

2015

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Vincenzo Antonio Pacheco

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Served medium rare: the effect of burnt remains on oviposition, survival and fitness of the local blow fly (Diptera: Calliphoridae) community

By

Vincenzo A. Pacheco

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

2015

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Served medium rare: the effect of burnt remains on oviposition, survival and fitness of the local blow fly (Diptera: Calliphoridae) community

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13 February 2015

DECLARATION OF ORIGINALITY

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ABSTRACT

Two local blow fly species, *Lucilia sericata* Meigen and *Phormia regina* Meigen (Diptera: Calliphoridae) were used to investigate the effects of burnt carrion on the oviposition behaviour of females and the survival and performance of larvae feeding on these remains. Burnt carrion may be encountered after homicides and forest fires. Increased levels of flame impingement leads to the presence of cracks in the skin and these sites may be suitable for blow fly oviposition. Both species demonstrated a preference for the cracks as oviposition sites. *Phormia regina* laid more eggs at the cracks, but *L. sericata* deposited more eggs on the head. Larval survivorship increased with increased flame impingement, despite a significant loss of consumable resource. The performance of the larvae was not affected by the severity of flame impingement, however, both species responded positively to interspecific interactions on burnt remains, resulting in larger adult blow flies.

DEDICATION

I dedicate this thesis to my grandparents: Gilberto Pacheco, Maria Clotilde Pacheco, Domenico Donato and Caterina Donato. These four people have inspired me to never give up, even in the face of adversity. Thank you for putting me through school and instilling in me that education is something that should always be valued.

ACKNOWLEDGEMENTS

I would like to start by thanking my supervisor, Dr. Sherah VanLaerhoven, for providing me with the opportunity to complete my Master's Degree. Additionally, I would like to thank my committee members, Dr. Oliver Love and Dr. Christina Semeniuk, for their support, guidance and, most importantly, patience during the duration of my degree. None of my research would have been possible without the generous donations by Ken Debrouwer and the Great Lakes Pig Company. I appreciate the help from all of the laboratory volunteers and work-study students, especially Lida Ngyuen-Dang.

The following people that I want to thank all have two things in common. First, they all played a part in talking me down from the thesis ledge. There were times throughout this process that I lost sight of the finish line, but these people were always there to point me in the right direction and encourage me. Second, these people embody the word family and without them, none of this would have been possible.

To those that encouraged me to lift heavier, lift faster and be stronger, I thank you. Working out became part of my life when the stress of graduate school became too much. Meeting my gym family was something I was not expecting, but without them this journey would have been much tougher. Thank you for the laughs, good eats and most importantly the amazing memories. A special thank you is extended to Michelle Marcuz for being my sounding board and listening to me vent uncontrollably throughout my time in graduate school.

Without my lab family, I would definitely not be where I am today. As people, I have the utmost respect for Krystal Hans and Meghan Vankosky. As lab mates, I will be forever grateful for all they have done in helping me accomplish all that I have. To Krystal, thank you for sacrificing both your mornings and evenings to help me with experiments, for all of the lunch outings, pig trips and conference adventures! To Meghan, thank you for sacrificing countless hours to help me with statistics – you really are a stats wizard – and your expert editing skills! To both of you, thank you for the laughs, for being patient, for listening and, most importantly, for being my friends.

Finally, I would like to thank the most important people in my life, the Pacheco Family. Before I started this degree, I took my family for granted. This experience has

changed that. Completing this degree has been both mentally and physically taxing and my family has been there for me every step of the way. To my mother Enza and my father Antonio, thank you for listening to me, supporting me and encouraging me to keep fighting. Finally, to my sister and my rock, Rebecca, thank you for the countless hours spent on FaceTime encouraging me to keep my head up and to focus on the finish line.

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CHAPTER 1:
BRIDGING THE GAP BETWEEN FORENSIC ENTOMOLOGY, INSECT ECOLOGY
AND BURNT CARRION

FORENSIC ENTOMOLOGY AND FIRE

Forensic entomology is the study of insects utilized in legal investigations. Although there are other sub categories of this field, the focus of this thesis is medicolegal entomology, which deals with cases in which arthropod fauna are found on or near victims of violent crimes or other unattended deaths (VanLaerhoven and Anderson, 2013). Forensic entomologists are most often asked to provide an estimate of the minimum postmortem interval (PMI), or a minimum time between death and discovery of the deceased (Cragg, 1955; Smith, 1986; Thomas and Mangan, 1989; Greenberg, 1991; Hall and Doisy, 1993; Smith and Wall, 1997b; Gião and Godoy, 1997; Byrd and Allen, 2001; Bourel *et al.*, 2003; Anderson, 2004; Arnaldos *et al.*, 2005; Byrd and Castner, 2010; Higley and Haskell, 2010; Wells and Lamotte, 2010). There are two accepted methods for estimating the PMI using insect evidence. The first utilizes the successional pattern of insects arriving at a body and relies upon the predictability of arrival of specific species at specific stages of decomposition (Orfila, 1848 [cited in Greenberg 1991]; Bergeret, 1855 [cited in Greenberg, 1991]; Bornemissaza, 1957; Payne, 1965; Catts and Goff, 1990). The second and more widely used method for calculating a PMI uses the development time, in accumulated degree hours (ADH), of the first arriving carrion insects to the body, which are usually blow flies (Diptera: Calliphoridae) (Catts and Goff, 1990; Anderson, 2004).

There are over 1000 species of known blow flies; all are characterized by their metallic appearance, with common colours including green, blue and black (Smith, 1986; Smith and Wall, 1997b; Byrd and Castner, 2010). Like all other Diptera, a blow fly has a single pair of wings used for flying and modified hindwings, the halteres, used to remain balanced while in flight (Byrd and Caster, 2010). Blow flies have excellent dispersal capabilities as demonstrated by their ability to travel upwards of 20 km daily (Greenberg, 1991). Due to their dispersal capabilities and quick arrival at carrion for oviposition, this

family of flies is considered the most beneficial to forensic entomologists when estimating a PMI (Cragg, 1955; Smith, 1986; Thomas and Mangan, 1989; Greenberg, 1991; Hall and Doisy, 1993; Smith and Wall, 1997b; Gião and Godoy, 1997; Byrd and Allen, 2001; Bourel *et al.*, 2003; Anderson, 2004; Arnaldos *et al.*, 2005; Byrd and Castner, 2010). The predictable rate of immature development of blow flies is one of the more critical types of evidence used by forensic entomologists. The blow fly lifecycle has six distinct stages that have been described in depth elsewhere (McGavin, 2001; Whitfield and Purcell III, 2013), and therefore, are summarized briefly. Female blow flies lay eggs, and the larvae that hatch, called maggots in the order Diptera, develop through three feeding instars, moulting their exoskeleton between each stage. As is the case with most immature insects, the larval stages are similar in appearance. The three feeding instars can be differentiated morphologically based on the number of posterior spiracle openings. Once the maggot has finished feeding in its third instar, it will wander away from the food source in search of a dry place to pupate. Post pupation, the adult fly emerges and the lifecycle starts again.

Insects such as blow flies are poikilotherms, and therefore, they are unable to control their own body temperature. Thus, the total time required for the development of a blow fly, from the egg state to adult emergence, is dependent on ambient temperature in the blow fly's habitat (Baskerville and Emin, 1969; Adams and Hall, 2003; Bourel *et al.*, 2003; Higley and Haskell, 2010). In general, as ambient temperatures increase the rate of insect development also increases, with the opposite also being true. The relationship between temperature and insect development can be visualized using a curvilinear and linear model (Higley and Haskell, 2010). As temperatures approach the upper and lower developmental thresholds insect development follows a curvilinear model, with a linear model predicting development between the temperature thresholds (Higley and Haskell, 2010). Beyond the temperature thresholds an insect does not experience further development; it either engages in diapause or dies. At the scene of an investigation, the insect evidence collected by a forensic entomologist should include specimens of the oldest stages of the blow fly present. These specimens result from the earliest blow fly colonization events, and therefore, accurately estimate the minimum PMI (Catts and Goff, 1990; Adams and Hall, 2003). Although temperature is the primary abiotic factor that

determines the rate of development of insects, other factors such as the presence of drugs, wrapping or burying of the remains, the temperature of the maggot masses and burning of remains (Goff, 1992; Anderson, 2010; Wells and Lamotte, 2010) can all impact the arrival time and development of blow flies.

The incidence of homicide by fire has increased in Canada since 2008, with the exception of 2012 (Statistics Canada, 2013). Homicide by fire, which is described by Statistics Canada (2013) as the result of either suffocation or direct burns, represents 3.1% of the 543 homicides in Canada during 2012, however, these statistics do not account for bodies burnt postmortem. In Ontario, there were 70 fire fatalities in 2012 (excluding fatalities resulting from fiery motor vehicle accidents), or 5.2 deaths for every one million residents, which were homicidal or suicidal in nature, or resulted from arson, among other causes (OFMEM, 2013). Studies have shown that burning human remains is most commonly used to eliminate trace evidence such as hair, bodily fluids or blood (Fanton *et al.*, 2006; Ubelaker, 2008; Gruenthal *et al.*, 2012). A study conducted in Lyon, France analyzed all autopsy records of bodies with signs of flame impingement over a period of ten years (Fanton *et al.*, 2006). Of 40 recorded cases, accidental death by fire occurred over 50% of the time, whereas criminal burnings were second most prevalent, accounting for 31% of the cases (Fanton *et al.*, 2006). Fanton *et al.* (2006) noted that soot found in the oesophagus and stomach can indicate that burning was conducted perimortem, or before death, as this would indicate the victim was still alive and breathing at the time of the fire. However, they deduced that the majority of criminal burnings are done postmortem, with victims rarely burnt alive (Fanton *et al.*, 2006).

Fire as a means of eliminating evidence is popular, but extreme measures must be taken to successfully eradicate evidence from a body. Even with temperatures reaching as high as 1500°C, modern crematoriums still aren't completely successful in eliminating all identifiable traces of human remains (Kennedy, 1996), as traces of bones and teeth remain (Murray and Jerome, 1993; Kennedy, 1996). Temperatures as high as 1200°C can destroy mitochondrial DNA found within bones, but can still leave histological features that can be used to identify a victim (Cattaneo *et al.*, 1999). To present a family with the finely powdered cremains that we are accustomed to seeing, a crematorium will pulverize, or blend, the remains after the cremation is complete (Kennedy, 1996). It is not until after

this step is complete that the remains will be rid of any identifiable pieces (Kennedy, 1996). It would be extremely difficult for a perpetrator who did not have access to a crematorium to sustain the extremely high temperatures required to completely destroy all traces of a human body (Anderson, 2010).

The transformational changes that a body undergoes during exposure to flames are well documented and understood (Kennedy, 1996; Bohnert *et al.*, 1998; Ubelaker, 2009). To help standardize the characterization of burnt remains, Glassman and Crow developed a scale referred to as the Crow-Glassman Scale (CGS) (Glassman and Crow, 1996). Prior to the publication of the CGS, a scale was used to describe the degree of burns to survivors (Glassman and Crow, 1996). This scale reflected the depth of the burn, the percentage of burns to the body and overall extent of burn damage to the victim (Glassman and Crow, 1996). However, this scale could not be easily applied to deceased victims of fires. The CGS (Table 1.1) has five distinct levels that outline the severity of burns to victims who have died due to the injuries sustained as a result of flame impingement. Level 1 is the least severe, synonymous with someone who has died via smoke inhalation, and Level 5 is the most severe and is synonymous with someone who has been cremated (Glassman and Crow, 1996).

There have been a number of studies that have investigated insect attraction to burnt remains and their utility in calculating an accurate PMI on these bodies (Avila and Goff, 1998; Introna *et al.*, 1998; Pai *et al.*, 2007; Chin *et al.*, 2008; Vanin *et al.*, 2013). Introna *et al.* (1998) documented two cases in which three burnt bodies were found inside cars. In each case the bodies were burnt postmortem to conceal the cause of death; in the first case the deceased individual was shot twice in the neck and in the second case the deceased persons were severely beaten and shot (Introna *et al.*, 1998). Although not specifically stated, all three of the bodies were burnt to Level 3 on the CGS as there was heavy charring, visible internal structures and dismembered limbs; each body contained large numbers of actively feeding larvae (Introna *et al.*, 1998). When calculated, the PMI estimates from both case studies were validated through eyewitness testimony (Introna *et al.*, 1998). Similar to these case studies, Pai *et al.* (2007) were also able to successfully validate a PMI estimate using flesh fly (Diptera:

Sarcophagidae) maggots collected from a female victim burnt postmortem to conceal the means of death. These three cases illustrate that insects can colonize and develop successfully on badly burnt bodies and that an accurate PMI can be estimated from insect evidence collected from flame-impinged bodies. However, if blow fly larvae are present on remains prior to and during flame impingement, the intense heats have been found to disturb the development of blow fly larvae resulting in inaccurate PMI estimates (Pacheco and VanLaerhoven, submitted)

Similarly, the recoverability of insect evidence after arson has been investigated (Anderson, 2005; Pacheco and VanLaerhoven, submitted). It is common for a perpetrator to return to the scene of a crime and dispose of any remaining evidence (Anderson, 2005). Arson is the most prevalent method of evidence elimination under those circumstances (Anderson, 2005). In arson scenarios, insect evidence is routinely overlooked (Anderson, 2005). To investigate such scenarios, Anderson (2005) placed four pig (*Sus scrofa* Linnaeus) carcasses outside. Acquiring human remains to conduct forensic entomology experiments in Canada can be extremely difficult, whereas domestic pig carcasses are easily obtainable, affordable and do not upset public opinion (Catts and Goff, 1992). Most importantly for a human analogue, pigs decompose in a manner that closely resembles human decomposition due to pigs and humans having similar skin composition, fat bodies at equivalent locations, omnivorous diets, similar internal structures/organs and similar chest cavities (Schoenly *et al.*, 2007). The pig carcasses decomposed under natural conditions and attracted insect colonizers, in order to represent a generalized situation in which a body might be dumped for disposal (Anderson, 2005). Each of the pigs was allowed to decompose until they reached the active decay stage, characterized by initial liquefaction of the body and the loss of flesh (Payne, 1965; Anderson, 2005). The pigs and their associated colonized insects were placed in a suburban house and four different arson scenarios were simulated, including dousing the pigs in gasoline to act as an accelerant and covering them with furniture (Anderson, 2005). Forensically useable insect evidence was collectable following all four arson situations, in which the temperatures ranged between 600°C and 1000°C (Anderson, 2005).

Although dead, the insect evidence collected in this study suggests that extreme temperatures are not enough to completely eliminate pre-colonized insects or deter the colonization of new individuals, as flies were attracted to the burnt remains after the flames were extinguished (Anderson, 2005). Additionally, the process of wrapping remains in blankets, for the purpose of concealment and easier transportation, has been found to protect pre-colonized insect evidence during periods of flame impingement that would result in CGS Level 2 burns (Pacheco and VanLaerhoven, submitted).

Although there have been no in-depth studies that try to explain how burnt remains affect blow fly oviposition behaviour or the consequent survivorship and fitness of blow fly offspring, the few studies available have both comparable and conflicting results. A study conducted at two different sites on the Hawaiian Island of Oahu showed that similar fauna colonize both burnt and un-burnt remains (Avila and Goff, 1998). Four pig carcasses, two of which were doused in gasoline and burnt to a Level 2 on the CGS, were left exposed outdoors to attract naturally colonizing fauna (Avila and Goff, 1998). At both study sites, it was observed that fauna were the same on the burnt and un-burnt pigs (Avila and Goff, 1998). For example, the blow fly *Lucilia sericata* Meigen (Diptera: Calliphoridae) was present on burnt carcasses at both study sites (Avila and Goff, 1998). Blow fly colonization occurred a day earlier on burnt remains when compared to un-burnt remains, with blow fly activity observed while remains were still on fire (Avila and Goff, 1998). The egg masses on the un-burnt remains were, on average, smaller than those on the burnt remains, with the majority of the egg masses on the burnt remains found in the mouth, front legs and abdomen (Avila and Goff, 1998). The authors believed that bodily fluids seeping through the cracks in the skin caused by the fire increased the attractiveness of the carcass to gravid females (Avila and Goff, 1998). These cracks not only acted as wounds, but also provided additional suitable oviposition locations to those described above (Avila and Goff, 1998).

Although the colonizing fauna was the same on burnt, using gasoline as an accelerant, and un-burnt pig carcasses, Chin *et al.* (2008) observed that there were

more adult flies on un-burnt carcasses with eggs being laid within the first hour; oviposition on the burnt remains only began during the second day of exposure, in direct contrast to Avila and Goff (1998). Interestingly, Chin *et al.* (2008) reported that there were larger egg and larval masses on the burnt remains, as was observed by Avila and Goff (1998), leading to increased surface and internal temperatures due to the activity of larger maggot masses which generate heat. These increased temperatures led to increased rates of larval development and carcass decomposition (Chin *et al.*, 2008).

Vanin *et al.* (2013) showed that carcasses burnt in between a Level 2 and Level 3 on the CGS, using wood without accelerant, were colonized by similar fauna when compared to carcasses that remained un-burnt, but in contrast to Avila and Goff (1998), flies arrived at burnt and control carcasses at approximately the same time. Additionally, it was observed that two blow fly species, *Phormia regina* Meigen (Diptera: Calliphoridae) and *L. sericata*, were able to colonize burnt remains (Vanin *et al.*, 2013). Vanin *et al.* (2013) also noticed cracks in the outer layers of skin that revealed internal structures, potentially increasing the number of suitable oviposition sites, as reported by Avila and Goff (1998). The authors also stated that as a carcass begins to burn, the composition of volatile molecules within the body start to change, altering the odour and attracting more insects (Vanin *et al.*, 2013).

INSECT ECOLOGY AND FIRE

In natural habitats, fires occur on a regular basis as a result of natural and anthropogenic causes. In Ontario there has been an annual average of 1098 forest fires in the past decade, accounting for just under 111 000 ha of land annually (Aviation, Forest Fire and Emergency Services, 2014). Despite the associated destruction, forest fires are a natural part of ecosystem regeneration and restoration (McCullough *et al.*, 1998; Nasi *et al.*, 2002). Forest fires are responsible for changes in species composition, altering biomass levels and changes in nutrient cycling; all of these factors combined contribute to overall ecosystem health in areas affected by fire (McCullough *et al.*, 1998; Nasi *et al.*,

2002).

In the aftermath of a forest fire, many organisms within the affected area will either be killed or displaced. The eventual return of displaced individuals, or replacement of deceased individuals via immigration into the affected habitat is important for both the growth and sustainability of the habitat. Studies have demonstrated that insect population levels are at their lowest both immediately after a fire and in the months that follow (Swengel, 2001). An insect's ability to survive a forest fire is largely dependent on its developmental stage, as immature insects are wingless and unable to escape the flames (Swengel, 2001). Insects with wings and those that are skilled fliers are believed to be the most successful at repopulating an area after a fire (Lamotte, 1975; Pippin and Nichols, 1996; Moretti *et al.*, 2004). Opinions differ, however, regarding how long it takes for insect populations to be at their highest levels following a fire. Orgeas and Andersen (2001) have stated that overall beetle numbers, species diversity and species richness are highest five years after a forest fire. Others, such as Force (1981), believe that insect species richness and population numbers are at their highest after the first year following a fire, when compared to both the second and third years. Force (1981) attributes this to an influx of both invader and generalist species. One positive effect of repeated burns in a single habitat is that populations of endangered species may increase, due to decreased competition with more dominant species (Moretti *et al.*, 2004).

Some insects, however, are the most successful immediately following a forest fire due to greater susceptibility or availability of their food source. For example, fires leave trees vulnerable to attacks by insects (McCullough *et al.*, 1998). Pyrophilous insects are attracted to fire (Klocke *et al.*, 2011). These insects rely on forest fires for food, reproduction and oviposition; and as such, they are known to be among the first arthropod colonizers after a fire-related disturbance (Wikars, 2002; Klocke *et al.*, 2011). One common fire-loving insect family is the Buprestidae, or jewel beetles (Coleoptera). Buprestidae have been observed to travel up to 50 miles (approximately 80.5 km) to find burnt wood as they prefer to reproduce on and oviposit in this medium (Linsley, 1943; Schmitz *et al.*, 1997; Schütz *et al.*, 1999; Klocke *et al.*, 2011). These beetles have been seen on logs that are still burning (Linsley, 1943; Klocke *et al.*, 2011), similar to blow flies observed near or on pig carcasses that are still burning (Avila and Goff, 1998).

Physiological studies have shown that Buprestidae possess both infrared receptors in their thoracic organs that detect heat and olfactory receptors in their antennae that detect smoke (Schmitz *et al.*, 1997; Schütz *et al.*, 1999; Klocke *et al.*, 2011).

There are a few published works on the response of Diptera to forest fires, however, none on the responses of blow flies specifically. Studies have yielded a wide range of results relating to the recovery rates, species diversity and species richness of Dipteran families, demonstrating that depending on the time of year a fire occurs and how frequent sampling occurs afterward, there are no noticeable differences in fly population numbers (Rice, 1932; Bulan and Barrett, 1971). However, conflicting results have also been found that indicate flies respond better post fire (Hurst, 1971; Nagel, 1973; Van Amburg *et al.*, 1981; Winter, 1984; Moretti *et al.*, 2004; Durska *et al.*, 2010). It is believed that blow flies should respond well post-fire due to increased availability of carrion (Shvidenko and Goldammer, 2001; United States National Park Service, 2014), fast oviposition times (Bourel *et al.*, 2003; Anderson, 2004; Byrd and Castner, 2010) and their ability to land on remains that are still on fire (Avila and Goff, 1998).

Blow flies use a well established two-step method to locate carrion both efficiently and effectively (Byrd and Castner, 2010). The first step involves scent detection using chemical receptors in their antennae, followed by the second step of visual detection (Byrd and Castner, 2010). Using taste receptors in their legs, feet and body, a blow fly will traverse carrion to determine if the resource is suitable for oviposition of its offspring (Kamal, 1958; Byrd and Castner, 2010). Oviposition is accomplished by a female fly extending her ovipositor, from her abdomen, onto a substrate where her eggs will be deposited (Byrd and Castner, 2010). Where possible, blow flies prefer to oviposit their clutches in or near the natural orifices of a body such as the nostrils, ear canals, mouth, anus and vagina. (Smith, 1986; Bourel *et al.*, 2003; Byrd and Castner, 2010). Attracted by the blood, female blow flies will oviposit in wounds such as scratches or cuts, which provides both sugar and protein and acts as a food source (Cragg, 1955; Thomas and Mangan, 1989; Hall and Doisy, 1993; Avila and Goff, 1998; Byrd and Allen, 2001; Bourel *et al.*, 2003; Byrd and Castner, 2010). Blow flies are also known to successfully oviposit on burnt remains (Avila and Goff, 1998; Introna *et al.*, 1998; Pai *et al.*, 2007; Chin *et al.*, 2008; Vanin *et al.*, 2013). It has been suggested that the preference of blow

flies for burnt remains is a result of bodily fluids seeping out of the body through the cracks created via flame impingement (Avila and Goff, 1998; Vanin *et al.*, 2013). That cracks in the skin are suitable for oviposition, and preferred sites for oviposition has yet to be rigorously tested.

