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Spatial and Temporal Variability in the Carrion Insect Community: Using Blow Flies (Family: Calliphoridae) as a Model System to Study Coexistence Mechanisms at Multiple Scales

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Spatial and Temporal Variability in the Carrion Insect Community: Using Blow Flies (Family: Calliphoridae) as a Model System to Study Coexistence Mechanisms at Multiple Scales

by

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DECLARATION OF ORIGINALITY

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ABSTRACT

Resource partitioning can lead to species coexistence. In a field study, temporal and spatial partitioning were examined by testing the effects of season and habitat on the structure of the blow fly community on domestic pig carcasses, *Sus scrofa domesticus* in southwestern Ontario, Canada. Blow fly communities did not differ between field and forest habitats, however there were seasonal differences. Fall was characterized by having more species and higher levels of species evenness, diversity, and niche overlap than spring and summer.

On a finer scale, effects of arrival order were examined in laboratory experiments with three blow fly species: *Phormia regina*, *Lucilia sericata*, and the introduced species *Chrysomya rufifacies*. Arrival order of adults was varied in combinations of two species: "*L. sericata* and *P. regina"* and "*L. sericata* and *C. rufifacies*". Both positive and negative priority effects were recorded, with species having altered colonization patterns temporally and spatially in response to presence of another species, even at low density (i.e. minimal competition). Blow flies sometimes selected oviposition sites other than the natural orifices predicted by previous studies, such as the neck and cheek regions or between legs. Delays in colonization, particularly for *P. regina* and *C. rufifacies*, occurred in response to the absence of heterospecifics. Additional experiments with larvae determined that *C. rufifacies* and *P. regina* benefitted from the presence of *L. sericata* due to predation (for *C. rufifacies*) or the presence of compound(s) that may aid in the digestion of the resource and increase nutrient availability (for *P. regina*).

In summary, adult and larval experiments indicate that species interactions and differences in arrival order can affect colonization times, the distribution of eggs over a

resource, larval interactions and offspring fitness. On a larger scale, temporal partitioning (i.e. seasonal effects) can promote coexistence in blow flies, however, spatial partitioning (i.e. habitat effects) was not evident. This study demonstrates the importance of ADD standardization, emphasizes the need to understand species interactions between native and non-native species, and highlights the need for more ecological studies regarding habitat and seasonal differences within the carrion community.

DEDICATION

I dedicate this dissertation to the many family members and significant people in my life that supported me throughout my graduate years. My parents, for giving me the love and support that only parents can give. I would not be where I am today without them and I am thankful they were given the chance to be my parents. To my brother, Mark, for always looking out for me and for being the best big brother I could have asked for. Thank you to all of my birth siblings (Tina, Tom, Tracy, Kara and Lauren), especially my research assistant Mandy, for giving me support and words of encouragement when I needed it the most. Each of you came into my life at different points during my Ph.D. and gave me the motivation to keep moving forward, always knowing that each of you would be there behind me. Thank you to my Robinson, for coming into my life at a point where I didn't think I needed help, not realizing it was when I needed it the most. Thank you for simply being there, accepting me and my work wholeheartedly, and for sharing your Al and all the joy she brings to your life.

Finally, I dedicate this work to my daughter, Alexandra. For her excitement about entomology, her willingness to get dirty and constant understanding of the time required for graduate work. Her childhood years were spent in the lab with flies and maggots, which was anything but typical, but she took it in stride and worked right along with me in every aspect of this project. Most importantly, I thank her for lending me her hands when mine weren't enough.

This work is the result of many friends and family that have played a role in helping me get to where I needed to go. I will be eternally grateful.

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LIST OF ABBREVIATIONS

ADD – accumulated degree days, incorporates time and temperature over a certain developmental threshold to represent insects' physiological time, values are calculated on a per day basis based upon mean daily temperatures (i.e. each degree day), then summed over multiple days (i.e. accumulated degree days). A minimum threshold of O°C was used in this study.

ADH – accumulated degree hours, represents the physiological time (i.e. incorporates time and temperature) for an insect, values are calculated on a per hour basis (i.e. each degree hour), then summed over multiple hours (i.e. accumulated degree hours). A minimum threshold of O°C was used in this study.

MTC – minimum time of colonization, used in this study to describe the minimum amount to time from the first oviposition event.

PIA – period of insect activity; begins at the onset of insect arrival until the body is discovered. PIA combined with the pre-colonization window determines the PMI.

PMI – also known as the postmortem interval, begins at the onset of death and continues until the body is discovered. PMI estimations attempt to determine time of death (PMI = PIA + pre-colonization window).

CHAPTER 1: GENERAL INTRODUCTION: THE IMPORTANCE OF THE CARRION INSECT COMMUNITY IN FORENSIC AND ECOLOGICAL RESEARCH

The Carrion System and Mechanisms for Coexistence

Ephemeral communities, like carrion, are model systems for investigating the processes that are important in determining both micro- and macro-community structure. After the death of an organism, a distinct insect community assembles on the resultant carrion. The patterns of assembly in this community change over time and space. These patterns are relatively predictable, as changes in community structure are highly correlated with the decomposition of the resource (Megnin 1894, Smith 1986, Morin 1999, Byrd and Castner 2001) but may be significantly influenced by both abiotic and biotic factors (Megnin 1894, Smith 1986, Schoenly and Reid 1987, Catts and Goff 1992, Schoenly et al. 2007, Wilson and Wolkovich 2011). Previous studies examining spatial or temporal community dynamics typically involve competitive interactions within a particular guild and have predominantly involved plant-based systems (Connell and Slatyer 1977) in which later successional species have been absent due to time constraints of the studies (Michaud and Moreau 2009). As an alternative, the carrion system and its community members can be easily manipulated through inclusion/exclusion of species to evaluate mechanisms and interactions between individuals, populations, species, and guilds. The carrion insect community is highly diverse and species can be easily classified into feeding guilds (see Braack 1987) based upon the type of resource consumed, such as sarcosaprophagous (muscle/soft tissue); coprophagous (gut or digestive material); dermatophagous (skin tissue); keratophagous (keratinous structures);

saprophagous (multiple tissue types and other decaying material), predaceous (feeding on other insects); parasitic (feeding on insect hosts that they kill during their immature development); and omnivorous (feeding at multiple trophic levels). The carrion system can be easily replicated, allowing one to experimentally evaluate the replicability of ecological patterns. These important aspects, in combination with relatively predictable patterns of succession, make the carrion system and its insect members an appropriate model for studying the ecological mechanisms that structure ecological communities over space and time (Schoenly and Reid 1987, Michaud and Moreau 2009, Tomberlin et al. 2011, 2012, Beasley et al. 2012, Barton et al. 2013).

Multiple mechanisms -- aggregation, competition, predation, cannibalism, parasitism, mutualism, inhibition and facilitation -- have been identified as influential in determining carrion insect community structure (Fuller 1934, Beaver 1977, Atkinson and Shorrocks 1981, Kneidel 1984, Atkinson 1985, Braack 1987, Hanski 1987, Ives 1991, Woodcock et al. 2002, Inouye 2005). In addition, the distribution, population dynamics and coexistence patterns of multiple species within a guild can be influenced by adaptations based on species-specific responses to stress (Kamal 1958). Carrion communities commonly have high levels of species diversity despite food limitation and intense competition (Kamal 1958). In several species of carrion flies, coexistence was due to differential responses between species to stressful conditions (Kamal 1958). This allowed for individual species to flourish within their optimal conditions and coexist in the community (Kamal 1958). Differential responses to abiotic conditions can alter the presence of, absence of, or interactions between community members (Tilman 1982, Stone et al. 1996, Chesson 2000, Chase and Leibold 2003) and thermal constraints can

influence species interactions and community structure (Cavender-Bares et al. 2009, Wittman et al. 2010, Lessard et al. 2011).

In patchy and ephemeral resources, such as carrion, high levels of species diversity and coexistence can occur despite the high levels of intra- and inter-specific competition or complete exhaustion of the resource (Atkinson and Shorrocks 1981, 1984, Hanski 1983, 1987, Shorrocks 1990, Shorrocks and Bingley 1994, Krijger et al. 2001, Von Zuben et al. 2001, Hattori and Shibuno 2013). Reduced availability of resources can lead to intra- and interspecific competition and reduce the fitness of organisms (Fox 2000, Fox and Czesak 2000). Although this may lead to the competitive exclusion of species with the same resource requirements, there are many other spatially-based mechanisms that allow for coexistence on shared resources including disturbance (Sousa 1979), predation (Dodd 1959, Philips 1974, Holt and Polis 1997), resource partitioning (Tilman 1982, Hattori and Shibuno 2013), interference (Schoener 1976), priority effects and the fugitive strategy (Hutchinson 1954, Kneidel 1983), the relative effects of interspecific to intraspecific competition such as aggregation (Shorrocks et al. 1979) and dispersion (Huffaker 1958) and non-equilibrium and variable dynamics (Hutchinson 1961). Patchy distributions of resources combined with heterogeneity in environmental conditions allows species to find more suitable resources and conditions on a microclimatic scale (Simberloff and Wilson 1969, Levins 1979, Chesson and Warner 1981, Sulkava and Huhta 1998, Barton et al. 2013, Hattori and Shibuno 2013). In the decomposer soil community, an increase in habitat patchiness caused an increase in species' acquisition of resources, which ultimately resulted in an increase in biodiversity and decomposition rate (Sulkava and Huhta 1998). Carrion beetles (Coleoptera:

Silphidae) partitioned themselves with respect to season, habitat, and diurnal activity, which contributed to high levels of species diversity and coexistence (Kocarek 2001). During late fall, when overall beetle numbers were low and competitive interactions were infrequent, some species were able to persist in areas they had been previously excluded from (Kocarek 2001). Because the carrion insect community is highly diverse, with many families of insects, like silphid beetles, containing multiple species within the same trophic guild, the same mechanisms that maintain diversity and coexistence within the carrion beetle assemblage may also be present in other carrion insect families.

Temporal variability, not just in phenology but also in arrival time, may change competitive or colonization abilities and resource use, resulting in priority effects that mediate coexistence. Early arriving species can exert a priority effect on later arriving species (Beaver 1977, Hanski and Kuusela 1977, Kneidel 1983, Shorrocks and Bingley 1994, Fukami et al. 2005, Korner et al. 2008, Moore and Franklin 2012, Von Gillhausen et al. 2013). With positive priority effects, later arriving species have an increased ability to colonize and gain fitness due to the presence of early arriving species. For example, in two species of saproxylic beetles, *Rhagium inquisitor* L. exerted a positive priority effect on *Acanthocinus aedilis* L., with *A. aedilis* producing more offspring when it followed or arrived simultaneously with *R. inquisitor* (Victorsson 2012). In contrast, in the case of negative priority effects, secondary colonizers suffer a decrease in colonization ability and/or fitness of the subsequent offspring. For example, in carrion-breeding dipteran communities developing on snail carcasses, the early arrival of *Megaselia scalaris* (Loew) resulted in its increased survival and it had strong negative impacts on other species present, specifically another phorid fly, *Megaselia aurea* (Aldrich), and

Drosophila tripunctata (Loew)) (Kneidel 1983). *Megaselia scalaris* acted like a fugitive species and by arriving earlier, it was able to persist in the community (Kneidel 1983). Species coexistence can be enhanced or weakened when priority effects are present.

Many dipteran families have evolved to utilize patchy, fragmented and ephemeral resources such as flowers and decaying fruits (Buck 1997, Shorrocks 1990), mushrooms (Atkinson and Shorrocks 1977, Shorrocks 1990, Shorrocks and Bingley 1994), both large and small carcasses (Beaver 1977, Buck 1997, Von Zuben et al. 2001), and dung (Buck 1997). Within the carrion insect community, blow flies (Diptera: Calliphoridae) are typically the first to colonize and assemble and do not require an additional species to be present for establishment. They consequently form the base of the community that subsequently develops (Baumgartner and Greenberg 1985, Greenberg 1991, Byrd and Castner 2001, Campobasso et al. 2001, Beasley et al. 2012, Barton et al. 2013). Most species within the blow fly family are considered members of the sarcosaprophytic guild, the larvae of which feed directly on decomposing animal tissue to complete their development (Braack 1987). Adult flies are anautogenous: they require additional feeding during the adult stage to obtain the nutrients required to produce eggs (Wall et al. 2002, Davies 2006). Adult flies in the wild have shortened life-spans (approx. 50 degree-days in *Lucilia sericata* (Meigen)) compared to longevity in captivity (e.g., 123 degree-days in *L. sericata* (Meigen) (Pitts and Wall 2004), and typically live long enough to lay a single batch of eggs only once during their lifetime (although some females may deposit oviposit 2-3 batches eggs) (Hayes et al. 1999, Davies 2006). Blow flies are important in returning nutrients back into the surrounding ecosystem while providing a resource for higher trophic levels (Beasley et al. 2012, Barton et al. 2013). Due to their

early arrival and their importance as an additional base resource in this system, much insight can be gained by examining the patterns and processes that govern calliphorid fly colonization and utilization of animal carcasses.

Blow fly species have been known to exhibit habitat and/or seasonal differences. *Lucilia sericata* (Meigen) has been collected more abundantly in open pasture habitats, while *Calliphora vicina* (Robineau-Desvoidy) is more abundant in woodland and hedgerow sites (Smith and Wall 1997). Further examination found asymmetric larval competition between these species, with *L. sericata* having lower abundance levels on carcasses where *C. vicina* was also present suggesting that the uneven distribution of adults between habitats was important in structuring the blow fly family (Smith and Wall 1997). Baumgartner and Greenberg (1985) also found that coexistence of more than 26 blow fly species along a transect in a small Andean forest was due to niche partitioning along various climatic zones and habitats. Early studies examining the distribution and dispersal of blow flies indicated that different blue bottle flies (Tribe: Calliphorini) have higher abundance levels in cooler months and habitats, whereas green bottle flies (Tribe: Luciliini) inhabit well-lit warmer habitats (Macleod and Donnelly 1958). Some species, such as the black bottle fly, *Protophormia terraenovae* (Robineau-Desvoidy) (Tribe: Phormiini), showed no significant trends with respect to season or habitat (Macleod and Donnelly 1958). Blow fly populations vary in abundance with habitat or season and, in addition, recruitment to a resource can vary with respect to attraction to particular types of bait (Baumgartner and Greenberg 1985). Davies (1999) determined that season, habitat, size and type of carcass influenced blow fly populations. Seasonal conditions and specific climatic conditions prevailing in a particular year can affect the arrival

pattern of many carrion insects, including blow flies (Archer 2003). Despite this variability, regular seasonal and habitat patterns within a species and differences between species suggest that within-guild partitioning does occur in blow flies at multiple spatial and temporal scales (Davies 1999, Archer 2003, Archer and Elgar 2003, Hwang and Turner 2005, Brundage et al. 2011, Benbow et al. 2013, Fremdt and Amendt 2014).

Spatial aggregation within and between resource patches can lead to coexistence. Although the consequences of aggregation within a resource have been well studied (Hanski 1981, Atkinson and Shorrocks 1984, Ives 1989, 1991, Spencer et al. 2002), the mechanisms underlying this behaviour, specifically with respect to clutch size, arrival order and oviposition decisions, such as where and when a female should deposit eggs, are not well understood (Hoffmeister and Rohlfs 2001). Optimal clutch size reflects a trade-off between maximizing female fecundity and offspring fitness (Lack 1947, Godfray et al. 1991, Kagata and Ohgushi 2004, Charnov and Morgan Ernest 2006). Oviposition decisions can be influenced by species interactions and the consequences of these decisions can be measured through offspring traits: their size, reproductive potential and fecundity, mortality and developmental rates (Fox and Czesak 2000, Hendry et al. 2001, Kagata and Ohgushi 2004). By measuring the size of insects, one can assess the direct and indirect effects of species interactions during various stages of development and understand how these factors affect the fitness of subsequent adults. There is a general interrelationship between adult body mass and individual egg mass (Rahn et al. 1975, 1985, Hendry et al. 2001, Creighton 2005). Maternal body size is positively correlated with egg size and clutch size and, thus, with fecundity and reproductive success (Jann and Ward 1999). Body size is an important variable

commonly used to measure effects of intra- and inter-specific competition in a variety of dipteran families, including house flies (Muscidae; Peters and Barbosa 1977), fruit flies **(**Drosophilidae; Atkinson 1979), mosquitoes (Culicidae; Barbosa et al. 1972), and black flies (Simuliidae; Malmqvist et al. 2004). Adult blow fly size is constrained by the amount of resources consumed by larvae (Mackerras 1933, Fuller 1934, Ullyett 1950, Goodbrood and Goff 1990, Marchenko 2001, Slone and Gruner 2007, Shiao and Yeh 2008, Reid 2012). Dipteran pupal size is highly correlated with adult body size (Jann and Ward 1999, Allen and Hunt 2001, Fischer et al. 2004). Honek (1993) determined that for oviparous and larviporous insects, there exists a potential 0.95 % increase in fecundity for each 1% increase in dry body weight across a wide range of species. Adult blow flies can be easily measured, which provides a viable method to investigate the effects of abiotic/biotic factors as well as the consequences of interactions between individuals, populations and guilds within the carrion community on estimates of fitness.

In many insects, immature stages have limited dispersal ability and are highly influenced by the oviposition decisions made by the parent female (Von Zuben et al. 2001, Gripenberg et al. 2010, Liu et al. 2012, Akol et al. 2013). This is particularly true in patchy and ephemeral resources, in which the number of eggs laid and resultant larvae produced within a patch is dependent upon competition and factors affecting populations and individuals in the previous or parental generation (i.e. immigration rates, dispersal, fecundity) (Von Zuben et al. 2001). Differences in oviposition strategies within and between species on patchy resources can lead to long-term coexistence of species populations (Atkinson and Shorrocks 1981, Ives 1988). By selecting more suitable oviposition sites that maximize offspring fitness, or by preferentially ovipositing with

conspecifics, females indirectly provide refuges for other species to oviposit and coexist within the system. This effect can extend from within a resource to between resource patches to encompass multiple spatial scales, and thus can promote local and regional coexistence (Inouye 1999). Dispersal between patches can also promote coexistence between predator and prey species (Huffaker 1958) as well as competitors (Inouye 1999).

Although the adult stage is important in influencing larval distribution patterns at the local (within a resource) or regional (between resources) scale, larval interactions and the mechanisms that govern them can have profound influences on individuals (i.e. reproduction, survival, dispersal), populations (i.e. population dynamics, stability, future recruitment), and overall community structure (Fuller 1934, Hassell 1975, Denno and Cothran 1975, Peters 1983, Forrest 1987, Allen and Hunt 2001, Boggs and Freeman 2005, HillesRisLambers et al. 2012, Kvist t al. 2013). Many different forms of interactions can occur between individuals, both intraspecific and interspecific, and with their abiotic and biotic environment. These interactions influence adaptations in insects that can differ between life stages or can act directly and/or indirectly to impact a single stage (e.g., juvenile or adult) (Kingsolver et al. 2011) to ultimately influence adult size, behaviour (Peters 1983) and/or population dynamics (McPeek and Peckarsky 1998). Since the strength of species interactions can vary with respect to life stage (Yodzis 1988, Paine 1992, McPeek and Peckarsky 1998), there is a need to understand species interactions during multiple life stages (Kingsolver et al. 2011, HilleRisLambers et al. 2012). It is likely that there are many factors that determine blow fly community structure at any particular point in space and time. Ecological studies are vital to understanding these mechanisms at multiple life stages, particularly considering the

importance of this insect family in forensic entomology.

Application to Forensic Entomology

An important application of the principles of carrion decomposition lies within the field of forensic entomology. Forensic entomology is the use of insects in criminal investigations, primarily to narrow down the post-mortem interval (PMI) in death investigations (Byrd and Castner 2001). Upon discovery of remains, insect samples are taken from a corpse and surrounding area (i.e. soil, leaf litter) and compared to known successional timelines. These timelines are created by compiling a day-to-day catalogue of insect species on multiple carcasses for a particular region, season or set of ecological and environmental conditions (Schoenly et al. 2007). Extensive research has shown that the carcass of a pig (~25 kg starting mass) is an acceptable model for a dead human body (Schoenly et al. 2007), having similar internal cavity dimensions, skin characteristics, fat distribution, gut fauna and insect successional patterns (Smith 1986, Catts and Goff 1992, Goff 1993, Anderson and VanLaerhoven 1996, Byrd and Castner 2001 Schoenly et al. 2006, 2007). These characteristics make the carrion system an appropriate model to test whether successional patterns are truly replicable. The ability of a forensic entomologist to estimate PMI is dependent upon the quality of data collected from baseline studies used to compare colonization times and successional patterns (Schoenly et al. 2006, 2007). Patterns of succession may vary in response to differences in environmental conditions such as habitat, size and type of carrion and climate (Anderson and VanLaerhoven 1996, VanLaerhoven and Anderson 1999, Woodcock et al. 2002, Archer and Elgar 2003, Schoenly et al. 2006). Understanding how these patterns are influenced

by such factors is pivotal for the use of successional studies in a forensic context.

The field of forensic entomology has progressed over the years and has moved from a qualitative to quantitative approach (Tomberlin et al. 2011, 2012). Though it is still important to document patterns of succession in carrion insects, more emphasis has been placed on understanding the mechanisms that drive these patterns as well as the factors that can alter assembly patterns or species presence/absence in the community. There is a need to validate the use of insects in legal investigations, which can only be done through the use of scientific experimental designs that incorporate true replicates to allow for the assessment of replicability in results (Tomberlin et al. 2012). The use of lab and field based studies focusing on ecological interactions between individuals, species, populations and abiotic factors would provide insight into the mechanisms explaining why observed patterns are occurring. Tomberlin et al. (2012) outlined specific criteria to consider when carrying out forensic entomological research. Those criteria arose from a report from the National Research Council (NRC), which called to validate the science used within the field of forensics. They include: a proper animal model (e.g., the pig); a consistent time of death and method of euthanasia; a consistent storage method and duration (none in this study); consistent period of time until carcass placement; sufficient number of replicates for statistical analyses; consistent timing (i.e. month) of study; and proper sampling to account for time of colonization as well as community progression throughout decomposition (Tomberlin et al. 2012). The studies described in this dissertation not only meet the criteria outlined by Tomberlin et al. (2012), but constitute the most comprehensive forensic entomological studies conducted to date. Although carrion communities and species interactions may differ between regions, the

experimental approaches used in this study can be easily repeated and provide a general template for future research examining community processes and species interactions.

Research Objectives

The first objective in this study was to examine the effects of habitat (i.e., spatial partitioning) and season (i.e., temporal partitioning) on the structure of the blow fly (Family Calliphoridae) community in southwestern Ontario (see Chapter 2). With respect to spatial and temporal partitioning, I hypothesize that if these mechanisms are important in the blow fly community, there will be distinct differences in community structure between habitats and/or seasons. If these processes are not important in structuring the community, then there would be no differences in community structure between habitats or seasons. Based upon previous literature, I would expect to see differences in species composition and community structure between seasons. However, given that blowflies have good dispersal abilities and are adapted to finding carrion as a resource, I would expect to find similar community composition and structure between habitats, as it would be equally likely for flies to reach carrion placed in nearby habitats.

Given the forensic application of the current research, dead domestic pigs, *Sus scrofa domesticus* L., were used, as recommended for forensic investigations (Schoenly and Reid 1987, Catts and Goff 1992, Goff 1993, Schoenly et al. 2006, 2007, Tomberlin et al. 2011, 2012). Pig carcasses were placed in forest and field habitats over three seasons (spring, summer and fall) in the Windsor-Essex region of Ontario. In order to standardize analyses, temperature data were transformed into accumulated degree-days (ADD), which is known to be important for insect behaviour, development, availability,

community composition and member interactions. Four community indices (number of species (S), Species Evenness (E), Simpson's Index of Diversity (1-D) and Levins' Standardized Niche Breadth (Ba)) were examined over four ADD quartiles**,** for two habitats (field and forest) and three seasons (spring, summer and fall), to determine the role of spatial and temporal partitioning in blow flies. Community composition was assessed through relative abundance and was examined through the use of Non-Metric Dimensional Scaling (NMDS) with Multi-Response Permutation Procedure (MRPP) to test if habitat and season were significant grouping factors for blow flies in Windsor/Essex County, Ontario.

The second objective in this study was to determine the role of priority effects in species interactions and their importance in mediating coexistence of blow fly species. This was investigated by manipulating blow fly species arrival order in two-species systems and by examining species' performance on piglet carcasses. Three blow fly species (Diptera: Calliphoridae), *Lucilia sericata* (Meigen), *Phormia regina* (Meigen) and *Chrysomya rufifacies* (Macquart), all species found on carrion in the Great Lakes Region, were selected to test the role of priority effects within the carrion insect community. For adult interactions (Chapter 3), the time and location of oviposition events were and total number of eggs laid by each species were recorded and compared. For larval interactions (Chapter 4), mortality rates, overall survival and several measures of adult size were recorded and compared. If there were no priority effects among blow flies, then there would be no effect of arrival order on subsequent populations of blowfly species, and no differences between single and dual species communities. If priority effects occur, then colonization by a species and/or larval interactions would be

influenced by arrival order (i.e. the presence of an additional species). These differences would be evident in the variables measured in this study.

This research examines patterns of blow fly community structure and composition over a large spatial and temporal scale combined with lab-based manipulative studies to examine species interactions of three calliphorid species on a finer spatial and temporal scale. Since blow flies utilize patchy and ephemeral resources, are easily reared and manipulated, have short life cycles, and are relatively common, they can be considered a model system to study the relative importance of spatial and temporal partitioning, species interactions (both intra- and inter-specific) and how these interactions may influence adult female oviposition decisions and larval interactions. The blow fly system can also be used to examine how these effects can cascade to influence the potential fitness of individuals, populations and community structure.

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CHAPTER 2: THE EFFECT OF SEASON AND HABITAT ON SUCCESSION AND COMMUNITY COMPOSITION OF BLOW FLIES (FAMILY: CALLIPHORIDAE) ON PIG CARCASSES

INTRODUCTION

Understanding species' abundance patterns and monitoring spatial and temporal changes in community structure is important in understanding the coexistence of multiple species that seem to utilize the same resources (Ives 1991, Tokeshi and Schmid 2002, Chave 2004, Inouye 2005, Razgour et al. 2011, HillesRisLambers et al. 2012, Barton et al. 2013). Coexistence can occur between species with high levels of niche overlap and shared life history characteristics (Barker 1971) provided the community has a high level of species evenness, which can lead to species' persistence and ecosystem stability (Collet et al. 2014, Pu et al. 2014). Resource partitioning over time and space allows for species coexistence and persistence in highly speciose communities.

Aggregation of competing species can be considered a type of niche partitioning on a spatial scale (Atkinson and Shorrocks 1984, Inouye 2005, Hattori and Shibuno 2013). Aggregation can occur both within a single local resource patch as well as between resource patches to promote coexistence on many spatial scales. High levels of larval aggregation, which resulted from adult female flies ovipositing among carcasses, provided a mechanism for coexistence by decreasing interspecific competition while increasing intraspecific competition (Atkinson and Shorrocks 1981, 1984, Atkinson 1985, Ives 1991, Woodcock et al. 2002, Inouye 2005). Thus, a less competitive species may be able to exploit and sustain its population in another resource patch within the ecosystem even if it is extirpated within an individual local resource patch (Atkinson and Shorrocks

1981, 1984, Atkinson 1985, Ives 1991, Woodcock et al. 2002, Inouye 2005, Hattori and Shibuno 2013). Although a less competitive species may experience local extinction events, it is able to persist in the community on a regional level demonstrating the importance of examining coexistence and population dynamics at multiple spatial scales (i.e. local as well as regional) (Inouye 2005, Hattori and Shibuno 2013).

Resource partitioning can also occur temporally. In temperate regions where distinct seasonal conditions exist, many species exhibit either preferences for particular seasons or may undergo diapause during periods of unfavourable weather conditions such as the heat of summer or to avoid freezing in winter (Morin 1999). Differences between species' establishment probabilities can influence community structure and can allow for many species to coexist within a particular space or time period (Hubbell 2001, HillesRisLambers et al. 2012). Temporal partitioning with respect to arrival order, in conjunction with differences in competitive/colonization abilities or resource use combined may result in priority effects which can also mediate coexistence (Kneidel 1983, Shorrocks and Bingley 1994, Von Gillhaussen et al. 2014). At smaller temporal scales, there may be diurnal as well as seasonal changes in community composition that interact with spatial resource partitioning of similar habitats to facilitate coexistence (Neilson 1978, Albrecht and Gotelli 2001). Resource partitioning (Tilman 1982, Chesson 2000, Chase and Leibold 2003, Razgour et al. 2011) combined with differential responses to abiotic conditions (Indermaur et al. 2009, Razgour et al. 2011) over various temporal and spatial can alter the presence, absence or interactions between community members and can promote coexistence between species.

The carrion insect community, and in particular the community of blow fly (Calliphoridae) species, is a model system frequently used to study community assembly patterns and mechanisms. Blow flies are among the first insects to colonize remains during the early stages of decomposition (Baumgartner and Greenberg 1985, Greenberg 1991, Byrd and Castner 2001, Campobasso et al. 2001, Michaud and Moreau 2013). They are generally common insects that have strong dispersal abilities. Multiple blow fly species usually utilize the same carrion resource (Kamal 1958, Smith 1986, Greenberg 1991, Michaud and Moreau 2013), but overall their diversity is moderate (i.e., ~10-20 species in any particular region (Kamal 1958, Macleod and Donnelly 1958, Denno and Cothran 1975). Individual species may exhibit habitat or seasonal associations (Macleod and Donnelly 1958, Denno and Cothran 1975, Hanski and Kuusela 1980, Kneidal 1984, Baumgartner and Greenberg 1985, Wells and Greenberg 1994, Smith and Wall 1997, Davies 1999, Archer 2003, Archer and Elgar 2003b, Brundage et al. 2011, Benbow et al. 2013), patterns that contribute to coexistence in blow flies. For example, Baumgartner and Greenberg (1985) determined that some species in Peru are preferentially attracted to particular types of bait, while other species vary in abundance with habitat or season. However, some species, such as the black bottle fly, *Protophormia terraenovae* (Robineau-Desvoidy) (Tribe: Phormiini), show no significant differences with respect to season or habitat (Macleod and Donnelly 1958). Despite the annual, habitat and resource variability that occurs in the carrion community (Archer 2003), distinct seasonal profiles of blow fly abundance often develop (Archer 2003, Archer and Elgar 2003b) and may contribute to species coexistence (Macleod and Donnelly 1958, Denno and Cothran 1975, Hanski and Kuusela 1980, Kneidal 1984, Wells and Greenberg 1994).

In this study I investigate temporal and spatial patterns in community structure and composition between two habitats (forest and field) and three seasons (spring, summer and fall), to determine if partitioning by habitat or season occurs in the blow fly community in southwestern Ontario. If spatial partitioning is not important, then there will be no significant difference in community indices or community composition (H_{O1}) between forest and field habitats. Similarly, if temporal partitioning is unimportant in the development of blow fly communities, then there will be no significant differences in community indices or community composition between spring, summer and fall seasons (H_{O2}). If either of these null hypotheses is rejected, then spatial and/or temporal associations of blowflies will result in differential patterns in community composition that contribute to persistence of some species in the overall landscape and regional species pool. For community composition analysis (NMDS and MRPP), if habitat proves not to be a significant grouping factor (H_{O3}), then there would be no partitioning among blow fly species over a large spatial scale. If season is not a significant grouping factor (H_{O4}), then there would be no partitioning among blow fly species over a broad temporal scale.

METHODS

Test Site Locations

Experimental test sites (A-F) were in the Windsor-Essex County region of Ontario, Canada. Site A was located at the Windsor Regional Airport; Site B at Ojibway Nature Preserve; Site C in McGregor Township; Site D near Harrow; Site E near Essex; and Site F near Amherstburg (see Figure 2.1). Domestic pig, *Sus scrofa domesticus* L., carcasses (described below) were placed in field and forest habitats at each of the sites, thus facilitating direct comparisons of habitat effects on the blow fly community. The field habitats were open sites with no tree cover, maximum light penetration and either tall grasses (Site A, B and E) or open agricultural fields (Sites C, D and F). Forest habitats were located more than 25 m from the forest edge and were completely covered by the forest canopy to limit edge effects. All forest habitats were classified as Carolinian deciduous forest (i.e. tree species composition of hickory, ash, chestnut, walnut, oak) (Site Assessment Report, Site A). The Windsor-Essex County Region is warmer than all other regions of Canada; it typically experiences a minimum of 223 days per year with maximum daily temperatures above 10 °C (Windsor-Essex County Development Commission 2006). Although Windsor-Essex County is highly urbanized, all test sites were located outside urban centres, in areas with fewer than 1000 people and less than 400 people km^{-2} (Statistics Canada 2001, 2011).

To ensure each pig carcass represented an independent replicate within each site, the forest and field carcass locations within each test site were separated by at least 100 m. This methodology was based on evidence from Anderson and VanLaerhoven (1996) that 50 m is sufficient isolation to eliminate olfactory interference. Each test site (A

through F) was located at least 3.5 km from others to capture a large spatial scale and to ensure independent colonization of carcasses by blow flies.

Experimental Design

Domestic pigs were used as surrogates for humans (Schoenly and Reid 1987, Goff 1993, Schoenly et al. 2006). It has been shown that decomposition processes that occur on pig carcasses (23-27 kg starting mass) are similar to those which occurs on human bodies (Schoenly et al. 2007), as domestic pigs and humans have similar internal cavity dimensions, skin characteristics, fat distribution and gut fauna (Smith 1986, Catts and Goff 1992, Goff 1993, Anderson and VanLaerhoven 1996, Schoenly et al. 2006, 2007). To maintain consistency across replicates, all pigs were female, 27.0 ± 4.1 kg mass at death, and were orientated similarly, with the head of each pig facing north and the dorsal side facing east (see Figure 2.2).

