larval densities.

**Materials and Methods**

**Colony Maintenance**

Colonies of adult *L. sericata* and *P. regina* were grown and maintained at 25 ±0.21°C (mean ± SE) on a 12D:12L cycle in aluminum mesh screen cages (46 by 46 by 46 cm) (BioQuip Products, Item ID: 1450C) at the University of Windsor, Windsor, Ontario, Canada. The colonies were established in 2005 at the University of Windsor, Windsor, Ontario by using wasp traps lined with paper towels and using liver as an attractant to trap flies. Wild flies are caught and added to the colonies each year. The colonies were provided with water in Erlenmeyer flasks with dental wicks to prevent drowning, sugar cubes and protein powder *ad libitum* in the form of a paste as a carbohydrate and protein source (Anderson, 2000). Egg masses of approximately 500 eggs were collected using 50 g of pork liver as an oviposition medium for 24h to establish experimental colonies of each species. The liver serves as a rich protein source for females to ensure complete ovarian development. Egg masses from each species
hatched at 18.9 ± 0.8hrs (Mean ± SE) at 25 ± 0. 21°C (Mean ± SE) for *P. regina* and 21 ± 2.5hrs (Mean ± SE) for *L. sericata* at 25 ± 0. 21°C (Mean ± SE) on a 12D:12L diel cycle and 2 to 3 hour-old larvae were transferred onto 50 grams of pork liver using a paint brush and then placed into 1-L Bernardin mason jars, one-third filled with wood shavings made from aspen (Top bedding, Item: large wood shavings). The wood shavings serve as a suitable pupation medium for the blow flies; the jars were covered with landscape tarp (Quest Brands Inc. ID: WBS 50) to allow gaseous exchange and a metal ring lid to prevent the escape of maggots.

**Experimental Design**

The single species temperature treatments were: *P. regina* only; (1) at 15°C, (2) at 25°C, (3) at 35°C, and (4) at 40°C. *L. sericata* only; (5) at 15°C, (6) at 25°C, and (7) at 35°C. Within each single species temperature treatment, five larval density treatments were tested (25, 50, 100, 200 & 400 larvae per jar). The larval densities and species treatment at each temperature were placed in a growth chamber (Conviron Adaptis A1000) programmed with a photoperiod of 12:12(L: D), 70% relative humidity. Each density and species treatment were replicated ten times within each temperature. Data loggers (Smart button, ACR Systems Inc.) were programmed to record the temperature in the growth chambers every 60 minutes. Internal maggot mass temperature was recorded every 24 hours using an infrared temperature gun (Measupro Infrared thermometer, IRT20). When the adult flies emerged in the rearing jars, they were killed and counted to determine the percent successful emergence of adult (survival) of each species at each specific density and temperature. Development time was converted to accumulated degree days (ADD) and analyzed using a generalized linear model. ADD was calculated
using the formula, ADD = D (T_m – T_min); where D = developmental time (days), T_m = the ambient (experimental) temperature (°C), and T_min = lower temperature threshold of species of interest, in our case L. sericata (10.6°C) (Grisendi et al., 2015) and P. regina (10°C) (Byrd and Allen 2001). The adult size of each blow fly at each density and temperature was determined by measuring the posterior cross-vein of the left wing. The wing vein measurement is taken from the length of the cross-vein dm-cu vein (Smith and Wall 1997). The cross-vein dm-cu is a short transverse vein that connects veins M and Cu_1 at the distal end of the wing (Bland and Jaques 2010).

**Statistical Analyses**

All analyses were completed in Statistica 7 (Data analysis software system, version 7. www.statsoft.com). Survival was arcsine transformed to meet the assumptions of normality and then analyzed using a three-way ANOVA to examine the effect temperature, density and the interaction between both variables on survival. A General Linear Model was used to analyze the effects of temperature, density, species and possible interactions on the adult female body size of P. regina and L. sericata; data was square root transformed (Bland and Altman 1996) to meet the assumptions of normality. Normality was tested for using the Kolmogorov-smirnov test, and data transformations were effective resulting in a p-value greater than 0.05. The effect of density, temperature, and species on total development time and the interaction between these factors were analyzed using a Generalized Linear Model (GLZ, distribution = Poisson, link=log). Significant levels were set as α = 0.05. Significant results were followed by mean comparison post hoc tests (Tukey), and a Bonferroni correction was used to correct for multiple tests.
Results

Survival to adult was influenced by an interaction between temperature, species, and density (ANOVA $F_{8,270} = 5.52, p < 0.001$) (Table 2.1, Figure 2.1). Variation in survival can be explained predominately by temperature (MS = 5.42) followed by density (MS = 0.195) and species (MS = 0.049). For *P. regina*, survival was highest at 25°C across all densities. At 35°C, survival was equivalent to that at 25°C only for low densities of 25 and 50 larvae/jar. Survival of *P. regina* at 15°C was higher overall than that at 35°C, as survival decreased with density at 35°C but no significant effects at 15°C. There was no survival of *P. regina* at 40°C, thus it was not included in further analysis. In contrast, density had less of an impact on an individual temperature on the survival of *L. sericata*. The exception was at the density of 25 larvae/jar where survival was equivalent at both 15°C and 25°C. Overall, *L. sericata* survival was highest at 25°C, followed by 15°C and lowest at 35°C. Effect of species was observed at low densities of 15°C and 25°C.

Temperature and species interacted to influence the ADD of *L. sericata* and *P. regina* (Table 2.2). Temperature (MS = 63014146) accounted for most of the variation in ADD, followed by species (MS = 16336133). There was no effect of density. Hence ADD was pooled within densities (Table 2.2). *Lucilia sericata* and *P. regina* accumulated fewer degree days at higher temperatures; the highest ADD was recorded at 15°C and the lowest at 35°C (Figure 2.2). *Phormia regina* required less ADD to fully develop and had a narrower ADD range than *L. sericata*. 
The effect of temperature, density, and species on the female adult body size of both species was analyzed using a general linear model. Adult body size was influenced by an interaction between species, density, and temperature \((F_{8,571} = 6.8, p < 0.001)\) (Table 2.3). Temperature (MS = 1.148) had the largest effect on adult body size, accounting for over four times more variation than density (MS = 0.301) and ten times more variation than species (MS = 0.1218) (Table 2.3). Overall, largest adults were observed at 25°C in lower larval densities for *P. regina*, followed by adults at 15°C in higher larval densities and adults at 35°C with lower larval densities for *P. regina*. Whereas, largest adults were observed at 25°C in lower larval densities followed by adults in lower densities at 15°C for *L. sericata* (Figure 2.3). Smaller adults were observed at higher densities at 35°C for *P. regina*. Whereas, smaller adults were observed at all densities at 35°C for *L. sericata* (Figure 2.3). Females of *L. sericata* were slightly larger than females of *P. regina*, but this was only evident at 50 larvae/jar and 200 larvae/jar at 15°C (Figure 2.3).

**Discussion**

Environmental conditions such as temperature can influence the life history of poikilotherms. They maintain their body temperature by absorbing heat from surrounding environment (Angiletta *et al.*, 2002; Bale *et al.*, Denlinger and Yocum 1998; Hoofmann *et al.*, 2003). In the current study, there is substantial evidence of changes in development in response to different environmental conditions. This plasticity represents a complex response that allows organisms to cope with environmental variability. Insects have a
temperature tolerance zone with minimum and maximum critical temperatures and the optimum range somewhere in between.

Insects developing at higher temperatures have increased rates of development due to increased metabolic rates and cellular processes (Srivastava and Omkar, 2003; Pervez, 2004). Our results agreed with observations from previous studies and our predictions; there was a negative linear relationship between accumulated degree days (ADD) and temperature, insects accumulated more thermal units each day when developing at higher temperatures and hence had shorter development times as they reached their required ADD for development faster. ADD is defined as the measurement of thermal unit required for the growth and development of insects (Byrd and Castner 2010), and according to our data, *P. regina* requires a minimum of 153 – 222 ADD for successful adult emergence, with a threshold temperature of 10°C. In contrast, *L. sericata* requires a minimum of 182 - 362 ADD for successful adult emergence, with a threshold temperature of 10.6°C. When converted back to development time (hrs), development of egg to adult (development time) was inversely proportional to temperature (Tamiru *et al.*, 2011; Yadav and Chaudhary, 1986; Muegge and Lambdin 1989; Levesque *et al.*, 2002; Mbapila *et al.*, 2002). We found that development time (time to adult emergence) decreased as developmental temperatures increased, except for the development of *P. regina* at 40°C with no adult emergence. Other studies have also reported no adult emergence at 40°C indicating that 40°C approaches the lethal upper temperature for *P. regina* (Melvin 1934; Bryd and Allen 2001). The rate of development differed between species, with *P. regina* developing faster than *L. sericata*. This observation is also in
concordance with the comparative study by Kamal (1958). We found no effects of density on the ADD and development time of *L. sericata* and *P. regina*.

While several life history traits were measured, survival was chosen as the best fitness measure as individuals must survive in order to reproduce and have an influence in their ecological communities. Temperature influenced survival, which is defined as the number of successfully emerged adults. Survival was strongly influenced by each species thermal range with both species having low survival at the upper threshold temperatures used in this study. The overall survival graphs of both species resemble a normal distribution graph with peak survival at optimal temperatures and decreased population at the lower and upper thermal thresholds as these represent zones of physiological stress. *Phormia regina* had higher survival in higher temperatures except at 40°C when compared to *L. sericata*, this is as a result of the broader thermal range associated with *P. regina* and could also indicate higher fitness as *P. regina* will survive and reproduce at higher temperatures where *L. sericata* can not. Strong effects of density were recorded for *P. regina* at 35°C as percent survival drastically decreased as larval population increased. An explanation for this could be as a result of increasing internal maggot mass temperature as density increases, or as a result of increasing rate of decomposition with temperature and increased population density on a limited resource. There is a positive correlation between density and temperature (Rivers *et al.* 2011); calliphorid larvae form dense larval feeding aggregates, and these aggregates can experience internal maggot temperatures higher than their developmental temperatures (up to 20°C higher). The adverse effects of density that we notice for *P. regina* at 35°C could be because of increased maggot mass temperatures. However, the internal maggot mass temperatures
recorded for all densities every 24hrs until pupation showed negligible changes. It is more likely that this adverse effect of increasing density is due to increased competition for food and an increase in the rate of decomposition of the resource due to high temperatures. Thus, a decrease in survival at higher densities is likely due to increased competition at higher temperatures. Individuals are competing for less resources at higher densities, and when combined with the rapid rate of decomposition associated with high developmental temperatures, there is competition for resource that is not only limited but also dissipating quickly. It is important to note that the severity of the effects is dependent on temperature, there is a higher influence of density on survival of both species at 35℃ in comparison to the effects of density at 25℃. The developmental thermal range mediated the effects of density on survival for these blow flies. Gosselin et al., (2010) observed increasing survival with increasing temperature of L. sericata between 12.5 to 30℃ and decreasing survival above 30℃. Hans (2016) observed similar findings for L. sericata between 15 to 30℃ with decreasing survival above 30℃. Our results agreed with previous studies for L. sericata, showing a drastic decrease in survival at 35℃.