One way to validate the assumption that cracks are a preferred oviposition site is to use experiments to test optimal oviposition theory (Jaenike, 1978). Blow flies do not practice parental care; once her clutch has been deposited, a female blow fly will not return to ensure her eggs are well protected and well taken care of. Optimal oviposition theory states that female insects will deposit their clutches in locations in which they believe their offspring will have the best chance of survival (Jaenike, 1978). Cracks may provide the greatest benefit for developing offspring by reducing the distance a first instar maggot would need to travel to find food, thereby conserving energy, which should increase larval survival. This, together with easy access to nutrient rich resources during the larval stages, should increase the fitness of the resulting adults.

Theoretically, a female needs to possess the ability to selectively choose oviposition locations that will benefit the development, survivorship and fitness of her offspring (Jaenike, 1978), but larval density within the oviposition location also impacts fitness. Blow flies participate in scramble competition, a form of exploitative competition (Park, 1962), as all larvae present share the carrion resource (Nicholson, 1954 in Prinkkilä and Hanski, 1995). Larval density has both beneficial and detrimental effects in blow flies such that there is likely an optimal density for different species. Increased larval densities, in the form of maggot masses, can be beneficial to developing larvae. Maggot masses keep temperatures elevated on carrion, which in turn help to decrease development times of feeding larvae, including the earlier instars that are more susceptible to temperature related fatalities (Baxter and Morrison, 1983; Catts, 1992; Catts and Goff, 1992; Turner and Howard, 1992; Ireland and Turner, 2006; Kheirallah *et al.*, 2007; Anderson, 2010). Additionally, increased larval densities can aid in an increased concentration of proteolytic enzymes, which help break down carrion tissues faster (Baxter and Morrison, 1983; S. dos Reis *et al.*, 1999; Ireland and Tuner, 2006; Kheirallah *et al.*, 2007). However, past an optimum level, as larval density continues to increase, overall development time increases, yet fitness (i.e., blow fly size) and

survivorship decreases (Hutton and Wasti, 1980; Goodbrod and Goff, 1990; So and Dudgeon, 1990; Prinkkilä and Hanski, 1995; Saunders and Bee, 1995; Smith and Wall, 1997a; Ireland and Turner, 2006; Kheirallah *et al.*, 2007), likely due to competition for nutritional resources (VanLaerhoven, in press).

Carrion is both an ephemeral and heterogeneous environment within which female flies choose an oviposition site, and the choice of where and how many eggs to deposit can have a significant impact on the overall population density and distribution of population density on the resource. In a study conducted with eastern tree-hole mosquitoes, *Ochlerotatus triseriatus* Say (Diptera: Culicidae), Ellis (2008) noted that larval density and fitness levels were proportional to one another, such that as larval density increased, fitness decreased with a corresponding increase in development time and a decrease in overall survivorship. When given a choice, the female mosquitoes avoided the most dense patches and oviposited in less dense patches (Ellis, 2008). The oviposition preference-offspring performance hypothesis is an extension of optimal oviposition theory and states that offspring fitness will be highest when females select optimal oviposition sites (Thompson, 1988; Ellis, 2008). In the case of the mosquitoes studied by Ellis (2008), optimal oviposition sites were those with low density, as important aspects of mosquito development were negatively correlated with increased larval density. A positive preference-performance relationship is noted when a female insect can ‘determine’ the larval density on a particular resource, with the opposite also being true (Ellis, 2008). Based on the predictions of the oviposition preference-offspring performance hypothesis, and the results of the mosquito study (Ellis, 2008), female blow flies should oviposit at sites where the larval density will be within an optimal range for fitness avoiding the highest density or lowest density sites. As it relates to burnt remains, cracks should receive more eggs, due to their aforementioned perceived benefits to larval offspring. If this is the case less intraspecific competition should be observed at the more traditional oviposition sites on burnt carcasses due to decreased larval density at those sites.

In blow flies it appears as though outcomes of species interactions are affected not just by larval density, but also by adult density. On carrion, there is the potential for two different types of competition: (1) competition for oviposition sites and (2) competition

for larval resources. Two species cannot utilize the same limited resource in the same manner at exactly the same time without competitive exclusion driving one species to extinction (Gause, 1934). The spatial and temporal aggregation of species on carrion is speculated to be two of the main mechanisms that regulate, both interspecific and intraspecific, competitive interactions (Fiene *et al.*, 2014; VanLaerhoven, in press). The lottery effect (Chesson and Warner, 1981) provides a means of temporal resource partitioning, whereby the females of a particular blow fly species who first encounter a carrion resource when it becomes available should be able to establish a dominant population (VanLaerhoven, in press). Burnt remains provide a unique carrion landscape in that they possess different oviposition sites from un-burnt remains that can be utilized depending on the severity of flame impingement, thus also providing a different spatial resource partitioning from un-burnt remains. Carrion colonizing insects, such as blow flies, who adapt and utilize these unique resource patches, such as cracks that develop in the skin, thereby changing the spatial resource partitioning, have the potential to be successful on altered resources. There have been conflicting reports of outcomes of competition or competitive exclusion of blow flies on carrion (Hanski, 1987) and these differences in resource partitioning, together with initial population density, may provide an explanation (VanLaerhoven, in press). Known as founder control (Mittelbach, 2012), the species with the greatest initial population density stands to benefit the most from a carrion resource and outcompete those with lower densities (VanLaerhoven, in press).

One factor that will determine the extent of competition, and therefore, the competitive exclusion of the weaker species, is the initial amount of carrion resource available (VanLaerhoven, in press). Consumer-resource theory (Tilman, 1982) predicts that on an essential limiting resource, such as carrion, two competing blow fly species of the same population density cannot both persist without one driving the other to extinction (VanLaerhoven, in press). The competitor that can maintain its population density, while using less of the resource, will stand the greatest chance of outcompeting the other (Tilman, 1982). In the case of burnt remains, as burn severity increases (i.e., higher levels on the CGS), the amount of consumable resource for developing blow flies decreases, potentially increasing competitive effects. Female blow flies should change how many eggs they oviposit in the presence of heterospecific females, depending on the

amount of resource present and the species' optimum larval density, in order to increase the chances of their offspring successfully developing to adult.

STUDY SYSTEM

Two species of blow fly were used to conduct the research described by this thesis, *L. sericata* and *P. regina*. Although both species are within the same family (Calliphoridae), these flies are classified into two different subfamilies and further into two different tribes (Hall, 1948; Hall and Townsend Jr., 1977). *Lucilia sericata* is classified in the subfamily Calliphorinae in the tribe Luciliini, whereas *P. regina* is classified in the subfamily Chrysomyinae in the tribe Phormiini (Hall, 1948; Hall and Townsend Jr., 1977). *Lucilia sericata*, the sheep blow fly or green bottle fly, has a metallic green appearance, which changes slowly with age to a dark copper (Smith, 1986). Contrary to its common name, the black blow fly, *P. regina*, is usually dark green to olive green in colour (Smith, 1986). Both species are native to southern Canada (Smith, 1986; Byrd and Castner, 2010) and have been observed colonizing a single carrion resource simultaneously, including burnt remains (Anderson and VanLaerhoven, 1996; VanLaerhoven and Anderson, 1999; Sharanowski *et al.*, 2008; Vanin *et al.*, 2013). Unfortunately, detailed information on their species interaction is lacking. Increased egg, and therefore larval densities, on altered carrion, such as burnt remains, could either magnify or reduce positive or negative species interactions.

Female *L. sericata* may lay up to 3000 eggs in their lifetime, and deposit roughly 300 eggs in a single clutch (Smith, 1986). For both species, oviposition may begin as early as five days post adult emergence (Smith, 1986; VanLaerhoven and Anderson, 2001). Female flies require foods rich in energy, including sugars and proteins; protein is essential for the successful development of a female's eggs (Vogt *et al.*, 1985; Erzinçioğlu, 1996). Carrion not only represent a suitable oviposition medium, they also act as a sugar and protein source, as indicated by both non-gravid and gravid females visiting these resources (Brodie *et al.*, 2014). Females of both species have been observed ovipositing on surfaces completely covered with eggs, regardless of available

space (Kamal, 1958). *Lucilia sericata* eggs can mature and hatch anywhere between 10 and 52 hours after oviposition, depending on temperature (Smith, 1986). For *L. sericata*, development through the three instars requires between three and 11 days, while the pupal stage has been recorded to last from four to 24 days (Smith, 1986). The egg eclosion time for *P. regina* can range between eight and-a-half to 24 hours, while the complete lifecycle can take from eight to 25 days (James, 1947; Kamal, 1958; Byrd and Allen, 2001).

Lucilia sericata can survive higher temperatures and are more prevalent during the warmer summer months (Smith, 1986). In saying that, *L. sericata* prefer ovipositing on carrion located within open fields where presumably temperatures are hotter as the eggs hatch earlier and then develop and emerge as adults faster in these conditions when compared to those in shaded areas (Cragg, 1995; Smith and Wall, 1997a; Smith and Wall, 1997b), yet prefer protected areas on carrion for egg oviposition (Byrd and Castner, 2010). Female *L. sericata* prefer ovipositing in the mouth, eyes and nostrils regardless of whether they colonize before or at the same time as *P. regina*, however when they were second to colonize after *P. regina*, they showed a preference for additional locations on the body including the head, abdomen and between the legs of carrion (Rosati, 2014). Additionally, wounds represent a wet environment where *L. sericata* prefer to oviposit (Kamal, 1958; Grassberger and Reiter, 2001). Cracks caused via flame impingement should be wetter and therefore may be a stronger attractant to *L. sericata* females.

Phormia regina prefer cooler temperatures and as a result, their populations often decline during the summer months in the southern United States (James, 1947; Byrd and Allen, 2001), yet remain dominant during the summer months in southern Ontario (Rosati, 2014). Female *P. regina* tend to perform a specific spatial routine before ovipositing: a female circles her desired oviposition location before moving in backwards and fluttering her wings, which is followed by the extension of the ovipositor (Kamal, 1958). Following the spatial routine, eggs are oviposited in clumps, which likely contributes to the greater variation in development time of *P. regina* eggs compared to *L. sericata* eggs (Kamal, 1958; Smith, 1986; Byrd and Castner, 2010). In contrast to *L. sericata*, *P. regina* show no preference for colonizing carrion located in shaded or open locations (Joy *et al.*, 2002). Recent studies, however, have shown that when *P. regina* colonize a carrion resource first they oviposit between the legs, on the abdomen and on

the head, while avoiding the mouth, ears and nostrils (Rosati, 2014). When *P. regina* arrives after or at the same time as *L. sericata* they switch their oviposition preference to inside the head (i.e., mouth, ears, nostrils) (Rosati, 2014).

In the literature, there are more documented cases of interspecific interaction effects on *L. sericata* than on *P. regina*. Overall, *L. sericata* is negatively affected by both intraspecific and interspecific competition (Cragg, 1995; Smith and Wall, 1997a; Smith and Wall, 1997b; Rosati, 2014), with decreased fitness and adult/larval survival (Ullyett, 1950; Hutton and Wasti, 1980; Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007). Regardless of their ability to survive under harsher conditions (Cragg, 1955), the presence of other blow flies species on carrion has been shown to reduce the size of emerging adults (Smith and Wall, 1997a). Increased interspecific larval density of *L. sericata* with other *Lucilia* and blow fly species can decrease the survival rates of larvae, and therefore, can have negative impacts on the size of flies, lifespan and fecundity of female flies (Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007). In general, female Dipteran species are smaller than males, however female *L. sericata* are known to have greater size plasticity (Prinkkilä and Hanski, 1995; Smith and Wall, 1997a). However, interspecific interactions between *L. sericata* and *P. regina* have not resulted in detrimental effects to survival and fitness to *L. sericata* at some larval densities tested (Hutton and Wasti, 1980; Rosati, 2014). Larval densities ranged from 10 to 210 larvae present on 25 g of resource (Hutton and Wasti, 1980) or between 200 and 400 larvae on fetal pig carcasses weighing more than 700 g (Rosati, 2014). Negative responses, such as decreased fitness and adult/larval survival as described above, have also been observed under circumstances where intraspecific interactions occur (Ullyett, 1950; Hutton and Wasti, 1980; Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007). Given these studies, *L. sericata* are likely to have a lower optimum larval density, but their developmental plasticity to successfully mature smaller adults in the presence of competition may allow them to persist in a wider range of larval densities.

Unlike *L. sericata*, female *P. regina* are smaller than males (Rosati, 2014). *Phormia regina* is slow to oviposit when in a single species dominated setting, whereas they oviposit much more rapidly in the presence of *L. sericata*, which might indicate *P.*

regina is facilitated by other blow fly species (Rosati, 2014). Larval survivorship rates and adult fitness levels of *P. regina* increase when in the presence of *L. sericata*, with no effect of density at the levels tested in the study (Rosati, 2014). Other research, however, has demonstrated adverse effects to *P. regina* when *L. sericata* are present, as entire larval populations have been observed to be eliminated (Hutton and Wasti, 1980). Larger conspecific larval densities have also resulted in lower adult emergence (Hutton and Wasti, 1980), however based on these studies, it is likely that the optimum larval density of *P. regina* is higher than that of *L. sericata*. Different from *L. sericata*, *P. regina* does not exhibit a great degree of developmental plasticity to successfully produce small adults. Therefore, although *P. regina* may have a higher optimum larval density, it likely has a narrower range of densities that it can successfully develop to adult.

RESEARCH OBJECTIVES

The need for research as it relates to the overall effect that burnt remains have on forensically important blow flies is evident. Homicide rates due to flame impingement have been increasing, and regularly occurring forest fires are a natural part of ecosystem regeneration. This thesis aims to demonstrate that burnt carrion affects both the potential colonization and consequently the survival and fitness of two local blow fly species. Using simulated human remains, the oviposition behaviours of *L. sericata* and *P. regina* were observed to test the prediction that flame impingement affects the number of eggs and oviposition location of forensically important blow flies. These choices should result in differing survival and fitness of the offspring that develop on burnt remains.

Increased flame impingement, and therefore, middle levels on the CGS, should result in the presence of cracks in the skin. Based on the optimal oviposition theory these cracks, and associated bodily fluids, should represent an ideal location for the development of both species of blow fly larvae when colonizing on their own. This should be observed as a shift in oviposition preference measured as an increased number of oviposition events at the cracks in these areas when compared to these areas of the body without cracks, with an associated reduction in the oviposition events in other areas of the body in the presence of cracks. Given the differential oviposition location

preferences of *L. sericata* and *P. regina* as reported in the literature, *L. sericata* will likely oviposit directly in the cracks, whereas *P. regina* will likely oviposit on bodies with cracks (middle of the CGS), but not directly in the cracks themselves. Along with this shift in oviposition preference, based on optimal oviposition theory, there should be an overall increase in survival and fitness of resulting adults that developed on bodies with cracks when compared to adults that have resulted from maggots developing on bodies without cracks or with fewer cracks (lower on the CGS).

However, based on the density-dependent aspects of the oviposition preference-offspring performance hypothesis, although the cracks might represent an ideal location, *L. sericata* will likely oviposit in cracks more often as previously predicted, but deposit fewer eggs due to its lower optimum larval density. In addition, due to the predicted reduction in carrion resource biomass associated with increased flame impingement (increasing in CGS), *L. sericata* will likely oviposit fewer offspring as carrion increase in CGS level. In contrast, *P. regina* will oviposit both more often and deposit more eggs on bodies with cracks and as bodies increase in CGS level due to its higher optimum larval density.

Based on the oviposition preference-offspring performance hypothesis, it is predicted that when both species colonize carrion simultaneously *L. sericata* will deposit fewer eggs overall, with cracks utilized when present, whereas *P. regina* will oviposit more eggs, with more oviposition events and with a greater degree of overlap with *L. sericata* oviposition location choices than when *P. regina* colonizes alone. As CGS level increases, *P. regina* survival and fitness will increase with CGS level due to its higher optimum larval density and facilitation by heterospecifics, whereas *L. sericata* fitness will decrease due to its lower optimum larval density and competition with heterospecifics but survival will remain unchanged due to its wider developmental plasticity.

The conclusion of this research should help bring the interactions between blow flies and burnt carrion to the forefront for both forensic entomologists and community ecologists.

Table 1.1: The five levels of the Crow-Glassman Scale (CGS) with a description of the degree of damage to a body represented by each level of the scale (adapted from Glassman and Crow, 1996).

CGS Level	Description of Remains
Level 1	Skin is blistered, hair singed, death results from smoke inhalation and the body is easily recoverable and identifiable
Level 2	The body remains recognizable and begins to char. Disarticulation can become a factor, with identification performed with the help of other forensic professionals
Level 3	Limbs start to separate from the torso, but due to the severity of the burn the head is unrecognizable making identification by sight almost impossible. A forensic anthropologist should be called in to help search for detached limbs. Identification made via dental records
Level 4	The body is highly charred and the head is no longer associated with the body, with severe cracking of the skull. A forensic anthropologist is needed to perform a meticulous search for fragmented pieces of the body, with identification coming through dental records
Level 5	This level can be synonymous with cremation. The body is unrecognizable as human and therefore unidentifiable. All bones are fragmented and fragile. Forensic professionals need to be on scene to process remains and to help in the identification process.

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CHAPTER 2:
THE OVIPOSITION BEHAVIOUR OF *LUCILIA SERICATA* MEIGEN AND
PHORMIA REGINA MEIGEN (DIPTERA: CALLIPHORIDAE) ON BURNT
CARRION

INTRODUCTION

Blow flies (Diptera: Calliphoridae) are one of the first insect species to arrive on carrion and are, therefore, important to forensic entomologists when estimating a postmortem interval (PMI), or the minimum time between death and discovery of a decedent based on insect behaviour and development (Cragg, 1955; Smith, 1986; Thomas and Mangan, 1989; Hall and Doisy, 1993; Smith and Wall, 1997a; Smith and Wall, 1997b; Gião and Godoy, 1997; Byrd and Allen, 2001; Bourel *et al.*, 2003; Arnaldos *et al.*, 2005; Nability *et al.*, 2006; Byrd and Castner, 2010; Higley and Haskell, 2010; Wells and Lamotte, 2010). There are a number of abiotic and anthropogenic factors that can alter the quality of carrion resources and potentially result in changes to the oviposition behaviour of female blow flies and therefore the colonization by blow flies. One important example is flame impingement, as the flames physically alter the carrion resource. These alterations can result in a delay in oviposition due to competition stress or resource quality. This has the potential to impact the precolonization interval of blow flies, which is usually presumed to be extremely short (Anderson and VanLaerhoven, 1996).

A few studies have been conducted to determine the effects of flame impingement on blow flies. For example, studies have shown that blow flies can successfully colonize and develop on burnt remains and are recoverable as evidence after arson scenarios (Avila and Goff, 1998; Anderson, 2005; Introna *et al.*, 2005; Pai *et al.*, 2007; Chin *et al.*, 2008; Vanin *et al.*, 2013; Pacheco and VanLaerhoven, submitted). Burnt remains have been found to attract similar fauna as remains that are unaffected by flame impingement (Avila and Goff, 1998; Introna *et al.*, 2005; Pai *et al.*, 2007; Chin *et al.*, 2008; Vanin *et al.*, 2013). In rare cases, blow flies have been attracted to remains that are still on fire (Avila and Goff, 1998). Given their ability to colonize burnt remains, blow flies collected

from these remains have been successfully used to provide accurate minimum PMI estimates as verified by witness testimony (Pai *et al.*, 2007; Introna *et al.*, 2008; Vanin *et al.*, 2013).

Burning is seldom the primary cause of death for homicide victims and is usually performed postmortem as a means to eliminate physical evidence or the original cause of death (Fanton *et al.*, 2006; Introna *et al.*, 2008; Ubelaker, 2008; Gruenthal *et al.*, 2012). Modern crematoriums are capable of reaching temperatures as high as 1500 °C, but can still leave remains that can be used to make a positive identification (Murray and Jerome, 1993; Kennedy, 1996). There are thorough reports of the processes a body undergoes when being burnt (Bohnert *et al.*, 1998). To standardize the appearance of a victim following the discovery of burnt remains, Glassman and Crow (1996) developed the Crow-Glassman Scale (CGS) that is now a common point of reference in forensic and criminal investigations. The CGS includes five levels, which detail the physical appearance of burnt remains (Glassman and Crow, 1996). An increase in CGS level is indicative of increased destruction to a burn victim due to flame impingement (Glassman and Crow, 1996). Level 1 is synonymous with someone who has succumbed to smoke inhalation with noticeable blistering of the skin and singed hair (Glassman and Crow, 1996). A body with noticeable charring and damage to limbs is representative of a burn victim classified as a Level 2 (Glassman and Crow, 1996). A burnt body classified as a Level 3 on the CGS, is indicative of limbs starting to disarticulate and more severe charring throughout, however, it is important to note that the head is still attached to the body at this level (Glassman and Crow, 1996). The higher the CGS level, the harder the identification process becomes (Glassman and Crow, 1996). Without access to commercial crematoriums, maintaining high temperatures and direct contact with fire for an extended amount of time would be incredibly difficult, and as a result, the use of flame impingement as a forensic countermeasure is not necessarily sufficient to destroy all of the evidence connected to the body, including insect evidence (Anderson, 2010).

The location of eggs, and of the consequent blow fly larvae, is also important to forensic entomologists as it allows them to recreate the context of a scenario, given the behaviour of the blow flies. Under normal circumstances, blow flies prefer to colonize and oviposit their eggs in the body's natural orifices (Smith, 1986; Bourel *et al.*, 2003;

Byrd and Castner, 2010). Wounds are known to attract ovipositing females (Cragg, 1955; Thomas and Mangan, 1989; Hall and Doisy, 1993; Byrd and Allen, 2001). Wounds expose blood and other tissues to blow flies, which act as a sugar and protein source (Avila and Goff, 1998; Byrd and Castner, 2010). Similar to wounds, cracks are believed to be attractive to blow flies as they expose bodily fluids and internal structures, making these sites optimal for instar maggots (Avila and Goff, 1998; Vanin *et al.*, 2007). It has been predicted that cracks in the skin, which are induced by high temperatures associated with flame impingement, should facilitate colonization and oviposition on burnt carrion resources (Avila and Goff, 1998; Vanin *et al.*, 2007). Oviposition events in atypical locations, such as cracks on burnt carrion, requires forensic entomologists to consider why the blow flies are straying from their normal oviposition locations when interpreting the insect evidence within the context of an investigation. Although it has been stated that cracks in burnt remains could be responsible for changes in oviposition behaviour (Avila and Goff, 1998; Vanin *et al.*, 2007), it has not been specifically investigated as of yet.

Ovipositing in cracks should reduce the amount of energy that developing blow fly larvae need to expend when feeding on carrion. When feeding, larvae use their mouth parts to penetrate the outer layer of skin, however, if the ovipositing female deposits her clutch in a crack, the tougher outer layer of their food source has already been pierced, reducing the energy required to access the nutritional resources. Optimal oviposition theory predicts that female insects deposit eggs where her offspring will have the greatest chance at survival (Jaenike, 1978). Following this prediction, and the predictions of Avila and Goff (1998) and Vanin *et al.* (2007), the cracks that result from flame impingement should provide female blow flies with a favourable oviposition site that will maximize their offspring's nutritional intake with less energy required. If female blow flies follow the predictions of optimal oviposition theory (Jaenike, 1978), there should be a noticeable shift in oviposition preference away from traditional oviposition sites, resulting in more oviposition events at the cracks. This should indicate a female preference for the cracks. Additionally, as burn severity increases (i.e., higher levels on the CGS), the number of cracks on the resource affected by flame impingement should increase, further increasing the oviposition events at the cracks at the expense of the

traditional oviposition sites.