Pig carcasses were placed in both habitats at each test site in spring, summer and fall to examine possible seasonal effects on the blow fly community. Day 0 for each experiment was 14 April 2005 (spring), 24 June 2005 (summer) and 3 October 2005 (fall). On Day 0 for each trial, 12 domestic pigs were killed on the farm at approximately 0900 h using a bolt gun pistol fired to the forehead. Pig carcasses were wrapped in a tarp to prevent exposure to insects and installed at test sites within 12 hrs following death in the spring season and within six hours from death in the summer and fall. At each test site, pig carcasses were inspected and any insect eggs, if present, were removed and the body area was rinsed with water and ethanol. Pigs were then dressed in t-shirts and shorts or underwear, as clothing has been found to influence the colonization and

succession of insects (Byrd and Castner 2001). Because wounds may influence successional patterns on animal carcasses (Greenberg 1991, Byrd and Castner 2001, Campobasso et al. 2001, Cross and Simmons 2010), a puncture wound was created in the lower left side of each pig's rib cage. A datalogger (ACR Smartbutton™) was inserted into the wound to measure internal carcass temperature at 60-minute intervals. An additional datalogger was placed at each test site, on the back of each malaise trap, approximately 1.0 m from the head of the pig and ~0.5m above the ground to record ambient temperatures at 60-minute intervals. To allow for weighing, carcasses were placed on a wired mesh platform (12.5 gauge) with a rebar frame. This allowed constant contact between the carcass and soil throughout the decomposition process. Mesh wiring (50 gauge) was placed over each pig and pinned into the ground using metal stakes to discourage scavengers. Malaise and pitfall traps were placed at each test site to collect flying and crawling insects, respectively, however, data from these collections were not included in blow fly community analyses. To quantify biomass loss, carcass weights were recorded weekly until only bones and adhering tissue remained and individual carcass weights did not change over two subsequent weighing events. This protocol was validated by De Jong et al. (2011) to measure temperature and biomass loss without causing significant disturbance to the decomposition process or community succession. Carcass weights were recorded for ten weeks during spring decomposition and five weeks for summer and fall trials.

Sampling began 24 hours after pig death at all test sites and continued daily until dipteran larvae completed feeding and reached the prepupal stage, which is characterized by them wandering away from the resource to find pupation sites (Greenberg 1990,

1991). This sampling frequency accounted for the majority of blow fly activity. However, though infrequent, a few female blow flies colonized carcasses during the later (i.e. advanced) stages of decay. If eggs and/or larvae were detected at this time, they were collected during the sampling of all carrion insects that was carried out every 2-3 days after prepupal wandering began; when carcasses reached the late advanced stage of decomposition they were sampled weekly until they reached the dry-remains stage and were no longer attractive to insects (Smith 1986, Greenberg 1991). At this time carcasses were removed and disposed of. At each sampling event pig carcasses and clothing were inspected for the presence of adult flies as well as egg masses and immature larvae. Non-colonized areas were not sampled, as there were no insects present in those regions. In areas where insect activity was present, adult flies were sampled by hand and placed directly into 70% ethanol, labeled, and later identified. Large larval masses (>1000 individuals) were sampled by collecting approximately 100 immature individuals from multiple locations. When small batches of eggs or larvae were located, ~5-10% were visually estimated and subsampled from one corner. This was repeated for each colonized region of the pig. When multiple egg masses or larval groups were present in the same region, they were sampled and reared separately as they may have been from different species. This sampling protocol is in agreement to Michaud and Moreau (2013) and Michaud et al. (2012), who suggested that hand sampling of ~10% of individuals in this way accurately reflects the dipteran community, while keeping the disruption to the developing insect community to acceptable levels (Michaud and Moreau 2013). Other than visual estimation of subsample sizes, there is no adequate way to quantify the amount of eggs removed without damaging or altering colonization patterns. However,

as proposed by Michaud and Moreau (2013) and Michaud et al. (2012), further studies should be done to determine upper threshold levels for sampling intensity.

After collection, each egg mass or group of larvae was placed in a non-sterile specimen container (120 ml) with pork liver and covered with a paper towel secured with an elastic rubber band. Eggs and larval masses remaining on the carcasses were left to develop undisturbed unless more eggs were laid in the same location or larvae began to converge into larger masses. Larger larval masses were also sampled by removing ~100 larvae, with small subsamples being taken throughout the mass at different depths and locations. This sample size and methodology is considered to be effective for sampling species within the community without significantly affecting insect succession (VanLaerhoven and Anderson 1999). Once daily sampling of each carcass ended, each egg or larval sample was transferred from its specimen container to a 1 L Bernardin™ Mason jar, the lower third of which was filled with vermiculite as a pupation medium. Larvae were fed pork liver *ad libitum* until the prepupal/wandering stage, at which point food was removed. Larvae pupated and adults emerged within the jars. After their death, adult flies were separated and identified to species. Specimens were pinned, labeled and placed in insect boxes; with large samples, representatives from each species were pinned and remaining specimens were counted and stored in scintillation vials.

During the fall sampling period, a large percentage of pupae entered diapause and did not complete development, thereby affecting community analyses. However, because rearing was carried out in jars kept outdoors under ambient conditions, any emergent adults reflected true fall populations, whereas dormant pupae would have overwintered and be reflective of the subsequent spring blow fly communities.

Degree days were calculated for each day by subtracting the lower developmental threshold temperature (set at 0ºC; see below) from the daily mean temperature at each site. Values for lower developmental thresholds vary with respect to species, within a species, geographic region, life stage and environmental conditions (i.e. photoperiod, fluctuating temperatures). Many of the lower threshold values for blow flies have been estimated through regression rather than determined experimentally (Nabity et al. 2006, Warren 2006, Anderson and Warren 2011). The use of theoretical rather than experimental values to determine developmental threshold temperatures can lead to errors in estimates of accumulated degree days (ADD) or hours (ADH) (Anderson and Warren 2011). The use of base threshold temperatures above 0°C can also lead to overestimates of the post-mortem interval (VanLaerhoven 2008). Given these considerations, I assigned a minimum base threshold value of 0°C. When the mean daily temperature was below 0ºC, a value of zero was assigned for degree-days on those dates. Accumulated degree days (ADD) were then calculated for each date within the study by adding to the ADD value for the previous day the current day's DD, in order to determine daily ADD values on a *per site* basis ($ADD_{site} = \Sigma\ DD_{1, 2, \ldots n}$; n = total number of experimental days).

Biomass loss was calculated as the remaining pig weight divided by initial carcass weight for each weight event during decomposition. In weeks when values exceeded the initial carcass weights (due to the weight of insects present in addition to the carcass weight), biomass loss was considered zero.

Community Indices

Four community indices were used to monitor changes in community structure: species richness (S, the number of species present within a sample from a pig at that particular ADD quartile), species evenness (E; Pielou 1966), Simpson's Index of Diversity (1-D; Simpson 1949), and Levins' Standardized Measure of Niche Breadth (B_A; MacArthur and Levins 1967). All diversity measures were calculated for each pig overall, and also for each ADD quartile: from 0 – 50 ADD, 50 – 100 ADD, 100 – 150 ADD and 150+ADD. These four quartiles were chosen to represent biologically significant time points during decomposition. The 1st quartile (0-50 ADD) represented early arriving species (i.e. the primary colonizers within the blow fly community) and typically corresponded to the first 24 to 48 hrs postmortem. The 2nd and 3rd quartiles (50-100 ADD, 100-150 ADD) represented the most active periods of larval activity and were characterized by the presence of multiple, large maggot masses and rapid tissue removal. The 4th quartile (150+ ADD) represented the remainder of the decomposition period, which was primarily characterized by few remaining larvae since the majority of larvae had reached the prepupal stage and had dispersed from the carcass. Although the spring decomposition was prolonged, the last degree-day section was still characterized by few larvae and occurred after the majority of larvae had dispersed.

Statistical Analyses

For all statistical tests, a significant effect was designated when $p<0.05$, or the appropriate value following a Bonferroni correction.

Analyses of temperature and biomass data were conducted using the statistical

software program IBM SPSS Version 21 (2012). Residuals for daily temperature met the homogeneity of variance assumption (Levene's test, $p>0.05$) (IBM SPSS Manual 21). Daily temperature data were analyzed using a general linear model with experimental day, test site, habitat and season as main effects (IBM SPSS Manual 21). All two-way and three-way interactions for test site, habitat and season were included in the model. Data for ADD were log transformed to improve fit for a generalized linear model with a gamma distribution and log link function (McCullagh and Nelder 1989, IBM SPSS Manual 21). "Days of decomposition" was used as a covariate and test site, habitat and season as main effects. Slope parameters were compared and a Bonferroni correction applied to p-values in order to examine differences between treatments.

Biomass loss typically follows an exponential or logarithmic decay pattern (Simmons et al. 2010, De Jong et al. 2011) and was analyzed using a generalized linear model with a gamma distribution and log link function on percentage of biomass remaining (McCullagh and Nelder 1989, IBM SPSS Manual 21), with ADD as a covariate and site, habitat and season as main effects. Slope parameters were compared and a Bonferroni correction applied to p-values to determine significant differences in biomass loss rates between treatments.

Statistical analyses on three out of four community indices (# species [S], species evenness [E] and Simpson's Index [1-D]), non-metric multi-dimension scaling (NMDS) and multi-response permutation procedure (MRPP) analyses were carried out using the statistical software program IBM SPSS Version 21 (2012). Stata 13 (Statacorp 2013) was used to analyze Niche Breadth (Ba) values as results presented from SPSS were not based upon bootstrapped estimates.

A repeated measures ANOVA was used in order to test for within-pig effects across ADD quartiles as well as between pig effects for habitat and season. Residuals were checked for normality using Shapiro-Wilks tests due to smaller sample sizes. Residuals were normal ($p>0.05$) for three out of four community indices (# species [S], species evenness [E] and Simpson's Index [1-D]). Mauchly's test was used to determine if there were any violations of the sphericity assumption, which was the case in all 3 indices ($p<0.05$). Thus, a Greenhouse-Geisser correction factor was used (Greenhouse and Geisser 1959). Residuals for Levins' Standardized Niche Breadth were not normal ($p<0.05$) and various transformation methods including the log transformation, log(x+1), natural logarithm, inverse transformation and square root transformation did not normalize the data. Therefore, a bootstrapped (k=1000, simple sampling) repeated measures ANOVA was used (Efron 1979). Pairwise comparisons were carried out *post-hoc* based on either on estimated means or bootstrapped estimated marginal means (when appropriate) in order to examine the differences in measures between ADD quartiles within each season as well as the differences between seasons within each ADD quartile. All *post hoc* tests and pairwise comparison p-values were corrected using the Bonferroni correction to prevent Type I error.

Community composition was examined by determining relative abundance for each species in each sample was calculated by dividing the number of individuals of a species by the total number of individuals in the sample. Non Metric Multi-Dimensional Scaling (NMDS) and Multi-Response Permutation Procedure (MRPP) were based on Euclidean distances (Zimmerman et al. 1985, McCune and Grace 2002, Cai 2006) and analyses were carried out using the statistical software program IBM SPSS Version 21

(2012). NMDS across a 2-dimensional ordination space was used to visualize differences in community composition between blow fly communities in forest and field habitats and between seasons (spring, summer and fall) using the relative abundance of blow fly species to compare community composition during decomposition. Each pig carcass was represented as a data point. To compare communities over time, the communities of the four decomposition quartiles described above were compared. MRPP analysis was carried out on relative abundance data over forest and field habitats to evaluate the efficiency of habitat as a grouping variable, and then again over spring, summer and fall seasons to evaluate the efficiency of season as a grouping factor for the blow fly community. This was done for each ADD quartile as well as overall where data for all quartiles and each species were combined. MRPP macro-codes were obtained from http://lcai.bol.ucla.edu/mrpp.txt and used as described by Cai (2006).

RESULTS

Analyses of Site Temperature and Rates of ADD Accumulation

Sites differed in microclimate, as evident in the significant interaction between site, habitat and season ($F_{14,15476}$= 5.167, $p < 0.001$, R^2=0.958) (adjusted α=0.0009). In general, field locations were warmer than forest locations, except during the fall season and at control sites in the summer when forest and field temperatures were similar. During the summer, temperatures were similar in the forest locations but differed in the field locations such that Control 1, 2 and Site C were similar to each other but cooler than Sites A, B, D, E and F. Sites B, D and F were cooler than Sites A and E. Site E was similar to Site A and both were warmer than all other sites. During the spring, there were site differences in both forest and field habitats. In the field locations, Control 1, 2 and Site C were similar to each other but cooler than Sites A, B, D, E and F (see Figure 2.3a). In the forest, Control 2 was warmer than the remaining sites (see Figure 2.3b). In order to account for site specific differences in temperature, further analysis in this chapter is based upon accumulated degree days (ADD).

Rates of accumulated degree days (ADD) differed between habitat and season with a two-way interaction (X^2= 6.863, df = 2, p = 0.032) (adjusted α=0.017). Habitat comparisons were made for season, however, when a Bonferroni correction was applied, differences between forest and field habitats were no longer statistically discernable. Parameter estimates (β_i) were compared in both forest and field habitats and there were differences between seasons in the rate of ADD accumulation, with coefficients being higher in the spring than summer followed by fall (see Figure 2.4).

Biomass Loss

Biomass loss was similar between test sites and between forest and field habitats, however, seasonal differences were present. There was a significant season*ADD interaction effect, with season explaining the variation in the model since no other interaction terms or main effects were significant (adjusted α=0.017) (see Table 2.1). Parameter estimates (β_i) were compared using summer as a reference category. Carcasses decomposed similarly in the fall and summer (X^2= 2.079, df = 1, p = 0.149), with slower decomposition in the spring than summer (X^2= 107.71, df = 2, p<0.0001) (see Figure 2.5). During the first few weeks of decomposition, spring carcasses retained their weight while summer and fall carcasses lost most of their biomass.

Overall Blow Fly Community Composition

Mean relative abundance was determined for each blow fly species within each season and habitat to illustrate overall community composition (see Table 2.2). The spring blow fly communities in both habitats were dominated by *P. regina* (>80% in forest and >90% in field), while the remaining 10 – 20 % of the community consisted of *Cynomya cadaverina* (Robineau-Desvoidy), *Calliphora terraenovae* (Macquart) *C. vicina*, *Calliphora vomitoria* (Linnaeus), *L. sericata*, *C. macellaria* and *P. terraenovae*. *Lucilia illustris* was only collected in forest locations while *L. coeruleiviridis* was present in field locations. The summer was also dominated by *P. regina* (>99% in forest and >95% in field), while the remaining blow fly community consisted of *C. macellaria*, *P. terraenovae*, *L. sericata* and *L. illustris* in both field and forest locations, however *L. coeruleiviridis* was only present in field locations. The fall blow fly community showed

less dominance by *P. regina* (<50% in forest, <40% in field), along with an increase in the abundance of several other blow fly species: *C. vomitoria, L. illustris* and *C. macellaria*. A small percentage of the community was comprised of *C. vicina*, *C. cadaverina*, *C. rufifacies*, *L. sericata* and *L. coeruleiviridis*.

It is noteworthy that blue bottle flies (*C. vicina, C. vomitoria*, *C. terraenovae* and *C. cadaverina*) were only present in the spring and fall seasons; none were collected during the summer. *Protophormia terraenovae* was not present in the fall, but was present in the spring and summer in both habitats, while *C. terraenovae* was only present in the field habitat. *Cochliomyia macellaria* was present but rare in the community in spring and summer seasons, however comprised a major part of the fall community; it was more abundant in forest than in field habitats. *Chrysomya rufifacies* was only present during the fall.

Community Indices: Main Effects and Interactions

For each community index, the effects of season were examined within each ADD quartile. The effects of ADD quartile had to be compared within each season due to the presence of a significant interaction between ADD quartile*season. There were no significant interactions between ADD quartile*season* habitat, ADD quartile*habitat or habitat*season (see Table 2.3).

Effect of Habitat on Community Indices Within ADD Quartiles

Habitat did not contribute significantly to the variability within the blow fly community: community indices were similar between forest and field habitats. There

was no significant effect of habitat on the number of species, Species Evenness (E), Simpson's Index of Diversity (1-D) or Standardized Niche Breadth (Ba) (see Table 2.3).

Effect of Season on Community Indices Within ADD Quartiles

There were seasonal differences in the number of blow fly species present and species evenness during decomposition, with more species in fall than summer for all four quartiles (adjusted α=0.0056). This trend also occurred between the fall and spring, except for the 3rd quartile when the number of species in the fall and spring communities were similar (see Figures 2.6a and 2.6b). Simpson's Index values reflected this same seasonal pattern, with diversity levels being highest in the fall and lowest in the spring and summer, except for the 3rd quartile when spring diversity levels were similar to fall values. There was greater diversity in the summer than spring during the 2nd and 3rd quartiles, however, levels were similar to summer during the 1st and 4th quartiles (see Figure 2.6c). Niche breadth values varied with respect to season, such that fall communities had higher niche breadth values than summer. Spring communities were similar to summer during the 1st and 4th quartiles and similar to the fall during the 2nd and 3rd quartiles (see Figure 2.6d).

Effect of ADD on Community Indices Within each Season

Community indices varied over ADD quartiles for each season. During the fall season, there were more species in the 2nd quartile (see Figure 2.6a) and higher levels of species evenness and diversity in the 2nd and 3rd quartiles (see Figure 2.6b,c). There were no differences in niche breadth values over any of the four ADD quartiles (see Figure

2.6d). During the spring season, the number of species was highest in the 4th quartile and lowest in the 1st quartile (see Figure 2.6a). Species evenness, diversity and niche breadth values were highest in the 2nd and 3rd quartiles (see Figure 2.6b,c,d). During the summer season, there were more species in the 2nd quartile (see Figure 2.6a). In contrast, there were no differences in species evenness, diversity levels or niche breadth values over the four quartiles (see Figure 2.6b,c,d).

Season and Habitat Differences in Blow Fly Community Composition

Similarity in blow fly community composition was examined for each ADD quartile and on an overall basis. It was based on the relative abundance of each blow fly species per pig carcass for each season and habitat (see Appendix A). There were distinct seasonal groupings within the blow fly community. MRPP analyses determined that blow fly communities could be differentiated into seasonal groups during the 1st (δ=0.088, T=-14.231, p<0.001), 2nd (δ=0.086, T=-14.732, p<0.001), 3rd (δ=0.086, T=-15.534, p<0.001) and 4th(δ=0.009, T=-6.745, p<0.001) quartiles (see Figure 2.7). Although there was significant differentiation between seasons during the 1st and 4th quartiles (see Figure 2.7a,d) there was less separation in data points than during the 2nd and 3rd quartiles. Spring and fall communities were both characterized by the presence of similar sets of species (i.e. *P. regina*, *C. vomitoria*).

Habitat differences were non-existent, as evidenced by the lack of groupings between forest and field habitats for any ADD quartile (1st: δ=0.011, T=0.538, p = 0.654, 2nd: δ=0.010, T=-0.563, p = 0.246, 3rd: δ=0.010, T=-0.710, p = 0.203 and 4th: δ=0.010, T=-1.726, p = 0.063) (see Figure 2.8).

NMDS analysis that examined pooled relative abundance values over all quartiles for each pig demonstrated differentiation between seasons (see Figure 2.9a), which was confirmed with MRPP analysis (δ=0.022, T=-13.480, $p<0.001$). However, when examining the blow fly community on a habitat basis, NMDS and MRPP analysis demonstrated that there was no differentiation between blow fly communities in forest and field habitats (δ=0.025, T=-0.867, $p = 0.167$) (see Figure 2.9b).

DISCUSSION

The Effects of Time, Season and Habitat on Blow Fly Community Composition

There was no evidence of spatial partitioning of the blow fly community between forest and field habitats in this study, as the number of blow fly species, diversity, niche breadth values, evenness and relative abundance between species did not vary between forest and field locations. Similar to my study, research has shown no significant habitat associations for certain blow fly species (Macleod and Donnelly 1957, Goddard and Lago 1985, Joy et al. 2002, Centeno et al. 2004, Horenstein et al. 2012). Joy et al. (2002) found *P. regina* in similar proportions on raccoon carcasses in sunlit and shaded areas in West Virginia, which is similar to the forest and field communities that developed during spring and summer trials in my study. Martinez-Sanchez et al. (2000) collected *C. vicina* equally in open pasture and wooded habitats. Horenstein et al. (2012) also found similar blow fly species in sun and shaded locations, while Matuszewski et al. (2008) found no differences in community composition between pine-oak, hornbeam-oak and alder forest habitat types in Central Europe; however, because these studies lacked replication, inferences that can be drawn from them are limited.

Previous research has led to conflicting ideas regarding habitat preferences in blow flies. For instance, there are a number of blow fly species that have been classified as eusynanthropic, or dependent upon human environments (Gordh and Headrick 2001) and urban (Smith 1986, Greenberg 1990, Ferreira and Barbola 1998, Hwang and Turner 2005, Horenstein et al. 2007). However, in my study, *C. vicina*, *L. sericata*, *C. rufifacies*, all documented as being urban species, were readily collected in multiple rural sites where the population density was less than 150 pal/km^2 (Organization of Economic Co-

operation and Development, Statistics Canada 2006). Blow flies that have previously been considered as urban or eusynanthropic have been reported to be increasing in occurrence in natural, rural regions (Smith and Wall 1997, Schnack et al. 1998, Martinez-Sanchez et al. 2000, Centeno et al. 2004, Horenstein et al. 2007) or their urban association is dissolving (Schnack et al. 1998, Jensen and Miller 2001, Horenstein et al. 2007, Eberhardt and Elliot 2008). Alternatively, the purported association of these species with urban areas may simply be an artifact of a species' point of introduction, which is commonly associated with human travel**,** rather than a true habitat preference.

Other studies have inferred habitat preferences of blow flies on the basis of them being collected in either sunny or shaded locations, or in habitats dominated by certain plant species. Horenstein et al. (2007) determined that *C. vicina* was found primarily (>97% of individuals collected) in the shade and showed a negative correlation with temperature, with abundance increasing with decreasing temperature. Smith and Wall (1997) found *C. vicina* was more abundant in woodland and hedgerow sites than in open pasture. However, as mentioned previously, these studies lack replication and consequently the habitat preference reported is not supported statistically. In my study, two species did demonstrate habitat preferences: *L. illustris* was only present in forest locations and *L. coeruleiviridis* was only present in field locations in the spring. However, both these species were collected in such low numbers that conclusions about their habitat associations are weakly supported. It is known that differences in species presence and abundance between habitats and seasons can contribute to higher diversity and species coexistence, as seen within the blow fly community on broad temporal and spatial scales (Atkinson and Shorrocks 1981, 1984, Atkinson 1985, Cruickshank and

Wall 2002a). Though I conclude that spatial partitioning between forest and field habitats did not occur in the blow fly community I studied, additional habitat types in the region (e.g., shores, swamps, marshes) should be examined.

Temporal partitioning of the blow fly community in the Windsor-Essex region was evident both within and between seasons, with distinct differences in number of blow fly species, diversity, niche breadth evenness and relative abundance. In the spring, the number of species present increased over time with highest numbers during the later ADD quartiles (i.e. 100 – 150 ADD and 150+ ADD), indicating that additional blow fly species joined the community throughout the process of decomposition. Species evenness, diversity and niche breadth were highest during the 2nd and 3rd quartiles, which indicates that during this time in decomposition (50-150 ADD) blow fly species are able to coexist in more even numbers that earlier or later. Later in decomposition, the spring blow fly community became dominated by one or two species, with an associated reduction in species evenness despite the increasing number of species present. In contrast, fall communities had a high number of species (i.e., 5-6)**,** evenness and diversity through the 1st and 2nd ADD quartiles, after which these measures of diversity steadily decreased. Niche breadth values declined throughout decomposition of the carcasses. In the last ADD quartile, larval blow fly interactions diminish as larvae leave the resource to pupate. This is evident by decreased species evenness, diversity and niche breadth in both spring and fall.

Community structure was dominated by *P. regina* in the summer and remained consistent during decomposition. This suggests that the summer blow fly community is determined early in decomposition and then maintained with low evenness, diversity, and

niche breadth values and no change in species number. Consequently, the summer blow fly community becomes the result of a "first-come, first-serve" basis, where the number of females available for colonization during those first few hours post-mortem determines the resultant community structure (primarily *P. regina* in this study). Interestingly, *C, macellaria* also arrived quickly and in large numbers, but the numbers of their offspring on the carcasses remained low.

The blow fly communities in spring and fall were comprised of more species than in summer, with higher species evenness in fall than in spring. This contrasts with results from Prado e Castro et al. (2012) who found a higher number of species present during summer in Portugal. Prado e Castro et al. (2012) also recorded fewer blow fly species in spring during the active stage of decay, whereas in my study the number of species increased during decomposition during the spring season. However during the fall and summer season, I observed the pattern reported by Prado e Castro et al. (2012): the number of species increased from the initial stages of decomposition (i.e. from 1st to 3rd quartiles), peaked in the bloated and active stages (i.e. the 2nd and 3rd quartiles in the fall and the 2nd quartile in the summer) and decreased in late decomposition (i.e. 4th quartile).

The seasonal differences in community indices that I quantified may be due to slower decomposition in the spring compared to summer or fall, with spring carcasses maintaining most of their biomass during the first few weeks of decomposition. Cooler temperatures lead to longer periods of time during which the resource is attractive and available for colonization by blow fly species; prolong larval development (Jensen and Miller 2001, Joy et al. 2002); and lower consumption rates, thereby extending resource availability. These effects could result in longer persistence of species and extended

colonization of the carcasses by blow fly females, which occurred during spring trials. The summer trend of few species, high dominance and low evenness could have resulted from higher summer temperatures and faster ADD rates, which drastically decreased the time that carrion was available for colonization and subsequent larval development as biomass was rapidly lost in this season compared to decomposition in the spring.

Many of the seasonal differences in species composition can be explained by the presence of blue bottle flies (*C. vicina, C. vomitoria*, *C. terraenovae* and *C. cadaverina*) only in the spring and fall seasons. Many studies have reported differences in the presence/absence and abundance of blue bottle species and suggest that these differences relate to temperature conditions, with increased abundance of Calliphorini species in colder seasons (Schroeder et al. 2003, Watson and Carlton 2005, Horenstein et al. 2007, Fremdt and Amendt 2014) and a decrease in the abundance during summer seasons (Hall and Doisy 1993). In my study, although I occasionally observed adult blue bottles on carcasses early in the morning during the summer, they failed to reproduce, and it has been suggested that adults may survive in cooler refugia while larvae or pupae may enter diapause at high ambient temperatures until more favourable conditions return (for *C. livida* and *C. vicina*, Introna et al. 1991; for *C. vomitoria*, Anton et al. 2011). Both of these responses of blue bottle flies deserve further study.

Another seasonal difference in species composition was the presence of *C. rufifacies* only during the fall. This species is not permanently established in Ontario and its availability within the regional species pool of blow flies is dependent upon its dispersal from the mid to southern U.S. states where populations of this species can successfully overwinter (Rosati and VanLaerhoven 2007). Consequently, its presence

only in the fall is not due to species interactions, but rather its regional availability. Other studies have shown that *Chrysomya* spp. dominate in the majority of seasons and carcasses they colonize due to the ability of their larvae to become facultative predators, i.e. they consume both the resource and other insect larvae (Baumgartner and Greenberg 1984, Goodbrood and Goff 1990, Wells and Greenberg 1992, 1994, Baumgartner 1993, Watson and Carlton 2005, Rosati and VanLaerhoven 2007). In my study, *C. rufifacies* did not dominate the blow fly communities in fall; however, this may have been due to the low population of this species in Windsor-Essex County at that time.

There have been many studies demonstrating differences in abundance patterns in blow flies that relate to particular habitats or seasons (Deonier 1940, Hall 1948, Ulyett 1950, Cragg 1955, Denno and Cothran 1975, Smith 1986, Hwang and Turner 2005, Watson and Carlton 2005, Brundage et al. 2011, Benbow et al. 2013, Fremdt and Amendt 2014). In my study, *P. regina* consistently dominated blow fly communities during the spring and summer. Similarly, Joy et al. (2002) found *P. regina* to be dominant on raccoon carcasses in sunlit and shaded areas in southwestern West Virginia, similar to spring and summer carcasses in both field and forest habitats in my study, and they recorded low numbers of *L. sericata*. In contrast, in my fall study *P. regina* did not dominate the carcasses, with this species comprising <50% of the blow fly community. *Cochliomyia macellaria* is another species that differs in dominance between different locations. For example, in southern Louisiana, poultry carcasses were dominated by *C. macellaria,* followed by *L. sericata* (Tessmer et al. 1995). In the current study, *C. macellaria* was the dominant species on carcasses in the field habitat in fall, but was rare in all other treatments despite its presence in spring and summer. The relative dominance

of a species can vary (Macleod and Donnelly 1957, Levot et al. 1979, Smith and Wall 1997; present study), however at present, there is a lack of knowledge regarding the mechanisms driving blow fly community structure. Additional studies similar to this one, to quantify spatial and temporal partitioning within regional blow fly communities, are needed. However, they need to be coupled with experimental studies to understand the mechanisms that result in the patterns in blow fly diversity.

The lack of habitat association that I observed may have been affected by blow fly flight and orientation patterns, which may be largely independent of the surrounding habitat. Prior to the detection of a food resource, different blow flies species (see Macleod and Donnelly 1957) take flights that vary randomly in direction and distance, resulting in them being widely distributed throughout the region. However, once chemical stimuli associated with a carcass such as decomposition byproducts, pheromones or kairomones are detected, the flies exhibit positive anemotaxis and positive chemotaxis that result in them arriving at the resource (Cruikshank and Wall 2002a,b). Some species may appear to have a preference for a certain habitat, whereas in fact they are present simply as a result of them remaining longer in environments that fall within upper and lower thresholds for light and/or temperature.

Seasonal differences in blow fly communities, as seen in this study, may be partially explained through understanding optimal conditions required by individual species for activity or development. Many studies have demonstrated that particular species of blow flies have species-specific minimum thresholds for development with respect to temperature and larval nutrition (Deonier 1940, Nielson and Nielson 1946, Kamal 1958, Levot et al. 1979, Greenberg and Tantawi 1993, Nabity et al. 2006). This

was also proposed by Cruickshank and Wall (2002a) when they suggested that the degree to which *Lucilia* sp. aggregated their populations over a landscape was driven by stimulus response mechanisms that resulted in them existing within environmental limits (Cruikshank and Wall 2002b). As stated previously, blue bottles are also known to decrease in abundance or undergo a summer diapause, demonstrating that upper thresholds can limit availability or activity of blow fly species. The interaction between thermal constraints and species interactions is recognized in community assembly (Cavender-Bares et al. 2009, Wittman et al. 2010, Lessard et al. 2011) and is an important aspect to explore within blow flies, especially when examining successional patterns and their use in a forensic context.

Relevance to Forensic Entomology and Future Research

The composition of insect communities is highly dependent upon temperatures, as individual species respond to temperatures differentially (Forrest and Thomson 2011, De Sassi et al. 2012). Moreover, the rate of development of immature insects is temperature dependent, with development generally increasing linearly as temperatures increase above some minimum lower threshold. This is recognized in the concept of degree days. Degree days (DD) and their accumulation over time (ADD) are consequently very important to consider when attempting to use the insect community on a carcass to make inferences about the time of death and post-mortem interval (Forrest and Thomson 2011). I examined temperature and ADD data to determine if these variables differed between sites, habitats and seasons. My analyses of temperature data demonstrated a significant site x habitat x season interaction, confirming that microclimatic differences can result in

site specific differences in temperature. Due to the cumulative nature of ADD calculations, small daily differences in temperature between sites or habitats become enhanced over time. For this reason, the development of blow fly communities over time is more accurate when interpreted with respect to site-specific ADD than simply to elapsed time. Forensic researchers have recently begun to examine decomposition and carrion insect communities on the basis of ADD (Michaud and Moreau 2009, 2011, 2012, Simmons et al. 2010, Archer 2014). However, despite that, insect successional data are still commonly presented on a calendar basis, a practice that ignores temperatures and their effects (Matuszewski et al. 2008, Prado e Castro et al. 2012, 2013, Azmi and Lim 2013, Pastula and Merritt 2013). By incorporating the use of ADD into forensic entomology studies, the variability in successional patterns that occurs when data are presented on a calendar basis can be reduced and standardized. My study emphasizes the importance of *ADD standardization due to site-specific differences*, which is of paramount importance since it is common practice in forensic investigations to simply use data from the nearest weather station to estimate PMI, a practice that fails to account for site-specific differences in temperatures.

Though this study detected no habitat differences, there were distinct seasonal differences in blow fly community composition and structure. These findings support the concept that examination of seasonal composition patterns may be an important PMI tool (as suggested by Hall and Doisy 1993). Developing regional seasonal profiles for PMI determination will be useful in narrowing down the seasonality of activity of an insect species (PIA, or period of insect activity), especially in cases where more than one year has passed since death and only remnants of the blow fly community remain (i.e. dead

adults, empty or unemerged puparia). Michaud and Moreau (2009) used the occurrence and absence data for key species in a carrion community to create a probability of occurrence matrix, which they then used to statistically validate the presence of a species within the community at a particular time. My study determined that there are changes in community composition due to time and temperature during decomposition (i.e. ADD quartile) as well as season. This conclusion is in agreement to other studies that have also demonstrated distinct seasonal differences in carrion assemblages (Centeno et al. 2002, Archer and Elgar 2003a,b, Tabor et al. 2005, Watson and Carlton 2005, Sharanowski et al. 2008, Moretti et al. 2011, Brundage et al. 2011, Horenstein et al. 2012, Benbow et al. 2013, Fremdt and Amendt 2014). However, in order to fully evaluate the use of blow fly community composition as a potential for determining the timing (seasonality) of colonization, research into how the community is structured in additional natural settings must be conducted.