The phenomenon where individuals that are developing at low temperatures exhibit larger body size is known as the temperature-size rule (Atkinson 1994; Angilletta et al., 2003). The adult body size of P. regina and L. sericata in this study indicates that temperature had a strong influence. Overall, the adult body size of P. regina and L. sericata follows that of a normal distribution graph, much like survival. Largest adults were observed at an optimum temperature and smaller adults at temperatures representing lower and upper limits, 15℃ and 35℃ respectively. These observations in body size do
not follow the temperature-size rule, and this could be due to limited availability of resources and the effect of temperature on the rate of decomposition of resources. A cost-benefit relationship could also explain this discrepancy in the adult body size, that is, for individuals to achieve a significant body size at low temperatures they must prolong development and postpone reproduction in comparison to individuals in warmer temperatures (Angilletta et al., 2003). However, because adult body size is strongly related to fecundity and high fecundity is encouraged by high temperature, this ultimately means that adult body size is affected by an individual’s favourable thermal range, resource availability, and resource allocation (Berger et al., 2008). The interaction between temperature and density on adult body size in this study was surprising and unexpected. The effects of density on body size was influenced by temperature with negative relationships at higher temperatures, 25°C and 35°C and a positive relationship at a lower temperature, 15°C for P. regina, and a negative relationship at 25°C and a positive relationship at 15°C for L. sericata. A decrease in resource availability can explain the decrease in adult body size with increasing densities. Higher internal maggot mass temperatures could also explain it but since maggot mass temperatures recorded for this study were not significant the plasticity in adult body size is likely due to changes in the levels of competition with density and by temperature as well. The positive relationship at 15°C could be seen as positive effects of many individuals piercing the resource explained by Greenberg and Kunich (2002) and a combination of lower intensity of competition at low temperatures. The rate of decomposition is much slower at 15°C than at other temperatures tested for this study; larvae benefit from multiple individuals not only piercing the resource but also releasing enzymatic secretions that aid in digestion.
of resource. Since gravid female blow flies oviposit in aggregates, that often vary in density (Rivers et al., 2011). Larvae can experience these effects of density on adult body size that can be positive or negative depending on the developmental temperatures. These effects of density can be intra-specific or inter-specific, in this case, the effects of density were observed under single species conditions (intra-specific). It is important to note that while there are suggested positive effects of aggregated oviposition and developing within larval aggregates, there are also adverse effects of density such as increased intra-specific competition with temperature as blow flies feed on carrion, which is an ephemeral resource and is consumed over time making it a limited resource. In addition, Tomberlin et al. (2011) proposes blow-fly adult size can be governed by phenotypes, genotype or phenotype-genotype interactions. In the present study, there are significant differences in body size with respect to developmental temperatures and larval population densities indicating that body size exhibits plasticity with different developmental conditions. Body size is determined by genotype and modified by environmental conditions resulting in phenotypic differences. Therefore, the adult body size observed for this research is likely as a result of an interaction between genotype and environment.

The relative contributions of each independent effect on the overall fitness of the blow flies was examined by investigating how much variation is attributed to each effect. Temperatures was responsible for most of the variation reported in survival and other life history traits measured; this is expected as blow flies are unable to regulate their body temperature and depend on the temperature of the ambient surroundings. The severity of the effects was a function of each species thermal range, seen in L. sericata at 35°C and
*P. regina* at 40℃. While population density affected survival and adult body size, much of the variation in these life history traits is attributed to changes in developmental temperatures. Temperature is an important factor in the development of insects, while individuals are affected by species interactions and population density, as insects are unable to regulate their body temperature, they are vulnerable and rely on the ambient temperature to ensure survival and reproduction.
REFERENCES


Hans, K.R., 2016. Using an ecological framework to resolve issues in forensic entomology: Exploring temperature mediation of species interactions within blow fly (Diptera: Calliphoridae) communities (Doctoral dissertation, University of Windsor (Canada)).


the black blowfly (Diptera: Calliphoridae). *Journal of Medical Entomology* 39: 392-397.


Table 2.1. Analysis of Variance (ANOVA) results to determine the effects of density and temperature on the percent survival of *L. sericata* and *P. regina*. Species was tested as a covariate. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects. For these analyses, *P. regina* was not tested at 40°C as there was zero survival.

<table>
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<th>F-ratio</th>
<th>P-value</th>
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<td>Species* Temperature</td>
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<tr>
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<td>0.194</td>
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Table 2.2. Generalized linear model (distribution = Poisson, link function = log) to analyze the effects of density, species, and temperature on the accumulated degree days of *L. sericata* and *P. regina*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects. For these analyses, *P. regina* was not tested at 40°C as there was no adult emergence at this temperature.

<table>
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<th>Wald Stat.</th>
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Table 2.3. General Linear Model (GLM) results to determine the effects of density and temperature on the female body size of *L. sericata* and *P. regina*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects.

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<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Mean (±S.E) % Survival

Density

35°C

25°C

15°C

P. regina

L. sericata

A (I, II)

B (II)

AB (I)

A (II)

B (II)

(I)

(II)

(I)

(II)

(I)

(II)

(I)

(II)

(I)

(II)

(I)

(II)

(I)

(II)

(I)

(II)

(I)

(II)

(I)
Figure 2.1. The mean (±S. E) percent survival of *P. regina* and *L. sericata* at 15 °C, 25 °C and 35 °C across densities (larvae/jar). There was an interaction between density, species and temperature (F$_{8, 270}$ = 5.516, *p* < 0.001). For these analyses, *P. regina* was not tested at 40°C. Asterisks represent difference between species, letters represent difference between densities and roman numerals represent differences between temperature. Means with an asterisk indicate a difference between species survival at that larval density and temperature. Means with the same letters indicate no significant differences in survival between densities within a species at a given temperature. Means with the same numerals show no significant differences in survival between temperatures at that density for a species. Bonferroni corrected *p*-value for these post hoc tests was set as $\alpha = 0.0125$. 
Figure 2.2. The mean (±S. E) accumulated degree days (ADD) for *L. sericata* and *P. regina* at all developmental temperatures tested. Density was not a significant effect, hence ADD was pooled within densities at each temperature for each species. There was an interaction of temperature and species ($F_{2, 293} = 387.86$, $p < 0.001$)
Mean (± S. E) Adult size of Adult Females

---

**P. regina**

- **P. regina 35 degrees**
- **P. regina 25 degrees**
- **P. regina 15 degrees**

**L. sericata**

- **L. sericata 35 degrees**
- **L. sericata 25 degrees**
- **L. sericata 15 degrees**
Figure 2.3. The mean (± S.E) female adult body size across all temperatures for *L. sericata* and *P. regina*. Body size was influenced by an interaction between, density, species and temperature (F\textsubscript{8,571} = 6.8, p< 0.001). For *L. sericata* at 35°C (y = -0.001x\textsuperscript{3} + 0.0156x\textsuperscript{2} - 0.0002x + 1.129, R\textsuperscript{2} = 0.82), 25°C (y = 0.0005x\textsuperscript{3} - 0.0074x\textsuperscript{2} + 0.0124x + 1.267, R\textsuperscript{2} = 0.98) and 15°C (y = 0.0059x\textsuperscript{3} + 0.094x\textsuperscript{2} + 0.4127x + 0.859, R\textsuperscript{2} = 0.67). For *P. regina* at 35°C (y = 0.0019x\textsuperscript{3} - 0.0269x + 0.0654x + 1.139, R\textsuperscript{2} = 0.96), 25°C (y = 0.0017x\textsuperscript{3} - 0.027x\textsuperscript{2} - 0.0908x + 1.2108, R\textsuperscript{2} = 0.93) and 15°C (y = -0.0031x\textsuperscript{3} + 0.0429x\textsuperscript{2} - 0.1677x + 1.2931, R\textsuperscript{2} = 0.97). Means with an asterisk indicate body size differences between temperatures at the same density. Means with the same letters indicate no significant differences in adult body size between densities within a species at a given temperature. Means with the same numerals show no significant differences in adult body size between species within a temperature. Bonferroni corrected p-value for these post hoc tests was set as α = 0.0125
CHAPTER 3
CONSEQUENCE OF TEMPERATURE, DENSITY AND SPECIES INTERACTION
ON THE DEVELOPMENT AND SURVIVAL OF BLOW FLIES (DIPTERA:
CALLIPHORIDAE).

Introduction

Ecological communities are made up of two or more species occupying the same geographical area at the same time (Agrawal et al., 2007; Ricklefs, 2008; Brooker et al., 2009). The communities are bound together by their shared environment and the network of influences they have on one another. Communities may be classified on a continuum of interactive to non-interactive communities depending on the availability of unused niche space. Non-interactive communities are made up of large amounts of niche space created by high mortality rates and decreased population due to changes in abiotic environments (Cornell and Lawton 1992). On the other hand, interactive communities constitute saturated niche space, resulting in strong species interactions between individuals on the same trophic level, either directly or indirectly using a mutual resource (Cornell and Lawton 1992). Gause’s law states that more than one species cannot utilize the same resource in the same way and at the same time without competitive exclusion of one species (Gause 1934). This means that species coexistence within communities is as a result of niche heterogeneity or spatial-temporal heterogeneity. Niche heterogeneity describes uneven and diverse habitats which offer different conditions for growth, and due to its patchiness, heterogeneous environments support higher species diversity (Bell et al., 2000). For example, the diversity and abundance of soil mites increased with an increase in soil complexity (Anderson 1978). Similarly, insect diversity increases with an
increase in the structural diversity of their plant hosts (Strong and Levin 1979; Moran 1980). Spatial heterogeneity involves species occupying different areas of shared resources to consequently reduce the levels of inter-specific competition that they might experience (Ives 1999). Temporal heterogeneity describes species temporal separation in utilizing shared resources, that is, species use daily, seasonal and multi-annual patterns of colonization, foraging, and migrations to utilize resources and decreases levels of competition (Rosenberg and Freedman 1994).

Interactions such as predation (parasitism and herbivory), competition, amensalism, facilitation (mutualism and commensalism) can occur within or between trophic levels and can change due to presence or absence of a species and on the quality of the shared resources. These interactions can affect life history traits in different insect species. Park (1954) worked extensively on the competition between two species of flour beetles Tribolium confusum Jacquelin du Val and Tribolium castaneum Herbst (Coleoptera: Tenebrionidae). Both species had decreased survival when grown together in comparison to when they were grown separately. Similarly, Shorrocks and Bingley (1994) showed that interspecific competition between two Drosophila spp. (Diptera: Drosophilidae) resulted in decreased survival, smaller adult size and increased development time.

Temperature can affect the strength of species interactions experienced by insects during development, and different temperatures can affect the competitive abilities of species within the same trophic level. Populations of flour beetles, T. confusum and T. castaneum grown together at two different temperatures 34°C and 24°C showed that at 34°C T. castaneum persisted whereas T. confusum was competitively excluded (Park
1954). However, when grown at 24°C *T. castaneum* survival decreased in comparison to *T. confusum* (Park 1954). These observations indicate that environmental conditions such as temperature influence species interactions and the outcome of these interactions.