The physical characteristics of oviposition sites, such as natural orifices or cracks in the skin, are not the only factors that might influence the oviposition decisions of female blow flies. Offspring fitness is also related to factors such as the presence and density of both heterospecific and conspecific larvae at the oviposition site. The effect of larval competition on the subsequent fitness of those individuals is probably species specific, in that some larvae might be better competitors than others. For example, Ellis (2008) conducted a series of studies to determine the effects of oviposition site quality and intraspecific competition on the selection of oviposition sites by female eastern tree-hole mosquitoes, *Ochlerotatus triseriatus* Say (Diptera: Culicidae). Ellis (2008) found that increased larval density of mosquitoes was correlated with a decrease in larval survivorship. Therefore, although resource rich sites were preferable as oviposition sites, if the larval density at those sites was also high, the subsequent fitness of the larvae was lower than the fitness of mosquito larvae at poor quality sites where larval density was low (Ellis, 2008). The oviposition preference-offspring performance hypothesis (Jaenike, 1978; Thompson, 1988) extends upon optimal oviposition theory to explain how oviposition site selection is related to offspring performance. Different measures of offspring fitness are better correlated to oviposition preference than others (Thompson, 1988), which is why it is important to consider other factors, such as density effects when assessing the oviposition decisions by female insects.

Similar to the eastern tree-hole mosquitoes, effects of density on offspring performance have been observed for blow flies (Hutton and Wasti, 1980; Goodbrod and Goff, 1990; So and Dudgeon, 1990; Prinkkilä and Hanski, 1995; Saunders and Bee, 1995; Smith and Wall, 1997a; Ireland and Turner, 2006; Kheirallah *et al.*, 2007). Contrary to mosquitoes, however, increased larval density in blow flies is known to play a positive role in their development such as with the increased temperatures in larval aggregations, or maggot masses, and the build-up of digestive enzymes that help in the digestion of carrion tissues that result when maggot density is high (Baxter and Morrison, 1983; Catts, 1992; Catts and Goff, 1992; Turner and Howard, 1992; S. dos Reis *et al.*, 1999; Ireland and Turner, 2006; Kheirallah *et al.*, 2007; Anderson, 2010). Therefore, it will be important to consider how female blow flies react to perceived larval densities at both

traditional oviposition sites and at the cracks on burnt remains when making oviposition decisions.

Lucilia sericata Meigen and *Phormia regina* Meigen are two common blow fly species in Southern Ontario, Canada. These two blow fly species have been reported to colonize the same carrion (Anderson and VanLaerhoven, 1996; VanLaerhoven and Anderson, 1999; Sharanowski *et al.*, 2008), which includes burnt carrion (Vanin *et al.*, 2013). These two species have demonstrated different preferences for oviposition locations on carrion (Rosati, 2014). *Lucilia sericata* prefers ovipositing in the mouth, on the eyes and in the nostrils of carrion (Rosati, 2014), indicating a preference for ovipositing in wetter locations (Grassberger and Reiter, 2001). However, if they colonize a resource after *P. regina*, their preferences change, and in addition to the above locations, they include additional parts of the head, abdomen and between the legs (Rosati, 2014). The opposite has been observed for *P. regina* as they prefer depositing their eggs on the head, but not inside the mouth, nostrils, or in the eyes, and they prefer the abdomen and between the legs when they are the first colonizer (Rosati, 2014). This indicates a preference for ovipositing in areas with less fluid. When they colonize after *L. sericata*, *P. regina* chooses to oviposit closer to *L. sericata* egg clutches, such as inside the head in areas like the ear canal and inside the mouth and nostrils (Rosati, 2014).

After oviposition, these two species have different strategies when faced with conspecific and heterospecific larvae. Increased interspecific and intraspecific larval densities are known to result in decreased larval survival and reduced size of *L. sericata* adults (Ullyett, 1950; Hutton and Wasti, 1980; Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007). However, in the presence of varying densities of *P. regina* larvae, there have been no detrimental effects to either larval survival or adult fitness of *L. sericata* (Hutton and Wasti, 1980; Rosati, 2014). The effects of interspecific competition on *P. regina* recorded in the literature are not consistent. For example, studies have shown an increase in larval survival in the presence of *L. sericata* (Rosati, 2014), whereas other studies have demonstrated absolute larval death (Hutton and Wasti, 1980). In interactions with conspecifics at higher larval densities, *P. regina* has been known to experience an increase in larval death (Hutton and Wasti, 1980).

The objective of this research was to investigate the effects that burnt remains

have on the oviposition behaviour of *L. sericata* and *P. regina*. Overall, the results of this research will elucidate the effects that different levels of burn severity to carrion, and the cracks that result, have on female blow fly oviposition preferences. To test this, burnt simulated human remains were exposed to both blow fly species in the presence of both conspecifics and heterospecifics, in order to determine if females exhibited an oviposition preference for the cracks that result from burning a body.

In general, based on optimal oviposition theory, it is predicted that between CGS levels there will be a shift in oviposition preferences as CGS level increases, due to females compensating for the formation (i.e., cracks in the skin), and in certain cases, the destruction of oviposition locations. Within CGS levels, it is predicted that there will be a shift in oviposition preference away from traditional oviposition sites towards the cracks.

More specifically, due to the previously described oviposition preferences seen in the literature and the development of cracks associated with an increase on the CGS, it is predicted that there will be an overall increase in the number of oviposition events on the cracks by *L. sericata*. This shift in oviposition preference away from the traditional sites, as described by optimal oviposition theory, will represent an ideal location for developing larvae. An increase in the number of oviposition events may not necessarily equate to an increase in the number of eggs oviposited by female *L. sericata* at the cracks due to the perceived decreased optimum larval density, as predicted by the oviposition preference-offspring performance hypothesis. With an increase in burn severity, as indicated by an increased CGS level, there should be a decrease in overall biomass available for consumption by developing larvae, which should result in fewer eggs being deposited by female *L. sericata* at the cracks.

Due to the previously described oviposition preferences and opposite to *L. sericata*, *P. regina* will demonstrate a preference for ovipositing on burnt remains, but will likely avoid laying eggs directly in the cracks, opting for sites close to the cracks instead. Female *P. regina* that deposit eggs close to the cracks would be making decisions to optimize offspring survival, as predicted by optimal oviposition theory. With fewer oviposition events at the cracks, *P. regina* should deposit a greater number of eggs on these locations due to a perceived increased optimum larval density, as predicted by the oviposition preference-offspring performance hypothesis.

Finally, when in the presence of heterospecifics, *L. sericata* should try to make use of all available cracks, but oviposit fewer eggs, whereas *P. regina* will oviposit more often resulting in a larger egg total. The oviposition sites selected by female *P. regina*, will resemble those of *L. sericata*, when compared to when *P. regina* is in the presence of conspecifics.

METHODS

I) Colony Maintenance

Colonies of both *L. sericata* and *P. regina* are maintained at the University of Windsor, Windsor, Ontario, Canada. The colonies were established in 2005 by trapping wild type flies in wasp traps (King Home and Garden Inc., Item ID: 56789) using pork liver as an attractant. On a yearly basis, new wild type flies are caught and added to the colonies to prevent the effects of inbreeding depression. The colonies are kept under controlled conditions following a 12 L: 12 D diel cycle with a mean temperature (\pm S.E.) of 25 ± 1 °C and 60 ± 5 % relative humidity. Adult blow flies are provided with sugar cubes and a paste made from instant milk powder to act as a carbohydrate and protein source, respectively (Anderson, 2000; Byrd and Allen, 2001; Nabity *et al.*, 2006). Water is provided in Erlenmeyer flasks containing cotton dental wicks. Colony cages are constructed of aluminium framing and aluminium mesh screening and measure 46 cm³ (BioQuip Products, Item ID: 1450C).

To maintain the colonies, eggs are obtained by providing pork liver (ca. 20-30 g) as a suitable oviposition medium for gravid females (Byrd and Allen, 2001). Once egg masses of at least 100 eggs are observed, the liver and eggs are removed and placed into a 1 L glass Mason jar lined with pine sawdust to absorb excess fluids and provide a dry pupation medium (Hutton and Wasti, 1980). The Mason rearing jars are sealed with gardening canvas (Quest Brands Inc., Item ID: WBS 50) and a ring lid to ensure that the developing maggots are contained within the jar, while still allowing gas exchange. Liver is added as needed to ensure that developing maggots have enough to eat (Anderson, 2000). Once adults begin emerging, they are released from the jar into the appropriate colony cage. Colony cages and rearing jars are checked daily.

II) Experimental Cage Treatments

Five days prior to scheduled burns, experimental colony cages were assembled as described below. Rearing jars containing adult blow flies, less than 24 h post emergence, were chilled in a refrigerator for approximately 15 minutes to decrease their activity, making sexual identification and transferring to cages easier (Ricker *et al.*, 1986). Flies were removed individually from the jars and sexed based on eye morphology: the compound eyes of a male blow fly appear to touch, while those of a female blow fly have a distinct spacing (Erzinçlioğlu, 1996). Three different species treatment cages were prepared containing 100 females and 50 males in the following combinations: single species treatments of (1) *L. sericata* only and (2) *P. regina* only and (3) a mixed species treatment with 50 females and 25 males of both species. In the mixed species cages, 50 females and 25 males of each species were used to maintain the population density across all species treatments. Within each species treatment, there were four levels of burn treatment (one cage each), which included a control (Level 0), Level 1, Level 2, and Level 3 of the CGS. As a result of the number of cages being limited, and because it was difficult to ensure that there were enough adult blow flies of the same age available to use at the same time, only one or two replicates of the species treatments could be conducted at a time. Therefore, species treatments were conducted when adult blow flies were available and were completed over a one year period (Table 2.1). In a pilot study, no oviposition events occurred on Level 4 and Level 5 of the CGS, therefore, those two levels were excluded from this experiment.

Flies in all cages had constant access to sugar cubes and water. During the five days prior to the scheduled burn, adult blow flies within the cages were allowed to feed on pork liver for 1 h per day. Protein meals are necessary to ensure maturation of the reproductive system, especially of female flies (Erzinçlioğlu, 1996); this procedure ensured females were gravid on the day of the burn (VanLaerhoven and Anderson, 2001). On the fourth and fifth days of protein feeding, the cages were shaken every five minutes to prevent gravid females from ovipositing on the liver.

III) Burning

The day prior to scheduled burn dates, four deceased and pre-weighed (639 to 2247 g) fetal pigs (*Sus scrofa domesticus* L., Artiodactyla: Suidae) were removed from

laboratory freezers and allowed to thaw overnight. The fetal pigs used in this experiment were obtained from a local farmer and had died from natural causes. On the day that the pig carcasses were burnt, three pigs were transported to Amherstburg, Ontario where the burns occurred in a steel fire pit measuring 89 x 55 cm. The fourth pig carcass remained at the University of Windsor as it was not burnt and acted as the control (Level 0). The fires used to burn the pigs were started with untreated scrap wood and newspaper and were ignited with a barbeque lighter. The fire was fed with scrap wood as needed.

Of the three pigs transported to the burn site, each was burnt to a different level on the CGS (Level 1, Level 2 and Level 3). Pigs were burnt in descending order of the CGS, starting with Level 3, to try to ensure that the time between the end of flame impingement and departure back to the University of Windsor was minimal. Generally, the greater the CGS level, the longer the duration of flame impingement. The fire was continuously scanned using an Omega OS423HT-LS non-contact infrared thermometer while each pig was exposed to flame impingement and the maximum temperature of the fire was recorded. Post-burn, each pig was weighed using a digital scale (Starfrit, Model #: 70200) to account for total biomass lost due to burning. Each pig was inspected for cracks caused by flame impingement and the position, length and width of any observable cracks was measured by hand and recorded. The pigs were placed in a plastic storage container and transported back to the University of Windsor. Five burns were completed for each species treatment across all CGS levels, for a total of 20 fetal pigs burnt per species treatment (Table 2.1).

IV) Surface Area (mm²) Estimation for Experimental Pig Carcasses

Another factor that could influence oviposition site selection is the available surface area for egg laying. For example the Senita moth, *Upiga virescens* Hulst (Lepidoptera: Pyralidae), has shown an affinity for ovipositing on the smaller internal structures (e.g., corolla tubes and anthers) of Senita cactus (*Lophocereus schottii*) flowers, when compared to the larger external structures such as the petals (Holland *et al.*, 2004). Mediums with larger surface areas have also been found to attract ovipositing insects, as larger cowpea seeds can facilitate more oviposition by the seed beetle (*Callosobruchus maculatus* Fabricius, Coleoptera: Bruchidae) (Cope and Fox, 2003). Therefore the role of surface area in determining blow fly oviposition site preference was tested. It was

assumed that if only surface area influenced blow fly preference for oviposition sites, the frequency of oviposition events would be directly proportional to the surface area. For example, if the head accounted for 50% of the total surface area, then 50% of the oviposition events should have occurred on the head. To test this prediction for each CGS Level, the surface area of each oviposition site on each pig carcass used in this study was estimated.

Estimates of surface area were made using regression equations, that were established by Pacheco *et al.* (submitted), which could be used to predict the surface area of an oviposition site on a pig, using the pig's weight. The locations of interest were identified during data collection based on a numerical scale (adapted from Rosati, 2014). These locations included the (1) head, (2) the legs or between the legs, (3) the abdomen, and (4) the cracks. Oviposition locations 1, 2 and 3 will be referred to as traditional oviposition sites for the remainder of this study. The methods used by Pacheco *et al.* (submitted) are given in brief below. The weights of 13 pigs were recorded (ranging from 454 g to 2177 g). Surface area estimates were made by photographing the oviposition sites using a Nikon D70 camera with an AF Micro-Nikkor 60 mm f/2.8D lens, perpendicular to the surface of the pig (at a 90° angle) (Pacheco *et al.*, submitted). A 15 cm ruler was used in each picture for scale. If necessary, larger surfaces of the carcasses were photographed in sections. The pictures were uploaded to a computer and processed using ImageJ™ (<http://imagej.nih.gov/ij/index.html>). A 10 mm line was drawn over the plastic ruler in each picture using the STRAIGHT line tool and calibrated used the ANALYZE > SET SCALE function. The oviposition sites were outlined using the POLYGON SELECTION tool and the corresponding surface area (mm²) of the selected region was calculated using the ANALYZE > MEASURE function. Measurements were also made in ImageJ™ to estimate the internal surfaces of the head, such as the mouth and inner ear canal (e.g., the volume of a right circular cone). After all locations of interest had been measured, the relationship between surface area and body weight was determined using regression analysis (SAS Institute, 2011).

The regression equations (head: $y = 13.68x + 3304.10$; legs: $y = 7.67x + 6532.30$; abdomen: $y = 4.23x + 3081.30$) (see Pacheco *et al.*, submitted) were then used to estimate the surface area of each oviposition location, based upon the post-burn weight of the pig

carcass. Due to a lack of photographs, each crack was assumed to be a rectangle and surface area was calculated by multiplying the length (mm) of the crack by the width (mm) of the crack. The total area of the carcass available for oviposition was determined by adding the surface area of all four locations together. The proportion of the surface area available for oviposition that each location accounted for was calculated (e.g. Fig 5, CGS Level 3, *L. sericata*: Head = 45.11%, Legs = 35.34%, Abdomen = 18.38%, Cracks = 1.17%). The mean proportion of the carcass that each location accounted for was calculated using $n = 5$ pigs that were burnt for that CGS level and species treatment.

V) Oviposition Observations and Calculations of Oviposition Events

After transportation back to the University of Windsor, all four pigs, including the control (Level 0), were randomly assigned to and placed into one of the experimental cages, containing reproductively mature adult blow flies. The pigs remained in the cages for a period of 24 h. Hourly checks were performed during daylight hours, according to the diel cycle in the rearing room (Table 2.1). Temperatures were recorded hourly using a SmartButton Temperature recorder (ACR Systems Inc., Item ID #: 01-0180). The mean temperatures in the rearing room during the 24 h oviposition period are provided in Table 2.1. During the hourly observations, the remains were checked for the presence of new eggs masses, which indicated successful oviposition events and the positions of the egg masses on the carcass were recorded using the four position location scale as described above.

Oviposition preferences of female blow flies was evaluated by asking several questions. First, did the site on the body affect oviposition preference within a CGS level? To answer this question, the proportion of oviposition events that occurred at each of the four sites (described above) was calculated by dividing the number of events at each site by the total number of events recorded on that pig. The proportions calculated for each pig ($n = 5$) were used to calculate the mean proportion of oviposition events at each site for each CGS level for each species treatment.

Second, did the presence of cracks at a specific site between CGS levels, alter the preference of female blow flies for that site? Using the abdomen as an example, this question asked if the presence of cracks on the abdomen made it more attractive than an abdomen without cracks. To answer this question, the oviposition events at a particular

oviposition site were totalled across a replicate, for the CGS levels being compared; oviposition events that occurred in the cracks were further separated based on where the cracks were located on the body, such as on the face, between the legs or on the abdomen. The proportion of oviposition events was then calculated by dividing the number of events that occurred either within the cracks or on the traditional oviposition site by the total number of oviposition events (as described above). The proportions calculated for each ($n = 5$) were used to calculate the mean proportion of oviposition events for each traditional oviposition site when it was compared to the mean oviposition proportion that occurred in cracks on the same location at a higher CGS level. Oviposition events were compared between CGS Level 1 and Level 2 and between CGS Level 1 and Level 3. For example, the total number of oviposition events on the abdomen for carcasses burnt to CGS Level 1 was compared to oviposition events that occurred in cracks found on the abdomen on CGS Level 2 carcasses. A comparison between CGS Levels 2 and 3 was not made, as both of these levels contained cracks and, therefore, this comparison would not help to answer whether or not the presence of cracks was a factor in the change in oviposition preferences of female blow flies. CGS Level 0 was also not included in these comparisons, as these carcasses were not exposed to flame impingement unlike the other three CGS levels and, therefore, did not have the same opportunity to develop cracks. This was repeated for each species treatment.

VI) Egg Numbers

The number of eggs on each experimental carcass was calculated following methods outlined by Rosati *et al.* (in review), which are described briefly below. After the 24 h oviposition observation period, the pigs were removed from their cages and each egg mass was photographed at a 90° angle, using a Nikon D70 camera with an AF Micro-Nikkor 60 mm f/2.8D lens. A 15 cm ruler was in each picture for scale. Depth measurements (mm) were taken at different points within each photographed egg mass and recorded. The photographs were uploaded to a computer and imported to ImageJ™. A 10 mm line was traced over the plastic ruler using the STRAIGHT line tool. The straight line was calibrated using the ANALYZE > SET SCALE function. Using the previously recorded depths, the corresponding area on each egg mass was outlined using the POLYGON SELECTION tool. The surface area of the outlined area was calculated

using the ANALYZE > MEASURE function. The depth of the outlined section of the egg mass was multiplied by the observed surface area (mm^2) value, resulting in the volume of the outlined egg mass section. The volumes of each section on an egg mass were added together to provide the overall volume of the egg mass. The total number of eggs in a blow fly egg mass was calculated using species-specific regression equations (*L. sericata*: $y = 4.11x + 12.40$; *P. regina*: $y = 4.71x + 3.40$) developed by Rosati *et al.* (in review) to use the volume (mm^3) of an egg mass to predict the number of eggs. To produce a regression equation for the mixed species treatment (mixed: $y = 4.57x + 0.10$), the data sets of each individual species (Rosati *et al.*, in review) were combined and a regression was performed using the PROC REG function in SAS (SAS Institute, 2011).

VII) Statistical Analysis

In the mixed species treatment, it was difficult to determine which species was responsible for each oviposition event, and consequently resulting egg numbers. To compensate for this, the mixed species data (i.e., oviposition events and egg numbers) was compared to data for *L. sericata* alone, and for *P. regina* alone in separate analyses.

Oviposition Observation Temperatures:

Replicates (i.e., burns) within each species treatment were not completed at the same time. Instead they were carried out over a period of one year. To determine if there was a difference between the temperatures experienced by ovipositing females during the 24 h period in which they were exposed to the fetal pig carcasses throughout the fifteen different burn dates (Table 2.1), an ANOVA was performed using the PROC GLM function in SAS (SAS Institute, 2011). If a difference was noted between the temperatures experienced across the three species treatments, means comparison tests were performed using the LSMEANS function in SAS to determine which species treatments experienced statistically different temperatures (SAS Institute, 2011).

Burning – Mean Biomass Loss:

All mean biomass loss (\pm S.E.) data was square root transformed to meet the assumptions of normality and homogeneity of variance (normality: Shapiro-Wilk's test: $p > 0.05$, homogeneity of variance: Bartlett's Test: $p > 0.05$; SAS Institute, 2011). To

determine if CGS level had an effect on the amount of biomass lost after a burn, a two-factor analysis of variance (ANOVA) was performed using the PROC GLM function in SAS (SAS Institute, 2011). All figures and means reported use back-transformed data.

Surface Area and Oviposition Site Selection:

A chi-squared analysis was performed using PROC FREQ in SAS (SAS Institute, 2011) to compare the frequency of observed oviposition events at each site to the proportion of the total surface area that each location accounted for. This analysis was used to determine if the surface area of site affected oviposition preference by female blow flies. For this analysis, if the proportion of oviposition events at a specific site was equal to the proportion of surface area represented by that site, it would be concluded that surface area did affect oviposition preference. Each of the four oviposition sites needed to have proportions that were positive, non-zero integers (SAS Institute, 2011). To ensure that observed oviposition frequencies with no oviposition events observed met this requirement, all data was coded by adding a single oviposition event to each site (Zar, 2010). Additionally, to ensure that the expected oviposition frequencies were positive, the data was coded by adding 0.1% to both observed and expected oviposition frequencies (Zar, 2010). All oviposition frequencies reported in Table 2.3 are the non-coded, original values.

Using the PROC REG function in SAS (SAS Institute, 2011), a linear regression was performed to determine if there was a relationship between the surface area of each oviposition site and the number of eggs oviposited.

As surface area had no influence on oviposition site selection (see Results), chi-square tests were used to compare the number of oviposition events that occurred on each site within each CGS level for each species treatment. Analyses were performed using PROC FREQ in SAS (SAS Institute 2011). For this analysis, oviposition sites with no oviposition events were excluded. For analyses with oviposition at all four sites, the expected frequency of oviposition for all sites was 25%; if sites were excluded due to lack of oviposition, the frequencies were adjusted as needed, based on the number of sites included in the analysis (for example, if oviposition occurred at three sites, the expected frequency for all sites was 33.3%). Significant chi-square tests were followed by ‘means

separation tests' for chi-square, or subdividing, following the procedure described by Zar (2010).

Again, if it was determined that surface had no influence on oviposition site selection, a separate chi-square analyses was also performed using PROC FREQ in SAS (SAS Institute, 2011), to compare the number of oviposition events, between CGS levels, that occurred on sites with no cracks to those same sites with cracks. For this analyses, as only two sites were compared between CGS levels, it was expected that the oviposition events at each site would be 50%. Traditional oviposition sites that did not contain cracks, and therefore had no oviposition events, were excluded from the comparisons. Including them in comparisons would require coding the data to include non-zero, positive integers, however this would result in a false positive result that would indicate cracks influenced shifts in oviposition preferences, when in fact there were no cracks (SAS Institute, 2011).