The information provided within this particular study is an important step in quantifying how the blow fly community is structured during different seasons. While it was conducted in southwestern Ontario, it is relevant to a considerably larger region around the Great Lakes. However, it was limited to a single year. Because annual variation has been shown to be an important variable in causing changes in community structure (Macleod and Donnelly 1957, Martinez-Sanchez et al. 2000, Archer 2003, Archer and Elgar 2003b), it would benefit from replication over multiple years. Studies within the field of forensic entomology should further quantify discrepancies between the use of ADD versus calendar date as a measure of elapsed successional time. I recommend that future studies depict data with respect to both calendar dates and ADD,

as the arrival and colonization of some species is more dependent on elapsed time, while others are more dependent on the state of the resource. Results from this study demonstrate that the blow fly community structure is influenced by season but not habitat. Forensic entomologists are now calling for more stringent experimental designs that incorporate ecological principles, to account for the complex interactions that may be present in carrion insect communities (Brundage et al. 2011, Tomberlin et al. 2011a,b, Michaud et al. 2012, Benbow et al. 2013, Moretti and Godoy 2013, Fremdt and Amendt 2014). Similar experiments to that reported here should be conducted over more regions, seasons, and habitats, in order to assess the replicability of these patterns under other conditions and to account for multiple interacting factors that can influence blow fly populations.

Figure 2.1. Test site locations in the Windsor/Essex County Region of south-west Ontario, Canada. Test sites are labeled A through F. Each site had both field and forest habitats for direct comparisons. Control sites were located within sites B (a nature reserve with high diversity, chosen to capture the regional species pool (Paeiro et al. 2010) and site D (mid-location to the four more southerly sites). Image courtesy of GoogleEarth™

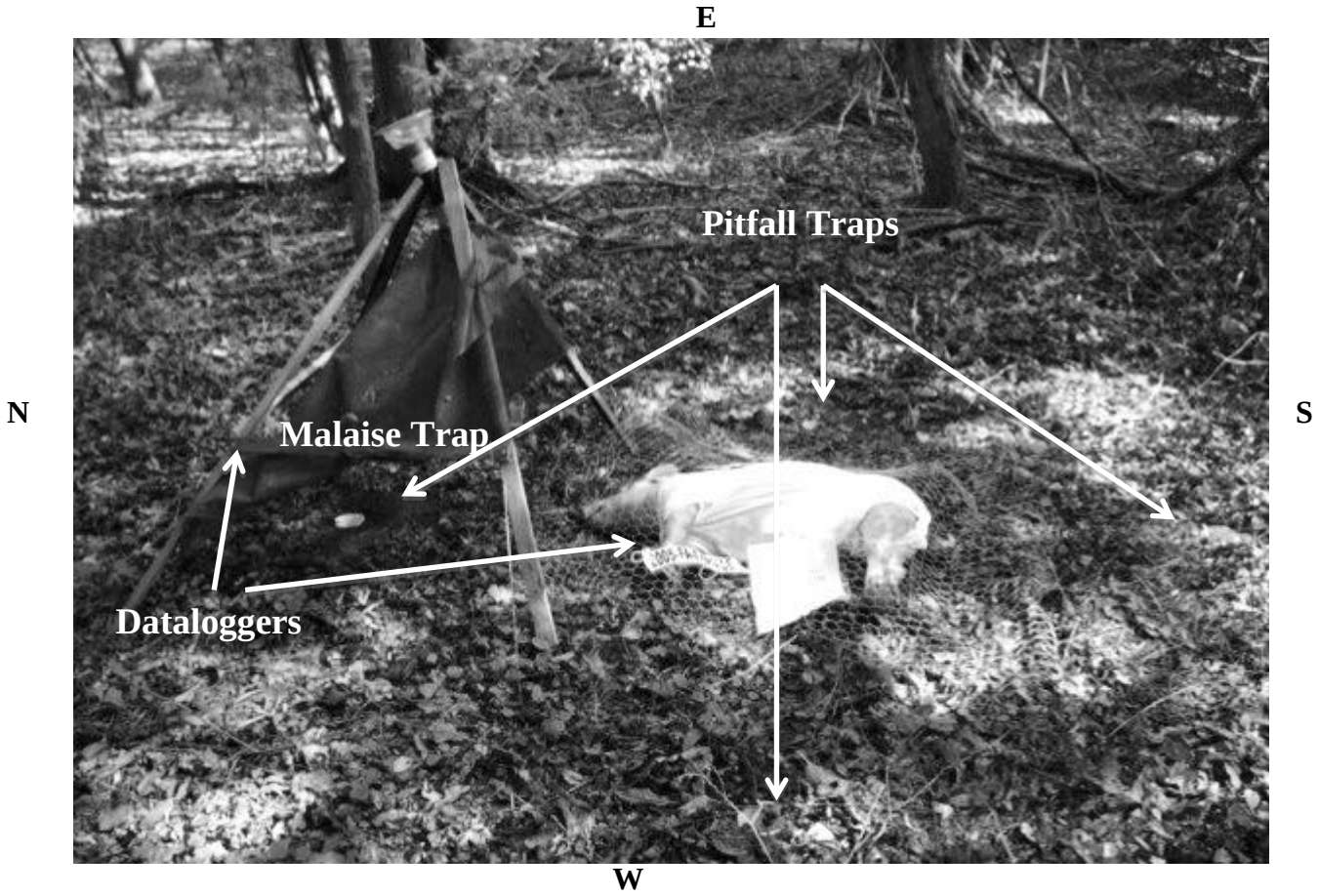

Figure 2.2. Experimental setup and carcass placement for each test site. Carcasses were all female, killed, dressed and placed with head facing north and back facing east. Pitfall traps were located 2.5m in the north, east, south and west directions. Malaise traps were placed at the head of each carcass. Dataloggers placed on the back of each malaise trap (ambient temperature) as well as within the chest cavity of each carcass (internal carcass temperature) recorded temperatures on an hourly basis. Control sites consisted of malaise and pitfall traps set up in the same manner, however, no carcass was placed within these locations.

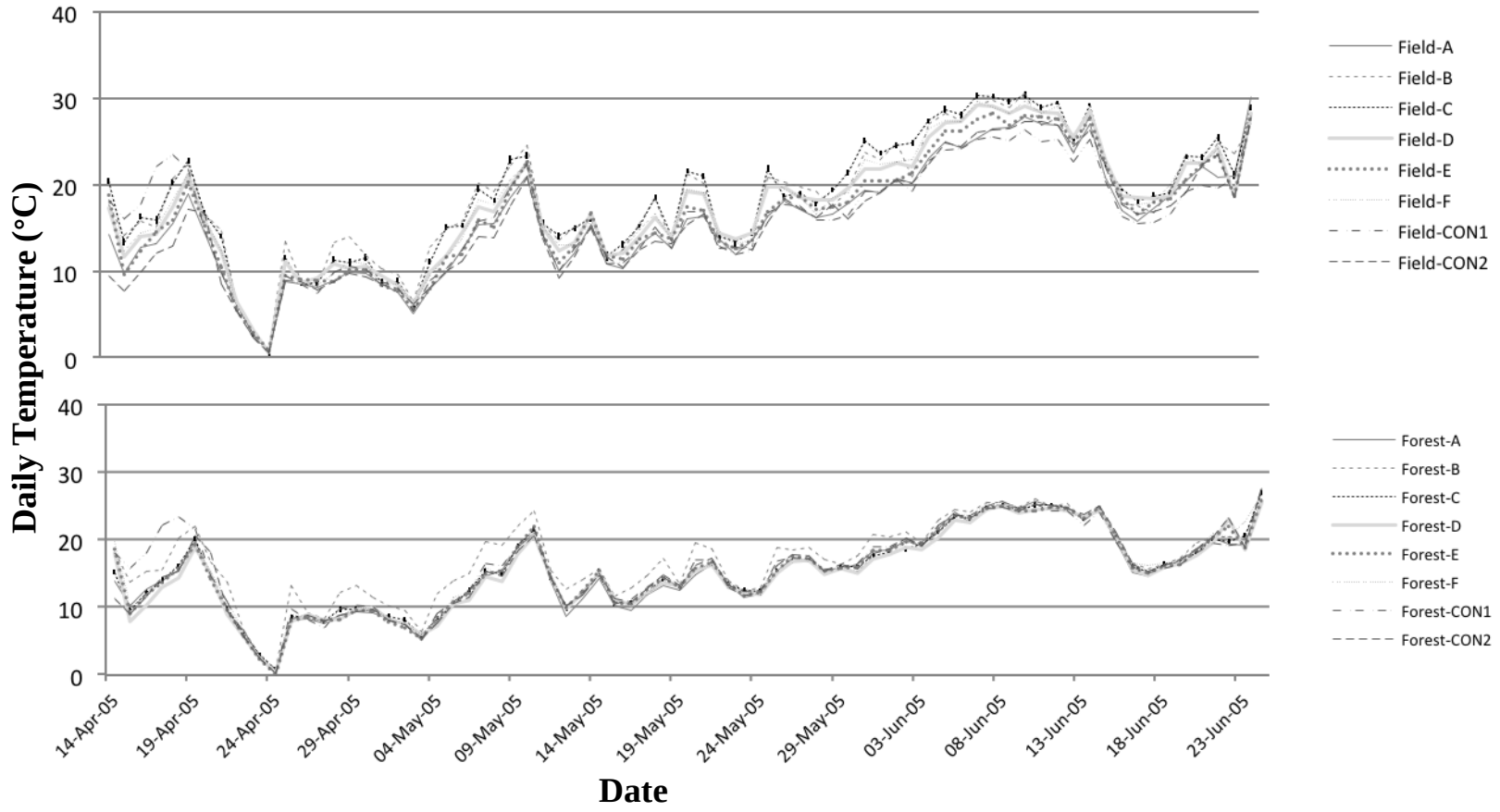

Figure 2.3. Daily ambient air temperatures (°C) for test sites in forest and field habitats from the onset of spring trials (April 14, 2005) until the onset of summer trials (June 24, 2005) located in the Windsor/Essex County Region of southwestern Ontario, Canada. There was a significant test site*habitat*season interaction (ANOVA: $p<0.001$), and pairwise comparison tests with a Bonferroni correction were used to determine test site differences in spring forest and field sites. Site differences also occurred in summer field sites (not presented here). There were no site differences ($p>0.05$) in summer forest sites or during the fall season for forest or field habitats, thus data is not presented.

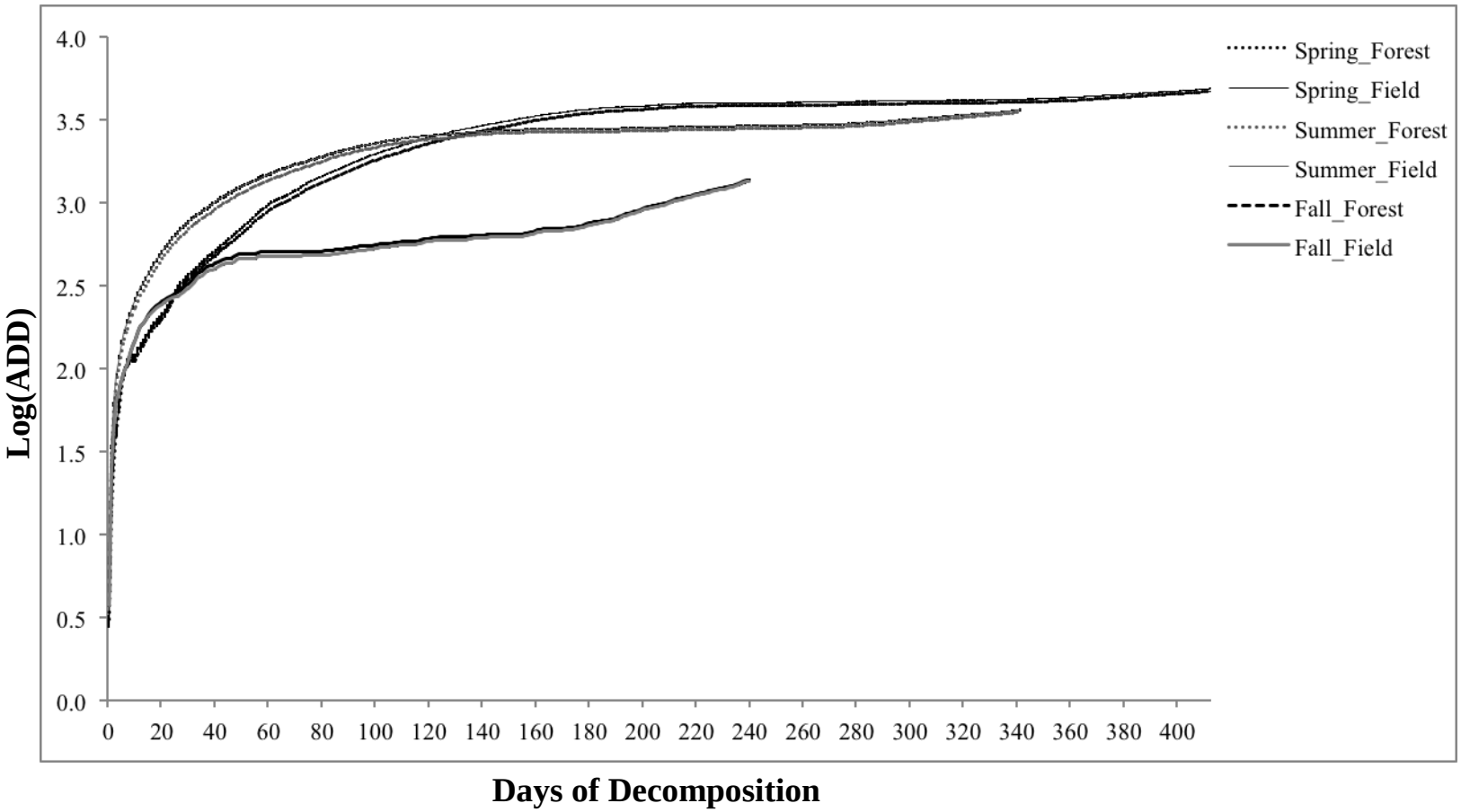

Figure 2.4. Mean (±1SE) accumulated degree days (ADD) for forest and field habitats from the onset of decomposition. Spring, summer and fall trials lasted 412, 342 and 241 days. A generalized linear model was used with a gamma distribution, log-link function and site, habitat, season as main effects and days of decomposition as a covariate. There was a significant test habitat*season interaction (p=0.032).

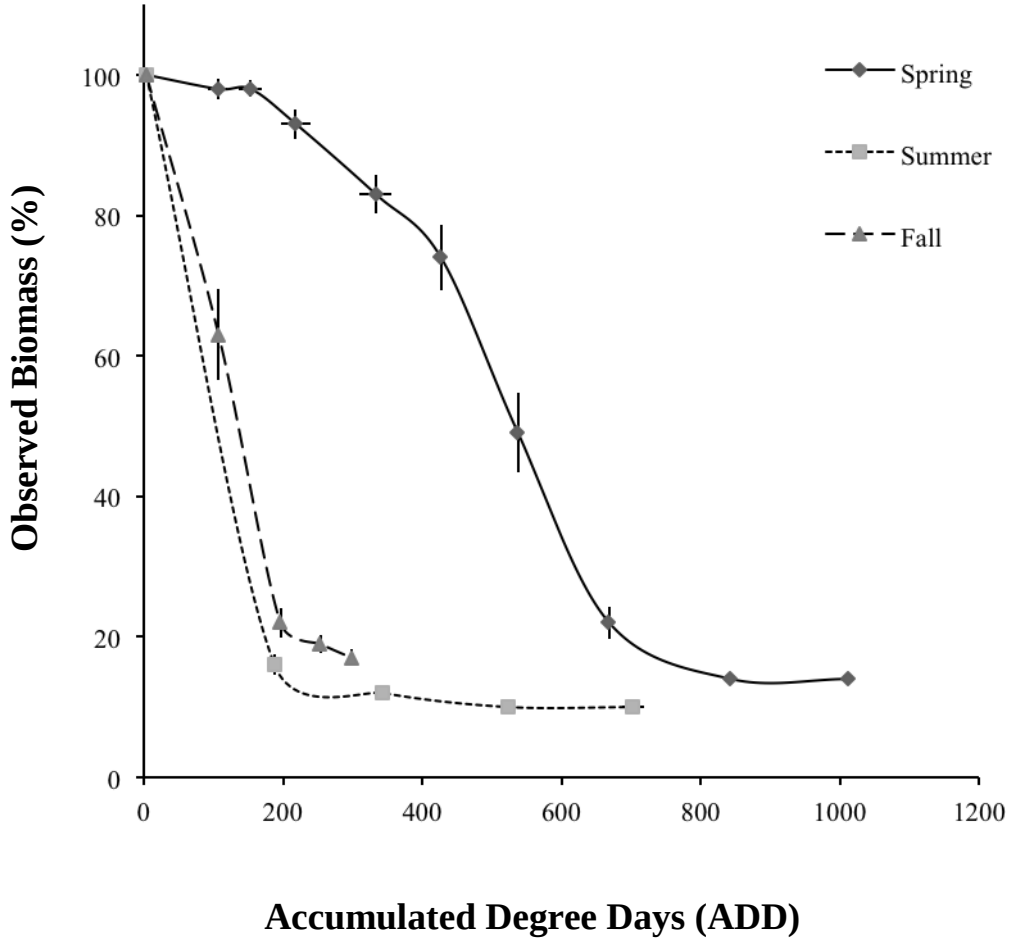

Figure 2.5. Biomass loss during decomposition over spring summer and fall seasons. A generalized linear model was used with a gamma distribution, log-link function and ADD as a covariate. Season and ADD were significant predictors ($p<0.001$). Each point represents remaining biomass mass means and bars represent standard errors of the means. There were no significant differences between test sites (A through F) or habitat (field and forest) ($p>0.05$), thus data were pooled.

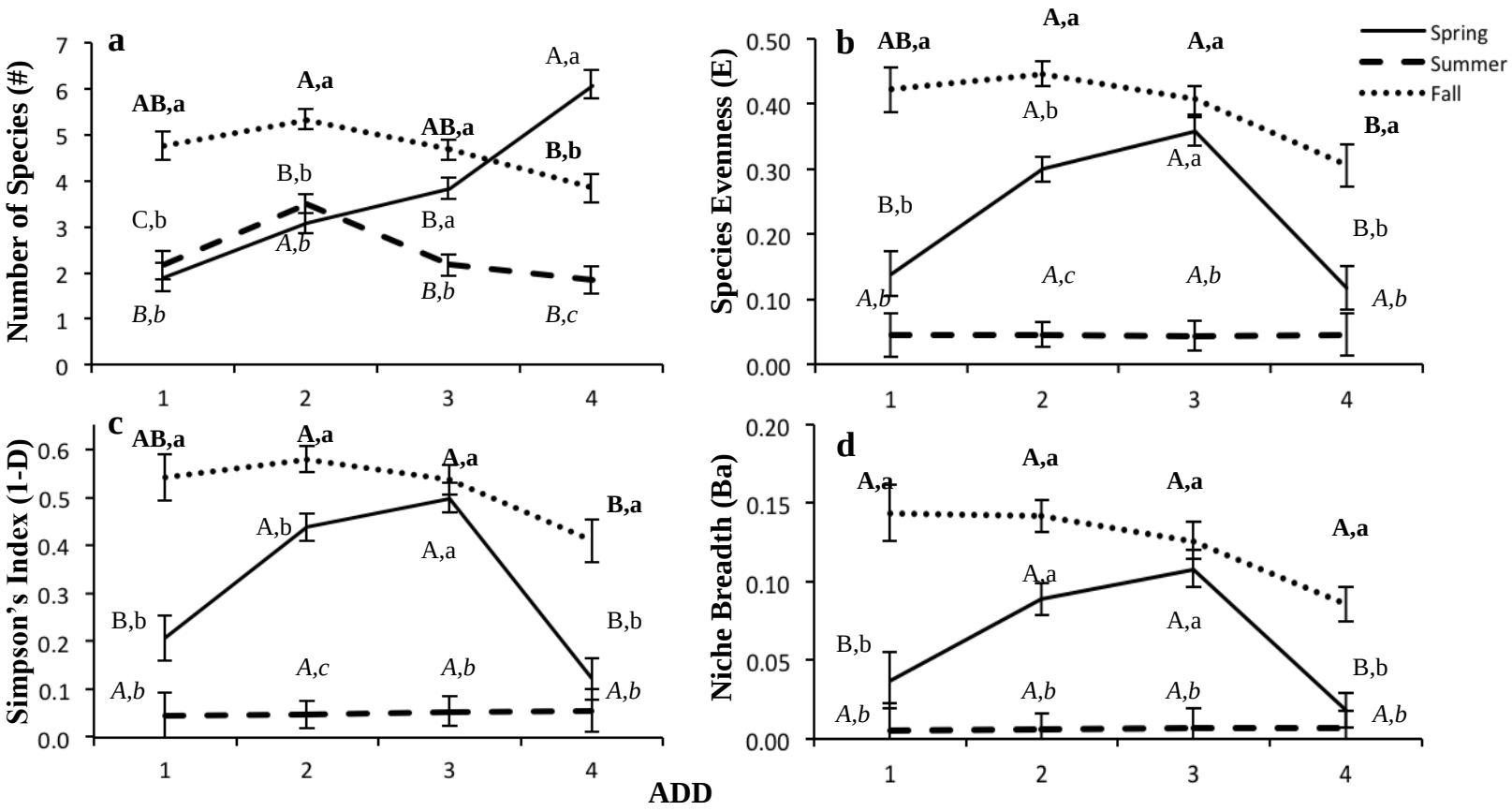

Figure 2.6. Community diversity indices (mean±1SE) for the blow fly community during spring, summer and fall seasons over four categories of accumulated degree days (ADD). **a** – Number of Species. **b** – Species Evenness. **c** – Simpson's Index of Diversity. **d** – Standardized Niche Breadth. A repeated measures ANOVA was used on each community index and pairwise comparison tests between means were used with a Bonferroni correction to determine differences among seasons or quartile. Means with the same letter do not differ significantly. Comparisons were made between ADD quartiles for each season and are denoted by capital letters while comparisons between seasons for each quartile are denoted by small letters. Summer comparisons are denoted in *italics*, Fall in **bold**. There were no significant effects due to habitat ($p>0.05$), thus data for forest and field habitats were pooled.

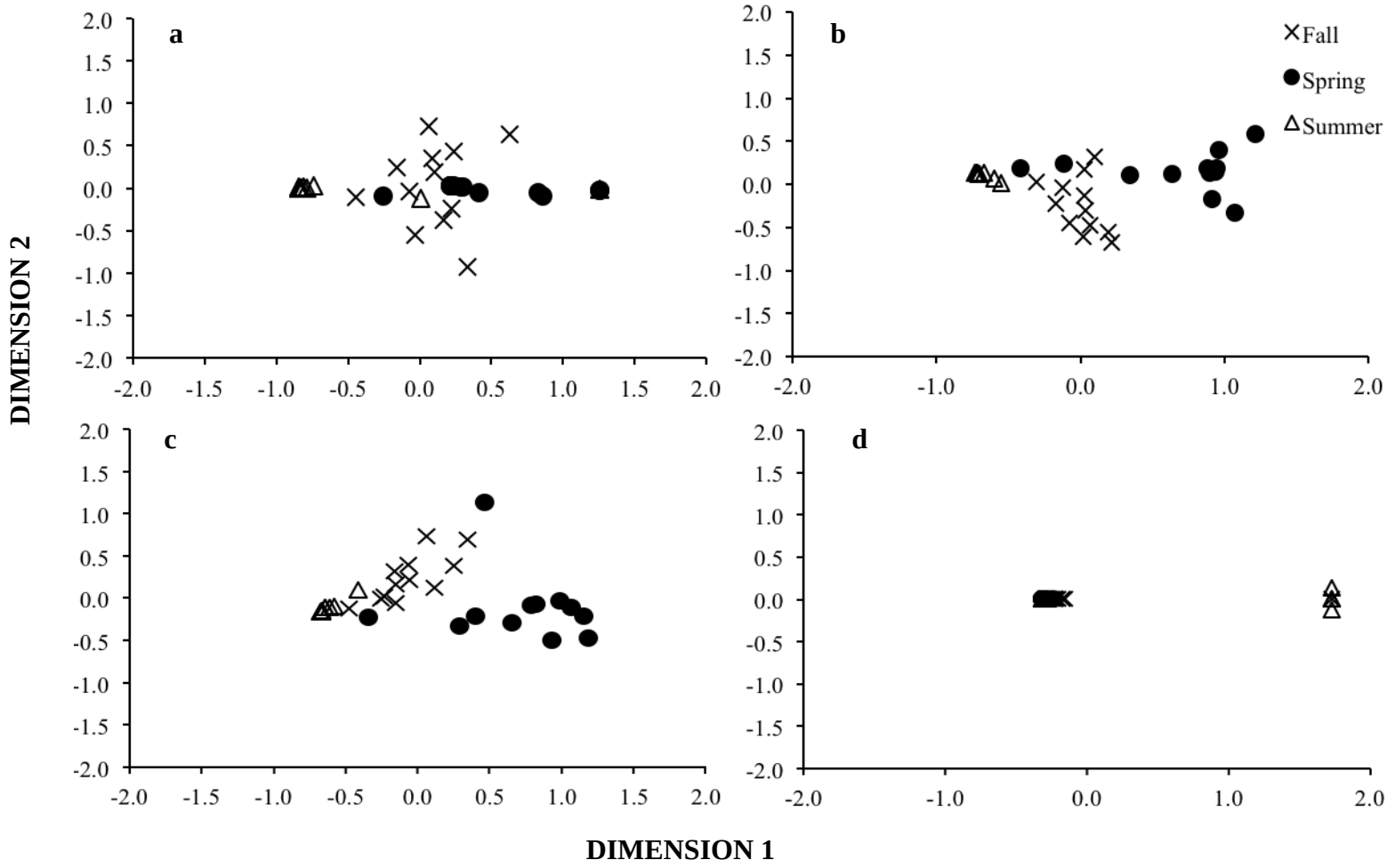

Figure 2.7. Non-metric multidimensional scaling of blow fly communities between seasons on a per pig carcass basis for each ADD quartile. **a** – 0 – 50 ADD. **b** – 50 – 100 ADD. **c** – 100 – 150 ADD. **d** – 150+ ADD. MRPP analysis determined season was a significant grouping factor in all four quartiles ($p<0.001$).

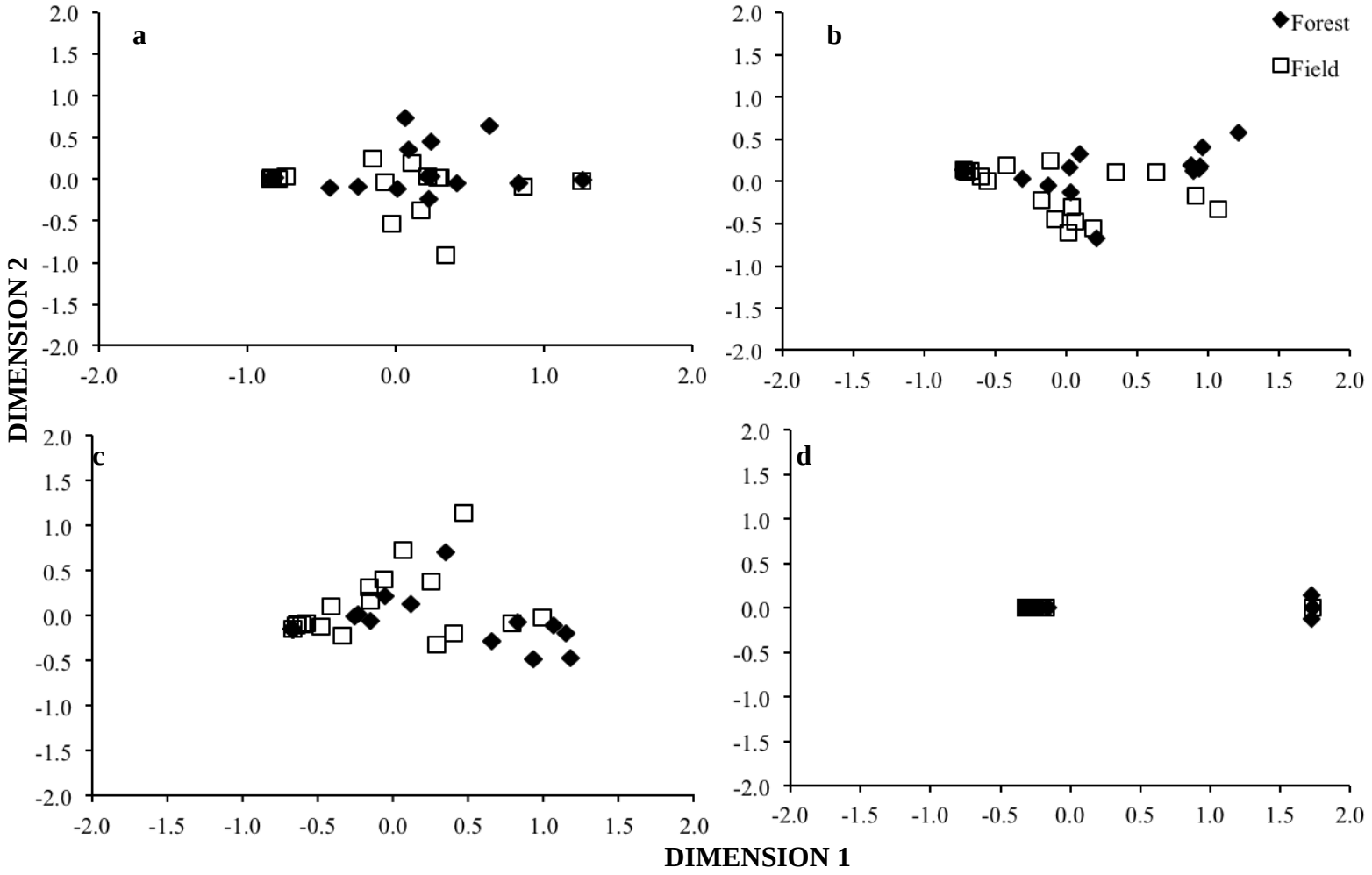

Figure 2.8. Non-metric multidimensional scaling of blow fly communities between habitats on a per pig carcass basis for each ADD quartile. **a** – 0 – 50 ADD. **b** – 50 – 100 ADD. **c** – 100 – 150 ADD. **d** – 150+ ADD. MRPP analysis determined habitat was not a significant grouping factor in any of the four quartiles ($p>0.05$).

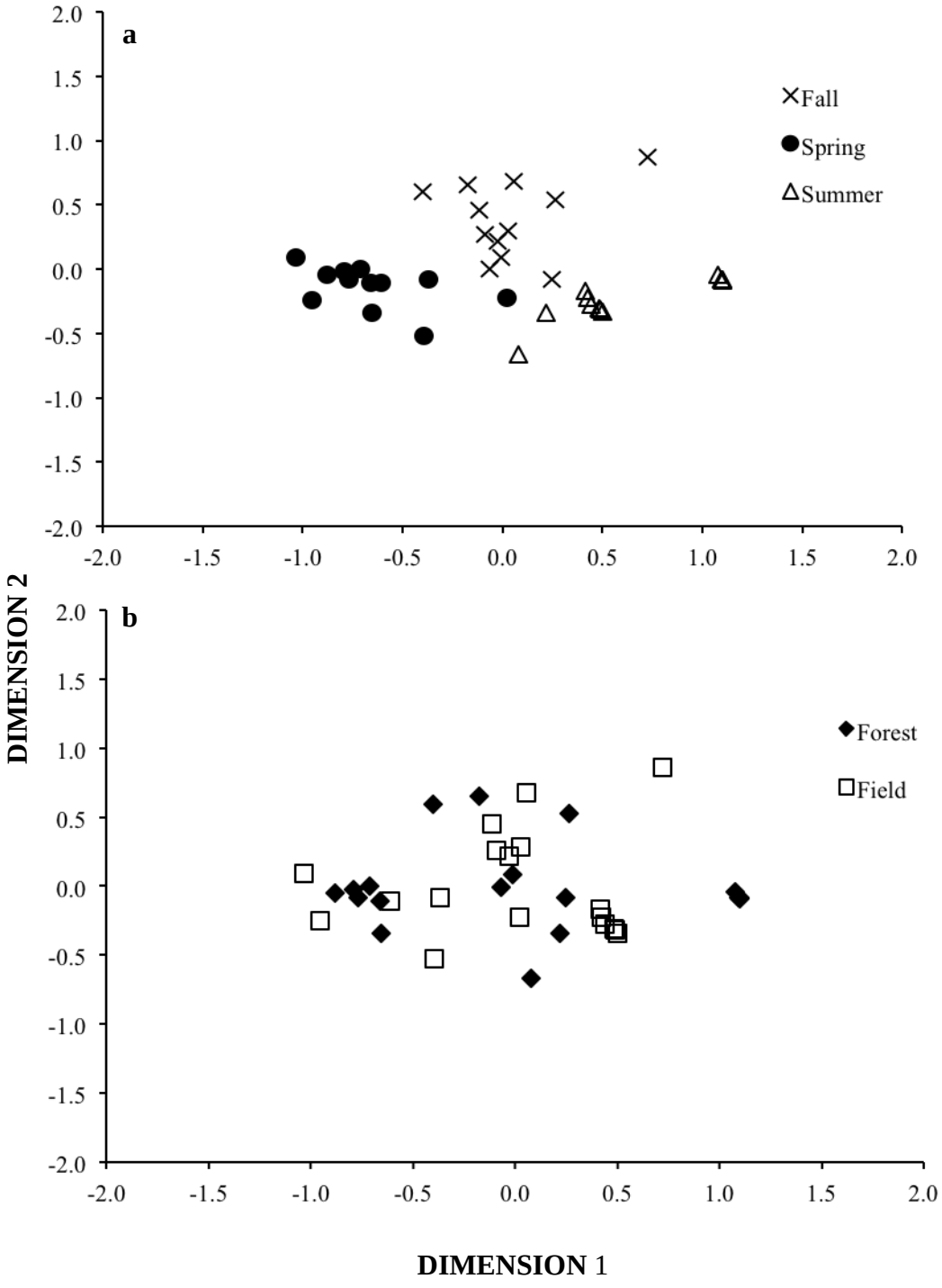

Figure 2.9. Non-metric multidimensional scaling of overall blow fly community composition between habitats and seasons on a per pig carcass basis. **a** – pigs are classified by season. **b** – pigs are classified by habitat. MRPP analysis determined season was a significant grouping factor ($p<0.001$), however, habitat was not ($p>0.05$).