Temperature effects on insect development differ in single species and multi-species cultures (Gilman *et al.*, 2010). Davis *et al.*, (1998) studied the effects of temperature on the interactions between three species; *Drosophila melanogaster* Meigen, *Drosophila simulans* Sturtevant and *Drosophila subobscura* Collin (Diptera: Drosophilidae). Mixed culture interactions resulted in a shift in the optimal thermal range of *D. melanogaster* and *D. subobscura* (Davis *et al.*, 1998). The effect of temperature can alter the metabolic rates and competitive ability of different species and ultimately influence the outcome of their species interactions.

Because insects are poikilotherms (unable to regulate their body temperature) and rely on environmental conditions to regulate their metabolic and developmental processes. Insect development occurs within a thermal range with minimum (T$_{min}$) and maximum limits (T$_{max}$), and an optimal range in-between (Huey and Kingslover 1989). These limits represent the lowest and highest temperature limits at which insect development can occur (Huey and Kingslover 1989). Several studies have reported a decrease in survival when species develop in temperatures higher than the species optimal range; *Aedes aegypti* Linnaeus (Diptera: Culicidae) (Tun-Lin *et al.*, 2000), *Ephestia cautella* Walker, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) (Arbogast 1981) and *Lucilia sericata* Meigen (Diptera: Calliphoridae) (Gosselin *et al.*, 2010) to name a few. Temperature influences the growth rate of insects by decreasing development time with increasing temperature (Byrd and Castner 2010). However,
development at temperatures higher than that of the optimum range can increase
development time such as demonstrated with *Aphis gossypii* Glover (Hemiptera:
Aphididae) (Kersting *et al.*, 1999) and *Cnaphalocrocis medinalis* Guenee (Lepidoptera:
Crambidae) (Park *et al.*, 2014).

Temperature influences the adult body size of insects with individuals developing
at low temperatures having a larger body size than individuals developing at higher
temperatures (Angilletta *et al.*, 2003). Understanding the effects of temperature on adult
body size is important because body size is strongly related to fecundity. Fecundity
fluctuates with female body size, which varies with developmental temperature (Berger *et
al.*, 2008). Under constant developmental temperature, fecundity has a positive linear
relationship with female adult body size (Rosenheim and Rosen 1992; Apostolos and
Robert 2008; Aung *et al.*, 2009).

Another factor that influences the outcome of species interactions is density.
Increase in the larval density in single species cultures on limited resources results in
negative effects on measured life history traits (Wrensch 1978; Hennerberry and Kishaba
1966), including reduced adult body size which also means decreased fecundity
(Credland *et al.*, 1986; Reeve *et al.*, 1998), reduced survival (Agnew *et al.*, 2002; chapter
2) and increased developmental times (Kersting *et al.*, 1999). Density mediated the
outcome of interspecific competition on two blow fly species; *Lucilia sericata* Meigen
and *Calliphora vicina* Robineau-Desvoidy (Diptera: Calliphoridae) (Smith and Wall
1997). The effects of inter-specific competition were more significant at densities greater
than ten larvae/g of liver for *L. sericata*. However, the opposite was true for *C. vicina
with greater effects in intra-specific than the interspecific competition (Smith and Wall
These results suggest that *C. vicina* is an inferior intraspecific competitor but superior interspecific competitor whereas *L. sericata* is an inferior interspecific competitor but a superior intraspecific competitor. Density mediates the outcome of species interactions by changing the intensity of species interactions experienced by the individuals present. Regardless of the interaction type, increasing larval density should be expected to increase the intensity of the species interaction experienced by individuals.

Given that carrion is a patchy and ephemeral resource, gravid females must maximize their offspring fitness by choosing to oviposit in areas that limit high levels of interspecific competition experienced by their offspring (Ives et al., 1999). Females oviposit in aggregates presumably to help reduce desiccation experienced by the eggs and promote larval development by shared digestive enzymes that encourage decomposition and inhibit competitive microbes (Greenberg and Kunich; Rivers et al., 2010; Brodie et al., 2015). This aggregated oviposition results in unsaturated niche space for other species to colonize, thereby supporting coexistence over a shared resource. This should allow highly competitive species to be able to coexist on a limited shared resource (Atkinson and Shorrocks 1981). These egg aggregates can also vary in location, density, species composition and ultimately influence the levels of inter- and intraspecific competition.

The blow fly life cycle is composed of eggs laid by gravid females that hatch into first instars after accumulating sufficient accumulated degree-days (ADD). ADD is defined as the measurement of thermal units required for the growth and development of insects (Byrd & Castner, 2010). After accumulating the minimum ADD, first instar larvae molt into the second instar by shedding their larval cuticle and mouthparts (James
1947). The larvae progress through second instar and third instar, molting in between until they achieve the post-feeding larval stage and move away from the food source in search of suitable pupation sites (James 1947). The pupal stage is the longest duration, after which, the adult fly emerges, leaving the empty puparium behind (James 1947).

Dos Reis et al. (1999) studied the effects of intraspecific and interspecific interactions on two blow fly species *Chrysomya putoria* Wiedemann and *Cochliomyia macellaria* Fabricius (Diptera: Calliphoridae) at constant temperature and observed that in single species cultures survival decreased with increasing density. However, in mixed-species cultures survival of *Ch. putoria* increased with increasing density, whereas survival of *Co. macellaria* was low in mixed cultures compared to single species cultures (Dos Reis et al., 1999). These results suggest that *Co. macellaria* is a competitively inferior species in the presence of *Ch. putoria* at 25℃ (Dos Reis et al., 1999). Similarly, observations in two necrophagous species *Hemipyrellia ligurriens* Wiedemann (Diptera: Calliphoridae) and *Boettcherisca formosensis* Kirner and Lopes (Diptera: Sarcophagidae) reared at different larval densities at a constant temperature for single and mixed species cultures, showed higher survivorship of *B. formosensis* in mixed cultures than single cultures and lower survivorship and smaller adults of *H. ligurriens* in mixed than single species cultures (So and Dudgeon 1990). These observations suggest that *B. formosensi* is a stronger interspecific competitor than *H. ligurriens* at the temperature tested (So and Dudgeon 1990).

Within the carrion insect community, *L. sericata* and *Phormia regina* Meigen (Diptera: Calliphoridae) are species both native to southern Canada and have been observed colonizing the same carrion (Anderson and VanLaerhoven 1996;
VanLaerhoven and Anderson 1999). *Lucilia sericata* is known as the sheep blow fly or the green bottle blow fly. It has a thermal range of 10°C to 32.5°C per Gosselin *et al.* (2010) and Roe and Higley (2015) and is an inferior interspecific competitor, exhibiting decreased survival and adult body size in mixed species treatments (Smith and Wall 1997). *Phormia regina* also known as the black blow fly and has a thermal range of 10°C to 40°C (Byrd and Allen 2001). It is thought to be facilitated in the presence of *L. sericata*. However, this facilitation is a function of temperature and turns to competition above 25°C (Hans 2016). There is limited research investigating their species interactions and even less investigating the possible effects of temperature and increased larval densities and how these factors might influence species interactions.

The focus of this chapter is investigating the role of both temperature and density in mediating the species interactions between *P. regina* and *L. sericata*. We measured several life history traits that determine population fitness including survival, development time by calculating accumulated degree days, and fecundity using adult body size as a proxy. Based on Chapter 2, we expect shorter development time with increasing developmental temperature for both species regardless of the ratio of species combination, within the range of temperatures tested herein. Based on our results from Chapter 2, we do not expect larval density to affect development time for both species. We expect to observe decreased development time for the species with the higher species proportion within species ratio combinations due to the possible effects of ‘founder control’ a phenomenon which assumes that the more abundant species present within a resource, will have a competitive advantage over other species present.
Given that the developmental temperature range for *L. sericata* is between 10°C – 32.5°C and *P. regina* is between 10°C - 40°C (Byrd and Allen 2002; Roe and Higley 2015; Hans 2016), we predict that survival will increase with increasing developmental temperatures but will decrease at the lower and upper thermal limits of each species. In Chapter 2, we observed lowest *L. sericata* survival at 35°C, as this temperature exceeds the thermal limit for this species, while *P. regina* had lower survival at 15°C, there was significantly higher survival when compared to survival of *L. sericata* at 35°C indicating that the lower thermal limit for this species is below 15°C. Based on Hans (2016) results, we predict that the presence of *P. regina* will have no effects on the survival of *L. sericata* at the temperatures tested, except at 35°C as this temperature exceeds the thermal limit for *L. sericata* and increased inter-specific interactions might result in decreased survival. As we observed, the lowest survival of *P. regina* at low density at 15°C and higher densities at 35°C (Chapter 2), we predict an interaction between density, temperature and species ratio for *P. regina*. We predict that *P. regina* will have higher survival when present within species ratios at higher proportions, however, this effect will decrease with increasing larval density due to increased competition but will overall be dependent on temperature such that below or above the *P. regina* threshold temperature we expect a negative relationship between survival, species ratio, and density. Similarly, we observed increasing survival of *L. sericata* with increasing larval density at 25°C but decreasing survival with increasing density at 15°C and 35°C (chapter 2), we predict an interaction between density, temperature and species ratio on the survival of *L. sericata*. Based on Hans (2016) results, we expect survival of *P. regina* to increase in the presence of *L. sericata* at 25°C and 35°C and decrease at 15°C in the
presence of *L. sericata* compared to when *P. regina* is on its own. We predict that whichever species has the higher density within the overall combined species density will have increased survival compared to the lower density species. However, we expect this competitive advantage to be influenced by temperature such that each species loses their competitive advantage at temperatures approaching or exceeding their upper and lower thermal limits. Given this, we expect an interaction between species ratio and temperature for both species. We predict that increasing larval densities will lead to a decrease in survival due to increased competition for a limited resource but again, based on our results in chapter 2 this will be influenced by a species developmental thermal range.

Based on our results in chapter 2, we predict that the adult body size will decrease with increasing developmental temperatures regardless of the ratio of species combination and will decrease with increasing larval density due to increasing competition for a limited resource. We predict that the species with higher density within the overall combined species density will have a larger adult body size at a given temperature, but this will be a function of temperature as both species have different temperature thresholds.

**Materials and Methods**

**Colony Maintenance**

Colonies of adult *L. sericata* and *P. regina* were grown and maintained at 25 ± 0.21°C (Mean ± SE) on a 12D:12L cycle in aluminum mesh screen cages (46 by 46 by 46 cm) (BioQuip Products, Item ID: 1450C) at the University of Windsor, Windsor, Ontario, Canada. The colonies were established in 2005 by using the king wasp traps (King home
and garden products, Item ID: 56789) lined with paper towels and using liver as an attractant. Wild flies are caught and added to the colonies each year. The colonies were provided with water in Erlenmeyer flasks with dental wicks to prevent drowning, sugar cubes and protein powder *ad libitum* in the form of a paste as a carbohydrate and protein source respectively (Anderson, 2000). Egg masses of approximately 500 eggs were collected using 50 grams of pork liver as an oviposition medium for 24h to establish experimental colonies of each species. The liver serves as a rich protein source for females to ensure complete ovarian development. Egg masses from each species hatched at 18.8 ± 0.8 hrs (Mean ± SE) for *P. regina* and 21± 2.5hrs (Mean ± SE) for *L. sericata* at 25 ± 0. 21°C (Mean ± SE) on a 12D:12L diel cycle and 2 to 3 hour-old larvae were transferred onto 50 grams of pork liver using a paint brush and then placed into 1-L Bernardin mason jars, one-third filled with wood shavings made from aspen (Top bedding, Item ID: large wood shavings). The wood shavings serve as a suitable pupation medium for the blow flies; the jars were covered with landscape tarp (Quest Brands Inc. ID: WBS 50) to allow gaseous exchange and a metal ring lid to prevent the escape of maggots.