Egg Numbers:

The effect of oviposition site, CGS level, and species treatment on the number of eggs was determined using a three factor ANOVA using the GLM procedure in SAS (SAS Institute, 2011). This was performed separately for communities with *L. sericata* and communities with *P. regina*. Before proceeding with the ANOVA, surface area of the oviposition locations was investigated as a potential covariate. First, the PROC REG function in SAS (SAS Institute, 2011) was used to determine if there was a relationship between the surface area of each oviposition site and the number of eggs oviposited. Following significant regression results, surface area was included in the three-factor ANOVA model as a covariate. If surface area was a significant factor in the PROC GLM analysis, surface area was included as a covariate for all analyses. Otherwise, surface area was removed from the model and the three-factor ANOVA was conducted with no covariate. The data was square root transformed to meet the assumptions of normality and homogeneity of variance (normality: Shapiro-Wilk's test, $p > 0.05$; homogeneity of variance: Bartlett's test, $p > 0.05$) (SAS Institute, 2011). All figures and means presented are for the back-transformed data.

RESULTS

I) Oviposition Observation Temperatures

The temperatures experienced by the ovipositing females across all species treatments ranged from 21.3 and 23.7°C, and were not statistically different from one another ($F_{2, 372} = 0.80, p = 0.4493$) (Table 2.1). The mean temperatures (\pm S.E.) experienced by ovipositing *L. sericata*, *P. regina* and flies in the mixed species treatment were 22.3 ± 0.1 °C, 22.5 ± 0.2 °C and 22.3 ± 0.1 °C, respectively.

II) Burning – Surface Area and Oviposition Site Selection

The mean fire temperature (\pm S.E.) of fetal pig carcasses burnt to Level 1, Level 2, and Level 3 on the CGS were 648.3 ± 16.8 °C, 696.3 ± 14.6 °C and 702.3 ± 19.4 °C, respectively. The amount of biomass lost increased as CGS level increased ($F_{1, 42} = 68.95, p < 0.0001, R^2 = 0.61$; Figure 2.1). The mean biomass lost for fetal pig carcasses burnt to CGS Level 1, Level 2 and Level 3 were 51.7 ± 12.9 g, 97.0 ± 14.9 g and 391.8 ± 45.1 g, respectively (Figure 2.1). The overall ANOVA revealed that there were differences between the amounts of biomass lost between CGS levels ($F_{2, 42} = 38.58, p < 0.0001$). There was no difference in the mean biomass loss between pigs burnt to CGS Level 1 and Level 2 ($p = 0.6755$), however pigs burnt to CGS Level 3 lost more biomass than those burnt to Level 1 and Level 2 ($p < 0.0001$; Figure 2.1).

The mean total surface area (\pm S.E.) available for oviposition across the five pigs used per burn level within each species treatment ranged from a mean of 44034.5 ± 2216.0 mm² to 56769.4 ± 1453.7 mm² (Table 2.2). The head comprised the greatest proportion of the surface area available for oviposition, followed by the legs, and finally the abdomen (Table 2.3). Cracks were only consistently present on CGS Level 2 and Level 3 carcasses, although some were present at Level 1 for pigs used in the mixed species treatments (Table 2.3). The number of cracks increased with CGS level starting at Level 1 such that pigs burnt to a Level 1, Level 2 and Level 3 on the CGS had a mean (\pm S.E.) of 0.1 ± 0.1 , 1.8 ± 0.3 and 3.8 ± 0.1 cracks per carcass, respectively. The majority of the cracks appeared in the crease where the legs meet the abdomen.

Surface area was not the primary determinant of oviposition site selection as the frequency of oviposition events at each site differed from the expected frequency of

oviposition events if selection had been based entirely on the surface area occupied of each site (Table 2.3). This was true for all species treatments across all burn levels. As surface area was not a primary factor influencing the oviposition site selection of female blow flies, the effect of oviposition site and CGS level on oviposition events was analysed. Blow flies exhibited an unequal preference for the different oviposition sites, regardless of CGS level and species treatment (Table 2.4). When pig carcasses were burnt to CGS Level 3, there was a shift in preference within CGS this particular level, of female blow flies (*L. sericata* alone, *P. regina* alone, and mixed species) to include oviposition in the cracks created via flame impingement, with a corresponding reduction in oviposition frequency at other locations (Table 2.4). On the control carcasses (Level 0) and those burnt to CGS Levels 1 and 2, *L. sericata* preferred to oviposit on the head (Table 2.4). However, the presence of cracks starting at CGS Level 2 resulted in a shift in oviposition frequency preference for *L. sericata* to include these cracks and at CGS Level 3, the majority of oviposition events occurred in the cracks (Table 2.4).

In contrast to *L. sericata*, despite the presence of cracks on carcasses burnt to CGS Level 2, *P. regina* did not shift to ovipositing in the cracks within a CGS level until the carcasses were burnt to CGS Level 3 (Table 2.4), with twice as many cracks present. With carcasses burnt to CGS Level 3, *P. regina* oviposited predominately on the cracks followed by the abdomen and lastly on the head (Table 2.4).

Despite the presence of cracks on some carcasses burnt to CGS Level 1 for the mixed species treatment, neither blow fly species shifted their behaviour to oviposit on the cracks. When more cracks were present on CGS Level 3 carcasses, the highest frequency of oviposition was observed on the cracks (Table 2.4). As observed in the single species treatments, the predominant oviposition location was the head when carcasses were burnt to low levels of the CGS.

There was a difference in the number of oviposition events by *L. sericata* between CGS levels on the legs of burnt carcasses when cracks were present and absent (Table 2.5 and Table 2.6). Specifically, on CGS Level 2 carcasses, *L. sericata* preferred to oviposit on the legs, but did not prefer to oviposit on the legs of CGS Level 1 carcasses (Table 2.5). The only difference between the carcasses at these two CGS levels was the presence of cracks on the legs of CGS Level 2 carcasses.

III) Egg Numbers

Regression analysis revealed a positive and significant relationship between egg number and surface area (*L. sericata*: $F_{1, 156} = 36.70$, $p < 0.0001$; *P. regina*: $F_{1, 155} = 23.82$, $p < 0.0001$). This relationship accounted for less than 20% of the variation in the data (*L. sericata*: $R^2 = 0.19$; *P. regina*: $R^2 = 0.13$). As expected, as the surface area of an oviposition site increased, total egg number also increased. Surface area was initially included in the three-factor ANOVA as a covariate, but had no significant effect (*L. sericata*: $p = 0.9863$; *P. regina*: $p = 0.8785$). Therefore, surface area was excluded from the model for the remainder of the analyses.

Species composition, CGS level and oviposition site had an effect on the number of *L. sericata* eggs deposited on pig carcasses exposed to *L. sericata* and *L. sericata* + *P. regina* ($F_{31, 126} = 2.97$, $p < 0.0001$) (Table 2.7). Specifically, the number of eggs deposited by *L. sericata* was influenced by an interaction of CGS level and oviposition site (Table 2.7; Figure 2.2). On carcasses burnt to CGS Level 3, where cracks were the most prominent, there were less eggs on the cracks, despite their being more oviposition events at the cracks (Table 2.4; Figure 2.2). Overall, there were more eggs oviposited on the head for all CGS levels (Figure 2.3), with an increase in eggs laid in the cracks for CGS Level 2 (Figure 2.2). There was no effect of species composition on the number of eggs laid by *L. sericata*.

Similarly, species composition, CGS level and oviposition site had an effect on the number of *P. regina* eggs deposited by *P. regina* and *P. regina* + *L. sericata* ($F_{31, 125} = 3.20$, $p < 0.0001$) (Table 2.7). CGS level, oviposition site and species composition interacted to influence the number of eggs oviposited by *P. regina* as indicated by the significant three-way interaction (Table 2.7, Figure 2.4). When exposed to carcasses burnt to CGS Level 3, *P. regina* deposited the highest number of eggs, while ovipositing them in the cracks (Figure 2.4D). In single species treatments, the mean number of eggs oviposited in the cracks on carcasses burnt to CGS Level 3 by *P. regina* was 61 times more than the three remaining locations combined. Even with cracks present on CGS Level 2 carcasses, there were no eggs oviposited at these locations (Figure 2.4C). With the exception of CGS Level 0 (Figure 2.4A), all remaining CGS levels had the most eggs oviposited on the head (Figure 2.3, Figure 2.4B, Figure 2.4C).

Phormia regina was the only species to have the number of eggs oviposited influenced by the presence of heterospecifics. There were noticeable shifts in the number of eggs oviposited at the four different oviposition sites by *P. regina* when in the presence of *L. sericata*; this was true for all CGS levels (Figure 2.4). On CGS Level 3 carcasses, *P. regina* went from ovipositing all of their eggs on the cracks when alone, to depositing almost no eggs at this location when in the presence of *L. sericata* (Figure 2.4D). Based on the number of eggs present when faced with heterospecifics, the oviposition location preferences for *P. regina* shifted to match those of *L. sericata* (Figure 2.2, Figure 2.4).

DISCUSSION

Previous studies have demonstrated that the surface area of possible oviposition sites affects the oviposition behaviour of some insects (Cope and Fox, 2003; Holland *et al.*, 2004). However, in this particular study, surface area was not a primary factor affecting oviposition site selection by female blow flies. Rather, other factors, such as the suitability of each oviposition site are probably driving the oviposition behaviour of *L. sericata* and *P. regina*. For example, other authors including Avila and Goff (1998) and Vanin *et al.* (2007) have suggested that cracks in the skin that result from burning help to facilitate blow fly oviposition and might act as suitable oviposition sites.

It was initially predicted that *L. sericata* would prefer to oviposit in the cracks, due to their preference for wet oviposition locations (Grassberger and Reiter, 2001). As expected, in the presence of conspecifics the cracks were the preferred oviposition locations of *L. sericata*, but only when carcasses were burnt to a CGS Level 3, as indicated by a higher oviposition frequency (i.e., more oviposition events) at this CGS level relative to the other levels of the CGS. There were also cracks present on carcasses burnt to CGS Level 2, but these cracks were not selected as oviposition sites. Rather, *L. sericata* showed a preference for ovipositing on the head, contrary to initial predictions. Cracks present on CGS Level 2 carcasses were not as severe, and therefore, were not as wet as those on CGS Level 3 carcasses, which could result in female *L. sericata* rejecting the cracks in favour of the head where natural orifices are generally wet. On carcasses where cracks did not affect preference, *L. sericata* females preferred to oviposit on the

head, which is similar to the results of previous research (Rosati, 2014). Similar to *L. sericata*, in the presence of conspecifics *P. regina* females demonstrated a preference for ovipositing on the cracks when carcasses were burnt to CGS Level 3, instead of simply near the cracks as predicted. Although cracks were also present on CGS Level 2 carcasses, they were avoided by *P. regina*. For all other CGS levels tested, *P. regina* preferred ovipositing on the head, followed by sites between the legs and on the abdomen, which supports initial predictions and previous research (Rosati, 2014).

As it was difficult in mixed species treatments to determine which oviposition events belonged to which species, only broad conclusions can be made. It is known that both species successfully oviposited in the mixed species treatments because adult flies of both species emerged and were collected at the end of the experiment. The conclusions that follow were made by comparing the oviposition frequencies in mixed treatments to those of the two single species treatments; mixed species oviposition patterns were more similar to those of *L. sericata* alone. Overall, female flies in mixed species treatment only oviposited on the cracks when presented with carcasses burnt to CGS Level 3. At all other CGS levels, they preferred to oviposit on the head. If the oviposition events belonged to *L. sericata*, these results match initial predictions for CGS Level 3, but not for CGS Level 2. When carcasses were un-burnt (control) or burnt to CGS Level 1, the results support those of previous observations (Rosati, 2014). If the oviposition events were a result of *P. regina* females, then the observations presented here match the initial prediction and support previous research that indicated *P. regina* would change their oviposition behaviours to match those of *L. sericata* (Rosati, 2014).

Cracks created due to flame impingement can occur at any location on the carcass, therefore, it was expected that a change in frequency of oviposition events on the same location of the carcass between CGS levels with and without cracks would also be an indication of a shift in female preference from traditional sites to the cracks. A shift of this nature was observed for *L. sericata*, in which case there were fewer oviposition events observed at the traditional sites in favour of the cracks when cracks were present between or on the legs. This only occurred, however, when comparing CGS Level 1 carcasses to CGS Level 2 carcasses. It is speculated that this was due to size of the cracks that formed on the legs. These cracks were generally smaller than those found on the

abdomen, which was a traditional oviposition location that did not see a shift towards higher oviposition frequencies. Larger cracks, such as those found on the abdomen, have the potential to exude more bodily fluids making them wetter compared to smaller cracks which do not exude a higher volume of fluids. Although *L. sericata* may prefer wetter sites, such as larger cracks found on CGS Level 3 carcasses, this preference can be balanced with the risks of egg and larval drowning. Overall, the influence of cracks on female site selection for oviposition, between CGS levels was species and site specific.

The choice of where to oviposit is only one aspect of oviposition behaviour as females can also choose how many eggs to deposit at each site. Therefore, in addition to the number of oviposition events at each site, the number of eggs deposited at each site was also used to evaluate how suitable female blow flies perceived the four different oviposition sites to be for their offspring. It was initially predicted that cracks would be the most suitable oviposition sites for *L. sericata*, resulting in more oviposition events at the cracks. However, across all CGS levels, including Level 3 where cracks were the preferred oviposition site in terms of oviposition events, more eggs were deposited on the head. This result was unexpected and contrary to initial predictions. This was also surprising given cracks are similar to wounds, which are known to be preferred by blow fly species, particularly *L. sericata* (Cragg, 1955; Thomas and Mangan, 1989; Hall and Doisy, 1993; Byrd and Allen, 2001; Grassberger and Reiter, 2001). More eggs being deposited on the head was also seen when the effects of CGS level were excluded. At the cracks, this result may indicate that the optimum larval density of *L. sericata* is lower, which is in agreement with initial predictions. As predicted, the amount of biomass on carcasses decreased as CGS level increased, however CGS level alone did not influence the amount of eggs deposited by female *L. sericata*. This did not agree with initial predictions. Also contrary to initial predictions, the amount of eggs oviposited by *L. sericata* was not influenced by interactions with herteospecifics. *Phormia regina* displayed more complex oviposition patterns compared to *L. sericata*, as their egg laying behaviour was influenced by a number of factors, including both the presence of heterospecifics and conspecifics. The only instance in which *P. regina* deposited more eggs in the cracks, compared to any other oviposition site, was in the presence of conspecifics and on carrion burnt to CGS Level 3, indicating a higher optimal larval

density of this species, which agrees with the initial predictions. Avila and Goff (1998) also reported seeing larger eggs masses, and therefore, more eggs in the mouth, on the abdomen and on the legs of burnt remains. This trend was also observed in this study, except for when *P. regina* was alone on carcasses burnt to CGS Level 3.

Looking at the combination of oviposition frequency and egg numbers, the results of this study indicate the suitability of the cracks as oviposition locations is species and CGS level specific. Optimal oviposition states that females will choose oviposition locations to benefit the survival of their offspring (Jaenike, 1978). Although *L. sericata* oviposited more frequently in the cracks on CGS Level 3 carcasses, they are not laying more eggs in these locations. The increased number of eggs at traditional oviposition locations, rather than cracks, suggests that cracks may not be beneficial for offspring, perhaps due to increased risks of drowning. That there were more oviposition events at the cracks suggests that females are visiting the crack more often, however, this may be to feed. This makes sense, as cracks provide easy access to both a protein and sugar source, which are both vital to blow fly survival and reproductive development (Erzinçlioğlu, 1996; Avila and Goff, 1998; Byrd and Castner, 2010). Other blow fly species, such as the screw worm fly, (*Cochliomyia hominivorax* Coquerel) have been observed visiting wounds more frequently to feed, rather than to oviposit (Guillot *et al.*, 1977; Thomas and Mangan, 1989). *Lucilia sericata* may prefer lower densities as predicted, and in conjunction with their increased developmental plasticity, can survive better when conditions are poorer, such as in the conditions found in cracks.

One possible explanation for the disparity in oviposition preference and deposited egg numbers observed in this study is skip oviposition (Mogi and Mokry, 1980). This particular oviposition behaviour has been observed in certain species of mosquitoes in which a female will deposit her batch of eggs across numerous suitable locations, rather than depositing them all in one location (Mogi and Mokry, 1980). Even if cracks were an optimal location for *L. sericata* oviposition, the benefits of egg aggregation previously discussed could lead to increased egg numbers at less optimal locations such as the legs or abdomen. Opposite to *L. sericata*, the cracks appear to be an optimal oviposition location for *P. regina*, but only on CGS Level 3 carcasses as there was an increase in both oviposition event frequency and egg numbers at the cracks for carcasses exposed to such

severe flame impingement. It was predicted that *P. regina* larvae prefer higher larval densities for the greatest chance at success. It appears that these conditions might be met by ovipositing on the cracks.

In theory, cracks should represent an optimal spot for oviposition. It is difficult for the larvae of certain forensically important flies, such as flesh flies (Diptera: Sarcophagidae), to pierce the skin of humans (Haufe and Nelson, 1957). Cracks created via flame impingement eliminate the need for immature larvae, especially first instar larvae, to pierce the skin. By skipping this step larvae should expend less energy, and thus decrease the amount of energy that developing larvae need to use when feeding. Although this may be the case, high larval mortality associated with the cracks was also observed, usually via drowning (V.A. Pacheco, personal observation). Therefore, seeping wounds, such as cracks, may also represent a potential hazard that can be detrimental to larval survivorship. Blow fly eggs stand a greater chance of survival in cracks than larvae, due to physiological characteristics that make them more resistant to wet conditions. Specifically, each egg possesses a central breathing groove made of a porous material that traps air and repels water in wet conditions (Erzinçlioğlu, 1996). However, studies have also shown that eggs containing un-hatched Dipteran larvae, including *L. sericata*, can still breathe and can inhale surrounding liquids, leading to death (Sikes and Wigglesworth, 1931; Kalis, 1938). Female blow flies might be aware of the potential dangers associated with bloody cracks, and therefore, make the decision to oviposit elsewhere if the cracks are too wet, providing their offspring with the greatest chance of survival as predicted by optimal oviposition theory (Jaenike, 1978).

Determining whether or not blow flies prefer ovipositing in cracks, rather than traditional locations was possible for carcasses burnt to CGS Levels 2 and 3. At these levels, particularly Level 3, cracks are prominent, however, cracking has been reported in studies where carrion was burnt to Level 2 (Avila and Goff, 1998; Vanin *et al.*, 2007). Some cracks were observed on carcasses burnt to CGS Level 1; however, these occurred when the carcasses were handled and no oviposition events were observed on these cracks. Although burning a carcass can lead to more oviposition sites due to the appearance of cracks, burning can also destroy other potential oviposition sites. Locations on the head, such as the ears, become badly burnt as the CGS level increases

and can lead to scenarios where oviposition is no longer possible at those sites.

This study attempted to verify the assumption that cracks on carrion, created as a result of flame impingement, provided suitable and alternative oviposition sites for ovipositing female blow flies. It has been experimentally shown, however, that the oviposition behaviour of *L. sericata* and *P. regina* is species and CGS level specific. Each blow fly species has a different strategy when dealing with burnt carrion. Cracks that result from flame impingement are not an optimal oviposition location for *L. sericata*, whereas cracks on CGS Level 3 carcasses are suitable for *P. regina*. However, the potential for offspring death via drowning in bodily fluids resulting from severe flame impingement might be a deterrent keeping female blow flies from depositing larger numbers of eggs on cracks. In the future, it would be helpful to test this assumption by manipulating fluid levels at the cracks and observing female preference for 'wet' and 'dry' cracks on equivalent locations of the carcass. Future work should also incorporate field validation tests, as the results obtained under controlled conditions might differ from those experienced under natural conditions.

Table 2.1: The dates of each burn for each species treatment and the time the carcasses were placed into the experimental cages (n = 5 burn events for each species treatment). Pig carcasses were removed from the experimental cages 24 h later, following the oviposition observation period. Daylight hours in the rearing room occur between 7am and 7pm. The mean temperature (\pm S.E.) in the rearing room and experienced by ovipositing female blow flies during the 24 h oviposition observation period are provided.

Replicate	Date	Time	Temperature ($^{\circ}$ C)
<i>Lucilia sericata</i>			
Burn 1	May 16-17, 2013	12 pm	22.2 \pm 0.2
Burn 2	June 5-6, 2013	11 am	22.4 \pm 0.2
Burn 3	Aug. 21-22, 2013	11 am	23.1 \pm 0.2
Burn 4	Sept. 3-4, 2013	11 am	21.5 \pm 0.0
Burn 5	Feb. 3-4, 2014	11 am	22.1 \pm 0.3
<i>Phormia regina</i>			
Burn 1	Aug. 5-6, 2013	12 pm	21.3 \pm 0.1
Burn 2	Aug. 5-6, 2013	12 pm	21.3 \pm 0.1
Burn 3	Sept. 15-16, 2013	12 pm	22.4 \pm 0.2
Burn 4	Feb. 26-27, 2014	10 am	23.7 \pm 0.2
Burn 5	Feb. 26-27, 2014	10 am	23.7 \pm 0.2
Mixed – <i>Lucilia sericata</i> and <i>Phormia regina</i>			
Burn 1	May 31 – June 1, 2013	11 am	22.4 \pm 0.1
Burn 2	Sept. 15-16, 2013	12 pm	22.4 \pm 0.2
Burn 3	Feb. 3-4, 2014	11 am	22.1 \pm 0.3
Burn 4	March 24-25, 2014	10 am	22.0 \pm 0.2
Burn 5	April 18-19, 2014	10 am	22.6 \pm 0.1

Table 2.2: The mean surface area (mm²) (\pm S.E.), estimated using ImageJ™, for each of the four oviposition sites and the total surface area available on a fetal pig carcass for each CGS level and a control (n = 5 for each burn level and each species treatment). The percentage that each oviposition site accounted for on each fetal pig is the second number provided in each cell of the table.