Table 2.1. Effect of accumulated degree days (ADD), site, season and habitat on biomass loss. Data were analyzed using a generalized linear model with a gamma distribution, log-link function and site, habitat, season as main effects and ADD as a covariate. Significant effects are in **bold.**

Source	X^2	df	P
Main Effects			
Site	1.218	5	0.943
Habitat	0.045	1	0.832
Season	**90.659**	**2**	**<0.001**
ADD	**695.198**	**1**	**<0.001**
Two-Way Interactions			
Site*Habitat	1.020	5	0.961
Site*Season	1.987	10	0.996
Site*ADD	0.827	5	0.975
Habitat*Season	0.367	2	0.832
Habitat*ADD	0.508	1	0.476
Season*ADD	**104.709**	**2**	**<0.001**
Three-Way Interactions			
Site*Habitat*Season	1.788	10	0.998
Site*Habitat*ADD	3.993	5	0.550
Site*Season*ADD	1.833	10	0.997
Habitat*Season*ADD	0.483	2	0.785
Four-Way Interaction			
Site*Habitat*Season*ADD	6.029	10	0.813

Table 2.2. Mean relative abundance (±1SE) of blow fly species for three seasons (spring, summer and fall) and two habitat types (forest, field) in Essex County, Ontario. Spring, summer and fall trials commenced on April 14, June 14 and October 3, 2005, respectively. − - indicates that species was not collected during sampling.

		TRIBE CALLIPHORINI											
		Calliphora terraenovae			*Calliphora vicina*			*Calliphora vomitoria*			*Cynomya cadaverina*		
Season	**Habitat**	Mean		SE	Mean		SE	Mean		SE	Mean		SE
Spring	Field	1.907	±	1.472	0.131	±	0.248	0.224	±	0.484	5.602	±	3.775
Spring	Forest	11.147	±	8.869	1.598	±	2.751	0.671	±	1.174	4.872	±	6.611
Summer	Field		−			−			−			−	
Summer	Forest		−			−			−			−	
Fall	Field	0.022	±	0.054	0.078	±	0.160	5.140	±	4.636	0.005	±	0.013
Fall	Forest		−		0.054	±	0.104	24.590	±	15.417	0.025	±	0.041
		TRIBE CHRYSOMYINI											
		Chrysomya rufifacies			*Cochliomyia macellaria*			*Phormia regina*			*Protophormia terraenovae*		
		Mean		SE	Mean		SE	Mean		SE	Mean		SE
Spring	Field		−					91.771	±	4.377	0.105	±	0.141
Spring	Forest		−		0.017	±	0.042	81.374	±	18.276	0.297	±	0.645
Summer	Field		−		4.129	±	4.132	94.994	±	4.573	0.013	±	0.032
Summer	Forest		−		0.384	±	0.414	99.280	±	0.355	0.022	±	0.035
Fall	Field	0.543	±	0.900	49.196	±	8.040	36.437	±	9.727		−	
Fall	Forest	0.045	±	0.069	10.162	±	7.634	50.716	±	11.830		−	
		TRIBE LUCILIINI											
		Lucilia coeruleiviridis			*Lucilia illustris*			*Lucilia sericata*					
		Mean		SE	Mean		SE	Mean		SE			
Spring	Field	0.023	±	0.027		−		0.238	±	0.221			
Spring	Forest		−		0.002	±	0.005	0.021	±	0.026			
Summer	Field	0.028	±	0.068	0.503	±	0.641	0.334	±	0.541			
Summer	Forest		−		0.255	±	0.334	0.058	±	0.125			
Fall	Field	0.028	±	0.057	4.546	±	3.321	4.005	±	5.694			
Fall	Forest	0.014	±	0.034	11.987	±	10.140	2.407	±	3.567			

Table 2.3. Effect of ADD, season and habitat on community indices for the blow fly community. Analyses were carried out using a repeated measures ANOVA with a Greenhouse-Geisser correction factor for Mean Number of Species, Species Evenness (E), and Simpson's Index of Diversity (1-D). A bootstrapped repeated measures ANOVA (k=1000) was used for Levins' Standardized Niche Breadth (Ba). Significant effects are in **bold.**

	# Species			Species Evenness (E)			Simpson's Index (1-D)			Niche Breadth (Ba)		
Source	df	F	P	df	F	P	df	F	P	df	F	P
ADD	**2.2**	**11.042**	**< 0.0001**	**1.9**	**15.232**	**<0.000**	**2.0**	**17.027**	**<0.000**	**3**	**17.447**	**<0.0001**
Season	**2**	**46.253**	**< 0.0001**	**2**	**90.383**	**<0.000**	**2**	**85.163**	**<0.000**	**2**	**75.855**	**<0.0001**
Habitat	1	0.341	0.561	1	1.332	0.258	1	1.389	0.248	1	0.820	0.364
ADD*Season	**4.3**	**27.519**	**< 0.0001**	**3.7**	**8.016**	**<0.000**	**4.0**	**8.637**	**<0.000**	**6**	**12.645**	**<0.0001**
ADD*Habitat	2.2	1.194	0.312	1.9	0.211	0.796	2.0	0.190	0.825	3	0.153	0.927
Season*Habitat	2	0.805	0.457	2	0.843	0.440	2	0.622	0.543	2	0.025	0.975
ADD*Habitat*Season	4.3	0.422	0.805	3.7	1.092	0.367	4.0	0.812	0.521	6	0.927	0.474
Error (within subject)	64.5			56.0			59.3			90		
Error (between subject)	30			30			30			30		

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CHAPTER 3: PRIORITY EFFECTS: THE POTENTIAL FOR COEXISTENCE DUE TO SPATIAL AND TEMPORAL CHANGES IN THE OVIPOSITION BEHAVIOUR OF ADULT BLOW FLIES (FAMILY: CALLIPHORIDAE)

INTRODUCTION

Spatial aggregation of offspring within a single resource, influenced by the choices of where a female should reproduce and how many offspring she should have, is a form of spatial resource utilization that promotes coexistence. In the case of insects, by preferentially ovipositing with conspecifics and on particular colonization sites within a resource, the resulting offspring may experience higher levels of intraspecific competition than interspecific competition (Ives 1991). This aggregated oviposition leaves unoccupied sites available for less competitive species to colonize, allowing them to coexist over the spatial scale of the single resource. By varying the levels of offspring density at different locations, and consequently the influence of intra- and interspecific competition within resource patches, long-term stability of highly competitive populations can occur (Atkinson and Shorrocks 1981, Ives 1988), despite multiple species exhibiting similar life history characteristics (Green 1986). High levels of diversity can be maintained when multiple interacting species have moderate competitive abilities or when dominance patterns differ spatially or temporally (MacArthur and Wilson 1967, Atkinson and Shorrocks 1981, Shorrocks and Bingley 1994). Competition can be a major factor in interactions between species, particularly in ephemeral resources (Atkinson and Shorrocks 1981, Ives 1988, Shorrocks and Bingley 1994).

Another form of competition, inhibition, can decrease the realized niche of one or more species within a wide diversity of taxa (Connell and Slatyer 1977). For example,

early arrival and establishment of native plant species can inhibit the invasibility of non-native plants by reducing the amount of space and resources available for the invasives (D'Antonio et al. 2001, Lulow 2006, Wainwright et al. 2012). Strong inhibitory effects created by large single-species patches of two highly competitive herbaceous plant species (*Setaria faberii* Herrm and *Erigeron annuss* L.) inhabiting old-field plant communities were reduced by heterogeneity in patch size, which decreased interspecific competition and mediated their coexistence (Facelli and Facelli 1993). In bacterial communities, inhibition between competitors led to coexistence due to local aggregations of populations combined with localized temporal extirpation (Blanchard et al. 2014). However, not all species interactions are negative. Facilitation can promote coexistence; it is the process in which the presence of one species enhances another by expanding the available niche of some individuals to allow for a greater ability to establish and persist within a community (Connell and Slatyer 1977). An example comes from two competing species of saproxylic beetles. The early or simultaneous arrival of *Rhagium inquisitor* L. increases the number of offspring in *Acanthocinus aedilis* L. compared to when this species is alone. The facilitation effected by the presence of *R. inquisitor* may increase the oviposition of *A. aedilis* or may enhance the quality of the larval food resource (Victorsson 2012). Due to the complexity of community assembly, it is important to consider the potential for both facilitory and inhibitory mechanisms when examining coexistence within a system.

Differences in arrival order of individuals within a community can result in both positive and negative interactions as well as affect the resultant community structure; these effects are referred to as priority effects (Beaver 1977, Hanski and Kuusela 1977,

Kneidel 1983, Shorrocks and Bingley 1994, Fukami et al. 2005, Korner et al. 2008, Moore and Franklin 2012). A species can inhibit further invasion of a resource patch if it successfully arrives and colonizes that patch first (Levin 1974, Sale 1977, 1980, Kneidel 1983, Shorrocks and Bingley 1994) because early colonizers may outcompete later arriving species through their use and depletion of the resource (Hanski and Kuusela 1977). In the case of *Drosophila* spp. (Diptera: Drosophilidae) on decaying mushrooms, species that arrived later experienced increased mortality, smaller offspring size and slower development and competitive interactions between species were drastically altered (Shorrocks and Bingley 1994). On the other hand, fugitive species can take advantage of their early arrival, allowing them to persist despite being less competitive (Hutchinson 1951, Levin 1974, Hanski 1983, Kneidel 1983, Shorrocks and Bingley 1994). Von Gillhaussen et al. (2014) determined that in greenhouses, early arrival of legumes into the system exerted an initial inhibitory effect on other legumes, while simultaneously facilitating the establishment of later arriving non-leguminous plants.

Given a patchy and ephemeral resource upon which typically only one or very few generations of insects can develop, selective pressure is exerted on gravid females to maximize their reproductive output and offspring fitness (Beaver 1977, Von Zuben et al. 2001, Creighton 2005), which can have consequences on population densities and community structure (Spencer et al. 2002, Kagata and Ohgushi 2004, Creighton 2005). Females can preferentially chose oviposition mediums that enhance offspring fitness (Scheirs et al. 2000, Scheirs and De Bruyn 2002, Roder et al. 2008, Woodcock et al. 2013). For example, dermestid beetle females preferred to oviposit on carrion tissue types that maximized their offspring fitness (Woodcock et al. 2013). Female mosquitoes,

Culiseta longiareolata (Macquart) (Diptera: Culicidae) had increased survival and larger populations due to a predator avoidance strategy, as demonstrated by females ovipositing in pools where the predator *Notonecta maculata* (Fabricius) (Hemiptera: Heteroptera) was absent (Spencer et al. 2002). Females may also aggregate their eggs due to the facilitory effects experienced by gregarious larvae that can acquire more resources when feeding in clumps. This is believed to be a result of either an Allee effect or a refuge-dependent Allee effect, where aggregated larvae have an advantage at finding refuges that exclude natural enemies such as predators and parasitoids (Hoffmeister and Rohlfs 2001). Females arriving at a patch already inhabited by a competitor may lay fewer eggs than in uninhabited patches (Parker and Courtney 1984, Yanagi et al. 2013) in order to diminish potential for negative competitive their larvae may experience (Ives 1989). Conversely, some females may lay more eggs in already inhabited patches if the previously established species is a weak competitor (Ives 1989, Visser 1996). Moreover, research has demonstrated that blow fly larvae may be facilitated by the presence of bacteria or by distinct changes in the bacterial community composition that are driven by the presence of blow fly species or other carrion insect species (Hobson 1931, Hollis et al. 1985, Esser 1990, Mumcuoglu et al. 2001). Female differences in oviposition behaviour can alter patterns of larval aggregation and competition, and in some systems stabilize and even promote species coexistence (Ives 1989, Heard and Remer 1997). Females can respond to changes in resource abundance by selectively altering the distribution of eggs laid on resources, thereby allowing for species coexistence when resources are scarce and patchily distributed (Heard and Remer 1997). Despite conflicting views on whether oviposition preferences directly lead to increased offspring

fitness (Thompson 1988, Fox and Czesak 2000), there is agreement that individual recruitment into a community is a crucial process as it establishes the initial population size of a species and, thus, has a great potential to affect subsequent community patterns and processes (Ives 1989, Encalada and Peckarsky 2006).

Within the carrion insect community, three blow fly species (Diptera: Calliphoridae), *Lucilia sericata* (Meigen), *Phormia regina* (Meigen) and *Chrysomya rufifacies* (Macquart), were selected to test the effects of arrival order of the species on the oviposition behaviour of female blow flies on dead piglets. Arrival order in two-species combinations (*L. sericata* and *P.regina*, *L. sericata* and *C. rufifacies*) was varied, with either one or the other species introduced before the other species, or both species introduced at the same time. Priority effects were measured on a temporal scale by the time taken for colonization as measured by female oviposition on the resource, and on a spatial scale by the number of eggs laid in each location on the resource. High colonization potential/ability would be evident in a large amount of eggs laid, the laying of eggs in highly desirable locations or a short amount of time taken to colonize. Within this study, a priority effect is deemed important if it is detected in at least one variable.

If priority effects do not influence the assembly of these species, then arrival order will have *no effect* on colonization potential (H_{null}). If there is a positive priority effect ($H_{1a,b}$), then the presence of one species will *increase* the colonization potential of the second species. Alternatively, a negative priority effect ($H_{2a,b}$) will be inferred if the presence of one species *decreases* the colonization potential of the other species.

As mentioned previously, priority effects can also be measured through changes in egg distribution. If colonization is unaffected by arrival order of two species on the

pig carcass, then site selection of female blow flies should be consistent regardless of arrival order and should follow one of two patterns: random or exponential. A random colonization pattern would indicate no preference in oviposition locations while an exponential pattern would indicate that females are laying eggs according to recognized oviposition preferences, with primary colonization sites being located in the natural orifices of the body, such as the eye, nose, ear, mouth, followed in preference by less desirable secondary locations, such as the anus or body crevices (Mann et al. 1990, Greenberg 1991, Campobasso et al. 2001, Mahon et al. 2004, Gruner et al. 2007, Cross and Simmons 2010). If these expected patterns fail to occur, this indicates an alternative preference which will be determined by further examination of egg-laying patterns.

Since colonization behaviour involves oviposition of females beyond the initial oviposition event, the role of priority effects on the total number of eggs laid and the distribution pattern of these eggs was also examined. If priority effects are not influencing the overall colonization of the resource, then there will be no differences between treatments. If a positive priority effect exists, more eggs would be laid or the benefiting species would shift its egg distribution from locations with moderate/low desirability to highly desirable locations when alone or first to arrive. The opposite trend would occur if a negative priority effect exists.

METHODS

Study Species

All three species, *Lucilia sericata*, *Phormia regina* and *Chrysomya rufifacies*, are effective dispersers (Illingworth 1927, Hall 1948, Greenberg 1991) and are present in the Great lakes Region. All three species are similar with respect to birth, death and dispersal rates (Subramanian and Mohan 1980, Greenberg 1991, Wall et al. 1992, Baumgartner 1993, Pitts and Wall 2004) and their larvae are sarcosaprophytic, feeding directly upon muscle and soft tissue. Although *C. rufifacies* can become a facultative predator during later instars, during the adult stage, the stage responsible for oviposition choices examined in this study, it is ecologically equivalent to *L. sericata* and *P. regina*. Details regarding individual species characteristics are provided in Appendix B.

Experimental Design

Laboratory blow fly colonies were maintained in cages (45 cm x 45 cm x 45 cm; described below) under a 16L:8D diel cycle, a temperature of 21°C and 50% humidity. Adult flies were fed *ad libitum* with granulated sugar, skimmed milk powder, and water in an Erlenmeyer flask plugged with a dental wick to prevent drowning. Experiments utilized the same conditions. Colonies of *P. regina* and *L. sericata*, maintained since 2005, were augmented annually with wild-type females collected from the Windsor area using King Wasp traps (www.kinghg.on.ca) baited with pork liver. *Chrysomya rufifacies* colonies were established from pupae collected from carcasses placed outdoors at the FLIES Facility at Texas A&M University in College Station, TX.

Fresh pork liver (35 g) was placed in each colony cage as an oviposition medium

for a period of 24 hrs or until sufficient eggs (approximately 10,000) were collected to set up the experiments. Eggs were divided and placed into multiple rearing jars containing approximately 200 larvae per jar. Each rearing jar consisted of a 1 L Mason jar filled 1/3 with wood shavings (NEPCO Beta Chip) as a pupation medium, pork liver as a food source, and a landscape tarp lid (Weed Barrier WPB 4006) to allow adequate ventilation. Rearing jars were then placed at room temperature or within a growth chamber (Powers Scientific Inc. Model DROS33SD Level 2) where temperature was manipulated from 15-28°C to ensure simultaneous adult emergence. During larval development, jars were checked daily and provided pork liver *ad libitum* until more than 70% of larvae pupated, at which time excess food was removed. Upon emergence, adult flies were sexed and placed into a mesh treatment cage. Since adult size of several species is positively correlated with fecundity (Calliphoridae: Fuller 1934, Wall et al. 2002; Scathophagidae: Jann and Ward 1999; Piophilidae: Bondurainsky and Brooks 1999), larvae were fed *ad libitum* to ensure adequate nutrition during larval development and upon emergence; only full sized and fully formed adults were selected for use in the experiments.

Silva et al. (2003) reported that *Lucilia* sp. may exhibit density dependent effects, with adult mortality increasing and female fecundity decreasing at high density. Moe et al. (2002) determined that maximum survival and reproductive rates occurred with an approximate density of 50 females per 24 cm^3. Based on these findings, my personal experience and my preliminary studies, 100 females and 50 males (see Table 3.2) were determined to be an appropriate population size within the confines of a rearing cage (45 cm x 45 cm x 45 cm^3) since it allowed for adequate access to the oviposition medium, yet minimized the influence of density dependent effects in order to ensure that each female

had an opportunity to lay a full complement of eggs.

Adults were provided with granulated sugar cubes and water *ad libitum*. To ensure that the minimum protein threshold for egg maturation was exceeded (see Wall et al. 2002), pork liver was placed within the cages using the following protocol: on Day 1 and Day 2, each cage was provided with 35 g of fresh liver for a 24 hr period to ensure maximum protein uptake for ovarian development; on Days 3-5, 35 g of liver was provided for only 3 hrs per day in order to maintain a high level of protein uptake by females while restricting the availability of the oviposition medium. By restricting access to liver on Days 3-5, female flies that were gravid beginning as early as Day 3 were largely prevented from laying eggs. To account for adult mortality during this pre-oviposition feeding period, dead adults were replaced on Day 4 with the same number of males and females that had been maintained under identical conditions. On Day 6, with most females (approximately 90%) gravid, piglets were placed in each treatment cage.

Each morning during the experiment (On Day 6 to Day 8 after emergence of the experimental flies) at approximately 9 am, newborn piglets (*Sus scrofa domesticus* L.) that had been dead for only one to two hours were collected from Robert Rivest Farms, Ltd. in Staples, ON. Because the profile of volatiles released from an entire carcass can change during decomposition (Vass et al. 1992), only very fresh carcasses were used. Piglets were weighed (range: 705-1208 g), rinsed with tap water and placental coverings removed prior to placement into the treatment cages. Setting up each experiment took approximately three hours, resulting in piglets being placed in treatment cages (with 100 or 50 females, depending on treatment condition) around 12 noon. They were checked hourly to record the timing and location of oviposition events.

During the night cycle (onset of scotophase: 2200 h), after approximately 12-17 hrs from introduction of the piglets into the treatment cages (from 0000 – 0500 hrs), each piglet was removed from the treatment cage to quantify colonization events. Pictures of each egg mass were taken using a NIKON D70 camera directed perpendicular to the piglet surface, with a 15 mm plastic ruler for scale. Depth measurements were taken at various points within each egg mass. Once all egg masses were documented and photographed, piglets were immediately placed back into their respective treatment cage. This procedure enabled documentation of egg masses prior to hatching while minimizing the disturbance to colonization behavior, since blow flies have low activity and seldom oviposit at night (Tessmer et al. 1995, Singh and Barti 2001, Amendt et al. 2008).

After 24 hrs postmortem, at approximately 0900 h and ~21 hrs from initial exposure, piglets were removed from the cages and any new eggs masses laid in the beginning of the second light cycle were recorded. Egg masses were documented with respect to location, parent species, size and changes in depth, except in the "*species together"* treatments in which parent species could not be differentiated. Once the data were recorded, piglets were either disposed of, (in the case of "*species only*" and "*species together*" treatments) or placed in a subsequent treatment cage, or Cage 2 of "*species vs. species*" (with 50 females) for the 24-48 hr postmortem interval, also with a 21 hr exposure window. All treatments described in Table 3.2 were replicated ten times.

Behavioural Observations

General observations and notable behaviours were recorded hourly. Female distribution on the carcass was recorded as the number of individuals on each region of

the body, as well as the number and location of any ovipositing females. No statistical analyses were carried out on these qualitative data; general patterns and notable behaviours (i.e. nocturnal oviposition, intra- or inter-specific interactions) are presented.

Time to First Colonization

A "colonization event" was defined as an egg was deposited by a female either directly on the resource or in the immediate surrounding area. For example, *P. regina* would commonly lay eggs on the paper underneath the carcass as well as on the body itself. Both instances were recorded as colonization events. Time elapsed until the first colonization event was recorded for each treatment and each species.

Location and Frequency of First Colonization

Egg mass locations were categorized using the criteria outlined in Table 3.3, based on published blow fly oviposition patterns (see Mann et al. 1990, Greenberg 1991, Campobasso et al. 2001, Mahon et al. 2004, Gruner et al. 2007, Cross and Simmons 2010) and my personal observations. As a general pattern, blow flies predominately lay eggs within natural orifices presumably because those locations offer protection for the eggs against predation and desiccation (Greenberg 1991, Campobasso et al. 2001, Cross and Simmons 2010). These locations were ranked as the most desirable sites (category 1), with other sites ranked down to the site with the lowest desirability or expected oviposition preference (category 8). Locations were further grouped into 3 desirability categories: high, moderate and low (see Table 3.3). Locations of low desirability are characterized by a lack of protection from desiccation, the need to travel to reach a more

humid location, or where skin in the mucous membranes is difficult to penetrate. With respect to surface area available for egg deposition, each site within high desirability locations has less surface area compared with moderate, with low desirability locations collectively having the greatest surface area. Thus, if the majority of eggs are laid in high desirability locations (as expected from the literature), this indicates a site preference rather than a reflection of the area available for colonization. The frequency of first colonization in each priority site/category was the number of piglets on which eggs were first laid in that particular site or desirability location.

Egg Measurements

Egg masses were documented with respect to location, parent species, size and changes in depth, except in the "*species together"* treatments where parent species could not be differentiated. The scale in the photographs allowed for calibration of images. Digital Image Analysis using Image J™ Software was carried out to estimate surface area for each mass or region. Egg mass volume (mm^3) was estimated by incorporating depth measurements and surface area according to the protocol outlined in Rosati et al. (unpublished data). The number of eggs laid was estimated using the regression equations developed by Rosati et al. (unpublished data).

$$\text{\# of eggs} = \frac{(\text{egg volume} + 3.210)}{0.269}$$

The overall distribution of eggs was examined on a per pig basis. Egg masses were grouped according to species and arrival order and oviposition site desirability.

Statistical Analyses

For all statistical tests, a significant effect was designated when $p<0.05$, or the appropriate adjusted p-value following a Bonferroni correction.

The effect of density of females per cage on the mean time to oviposition and percentage of eggs laid in each desirability level was examined using Independent sample t-tests for each species by comparing "*species only*" (n = 100 females) and "*species first*" (n=50 females) treatments. The effect of density on the percentage of eggs laid in locations differing in desirability and in 8 different body sites for each species was tested using an ANOVA with desirability level or body site and treatment as main effects. The effect of density on the frequency of first oviposition location was examined using a Fisher's exact test due to small cell counts (<5) and fixed column totals (Fisher 1922, SPSS Manual V21). For *P. regina* and *C. rufifacies*, desirability levels 1 and 2 were pooled and for *L. sericata* desirability levels 2 and 3 were pooled to eliminate zero cell counts and to create 2x2 tables for analyses. The effect of species combination and arrival order on the mean time to colonization of *L. sericata* was examined using an ANOVA. Data were pooled if there were no differences ($p>0.05$) between "with *P. regina*" and "with *C. rufifacies*", or between "*species only*" and "*species first*" treatments.

For all species, residuals for mean time to colonization were not normal (Shapiro-Wilks test, $p < 0.001$) and transformation methods including the log, ln, inverse, square root, or e^x did not improve normality. Time to first colonization event was analyzed using a bootstrapped (k=1000) linear mixed model ANOVA (Efron 1979, SPSS Manual V21), with time to colonization as a dependent variable and arrival order and species as

fixed main effects. Bootstrapped pairwise comparisons were used to determine differences between species (within each arrival order) and between arrival orders (within each species) with a Bonferroni correction for p-values to correct for multiple hypothesis testing (SPSS Manual v21).

Location of first oviposition was analyzed using a log-linear model to test for interactions between species, arrival order, and desirability of egg locations. Planned comparisons were carried out testing the distribution of high, moderate or low desirability levels against two expected distributions: equal (i.e. no preference for locations) or exponential (i.e. expected pattern according to previous literature) (Mann et al. 1990, Greenberg 1991, Byrd and Caster 2001, Campobasso et al. 2001, Mahon et al. 2004, Gruner et al. 2007, Cross and Simmons 2010). Expected values consisted of 33% of eggs laid in each priority location for an equal distribution, or 90% high, 7% moderate and 3% low to simulate an exponential distribution. Binomial tests were carried out within each species and arrival order and used *post hoc* to examine preferences in location desirability for each pairwise comparison (i.e. high vs. moderate, high vs. low, moderate vs. low).

The percentage of eggs laid in each desirability location and body site was calculated in order to standardize data on a per pig basis. "*Species only*" and "*species first*" treatment data were pooled as there were no differences between these treatments on percentage of eggs laid in each desirability location or body site ($P > 0.05$) (i.e. *P. regina only* and *P. regina first* values were combined). Data for mean total number of eggs laid were not pooled for *first* and *only* treatments because the number of females flies in these treatments differed. Data and residuals for percentage of eggs and mean number of eggs laid were not normal (Shapiro-Wilks test $p<0.05$) and log, ln, inverse,

square root, e^x transformation methods did not improve normality of the residuals. Consequently, bootstrapped (k = 1000) ANOVA (Efron 1979, SPSS Manual V21) was used to test for effects of treatment and location desirability or body site on percentage of eggs laid in each location and for the effects of treatment on mean number of eggs laid. Pairwise comparisons were carried out based on bootstrapped estimated marginal means in order to examine the differences in percentage of eggs laid between different regions desirability and site) of the carcasses within each treatment. A Bonferroni correction was used to correct for multiple hypothesis testing on the same data set (SPSS Manual V21). A one-way ANOVA was used to test the effect of treatment on mean total number of eggs, with a Games-Howell *post-hoc* test (Games and Howell 1976) to test for differences between means due to heterogeneity of variances and unequal sample sizes.

RESULTS

Effects of Density and Species Combination

Density of females (100 vs 50 females) did not affect mean time to first colonization; the percentage of eggs laid in high, moderate and low locations; or the percentage of eggs laid in each body site for any species ($p>0.05$) (see Table.3.4). Nor did density of females affect frequency of first oviposition locations for *P. regina* ($p = 0.141$), *C. rufifacies* ($p = 0.628$) and *L. sericata* ($p = 0.162$), thus, all data for "*species only*" treatments and "*species first*" treatments were pooled. For *L. sericata*, there was no significant interaction between treatment combination and arrival order ($F_{2, 68} = 0.034$, $P = 0.966$) and no differences between species combination on mean time to colonization (see Table 3.4). Therefore, data were pooled for *L. sericata* second "with *P. regina*" or *"*with *C. rufifacies*" and for *L. sericata* together "with *P. regina*" or "with *C. rufifacies*".

Time of Primary Colonization

There was a significant interaction between species and arrival order ($F_{4,153} = 5.684$, $p < 0.001$), therefore, interspecific comparisons were made between species within each arrival order and intraspecific comparisons were made between arrival orders within each species (adjusted $\alpha=0.006$) (see Table 3.5). Whenever species were introduced simultaneously or when another species had previously colonized the resource, all species laid their eggs within three hours of exposure. However, interspecific differences in colonization times occurred when a species was introduced first, with *P. regina* and *C. rufifacies* exhibiting delayed colonization. Only *C. rufifacies* first had an intermediate time to first oviposition, compared to quicker oviposition when arriving with *L. sericata*

or delayed oviposition when arriving before *L.* sericata. In contrast, *L. sericata* did not demonstrate intraspecific differences due to arrival order and consistently laid eggs within the first two hours of exposure, regardless of treatment conditions. *Chrysomya rufifacies* also took more time to oviposit when introduced to the piglets before *L. sericata*, less time when introduced simultaneously, and intermediate when second.

Location of Primary Colonization

There was a significant interaction of species and desirability (X^2= 90.879, df = 6, $p < 0.0001$). The three-way interaction term between species, arrival order and desirability was not significant (X^2= 19.677, df = 12, p = 0.074); however, this probability was considered sufficiently high to warrant examination of desirability differences for each species and arrival order. The distribution of first oviposition locations was compared against two expected distributions: equal (random) and exponential (see above). Frequency of first oviposition locations followed an equal distribution ($p > 0.05$) when *P. regina* was together with *L. sericata*, when *C. rufifacies* followed *L. sericata*, and when *L. sericata* colonized after *P. regina*. No other treatments yielded an equal distribution pattern. Only *L. sericata* followed the exponential distribution ($p > 0.05$), but not when females colonized after *P. regina* (see Figure 3.1).

Preferences for primary colonization sites existed, however, the effect of arrival order varied for each species. Arrival order only mattered when *P. regina* was first, such that the primary colonization sites were in moderate and low locations with no eggs laid in highly desirable sites (see Figure 3.1a). When *C. rufifacies* was first or together with *L. sericata*, more eggs were laid in moderate than high desirability locations (first: X^2=

7.143, df = 1, p = 0.008; together: X^2= 4.500, df = 1, p = 0.034), with no differences between locations when they followed *L. sericata* (p > 0.05) (see Figure 3.1b). When *L. sericata* was first or together with *P. regina*, more females oviposited in high desirability locations (first: high vs moderate: X^2= 19.174, df = 1, p < 0.001; high vs low: X^2= 19.174, df = 1, p < 0.001; together: high vs moderate: X^2= 6.400, df = 1, p = 0.011; none in low), however, when *L. sericata* was second, this preference was not present (p > 0.05) (see Figure 3.1c). When *L. sericata* was first with *C. rufifacies*, females only laid eggs in high desirability locations; however, when it was second or together, there were no differences in high and moderate locations (p > 0.05) (see Figure 3.1d).

Examination of eight different body sites demonstrated that when first, *P. regina* laid in locations of low or moderate preference such as the head, umbilical regions and between the legs; however, when second or together with *L. sericata*, while continuing to predominantly oviposit in moderate and low preference locations, a few females oviposited in high desirability locations such as the eyes, mouth, nostrils and ears (see Figure 3.2a). This trend was also noted in *C. rufifacies*, with the exception of a few females laying eggs in piglet mouths when first (alone) (see Figure 3.2b). *Lucilia sericata* females laid their first eggs in the mouth, eyes and nostrils, regardless of arrival order, however, some females oviposited in the head, umbilical and leg regions when second (after *P. regina*) (see Figure 3.2c,d).

Total Number of Eggs

There were differences between treatments in the overall number of eggs laid ($F_{8,85}$= 2.206, p = 0.038) (see Figure 3.3), with *P. regina* females laying the least amount

of eggs when they colonized the resource alone. The highest number of eggs laid occurred in the *L. sericata* vs. *P.* regina (i.e. *L. sericata* first), *P. regina* vs. *L. sericata,* and *C. rufifacies* vs. *L. sericata* treatments. The rest of the treatments were intermediate in their effects.

Distribution of Colonization Sites Based on Total Oviposition

Although the total number of eggs laid was consistent across treatments, the distribution of the eggs over the resource differed, as evident in the interaction between arrival order and species selection of oviposition sites ($F_{16, 375}$= 8.658, $p < 0.001$) (adjusted α=0.017) (see Table 3.6). With respect to desirability levels, arrival order did not influence *P regina* and *C. rufifacies*, with both species preferring to lay eggs in moderate and low desirability locations. *Lucilia sericata*, on the other hand, altered its egg laying behaviour depending on arrival order. When *L. sericata* was first (alone) or when it was second with *C. rufifacies*, females laid more eggs in high desirability locations. There was a preference shift when *L. sericata* was second or together with *P. regina*, with females laying more eggs in moderate than in highly desirable locations.