**Experimental Design**

Species treatments were a full factorial design with three developmental temperatures of 15°C, 25°C and 35°C, five larval densities of 25, 50, 100, 200 and 400 larvae per 50g of pork liver and finally three ratios of species combinations of 75% *P. regina* to 25% *L. sericata*, equal 50:50 ratio and 25% *P. regina* to 75% *L. sericata*. The larval densities and the ratio of species combinations were placed in a growth chamber (Conviron Adaptis A1000) programmed with a photoperiod of 12:12(L: D), 70% relative
humidity at the appropriate temperature treatment. Each density and ratio of species combination was replicated ten times within each temperature treatment. Data loggers (Smart button, ACR Systems Inc.) were programmed to record the temperature in the growth chambers every 60 minutes. Internal maggot mass temperature was recorded every 24 hours using an infrared temperature gun (Measupro Infrared thermometer, IRT20). When the adult flies emerged in the rearing jars, they were killed and counted to determine the percent survival (adult emergence) of each species at each specific density, the ratio of species combination and temperature. Development time was converted to accumulated degree days (ADD) and analyzed using a generalized linear model. ADD was calculated using the formula, ADD = D (T_m – T_min); where D = developmental time (days), T_m = the ambient(experimental) temperature (°C), and T_min = lower temperature threshold of species of interest, in our case L. sericata (10.6°C) (Grisendi et al., 2015) and P. regina (10°C) (Byrd and Allen 2001). The adult size of each blow fly at each density and temperature was determined by measuring the posterior cross-vein of the left wing. The wing vein measurement was taken from the length of the cross-vein dm-cu vein (Smith and Wall 1997). The cross-vein dm-cu is a short transverse vein that connects veins M and Cu1 at the distal end of the wing (Bland and Jaques 2010).

**Statistical Analyses**

All analyses were completed in Statistica 7 (Data analysis software system, version 7. www.statsoft.com). Survival was arcsine transformed to meet the assumptions of normality and was analyzed using a three-way ANOVA, to examine the effects of temperature, density, species ratio and the interaction between these variables on the survival of both species. General Linear Model (GLM) was used to examine the effect of
density, temperature, species ratio and the interactions of these variables on the adult body size of both species; data were square root transformed (Bland and Altman 1996) to meet the assumptions of normality. Normality was tested for using the Kolmogorov-smirnov test, and data transformations were effective resulting in a p-valeur greater than 0.05. The effect of density, temperature, and species on total development time and the interaction between these factors were analyzed using a Generalized Linear Model (GLZ, distribution = Poisson, link=log). Significant level was set at α = 0.05. Significant results were followed by mean comparison post hoc tests (Tukey), and a Bonferroni correction was used to correct for multiple tests.

To measure the interaction effects of L. sericata on the survival of P. regina, the survival of P. regina in single species treatments (chapter 2) was compared to the survival of P. regina in mixed species treatments using an ANOVA. Similarly, we examined the interaction effects of P. regina on the survival of L. sericata by comparing the survival of L. sericata in single species treatments (chapter 2) to the survival of L. sericata in mixed species treatments (chapter 3) using an ANOVA. Significant level was set at α = 0.05.

To determine if the presence of heterospecifics versus conspecifics influences the development time of L. sericata and P. regina; we compared the development times in single species treatments (chapter 2) to the development time in mixed species treatments (chapter 3) for both species, L. sericata and P. regina using a Generalized Linear Model (GLZ, distribution = Poisson, link = log). This analysis was done for all ratios of species combinations, and significant levels were set at α = 0.05.
The adult body size of *P. regina* in mixed species treatments (chapter 3) was compared to the adult body size in single species treatments (chapter 2) using an ANOVA. Similarly, the adult body size of *L. sericata* in single species treatments (chapter 2) was compared to mixed species treatments (chapter 3) using an ANOVA to determine if adult body size is affected by development in the presence of heterospecific versus conspecifics. All ratios of combinations for each species were compared to the single species counterpart, and significant levels were set at $\alpha = 0.05$.

**Results**

**Survival**

The survival of *P. regina* was influenced by an interaction between density, temperature and species ratio (ANOVA: $F_{16,405} = 2.025$, $p = 0.011$) (Table 3.1). The relative impact of each factor in explaining the variability in survival was determined by examining the mean squares effect of each factor. Temperature explained the most variation in survival (MS = 13.81), followed by density (MS = 0.325) and finally species ratio (MS = 0.133) (Table 3.1). Survival at 15°C was neither affected by density or by species ratio (Figure 3.1). Within a temperature, there was no difference in the survival of *P. regina* between the species ratios at each density, except at 35°C with 200 larvae/jar and at 25°C with 50 larvae per jar. At 35°C with 200 larvae/jar, the survival increased with decreasing ratio of *P. regina*. At 25°C with 50 larvae per jar, *P. regina* survival was higher at the unequal species treatment ratios and lowest at the equal species ratio treatment.

At 35°C, there was no effect of density on survival of *P. regina* within any species ratio (Figure 3.1). There was also no effect of density on survival of *P. regina* at the two
unequal species ratio treatments at 25°C. However, within the equal 50:50 *P. regina:* *L. sericata* species ratio treatment, survival was lower at 50 larvae/jar compared to all higher densities.

Survival was highest for the 25:75 *P. regina:* *L. sericata* species ratio treatment at 25 and 35°C than at 15°C with no effect of density. This was also true for the equal 50:50 species ratio treatment except at 50 larvae/jar at 25°C which had lower survival and was not different than the 50:50 species ratio treatment across all densities at 15°C (Figure 3.1).

The survival of *L. sericata* was influenced by a three-way interaction between density, temperature and species ratio (ANOVA: $F_{16, 405} = 2.58, p < 0.001$) (Table 3.1), with temperature explaining most of the variation (MS = 15.87), followed by density (MS = 1.32) and then species ratio (MS = 0.033). Overall, the survival of *L. sericata* was unchanged by density at 25°C (Figure 3.2), and there was no effect of species ratio at 15°C or 35°C. At 35°C, only a density of 400 larvae/jar had increased survival compared to other densities. Survival was lower overall at 35°C compared to other temperatures, except at the two lowest densities at 15°C which was also lower overall. At 15°C, survival was higher at larval density of 100 larvae/jar and above, equivalent to survival across all treatments at 25°C. An effect of species ratio was only evident at 25°C, at 50 larvae/jar, with highest survival for species ratio 50% *P. regina* to 50% *L. sericata* compared to unequal species ratio treatments (Figure 3.2).

Survival of *P. regina* in mixed species treatments was pooled across species ratios and compared to survival of *P. regina* in single species treatments to measure interaction
effects of *L. sericata* on *P. regina*. Survival of *P. regina* was affected by an interaction between density, species treatment, and temperature (ANOVA: F\(_{8,570} = 4.18, p < 0.001\)) (Table 3.2) with temperature explaining most of the variation (MS = 6.73), followed by species treatment (MS = 1.67) and density (MS = 0.055) (Table 3.2). Survival of *P. regina* was higher in mixed species treatments than in single-species treatments at most densities at 35°C. At 25°C, survival was higher at higher densities in mixed species treatments when compared to *P. regina* on its own. At 15°C survival was lower in mixed species treatments than in single-species treatments in all densities except an intermediate density of 100 larvae/50 g (Figure 3.3).

Survival of *L. sericata* in mixed species treatments was compared to survival in single species. There was an interaction between density, species treatment and temperature (ANOVA: F\(_{8,570} = 6.99, p < 0.001\)) (Table 3.4). Temperature had the most influence on the survival of *L. sericata* (MS = 17.83) compared to density (MS = 0.26) and species treatment (MS = 0.013). Survival was the same at the lowest density, changing to higher at the highest density of 400 larvae/jar for mixed species compared to *L. sericata* on its own at 35°C (Figure 3.4). Survival was highest overall at 25°C with no differences between *L. sericata* alone or mixed species treatments. At 15°C, survival was intermediate compared to 25°C and 35°C. Survival also increased with increasing larval densities in mixed species treatments and yet density had no influence on survival at 15°C for *L. serciata* alone.

**Development**

Development time as measured in accumulated degree days (ADD). ADD of *P. regina* was influenced by an interaction between species ratio, density, and temperature
Temperatures explained most of the variation in development time (MS effect = 323377) followed by species ratio (MS effects = 1748), and then density (MS = 248) (Table 3.3). Overall, mean ADD of *P. regina* decreased with increasing developmental temperatures at all temperatures tested, and at all densities with the exception of 400 larvae/jar at 25°C, which was not different from ADD at 35°C. (Fig 3.5). There was no effect of density within a species ratio at 15°C or 35°C. At 25°C, mean ADD was highest at the lowest larval density, decreasing at 50 or 100 larvae/jar depending on the species ratio, to exhibit lower mean ADD at the higher larval densities. At 35°C for species ratios of 25% *P. regina* to 75% *L. sericata* at the highest two larval densities, mean ADD was greater than that for equal or 75% *P. regina* to 25% *L. sericata* treatments.

The development time measured in ADD of *L. sericata* was affected by a three-way interaction between species ratio, density, and temperature (GLZ, *p* <0.001) (Table 3.3). Most of the variation in ADD can be attributed to developmental temperatures (MS =146571), followed by species ratio (MS = 93315) and density (MS = 18873) (Table 3.3). *Lucilia sericata* took longer to develop (more ADD) at decreasing developmental temperatures for all temperatures tested (Fig 3.6). The longest development was at 15°C with no effect of species ratios or density. At 25°C, there was no effect of density on development, and species ratio only impacted development at 50 larvae/jar. In contrast, at 35°C, both species ratio and density affected development, yet no one species ratio or density affected development the same way.

We examined the effects of heterospecifics on the development of *L. sericata* and *P. regina* by comparing the ADD of single species to mixed species treatments of both...
species. For *P. regina*, species ratios were pooled and compared to single species
development time, and there was a three-way interaction between density, species
treatment and temperature (GLZ, *p* <0.001) (Table 3.4). Temperature was responsible for
most of the variation observed in ADD (MS = 21956982) followed by species treatment
(MS = 37816) and density (MS = 1141)(Table 3.4). Accumulated degree days decreased
as developmental temperature increased. However, *P. regina* accumulated more degree
days when developing in the presence of *L. sericata* than on its own, and this was true for
all densities tested at 15°C, lower densities at 25°C and highest density tested at 35°C.
(Figure 3.7).