CGS Level	Oviposition Sites				
	Total	Head	Legs	Abdomen	Cracks
<i>Lucilia sericata</i>					
Level 0	46860.8 \pm 6265.4	21451.9 \pm 3349.8	16711.6 \pm 1878.9	8697.3 \pm 1036.6	--
	100%	45.7%	35.7%	18.6%	
Level 1	46952.0 \pm 2380.0	21501.2 \pm 1272.5	16739.3 \pm 713.8	8712.5 \pm 393.8	--
	100%	45.8%	35.7%	18.6%	
Level 2	46403.5 \pm 2015.7	21112.6 \pm 1072.7	16557.7 \pm 572.7	8592.3 \pm 332.0	141.0 \pm 47.6
	100%	45.5%	35.7%	18.5%	0.3%
Level 3	44034.5 \pm 2216.0	19769.0 \pm 1188.4	15767.7 \pm 666.6	8176.5 \pm 367.8	321.3 \pm 93.4
	100%	44.9%	35.8%	18.6%	0.7%
<i>Phormia regina</i>					
Level 0	56769.4 \pm 1453.7	26749.6 \pm 777.2	19683.2 \pm 8802.6	10336.7 \pm 240.5	--
	100%	47.1%	34.7%	18.2%	
Level 1	46528.1 \pm 3779.8	21274.0 \pm 2020.9	16611.9 \pm 7429.0	8642.2 \pm 625.4	--
	100%	45.7%	35.7%	18.6%	
Level 2	48286.2 \pm 4081.3	22177.1 \pm 2178.8	17118.4 \pm 7655.6	8921.7 \pm 674.3	69.1 \pm 15.7
	100%	45.9%	35.5%	18.5%	0.1%
Level 3	49645.4 \pm 3612.2	22707.9 \pm 1916.8	17416.1 \pm 7788.7	9086.0 \pm 593.2	435.4 \pm 67.0
	100%	45.7%	35.1%	18.3%	0.9%
Mixed – <i>Lucilia sericata</i> and <i>Phormia regina</i>					
Level 0	51206.1 \pm 6753.8	23775.1 \pm 3610.9	18014.7 \pm 2025.4	9416.2 \pm 1117.4	--
	100%	46.4%	35.1%	18.4%	
Level 1	48585.5 \pm 2824.0	22344.0 \pm 1506.6	17212.0 \pm 845.1	8973.3 \pm 466.2	56.1 \pm 39.0
	100%	46.0%	35.4%	18.5%	0.1%
Level 2	52900.6 \pm 2433.3	24601.5 \pm 1313.2	18478.3 \pm 736.6	9672.0 \pm 406.4	148.8 \pm 51.9
	100%	46.5%	34.9%	18.3%	0.3%
Level 3	47993.5 \pm 4519.2	21897.9 \pm 2415.7	16961.8 \pm 1355.0	8835.3 \pm 747.6	298.4 \pm 44.4
	100%	45.6%	35.3%	18.4%	0.6%

Table 2.3: The mean expected oviposition frequencies (%) (\pm S.E.), based on the total surface area of each fetal pig and the area of the carcass occupied by each oviposition site. Within each cell the observed oviposition frequencies are reported beneath the expected frequencies. One oviposition event was added to all observations, so that sites with no oviposition events could be included in the analysis, so d.f. =3 for all analyses; non-coded frequencies are reported. For all CGS levels, $\alpha = 0.05$.

CGS Level	Oviposition Sites				X^2	d.f.	p-value
	Head	Legs	Abdomen	Cracks			
<i>Lucilia sericata</i>							
Level 0	45.7 \pm 0.9	35.7 \pm 0.7	18.6 \pm 0.2	0	695.9741	3	< 0.0001
	78.5 \pm 7.5	13.7 \pm 6.5	7.8 \pm 3.7	0			
Level 1	45.8 \pm 0.4	35.7 \pm 0.3	18.6 \pm 0.1	0	716.2700	3	< 0.0001
	70.5 \pm 7.8	7.5 \pm 5.0	22.0 \pm 9.3	0			
Level 2	45.5 \pm 0.4	35.7 \pm 0.3	18.5 \pm 0.1	0.3 \pm 0.1	1693.3540	3	< 0.0001
	60.8 \pm 12.2	16.7 \pm 10.5	0	22.5 \pm 15.0			
Level 3	44.9 \pm 0.4	35.8 \pm 0.3	18.6 \pm 0.1	0.7 \pm 0.2	1074.0716	3	< 0.0001
	33.3 \pm 9.1	10.0 \pm 10.0	12.2 \pm 9.7	44.4 \pm 17.3			
<i>Phormia regina</i>							
Level 0	47.1 \pm 0.2	34.7 \pm 0.1	18.2 \pm 0	0	1060.3225	3	< 0.0001
	50.8 \pm 20.1	38.6 \pm 15.9	10.5 \pm 5.4	0			
Level 1	45.7 \pm 0.8	35.7 \pm 0.6	18.6 \pm 0.2	0	742.2973	3	< 0.0001
	64.0 \pm 12.3	20.1 \pm 6.3	15.8 \pm 8.2	0			
Level 2	45.9 \pm 0.6	35.5 \pm 0.5	18.5 \pm 0.2	0.1 \pm 0	413.0074	3	< 0.0001
	51.4 \pm 13.5	34.7 \pm 12.8	13.9 \pm 7.0	0			
Level 3	45.7 \pm 0.5	35.1 \pm 0.3	18.3 \pm 0.1	0.9 \pm 0.1	1751.6439	3	< 0.0001
	14.0 \pm 6.4	0	29.3 \pm 18.1	56.7 \pm 23.3			
Mixed – <i>Lucilia sericata</i> and <i>Phormia regina</i>							
Level 0	46.4 \pm 1.3	35.1 \pm 0.9	18.4 \pm 0.3	0	1274.5941	3	< 0.0001
	72.3 \pm 9.0	21.8 \pm 0.1	5.9 \pm 3.8	0			
Level 1	46.0 \pm 0.5	35.4 \pm 0.4	18.5 \pm 0.1	0.1 \pm 0.1	370.4798	3	< 0.0001
	74.9 \pm 11.3	13.0 \pm 8.2	12.1 \pm 5.2	0			
Level 2	46.5 \pm 0.4	34.9 \pm 0.2	18.3 \pm 0.1	0.3 \pm 0.1	1300.9476	3	< 0.0001
	55.6 \pm 16.0	13.7 \pm 5.7	10.1 \pm 4.6	20.6 \pm 12.9			
Level 3	45.6 \pm 0.7	35.3 \pm 0.5	18.4 \pm 0.2	0.6 \pm 0.1	1822.2180	3	< 0.0001
	41.6 \pm 14.4	0	6.5 \pm 4.0	51.9 \pm 15.7			

Table 2.4: The mean oviposition frequencies (%) (\pm S.E.) observed within each CGS level for each species treatment. For analysis, the expected oviposition frequency was adjusted depending on how many sites had oviposition events. In each row of the table, oviposition frequencies with different letters were statistically different. For all CGS levels, $\alpha = 0.05$.

CGS Level	Oviposition Sites				X^2	d.f.	p-value
	Head	Legs	Abdomen	Cracks			
<i>Lucilia sericata</i>							
Level 0	78.5 \pm 7.5 a	13.7 \pm 6.5 b	7.8 \pm 3.7 c	0	92.4267	2	< 0.0001
Level 1	70.5 \pm 7.8 a	7.5 \pm 5.0 c	22.0 \pm 9.3 b	0	65.4388	2	< 0.0001
Level 2	60.8 \pm 12.2 a	16.7 \pm 10.5 c	0	22.5 \pm 15.0 b	34.5675	2	< 0.0001
Level 3	33.3 \pm 9.1 b	10.0 \pm 10.0 d	12.2 \pm 9.7 c	44.4 \pm 17.3 a	33.4286	3	< 0.0001
<i>Phormia regina</i>							
Level 0	50.8 \pm 20.1 a	38.6 \pm 15.9 b	10.6 \pm 5.4 c	0	25.5392	2	< 0.0001
Level 1	64.1 \pm 12.3 a	20.1 \pm 6.3 b	15.8 \pm 8.2 c	0	42.7796	2	< 0.0001
Level 2	51.4 \pm 13.5 a	34.7 \pm 12.8 b	13.9 \pm 7.0 c	0	21.1091	2	< 0.0001
Level 3	14.0 \pm 6.4 c	0	29.3 \pm 18.1 b	56.7 \pm 23.3 a	28.0603	2	< 0.0001
Mixed – <i>Lucilia sericata</i> and <i>Phormia regina</i>							
Level 0	72.3 \pm 9.0 a	21.8 \pm 10.1 b	5.9 \pm 3.8 c	0	72.2914	2	< 0.0001
Level 1	74.9 \pm 11.3 a	13.0 \pm 8.2 b	12.1 \pm 5.2 c	0	77.8217	2	< 0.0001
Level 2	55.6 \pm 16.0 a	13.7 \pm 5.7 c	10.1 \pm 4.6 d	20.6 \pm 12.9 b	52.1476	3	< 0.0001
Level 3	41.6 \pm 14.4 b	0	6.5 \pm 4.0 c	51.9 \pm 15.7 a	34.0339	2	< 0.0001

Table 2.5: The mean (\pm S.E.) oviposition frequencies (%) and chi-square results to determine the effect of cracks at each of the traditional oviposition sites between CGS Level 1 and Level 2 for each species treatment. All *P. regina* comparisons were omitted due to the absence of oviposition events on cracks. For all oviposition sites, $\alpha = 0.05$.

Oviposition Site	Oviposition Frequency	X^2	d.f.	<i>p</i> -value
<i>Lucilia sericata</i>				
Legs	37.5 \pm 20.0	5.0000	1	0.0253
Cracks on legs	66.7 \pm 22.4			
Abdomen	53.3 \pm 16.2	8.8844	1	0.0029
Cracks on abdomen	26.7 \pm 11.3			
Mixed – <i>Lucilia sericata</i> and <i>Phormia regina</i>				
Legs	31.1 \pm 20.3	12.3432	1	0.0004
Cracks on legs	8.9 \pm 8.9			
Abdomen	58.3 \pm 18.7	1.6667	1	0.1967
Cracks on abdomen	41.7 \pm 15.8			

Table 2.6: The mean (\pm S.E.) oviposition frequencies (%) and chi-square results to determine the effect of cracks at each of the traditional oviposition sites between CGS Level 1 and Level 3 for each species treatment. For all oviposition sites, $\alpha = 0.05$.

Oviposition Site	Oviposition Frequency	X^2	d.f.	p -value
<i>Lucilia sericata</i>				
Legs	33.3 \pm 12.1	2.2244	1	0.1358
Cracks on legs	46.7 \pm 22.6			
Abdomen	55.0 \pm 17.4	1.0000	1	0.3173
Cracks on abdomen	45.0 \pm 17.4			
<i>Phormia regina</i>				
Head	97.8 \pm 2.2	91.3171	1	< 0.0001
Cracks on head	2.2 \pm 2.2			
Legs	36.6 \pm 17.2	0.5882	1	0.4431
Cracks on legs	43.4 \pm 18.2			
Abdomen	48.6 \pm 22.4	3.6722	1	0.0553
Cracks on abdomen	31.4 \pm 20.4			
Mixed – <i>Lucilia sericata</i> and <i>Phormia regina</i>				
Legs	34.3 \pm 21.5	1.6302	1	0.2017
Cracks on legs	45.7 \pm 22.8			
Abdomen	33.3 \pm 16.0	0.7393	1	0.3899
Cracks on abdomen	26.7 \pm 13.8			

Table 2.7: Effect of species composition, CGS level and oviposition site on the number of eggs *L. sericata* and *P. regina* oviposited. Statistically significant effects are given in bold font. For all effects, $\alpha = 0.05$.

Effect	d.f.	F – value	p – value
<i>Lucilia sericata</i>			
Species composition	1	0.56	0.4569
CGS level	3	0.60	0.6141
Oviposition site	3	20.35	< 0.0001
Species composition * CGS level	3	0.19	0.9042
Species composition * Oviposition site	3	0.51	0.6788
CGS level * Oviposition site	3	2.34	0.0178
Species composition * CGS level * Oviposition site	9	0.60	0.7940
<i>Phormia regina</i>			
Species composition	1	0.06	0.8132
CGS level	3	0.94	0.4240
Oviposition site	3	11.33	< 0.0001
Species composition * CGS level	3	0.14	0.9352
Species composition * Oviposition site	3	1.98	0.1206
CGS level * Oviposition site	3	3.90	0.0002
Species composition * CGS level * Oviposition site	9	2.18	0.0275

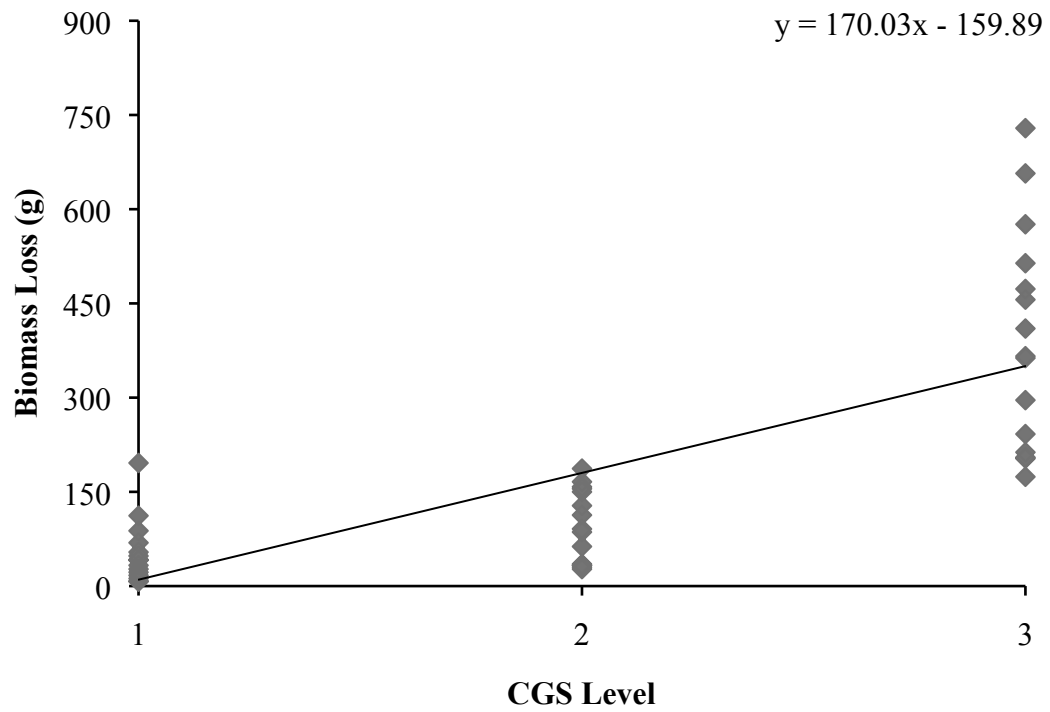


Figure 2.1: The total biomass lost for pig carcasses burnt to each CGS level used in this experiment. There was a significant, linear relationship between an increase in CGS level and the total biomass lost ($F_{1,42} = 68.95$, $p < 0.0001$, $R^2 = 0.61$).

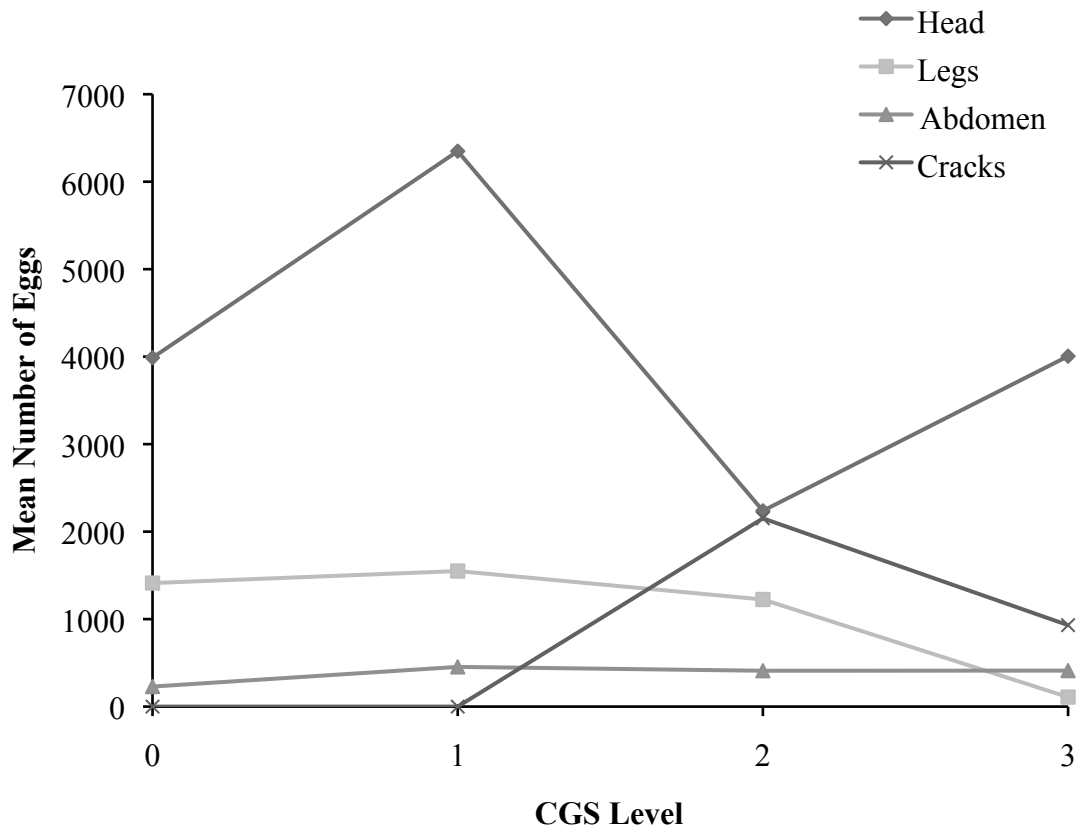


Figure 2.2: The effect of the two-way interaction between CGS level and oviposition site on the mean number of eggs oviposited by female *L. sericata* ($F_{9,126} = 2.34$, $p = 0.0178$). Note that differences in the number of eggs deposited at each oviposition site are much lower at CGS Level 2 than at the other CGS levels.

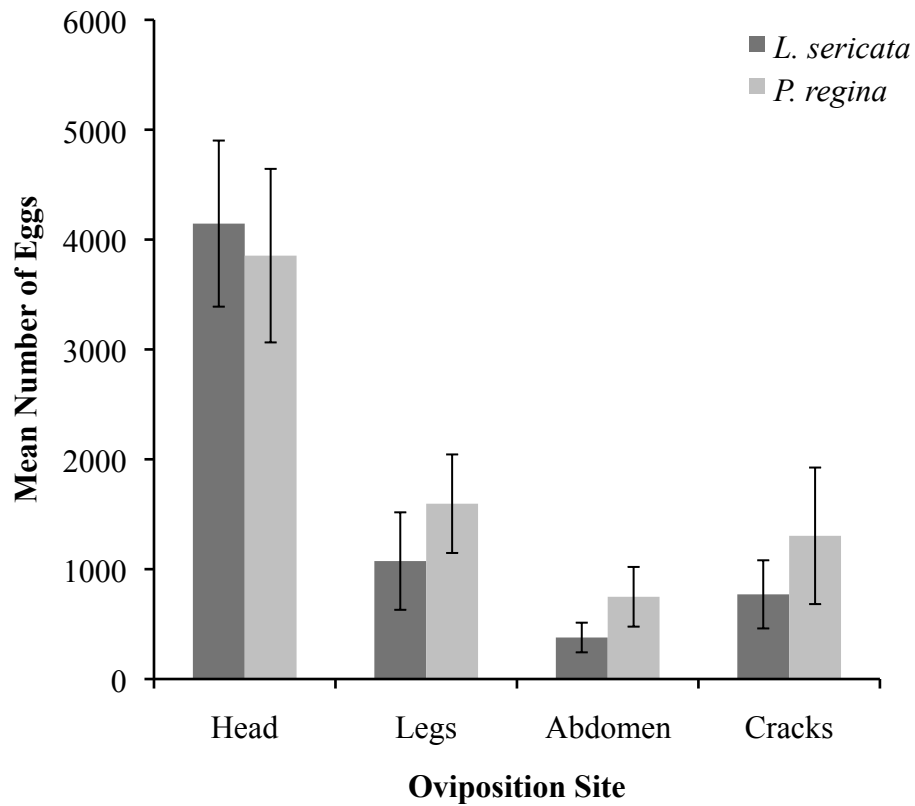


Figure 2.3: The mean number of eggs (\pm S.E.) on each oviposition site across all CGS levels, for *L. sericata* and *P. regina*. There was a significant effect of oviposition site and the number of eggs oviposited for *L. sericata* ($F_{3, 126} = 20.35, p < 0.0001$) and *P. regina* ($F_{3, 125} = 11.33, p < 0.0001$).

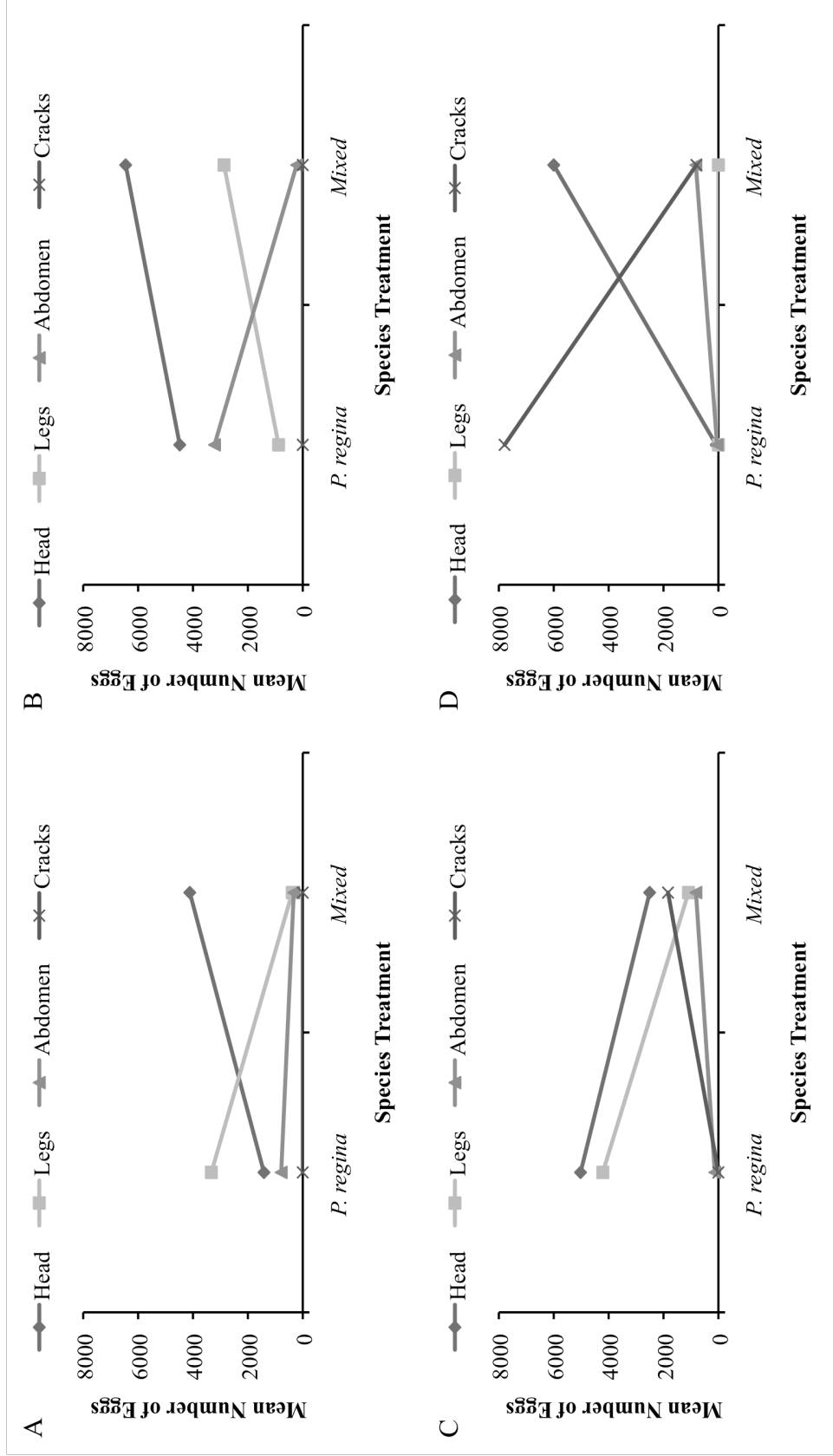


Figure 2.4: The effects of the three-way interaction of species composition, CGS level and oviposition site on the number of eggs *P. regina* oviposited ($F_{9, 125} = 2.18, p = 0.0275$). CGS level presented in panels A, B, C and D, represent CGS Level 0, Level 1, Level 2 and Level 3, respectively.