With respect to the percentage of eggs laid on various sites on each pig, preferences varied due to species, arrival order and site location ($F_{56,1000}$= 4.097, $p < 0.001$). Comparisons were made intra-specifically between treatments to determine if there were preferences within each species in body sites (adjusted α=0.002) (see Table 3.7). *Phormia regina* females laid the majority of their eggs evenly over most of the body (sites 3 to 8) and very few eggs in the mouth, eyes, and nostrils (site 1). When second, *P. regina* females shifted their preferences to the head region (site 3, near sites

already colonized by *L. sericata*) and along the body (site 8). Similarly, *C. rufifacies* females laid very few eggs in the ear, anus, and natural orifices (sites 1, 2 and 5) with the majority of eggs distributed over the body (sites 3,4, 6-8). When *C. rufifacies* were exposed to the resource after colonization by *L. sericata*, they laid most of their eggs near regions heavily colonized by *L. sericata*, such as between the legs and, near the head and umbilical regions. When *L. sericata* was introduced first or after *C. rufifacies*, females laid most of their eggs in the mouth. However, when *L. sericata* followed *P. regina*, females shifted their behaviour to oviposit evenly over the body, rather than laying most of their eggs in the natural orifices.

When two species colonized together (i.e. simultaneously: *L. sericata* and *P. regina*; *L. sericata* and *C. rufifacies*), there was a similar distribution of eggs over all oviposition sites, except for the higher amount of eggs located over the body (site 8) and fewer eggs in the ear canals in the *L. sericata* and *C. rufifacies* treatment. Though the anus is commonly thought to be a secondary site regularly colonized after the mouth/ear/nostrils are occupied (Mann et al. 1990, Greenberg 1991, Campobasso et al. 2001, Mahon et al. 2004, Gruner et al. 2007, Cross and Simmons 2010), this region was not colonized by *C. rufifacies*/*L. sericata* when they were together.

Behavioural Observations on Blow Fly Colonization

Oviposition behaviour was consistent and rapid for *L. sericata* regardless of arrival order. The majority (> 80%) of gravid females approached the piglet within minutes of it being placed within the treatment cages, with females laying eggs within the first 30 minutes of resource exposure. Oviposition continued by additional females over

the next few hours and then abruptly diminished, with the majority of them leaving the resource to groom or feed on sugar after approximately 3 hrs of exposure. During the rest of the photophase, very few females visited (< 20%) or oviposited on (< 10%) the resource. After the 8 hrs of scotophase, a large proportion (40 to 60%) of *L. sericata* females revisited the resource for a second wave of oviposition, however, these colonization sites were typically in moderate and low desirability locations, while the first wave of colonization occurred in high desirability locations.

The colonization behaviour of *P. regina* differed from that of *L. sericata*, and was dependent on arrival order. When *P. regina* was by itself (i.e. alone or introduced first) with the pig carcass, very few females (< 15%) visited the resource immediately. The majority of females remained on the sides of the cages and exhibited "bubble-blowing" behaviour, during which they extended their proboscis along with a liquid droplet (see Figure 3.4), followed shortly thereafter by extension of the ovipositor. They held this position for approximately 10 sec before repeating this behavioural cycle approximately 8-12 times. They then rested, groomed, repositioned themselves and began "bubble-blowing" again. Over successive bouts of this behavioural sequence, the females' abdomens swelled remarkably (see Figure 3.5). Most females carried out bubble-blowing behaviour for approximately 3-4 hrs prior to visiting the resource, while only a few visited the resource repeatedly and probed one or more locations with their proboscis. These locations corresponded to the site of the first oviposition event, which usually consisted of one female leaving a droplet of fluid from its ovipositor following which it (or another female) deposited a single egg. After this first oviposition event, additional females would probe around the area with their probosces and leave additional

droplets from their ovipositors, until eventually another (or the same) female laid an additional egg. This continued until either the number of eggs laid or the number of females that had deposited a droplet at the site seemed to reach a threshold, at which point multiple females began depositing clusters of eggs. Group oviposition usually occurred within 6-9 hrs after exposure to the piglet and lasted for the next 4-5 hrs. Secondary waves of mass oviposition events were not frequent with *P. regina* and colonization was much slower overall than for *L. sericata*, with the delay in oviposition corresponding to bubble-blowing and ovipositor-droplet marking behaviours. In contrast to *L. sericata*, *P. regina* usually laid their eggs in moderate to low priority locations.

When *P. regina* females were in the presence of *L. sericata* or presented with the resource already colonized by *L. sericata*, females immediately visited and inspected the resource, and oviposition usually occurred within the first 3 hrs, similar to the oviposition behaviour exhibited by *L. sericata*. Typically, *P. regina* females oviposited on or near locations where *L. sericata* eggs were present. After the initial wave of oviposition, females then retreated to the sides of the cages, underwent bubble-blowing behaviour and typically (in approximately 50% of cages) participated in a secondary wave of colonization after the scotophase.

Chrysomya rufifacies behaved in much the same manner as *P. regina*, exhibiting bubble-blowing and droplet marking behaviours. However, *C. rufifacies* females repeatedly laid eggs directly on top of *L. sericata* eggs.

Nocturnal oviposition was an unexpected behaviour. Cages were checked every 2 hrs during the scotophase with the use of a night-vision camera, at which times female distribution over the carcass was recorded. Actively ovipositing females and new eggs

were recorded; if they were observed, these were re-checked hourly until ovipositing ceased. Fly behaviour and movement was considerably slower during the scotophase and females typically remained in one position for long periods of time (1-2 hrs). Though infrequent (in 14 out of 128 cages and only one to five females per cage), all three blow fly species exhibited nocturnal oviposition. Most females that exhibited this behaviour had begun ovipositing during photophase or had recently oviposited and were still at the site of the eggs. Infrequently a few females (one to three) crawled onto the resource and oviposited in independent locations. However, the majority of the females (~95%) were motionless during the dark cycle, exhibiting very little activity. No females actively flew towards the resource in the dark.

DISCUSSION

Time to Oviposition and Arrival Order

Priority effects played a role in the colonization behaviour of *P. regina* and *C. rufifacies* in this study. Both species were facilitated by the presence of *L. sericata*, as evidenced by the decrease in the amount of time required to colonize the resource when females either followed or were in the presence of *L. sericata*. *Lucilia sericata* was relatively unaffected by the presence of other species, with females consistently colonizing a resource within the first few hours of exposure. The time required for blow flies to find and successfully colonize a carcass underlies their use in the determination of the minimum time of colonization (MTC) and the estimation of the post-mortem interval (PMI) (Rodriguez and Bass 1983, Greenberg 1991, Campobasso et al. 2001, Mahon et al. 2004, Tomberlin et al. 2011). *Phormia regina* and *C. rufifacies* did not exhibit typical blow fly behaviour and delayed their colonization when alone or first on the piglet carcasses. However, this delay was not seen in treatments where *C. rufifacies* or *P. regina* followed or arrived at the same time as *L. sericata*. In these treatments, both species behaved like *L.* sericata, with females colonizing the resource quickly and exhibiting rapid group oviposition within the first few hours after exposure.

It has been debatable whether or not certain blow fly species exhibit a delay in colonization upon arrival at a resource, yet the presence or absence of a delay can have profound implications for calculating the MTC. It is critical to understand the factors influencing the pre-colonization interval (i.e. the interval between death and arthropod colonization) (Tomberlin et al. 2011). *Lucilia sericata* typically colonizes remains within the first few hours postmortem (Fuller 1934, Hall and Doisy 1993,

Watson and Carlton 2005, Michaud and Moreau 2009); however, in the United Kingdom, it has been documented to exhibit a delay as well as a preference for aged carrion (Fisher et al. 1998, Eberhardt and Elliot 2008). Though my study emphasizes the role of *L. sericata* as an immediate colonizer of carrion, inhibitory species may exist that would exert a negative priority effect on *L. sericata* to potentially cause a delay in colonization. *Chrysomya rufifacies* coexists with many other blow fly species in its native range (Baumgartner 1993, Eberhardt and Elliot 2008) and is known to colonize a resource after prior establishment by another species (Watson and Carlton 2005, Yang and Shiao 2012). In contrast, in North America it becomes a dominant species within the community that can extirpate native species (Wells and Greenberg 1992, Baumgartner 1993, Rosati and VanLaerhoven 2007), and its colonization is sometimes but not always delayed (Byrd and Butler 1997, Byrd and Castner 2001, Lang et al. 2006, Gruner et al. 2007, Eberhardt and Elliot 2008, Yang and Shiao 2012). *Phormia regina* has also been noted to have a delay in colonization (Illingworth 1927, Gruner et al. 2007, Watson and Carlton 2005, Michaud and Moreau 2009), however, it can also be a primary colonizer (Greenberg 1991, personal observations). My results demonstrate *that P. regina and C. rufifacies* can exhibit delayed or immediate colonization depending upon interactions with another species.

Since individual species can exhibit both delays in colonization and immediate colonization, both in field and laboratory investigations, extreme caution must be exercised when incorporating a species' colonization delay into PMI estimates. Colonization behaviour is just one of many behaviours that can differ and influence the ecology of blow fly species in different regions. Further caution should be exercised

when extending conclusions based upon blow fly behaviour from one region to another. Additionally, while laboratory studies such as mine enable detailed understanding of two- or three-species interactions, inferences made from them are somewhat restricted because of the many environmental factors, more diverse blow fly communities and complex species interactions present in natural environments that cannot be accounted for within a laboratory setting. Field validation of the results from my lab experiments would help to determine if these results are also applicable in natural settings. Additional experimental conducted both in field and lab settings are warranted to fully understand the ecology of blow flies and how it affects their interactions. This is especially true in light of the importance of these species in forensic investigations.

Location of Oviposition Events and Arrival Order

Arrival time affected oviposition site selection of the blow flies used in this study. In the absence of other species, only *L. sericata* demonstrated the expected preference for moist protected sites such as the natural orifices of the face. However, it shifted its oviposition site selection to less desirable locations when colonizing after *P. regina*, a priority effect that would be predicted by competition theory as it relates to an ephemeral resource such as carrion (Lotka 1925, Diamond 1975, Beaver 1977, Tilman 1982, Woodcock et al. 2002). *Phormia regina* and *C. rufifacies* both shifted their oviposition locations from low desirability locations when they were the only species present to moderate or high desirability locations when second or in the presence of *L. sericata*, often ovipositing directly next to or on those sites already colonized by *L. sericata*. It is unknown why these two species would choose less desirable sites when highly desirable

sites are unoccupied, but this demonstrates that the assumption within the forensic entomology literature that all fly species prefer the moist protected sites of the orifices (Mann et al. 1990, Greenberg 1991, Campobasso et al. 2001, Mahon et al. 2004, Gruner et al. 2007, Cross and Simmons 2010) is not supported by experimental evidence.

Previous research has indicated that gravid female *C. rufifacies* and *P. regina* prefer to oviposit on previously colonized areas (Wilton 1954, Watson and Carlton 2005, Yang and Shiao 2012). This contradicts the assumption that competition is the driving mechanism behind oviposition site selection by *P. regina* and *C. rufifacies*. Recent research conducted by Reid (2012) determined that both *P. regina* and *C. rufifacies* larvae perform better in the presence of additional species, specifically L. *sericata*, than when each species completes its larval development alone. *Chrysomya rufifacies* becomes a facultative predator during later larval stages (Wells and Greenberg 1992, Baumgartner 1993) and therefore gains the advantage of having an additional food source if females oviposit near egg masses of other species.

I observed that *Phormia regina* and *C. rufifacies* spent more time evaluating the suitability of the resource, which was evident in the time spent bubble-blowing and marking with ovipositor droplets prior to laying eggs. Although bubble-blowing behaviour has been observed in both male and female *Phormia regina* (Stoffolano et al. 2008), I observed it in all three blow fly species I studied. Bubbling has also been observed in other higher order Dipterans such as horseflies (Tabanidae) (Hewitt 1912), *Rhagoletis pomonella* (Walsh) (Tephritidae) (Hendrichs et al. 1992) and flesh flies (Sarcophagidae) (Dacks et al. 2003). Bubbling *P. regina* flies have larger crop volumes than non-bubbling individuals (Stoffolano et al. 2008). Bubbling may enable flies to

concentrate crop solute and eliminate excess water, suggesting a primary digestive function for this behaviour (Hendrichs et al. 1992, Stoffolano et al. 1995, 2008, Stoffolano and Haselton 2013). This behaviour may have that function in *L. sericata*, as I observed it primarily after oviposition. However, in *C. rufifacies* and *P. regina*, when they were the only species present, bubbling occurred during the delay phase prior to oviposition which is suggestive of an alternative function. In other dipterans there is a link between regurgitation of crop contents and the dissemination of *Escherichia coli* bacteria (Sasaki et al. 2000), various pathogens (Greenberg 1971, Maldonado and Centeno 2003) and pheromones (Headrick and Goeden 1994, Walse et al. 2008). Density-dependent constraints for colonization have been demonstrated in *Chrysomya bezziana* (Villeneuve), in which oviposition rates declined exponentially with increasing numbers of females present (i.e. female catch rates) (Mahon et al. 2004). Similarly, Lam et al. (2007) demonstrated that bacteria that originated within adult female *Musca domestica* (Linnaeus) proliferated on the surface of deposited eggs and inhibited further oviposition once a bacterial density threshold was reached. Flies exhibited immediate induction of oviposition stimulated by pheromones from gravid females, followed by delayed inhibition in late arriving females that is mediated by bacterially derived cues on eggs, which in turn reduced larval competition and ensured conditions conducive to offspring development (Lam et al. 2007). The mechanisms underlying induction and inhibition of oviposition in other species remain largely unstudied. Recent research suggesting the occurrence of a conspecific contact signal and/or pheromone that induces aggregation in blow fly larvae (Boulay et al. 2013) makes it reasonable that such signals could affect the behaviour of adult blow flies as well.

Bubble-blowing behaviour was followed by grooming that may transfer bacteria or pheromones to the ovipositor and subsequently to the resource in droplets of fluid prior to oviposition. Additional visits to droplet deposition sites by subsequent females could assist them in reaching a bacterial or chemical "threshold" that must be met before other females deem a site suitable for oviposition. I hypothesize that bubble-blowing behaviour is involved in (a) the evaluation of the resource for oviposition suitability; (b) transfer of bacteria to the resource that make it a more suitable environment for the eggs and/or larvae; (c) a marking pheromone applied to the resource that increases in concentration through additional fluids deposited by conspecifics until a threshold for oviposition is surpassed. Given the digestive function that has been documented previously, I would also extend this concept and hypothesize that this behaviour may affect eggs. For example, it may affect the water content of eggs or the chemistry of the egg chorion, resulting in more resistant eggs with improved abilities to withstand desiccation. Other fly species may affect these relationships. For example, bacteria they deposit and/or alterations to oviposition sites that they induce could influence offspring survival. Specifically, in my research, *C. rufifacies* and *P. regina* females oviposited immediately and more rapidly when heterospecifics were present, suggesting that such effects by heterospecifics on blow fly colonization are possible. To better understand the function of bubbling behaviour in blow flies and other dipterans, further investigation are warranted of (a) the chemical and bacterial composition of the bubbles, (b) the potential for transfer of bacteria, chemical cues or pheromones between the proboscis and ovipositor, (c) behaviours of flies in act of bubbling, and (c) microscopic examination of egg characteristics.

The distribution of eggs can play an important role in the coexistence of multiple species on a resource (Atkinson and Shorrocks 1981, Ives 1988, 1989, 1991, Chesson 1991, Hoffmeister and Rohlfs 2001). The extent to which eggs are aggregated or dispersed is influenced by olfactory cues from a variety of sources (Eddy et al. 1975, Adams et al. 1979, Hammack 1984, Esser 1990), attraction cues such as volatiles or bacterial communities present during decomposition of the carcass (Ashworth and Wall 1994, Vogt and Woodburn 1994, Mahon et al. 2004, Tomberlin 2012), and visual recognition of larvae (Yang and Shiao 2012). Before and during oviposition, gravid females may be induced to aggregate or commence oviposition in a site by chemical stimuli (Barton-Browne et al. (1969), such as a cuticular lipid (Emmens 1981), chemicals emitted from ovipositing females (Esser 1990), or a marking pheromone (Prokopy 1972). This suggests that the aggregated response of blow fly females and egg distributions noted in this study could result from semiochemicals that may be important to induce and regulate blow fly oviposition behaviour.

Blow flies are short-lived and oviposit on ephemeral resources, traits that exert strong selective pressures on life history characteristics and egg laying strategies (Cruickshank and Wall 2002a,b, Davies 2006). Blow flies quickly orient to dead animals in large numbers and typically exhibit aggregated oviposition (Barton-Browne et al. 1969, Ashworth and Wall 1994, Tomberlin et al. 2011). However there were many instances observed in this study, especially in *P. regina* and *C. rufifacies* when by themselves, when individual females would lay a single egg in a particular location, often in locations of moderate or low desirability. This strategy appears to be suboptimal since those sites are not protected from predators or desiccation. This highlights our

incomplete understanding of the many factors during the pre-colonization window that may affect oviposition decisions by individual females (Hoffmeister and Rohlfs 2001).

Unexpected Observations

Forensic entomologists generally assume that blow flies do not oviposit at night. In fact, this plays an important role in the calculation of the MTC (Erzinçlioğlue 1966, Nuorteva 1977). In numerous research investigations, nocturnal oviposition did not occur (Nuorteva 1977, Tessmer et al. 1995, Haskell et al. 2002, Spencer 2003). In contrast, although it occurred infrequently, I recorded nocturnal oviposition under complete darkness for all three species. Other studies have also demonstrated that oviposition can occur at night (Green 1951, Greenberg 1990, Singh and Bharti 2001, Amendt et al. 2008), in low-light conditions (Baldridge et al. 2006) or under circumstances with unusually high night temperatures, previous presence of gravid females, and after females have surpassed stimulus thresholds (Wooldridge et al. 2007, Amendt et al. 2008, Zurawski et al. 2009, Berg and Benbow 2013, George et al. 2013). My observations that females did not actively fly but crawled towards the resource supports the findings of Wooldridge et al. (2007), that the flight activity of *C. vicina* and *L. sericata* decreased with decreasing light intensity. They determined that random flight, rather than directed flight, can occur in low/no light conditions and that the probability of oriented flight leading to oviposition on a corpse was low. This was also demonstrated by Zurawaski et al. (2009) who determined that adult flies had no flight capabilities under complete darkness.

Conclusions and Future Directions

This study demonstrated that priority effects differ depending on the spatial or temporal scale examined as well as the species studied and their order of arrival. This supports previous research that priority effects are important in structuring carrion insect communities (Beaver 1977, Hanski and Kuusela 1977, Shorrocks and Bingley 1994, Morin 1999, Bruno et al. 2003). *Chrysomya rufifacies* and *P. regina* experienced positive priority effects spatially and temporally from the presence of *L. sericata*, while *L. sericata* experienced negative priority effects spatially but temporally were unaffected by arrival order. Some of the blow fly species I studied followed neither a random nor expected pattern of oviposition. Instead, *P. regina* and *C. rufifacies* exhibited preferences for less desirable oviposition locations, and often preferred to lay eggs on or near eggs of a previously established species. Given this finding, it would be important to extend this study to examine the fitness consequences of oviposition decisions. This would confirm whether or not adult blow flies behave optimally to maximize offspring fitness. It would also be important to distinguish the eggs of each species in order to more precisely assess priority effects in blow flies, which could not be done in the experimental treatments with two species.

The oviposition strategy of *L. sericata*, to arrive and colonize early in the most desirable locations when alone, and then to shift to less desirable locations following colonization by *P. regina*, suggests that *L. sericata* may act as a fugitive species. Through these behaviours female *L. sericata* increase their offspring survival and fitness by monopolizing the resource early in decomposition. Other fugitive species have been observed within the carrion community and this strategy can be a mechanism for species

coexistence (Hanski and Kuusela 1977, Kneidel 1983, Shorrocks and Bingley 1994). However, further studies examining offspring fitness of *L. sericata* in association with other blow fly species and under different regimes of time alone and together could provide insight into whether or not *L. sericata* acts as a fugitive species within the carrion insect community.

On a community level, my studies were simplified and controlled. Clearly, as additional community members within and between guilds are added, as spatial and temporal scales are varied, and as abiotic conditions are altered to reflect more natural and more variable conditions, the complexity of the mechanisms that govern community assemblages will drastically increase. Results from this study provide a base for understanding a number of simple patterns of assembly which can be examined further to understand larger patterns of assembly within the carrion community. Small-scale manipulative studies, such as the manipulation of arrival order in my study, that incorporate multiple study parameters, such as the spatial aggregation and effects of arrival order examined in this study, can provide unique insight into understanding the factors that structure ecological communities (Gilbert and Owen 1990, Drake 1991, Farrell 1991, Levin et al. 2001, Alonso et al. 2006, HilleRisLambers et al. 2012). Incorporating multiple variables and study scales, both temporal and spatial, is a necessary step to continue to expand our understanding of processes that govern complex species and community interactions and how these processes change over time and space, which is particularly important given the forensic importance of the carrion insect community.

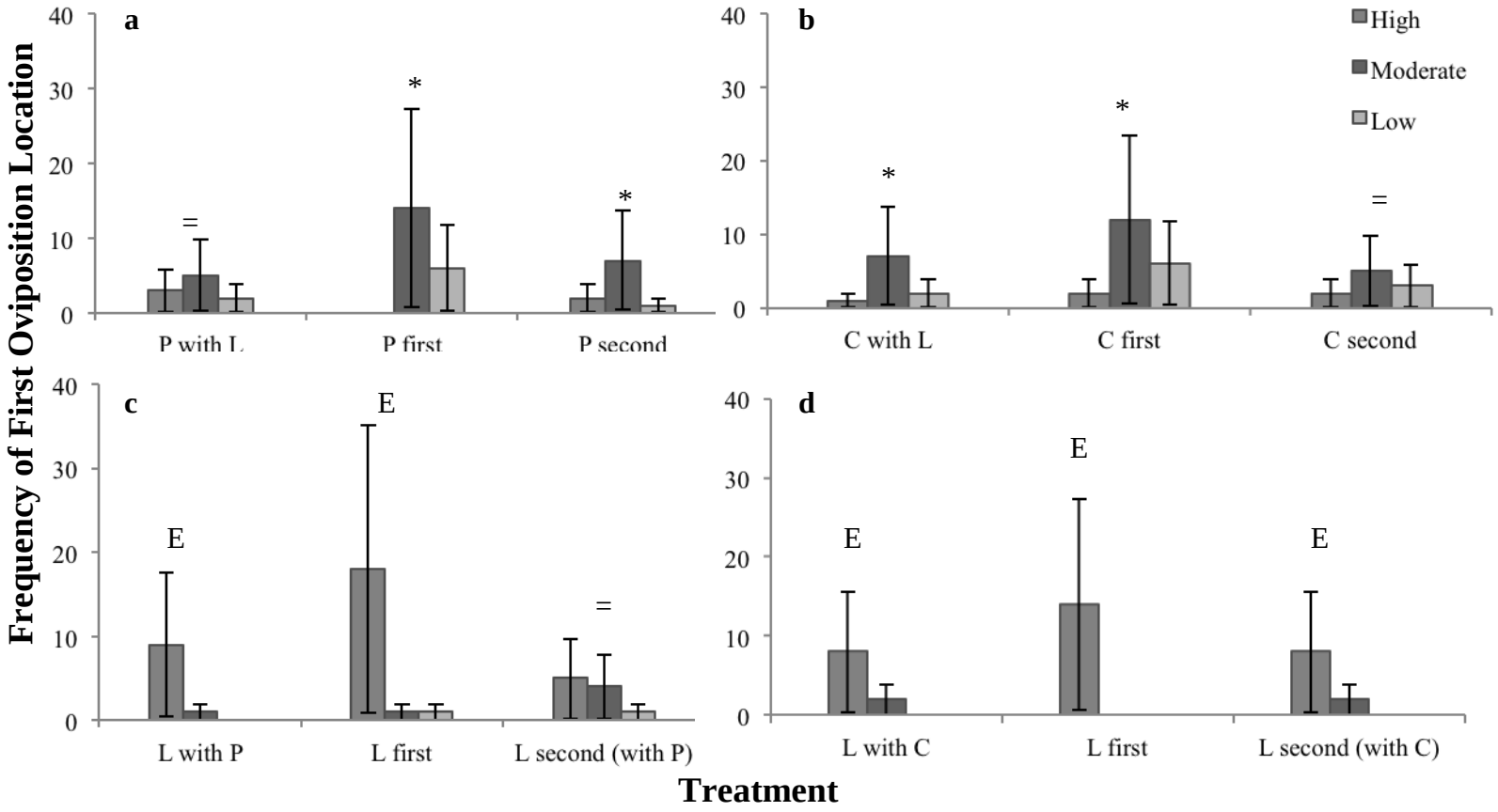

Figure 3.1. Effect of species and arrival order on location of first oviposition site for three blow fly species: *Phormia regina* (Meigen) (P), *Chrysomya rufifacies* (Macquart) (C) and *Lucilia sericata* (Meigen) (L). Data were grouped according to oviposition desirability (high, moderate, low). Frequency of first oviposition location ± 95% confidence intervals was determined for each treatment condition. Binomial tests were used to determine if there were any preferences in site locations. E – denotes distribution follows an exponential pattern and a preference for high desirability sites. = – denotes distribution follows an equal pattern and no site preferences. . * - denotes more females selected sites in that desirability level for the first oviposition event.

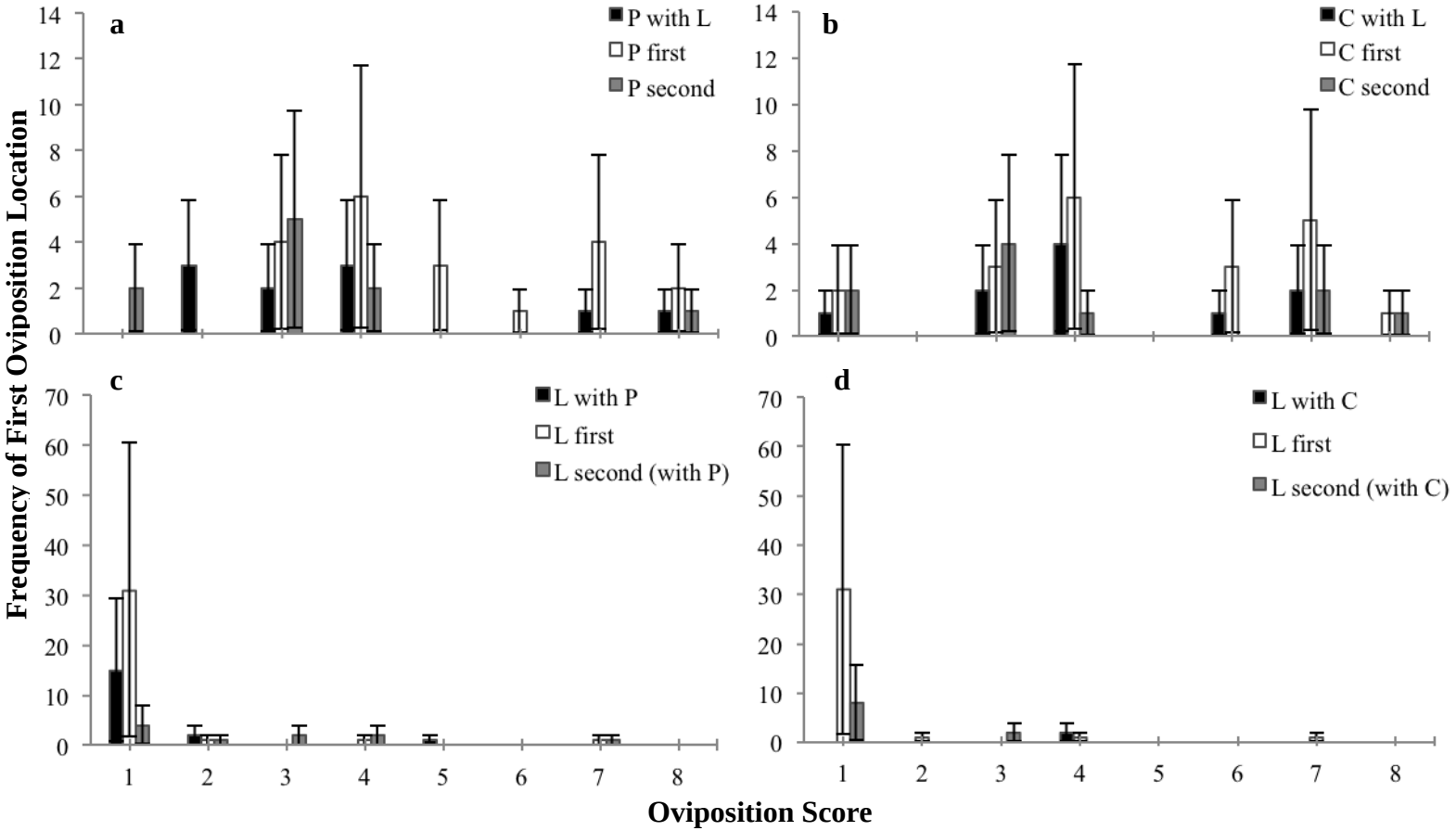

Figure 3.2. Location of first oviposition site for three blow fly species*: Phormia regina* (P), *Chrysomya rufifacies* (C) *and Lucilia sericata* (L). Data were grouped according to oviposition score. Frequency of first oviposition location ± 95% confidence intervals were determined for each treatment condition. **a** - *P. regina*, **b** - *C. rufifacies*, **c** - *L. sericata* (with *P. regina*) and **d** - *L. sericata* (with *C. rufifacies.*

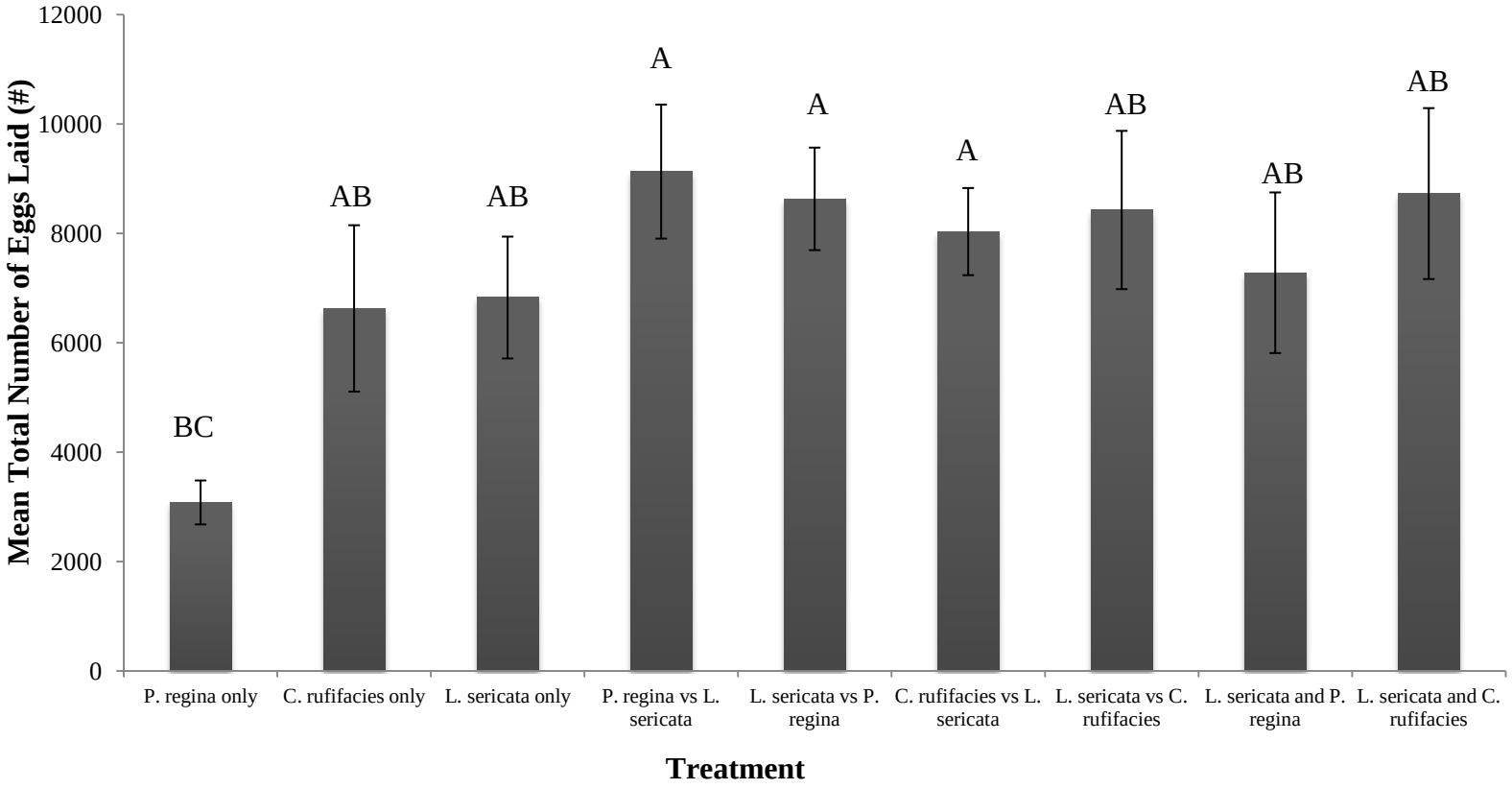

Figure 3.3. Effect of species and arrival order on mean number of eggs laid for *Chrysomya rufifacies*, *Phormia regina*, and *Lucilia sericata*. A one-way ANOVA was used to determine effect of treatment on the mean number of eggs laid. A Games-Howell post-hoc test was used determined differences among treatments. Means with the same letter do not differ significantly ($p>0.05$).