For *L. sericata*, species were pooled and compared to single species treatments,
the interaction between species treatments and temperature (GLZ, *p* < 0.001) (Table 3.4)
(Figure 3.8) influenced development as measured in ADD by *L. sericata*. Temperatures
explains most of the variation in ADD (MS = 1.24x10^8), followed by species treatment
(MS = 27246) (Table 3.4). Density did not affect development (*p* =0.098), ADD was
pooled within densities. ADD decreased with developmental temperature, and *L. sericata*
had higher ADD when developing in the presence of *P. regina* at all densities at 15°C but
no significant changes in ADD at 25°C and 35°C based on species treatment.

**Adult body size**

The female adult body size of *P. regina* was influenced by an interaction between
species ratio and temperature (GLM, *p* = 0.006) (Table 3.5). Variation in body size was
mainly due to species ratio (MS = 0.065). Density did not affect female adult body size (*p*
= 0.97), and hence body size was pooled withing densities for all species ratios. The
largest adults were observed in species ratio 50% *P. regina* to 50% *L. sericata* across all
temperatures tested and 25°C appeared to be the optimum temperature for largest female body size (Figure 3.9).

The female adult body size of *L. sericata* was influenced by an interaction between temperature, density and species ratio (GLM, \( p < 0.001 \)) (Table 3.5). Significant variation in body size was explained predominately by temperature (MS = 0.76), followed by density (MS = 0.172). Overall, the female adult body size decreased at the higher densities at 35°C for all species ratios analyzed. Density had no effect on body size of unequal species ratio treatments at 25°C, however, body size increased with increasing larval density at the equal species ratio treatment. At 15°C, there was no effects of density or species ratio on body size (Figure 3.10).

Female adult body size of *P. regina* in mixed species treatments was compared to adults in single species treatments. For females, a three-way interaction between temperature, species treatment, and density influenced the adult body size of *P. regina* for all species ratio at all temperatures tested \((p < 0.001)\) (Table 3.7). At all species ratios, variation in body size was largely due to species treatments, followed by temperature and finally, density (Table 3.7). Overall, we observed that adult body size changed between mixed species and single species treatments regardless of developmental temperature (Figure 3.11-3.13). Adult body size was larger in mixed species treatments compared to single species treatments at all temperatures tested for all species ratios but differed in the effect of density. At 35°C, species ratio 75% *P. regina* to 25% *L. sericata* had a decreased body size at 200 larvae/jar but no effect of density in equal species ratio and at 25% *P. regina* to 75% *L. sericata* (Figure 3.11). Mixed species body size was larger at higher densities for all species ratio at 35°C. At 25°C, adult body size decreased for
species ratio 25% *P. regina* to 75% *L. sericata* at 400 larvae/jar and no trends at equal 50:50 ratios and *P. regina* biased ratio (Figure 3.12). Mixed body size was larger at higher densities at 25°C. Finally, at 15°C, adult body size decreases for species ratio 75% *P. regina* to 25% *L. sericata* and equal species ratio at 200 larvae/jar, but at *L. sericata* biased ratio there was no effect of density (Figure 3.13).

The effect of hetero-specifics on the female adult body size of *L. sericata* was analyzed by comparing females in single species to females in mixed species treatment. Temperature, density, and species treatment interacted to influence the adult body size of females for all species ratio of combination (*p* < 0.001) (Table 3.6). At all species ratios, variations in body size was largely due to temperature followed by species treatments and finally, density (Table 3.6). When *L. sericata* developed with hetero-specifics, adult body size was larger at 15°C and 25°C, adult body size decreased with increasing larval density at 35°C for species ratio 75% *P. regina* to 25% *L. sericata* and 50% *P. regina* to 50% *L. sericata* (Figure 3.14) but increased at 25°C for all species ratio in mixed species treatments (Figure 3.15). At 15°C, density had no effect on adult body size for all species (Figure 3.16) Smaller adults were observed in single species treatments at higher densities at 25°C and 35°C (Figure 3.14-3.16).

**Discussion**

Carrion can be described as an interactive community, as it is often saturated with different carrion insects that exhibit strong interactions between individuals within or between trophic levels (Cornell and Lawton 1992). These strong species interactions can be influenced by factors such as temperature and density (Smith and Wall 1997; Gilman
et al., 2010). However, few studies have extensively investigated the influence of temperature and density on species interactions within blow fly communities and the effects on the development and fitness of blow flies.

Previous studies have suggested that *P. regina* benefits from the presence of *L. sericata* depending on the developmental temperature (Hans 2016). The results of this study agree with that observation, demonstrating increased survival of *P. regina* in the presence of *L. sericata* at higher temperatures. Yet, below 25℃ survival of *P. regina* was negatively affected when developing with *L. sericata*. These results could mean that *P. regina* not only has decreased competitive ability at lower temperatures, perhaps there are decreased enzymatic secretions by *L. sericata* at these temperatures as well due to decreased rate of development.

The positive effects of density and *L. sericata* on the survival of *P. regina* at the highest temperature tested was unexpected as we observed decreased survival of *P. regina* in chapter 2 with increasing larval density due to increased levels of competition on a limited resource (Wrensch 1978; Credland *et al.*, 1986; Goodbrod and Goff, 1990; Reeve *et al.*, 1998; Agnew *et al.*, 2002). These positive effects could be as a result of decreased interspecific interactions at this temperature, as 35℃ exceeds the temperature threshold for *L. sericata*. At this high temperature, there is decreased survival of *L. sericata* which decreases the overall density within the jar, decreasing the levels of inter-specific interactions and decreased intra-specific interactions experienced by *P. regina* as well as there are lower densities of *P. regina* left after the exclusion of *L. sericata* when compared to the single species cultures. Improved survival of *P. regina* at this temperature could be a combination of benefits from the enzymatic secretions of *L.
sericata and also increased resource availability due to exclusion of L. sericata. Overall, the development of P. regina in the presence of L. sericata resulted in a shift in the optimal developmental temperature range of P. regina from 25°C in single species culture to 35°C in mixed species culture.

Greenberg and Kunich (2002) suggested a possible mechanism to explain the coexistence of different species on a limited resource; larvae benefit from larval aggregations by multiple individuals penetrating tissues and releasing enzymatic secretions that aid digestions. However, these might not be the only positive effects experienced by larvae within multi-species larval aggregates. We suspect that antimicrobial properties of the larval sections of L. sericata might contribute to the increased survival of P. regina by acting against competing microbes and bacteria such as Staphylococcus aureus Rosenbach and Staphylococcus epidermis Evans (Bacillales: Staphylococcaceae). This reduces overall competition that might affect the development and survival of P. regina (Kerridge et al., 2005). Both bacteria species are part of the normal flora, commonly found on skin, hair, nose, and respiratory tract of organisms such as humans and animals and are likely present at even higher concentrations after death (Kerridge et al., 2005).

Previous studies have suggested that L. sericata is a poor interspecific competitor, displaying low survival during development with other blow fly species (Hutton and Wasti 1980; Smith and Wall 1997; Kheirallah et al., 2007). However, our analysis suggests that survival of L. sericata is unchanged when developing with P. regina but increases with larval density. We suspect that while there are no positive influences on the survival of L. sericata by P. regina, perhaps L. sericata is a better interspecific
competitor than previously assumed and *L. sericata* benefits from the decreased intra-specific interactions as species densities in mixed cultures are less than single species cultures.

Atkinson (1994) proposed the temperature-size rule, which states that the adult body size of an individual is a product of their environmental conditions such as temperature. Thereby, individuals developing at low temperatures exhibit larger adult body size (Angilletta *et al.*, 2003). The influence of temperature on the adult body size of *L. sericata* was evident within all ratios of combinations, and largest females were observed at 25°C when compared to other temperatures. This discrepancy with the temperature-size rule can be explained limited resource availability and energy allocation, that is, prioritizing faster development time over larger adult body size. At lower temperatures, individuals have longer development time, the rate of decomposition is lower, and the resource is not utilized as rapidly. For individuals to achieve a significant size, they must postpone development. Longer development increases their risk of predation and potentially increased competition with other carrion insects as they continue to colonize the resource.

Overall female body size of *P. regina* and *L. sericata* increased in the presence of hetero-specifics than conspecifics at all temperatures tested. This could mean that the enzymatic and antibiotic secretions of *L. sericata* might not only prevent bacterial growth but also aid in the breakdown of food resources, making it more readily available and easy to utilize. Females can use this opportunity to allocate more resource to their body size thereby increasing their fecundity. Given that survival of *L. sericata* was not positively or negatively affected by the presence of *P. regina*, it was interesting to detect
larger adult body size of *L. sericata* during development with *P. regina*. This could suggest that *L. sericata* benefits from decreased levels of intra-specific interactions while successfully utilizing the resource through the breakdown by enzymatic secretions. Interestingly, there was no effect of temperature on the female adult body size of *P. regina*; this could suggest that the positive effects of one species on the other were enough to increase adult body size such that the effects of temperature were minimized. The enzymatic and antibiotic secretions of *L. sericata* might facilitate the breakdown of food resources, making it more readily available and easy to utilize by *P. regina*.

Both blow fly species took longer to develop (more ADD) in mixed species treatments than in single species treatments, and this could be a trade-off between development rate and body size as individuals in mixed species treatments had larger adult body size than individuals in single treatments at the same temperature. Perhaps this increase in development time is not a negative effect but a cost to development to allow individuals prolong development while allocating more resources to body size. Females can use this opportunity to allocate more resource to their body size thereby increasing their fecundity.

As observed in chapter 2, temperature was responsible for the large variations in the life history traits measured. This is easily explained by the poikilothermic nature of insects and their dependence on surrounding temperatures to inform their developmental decisions. Developmental cues, enzymes, and proteins might only be activated at the lower threshold temperature of each insect and rate of metabolism and development increases as developmental temperature increases and halts when developmental temperatures exceed the upper thermal threshold as a result of enzyme degradation.
protein denaturing and membrane disruption (Chown and Nicholson 2004; Hochachka and Somero 2002). Temperature also accounted for much of the variation when single species cultures were compared to mixed species; this was true for all life history traits measured except adult body size of L. sericata and P. regina. Most of the variation observed for this life history trait was as a result of different species treatments, single species versus mixed species.

*Phormia regina* and L. sericata have been reported to inhabit and feed on the same carrion (Anderson and VanLaerhoven 1996; VanLaerhoven and Anderson 1999). This means that they often interact with one another and the outcomes of these interactions can influence their development and fitness negatively or positively. We incorporated different species ratios to test the ‘founder control’ phenomenon which assumes that the first arriving species, usually the more abundant species will have the greatest opportunity to dominate a resource, thereby giving it a competitive advantage over other species present (Mittelbach 2012). From our study, we can determine that the possible effects of ‘founder control’ will be dependent on a species thermal range, that is, within a favorable range, they can exert competitive advantage when present in higher proportions than other species. However, beyond these favourable conditions, individuals have no competitive advantage regardless of the proportions of each species is available. While the present study does not incorporate temporal priority effects, it provides useful information on the possible mechanisms that support species coexistence on a limited resource.