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CHAPTER 3:
SPECIES INTERACTIONS AND BURNT RESOURCES IMPACT SURVIVAL
RATES AND OFFSPRING PERFORMANCE OF *LUCILIA SERICATA* MEIGEN AND
PHORMIA REGINA MEIGEN (DIPTERA: CALLIPHORIDAE)

INTRODUCTION

Species interactions and natural disasters, such as forest fires, can act as stressors that may result in changes to animal fitness and survivorship. Although destructive, forest fires are beneficial ecosystem processes that induce ecosystem regeneration, increase biomass levels, and alter species composition and nutrient cycles (McCullough *et al.*, 1998; Nasi *et al.*, 2002). Over the past decade in Ontario, there have been an average of 1094 fires per year, which accounts for just under 110 000 ha of land affected (Aviation, Forest Fire and Emergency Services, 2014).

Although forest fires are perceived to have negative consequences, some animals have adaptations that allow them to benefit from fire. For example, certain insect families, such as jewel beetles (Coleoptera: Buprestidae), are defined as pyrophilous as they are attracted to fires (Linsley, 1943; Schmitz *et al.*, 1997; Schütz *et al.*, 1999; Klocke *et al.*, 2011). These beetles are attracted to fires as they rely on freshly burnt wood for food, mating opportunities, and oviposition sites (Wikars, 2002; Klocke *et al.*, 2011). Jewel beetles are known to travel up to 50 miles (80.5 km) to find burnt wood (Linsley, 1943; Schmitz *et al.*, 1997; Schütz *et al.*, 1999; Klocke *et al.*, 2011) and have been observed on logs that are still burning (Linsley, 1943; Klocke *et al.*, 2011). Similarly, blow flies (Diptera: Calliphoridae) have been observed on carrion that was still on fire (Avila and Goff, 1998). Aside from jewel beetles, there have been conflicting reports with regard to the effects of forest fires on insect populations (Swengel, 2001).

Researchers have investigated the responses of Dipterans to forest fires. Depending on both the season and sampling time, there have been no noticeable differences in fly populations observed after a forest fire (Rice, 1932; Bulan and Barrett, 1971; Hurst, 1971; Winter, 1984), however, other reports have shown that fly populations rebound faster after a fire (Hurst, 1971; Nagel, 1973; Van Amburg *et al.*, 1981; Winter,

1984; Moretti *et al.*, 2004; Durska *et al.*, 2010). For example, quick succession of plants after a fire increases the rate at which anthomyiid flies (Diptera: Anthomyiidae) and hover flies (Diptera: Syrphidae) re-establish their population numbers, as they rely on plants as a food source (Van Amburg *et al.*, 1981).

In 1988, devastating forest fires swept through Yellowstone National Park destroying 793 000 acres, or 36% of the area of the Park (United States National Park Service, 2014). These fires were directly responsible for the deaths of 345 elk, 36 deer, 12 moose, six black bears and nine bison (United States National Park Service, 2014). Similarly, forest fires in Russia have contributed to the death of 70 - 80% of squirrel populations, 15 - 25% of boar populations and 90% of mouse and rodent populations (Shvidenko and Goldammer, 2001). This increase in the carrion populations, as a result of fire in an area, can be highly attractive to carrion colonizing insects such as blow flies, which are capable of utilizing burnt remains as oviposition sites (Avila and Goff, 1998; Introna *et al.*, 1998; Pai *et al.*, 2007; Chin *et al.*, 2008; Vanin *et al.*, 2013; Chapter 2). Although blow flies are able to colonize burnt carrion (Chapter 2), their survival might be limited. Compared to un-burnt carrion, burnt carrion does not have the same biomass, and therefore, there is less consumable material for developing blow fly larvae. Although burnt carrion is attractive to female blow flies (Chapter 2), it might also have negative impacts on larval development and survival due to the magnification of interspecific and intraspecific competition associated with more limited resources. It is believed that until this study, there have been no publications that experimentally examine the survivorship rates and fitness levels of blow flies that have colonized burnt remains

Due to the scarcity of carrion resources and its ephemeral and patchy nature, egg aggregation is often observed in blow fly species (Ireland and Turner, 2006). The aggregation of eggs by female blow flies can benefit developing larvae as larger larval masses (maggot masses) can protect the maggots via thermal regulation by increasing the rate of development, as maggot masses have increased temperatures (Baxter and Morrison, 1983; Catts, 1992; Catts and Goff, 1992; Turner and Howard, 1992; Ireland and Turner, 2006; Kheirallah *et al.*, 2007; Anderson, 2010). This is important when ambient temperatures pose a risk to continued development. Essentially, maggot masses can rely on the heat they generate to ensure continued growth (Catts, 1992; Catts and

Goff, 1992; Anderson, 2010). Vulnerable stages, such as first instar larvae, also develop more quickly in maggot masses than when alone; this increased rate of development may help ensure their survival (Catts, 1992). Maggot masses and decreased developmental times of maggots have been observed on burnt carrion (Chin *et al.*, 2008). Larval aggregation also contributes to the faster breakdown of carrion resources as many maggots feeding in the same area increases the local concentration of proteolytic enzymes, which help in the pre-digestion process (Baxter and Morrison, 1983; S. dos Reis *et al.*, 1999; Ireland and Tuner, 2006; Kheirallah *et al.*, 2007).

Although there are clear benefits to larval aggregation, it is a risk for females to deposit large egg masses as large numbers of larvae can also have detrimental effects on blow fly offspring as a result of overcrowding and competition for limited resources (Ireland and Turner, 2006). Optimal oviposition theory (Jaenike, 1978) predicts that by depositing their clutches in locations that provide their offspring with the best chance at survival, female insects can increase their fitness. The selection of the oviposition site is important because like most insects, female blow flies do not play an active role in the development of their offspring as they do not practice parental care (Jaenike, 1978; Tallamy, 1999). The oviposition preference-offspring performance hypothesis is an extension of optimal oviposition theory and aims to determine if there are correlations between offspring performance (development, survivorship, fecundity, etc) and site selection by the female parent (Jaenike, 1978; Thompson, 1988; Ellis, 2008). Unfortunately, not all metrics of offspring performance correlate well with female fitness preference (Thompson, 1988; Jaenike, 1990; Mayhew, 1997). One important factor that can affect the correlation of female preference and offspring performance is larval density. Increased larval density has been observed to lead to a decrease in survival (Ellis, 2008). Therefore, while female insects might exhibit a preference for specific oviposition sites, those sites might not always be the best for the offspring. This might be particularly true for Diptera, including blow flies, as studies have shown density dependent effects on the survival and consequently the fitness of different larvae in habitats of varying quality (Hutton and Wasti, 1980; So and Dudgeon, 1990; Prinkkilä and Hanski, 1995; Saunders and Bee, 1995; Smith and Wall, 1997a; Ireland and Turner, 2006; Kheirallah *et al.*, 2007; Ellis, 2008).

The blow flies *Lucilia sericata* Meigen and *Phormia regina* Meigen colonize the same carrion, including burnt remains (Anderson and VanLaerhoven, 1996; VanLaerhoven and Anderson, 1999; Sharanowski *et al.*, 2008; Vanin *et al.*, 2013). Previously published reports have suggested that *L. sericata* is negatively affected by both interspecific and intraspecific competition (Ullyett, 1950; Hutton and Wasti, 1980; Cragg, 1995; Prinkkilä and Hanski, 1995; Kheirallah *et al.*, 2007; Smith and Wall, 1997a). *Lucilia sericata* have been shown to be inferior competitors when faced with interspecific competition from other blow fly species (Prinkkilä and Hanski, 1995; Smith and Wall, 1997a). In larger egg and larval densities with heterospecifics, *L. sericata* had a decrease in larval survival and adult fitness (Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007). Instances in which *L. sericata* have responded positively to, or have benefited from interspecific competition have occurred when they compete with *P. regina* (Hutton and Wasti, 1980; Rosati, 2014), with development and survival remaining unaffected. When competing with conspecifics (intraspecific competition), *L. sericata* larval survival and adult size has also been shown to decrease as larval and egg densities increase (Hutton and Wasti, 1980; Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007). It is common for female Dipterans to be smaller than their male counterparts, however, *L. sericata* females are known to be larger than males (Prinkkilä and Hanski, 1995; Smith and Wall, 1997a).

Phormia regina has also demonstrated mixed responses to larval density (Hutton and Wasti, 1980; Rosati, 2014). In the presence of heterospecifics, more specifically *L. sericata*, Hutton and Wasti (1980) observed complete elimination of *P. regina* larvae, regardless of density levels. However, the opposite was noted in recently completed studies by Rosati (2014), that demonstrated *P. regina* larvae survival and adult fitness were increased in the presence of *L. sericata*. In instances of higher conspecific larval density, *P. regina* has demonstrated negative effects in the form of lower adult emergence (Hutton and Wasti, 1980). Adult males were found to be larger in the lower larval densities, whereas female larval mortality, adult survival and fitness were not affected by intraspecific competition (Rosati, 2014).

Taken together, these previous studies suggest that the optimum larval density for *L. sericata* should be lower than that of *P. regina*. However, because of their ability to

successfully develop to adult and produce smaller adults in the presence of limited resources, higher larval density should impact fitness but not survival. In contrast, these previous studies suggest *P. regina* is less able to successfully develop to adult in the presence of higher competition for resources but may have a higher optimum larval density.

It has been shown that body size is directly related to numerous life history benefits, such as dispersal capabilities, mating success and egg load and development (Vogt *et al.*, 1985; Ellers *et al.*, 1998; Stoffolano Jr. *et al.*, 2000; Wardhaugh, 2001). For example, the female parasitic wasp *Asobara tabida* Nees (Hymenoptera: Braconidae) has been shown to disperse further as body size increases, while facilitating an increased egg capacity (Ellers *et al.*, 1998). There have also been reports of blow flies, specifically *P. regina*, benefiting from increased body size, as a larger head width has been correlated to greater mating success due to increased aedeagus size (Stoffolano Jr. *et al.*, 2000). Female body size has a positive relationship to egg maturation and number of developing oocytes, and therefore, egg load in *Lucilia* species (Vogt *et al.*, 1985; Wardhaugh, 2001).

The objective of this research was to investigate the effects that burnt remains have on both blow fly survival and fitness, while in the presence of both heterospecifics and conspecifics. It is predicted that as burn severity increases, as described by the Crow-Glassman Scale (CGS) (Glassman and Crow, 1996), the amount of consumable biomass will decrease. This, in turn, may increase competition for available resources. As previous studies have demonstrated, an increase in egg density, through egg aggregation by ovipositing females, may enhance these competitive effects.

Optimal oviposition theory leads to the prediction that *L. sericata* will oviposit on burnt carcasses, and when available, will oviposit directly in the cracks that result from severe flame impingement (Chapter 2). In the presence of conspecifics, this should lead to an increase in both larval survival and emerged adult fitness on carcasses with cracks (higher on the CGS), compared to those without cracks (lower on the CGS). The same trend is expected to be observed for *P. regina* when in the presence of conspecifics.

Finally, the oviposition preference-offspring performance leads to the prediction that the combination of increased flame impingement, heterospecific competition and an estimated lower optimum larval density will result in the decreased fitness of *L. sericata*,

whereas larval survival will remain unaffected as a result of increased developmental plasticity. In contrast, due to the facilitation from heterospecifics in the form of *L. sericata*, and a higher optimum larval density, *P. regina* will experience both increased larval survival and adult fitness when carcasses experience greater levels of flame impingement (higher levels on the CGS).

METHODS

I) Colony Maintenance

Lucilia sericata and *P. regina* colonies are maintained throughout the year at the University of Windsor, Windsor, Ontario, Canada. Blow flies are reared as described in Chapter 2. The colony cages are held under controlled laboratory conditions with a 12L:12D photoperiod, with mean temperatures (\pm S.E.) of 25 ± 1 °C, and 60 ± 5 % relative humidity. Adult blow flies are provided with sugar cubes, a paste made from instant milk powder and water (Anderson, 2000; Byrd and Allen, 2001; Nabity *et al.*, 2006). Adult flies for the experiments described below were all obtained from these source colonies by collecting eggs deposited by female flies on pork liver (ca. 20-30g) (Byrd and Allen, 2001).

II) Experimental Cage Treatments

Experimental cages were constructed five days prior to scheduled burn dates as thoroughly detailed previously (Chapter 2), and as briefly described herein. Newly emerged adult blow flies from the source colonies in rearing jars were placed in a refrigerator for 15 minutes to decrease their movement (Ricker *et al.*, 1986). Chilling streamlines the processes of physical manipulation and sexual identification (Ricker *et al.*, 1986). Males can be differentiated from females by the morphology of their compound eyes; the eyes of a male fly touch, while the eyes of a female fly do not (Erzinçlioğlu, 1996). Three species treatments were utilized for this study: (1) *L. sericata* only, (2) *P. regina* only and (3) *L. sericata* plus *P. regina*. For single species treatments, 100 females and 50 males were removed from rearing jars and added to the appropriate cage. To ensure population densities were consistent, mixed species treatments were composed of 50 females and 25 males of each species. Each replicate contained four

experimental cages; one cage each for the three carcasses burnt to CGS Levels 1, 2, and 3 and for a control carcass (Level 0) that was not exposed to flame impingement. Flies within each experimental cage were fed sugar and water *ad libitum*. Each experimental cage was protein fed by supplying pork liver (ca. 20-30g) for 1 h each day, for five days prior to burns; this was necessary to ensure females were gravid on the day the carcasses were burnt (VanLaerhoven and Anderson, 2001). As a result of adult blow fly availability and cage constraints, replicates were completed over a period of one year (Table 3.1).

III) Burning

Four deceased fetal pigs (*Sus scrofa domesticus* L.), weighing between 639 and 2247 g, were thawed one day prior to being burnt, as previously described (Chapter 2). All burns were conducted in a steel fire pit, measuring 89 x 55 cm, in Amherstburg, Ontario, Canada. Fires used to burn the pig carcasses were constructed using untreated scrap wood and newspaper. Pigs were burnt in descending order of the CGS, starting with Level 3. After being removed from the fire, a digital scale (Starfrit, Model #: 70200) was used to weigh each pig carcass in order to determine the total biomass lost.

IV) Larval Development and Sorting

Following the 24 h oviposition observation period (Chapter 2), the fetal pigs and their associated egg masses were removed from their cages and transported to the greenhouse on top of the Biology Building at the University of Windsor. Pig carcasses were placed inside separate glass aquaria that measured 90 x 32 x 61 cm and were lined with 2 to 3 cm of pine wood shavings. The shavings act as a dry pupation medium for wandering larvae (Hutton and Wasti, 1980). All aquaria were sealed with silicone caulking and landscape tarp (Quest Brands Inc., Item: WBS 50), which allowed for air exchange while still preventing any natural colonization by insects from outside the aquaria. The carcasses remained in the aquaria until after adult flies had emerged and died. The mean temperatures experienced by the developing blow flies within the greenhouse are reported in Table 3.1. Once all adult flies had emerged and died, the contents of the aquaria were collected and the dead adults were sorted by species and counted.

V) Egg Numbers and Survival Rates

Survival estimates were made by comparing the number of emerged adult flies to the number of eggs deposited by female blow flies using the regression equations developed by Rosati *et al.* (in review). Prior to being placed into the aquaria, all egg masses on each fetal pig were photographed using a Nikon D70 camera with an AF Micro-Nikkor 60 mm f/2.8D lens. Each picture was taken at approximately 90° with a 15 cm ruler in the photograph as a scale. Once photographed, each egg mass had depth measurements taken following Rosati *et al.* (in review) and as described in Chapter 2. The pictures were uploaded to a computer and surface area measurements were calculated using ImageJ™ (<http://imagej.nih.gov/ij/index.html>) as described by Rosati *et al.* (in review) (Chapter 2). The volume (mm³) of each egg mass was calculated by multiplying the surface area (mm²) by the depth. To estimate the number of eggs on each carcass the volume of each egg mass was substituted into the appropriate regression equations (*L. sericata*: $y = 4.11x + 12.40$; *P. regina*: $y = 4.71x + 3.40$). A regression equation for the combination of *L. sericata* and *P. regina* (mixed: $y = 4.57x + 0.10$) egg masses, as experienced in the mixed treatment, was developed using the data set from Rosati *et al.* (in review). The total number of eggs on a carcass was obtained by summing the number of eggs in each egg mass. This was repeated for each pig (n = 5) for each species treatment for each CGS level.

To calculate the survival rate of adult blow flies on each carcass, the number of emerged adult blow flies was compared to the estimated number of eggs as determined by the regression equations described previously. Adult blow flies were defined as ‘survivors’ for this study as this is the only life stage that can pass on its genetic material to future generations.

VI) Fitness Measurements

The length of the posterior cross-vein (dm-cu) (Figure 3.1) is a common measure of adult size, and therefore, fitness due to increased mating success, dispersal to find resources and egg load (Vogt *et al.*, 1985; Smith and Wall, 1997a; Smith and Wall, 1997b; Ellers *et al.*, 1998; Hayes *et al.*, 1998; Stoffolano Jr. *et al.*, 2000; Wardhaugh 2001; Clark *et al.*, 2006; Ireland and Turner, 2006). The posterior cross-vein has been used in previous studies involving *L. sericata* and has become a standard due to its

durability when a wing is damaged (Smith and Wall, 1997a; Smith and Wall, 1997b; Hayes *et al.*, 1998; Clark *et al.*, 2006; Ireland and Turner, 2006).

The posterior cross-vein (dm-cu) was measured for a randomly selected subsample of 25 male and 25 female adult blow flies from each species treatment for each CGS level. If 25 blow flies of each sex were not available, due to damaged samples or a lack of emerged adults, all available flies were sampled. For measurement, the left wing was excised and the posterior cross-vein was measured using a Meiji EMZ Zoom Stereo Microscope with an ocular micrometer. Measurements were taken to the nearest hundredth of a mm. The mean wing vein length of each sex and species was calculated for all replicates for each species treatment and CGS level.

VII) Statistical Analysis

Burning – Mean Biomass Loss:

A square root transformation was applied to the biomass loss data so that the data would meet the assumptions of homogeneity of variance (Bartlett's Test: $p > 0.05$; SAS Institute 2011) and normality (Shapiro-Wilk's test: $p > 0.05$; SAS Institute, 2011). The relationship that CGS level had on biomass loss was determined using linear regression in SAS, using the PROC REG function (SAS Institute, 2011). If there was a significant and positive relationship between CGS level and mean biomass loss, the means were compared, using the PROC GLM procedure with the LSMEANS and PDIFF statements in SAS, to determine which CGS levels were different from one another (SAS Institute, 2011). Figures and means are presented using the back-transformed data.

Development Temperatures:

Due to colony and cage constraints, replicates of each species treatment at each burn level could not be conducted simultaneously and ambient temperatures in the greenhouse during larval development differed over time (Table 3.1). To determine if temperature differences during larval development were statistically different between species treatments, analysis of variance (ANOVA) analyses was conducted using the GLM procedure in SAS (SAS Institute, 2011). If different, means were compared using the LSMEANS and PDIFF statements in SAS (SAS Institute, 2011).

Survival Rate:

Some survivorship estimates were greater than 100% (Table 3.2), which is theoretically impossible. To correct for this, the Dixon's test for statistical outliers (Dixon and Massey, 1969) was used to determine if any of the survivorship estimates for each species treatment and CGS level greater than 100% represented statistical outliers. If datasets contained outliers, the data was Winsorized rather than trimmed, to prevent loss of power (Table 3.2) (Barnett and Lewis, 1994). Rates over 100% that were not considered outliers (e.g., Mixed species, CGS Level 2; Table 3.2) could not be Winsorized; this was unexpected, but these values were accepted as true data and remained in the data set. In data sets where two survivorship values were statistical outliers (e.g. Mixed species, CGS Level 3; Table 3.2), these data could not be Winsorized, as in a dataset with $n = 5$, Winsorizing would result in data sets that contained five identical survivorship rates.

Due to the inability to distinguish the eggs to species, both individual species treatment and mixed species treatment survival rates were included in each individual species dataset to test survival for each species. Mean survivorship rates for most species treatments and CGS burn levels did not meet the assumption of homogeneity of variance (Bartlett's Test: $p < 0.05$; SAS Institute 2011), but did meet the assumption of normality (Shapiro-Wilk's test: $p > 0.05$; SAS Institute 2011). As the transformed data still did not meet the assumptions of parametric tests, the Kruskal-Wallis non-parametric ANOVA equivalent was used to determine if species composition or CGS level had an effect on survivorship (NPAR1WAY procedure, SAS Institute 2011). For significant Kruskal-Wallis results ($p < \alpha = 0.05$), the mean survivorship values were evaluated using Wilcoxon's paired t -test equivalent as a *post hoc* test. The Wilcoxon's signed-rank test was used to perform comparisons between CGS level survivorship rates within single species treatments using the PROC NPAR1WAY procedure (SAS Institute, 2011). For each species treatment, the mean survivorship rates at CGS Level 0 and Level 1, Level 1 and Level 2 and Level 2 and Level 3 were compared. For each pair of Wilcoxon's signed-rank tests, $\alpha = 0.016$.

To test for the effects of species interactions on survivorship rates, a Kruskal-Wallis test was performed using the NPAR1WAY procedure in SAS (SAS Institute, 2011). For this analysis, the data sets for individual species treatments (i.e., *L. sericata* only or *P. regina* only) were combined with the data set from the mixed species treatment. This was performed due to the difficulty in determining which eggs in an egg mass belonged to which species. The identical overall egg total in mixed species treatments was used to determine the survivorship rates for both *L. sericata* and *P. regina*.

Using the PROC REG function in SAS, a linear regression was used to determine if there was a relationship between CGS level and mean survival rate (SAS Institute, 2011).

Since total egg number oviposited on each pig carcass differed with CGS level for the different species treatments (Chapter 2), and the previous test indicated that survival rate differed with CGS level for each species treatment, a relationship between total egg number per CGS level and survival rate per CGS level was tested. Although this was initially tested using a linear regression for each species treatment on a per pig basis using the PROC REG function in SAS (SAS Institute, 2011), due to the low replication and high variance all results were insignificant and uninformative. Instead, mean values per CGS level were utilized in the linear regression to reduce the variability to explore the trends, while explicitly recognizing that utilizing the mean values artificially increases the fit of the regression to these data (Camacho Mtz-Vara De Rey *et al.*, 2001).