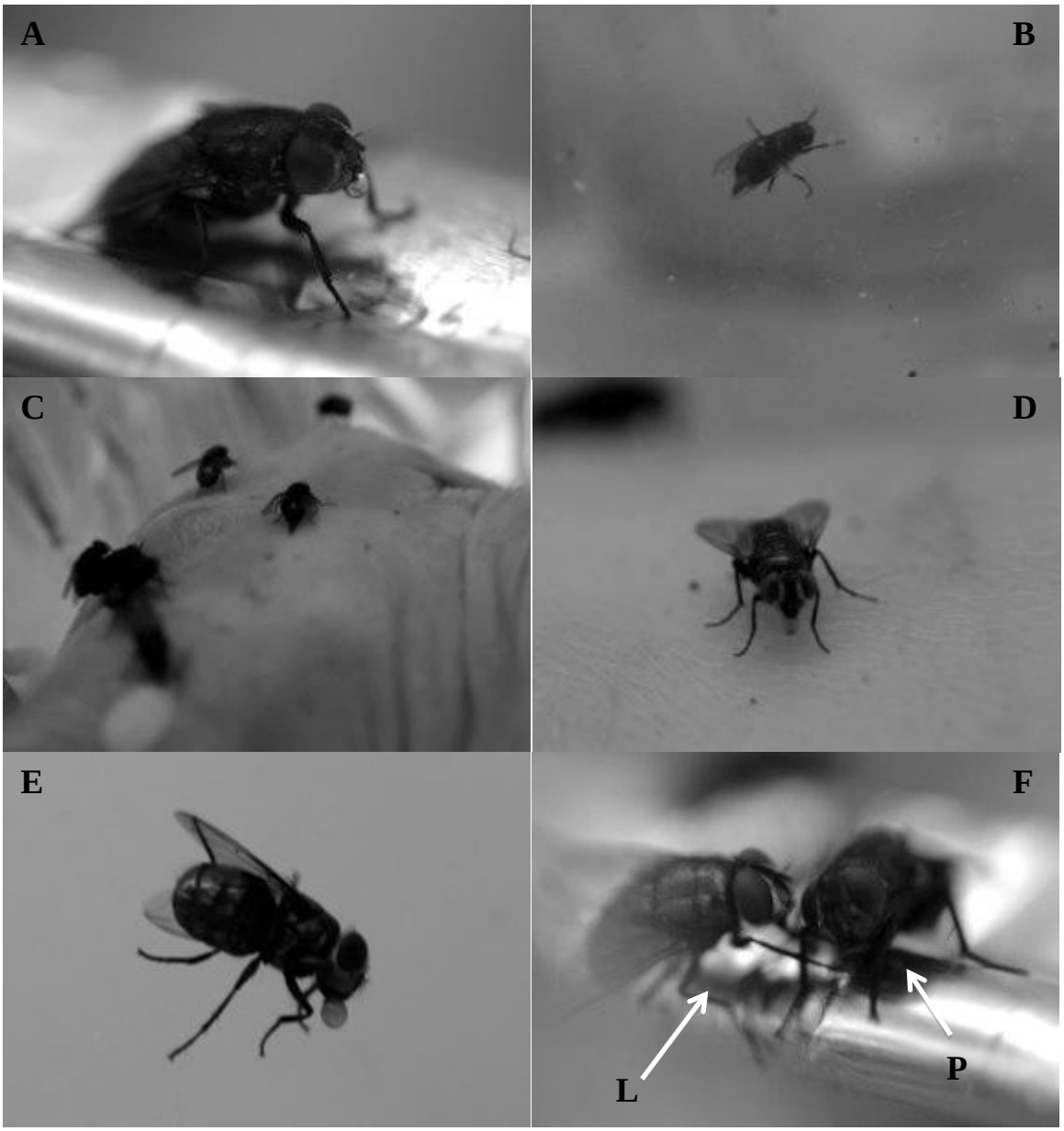

Figure 3.4. Bubble blowing behaviour exhibited in three blow fly species, *Chrysomya rufifacies* (Macquart), *Lucilia sericata* (Meigen) and *Phormia regina* (Meigen). **A** – *P. regina* with droplet extending from proboscis. **B** – *P. regina* extending ovipositor following proboscis extension. **C** – *P. regina* dragging ovipositor prior to ovipositioning on head of piglet carcass *Sus scrofa* (Linnaeus). **D** – *L. sericata* with droplet extending from proboscis. **E** – *C. rufifacies* with droplet extending from proboscis. **F** – *P. regina* (P) and *L. sericata* (L) interacting immediately after bubble blowing by *P. regina*. *Photos taken by J. Rosati*.

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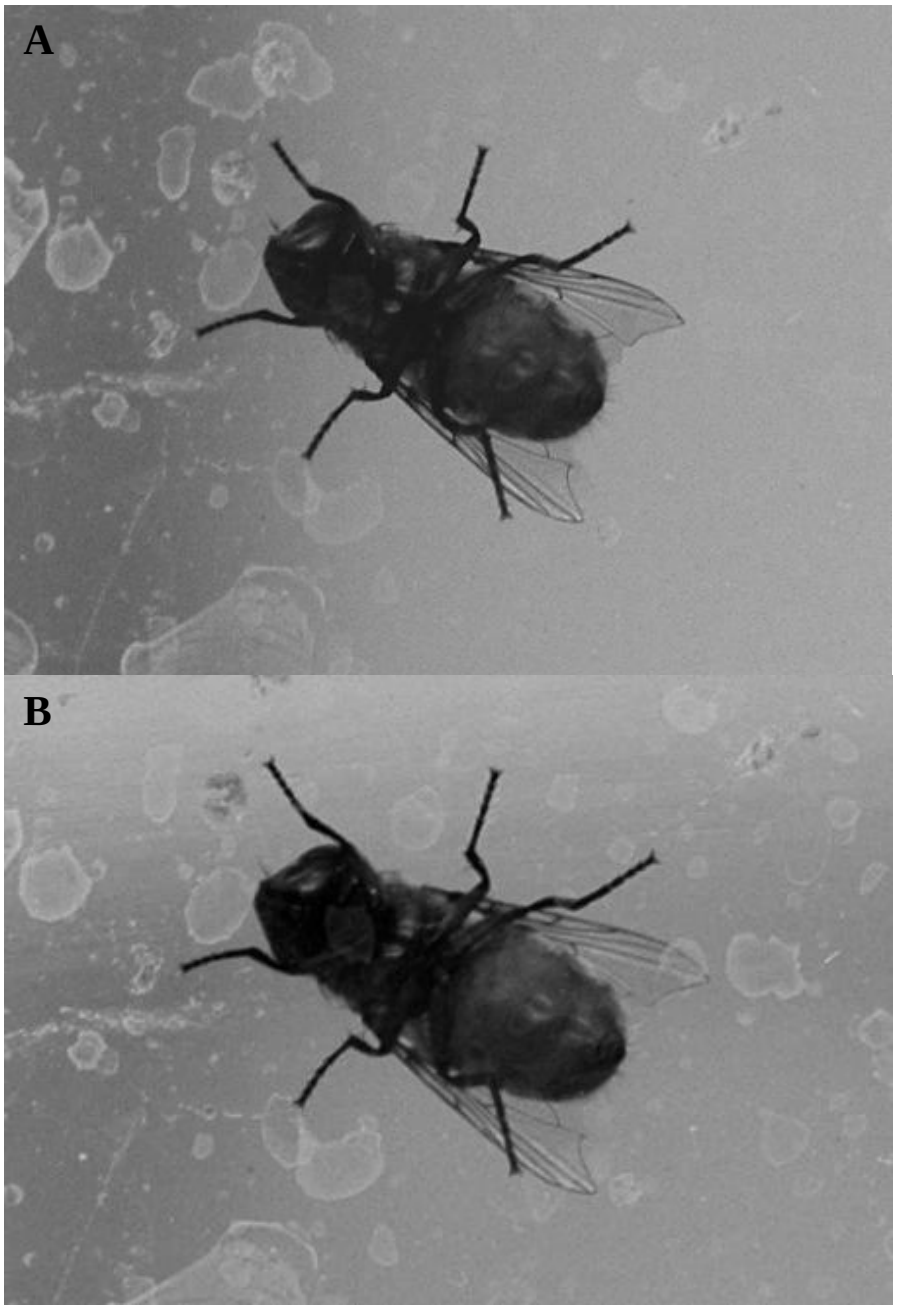

Figure 3.5. Bubble blowing behaviour in *Chrysomya rufifacies* (Macquart). Female shown here is approximately six hours after introduction of piglet carcass *Sus scrofa* L. into cage. During the six-hour window of exposure, the female has undergone multiple bubble blowing sessions. **A** – female beginning another session, abdomen slightly distended. **B** – female post-session, abdomen more distended. *Photos taken by J. Rosati*.

Table 3.1. Hypotheses and predicted outcomes for experiments testing the effect of arrival order on colonization potential of *Lucilia sericata* (Meigen) and *Phormia regina* (Meigen). H_1 represents positive priority effects, H_2 represents negative priority effects which were tested against the null hypothesis (H_{null}). Outcomes are described as high, moderate, or low or increased/decreased with respect to colonization potential (measure by time, location and amount of eggs deposited). Treatment conditions consist of each species being allowed to colonize independently (*L. sericata* only, *P. regina* only), both species colonizing simultaneously *(L. sericata* and *P. regina*) or one species colonizing first, followed by the second species (*L. sericata* first followed by *P. regina* in the *L. sericata* vs. *P. regina* treatment, and vice versa for the *P. regina* vs. *L. sericata* treatment). **LS** – *Lucilia sericata*, **PR** – *Phormia regina*. *(Note – predicted outcomes for L. sericata and C. rufifacies experiments would follow this outline).*

Hypotheses	***L. sericata* only**	***P. regina* only**	***L. sericata* vs. *P. regina***	***P. regina* vs. *L. sericata***	***L. sericata and P. regina***
H_{null}: Neutral	high	high	LS – high PR – high	LS – high PR – high	LS – high PR – high
$H_{1a:}$+ve priority effect (LS on PR)	high	low/moderate	LS – high PR – increased	LS – high PR – low/moderate	LS – high PR – increased
H_{1b}: +ve priority effect (PR on LS)	low/moderate	high	LS – low/moderate PR – high	LS – increased PR – high	LS – increased PR – high
H_{2a}: -ve priority effect (LS on PR)	high	high	LS – high PR – decreased	LS – high PR – high	LS – high PR – decreased
$H_{2b:}$ -ve priority effect (PR on LS)	high	high	LS – high PR – high	LS – decreased PR – high	LS – decreased PR – high

Table 3.2. Density of male and female blow flies (Diptera: Calliphoridae) of *Lucilia sericata* (Meigen), *Phormia regina* (Meigen) and *Chrysomya rufifacies* (Macquart) within each treatment at each time interval (10 replicates per treatment). The two time intervals for colonization in the "vs" treatments were 0-24 and 24-48 hrs post-mortem, with post-mortem referring to the time since death of the piglets, *Sus scrofa domesticus* (L.). Treatment density was maintained at 100 females, 50 males in the following species compositions.

	0-24hr Post-mortem exposure		***24-48hr Post-mortem exposure***	
Treatment	**# Female**	**# Male**	**# Female**	**# Male**
L. sericata only	100L	50L	n/a	n/a
P. regina only	100P	50P	n/a	n/a
C. rufifacies only	100C	50C	n/a	n/a
L. sericata and *P. regina*	50L, 50P	25L, 25P	n/a	n/a
L. sericata and *C. rufifacies*	50L, 50C	25L, 25C	n/a	n/a
L. sericata vs. *P. regina*	50L	25L	50P	25P
P. regina vs. *L. sericata*	50P	25P	50L	25L
L. sericata vs. *C. rufifacies*	50L	25L	50C	25C
C. rufifacies vs. *L. sericata*	50C	25C	50L	25L

Table 3.3. Scoring system used to classify blow fly egg masses with respect to body site, in which a score of 1 corresponds to a most desirable location and a score of 8 corresponds to a least desirable location. Scores were also classified according to oviposition location desirability (high, moderate, low).

SCORE	BODY SITE	DESIRABILITY LEVEL
1	mouth/eye/nostril	*high*
2	ear	*high*
3	head	*moderate*
4	belly/umbilical	*moderate*
5	anus	*moderate*
6	neck	*moderate*
7	between legs	*low*
8	rest of body	*low*

Table 3.4. The effect of density on mean time to oviposition and percentage of eggs laid in each site and each desirability level for each species and the effect of species combination on *L. sericata*. An Independent samples t-test was used for mean time to colonization with equal variances assumed for *P. regina* and *L. sericata* and unequal variances for *C. rufifacies*. An ANOVA was used with treatment as a main factor for percentage of eggs laid in each desirability location or oviposition site or species combination and arrival order for mean time to colonization of *L. sericata*. There were no significant differences ($p>0.05$) between species alone and first treatments, therefore data were pooled for subsequent analyses.

	Effect of Species Combination			**Effect of Density**								
	Mean Time to Colonization			Mean Time to Colonization			% in Desirability Location			% in Each Site Location		
Species	df (source,error)	F	P	df	t	P	df (source,error)	F	P	df (source,error)	F	P
Phormia regina	n/a	n/a	n/a	18	0.268	0.611	2,54	1.553	0.221	7, 144	1.664	0.122
Chrysomya rufifacies	n/a	n/a	n/a	18	0.996	0.335	2,54	0.716	0.493	7, 144	0.369	0.919
Lucilia sericata	1,68	1.453	0.235	32	0.924	0.363	2,96	0.071	0.931	7, 257	0.522	0.808

Table 3.5. Effect of arrival order and species on mean time to colonization (hrs) for three blow fly species: *Phormia regina* (Meigen), *Chrysomya rufifacies* (Macquart) and *Lucilia sericata* (Meigen). Mean time to colonization ± SE was measured from the beginning of exposure of gravid females to piglets as a resource (time=0 hrs) to the first oviposition event (hrs) for each arrival order within each treatment condition. "Species only" treatments were pooled with "species first" treatments. For *L. sericata*, treatments were pooled for arrival order (over both species combinations). A bootstrapped (k=1000) ANOVA was used and pairwise comparison tests based on bootstrapped means were used with a Bonferroni correction to determine differences among treatments. Means with the same letter do not differ significantly. Capital letters denote comparisons between species and small letters denote comparisons within species.

		Time to Colonization (hrs)					
Species	**Arrival Order**	**Mean± SE**		**Minimum**	**Maximum**	**σ^2**	**σ**
Phormia regina	Together	1.90±0.31	^{AB,b}	1	3	0.99	0.99
	First	5.65±1.23	^{A,a}	1	19	30.35	5.51
	Second	1.50±0.27	^{A,b}	1	3	0.72	0.85
Chyrsomya rufifacies	Together	1.70±0.21	^{A,b}	1	3	0.46	0.68
	First	10.70±1.80	^{A,a}	2	30	65.06	8.07
	Second	3.70±1.48	^{A,ab}	1	16	22.01	4.69
Lucilia sericata	Together	1.05±0.05	^{B,a}	1	2	0.05	0.22
	First	1.09±0.05	^{B,a}	1	2	0.08	0.29
	Second	1.25±0.14	^{A,a}	1	3	0.41	0.64

Table 3.6. Effect of species and arrival order on mean percentage of eggs (%) laid by *Phormia regina*, *Chrysomya rufifacies* and *Lucilia sericata* across high, moderate and low desirability oviposition locations. Mean percentage of eggs laid (%) + SE was measured on a per pig basis. A bootstrapped (k=1000) ANOVA was used and pairwise comparison tests based on bootstrapped means were used with a Bonferroni correction to determine differences among treatments. Means with the same letter do not differ significantly (p>0.05). Letters a through c were used to denote comparisons *within* each treatment with a-denoting a higher value (i.e. comparisons were made *between* desirability levels).

	Desirability Level		
Treatment	**% High**	**% Moderate**	**% Low**
Phormia regina first	7.10 ± 4.47^{b}	51.49 ± 7.24^{a}	41.41 ± 7.15^{a}
Phormia regina second	6.87 ± 4.29^{b}	56.06 ± 9.49^{a}	37.07 ± 10.07^{a}
Chrysomya rufifacies first	3.59 ± 2.76^{b}	46.60 ± 8.01^{a}	49.82 ± 7.40^{a}
Chrysomya rufifacies second	11.92 ± 7.21^{b}	40.08 ± 9.95^{a}	48.00 ± 9.29^{a}
Lucilia sericata first	50.16 ± 4.86^{a}	31.35 ± 3.87^{b}	18.49 ± 2.74^{c}
Lucilia sericata second (with *P. regina*)	12.07 ± 5.54^{c}	56.78 ± 6.54^{a}	31.15 ± 6.73^{b}
Lucilia sericata second (with *C. rufifacies*)	53.98 ± 8.44^{a}	25.52 ± 6.97^{b}	20.51 ± 6.03^{b}
L. sericata and *P.regina*	31.05 ± 8.04^{a}	46.78 ± 7.54^{a}	22.16 ± 5.30^{b}
L. sericata and *C. rufifacies*	18.01 ± 4.11^{b}	33.41 ± 9.25^{b}	48.58 ± 9.44^{a}

Table 3.7. Effect of species and arrival order on mean percentage of eggs (%) laid by *Phormia regina*, *Chrysomya rufifacies* and *Lucilia sericata* across individual oviposition locations (see Methods; Table 3.3). Mean percentage of eggs laid (%) + SE was measured on a per pig basis. A bootstrapped (k=1000) ANOVA was used and pairwise comparison tests based on bootstrapped means were used with a Bonferroni correction to determine differences among treatments. Means with the same letter do not differ significantly (p>0.05). Letters a through d were used to denote comparisons *within* each treatment with a-denoting a higher value (i.e. comparisons were made *between* desirability levels).

	HIGH DESIRABILITY		**MODERATE DESIRABILITY**				**LOW DESIRABILITY**	
Species	**% Score 1**	**% Score 2**	**% Score 3**	**% Score 4**	**% Score 5**	**% Score 6**	**% Score 7**	**% Score**
Phormia regina first	1.94±1.13^{d}	5.16±4.23^{cd}	18.48±6.13^{ab}	11.89±4.53^{abc}	8.32±3.17^{bc}	12.80±4.82^{abc}	15.74±4.94^{abc}	25.67±5.72^{a}
Phormia regina second	1.25±1.24^{b}	5.62±4.29^{b}	38.84±8.78^{a}	5.05±2.60^{b}	2.64±2.10^{b}	9.53±4.55^{b}	2.79±1.68^{b}	34.28±9.61^{a}
Chrysomya rufifacies first	2.73±2.68^{cd}	0.85±0.83^{d}	21.74±7.91^{ab}	11.98±4.31^{bc}	0.00±0.00^{d}	12.88±5.81^{abc}	20.92±5.41^{ab}	28.89±6.54^{a}
Chrysomya rufifacies second	11.22±7.31^{abc}	0.70±0.47^{cd}	17.61±8.96^{ab}	6.93±2.74^{b}	0.00±0.00^{d}	15.54±10.06^{ab}	27.85±11.06^{a}	20.14±7.62^{a}
Lucilia sericata first	42.98±5.54^{a}	7.18±1.85^{b}	11.93±2.80^{b}	9.80±2.09^{b}	1.06±0.57^{c}	8.56±3.23^{b}	7.80±2.09^{b}	10.69±1.99
Lucilia sericata second (with *P. regina*)	11.61±5.60^{a}	0.46±0.37^{b}	20.89±4.78^{a}	12.73±3.82^{a}	7.50±5.65^{ab}	15.66±9.16^{a}	13.04±4.75^{a}	18.11±5.51^{a}
Lucilia sericata second (with *C. rufifacies*)	53.69±8.29^{a}	0.28±0.28^{c}	6.23±5.25^{bcd}	19.16±5.29^{b}	0.00±0.00^{d}	0.12±0.12^{d}	14.82±6.45^{bc}	5.69±2.77^{c}
L. sericata and *P.regina*	14.08±4.90^{a}	16.97±6.72^{a}	18.43±8.91^{a}	11.00±3.93^{b}	6.31±2.63^{a}	11.04±4.05^{a}	6.31±3.60^{a}	15.85±4.96^{a}
L. sericata and *C. rufifacies*	16.84±4.26^{ab}	1.17±0.81^{cd}	7.27±5.91^{bc}	16.64±5.90^{ab}	0.00±0.00^{d}	9.50±8.15^{abc}	18.19±6.84^{ab}	30.39±9.01^{a}

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CHAPTER 4: PRIORITY EFFECTS: THEIR EFFECTS ON COEXISTENCE OF LARVAL BLOW FLIES (FAMILY: CALLIPHORIDAE)

INTRODUCTION

Communities are complex assemblages of diverse species that coexist through various mechanisms. While considerable effort has focused on differences in competitive abilities as the primary factor that enables coexistence (Hutchinson 1951, Levin 1974, Kneidel 1984), it has long been recognized that competition through exclusion can also reduce diversity (Gause 1934). Coexistence can also be affected if dominance differs over spatial or temporal scales. Temporal partitioning in species' arrival times is one factor that can mediate dominance (MacArthur and Wilson 1967, Atkinson and Shorrocks 1981, Shorrocks and Bingley 1994). For example, by colonizing a resource patch first, a species can resist subsequent invasion of the patch by other species (Levin 1974, Sale 1977, Kneidel 1983, Shorrocks and Bingley 1994, Wainwright et al. 2012). Alternatively, an early arriving species may alter its environment in ways that enhance the performance of late arriving species (Connell and Slatyer 1977, Victorsson 2012). Priority effects occur when an early arriving species exerts an effect on a later arriving species or vice versa (Beaver 1977, Connell and Slatyer 1977, Shorrocks and Bingley 1994, Fukami et al. 2005, Wainwright et al. 2012). Priority effects can be negative, as in the first situation described above, or positive (second situation described).

Within the carrion insect community, positive priority effects occur when the presence of early arrivers such as blow flies increase recruitment of later arriving species by exposing previously restricted food sources, such as bone, ligaments and internal organs, or by altering the bacterial community in ways that make the resource more

attractive or suitable to later arriving species (Hobson 1931, Hollis et al. 1985, Esser 1990, Mumcuoglu et al. 2001, Beasley et al. 2012, Tomberlin et al. 2012, Barton et al. 2013). Early arrival can also act to mediate subsequent interactions in the carrion community. For example, differences in arrival times of *Drosophila* spp. (Diptera: Drosophilidae) altered competitive interactions in decaying mushrooms, with increased mortality, smaller offspring size and longer developmental times in later arriving species (Shorrocks and Bingley 1994). Despite having low competitive abilities, by arriving quickly at resources, fugitive species can survive and sometimes dominate their community (Hutchinson 1951, Levin 1974, Hanski 1983, Kneidel 1983, Shorrocks and Bingley 1994). Early colonizers can gain a competitive advantage over later species when maturing larvae completely consume the resource (Hanski and Kuusela 1977) or prey upon competitors (Wells 1991, Wells and Greenberg 1992). To add to the complexity of these interactions, in the simplest two-species community the effects of each species on the other can be positive, negative, or neutral, and those outcomes may vary depending on the amount of time separating the arrival of the two species on the resource.

Within the carrion insect community, blow flies are among the most abundant taxa. Most blow fly species fall within the sarcosaprophytic guild, which includes those species that feed directly on decomposing carrion tissue (Braack 1987). Developing larvae generally experience high competition for food, given that multiple females often lay more eggs than can be supported fully by the resource (Ullyett 1950, Kneidel 1984). Additionally, there are a few blow fly species that exhibit alternative feeding strategies, such as non-native invasive *Chrysomya* species that have facultatively predaceous larvae

that feed on both the carrion and potentially competing larvae (Wells 1991, Wells and Greenberg 1992). Such intraguild predation can lead to exclusion, coexistence or alternative stable states within a community (Polis et al. 1989, Polis and Holt 1992). The intraguild prey larvae may respond in ways that enhance their persistence in the food web (Ingram et al. 2012), such as larval aggregation or dispersal on the carcass (Watson and Carlton 2005, Rosa et al. 2006).

The evolutionary consequences of intraguild predation are still unknown (Ingram et al. 2012). The success of many invasive species, such as *Chrysomya* spp., may be due to their wide diet breadth and their high reproductive, dispersal, and competitive abilities. Invasive species may also benefit more from an earlier arrival time than native species by being more apt to dominate their resource (Dickson et al. 2012, Wainwright et al. 2012). The continued range expansion and establishment of *Chrysomya* species could have a significant negative impact on many native insects that feed on carrion, ultimately disturbing native community structures and even endangering some populations (Rosati and Vanlaerhoven 2007). Much research has gone into studying various *Chrysomya* species, including *Chrysomya rufifacies* (Macquart), *C. albiceps* (Wiedemann), *C. megacephala* (Fabricius), *C. chloropyga* (Wied.) and *C. putatoria* (Wied.), with the presence of one or more of these species leading to a decline in numbers of ecologically similar species, including *Cochliomyia macellaria* (Fabr.) (Baumgartner and Greenberg 1984, Wells 1991, Wells and Greenberg 1992, Faria et al. 2004), *Lucilia eximia* (Wied.) (Baumgartner 1993), *Lucilia cuprina* (Wied.) (Tillyard and Seddon 1933), *Lucilia sericata* (Illingworth 1923) and *Calliphora stygia* (Fabr.) (McQuilland et al. 1983) (Rosati and VanLaerhoven 2007). By examining interactions between invasive and

native blow fly species, we can further identify the mechanisms that contribute to the stability of their coexistence.

Within communities on patchy and ephemeral resources such as carrion, complex interactions exist that can differ in strength between larval and adult stages (Kingsolver et al. 2011, McPeek and Peckarsky 1998, Paine 1992, Yodizis 1988). In most insects, immature stages have limited dispersal ability, and are strongly influenced by oviposition decisions of the parent female (Liu et al. 2012, Gripenberg et al. 2010, Von Zuben et al. 2001). Yet it is the larvae that must acquire all the nutrients required for development to the adult stage (Kvist et al. 2013). Direct and indirect larval interactions may influence adult size, reproduction, dispersal, behaviour, population dynamics and community structure (Kvist et al. 2013, Liu et al 2012, Boggs and Freeman 2005, Allen and Hunt 2001, McPeek and Peckarsky 1998, Peters 1983, Denno and Cothran 1975, Hassell 1975, Fuller 1934). Studies that elucidate both positive and negative interactions between species, particularly during the larval stages of their development, are necessary to understand the mechanisms that govern community structure. Priority effects resulting from the interactions between larvae of two or more species are important to identify as they facilitate our understanding how species successfully invade and establish within a community.

Three blow fly species were selected for study: *Lucilia sericata* (Meigen), *Phormia regina* (Meigen) and *Chrysomya rufifacies* (Macquart) (see Appendix B for species information). The first objective of this study is to quantify priority effects by introducing blow fly larvae onto piglet carcasses (*Sus scrofa domesticus* L.) either alone or first, second, or at the same time as another species. Larval performance is measured

through larval mortality rate; overall survival rate from first instar to adult; and adult size as a measure for fitness. If priority effects are not present, there would be no differences amongst treatments when a species is first, second, or together with another species (H_0). A positive priority effect ($H_{1a,b}$) would be confirmed if when one species is placed on the carrion at the same time or following another species, either experiences enhanced larval performance compared to treatments in which it is placed first or alone on the carrion resource. Conversely, a negative priority ($H_{2a,b}$) would be present in either of two species if arrival order causes a decrease in larval performance. Refer to Table 4.1 for predictions.

The second objective of this study is to investigate factors influencing coexistence. With *C. rufifacies* whose larvae are known to be a facultatively predaceous, if *C. rufifacies* does prey upon *L. sericata*, then *L. sericata* will have low larval performance in the presence of *C. rufifacies*, while *C. rufifacies* will have enhanced larval performance when *L. sericata* is present. In the case of the two native species studied, in which *P. regina* experiences positive facilitation when in the presence of *L. sericata* (see Chapter 3), *P. regina* larvae were provided with "washes" from actively feeding *L. sericata* larvae to investigate possible mechanisms underlying the positive facilitation effect. If facilitation of *P. regina* by *L. sericata* is present, then one or more wash treatments will result in higher survival and adult fitness for *P. regina*.

METHODS

Laboratory colonies of all three blow fly species were maintained under a 16L:8D diel cycle at an approximate temperature of 21°C and 50% relative humidity. Larval wash experiments were carried out in growth chambers set to the same conditions. Priority effect experiments were conducted from April 10, 2008 to April 24, 2010, in large aquaria placed in a greenhouse, that experienced ambient light cycles; the photophase varyied seasonally from 9 to 15 hrs (Time and Date AS 1995-2014: http://www.timeanddate.com/worldclock/) and mean temperature of 20.6 ± 8.05°C. Every trial included each treatment condition between each species combination (i.e. *L. sericata* and *P. regina*, and *L. sericata* and *C. rufifacies*) to ensure differences between treatments were not due to variability in greenhouse conditions (i.e. light levels, temperature, humidity, day length).

Colonies of *P. regina* and *L. sericata*, maintained since 2005, were supplemented annually with wild-type females collected from the Windsor area using King Wasp traps (www.kinghg.on.ca) baited with pork liver. Laboratory colonies of *C. rufifacies* were established from pupae collected from carcasses placed outdoors at the FLIES Facility at Texas A&M University, College Station, TX and imported to Canada. Adult flies in all source colonies were fed *ad libitum* granulated sugar, skimmed milk powder, and water in an Erlenmeyer flask closed with absorbent dental wicks. Fresh pork liver (50 g) was placed in each colony cage for egg collection and was replaced as required to obtain an adequate number of eggs (>5,000) over a period of three hours. Individual *L. sericata* larvae must consume ~0.5 g of liver to reach their optimal size (Reid 2012). When food is limited, blow fly larvae have lower mass and both pupae and adults are smaller

(Simkiss et al. 1993). Most trials in this study involved 400 larvae of one species or two species combined. The exception was the low density treatment that utilized only 200 larvae of one species. Based upon Reid's (2012) estimate for food requirements, a minimum mass of 200 g of resource should be provided for 400 larvae to ensure adequate larval nutrition. To ensure that experimental effects were due to priority effects and not competition or resource limitation, excess resource was provided through the use of whole piglet carcasses (>700 g).

Arrival Order and Larval Interactions

Frozen piglets from Robert Rivest Farms, Ltd. in Ruscom Station, ON, were removed from the freezer approximately 24 hrs prior to use, thawed and warmed to room temperature. Upon hatching, first instars were transferred to the left cheek region of piglets (*Sus scrofa domesticus* L.) using a fine-tipped paintbrush (0.5 mm) according to the treatments outlined in Table 4.1. The left cheek region was used based on its commonality as an oviposition location for all three species (see Chapter 3); the elimination of variability that would have been introduced if larvae were placed in various sites on the piglet's body; and the choice it provided larvae of nearby alternative feeding sites, including moist natural orifices (eyes, nose, ears, mouth) that larvae could reach quickly prior to desiccation.

For the two species (*"versus"*) treatments, 200 larvae of one species were transferred and allowed to feed for 24 hrs, followed by the addition of 200 larvae of species 2. For the single species ("*species only*") treatments, 400 larvae of a single species were transferred to a piglet. Low density treatments involving only 200 larvae of

a species per piglet were included to detect density effects (in "species only" treatments) and temporal effects (in two species treatments). Once larvae had been transferred, each piglet was placed in a greenhouse within a large glass aquarium filled with approximately 5cm of rearing medium (NEPCO Beta Chip wood shavings) and covered with a landscape tarp lid (Weed Barrier WPB 4006) that was sealed into place with a silicon based sealant (Project 1 6800 Series-aquarium sealant). Piglets were weighed at the beginning and end of each experiment, with the end of the experiment designated when adult flies had fully eclosed and died due to lack of water. Temperature was recorded hourly through the experiment using a datalogger (SmartButton, ACR Systems Inc.) placed in the center of the greenhouse. Pupal mortality (number of pupae from which adults failed to eclose) and emergence mortality (partially emerged or improperly formed adults) were recorded. Larval mortality was estimated by taking the number of larvae introduced in the treatment and subtracting the number of fully-formed adults, pupal mortality, and emergence mortality from the total number of larvae introduced, and then dividing by the total number of larvae introduced. Survival rate was determined by counting adults that emerged successfully and dividing by the total number of larvae placed on a piglet, yielding a value that represents the total larvae introduced – [larval death + pupal death + emergent death]. Treatments were replicated 10 times, except for the "*L. sericata* only" treatment and low-density treatments where 20 and 19 reps were carried out, respectively since these treatments were performed under the same experimental conditions for each species combination.

Mechanism of Facilitation of *L. sericata* on *P. regina*

This experiment was carried out to determine if the facilitation experienced by *P. regina* larvae in the presence of *L. sericata* larvae documented in this study is due to (i) bacteria or (ii) chemical exudates from bacteria, or actively feeding *L. sericata* larvae. Sterile and non-sterile washes were prepared from actively feeding *L. sericata* larvae and administered to feeding *P. regina* larvae. Two controls were used: untreated *P. regina* larvae as a true control and *P. regina* larvae administered water as a sham treatment to control for possible effects due to greater moisture content or rehydration of the food resource resulting from application of the experimental treatments.