The results of this study are particularly important as *P. regina* and *L. sericata* are carrion flies that arrival within minutes after death and are reliable forms of calculating
post-mortem interval (PMI) for homicide and suicide victims (Catts and Goff 1992). It is important to investigate biotic and abiotic factors that can influence their colonization and development as forensic entomologists rely on their predictable patterns and behaviours during forensic investigations (Catts and Goff 1992). In addition, both flies, especially *L. sericata* have been implicated in myiasis and costs the agricultural industry thousands in profits every year (Marshall *et al.*, 2011). Investigating factors that affect their life history traits is important in understanding and proposing solutions to decrease the incidence of myiasis for sheep farmers.

Future directions for this research would be to study the outcome of species interactions on these blow fly species at fluctuating temperatures because development under fluctuating temperatures differs from constant temperatures in single species treatments (Greenberg 1991; Byrd and Allen 2001). It might also be beneficial to examine how fluctuating temperatures affect the fecundity of these blow fly species at both single and mixed species treatments.
REFERENCES


Hans, K.R., 2016. Using an ecological framework to resolve issues in forensic entomology: Exploring temperature mediation of species interactions within blow fly (Diptera: Calliphoridae) communities (Doctoral dissertation, University of Windsor (Canada)).


Jaeger, T.F., 2008. Categorical data analysis: Away from ANOVAs (transformation or not) and towards logit mixed models. *Journal of memory and language* 59: 434-446.


Table 3.1. Analysis of Variance (ANOVA) results to determine the effects of temperature, density and species ratio, and the interaction of these effects on the survival of *P. regina* and *L. sericata*. Significant effects are indicated in bold font; $\alpha = 0.05$ for all effects.

<table>
<thead>
<tr>
<th>Effects</th>
<th>D.F</th>
<th>MS effect</th>
<th>F ratio</th>
<th>p – value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phormia regina</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
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<tr>
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<td>4.27</td>
<td>0.02</td>
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<tr>
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<tr>
<td>Density * Species ratio</td>
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<td>0.159</td>
</tr>
<tr>
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<td>&lt; 0.001</td>
</tr>
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<td>4.28</td>
<td>0.002</td>
</tr>
<tr>
<td>Density* Temperature* Species ratio</td>
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<td>2.025</td>
<td>0.011</td>
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<td><strong>Lucilia sericata</strong></td>
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</tr>
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<tr>
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<td>Density* Temperature* Species ratio</td>
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<td>0.062</td>
<td>2.58</td>
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Table 3.2. Analysis of Variance (ANOVA) results to determine the effects of temperature, density and species treatment, and the interaction of these effects on the survival of *P. regina* and *L. sericata*. Survival was pooled across species ratios and compared to single species treatments. Significant effects are indicated in bold font; α = 0.05 for all effects.

<table>
<thead>
<tr>
<th>Effects</th>
<th>D.F</th>
<th>MS effect</th>
<th>F ratio</th>
<th>p – value</th>
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<td>Density* Temperature* Species Treatment (single/mixed)</td>
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<tr>
<td><strong>Lucilia sericata</strong></td>
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Table 3.3. Generalized linear model (GLZ, distribution = poisson, link function = log) results to determine the relationship between density, species ratio and temperature on accumulated degree days (ADD) of *L. sericata* and *P. regina* at all temperatures.

Significant effects are indicated in bold font; α=0.05 for all effects.

<table>
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<th>Wald Stat.</th>
<th>p-value</th>
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<td><em><em>Density</em> Species ratio * Temperature</em>*</td>
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<td>6135</td>
<td>23.07</td>
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Table 3.4. Generalized linear model (GLZ, distribution = poisson, link function = log) results to determine the relationship between density, species treatments (mixed/single) and temperature on accumulated degree days (ADD) of *P. regina* and *L. sericata* at all temperatures. Significant effects are indicated in bold font; $\alpha=0.05$ for all effects.

<table>
<thead>
<tr>
<th>Effects</th>
<th>Wald Stat.</th>
<th>MS effect</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
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<tr>
<td>Species treatment (single/mixed)</td>
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<tr>
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</tr>
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<td>Species treatment (single/mixed)</td>
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<td>Density</td>
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<td>372885</td>
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<td><strong>124375446</strong></td>
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<td>Density * Temperature</td>
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<tr>
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<td>18735</td>
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Table 3.5. General linear model (GLM) results to test the relationship between density, species ratio and temperature on female body size of *P. regina* and *L. sericata* at all temperatures. Significant effects are indicated in bold font; $\alpha=0.05$ for all effects.

<table>
<thead>
<tr>
<th>Effects</th>
<th>D.F.</th>
<th>MS effect</th>
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<th>p-value</th>
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<tbody>
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<td><strong>Phormia regina</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species ratio</td>
<td>2, 701</td>
<td>0.065</td>
<td>5.48</td>
<td>0.004</td>
</tr>
<tr>
<td>Density</td>
<td>1, 701</td>
<td>0.0002</td>
<td>0.001</td>
<td>0.97</td>
</tr>
<tr>
<td>Temperature</td>
<td>1, 701</td>
<td>0.004</td>
<td>0.338</td>
<td>0.56</td>
</tr>
<tr>
<td>Species ratio * Density</td>
<td>2, 701</td>
<td>0.029</td>
<td>2.46</td>
<td>0.08</td>
</tr>
<tr>
<td><em><em>Species ratio</em> Temperature</em>*</td>
<td>2, 701</td>
<td>0.0616</td>
<td>5.19</td>
<td>0.006</td>
</tr>
<tr>
<td>Density * Temperature</td>
<td>1, 701</td>
<td>0.0113</td>
<td>0.95</td>
<td>0.33</td>
</tr>
<tr>
<td>Density* Species ratio * Temperature</td>
<td>2, 701</td>
<td>0.0413</td>
<td>3.48</td>
<td>0.055</td>
</tr>
<tr>
<td><strong>Lucilia sericata</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species ratio</td>
<td>2, 642</td>
<td>0.001</td>
<td>0.09</td>
<td>0.915</td>
</tr>
<tr>
<td>Density</td>
<td>1, 642</td>
<td>0.172</td>
<td>19.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>1, 642</td>
<td>0.760</td>
<td>85.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species ratio * Density</td>
<td>2, 642</td>
<td>0.048</td>
<td>5.40</td>
<td>0.005</td>
</tr>
<tr>
<td><em><em>Species ratio</em> Temperature</em>*</td>
<td>2, 642</td>
<td>0.034</td>
<td>3.84</td>
<td>0.009</td>
</tr>
<tr>
<td>Density * Temperature</td>
<td>1, 642</td>
<td>0.161</td>
<td>18.13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density* Species ratio * Temperature</td>
<td>2, 642</td>
<td>0.073</td>
<td>8.22</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3.6. Analysis of Variance (ANOVA) results to determine the effects of density, species treatment (single/mixed) and temperature on adult body size of female *P. regina* at all temperatures. Significant effects are indicated in bold font; $\alpha=0.05$ for all effects.

<table>
<thead>
<tr>
<th>Effects</th>
<th>D.F.</th>
<th>MS effect</th>
<th>F ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>4, 511</td>
<td>0.24</td>
<td>32.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed)</td>
<td>1, 511</td>
<td>1.93</td>
<td>264.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>2, 511</td>
<td>0.35</td>
<td>47.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Density</td>
<td>4, 511</td>
<td>0.12</td>
<td>16.94</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density* Temperature</td>
<td>8, 511</td>
<td>0.10</td>
<td>14.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Temperature</td>
<td>2, 511</td>
<td>0.24</td>
<td>32.71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density* Species treatment (single/mixed) * Temperature</td>
<td>8, 511</td>
<td>0.03</td>
<td>4.57</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effects</th>
<th>D.F.</th>
<th>MS effect</th>
<th>F ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>4, 519</td>
<td>0.20</td>
<td>25.81</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed)</td>
<td>1, 519</td>
<td>2.52</td>
<td>324.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>2, 519</td>
<td>0.43</td>
<td>56.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Density</td>
<td>4, 519</td>
<td>0.13</td>
<td>16.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density* Temperature</td>
<td>8, 519</td>
<td>0.03</td>
<td>3.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Temperature</td>
<td>2, 519</td>
<td>0.16</td>
<td>21.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density* Species treatment (single/mixed) * Temperature</td>
<td>8, 519</td>
<td>0.07</td>
<td>9.57</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Effects</th>
<th>D.F.</th>
<th>MS effect</th>
<th>F ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>4, 493</td>
<td>0.186</td>
<td>17.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed)</td>
<td>1, 493</td>
<td>2.53</td>
<td>233.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>2, 493</td>
<td>0.61</td>
<td>71.85</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Density</td>
<td>4, 493</td>
<td>0.19</td>
<td>17.84</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density* Temperature</td>
<td>8, 493</td>
<td>0.16</td>
<td>15.28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Temperature</td>
<td>2, 493</td>
<td>0.07</td>
<td>8.36</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density* Species treatment (single/mixed) * Temperature</td>
<td>8, 493</td>
<td>0.11</td>
<td>11.52</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3.7. Analysis of Variance (ANOVA) results to determine the effects of density, species treatment and temperature on adult body size of female *L. sericata* at all temperatures. Significant effects are indicated in bold font; α=0.05 for all effects.