Fitness Measurements:

The wing vein data met the assumptions of normality and equality of variance (Shapiro-Wilk's test: $p > 0.05$, Bartlett's test $p > 0.05$) (SAS Institute, 2011). Mean wing vein lengths of both species were compared using a three factor ANOVA using the PROC GLM function in SAS (SAS Institute, 2011). The three factors were CGS level, species composition and sex. To test for the effects of species interactions on fitness levels, the data sets for individual species treatments (i.e., *L. sericata* only or *P. regina* only) were compared to the data set from the mixed species treatment.

RESULTS

I) Burning – Mean Biomass Loss

As CGS level increased so did the mean amount of biomass lost, as there was a positive and significant linear relationship between the two ($F_{1, 42} = 68.95, p < 0.0001, R^2 = 0.61$) (Figure 3.2). The amount of biomass lost between CGS levels was statistically different (ANOVA: $F_{2, 42} = 38.58, p < 0.0001$). The mean amount of biomass lost, post flame impingement, of fetal pig carcasses burnt to CGS Level 1, Level 2 and Level 3 were 51.7 ± 12.9 g, 97.0 ± 14.9 g and 391.8 ± 45.1 g, respectively (Figure 3.2). Mean biomass lost between carcasses burnt to CGS Level 1 and CGS Level 3 was significantly different ($p < 0.0001$; Figure 3.2). Similarly, the mean biomass lost between CGS Level 2 was statistically different from that lost at CGS Level 3 ($p < 0.0001$; Figure 3.2). The biomass loss between carcasses burnt to CGS Level 1 and Level 2 were not statistically different ($p = 0.6755$).

II) Development Temperatures

Overall, the temperatures experienced by the developing blow fly larvae in the greenhouse, across the three species treatments, varied ($F_{2, 7572} = 125.44, p < 0.0001$) and ranged between 19.9 and 30.4°C (Table 3.1). Temperatures experienced by developing *L. sericata* larvae differed from developing *P. regina* larvae and those in the mixed species treatment ($p < 0.0001$). However, the temperatures experienced between developing *P. regina* larvae and those in the mixed species treatment did not differ from one another ($p = 0.4551$).

III) Survival Rates

An increase in CGS level led to an increase in survivorship rates, with an overall significant linear relationship between CGS level and survivorship rate. This was true for both species treatments (*L. sericata*: $F_{3, 38} = 11.26, p = 0.0018, R^2 = 0.21$; *P. regina*: $F_{3, 36} = 7.73, p = 0.0084, R^2 = 0.15$) (Figure 3.3).

A direct effect of CGS level on the mean survivorship rate of *L. sericata* larvae was observed (Kruskal-Wallis test: $X^2 = 12.419, \text{d.f.} = 3, p = 0.0059$; Figure 3.3). Survivorship was only different between CGS Level 1 and Level 2 ($p = 0.0038$). Species composition (i.e. the presence of *P. regina*) did not have a significant effect on the

survivorship of *L. sericata* larvae (Kruskal-Wallis test: $X^2 = 2.0563$, d.f. = 1, $p = 0.1516$).

Similar to *L. sericata*, CGS level had an effect on the mean survivorship rate of *P. regina* larvae (Kruskal-Wallis test: $X^2 = 9.0902$, d.f. = 3, $p = 0.0281$). Survivorship rates increased with CGS level (Figure 3.3). Similar to *L. sericata*, survivorship was only different between CGS Level 1 and Level 2 ($p = 0.0105$). Species composition did not have a significant effect (Kruskal-Wallis test: $X^2 = 0.1054$, d.f. = 1, $p = 0.7454$) on the larval survivorship of *P. regina*.

Due to the low replication and high variability, there was no significant linear relationship between the number of eggs oviposited on a carcass and survival rate across the CGS levels for each species treatment (*L. sericata*: $F_{1,2} = 12.81$, $p = 0.0700$, $y = -0.01x + 129.34$, $R^2 = 0.86$; *P. regina*: $F_{1,2} = 4.24$, $p = 0.1758$, $y = 0.01x - 34.82$, $R^2 = 0.68$; Mixed: $F_{1,2} = 0.1824$, $p = 0.7109$; $y = -0.004x + 86.89$, $R^2 = 0.08$; Figure 3.4). Although it is speculative, the trends in these data suggest that the survival rate of *L. sericata* may decline with increasing egg density, whereas the survival rate of *P. regina* may increase with increasing egg density (Figure 3.4).

IV) Fitness Measurements

Species composition significantly affected the size of *L. sericata*, but CGS level did not (Table 3.3). The mean posterior cross vein length (\pm S.E.) of flies in single species treatments measured 1.23 ± 0.01 mm, while those in mixed treatments measured 1.27 ± 0.01 mm. The sex of the flies, regardless of species treatment, also had a significant effect on the posterior cross vein length for *L. sericata* (Table 3.3). The mean posterior cross vein length (\pm S.E.) for males and females was 1.21 ± 0.01 mm and 1.29 ± 0.01 mm, respectively.

Similarly, species composition significantly affected the size of *P. regina* but, CGS level did not (Table 3.3). The mean posterior cross vein length (\pm S.E.) of flies in single species treatments measured 1.32 ± 0.01 mm, while those in mixed treatments measured 1.38 ± 0.02 mm. Regardless of species treatment, the sex of the flies had a significant effect on the posterior cross vein length for *P. regina* (Table 3.3). The mean length of the wing vein (\pm S.E.) for males and females was 1.38 ± 0.01 mm and 1.32 ± 0.01 mm, respectively.

DISCUSSION

Carrion resources that have been altered from natural disturbances, such as forest fires, are expected to have an impact on the survival, and consequently the fitness of developing blow flies. However, there have been no published studies that have investigated how burnt tissues impact the survival and fitness of blow flies. Therefore, the purpose of this research was to fill this knowledge gap by exposing burnt carrion to gravid female *L. sericata* and *P. regina*.

As expected, the survival rates of *L. sericata* increased as CGS level increased. There were fewer eggs deposited by females on the higher levels of the CGS (Chapter 2) and this decreased egg density was expected to relate to increased survival by decreasing competition between conspecific larvae on a more limited resource, however this prediction was not supported. Although there was less consumable resource available as CGS level increased it was previously observed that CGS level and species composition alone did not influence the number of eggs oviposited by female blow flies of either species (Chapter 2). Therefore, this decrease in egg density may be the only characteristic of female oviposition behaviour that is used to compensate for the loss of consumable resource available as CGS level increased, as fewer eggs result in less competition between conspecific larvae. Given the variation that is incurred due to the small sample size present in this study, although there is support for this speculation, it cannot be confirmed that egg density was the major factor to influence survival rates. Decreases in larval density may benefit *L. sericata*, which was observed when comparing the mean egg density and survival rates in this particular study. The response of *L. sericata* to conspecifics, as it relates to larval survivorship, on burnt carrion in this study agrees with the work of others (Hutton and Wasti, 1980; Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007) and agrees with the initial predictions. In addition, no effects of interspecific competition on *L. sericata* were observed in this study, as the presence of *P. regina* did not influence survival on either burnt or un-burnt remains, which agrees with the predictions. This result also agrees with the findings of Hutton and Wasti (1980) and Rosati (2014). The increased developmental plasticity of this particular species may have contributed to its ability to survive when dealing with

competition stress from heterospecifics.

Contrary to *L. sericata*, the survival patterns of *P. regina* were more complex. Similarly, the survival of *P. regina* increased as CGS level increased. Again, this agreed with initial predictions. Survivorship rates on carcasses burnt to CGS Level 2 were highest, despite the fact that the highest egg density was also observed on these carcasses. Additionally, survival and egg density (Chapter 2) at CGS Level 3 was second only to Level 2. *Phormia regina* can adapt to higher larval densities, as was illustrated by the potential trend observed between mean egg number and survival rates. An increase in conspecific larval density has previously been shown to decrease adult emergence (Hutton and Wasti, 1980), but on burnt remains the opposite was noted and supported initial predictions. The stress due to increased conspecific larval density, decreased consumable carrion resource and burnt remains did not appear to affect the survivorship of *P. regina*. Similar to *L. sericata*, the presence of heterospecifics did not influence the emergence rates of *P. regina*, which was unexpected but did agree with results recently observed by Rosati (2014), while disagreeing with Hutton and Wasti (1980). Of the two species, *P. regina* appears to respond better to adverse conditions given their higher survival on severely burnt remains and in larger larval densities.

As expected, as CGS level increased the mean amount of biomass lost also increased. Overall, survivorship on carcasses burnt to CGS Level 2 and Level 3 was greater than those on CGS Level 1 carcasses and on the un-burnt control carcasses that experienced no biomass loss, even though less consumable resources were available for developing larvae on CGS Level 2 and Level 3 carcasses. There are a number of important physical changes to burnt carrion between CGS Level 1 and Level 2, including cracking, that might also contribute to the differences in survivorship observed in this study. Carrion burnt to CGS Level 2 have cracks present (Chapter 2), with even more on carcasses burnt to CGS Level 3 (Glassman and Crow, 1996; Chapter 2). Eggs laid in these cracks might have a better chance of survival but this is likely to be balanced by the potential for eggs and larvae to drown in the bodily fluids that seep from the cracks. There are more bodily fluids as the cracks get larger and more extensive. Cracks on CGS Level 2 are not as deep or severe as those found on CGS Level 3 carcasses and the greater cracking of Level 3 carcasses may explain the increased variability in survival. The

differences in temperatures experienced by the developing larvae across species treatments could also explain some of the variability in survival rates, given the different temperature thresholds of the species in the study.

Unlike survivorship and contradictory to initial predictions, *L. sericata* adult size was not affected by CGS level, which suggests that burnt carrion does not affect the fitness of this species. Species composition had a significant effect on the fitness of adult *L. sericata*. *Lucilia sericata* adults were significantly smaller in the presence of conspecifics in this study. Previous studies indicated that *L. sericata* experience stress due to intraspecific competition (Ullyett, 1950; Hutton and Wasti, 1980; Prinkkilä and Hanski, 1995; Smith and Wall, 1997a; Kheirallah *et al.*, 2007) and the results in this study support these observations. However, when in the presence of heterospecifics across different larval densities, *L. sericata* larvae have been shown to be larger (i.e., increased fitness) (Hutton and Wasti, 1980; Rosati, 2014). An increase in the adult size of *L. sericata* in the presence of *P. regina* was observed in this particular study, which supports previous findings that have shown that interactions with *P. regina* can lead to positive responses (Hutton and Wasti, 1980; Rosati, 2014). The adult size ranges of emerged *L. sericata* in this particular study were found to match previously reported adult size ranges (0.88-1.40 mm) based on the length of the posterior cross-vein (Smith and Wall, 1997a; Smith and Wall, 1997b; Clark *et al.*, 2006). The mean size of female *L. sericata* was greater than the mean size of males, which agrees with the results of previous work (Prinkkilä and Hanski, 1995; Smith and Wall, 1997a).

The size of *P. regina* adults was also unaffected by CGS level, but rather by species composition, which is contradictory to initial predictions. Adults were smaller in the presence conspecifics and larger in the presence of heterospecifics, agreeing with initial predictions. The results of this study contradict those of others that have observed that entire populations of *P. regina* were eliminated when in competition with *L. sericata* (Hutton and Wasti, 1980) and confirms the findings that *P. regina* are more successful in development when engaging in species interactions with *L. sericata* (Rosati, 2014). The mean size of male *P. regina* was greater than the mean size of females, which agrees with previous findings (Rosati, 2014). Given a lack of published adult size ranges for *P. regina* based on posterior cross-vein length, the flies measured in this study fall within

ranges (1.00-1.43 mm) observed in the colony cages maintained at the University of Windsor. Overall, these results indicate that *P. regina* is positively affected by the presence of other blow fly species, such as *L. sericata*.

The positive responses observed for both species in the presence of heterospecifics, as seen through increased adult wing vein length and therefore assumed body size, can be attributed to either facilitation or competition. Facilitation can arise due to such mechanisms as larval enzyme and protein secretions during resource consumption, which has been shown to increase the rate of development in addition to increasing survival rates (Rosati, 2014). Due to the ephemeral nature of carrion, the competitive aspect of multiple species utilizing this resource seems problematic, however, one potential mechanism for coexistence on carrion is resource partitioning (Denno and Cothran, 1975; Ives, 1991). Perhaps larvae consume more resource in the presence of competitors, leading to an increase in body size, and therefore wing vein length. Seeing as CGS level did not affect the size of either blow fly species, it is unlikely that development on burnt carrion is a factor that would influence the fitness of either species. Rather, it is believed that egg, and therefore larval densities, had the greatest impact on larval development and size, and would have the greatest impact on the fitness of these two blow fly species.

It is already known that blow flies can locate and oviposit on carrion quickly (Cragg, 1955; Smith, 1986; Thomas and Mangan, 1989; Hall and Doisy, 1993; Smith and Wall, 1997b; Gião and Godoy, 1997; Byrd and Allen, 2001; Arnaldos *et al.*, 2005; Byrd and Castner, 2010). The results of this study show that blow flies also have the potential to be successful colonizers of an environment following natural disturbances, such as a forest fire, given their propensity to successfully develop as larvae and survive to adulthood when feeding on burnt remains. This is important to note, as burnt carrion populations are known to increase after disturbances (Shvidenko and Goldammer, 2001; United States National Park Service, 2014). Blow flies have a greater chance of survival on burnt carrion when the resource has minimal damage due to flame impingement (equivalent to CGS Level 2 and Level 3; Glassman and Crow, 1996). Additionally, a larger presence of blow flies should help attract a wider diversity of other animals to an area after a disturbance, especially those that feed upon the larvae, such as birds.

This study was conducted in closed arenas under artificial conditions in which the

number of blow flies present and the amount of time females were allowed to oviposit was limited. In a natural setting, a larger number of blow flies might utilize the same carrion resource and oviposition may also occur over a longer period of time. When they arrive first to carrion, the blow fly, *Lucilia coeruleiviridis* Macquart (Diptera: Calliphoridae) has been known to deposit an overabundance of eggs on a resource (Kneidel, 1984). This allows *L. coeruleiviridis* to outcompete species that arrive to the carrion later (Kneidel, 1984). Additionally, the size of carrion resource might impact the interactions experienced by the individuals present. Smaller carrion, such as the fetal pig carcasses used in this study might represent an environment where stress due to competition is higher because there is less resource available, or decreased because there are fewer oviposition events (Kneidel, 1984). Larger carrion resources may also lead to higher levels of competition (Kneidel, 1984). Regardless of the cause, increased competition can lead to an overall decrease in species diversity in a habitat (Kneidel, 1984).

Additionally, due to the nature of the closed arenas, this study was a no choice oviposition experiment, as females were only allowed to oviposit on the carrion presented to them. In the field, the availability of burnt remains might be higher providing females with the opportunity to select from a more diverse variety of burnt remains. In turn, this could change egg and larval densities, as well as species interactions, potentially resulting in different fitness levels and survival rates than what was observed in this study. A final drawback to utilizing controlled conditions for this particular experiment was the use of a metal tray to hold the carcass inside each experimental cage. The trays were used to make transportation of the carcasses to aquaria easier. These metal trays facilitated the collection of blood and internal contents, resulting in larger than normal drowning risks for developing larvae. If this experiment were repeated in the field, secreted bodily fluids would be absorbed into the ground, which would reduce the risk of drowning.

Interestingly, survival rates greater than 100% were recorded in this study and the effect of these observations on the results of the study were magnified because of the small sample size of the study. Future studies should consider increasing the sample size for each species treatment and for each CGS level. Winsorizing the data represents a suitable statistical technique for dealing with outliers (Barnett and Lewis, 1994), but for

small datasets, Winsorizing the data may not be a suitable option. One possible explanation for such large survival estimates is that the regression equations developed by Rosati *et al.* (in review) underestimated the number of blow fly eggs present in an egg mass, leading to inflated survival rates. A second, more feasible, explanation for such large survival rates is cryptic oviposition. Specifically, unlike the work by Rosati *et al.* (in review), in which all eggs were laid on liver, and were countable, it is possible that not all eggs laid on the pig carcass were countable. It is difficult to examine all internal cavities, such as the inner ear canal, thoroughly and take quality photographs for estimation purposes without risking the integrity of deposited egg masses. It would be informative for future work in this area to determine how many eggs are laid inside body cavities such as the inner ear canal, in order to determine if cryptic oviposition occurs, and to establish a method of estimating the actual number of eggs laid on a more complex three-dimensional surface.

Carcasses burnt to CGS Level 2 and Level 3 provided the greatest chance of survival for developing *L. sericata* and *P. regina* larvae in this study. Although carcasses at these levels experience the greatest loss of biomass as a result of burning, it is not enough to significantly impact the success of developing larvae. Instead it is believed that decreased larval density, in the case of *L. sericata*, may contribute to the greater success of *L. sericata* larvae. Larval density did not appear to impact the success of *P. regina* larvae as instances of higher larval densities still resulted in greater survival totals. Fitness levels were not affected by CGS level, but were affected by species composition. *Lucilia sericata* and *P. regina* responded negatively to conspecifics as shown by decreased adult size when compared to their larger size in the presence of heterospecifics. Overall, the results of this study give us reason to believe that blow flies would respond well after a natural disaster, such as a forest fire, by colonizing carrion that have died as a result of flame impingement. In the future, outdoor validation studies should be performed to ensure that the results found in controlled conditions described here are similar to those observed in the field.

Table 3.1: The dates of the developmental period and mean temperatures (\pm S.E.) experienced by ovipositing female blow flies and developing larvae during that time. The carcasses were burnt on the morning of the first day listed in each row. The mean temperatures (\pm S.E.) experienced by developing *L. sericata*, *P. regina* and mixed species larvae were 27.5 ± 0.1 °C, 25.4 ± 0.1 °C and 25.2 ± 0.1 °C, respectively. Overall, temperatures experienced by the larvae across each species treatment were different (ANOVA: $F_{2, 7572} = 125.44$, $p < 0.0001$). For each species treatment and CGS level, $n = 5$. Species treatments with different letters indicate a statistical difference ($\alpha = 0.05$).

Replicate	Date	Temperature (°C)
<i>Lucilia sericata</i> ^a		
Burn 1	May 16 – June 6, 2013	28.2 \pm 0.3
Burn 2	June 5 – June 26, 2013	29.5 \pm 0.2
Burn 3	Aug. 21 – Sept. 11, 2013	30.4 \pm 0.2
Burn 4	Sept. 3 – Sept. 24, 2013	24.7 \pm 0.2
Burn 5	Feb. 3 – Feb. 24, 2014	24.3 \pm 0.1
<i>Phormia regina</i> ^b		
Burn 1	Aug. 5 – Aug. 26, 2013	26.4 \pm 0.2
Burn 2	Aug. 5 – Aug. 26, 2013	26.4 \pm 0.2
Burn 3	Sept. 15 – Oct. 6, 2013	24.0 \pm 0.2
Burn 4	Feb. 26 – March 19, 2014	25.1 \pm 0.2
Burn 5	Feb. 26 – March 19, 2014	25.1 \pm 0.2
<i>Mixed – Lucilia sericata and Phormia regina</i> ^b		
Burn 1	May 31 – June 21, 2013	28.4 \pm 0.3
Burn 2	Sept. 15 – Oct. 6, 2013	24.0 \pm 0.2
Burn 3	Feb. 3 – Feb. 24, 2014	24.3 \pm 0.1
Burn 4	March 24 – April 14, 2014	29.3 \pm 0.3
Burn 5	April 18 – May 9, 2014	19.9 \pm 0.2

Table 3.2: The original and Winsorized survival rates (%) across the five replicates for each species treatment and each CGS level. Survival rates with an asterisk (*) indicate outliers as determined by the Dixon’s test. CGS levels without a Winsorized dataset either contained no statistical outliers or could not be Winsorized due to the sample size of the study.

CGS Level	Original Survival Rates	Winsorized Survival Rates
<i>Lucilia sericata</i>		
Level 0	19.2, 32.7, 55.3, 55.4, 1117.1*	32.7, 32.7, 55.3, 55.4, 55.4
Level 1	23.2, 34.2, 44.3, 50.2, 54.8	————
Level 2	44.8, 59.2, 76.9, 80.4, 246.2*	59.2, 59.2, 76.9, 80.4, 80.4
Level 3	47.1, 53.9, 58.7, 110.7*, 164.9*	————
<i>Phormia regina</i>		
Level 0	1.8, 16.2, 45.7, 49.1, 64.1	————
Level 1	15.5, 29.5, 44.7, 45.4, 179.5*	29.5, 29.5, 44.7, 45.4, 45.4
Level 2	42.0, 44.9, 54.5, 129.9*, 149.0*	————
Level 3	31.5, 34.3, 52.8, 88.6, 407.9*	34.3, 34.3, 52.8, 88.6, 88.6
Mixed – <i>Lucilia sericata</i> and <i>Phormia regina</i>		
Level 0	5.0, 5.3, 38.7, 59.8, 60.5	————
Level 1	25.2, 29.6, 29.7, 41.9, 53.3	————
Level 2	35.6, 53.3, 77.6, 80.9, 109.4	————
Level 3	6.0, 16.0, 47.8, 134.6*, 175.4*	————

Table 3.3: The results of the analysis of variance (ANOVA) to determine the effects of species composition, CGS level and gender, and the interactions therein, on the length of the posterior cross vein (cm-du) for *L. sericata* and *P. regina*. Statistically significant effects are indicated in bold font. For all effects, $\alpha = 0.05$.

Effect	d.f.	F Value	<i>p</i> - value
<i>Lucilia sericata</i>			
Species composition	1	16.69	0.0001
CGS level	3	0.98	0.4088
Sex	1	65.00	< 0.0001
Species composition * CGS level	3	1.78	0.1601
CGS level * Sex	3	0.13	0.9404
Species composition * Sex	1	0.03	0.8668
Species composition * CGS level * Sex	3	0.16	0.9251
<i>Phormia regina</i>			
Species composition	1	6.30	0.0147
CGS level	3	1.15	0.3343
Sex	1	9.08	0.0038
Species composition * CGS level	3	1.20	0.3161
CGS level * Sex	3	0.04	0.9903
Species composition * Sex	1	0.03	0.8602
Species composition * CGS level * Sex	3	0.06	0.9812

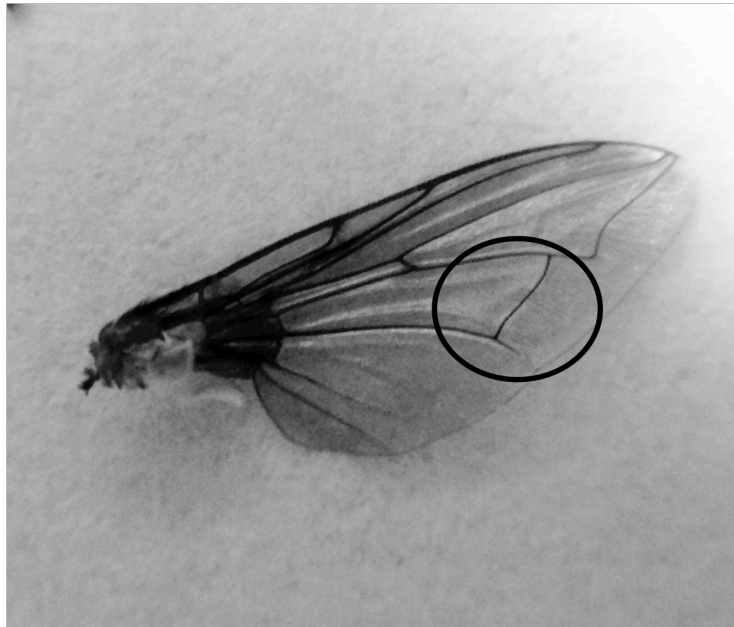


Figure 3.1: The posterior cross vein (cm-du), pictured here (circled), was measured to the nearest hundredth of a millimetre, as an indicator of adult fly size and used as a proxy of fitness for both *L. sericata* and *P. regina*.