Aqueous "washes" of *L. sericata* larvae were prepared as follows. Eggs of *L. sericata* were collected from adult colony cages as described previously. Upon hatching, 400 larvae were placed within each of eight 1 L Mason jars filled 1/3 with wood shavings (NEPCO Beta Chip) as a pupation medium and containing 100 g of pork liver as a food source placed on aluminum foil. Holes were punched into the foil to allow fluids to drain, thereby preventing larval drowning. Each jar was covered with a landscape tarp lid (Weed Barrier WPB 4006) for ventilation and placed within a growth chamber (Conviron Adaptis A1000IN) with a temperature of 25.0 ± 0.1°C and a relative humidity of 40 ± 1%. A diel cycle of 16L:8D was maintained. Three washes were prepared at three different points in *L. sericata* development: Wash 1 – when larvae moulted to the second instar; Wash 2 – when larvae moulted to the third instar; and Wash 3 – the mid-point during the third instar. A "wash" was prepared by pouring 50 ml of sterile, deionized water over each group of 400 feeding larvae and collecting the liquid in a 1000 ml beaker. Washes from all the *L. sericata* rearing jars were pooled, then centrifuged at

21°C and 14000 rpm for 15 minutes to separate out debris (i.e. blood cells, liver tissue, etc.). The resulting supernatent was divided in two portions, with half reserved for the sterile (ultrafiltered) wash treatment and the other half for the non-sterile wash treatment**.** The sterile wash was prepared by filtering it through a sterile vacuum filtration system with a 0.10 µm pore size polyethersulfone membrane (Nalgene* Rapid-Flow* Sterile Disposable Filter Units with PES Membrane, 250ml, 75mm diameter membrane: http://www.thermoscientific.com) to remove bacteria. The sterile wash could have contained chemicals produced by the *L. sericata* larvae and/or from bacteria associated with the liver and larvae.

Eggs of *P. regina* were collected on two dates, October 7 and November 3, 2011, by placing 35 g of pork liver in three colony cages. Liver was replaced every 3 hrs until a suitable amount of eggs (>3000) were collected over a short period of time in order to ensure uniformity in hatch times. Upon hatching, 50 larvae were placed within each rearing jar with 50 g of pork liver to provide excess food resources to eliminate competition (as stated previously, mean consumption is approximately 0.5 g/larva; Reid 2012). Ten jars were prepared for each of 4 treatments for a total of 40 jars on each start date. Treatments (10 mL per application; sterilized larval wash, unsterilized larval wash, water sham, and control) were applied to larvae three times, on Day 1 (1-day-old first instars); Day 3 (second instars; and Day 5 (third instars). The sham treatment consisted of *P. regina* larvae feeding with 10 mls of deionized water periodically added. Jars within each treatment were then divided equally between two growth chambers (Conviron Adaptis A1000IN) with a temperature of 25.0 ± 0.1°C, relative humidity of 40 ± 1% and diel cycle of 16L:8D. The developmental stage of the larvae was recorded

every 12 hrs until larvae entered the pre-pupal (wandering) stage. At this time the liver was removed and the larvae were checked every 6 hrs to for accurate recording of pupation time (in *P. regina*, wandering is reduced and pupation occurs quickly; Greenberg 1990, Nabity et al. 2006, Reid 2012, personal observations). Pupae from individual rearing jars were removed daily, placed into 100 ml Petri dishes and returned to the growth chamber until adult emergence. Temperature was recorded hourly for each chamber using a datalogger (SmartButton, ACR Systems Inc.). Larval, pupal and emergence mortality and survival rate were recorded (as described above).

The durations of several developmental "milesones" were recorded on a per jar basis: (a) egg hatching to first individual moulting to 2nd instar (i.e. minimum duration of 1st instar stage); (b) first individual moulting to 2nd instar to first individual moulting to 3rd instar (i.e. duration of 2nd instar stage); (c) first larva moulting to 3rd instar to first larva observed wandering away from food (duration of 3rd instar stage); (d) first larva observed wandering to first pupation event (i.e. duration of wandering stage); (e) first to last pupation event (i.e. period of pupation events); (f) first pupation event to first adult emergence (i.e. duration of pupation); and (g) first adult emergence to last adult emergence (i.e. period of emergence events). Twenty replications were conducted for each treatment.

Data related to development were converted to degree hours, determined each hour by subtracting the lower developmental threshold temperature (0^{o}C) from the temperature recorded by the datalogger. These values were summed over the number of hours reflected in each of the developmental variables to yield a corresponding value of accumulated degree hours (ADH). The lower developmental threshold values were set to

0ºC because they are known to vary with species, populations**,** geographic region, life stage and environmental conditions (i.e. photoperiod, fluctuating temperatures) (Warren 2006, VanLaerhoven 2008, Anderson and Warren 2011). Additionally, the lower threshold values for the populations of the blow fly species I studied have never been determined experimentally. Consequently, the conservative value of 0ºC is preferred.

Fitness Measurements

Reid (2012) determined that tibia length, thorax length and wing length were all correlated with adult fitness (R^2>0.90 for all three variables), which was measured by the number of chorionated and immature eggs present in female *L. sericata*. Consequently, I measured all three variables as proxies for fitness for both sexes when possible. Hind tibia length was measured from the point of attachment to the femur to the attachment of the basitarsus; thorax length was measured along the midline from the anterior end near the head to the posterior end of the scutellum; and wing length was measured from the distal margin of the basicosta to the apex of the wing. Flies were placed under a compound microscope at 10X magnification and measured with an ocular scale calibrated with a stage micrometer. For arrival order experiments (i.e. using piglet carcasses), 15 male and 15 female offspring reared from each pigs were randomly selected and thorax, wing and tibia lengths of each fly were measured. For the larval wash experiment (e.g. *P. regina* larvae treated with washes from feeding *L. sericata* larvae), when possible, 10 males and 10 females were randomly selected per jar and the same body parts were measured.

Statistical Analyses

For all statistical tests, a significant effect was designated when $p<0.05$, unless a Bonferroni correction was necessary. Piglet carcass weights were examined for arrival order experiments using a bootstrapped (k=1000) univariate ANOVA due to non-normality of the data and residuals (Efron 1979, SPSS Manual V21). Residuals were normal for survival rate (Shapiro-Wilks test, $p > 0.05$) but non-normal (Shapiro-Wilks test, $p < 0.001$) for larval mortality, thus a square root transformation was applied to larval mortality to improve normality and homogeneity of variance (SPSS Manual V21). A MANOVA was used to test the effect of treatment (arrival order, high and low density) on survival rate and square root larval mortality for each species (SPSS Manual V21). The homogeneity of variances assumption was not violated, however, there were unequal sample sizes, thus Tukey-Kramer tests (Tukey 1953, Kramer 1956, SPSS Manual V21) were used *post hoc* to differentiate between treatments.

For larval wash experiments, larval mortality and survival rates were normal Shapiro-Wilks test, $p > 0.05$), thus a one-way MANOVA was used to test for growth chamber effects. There was no significant effect of rearing chambers ($p>0.05$), thus data from different chambers were pooled. A one-way MANOVA was used to test for wash treatment effects on larval mortality and survival rates. Residuals were normal and variances were equal, therefore a one tailed Dunnett's *post hoc* test (Dunnett 1955, SPSS Manual V21) was used to test for differences between control and treatment conditions for larval mortality.

With respect to fitness estimates, a linear mixed model analysis was used, with each pig considered as a replicate (SPSS Manual V21). Analyses were carried out within

each species and sex to determine if there were differences in body size due to any of the treatments. Treatments included either arrival order and density in experiments regarding arrival order, or wash treatment in experiments regarding coexistence mechanisms between *L. sericata* and *P. regina*. Estimated marginal means were compared using mean pairwise comparisons tests within each species and within each sex to differentiate between treatment effects, and a Bonferroni correction was applied to adjust for multiple hypotheses tested with a single data set (SPSS Manual V21).

For the wash experiment, treatment effects on the minimum time to moult into each developmental stage and the duration of each stage were analyzed using bootstrapped (k=1000) MANOVAs due to non-normality of the response variables and their residuals (Efron 1979, SPSS Manual V21). Growth chamber effects on development were tested in the same manner and there were no significant differences between rearing chambers ($p>0.05$), thus data were pooled. Estimated marginal means were compared using mean pairwise comparison tests within each species and within each sex to differentiate between treatment effects; a Bonferroni correction was applied.

RESULTS

The Effect of Arrival Order on Larval Interactions

Pig carcass weights did not differ between treatments ($F_{1,125}$=1.738, p = 0.072). Larval mortality and survivorship to adult varied with respect to treatment for *P. regina* and *L. sericata*, yet remained consistent for *C. rufifacies* (*P. regina:* Wilk's λ = 0.425, $F_{8, 84}$= 5.598, p < 0.0001; *L. sericata*: Wilk's λ = 0.776, $F_{14, 174}$= 3.530, p < 0.0001 and *C. rufifacies*: Wilk's λ = 0.607, $F_{8, 86}$= 1.454, p = 0.186). The presence of *L. sericata* altered *Phormia regina* larval mortality and adult survival ($F_{4, 47}$= 12.254, p < 0.0001 and $F_{4,47}$= 8.148, p < 0.0001) (see Figure 4.1a). *Phormia regina* had lower larval mortality and higher survivorship to adult in the presence of *L. sericata*, both after and simultaneously with *L. sericata*, which was evident in the lower mortality and higher survival than when *P. regina* was alone. There were no differences between *P. regina* only (400 larvae) and low density treatments (200 larvae), thus, density at the levels used in this experiment did not affect *P. regina* survival or larval mortality.

For *Chrysomya rufifacies*, rates for larval mortality and survival to adult were consistent over all treatments. Changes in arrival order or density did not result in any differences in larval mortality ($F_{4, 48}$=1.058, p = 0.389) or survival ($F_{4,48}$= 1.494, p = 0.221) (see Figure 4.1b).

Mortality for *L. sericata* varied due to treatment ($F_{7,95}$=6.323, p < 0.001), with larvae having higher mortality when they preceded or followed *C. rufifacies* than when they preceded or were introduced simultaneously with *P. regina*. There were no differences between remaining treatments (see Figure 4.1c). Arrival order changed survival to adult ($F_{7,95}$=4.486, p < 0.001) with larvae having lower survival when they

preceded or followed the arrival of *C. rufifacies* and highest survival when they preceded *P. regina*. *Lucilia sericata* experienced lower mortality when larvae followed *P. regina* and higher survival when they preceded *P. regina*. There were no significant differences among any other treatment pairs. The presence of the predator *C. rufifacies* lowered the survival *of L. sericata*, however, simultaneous colonization with *C. rufifacies* resulted in higher survival to adult of *L. sericata* than when it preceded or followed *C. rufifacies*. There were no differences between low-density treatments, indicating that density at the levels used in this experiment did not influence larval *L. sericata* (see Figure 4.1c).

The effect of different species combinations and larval densities on fitness measures (wing, thorax and tibia) was studied within each sex. For *Phormia regina*, treatments did not affect wing length and tibial length in females (wing: $F_{4,42.3}$= 0.691, p = 0.602; tibia: $F_{4,42.8}$= 2.378, p = 0.067) or males (wing: $F_{4,42.0}$= 0.843, p = 0.506; tibia: $F_{4,42.2}$= 1.808, p = 0.145), however, thorax length was affected (females: $F_{4,42.9}$=2.662, p = 0.045; males: $F_{4,42.2}$=3.630, p = 0.012) (see Figure 4.2). Females and males had smaller thoraces when reared alone and females had larger thoraces when preceded by *L. sericata*, demonstrating a positive priority effect due to *L. sericata*. Males were larger in low density treatments, suggesting density has some effect on fitness measures.

Chrysomya rufifacies was affected by treatment in all three fitness measures (see Figure 4.3). Adult females were larger in treatments when *L. sericata* was present (wing: $F_{4,41.0}$= 5.316, p = 0.002; thorax: $F_{4,39.7}$= 8.198, p<0.001 and tibia: $F_{4,36.2}$= 11.436, p < 0.001). Males were also larger, however, they had largest wings and thoraces when second and together, intermediate when first and in low density conditions, and smallest when alone (wing: $F_{4,43.7}$= 4.286, p = 0.005; thorax: $F_{4,42.2}$= 5.703, p <0 .001). Males had

largest tibiae when *L. sericata* was present ($F_{4,43.8}$= 10.162, $p < 0.001$). In summary, *Chrysomya rufifacies* males and females had higher fitness when *L. sericata* was present, regardless of arrival order. There were no differences between *C. rufifacies* when alone (higher density) and low-density treatments, indicating that density did not affect body size in this species (see Figure 4.3).

Effects of arrival order were present in *L. sericata* females for all three fitness measures, while males were only affected in tibial length (see Figure 4.4). Females had larger wings when alone, smaller when introduced after *P. regina*, and no differences amongst remaining treatments. Females had longer thoraces when alone or in low density conditions and smallest when together with *P. regina*, with no differences between remaining treatments. The presence of *C. rufifacies* had positive effects on *L. sericata*, resulting in increased tibia length in both sexes. Priority effects were evident between *L. sericata* and *P. regina*, with males and females having smaller tibiae when *L. sericata* followed or was introduced simultaneously with *P. regina*. There were no density effects in *L. sericata*, with females and males being larger when alone.

Phormia regina exerted a negative effect on *L. sericata*, with *L. sericata* being smaller when reared in the presence of *P. regina*. However, this negative effect was lessened if *L. sericata* was first in arrival order, demonstrating that priority effects are present for *L. sericata*. *Lucilia sericata*, in turn, had a positive effect on *P. regina*, with *P. regina* adults being larger when reared in the presence of *L. sericata*. Although this benefit was present whenever *L. sericata* was present, these benefits were greater when *P. regina* was introduced simultaneously or after introduction of *L. sericata*; this also demonstrates a priority effect for *P. regina*. When examining interactions between *L.*

sericata and *C. rufifacies*, there was a positive effect on surviving adult size with *L. sericata* having larger tibiae when in the presence of *C. rufifacies*, regardless of arrival order. *Chrysomya rufifacies* was larger in all treatments where an additional species was present, and was smaller when it was alone, regardless of initial population densities. This indicates priority effects do not exist with respect to adult size between these two species.

Lucilia sericata Larval Wash Experiment

Data between growth chambers were pooled, due to lack of differences between chambers with respect to larval mortality or survival rates (Wilk's $\lambda = 0.972$, $F_{2,75} = 1.099$, $p = 0.339$) or development (minimum ADH: Wilk's $\lambda = 0.928$, $F_{5,74} = 1.151$, $p = 0.341$; duration of stages: Wilk's $\lambda = 0.890$, $F_{6,73} = 1.510$, $p = 0.187$).

For *P.* regina, survival to adult was the same over all treatments ($F_{3,79} = 1.568$, $p = 0.204$) (see Figure 4.5). Larval mortality differed, ($F_{3,79} = 2.966$, $p = 0.037$), with lower mortality rates when the wash was administered (see Figure 4.5).

The administration of larval washes from *L. sericata* (sterile and non-sterile) affected *P. regina* larval development (Wilk's $\lambda = 0.609$, $F_{15,199} = 2.608$, $p = 0.001$). The presence of the wash enhanced larval development of *P. regina* as evidenced by lowered minimum ADH required to reach the 2nd instar ($F_{3,79} = 4.039$, $p = 0.010$), 3rd instar ($F_{3,79} = 8.178$, $p<0.0001$), prepupal ($F_{3,79}=5.527$, $p = 0.002$) and pupal stages ($F_{3,79}=6.368$, $p = 0.001$). There were no differences between water and filtered wash treatments in the minimum ADH for pupal duration. These effects were transient, with no differences in the minimum ADH required for emergence for any of the treatment conditions ($F_{3,79}=$

1.286, p = 0.285) (see Figure 4.6). The administration of water did not affect larval development relative to the controls (p>0.05).

The application of the larval washes affected the duration of *P. regina* larval development (Wilk's $\lambda = 0.635$, $F_{18,201} = 1.948$, $p = 0.014$), particularly during the early stages (first instar: $F_{3,79} = 3.898$, $p = 0.012$; second instar: $F_{3,79} = 9.136$, $p < 0.001$). First instar *P. regina* larvae developed faster in the unfiltered and sterile wash treatments than the control treatments, however, the water treatment was similar to the filtered wash (see Figure 4.6). Larvae also had faster development during the second instar stage in the wash treatments (sterile and non-sterile) than both water and control conditions. These effects were not evident in the later developmental stages (third instar: $F_{3,79}=1.631$, $p = 0.189$; wandering: $F_{3,79}=1.811$, $p = 0.152$; pupation: $F_{3,79}=0.782$, $p = 0.508$ and emergence: $F_{3,79}=0.482$, $p = 0.695$).

Administration of the larval wash led to an increase in size of adults of both sexes of *P. regina* (see Figure 4.7). Treatments comparisons were made within each sex. For both sexes, tibiae were significantly longer in the sterile and unfiltered washes than the control and water treatments (males: $F_{3,39.4} = 7.582$, $p<0.001$; females: $F_{3,38.8} = 6.538$, $p = 0.001$). Similar responses were observed for thorax length (males: $F_{3,37.3} = 21.143$, $p<0.001$; females: $F_{3,38.0} = 16.079$, $p<0.001$, with both sexes having longer thoraces in the sterile and non-sterile washes. Wing length also differed between treatments (males: $F_{3,39.4} = 7.582$, $p<0.001$; females: $F_{3,38.8} = 6.538$, $p = 0.001$); females and males both had longer wings in the treatment receiving the sterile wash, smaller wings in the water (females) and control (males) treatments with adults from the unfiltered wash having intermediate sized wings.

Behavioural Observations

In control and water sham jars, when *P. regina* larvae reached the second instar, the majority of larvae (~ 90%) migrated away from the food source into the surrounding medium, residing under or adjacent to the resource for 1-2 days, after which they would return to the resource and continue feeding. When sterile or unfiltered washes, were administered the larvae did not exhibit this behaviour. Rather, they continued to feed on the resource and only left when they no longer required food and wandered away in search of a pupation site. Also, larvae in the jars that received sterile and unfiltered washes produced a foam-like substance during feeding. This was first observed during the second instar and continued into the third instar. This "foaming", if present in the control and water treatments, was not observed until the mid-to-late third instar.

DISCUSSION

Effect of Density

In my study, there were no differences between low density treatments (200 individuals per piglet carcass) compared to when species were alone (400 individuals per piglet) in either *Chrysomya rufifacies* or *Lucilia sericata*. A slight density effect was detected in *Phormia regina*, but only in male size, with males being larger in low density treatments. Density had no effect on male survival or larval mortality, or female size, survival or larval mortality. Density is frequently an important factor to consider in blow fly studies (Mackerras 1933, Goodbrood and Goff 1990, Simkiss et al. 1993, Marchenko 2001, Slone and Gruner 2007, Shiao and Yeh 2008, Reid 2012). In *C. rufifacies*, high densities during the larval stage can decrease development time (if > 600 larvae per 60 g of resource), decrease adult size (if > 320 larvae per 60 g resource) and increase mortality (if > 160 larvae per 60 g of resource) (Shaio and Yeh 2008). Since the densities used in this experiment were low (maximum 400 larvae) relative to resource amount (piglet carcass weights > 700 g), competitive effects were largely eliminated.

Larval Interactions Between Native Species – *L. sericata* and *P. regina*

This study identified both positive and negative interactions between these two native species. Previous studies between the native species *L. sericata* and *P. regina* determined that larvae that consumed the same resource did not coexist due to high levels of interspecific competition that led to the elimination of *P. regina* and the dominance of *L. sericata* (Hutton and Wasti 1980). My study contradicts those results: *P. regina* benefitted from the presence of *L. sericata*, with higher larval survival and larger adults.

However, given the large amount of resources in excess of larval requirements, my results indicate that the decrease in adult size was due to inhibitory effects exerted by *P. regina* rather than direct competition. Other research has demonstrated that *L. sericata* experiences negative effects of competition within highly diverse communities, with smaller adults emerging from these situations (Fuller 1934, Smith and Wall 1997, Lang et al. 2006). Therefore, positive and negative influences have both been identified between these two native species and further study is needed to determine the true nature of coexistence between these species. Coexistence between species may be due to spatially and temporally divergent strategies in resource exploitation (Denno and Cothran 1975).

Differences in arrival order can alter these positive and negative interactions within a community. My experiments determined that when *L. sericata* arrived early, the development of the larvae and size of the adults was enhanced relative to when they were reared simultaneously or after the arrival of a second species. This strategy of rapid detection and orientation to resources has been recognized as an important mechanism structuring carrion communities (Hutchinson 1965, Beaver 1977, Kneidel 1983). A species can inhibit further invasion of a patchy resource through early arrival and colonization (Levin 1974, Sale 1977, Kneidel 1983, Shorrocks and Bingley 1994). Alternatively, it may exert a competitive advantage over later-arriving species through the consumption and depletion of the resource (Hanski and Kuusela 1977). Differences in arrival order can determine community patterns, with priority effects in some cases allowing a species to persist within a community even it follows a suboptimal arrival order (Shorrocks and Bingley 1994). The presence of *L. sericata* led to increased survival and adult fitness for *P. regina*, especially when *L. sericata* larvae established on

the resource prior to *P. regina*, leading to the conclusion that *L. sericata* exerted a positive priority effect on *P. regina*. *Lucilia sericata* had reduced survival and adult size in the presence of *P. regina*, a negative priority effect of *P. regina* on *L. sericata*; however, these effects were minimized when *L. sericata* larvae were placed on the carcass a day before the *P. regina* larvae were added.

A potential mechanism for the facilitory effects of *L. sericata* on *P. regina* was demonstrated with *P. regina* larvae having lower mortality, shorter development time during the early instar stages and higher adult fitness when administered washes from actively feeding *L. sericata* larvae. The larval wash experiments confirmed that one or more compounds, possibly proteins, produced by feeding *L. sericata* larvae confer benefits on co-occurring *P. regina* larvae. These benefits include more rapid development of second instars, an overall increase in adult size (fitness), and more continuous contact time with the resource. During the first and early second instar stages, blow fly larvae can only take in liquefied food, as their mouthparts are not adapted for mastication (Guyenot 1907, Hobson 1931). It has been suggested that the liquefaction of tissue by maggots is due to presence of pepsin-based enzymes (Hobson 1931), however this has not been proven experimentally. My results are consistent with that hypothesis, indicating that *L. sericata* larvae secrete compounds that break down the resource and make feeding by *P. regina* more efficient.

Alternatively, it has been proposed that this facilitation may be bacterial in nature (Hobson 1931). The sterile wash used in this experiment helps to differentiate between these two mechanisms. When bacteria were removed by filtration (the sterilized wash), *P. regina* larvae had greater fitness than in the unfiltered wash, indicating that the

presence of bacteria diminished the benefits to *P. regina* (compared to the non-sterile wash). This reduction in benefits may result from resource competition between the bacterial community (on the carcass or associated with *L. sericata* larvae) and *P. regina* larvae. *Lucilia sericata* along with other blow fly species are known to produce antibacterial compounds that may reduce bacterial populations and decrease competition between bacteria residing on and consuming the resource, thereby allowing the resource to be available to fly larvae for a longer period of time (Mumcuoglu et al. 2001).

I conclude from this experiment that a facilitory compound(s) caused an increase in *Phormia* fly size (fitness) and more rapid second instar development. The compound(s) could be antimicrobial in nature and, when present, may act to reduce resource competition between *P. regina* and bacterial communities. Bacterial cues play important but still poorly understood roles within the blow fly community (Esser 1990, Ashworth and Wall 1994, Vogt and Woodburn 1994, Mumcuoglu et al. 2001, Mahon et al. 2004, Ahmad et al. 2006, Tomberlin et al. 2012, Davis et al. 2013) and other carrion insects (Hollis et al. 1985, Burkepile et al. 2006, Lam et al. 2007, Rozen et al. 2008). However, experimental results are conflicting depending on the insect species studied and the variables quantified. Isolation of the facilitory compound(s) associated with *L. sericata* washes would help to clarify the role that bacteria, facilitory compounds and/or anti-microbial compounds have in this carrion systems and may contribute to the broader understanding of coexistence in blow flies.

Phormia regina and *L. sericata* are broadly sympatric species (Chapter 2) with a long history of interactions. The results from my experiments on adult priority effects support the idea they have evolved mechanisms that promote their coexistence. For

example, blow flies have minimum nutritional and developmental thresholds that need to be met in order to successfully develop and reproduce (Levot et al. 1979, Tarone et al. 2011). By evolving the ability to utilize a compound(s) produced by *L. sericata*, as discussed in the previous paragraph, *P. regina* larvae could more quickly meet their minimum nutritional requirements and complete development, thus contributing to its persistence within the blow fly community. Conversely, *L. sericata* appears to gain an advantage over *P. regina* by colonizing food resources more quickly. Added to these interactions, many insects are known to respond to larval hardships by having smaller adult size (Honek 1993, D'Amico et al. 2001, Chown and Gaston 2010). Ullyett (1950) examined competition in blow fly populations and demonstrated that *L. sericata* larvae persisted within the community due to their ability to persist despite a strong reduction in adult size. There is a large amount of behavioural and genetic plasticity within *L. sericata* populations (Gallagher et al. 2010, Picard and Wells 2010, Tarone et al. 2011), which also may play an important role in its ability to persist within the carrion insect community under harsh and very competitive circumstances.

Larval Interactions Between Non-Native Species – *L. sericata* and *C. rufifacies*

My experiments on the interactions between *L. sericata* and *C. rufifacies* demonstrated that *C. rufifacies* benefited from the presence of *L. sericata*, regardless of arrival order. The consistently greater size and lower mortality of *C. rufifacies* regardless of the presence of *L. sericata* indicate that priority effects in this two-species system are unimportant for larvae of *C. rufifacies*. *Lucilia sericata* did experience a priority effect with *C. rufifacies*. Although *L. sericata* was negatively affected by the presence of *C.*

rufifacies through higher larval mortality and decreased survival, these effects were minimized when *L. sericata* was introduced simultaneously with *C. rufifacies*, in which case adult size was not negatively affected.

Chrysomya rufifacies has the ability to become a facultative predator during the second and third larval instars, feeding upon other insect larvae in the system as well as on the resource itself. When *L. sericata* colonizes after *C. rufifacies*, it completes its entire development while *C. rufifacies* is in its predatory stages (second and third instar) and consequently is under strong selection to evolve adaptations that enhance its survival. Despite a decrease in the survival of *L. sericata* when *C. rufifacies* is present, these negative effects are reduced when it establishes simultaneously with *C. rufifacies*. Reid (2012) examined developmental rates in communities consisting of *L. sericata* and *C. rufifacies* and determined that although *L. sericata* spent more time in the wandering stage and had slower overall development, it spent less time than *C. rufifacies* in the first, second and third instar stages. Intraguild predation, as seen in *C. rufifacies*, may lead to evolutionary responses in prey species (Palkovachs and Post 2009, Post and Palkovachs 2009, Schoener 2011, Ingram et al. 2012). The rapid larval development of *L. sericata*, its extended wandering stage (Greenberg 1990, Tarone et al. 2011, Reid 2012, personal observations), and its ability to successfully complete development at much reduced size (Fuller 1934, Lang et al. 2006, Tarone et al. 2011) are all pre-adaptations that enhance their ability to escape predation and achieve a moderate overall fitness when larvae become established simultaneously with *C. rufifacies*. The selection pressures exerted by *Chrysomya* may result in further refinements of these and other adaptations that enhance coexistence.

As stated previously, my results support the idea that the sympatric species, *P. regina* and *L. sericata*, have evolved mechanisms that promote their coexistence. However, *C. rufifacies*, an Australasian species, does not share this history. *Chrysomya rufifacies* is known to induce early wandering in multiple blow fly species in North America (Watson and Carlton 2005, Shiao and Yeh 2008, Swiger et al. 2014), a behaviour that involves trade-offs between survival, risk of predation and offspring size. This species is also an aggressive, facultative predator (Wells 1991, Wells and Greenberg 1992, Baumgartner 1993, Wells and Kurahashi 1997, Flores 2013), and larval populations develop faster and emergent adults do better in the presence of prey species (Ulyett 1950, Shiao and Yeh 2008, Reid 2012, Flores 2013). Because blow flies have minimum nutritional and developmental thresholds (Levot et al. 1979, Tarone et al. 2011), omnivory may provide *C. rufifacies* with higher nutrient quality through their prey. This could result in decreased developmental duration by meeting the minimum nutritional threshold for pupation earlier or larger larvae when the maximum developmental threshold for pupation is reached. Determining the factors that contribute to these thresholds, the details of how species interactions influence them, and their fitness consequences of these interactions is important in the application of blow fly development to estimation of periods of insect activity in forensic entomology.

Chrysomya rufifacies is one of many exotic *Chrysomya* species that have invaded North America over the past 30 years. Within their native range, *Chrysomya* exist within a diverse community of other calliphorid species (Baumgartner 1993, Shiao and Yeh 2008). This is not presently what is observed with North American blow flies (Illingworth 1923, Tillyard and Seddon 1933, McQuilland et al. 1983, Wells 1991, Wells

and Greenberg 1992, Baumgartner 1993, Wells and Kurahashi 1997, Flores 2013, Swiger et al. 2014). The presence of one or more exotic *Chrysomya* species can alter the mechanism(s) responsible for coexistence between native blow flies (Ullyett 1950, Faria et al. 2004, Rosa et al. 2006) and has been associated with a decline in populations of ecologically similar species (Rosati and VanLaerhoven 2007, Swiger et al. 2014). Given that *C. rufifacies* produces unisexual progeny within a clutch, it should be a poor colonizer (Wells 1991); however, its aggressive larval interactions, predatory behaviour and repeated introduction to North America through anthropogenic forces have influenced the establishment and continued range expansion of this and other invasive species (Wells 1991, Baumgartner 1993). Native species within a guild have some potential to resist and even inhibit invasion by introduced species in the same guild (Fargione et al. 2003). In the case of *C. rufifacies*, its facultative predation on larvae of other blow fly species may enhance its ability to overcome the resistance to invasion provided by *L. sericata* and other members within sarcosaprophytic guild. Compensatory mechanisms within prey species have been documented in another native species: later arrival in *Cochliomyia macellaria* led to increased survival of its larvae in the presence of *C. rufifacies* larvae (Flores 2013). Consumptive effects (through direct interactions) and non-consumptive effects (through indirect interactions) caused by generalist and invasive predators can alter patterns of coexistence, invasion resistance, distribution within the landscape and population interactions (Orrock et al. 2008). This topic should be examined further through the study of multiple blow fly species at various densities on limited resources (animal carcasses) to determine the mechanisms that exist within the sarcosaprophytic guild that increase resistance to invasions.

Conclusion and Future Directions

The experiments outlined in this chapter examined larval interactions of three calliphorid species feeding on carrion, specifically piglet carcasses. There were species-specific differences in arrival order on larval interactions, mortality and survival as well as fitness effects on adults. Positive and negative priority effects were present over different spatial and temporal scales, allowing me to conclude that priority effects are important in the assembly of blow flies. Due to the inconsistency of effects over spatial and temporal scales, and given that both facilitatory and inhibitory mechanisms are present in blow flies, research needs to be directed towards further understanding priority effects within the carrion community, particularly when species interactions and priority effects could lead to changes in larval behaviour and development. Factors that influence larval development need to be identified and evaluated, and their effects determined as they could directly influence the interpretation of data collected by forensic scientists to calculate periods of insect activity (Tomberlin et al. 2011). Further studies examining the direct and indirect effects of various species combinations and arrival orders will provide much needed support and validation for the use of blow flies in estimating the period of insect activity in forensic investigations.

In my experiment, when *L. sericata* was the only species present, larvae aggregated in protected regions of the carcass such as the head or internal body cavities. However, when *C. rufifacies* was present, *L. sericata* larvae experienced high levels of predation from *C. rufifacies*. In this situation the larvae did not aggregate to the same degree, were located away from sites on the head commonly occupied by *C. rufifacies*, were present in smaller patches and dispersed over multiple locations over the carcass,

and began to wander earlier. My use of full piglet carcasses provided larvae with a large total surface area and volume, thus allowing for peripheral pockets to which some *L. sericata* could move, aggregate and avoid predation. Bartholo de Andrade et al. (2002) documented similar behavioural response of *C. macellaria* larvae in the presence of predatory *Chrysomya albiceps.* The increased adult size of *L. sericata* in the presence of *C. rufifacies* probably reflects lower levels of intraspecific competition in the sites on the carcass to which they moved. Given that localized aggregation can promote coexistence, the disruption of aggregation may have consequences that cascade through subsequent trophic levels (Finke and Denno 2006). Detailed studies on spatial aggregation of the species interacting on carrion are lacking, though it is recognized important to consider in carrion and other ephemerally based resources (Atkinson and Shorrocks 1981, 1984, Atkinson 1985, Ives 1988 1991, Kouki and Hanski 1995, Barton et al. 2013, Fiene et al. 2014). Research measuring the distribution of predator and prey populations within the resource and extending this to examine patterns between resources could provide insight into how aggregation and species interactions effect changes within blow fly and carrion communities.