<table>
<thead>
<tr>
<th>Effects</th>
<th>D.F.</th>
<th>MS effect</th>
<th>F ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>75% <em>P. regina</em> to 25% <em>L. sericata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>4, 485</td>
<td>0.014</td>
<td>2.9</td>
<td>&lt;0.022</td>
</tr>
<tr>
<td>Species treatment (single/mixed)</td>
<td>1, 485</td>
<td>0.71</td>
<td>142</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>2, 485</td>
<td>0.69</td>
<td>138.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Density</td>
<td>4, 485</td>
<td>0.008</td>
<td>1.6</td>
<td>0.178</td>
</tr>
<tr>
<td>Density * Temperature</td>
<td>8, 485</td>
<td>0.004</td>
<td>0.8</td>
<td>0.587</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Temperature</td>
<td>2, 485</td>
<td>0.054</td>
<td>10.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density * Species treatment (single/mixed) * Temperature</td>
<td>8, 485</td>
<td>0.033</td>
<td>6.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>50% <em>P. regina</em> to 50% <em>L. sericata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>4, 515</td>
<td>0.028</td>
<td>4.5</td>
<td>0.0013</td>
</tr>
<tr>
<td>Species treatment (single/mixed)</td>
<td>1, 515</td>
<td>1.804</td>
<td>293</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>2, 515</td>
<td>1.54</td>
<td>250.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Density</td>
<td>4, 515</td>
<td>0.03</td>
<td>5.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density * Temperature</td>
<td>8, 515</td>
<td>0.014</td>
<td>2.2</td>
<td>0.025</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Temperature</td>
<td>2, 515</td>
<td>0.06</td>
<td>9.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density * Species treatment (single/mixed) * Temperature</td>
<td>8, 515</td>
<td>0.08</td>
<td>13.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25% <em>P. regina</em> to 75% <em>L. sericata</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>4, 486</td>
<td>0.01</td>
<td>1.82</td>
<td>0.123</td>
</tr>
<tr>
<td>Species treatment (single/mixed)</td>
<td>1, 486</td>
<td>0.80</td>
<td>30.47</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temperature</td>
<td>2, 486</td>
<td>1.03</td>
<td>130.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Density</td>
<td>4, 486</td>
<td>0.05</td>
<td>7.42</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density * Temperature</td>
<td>8, 486</td>
<td>0.014</td>
<td>2.61</td>
<td>0.008</td>
</tr>
<tr>
<td>Species treatment (single/mixed) * Temperature</td>
<td>2, 486</td>
<td>0.06</td>
<td>28.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Density * Species treatment (single/mixed) * Temperature</td>
<td>8, 486</td>
<td>0.025</td>
<td>4.14</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3.8. Polynomial equations and $R^2$ values for the effects of density female body size of *P. regina* and *L. sericata*. $\alpha = 0.05$, Significant effects in bold.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equation of Line</th>
<th>$R^2$</th>
<th>$P$ - value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phormia regina</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR; 75% PR/25%LS</td>
<td>$y = -0.0401x^2 + 0.17x + 1.0801$</td>
<td>0.005</td>
<td>0.20</td>
</tr>
<tr>
<td>PR; 50% PR to 50% LS</td>
<td>$y = -0.0504x^2 + 0.1861x + 1.1039$</td>
<td>0.009</td>
<td>0.209</td>
</tr>
<tr>
<td><strong>PR; 25% PR to 75% LS</strong></td>
<td>$y = -0.0353x^2 + 0.136x + 1.1551$</td>
<td><strong>0.072</strong></td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td><strong>Lucilia sericata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>35°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS; 75% PR to 25% LS</td>
<td>$y = 0.001x^3 - 0.0131x^2 + 0.0281x + 1.1751$</td>
<td>0.147</td>
<td>0.002</td>
</tr>
<tr>
<td>LS; 50% PR to 50% LS</td>
<td>$y = 0.0028x^3 - 0.0484x^2 + 0.2219x + 0.9269$</td>
<td>0.044</td>
<td>0.015</td>
</tr>
<tr>
<td>LS; 25% PR to 75% LS</td>
<td>$y = 0.0017x^3 - 0.0263x^2 + 0.1136x + 0.9771$</td>
<td>0.152</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td><strong>25°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS; 75% PR to 25% LS</td>
<td>$y = -0.0005x^3 + 0.0059x^2 - 0.0041x + 1.2079$</td>
<td>0.127</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>LS; 50% PR to 50% LS</td>
<td>$y = -0.0005x^3 + 0.0037x^2 + 0.0159x + 1.2639$</td>
<td>0.189</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>LS; 25% PR to 75% LS</td>
<td>$y = -0.0027x^3 + 0.041x^2 - 0.1672x + 1.3726$</td>
<td>0.230</td>
<td>0.002</td>
</tr>
<tr>
<td><strong>15°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS; 75% PR to 25% LS</td>
<td>$y = -0.0009x^3 + 0.0152x^2 - 0.0744x + 1.3778$</td>
<td>0.021</td>
<td>0.63</td>
</tr>
<tr>
<td>LS; 50% PR to 50% LS</td>
<td>$y = -0.0001x^3 + 0.0017x^2 - 0.012x + 1.3245$</td>
<td>0.018</td>
<td>0.22</td>
</tr>
<tr>
<td>LS; 25% PR to 75% LS</td>
<td>$y = -0.0026x^3 + 0.0409x^2 - 0.1735x + 1.4061$</td>
<td>0.014</td>
<td>0.48</td>
</tr>
</tbody>
</table>
Table 3.9. Polynomial equations and $R^2$ values for the effects of density on female body size of *P. regina* when developing on their own and in the presence of heterospecifics. $\alpha = 0.05$, Significant effects in bold

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equation of Line</th>
<th>$R^2$</th>
<th>$P$ - value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phormia regina</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>35°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR; 75% PR/25% LS</td>
<td>$y = 0.0391x^3 - 0.3477x^2 + 0.8624x + 0.7085$</td>
<td>0.371</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>PR; 50% PR/50% LS</td>
<td>$y = 0.0104x^3 - 0.0884x^2 + 0.2046x + 1.136$</td>
<td>0.008</td>
<td>0.246</td>
</tr>
<tr>
<td>PR; 25% PR/75% LS</td>
<td>$y = -0.0018x^3 + 0.0322x^2 - 0.1574x + 1.4077$</td>
<td>0.145</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>25°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR; 75% PR/25% LS</td>
<td>$y = 0.0034x^3 - 0.0417x^2 + 0.1567x + 1.0965$</td>
<td>0.015</td>
<td>0.774</td>
</tr>
<tr>
<td>PR; 50% PR/50% LS</td>
<td>$y = -0.0069x^3 + 0.0536x^2 - 0.1122x + 1.3437$</td>
<td>0.013</td>
<td>0.196</td>
</tr>
<tr>
<td>PR; 25% PR/75% LS</td>
<td>$y = -0.0472x^3 + 0.3942x^2 - 0.9571x + 1.9359$</td>
<td>0.402</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td><strong>Phormia regina</strong></td>
<td></td>
<td>0.146</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>15°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR; 75% PR/25% LS</td>
<td>$y = -0.0031x^3 + 0.0035x^2 + 0.0618x + 1.1252$</td>
<td>0.041</td>
<td>0.160</td>
</tr>
<tr>
<td>PR; 50% PR/50% LS</td>
<td>$y = 0.0107x^3 - 0.113x^2 + 0.3348x + 1.0115$</td>
<td>0.025</td>
<td>0.206</td>
</tr>
<tr>
<td>PR; 25% PR/75% LS</td>
<td>$y = 0.0089x^3 - 0.0951x^2 + 0.3416x + 0.8604$</td>
<td>0.1677</td>
<td>0.027</td>
</tr>
<tr>
<td><strong>Phormia regina</strong></td>
<td></td>
<td>0.0008</td>
<td>0.356</td>
</tr>
</tbody>
</table>

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Table 3.10. Polynomial equations and $R^2$ values for female body size of *L. sericata* when developing on their own and in the presence of heterospecifics.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Equation of Line</th>
<th>$R^2$</th>
<th>$P$ - effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>35°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lucilia sericata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS; 75% PR/25% LS</td>
<td>$y = 0.0081x^3 - 0.0645x^2 + 0.1146x + 1.133$</td>
<td>0.147</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LS; 50% PR/50% LS</td>
<td>$y = 0.0226x^3 - 0.2275x^2 + 0.6544x + 0.6538$</td>
<td>0.044</td>
<td>0.045</td>
</tr>
<tr>
<td>LS; 25% PR/75% LS</td>
<td>$y = 0.0137x^3 - 0.1258x^2 + 0.3427x + 0.8355$</td>
<td>0.312</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>25°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lucilia sericata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS; 75% PR/25% LS</td>
<td>$y = -0.0041x^3 + 0.0298x^2 - 0.0349x + 1.2184$</td>
<td>0.127</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LS; 50% PR/50% LS</td>
<td>$y = -0.0037x^3 + 0.0205x^2 + 0.0141x + 1.2522$</td>
<td>0.189</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LS; 25% PR/75% LS</td>
<td>$y = -0.0214x^3 + 0.1962x^2 - 0.5145x + 1.5835$</td>
<td>0.230</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>15°C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lucilia sericata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS; 75% PR/25% LS</td>
<td>$y = -0.0073x^3 + 0.0718x^2 - 0.2151x + 1.4684$</td>
<td>0.021</td>
<td>0.63</td>
</tr>
<tr>
<td>LS; 50% PR/50% LS</td>
<td>$y = -0.0011x^3 + 0.0084x^2 - 0.0317x + 1.3384$</td>
<td>0.018</td>
<td>0.22</td>
</tr>
<tr>
<td>LS; 25% PR/75% LS</td>
<td>$y = -0.0207x^3 + 0.1948x^2 - 0.5263x + 1.6231$</td>
<td>0.014</td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Lucilia sericata</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS; 75% PR/25% LS</td>
<td>$y = -0.0023x^3 + 0.0147x^2 - 0.0199x + 1.1732$</td>
<td>0.128</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Mean (± S.E) % Survival of *P. regina*

35°C

- ▲ 75PR/25LS *P. regina*
- ● 50PR/50LS *P. regina*
- ○ 25PR/75LS *P. regina*

25°C

15°C
Figure 3.1. The mean (± S.E) % survival of *P. regina* at temperatures 35°C, 25°C and 15°C. Survival of *P. regina* was affected by an interaction between density, species ratio and temperature ($F_{16, 405} = 2.025$, $p = 0.011$) Means with an asterisk indicate a difference between species ratios at that larval density. Means with the same letters indicate no significant differences in survival between densities within a species ratio within a temperature. Means with the same numerals show no significant differences in survival between temperatures within a species ratio within a density. Bonferroni corrected $p$-value for these post hoc test was set as $\alpha = 0.0167$
Mean (± S.E.)% Survival *L. sericata*