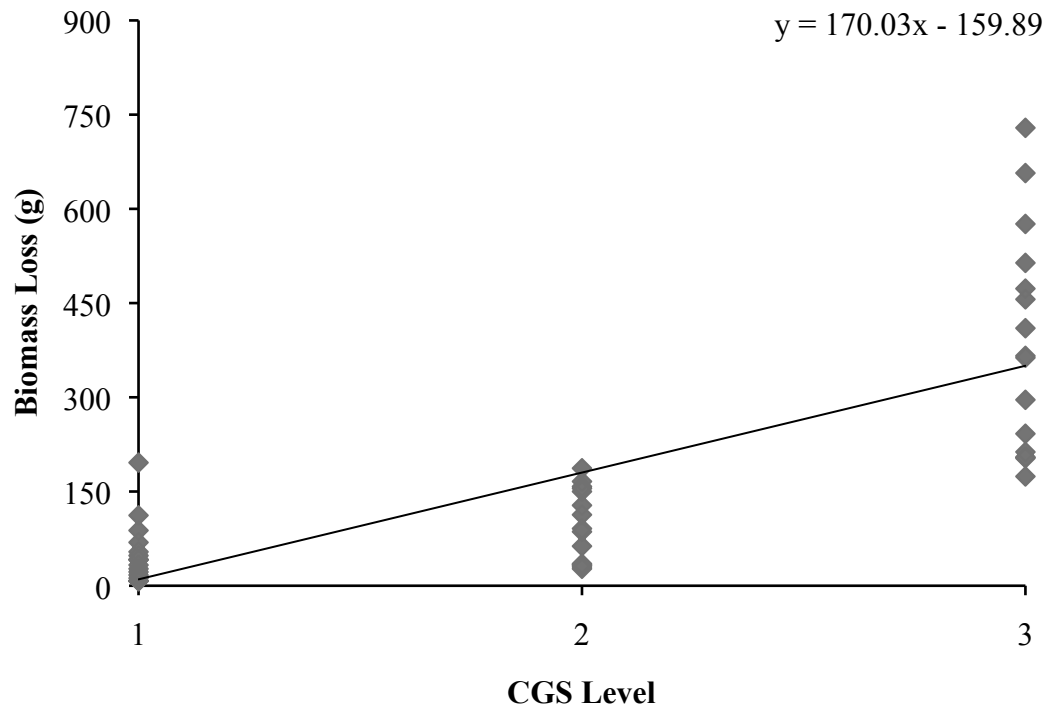


Figure 3.2: The total biomass lost after pig carcasses were burnt to Level 1, Level 2 and Level 3 on the CGS. A positive, linear relationship was observed ($F_{1,42} = 68.95$, $p < 0.0001$, $R^2 = 0.61$) between CGS level and the amount of biomass lost after flame impingement.

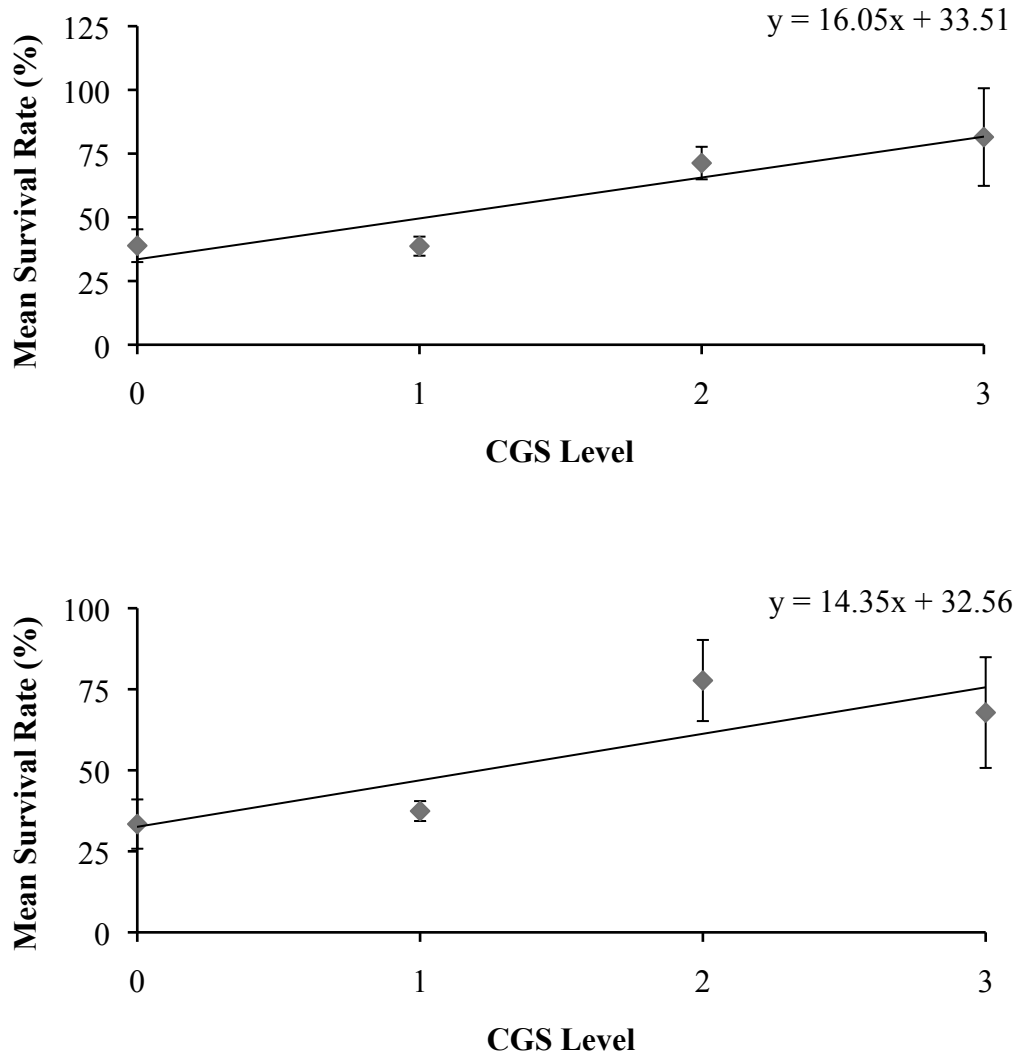


Figure 3.3: The mean (\pm S.E.) survival rates for *L. sericata* (top) and *P. regina* (bottom) larvae on fetal pig carcasses burnt to Levels 1 to 3 on the CGS and for the un-burnt controls (Level 0). For both species, survival rates between CGS Level 1 and Level 2 were significantly different (*L. sericata*: $p = 0.0038$; *P. regina*: $p = 0.0105$).

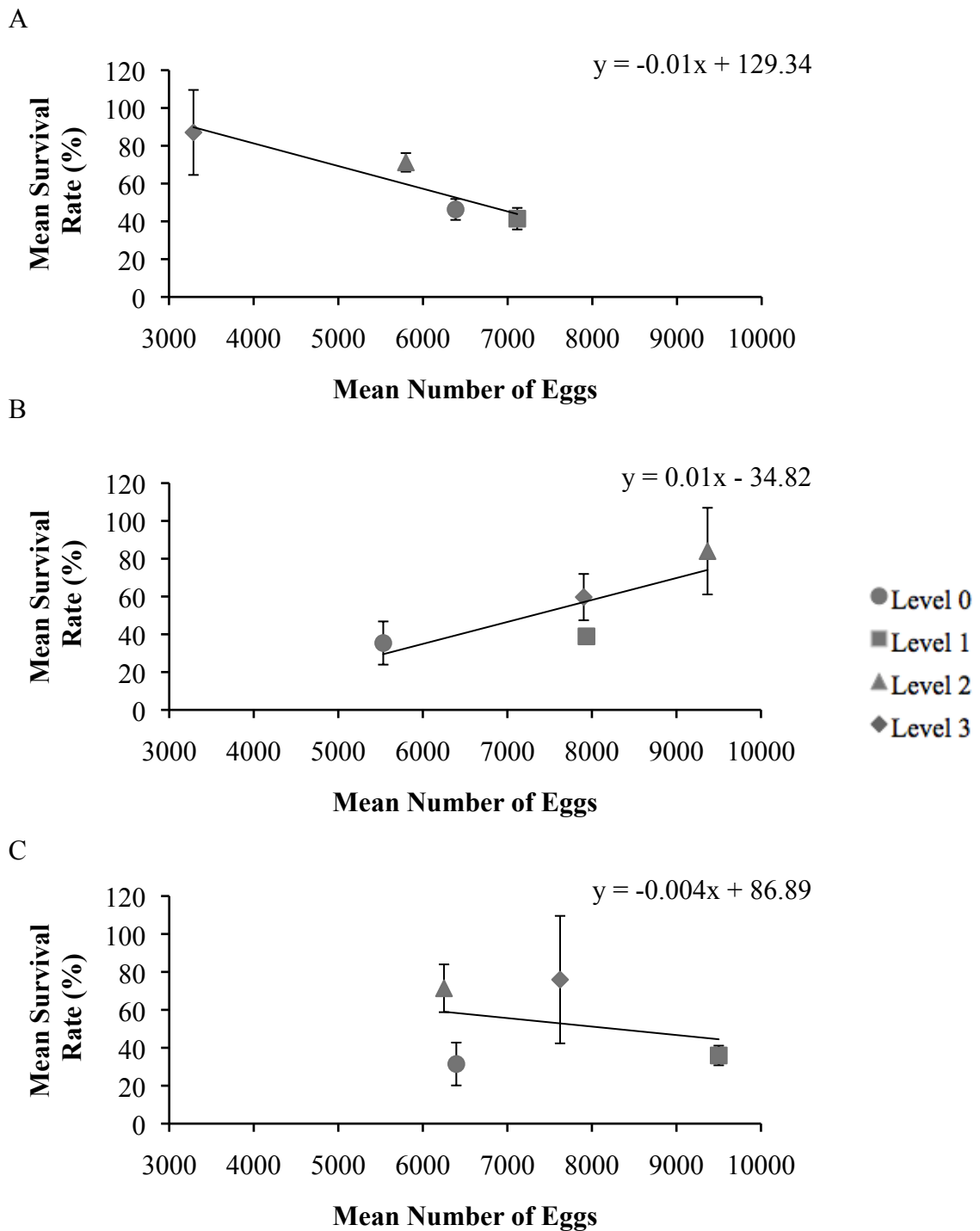


Figure 3.4: The relationship between mean number of eggs oviposited by female blow flies and mean (\pm S.E.) survival rate to the adult stage across CGS level. Panels A, B and C represent *L. sericata*, *P. regina* and mixed species treatments, respectively. None of the relationships were statistically significant due to the small sample size ($n = 5$ for each CGS level) and the variation within each CGS level, for each species.

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CHAPTER 4:
BLOW FLIES AND BURNT CARRION: WHAT HAVE WE LEARNED AND
WHERE DO WE GO FROM HERE?

FIRE TRENDS, OVIPOSITION THEORIES AND RESEARCH MODEL

Burnt carrion can result from a number of different scenarios. For example, fire related crimes such as arson and homicide or the use of fire as a forensic countermeasure may leave behind burnt remains that can be utilized by carrion insects. Except for 2012, the number of deaths resulting from flame impingement has been increasing in Canada (Statistics Canada, 2013). An increase in fire related homicides might lead to an increase in the incidence of burnt carrion that can be colonized by forensically important insects, such as blow flies (Diptera: Calliphoridae). Natural disasters, such as forest fires, may also lead to an increase in burnt remains, most commonly in the form of burnt animal carcasses, rather than human remains (Shvidenko and Goldammer, 2001; United States National Park Service, 2014). Blow fly oviposition on burnt carrion has been reported in the literature (Avila and Goff, 1998; Introna *et al.*, 1998; Pai *et al.*, 2007; Chin *et al.*, 2008; Vanin *et al.*, 2013). Due to their predictable life history and early arrival at carrion resources, blow fly development and behaviour is heavily utilized by forensic entomologists when calculating a minimum postmortem interval estimate (Cragg, 1955; Smith, 1986; Thomas and Mangan, 1989; Hall and Doisy, 1993; Smith and Wall, 1997b; Gião and Godoy, 1997; Byrd and Allen, 2001; Bourel *et al.*, 2003; Arnaldos *et al.*, 2005; Byrd and Castner, 2010). However, there has been no research conducted to experimentally determine if burnt remains alter the oviposition behaviour, and consequently larval survival and fitness, of blow flies.

Characterizing burnt remains has become easier with the introduction of the Crow-Glassman Scale (CGS) that is used to classify the severity of fire damage to a burn victim based on their appearance (Glassman and Crow, 1996). As the CGS level of a burnt body increases, the physical appearance of the remains also changes (Glassman and Crow, 1996). Among the more noticeable changes are the development of cracks in the skin and the dismemberment of limbs (Glassman and Crow, 1996). Cracks are prominent

at CGS Levels 2 and 3 (Avila and Goff, 1998; Chapter 2), and reveal internal structures and bodily fluids that contain sugars and proteins that attract blow flies (Avila and Goff, 1998; Byrd and Castner, 2010). It has been suggested that the cracks in the skin that result from flame impingement might represent optimal oviposition locations on burnt remains and as a result, may act to facilitate blow fly oviposition (Avila and Goff, 1998; Vanin *et al.*, 2013). Using optimal oviposition theory, which states that female preference for oviposition sites should maximize offspring performance (Jaenike, 1978; Thompson, 1988; Ellis, 2008), as a research model, the goal of this research was to determine if cracks on burnt carcasses were preferred by female blow flies for oviposition and how oviposition on burnt carcasses affected the resultant offspring. To conduct this research, two species of blow fly local to Ontario, Canada were used as a model species: *Lucilia sericata* Meigen and *Phormia regina* Meigen (Diptera: Calliphoridae).

BLOW FLIES OVIPOSITED ON BURNT CARRION AND THE CRACKS ON BURNT CARRION AFFECTED FEMALE PREFERENCE

Oviposition was observed on all pig carcasses included in this study, regardless of the degree of flame impingement. An increase in oviposition events at the cracks was observed for *L. sericata*, *P. regina* and in mixed species treatments, but only when carrion were classified as Level 3 on the CGS (Chapter 2). At this particular CGS level, there was a shift in oviposition preference away from traditional oviposition sites towards the cracks created via flame impingement. According to optimal oviposition theory (Jaenike, 1978), such a shift in oviposition preference indicates that female blow flies perceive the cracks as a beneficial habitat for their offspring. Cracks were present on carcasses classified as Level 2 on the GCS (Chapter 2), but no preference for cracks was observed. This might be explained several ways. For example, CGS Level 3 carcasses sustain more damage than Level 2 carcasses, so physical characteristics on the traditional locations might be missing or compromised at Level 3, increasing the attractiveness of the cracks. The reason for the difference might also be directly connected to the prominence or availability of the cracks on CGS Level 3 carcasses compared to Level 2 carcasses.

For a forensic entomologist, understanding where female blow flies oviposit can

help investigators to better understand the series of events connected with the death of a decedent. When forensic entomologists observe abnormal oviposition behaviour such as oviposition in an atypical location on the body, it can expand the scope of an investigation, as this abnormal behaviour must be explained. This change in oviposition behaviour can be the difference between a suspicious death being ruled either a suicide or a homicide. Not only is it beneficial to understand these oviposition behaviours when carrion are unaltered, but it is also beneficial when carrion are altered such as those exposed to flame impingement. From an ecological point of view, it is important to understand what factors influence the re-colonization behaviours of animals. Identifying that after natural disturbances, such as a forest fire, that altered carrion are not influencing the oviposition success of insects is encouraging. The oviposition success of blow flies on burnt carrion in this study provides evidence to suggest that blow flies may be an important taxa for the initiation of the re-colonization and succession processes in an affected ecosystem.

Although they would be extremely difficult to setup due to safety concerns and logistical problems, the use of field validation studies would help expand on the research presented in this thesis. These field validation studies would be beneficial to both forensic entomologists, as suspicious deaths occur year round, and to ecologists, as natural disturbances also occur throughout the year. Field validation studies could be organized in two ways. The first would be the arrangement of a controlled burn in an area already scheduled for routine maintenance, such as the tall-grass prairie ecosystem at the Ojibway Nature Centre in Windsor, Ontario, Canada. Within this controlled burn, pre-deceased carrion would be randomly placed in the area receiving the treatment and observations of insect behaviour would take place once the area was deemed safe. The second method would involve data collection after naturally occurring disturbances. This second method would most accurately represent natural conditions and provide an important setting in which to investigate blow fly responses to disturbances. If possible, field validation studies should be conducted in different ecosystems and during different seasons as these variables may change the observed results. By conducting these experiments in the field, the nature of the experiments change from a no-choice oviposition study, as conducted in the present study, to one in which female blow flies

can choose from a variety of carcasses burnt to varying levels on the CGS. Additionally, the present study utilized burnt fetal pig carcasses as the carrion in which the blow flies were exposed too. Future studies should also consider using larger pigs that more closely resemble the mean weight of an adult, for forensic entomology based studies, or a fully developed animal for ecology based studies. This would be beneficial, as larger individuals burn differently due to musculature and fat content (Nicholson, 1993), potentially resulting in different results from what have been shown within this thesis.

It is important to note that cracks resulting from flame impingement and wounds, such as those resulting from gunshots or cuts, have similar physical characteristics. Depending on the severity of physical damage experienced by a carcass that is burnt, it may be beneficial to distinguish between wounds that existed before burning to those that result from burning in cases where burning is used as a forensic countermeasure. Blow fly preference for wounds on a burnt carcass may also change. It would be informative to conduct further studies in which fetal pig carcasses are wounded before burning to determine how flame impingement affects pre-existing wounds and if female preference for those sites is affected when cracks form during burning.

PRESENCE OF CONSPECIFICS AND HETEROSPECIFICS AFFECTED FEMALE PREFERENCE

Although female blow flies did oviposit at the cracks on burnt carrion, overall, they laid fewer eggs at the cracks than at other sites (Chapter 2). For *L. sericata*, this result suggests that cracks may not be as optimal for oviposition as expected based on the number of oviposition events that were observed. It is predicted that very wet cracks might represent a risk to larvae. Thus, females lay fewer eggs at risky sites, but still deposit some eggs at those sites as they might prove to be beneficial rather than risky. The difference in egg numbers deposited at the various locations on carrion might also be the result of changes in larval densities between locations and female perception of those changes. Specifically, certain sites might be able to support larger larval populations than others and female blow flies recognize this when ovipositing. The results of this study suggest that *L. sericata* prefer sites of lower density when subjected to adverse conditions, such as those that might be found in the cracks where the risks of larvae

drowning are high. It is also likely that *L. sericata* engage in skip oviposition, a process in which females spread eggs within their clutch over numerous suitable locations (Mogi and Mokry, 1980). This type of behaviour would result in variability in egg number between locations, similar to that observed in this study. As expected, these results may indicate the optimum larval density, at the cracks, for *L. sericata* is lower. Contrary to *L. sericata*, *P. regina* oviposited large numbers of eggs at the cracks when they were the only species present and carcasses were burnt to a CGS Level 3 (Chapter 2). This particular species performs better when larval densities are high, so an increase in the number of eggs present at the cracks should not have a negative effect on their development.

These results provide forensic entomologists and ecologists with research that would suggest the suitability of cracks for oviposition is dependent on the species of blow fly present and on the degree of damage to the carcass, as indicated by CGS level. Specifically, given different circumstances, different blow fly species use different oviposition strategies to ensure their offspring have the best chance of eclosion and survival. It also demonstrates that competitive species interactions do not always play a role in the success of certain blow fly species, particularly *L. sericata*. As previously mentioned, aside from the presence of heterospecifics and conspecifics, the drowning potential associated with seeping bodily fluids may prevent oviposition in cracks or wounds. It would be interesting for future studies to investigate if this is truly the case by manipulating the volume of fluid present at the cracks on burnt carrion and observing the oviposition behaviour of female blow flies in both choice and no-choice experiments.

BURNT CARRION AFFECTED FEMALE FITNESS BUT NOT OFFSPRING PERFORMANCE

Larval survival in this study increased as the degree of flame impingement increased (Chapter 3). This result was surprising, as carcasses classified as Level 2 and Level 3 on the CGS lost the most biomass (Chapter 2 and 3), reducing the amount of resource available for developing maggots. For *L. sericata* this may have been influenced by the decrease in larval competition due to a decrease in egg density as CGS level

increased (Chapter 2). For *P. regina*, higher egg density and subsequent larval density was observed as CGS level increased, but this did not impact their survival. This could potentially be explained by a higher optimal larval density of *P. regina*, which contradicts the results of others (Hutton and Wasti, 1980). It appears that of the two blow fly species used in this thesis, *P. regina* is more robust given its ability to survive on altered carrion even when faced with higher larval densities, and therefore, greater potential larval competition. Overall, these results indicate that the fitness of a female blow fly is not compromised if she chooses to oviposit on burnt carrion.

The size of the adult blow flies that emerged from the larvae feeding on burnt carrion was not affected by the level of flame impingement. Size, measured using the posterior cross-vein as a proxy indicator, is a measure of offspring performance and can be used to predict the future fitness of those individuals (Smith and Wall, 1997a; Smith and Wall, 1997b; Hayes *et al.*, 1998; Clark *et al.*, 2006; Ireland and Turner, 2006). The results of this study suggest that the performance of larvae feeding on burnt carrion is not affected by the degree of damage caused by the flames. It also suggests that the subsequent fitness of the adults emerging from the larvae is unlikely to be affected by consuming burnt carrion. It is important to note that both *L. sericata* and *P. regina* larvae that developed in the presence of heterospecific larvae were larger as adults than larvae that developed among conspecifics. This would suggest that both species were facilitated by heterospecifics.

These results indicate that blow flies can be successful colonizers after a natural disturbance, particularly a forest fire, in which burnt carrion remain. This is encouraging for ecologists as it can help provide a better understanding of succession patterns that occur after natural disturbances. The development of maggots on burnt carrion can lead to an influx of other animals to an affected area, particularly those that rely on maggots for food. However, due to the significant amount of time it took to sort and count the adult flies that emerged after developing on the burnt carcasses in this study and other time constraints, the sample size for this study was low. An overall benefit to all future research would be to increase sample size. By increasing the number of burns performed for each of the species treatments, and therefore, increasing the number of burnt remains, the power available for all statistical analyses would increase.

In conclusion, the results of these studies provide an important basis for forensic investigations that involve burnt carcasses and have important implications to ecological research involving patterns of succession following fire related disturbances. However, not all of the results supported the predictions of the study, and new questions have arisen. Further research in this system is needed to answer these questions. Specifically, field validation studies and choice studies would be valuable, as would an assessment of female perception of risk at the cracks as moisture levels change. The results of this study will be important in informing the questions asked in improving the experimental design. Altogether, this research, in conjunction with past and future work will benefit both forensic entomologists and ecologists.

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