**35°C**

- 75PR/25LS L. sericata
- 50PR/50LS L. sericata
- 25PR/75LS L. sericata

**25°C**

**15°C**
Figure 3.2. Mean (± S.E) % Survival of *L. sericata* at temperatures 35°C, 25°C and 15°C. The interaction between density, species ratio and temperature (F_{16, 405} = 2.58, p < 0.001) influenced the survival of *L. sericata*. Means with an asterisk indicate a difference between species ratios at that larval density. Means with the same letter indicate no significant differences in survival between densities within a species ratio and within a temperature. Means with the same numerals show no significant differences in survival between temperatures within species ratio at that density. Bonferroni corrected *p*-value for these post hoc tests was set as *α* = 0.0167
Figure 3.3. Mean (± S.E) % Survival across all temperatures of *P. regina* when developing alone and mixed with *L. sericata*. Species ratios were pooled within each larval density and compared to *P. regina* on its own. The interaction between temperature, species treatment and density (*F*<sub>8,570</sub> = 4.18, *p* < 0.001) influenced the survival of *P. regina*. 
Figure 3.4. Mean (± S.E) % Survival across all temperatures of *L. sericata* when developing alone and mixed with *P. regina*. Species ratios were pooled within each larval density and compared to *L. sericata* on its own. The interaction between temperature, species treatment and density (F$_{8,570} = 6.99$, $p < 0.001$) influenced the survival of *L. sericata*. 
Figure 3.5. The mean (± S.E) accumulated degree days of *P. regina* at three temperatures and all species ratio. Accumulated degree days was affected by an interaction between species ratio, temperature and density (GLZ, Wald stat. = 21.90, *p* < 0.001). Means with asterisks indicate difference in species ratio at that temperature, at the same density. Means with the same letter indicate no significant differences in ADD between densities within a species ratio and within a temperature. Means with the same numerals show no significant differences in ADD between temperatures within species ratio at that density. Bonferroni corrected *p*-value for these post hoc test was set as α = 0.0167.
Figure 3.6. The mean (± S.E) accumulated degree days of *L. sericata* at three temperatures and all species ratio. Accumulated degree days was affected by an interaction between species ratio, temperature and density (GLZ, Wald stat. = 23.07, *p* < 0.001). Means with asterisks indicate differences in ADD within species ratios at that temperature, within a density. Means with the same letter indicate no significant differences in ADD between densities within a species ratio and within a temperature. Means with the same numerals show no significant differences in ADD between temperatures within species ratio at that density. Bonferroni corrected *p*-value for these post hoc test was set as *α* = 0.0167
Figure. 3.7. Mean (± S.E) Accumulated degree days (ADD) at all temperatures for *P. regina* when developing alone and mixed with *L. sericata*. ADD was pooled within the species ratios at each density. There was an interaction between species treatments (single/mixed), density and temperature (GLZ, Wald stat. = 21.49, \( p = 0.001 \)). Means with asterisks indicate differences in ADD within species treatments at that temperature, within a density. Means with the same letter indicate no significant differences in ADD between densities within a species ratio and within a temperature. Means with the same numerals show no significant differences in ADD between temperatures within species ratio at that density. Bonferroni corrected \( p \)-value for these post hoc test was set as \( \alpha = 0.0167 \).
Figure 3.8. Mean (± S.E) Accumulated degree days (ADD) at all temperatures for *L. sericata* when developing alone and mixed with *P. regina*. ADD was pooled within the species ratios at each density. There was an interaction between species treatments (single/mixed) and temperature (GLZ, Wald stat. = 19.16, \( p = 0.001 \)). Means with asterisks indicate differences in ADD between species treatments at that temperature. Means with the same letter indicate no significant differences in ADD between temperatures within a species treatment. Bonferroni corrected \( p \)-value for these post hoc test was set as \( \alpha = 0.05 \).
Figure 3.9. Mean (± S.E) female adult body size across all ratios of species combination for *P. regina* when developing at all temperatures. Adult body size was pooled within densities at each species ratio. The interaction between species ratio and temperature affect the adult body size of *P. regina* ($F_{2,701} = 5.19, p = 0.006$).
**Figure 3.10.** Mean female adult body size (± S.E) across all ratios of species combination *L. sericata* when developing at all temperatures. Adult body size of *L. sericata* was influenced by an interaction between temperature, species ratio and density ($F_{2,642} = 9.15, p < 0.001$). Means with asterisks indicate difference within species ratio at that temperature and density. Means with the same letter indicate no significant differences in body size between densities within a species ratio and within a temperature. Means with the same numerals show no significant differences in body size between temperatures within species ratio at that density. Bonferroni corrected $p$-value for this post hoc test was set as $\alpha = 0.0125$.
Figure 3.11. Mean female adult body size (± S.E) across all species ratio at 35°C for *P. regina* when developing alone and in the presence of *L. sericata*. There is an interaction between density, species treatment and temperature on the adult body size of *P. regina* (*p* < 0.001). Means with asterisks indicate difference between species treatment within densities at 35°C. Means with the same letter indicate no significant differences in body size between densities within a species ratio and within a treatment. Means with the same numerals show no significant differences in body size between species ratio within species treatment at that density. Bonferroni corrected *p*-value for this post hoc test was set as *α* = 0.0125
Figure 3.12. Mean female adult body size (± S.E) across all species ratio at 25°C for *P. regina* when developing alone and in the presence of *L. sericata*. There is an interaction between density, species treatment and temperature on the adult body size of *P. regina* (*p* < 0.001). Means with asterisks indicate difference between species treatment within densities at 25°C. Means with the same letter indicate no significant differences in body size between densities within a species ratio and within a treatment. Means with the same numerals show no significant differences in body size between species ratio within species treatment at that density. Bonferroni corrected *p*-value for this post hoc test was set as $\alpha = 0.0125$. 

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Mean (± S.E) Adult Body Size of *P. regina* Density

75%PR/25%LS

Mixed

Poly. (*P. regina*)

Poly. (Mixed)

50%PR/50%LS

25%PR/75%LS
Figure 3.13. Mean female adult body size (± S.E) across all species ratios at 15°C for *P. regina* when developing alone and in the presence of *L. sericata*. There is an interaction between density, species treatment and temperature on the adult body size of *P. regina* (*p* < 0.001). Means with asterisks indicate difference between species treatment within densities at 15°C. Means with the same letter indicate no significant differences in body size between densities within a species ratio and within a treatment. Means with the same numerals show no significant differences in body size between species ratio within species treatment at that density. Bonferroni corrected *p*-value for this post hoc test was set as \( \alpha = 0.0125 \)
Figure 3.14. Mean (± S.E) female adult body size across all species ratios for *L. sericata* at 35°C when developing alone and in the presence of *P. regina*. Body size of *L. sericata* females are affected by three-way interactions between, temperature, density and species treatments (*p* <0.001). Means with asterisks indicate difference between species treatment within densities at 35°C. Means with the same letter indicate no significant differences in body size between densities within a species ratio and within a treatment. Means with the same numerals show no significant differences in body size between species ratio within species treatment at that density. Bonferroni corrected *p*-value for this post hoc test was set as *α* = 0.0125.
Figure 3.15. Mean (± S.E) female adult body size across all species ratios for *L. sericata* at 25°C when developing alone and in the presence of *P. regina*. Body size of *L. sericata* females are affected by three-way interactions between, temperature, density and species treatments (*p* < 0.001). Means with asterisks indicate difference between species treatment within densities at 25°C. Means with the same letter indicate no significant differences in body size between densities within a species ratio and within a treatment. Means with the same numerals show no significant differences in body size between species ratio within species treatment at that density. Bonferroni corrected *p*-value for this post hoc test was set as *α* = 0.0125.
Figure 3.16. Mean (± S.E) female adult body size across all species ratios for *L. sericata* at 15℃ when developing alone and in the presence of *P. regina*. Body size of *L. sericata* females are affected by three-way interactions between, temperature, density and species treatments (*p* <0.001). Means with asterisks indicate difference between species treatment within densities at 15℃. Means with the same letter indicate no significant differences in body size between densities within a species ratio and within a treatment. Means with the same numerals show no significant differences in body size between species ratio within species treatment at that density. Bonferroni corrected *p*-value for this post hoc test was set as $\alpha = 0.0125$. 

*50%PR/50%LS*

*75%PR/25%LS*

*25%PR/75%LS*
CHAPTER 4

SUMMARY: UNDERSTANDING THE MECHANISMS OF SPECIES COEXISTENCE.

Community ecologists try to understand the mechanisms associated with species coexistence; investigate the effects of abiotic and biotic factors on species interactions and understand how these might mediate species interactions. Researchers often used different model systems to study this such as plant communities (Connell and Slayter 1977). The use of carrion as a model system for the community is beneficial because it is ephemeral and can be easily replicated and manipulated and can provide valuable insight into community assembly patterns and mechanisms of coexistence (Tomberlin et al., 2011).

Carrion communities are often composed of multiple species feeding on a single resource, contrary to Gause's law of competitive exclusion (Fuller, 1934; Gause, 1934; Hanski, 1980). From the results of this study; we suspect the coexistence is as a result of niche heterogeneity, spatial heterogeneity, temporal heterogeneity and species interactions. Carrion is a patchy resource that provides different areas with different conditions for growth. Female blow flies are known to lay their eggs in different locations on the carrion, *Lucilia sericata* in moist locations such as the eyes, nose, mouth, and *P. regina* in more dry locations such as the legs, stomach, head (Pacheo 2015). Different blow fly species have different seasonal distributions, as earlier stated in chapter 2, *P. regina* is found in the summer, spring and fall (Macleod and Donnelly 1958) whereas *L. sericata* is found in the summer and spring (Baumgartner and Greenberg 1985). In this study, we observed facilitative effects of *L. sericata* on the survival and
adult body size of *P. regina*. These characteristics suggest that carrion patchiness, female blow fly oviposition choices, seasonal distribution and positive interactive effects contribute to the coexistence of these two blow fly species and perhaps other blow fly species on a limited resource such as carrion.

Understanding the factors that contribute to the fitness of these flies involves investigating life history traits that are related to fitness such as survival and fecundity. Analysis of adult body size, ADD, and survival of both species suggests that *P. regina* might have higher fitness than *L. sericata*. This would be in line with the hotter is better rule proposed by Kingslover (2008). This rule proposes that species with higher optimum temperature thresholds will have higher fitness in comparison to other species. This idea is also supported by the oviposition behaviour of *P. regina*, which has higher fecundity than *L. sericata* at different temperatures (Hans 2016). This research also sheds light on the factors that might affect the temperature-size rule. Individuals from this study did not follow the temperature size rule because of limited resources and different rates of decomposition based on temperature. Individuals developing at low temperatures allocate more resources to development rate while limiting their adult body size due to limited resources available at these temperatures.

Overall, future directions of this research would be to incorporate fluctuating temperatures, as research has shown changes in development due to fluctuating temperatures (Greenberg 1991; Byrd and Allen 2001). It would also be beneficial to understand how fluctuating temperatures might affect fecundity during intra-specific and inter-specific conditions. Overall the results of this study have aided in describing the thermal performance range for the species tested. Paralleling research form Gosselin et
al., (2010), Hans (2016), Byrd and Allen (2001) and this present study, *L. sericata* has an optimum temperature range of about 20 to 30°C whereas *P. regina* has an optimum temperature range higher than this of about 25 to 35°C. Given that fecundity is supported by temperature, such that higher temperatures induce higher egg maturation rates, *P. regina* should overall have higher fecundity than *L. sericata*. This idea is strongly supported by Hans (2016) by measuring the mean egg load of both species over a range of developmental temperatures. *P. regina* persists at temperature that are detrimental to *L. sericata*, and had higher fecundity than *L. sericata*; all these suggest that *P. regina* has a higher fitness in comparison to *L. sericata*. It would be interesting to study the “hotter is better” rule in different blow fly species. Do species with higher optimal temperatures have higher fecundity, shorter development times and ultimately higher fitness? It is important to note that the positive effects of temperature of life history traits such as survival, development time and fecundity end when the optimum thermal temperatures are exceeded. Egg load (fecundity) and survival increased with increasing developmental temperatures until optimum temperatures were exceeded, this observation was accurate for *L. sericata* and *P. regina* (Hans 2016). The time to oviposition of *L. sericata* decreased with increasing developmental temperature until the optimum 30°C was exceeded (Hans 2016). This could mean that the relationship between temperature and life history traits is non-linear. The effects of density in this study aimed to observe the effects of intra-specific competition within small populations of *L. sericata* and *P. regina*. The effect of density(competition) is often maximized by the environmental temperatures, either by increasing the internal maggot mass temperatures experience by blow fly larvae or by increasing the rate of decomposition of an already limited resource.
It will be worthwhile to investigate the effects of density on fecundity; we would expect reduced fecundity due to adult body size decreases with density. Does the relationship between fecundity and body size hold true with changing larval densities?

In conclusion, the results of these study provide useful information for understanding the potential mechanisms involved in species coexistence in ephemeral resources. It also provides information for forensic entomologists, as many studies do not incorporate the possible effects of species interactions and density on the development of blow flies. These effects are often overlooked and with more information can improve the field of forensic entomology.
REFERENCES


Hans, K.R., 2016. Using an ecological framework to resolve issues in forensic entomology: Exploring temperature mediation of species interactions within blow...
fly (Diptera: Calliphoridae) communities (Doctoral dissertation, University of Windsor (Canada)).


Pacheco, V.A. 2015. Served medium rare: the effect of burnt remains on oviposition, survival, and fitness of the local blow fly (Diptera: Calliphoridae) community. (Master’s Thesis, University of Windsor (Canada)).


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