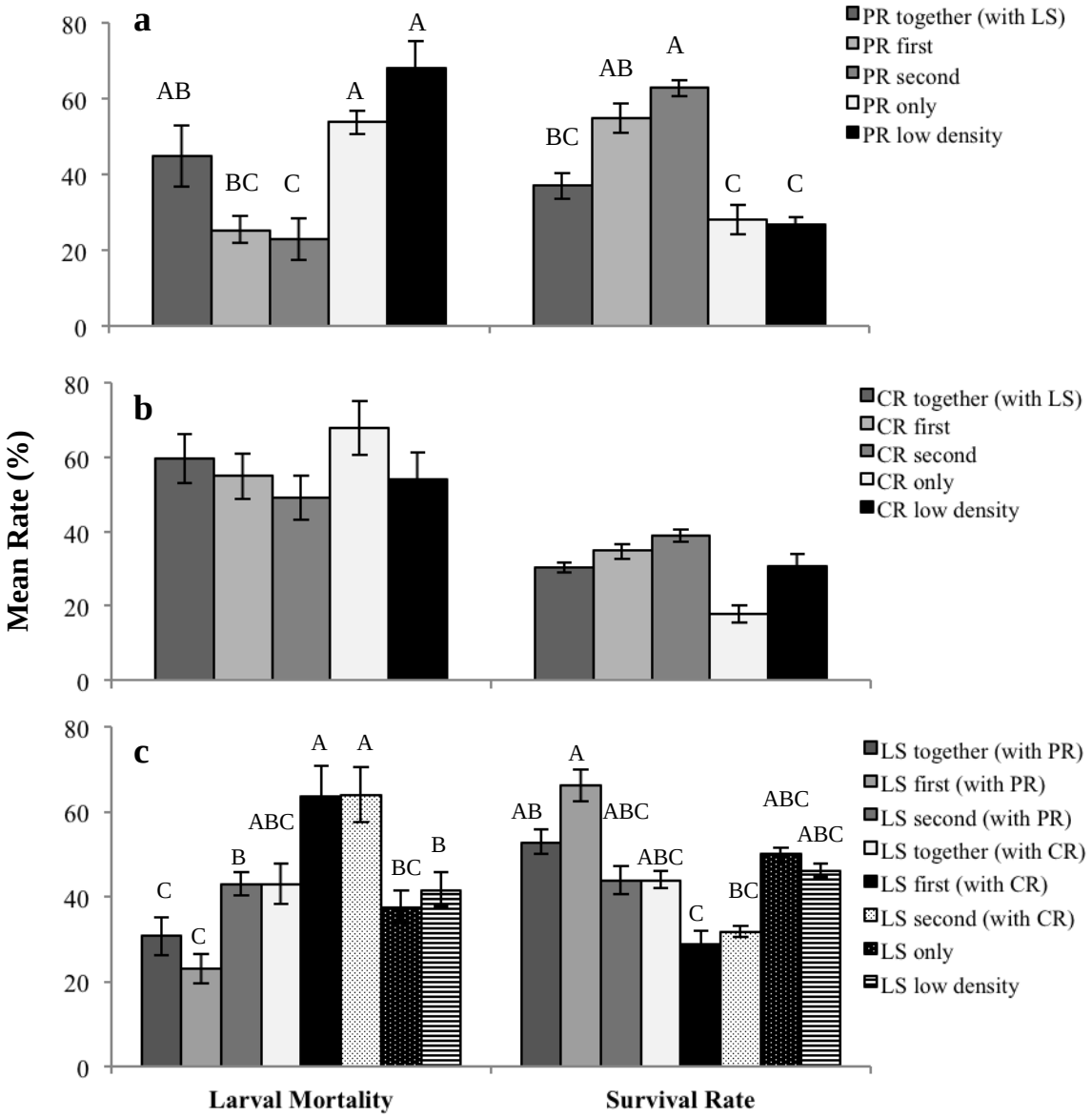

Figure 4.1. Mean larval mortality and survival rates (% ± SE) for three blow fly species, *Phormia regina* (Meigen) (PR, fig. a), *Chrysomya rufifacies* (Macquart) (CR, fig. b) and *Lucilia sericata* (Meigen) (LS, fig. c) for various arrival orders (first, second, together) and species compositions (*L. sericata* and *P. regina*, *L. sericata* and *C. rufifacies*, with two larval densities). Larvae were placed and reared on piglet carcasses (*Sus scrofa domesticus* Linnaeus). A MANOVA was used to test for effects of treatment on larval mortality and survival and rates were compared across treatments within each species using a Tukey-Kramer test for unequal sample sizes with an overall $p \leq 0.05$ significance level. Means with different letters indicate significant differences between treatments. There was an effect of treatment on larval mortality of *P. regina* and *L. sericata* ($p < 0.05$). There was no significant effect of treatment on survival rate or larval mortality of *C. rufifacies* ($p > 0.05$).

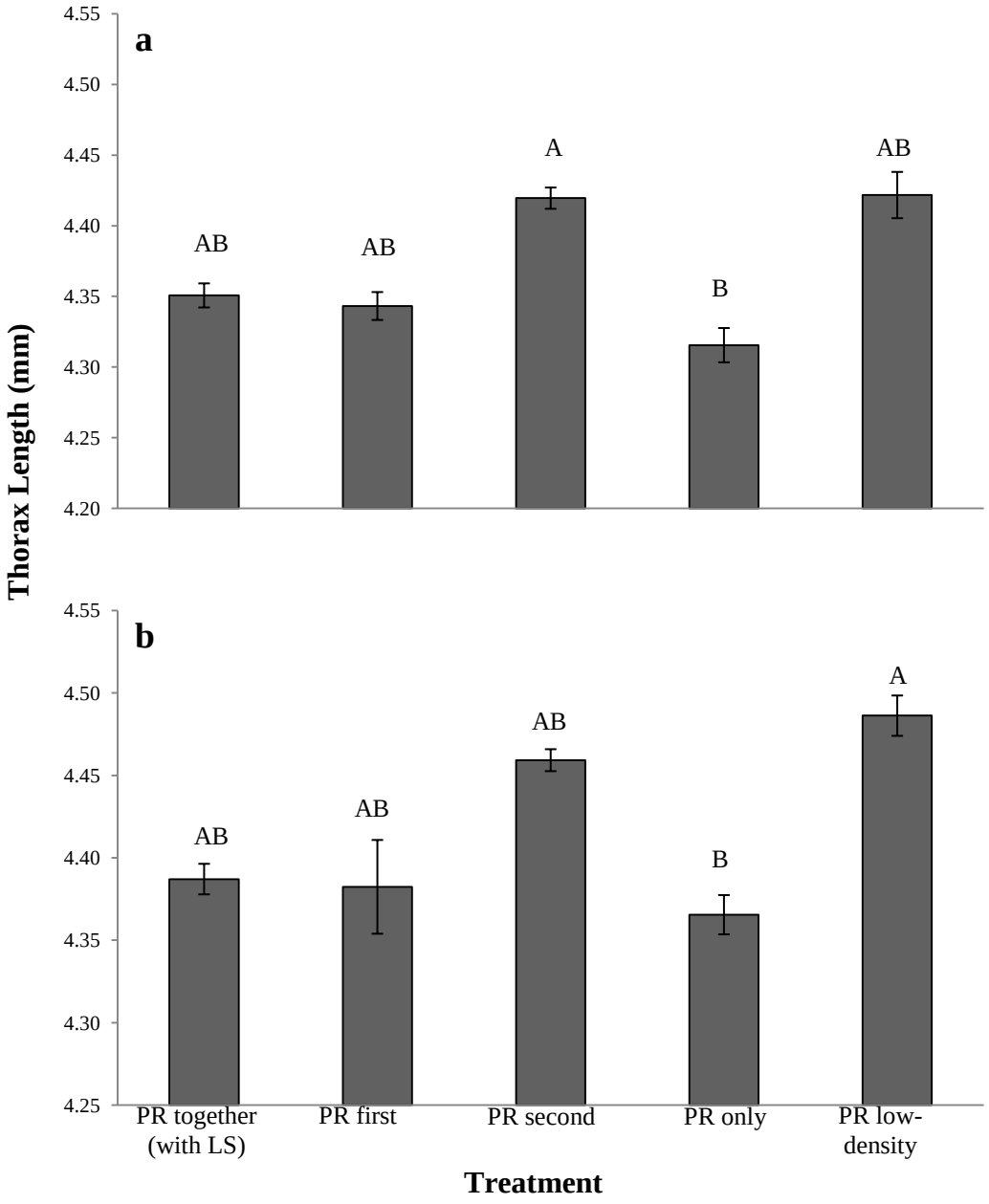

Figure 4.2. Mean thorax length (mm) ± SE of *Phormia regina* (PR) adult females and males for different arrival orders (together, first, and second with *L. sericata*) and density (400 larvae and 200 larvae per piglet). Comparisons were made within a sex and between treatments. A mixed linear model was used to test for main treatment effects ($p \leq 0.05$). Pairwise comparisons tests with a Bonferroni correction were used to test for significant differences among treatment means while maintaining an overall p-value of 0.05. Means with the same letter did not differ. There were no significant differences between treatments in male or female tibia or wing length, thus only thorax length is presented. **a** – females. **b** – males.

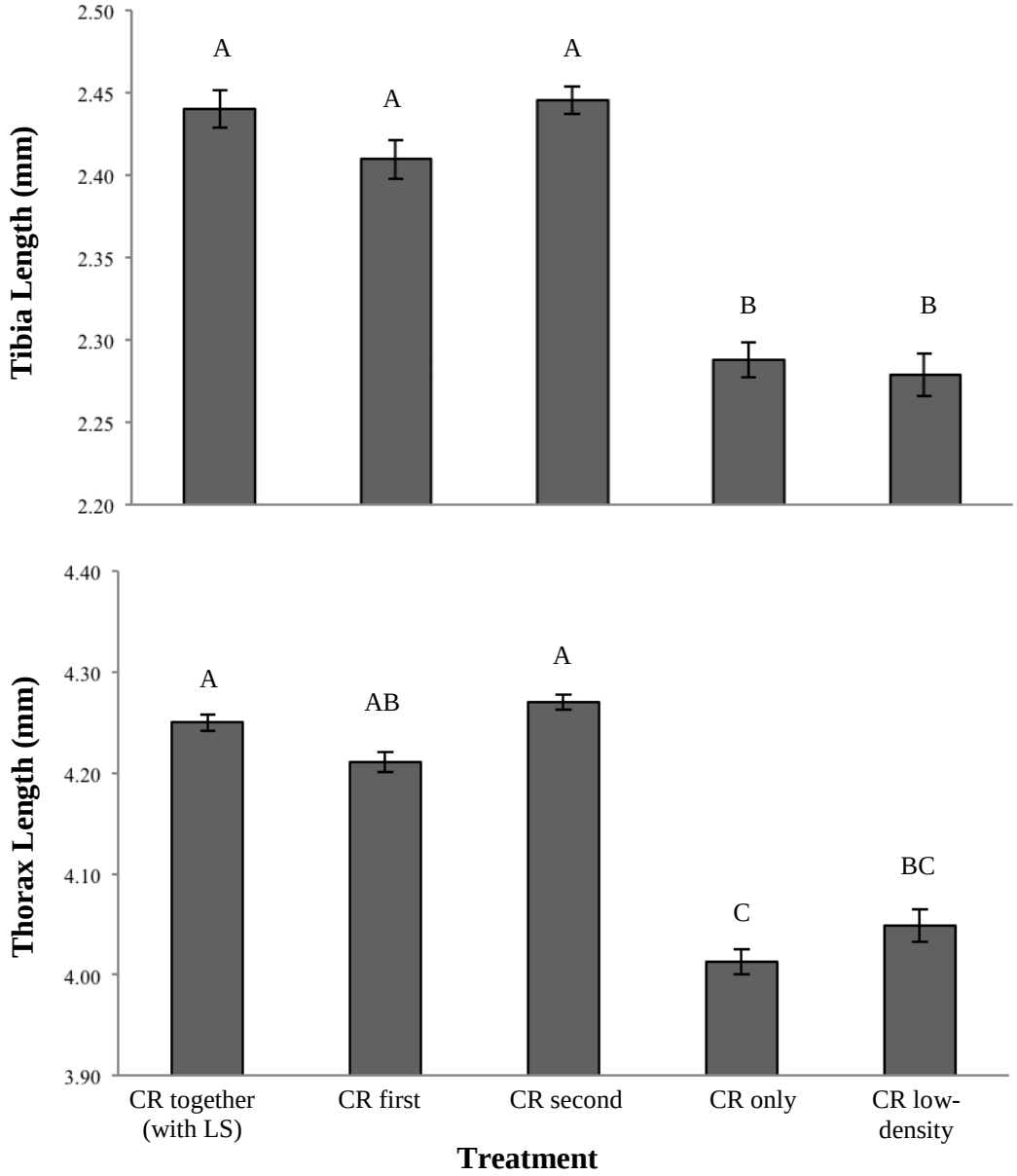

Figure 4.3. Mean tibia and thorax (mm) $\pm$ SE of *Chrysomya rufifacies* (CR) adult females for different arrival orders (together, first, and second with *L. sericata*) and density (400 larvae and 200 larvae per piglet). Comparisons were made within a sex and between treatments. A mixed linear model was used to test for main treatment effects ($p \leq 0.05$). Pairwise comparisons tests with a Bonferroni correction were used to test for significant differences among treatment means while maintaining an overall p-value of 0.05. Means with the same letter did not differ. Treatment effects for tibia, thorax and wing length were similar for males and females, thus only female data is presented. Wing length and tibia length for females were similar, thus only tibia length is presented.

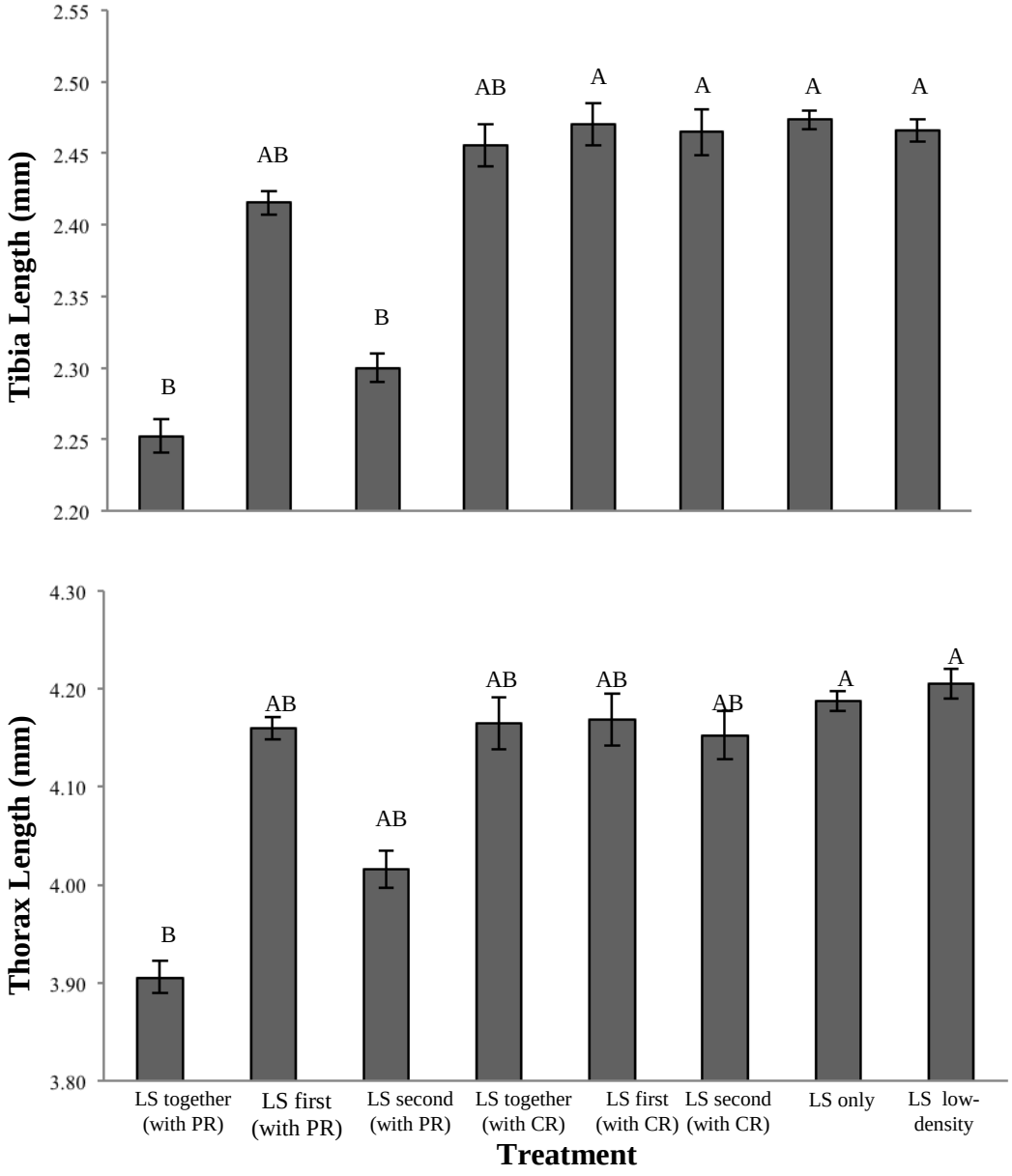

Figure 4.4. Mean thorax and tibia length (mm) ± SE of *Lucilia sericata* (LS) females for different arrival orders (together, first, and second with *L. sericata*) and density (400 larvae and 200 larvae per piglet). Comparisons were made within a sex and between treatments. A mixed linear model was used to test for main treatment effects ($p \leq 0.05$). Pairwise comparisons tests with a Bonferroni correction were used to test for significant differences among treatment means while maintaining an overall p-value of 0.05. Means with the same letter did not differ. There were no significant differences between treatments for male thorax or wing length. Treatment effects for tibia length were similar for males and females, thus only female data is presented. Wing length and thorax length for females were similar, thus only thorax length is presented.

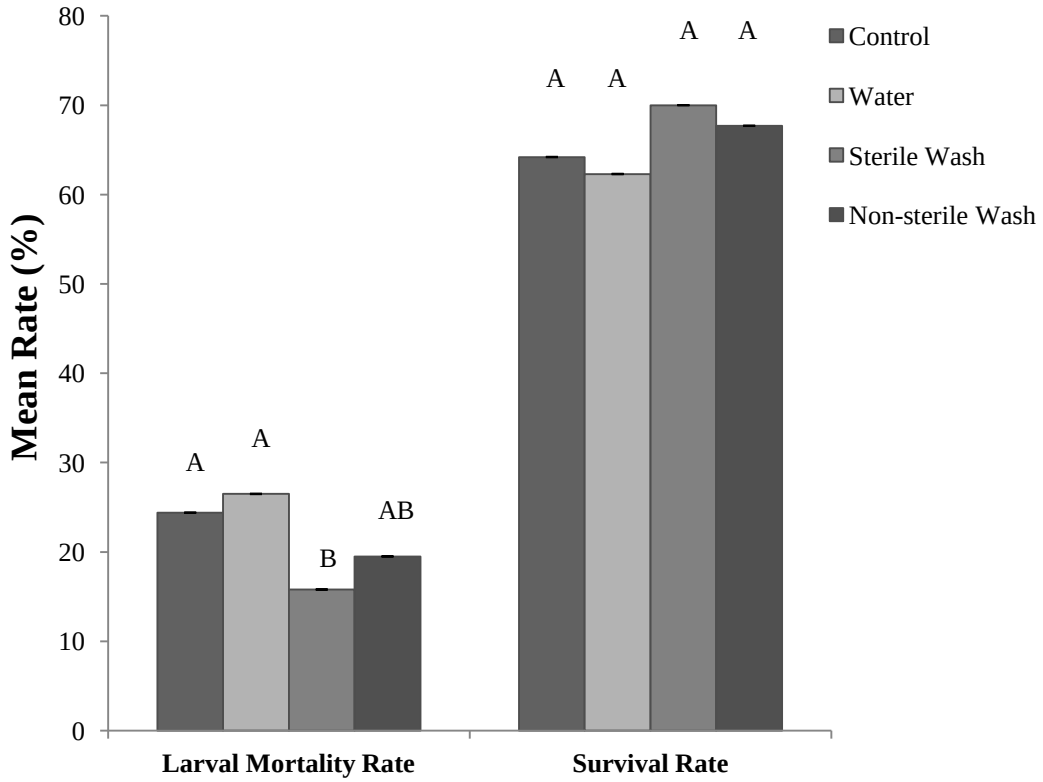

Figure 4.5. Effect of *L. sericata* larval wash on *P. regina* larval mortality and survival (mean % ± SE). A MANOVA was used to test for treatment effects and one-tailed Dunnett's tests (<control) were used to determine differences between treatments and controls. Means with different letters denote a significant difference ($p \leq 0.05$). A – denotes significantly higher. Experiments were carried out at 25.0 ± 0.5°C and 40 ± 1.0% relative humidity.

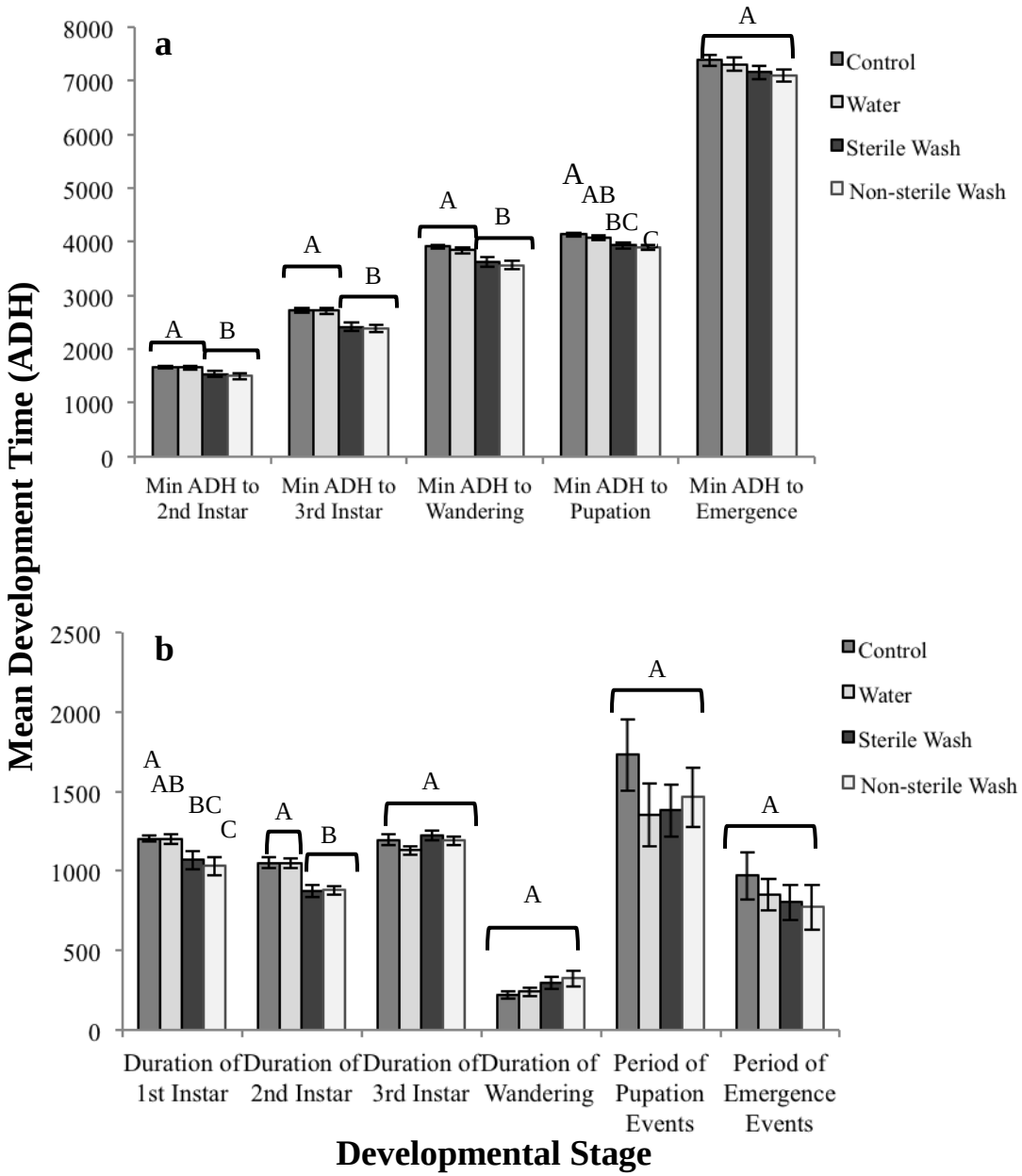

Figure 4.6. **a** – Effect of *L. sericata* larval wash on *P. regina* larvae mean minimum ADH ± SE to reach developmental stages. **b** – Effect of *L. sericata* larval wash on ADH for each larval stage (mean ± SE). Duration in each stage was measured from the first individual reaching the stage until the last individual leaving the stage. A bootstrapped (k=1000) MANOVA was used to test for effects of treatment and bootstrapped pairwise comparison tests with a Bonferroni correction were used to test for significant differences among treatments while maintaining an overall p value of 0.05. Means with the same letter did not differ. A minimum developmental threshold of 0°C was used in ADH calculations.

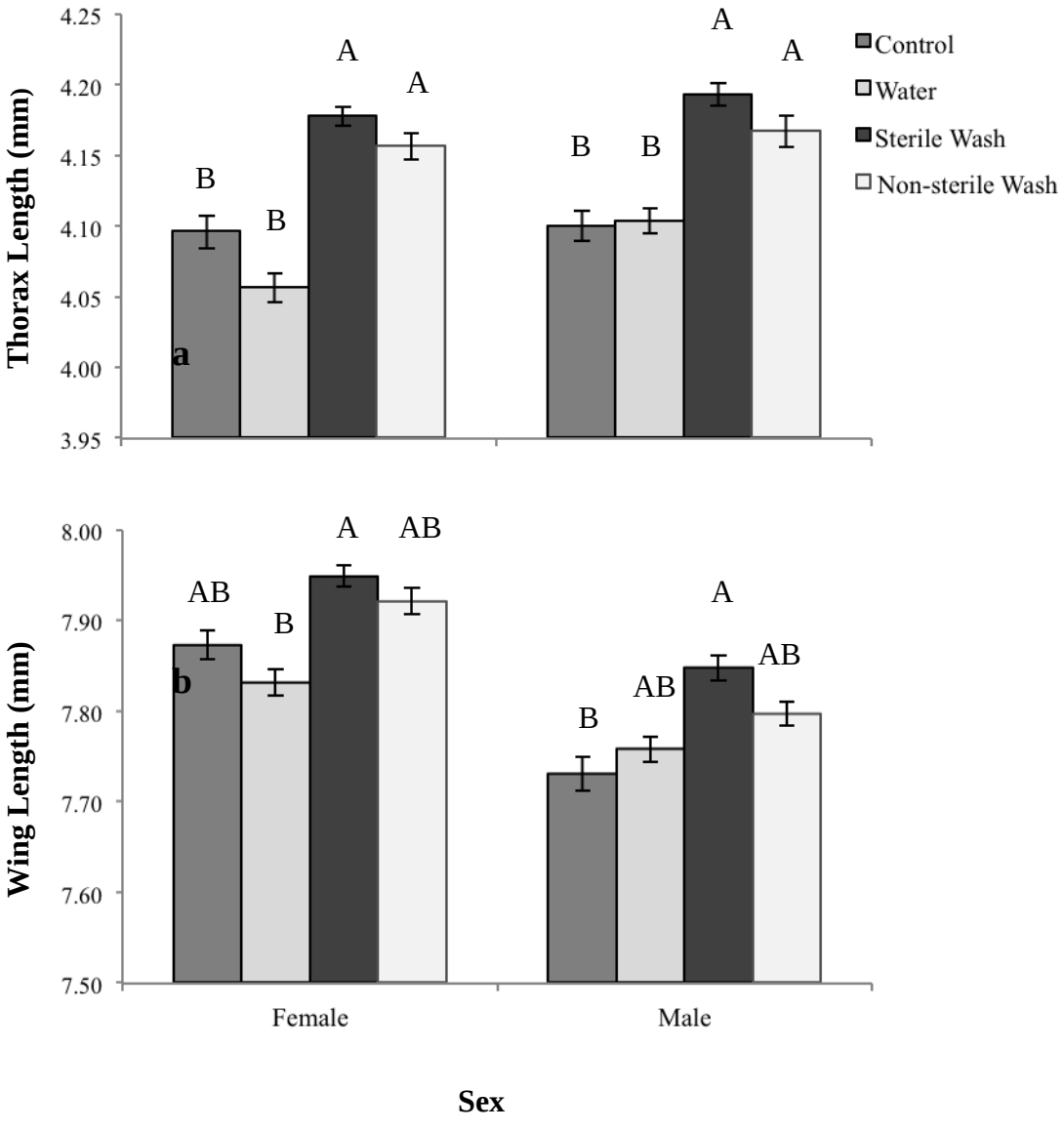

Figure 4.7. Mean thorax and wing length (mm) ± SE of *Phormia regina* adult females and males for treatments with (sterile or unfiltered) or without (control or water) *Lucilia sericata* larval wash. There was a significant effect of sex on size, thus comparisons were made within each sex and between treatments. A mixed linear model was used with a significance level of $p \leq 0.05$ to test for significant effects of treatment and pairwise comparison tests with a Bonferroni correction were used to test for significant differences among treatments while maintaining an overall p value of 0.05. Means with the same letter did not differ. Treatment effects for mean tibia length were similar to thorax length, therefore, only thorax length (**a**) and wing length (**b**) are presented.

Table 4.1: Hypotheses and predicted outcomes for experiments testing the effect of arrival order on larval development of *Lucilia sericata* (Meigen) and *Phormia regina* (Meigen). H_1 represents positive priority effects, H_2 represents negative priority effects that were tested against the null hypothesis (H_{null}). Treatment conditions consist of larvae developing alone (*L. sericata* only, *P. regina* only), one species developing first, followed by the second species (*L. sericata* first followed by *P. regina* in the *L. sericata* vs. *P. regina* treatment, and vice versa for the *P. regina* vs. *L. sericata* treatment) or larvae developing simultaneously *(L. sericata* and *P. regina*). Outcomes are described as high, low or increased/decreased with respect to larval performance (measured by larval mortality, survival and adult size). Outcomes for one species treatments are described in order to illustrate potential outcomes should priority effects be evident. **LS** – *Lucilia sericata*, **PR** – *Phormia regina*. *(Note – predicted outcomes for L. sericata and C. rufifacies experiments would follow a similar outline.*

Hypotheses	***L. sericata* only**	***P. regina* only**	***L. sericata* vs. *P. regina***	***P. regina* vs. *L. sericata***	***L. sericata and P. regina***
H_{null}: Neutral	high	high	LS – high PR – high	LS – high PR – high	LS – high PR – high
$H_{1a:}$+ve priority effect (LS on PR)	high	low/moderate	LS – high PR – increased	LS – high PR – low/moderate	LS – high PR – increased
H_{1b}: +ve priority effect (PR on LS)	low/moderate	high	LS – low/moderate PR – high	LS – increased PR – high	LS – increased PR – high
H_{2a}: -ve priority effect (LS on PR)	high	high	LS – high PR – decreased	LS – high PR – high	LS – high PR – decreased
$H_{2b:}$ -ve priority effect (PR on LS)	high	high	LS – high PR – high	LS – decreased PR – high	LS – decreased PR – high

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CHAPTER 5: THESIS SUMMARY: THE ROLE OF SPATIAL AND TEMPORAL PARTITIONING, PRIORITY EFFECTS AND MECHANISMS OF COEXISTENCE IN THE BLOW FLY COMMUNITY

There is a common goal in ecology: to understand the basis for community assembly patterns; to understand patterns and causes of coexistence within a resource or landscape; and to be able to predict these patterns within a given community or region (HilleRisLambers et al. 2012). Many model systems have been used to investigate mechanisms of coexistence and community assembly, ranging from plant communities (Clements 1916, Connell and Slatyer 1977, Tilman 1982, Drake 1991, Ejrnaes et al. 2006) to intertidal communities (Farrell 1991, Morin 1999) and coral reef assemblages (Sale 1980, Chesson and Warner 1981). However, there has been very little focus on carrion as a model system despite its longstanding recognition in the field of ecology as a valid tool for investigating ecological principles (Megnin 1894, Elton 1927, Whittaker 1953, Atkinson and Shorrocks 1981, Schoenly and Reid 1987, Michaud and Moreau 2009, Tomberlin et al. 2011a,b, Beasley et al. 2012, Barton et al. 2013). Communities that take years to develop, like those commonly studied in community assembly research, are limited in that important events or assembly steps that already occurred and may influence subsequent patterns can no longer be observed, despite the integral role they play in the resultant community structure (Drake 1991). Studying succession stages over a short period of time will fail to detect those priority effects. The carrion system, on the other hand, is easily replicated and manipulated, ephemeral in nature and exhibits rapid dynamics (Schoenly and Reid 1987). It can provide valuable insight into the many processes and mechanisms that underlie community assemblages (Tomberlin et al.

2011a,b, Beasley et al. 2012, Barton et al. 2013). There is a need for studies that manipulate and evaluate biotic and abiotic factors involved in coexistence and the assembly of ecological communities (HilleRisLambers et al. 2012), and successional and other ecological studies on ephemeral resources, such as carrion, can be used to investigate these processes. Carrion systems are valuable due to the many ecological interactions that are present, such as competition, priority effects, facilitation, etc., which can influence multiple levels of the community at local and regional scales.

The Calliphoridae family of flies, which comprises a large part of the sarcosaprophytic guild, was examined over three seasons (spring, summer and fall) and over two habitat types (open field and deciduous forest) to determine their effects on spatial and temporal partitioning in blow flies. Furthermore, a finer scale lab-based manipulative approach was used to examine interactions between three blow fly species, *Phormia regina* (Meigen), *Lucilia sericata* (Meigen) and *Chrysomya rufifacies* (Macquart). Interactions between two native blow fly species, *L. sericata* and *P. regina*, were examined as well as interactions between the native *L. sericata* and an invasive blow fly species, *C. rufifacies*. Experiments were conducted at the adult and larval stages, to fully evaluate species interactions at multiple life stages.

My field research focused on the role of seasonal and temporal partitioning during decomposition and how these factors affect blow fly community structure. Blow fly community indices were examined for the effects of season, habitat and carcass age. These included total number of species (S), species evenness (E), Simpson's Index of Diversity (1-D) and Standardized Niche Breadth (Ba). These indices were examined over time, which was represented by four quartiles of accumulated degree days (0-

50ADD, 50-100ADD, 100-150ADD and 150+ ADD). I uncovered distinct seasonal differences in the blow fly community (Chapter 2; see Figure 2.6, 2.9a), which supports previous research that has demonstrated that seasonal partitioning exists within the carrion community (Macleod and Donnelly 1958, Denno and Cothran 1975, Hanski and Kuusela 1980, Kneidal 1984, Wells and Greenberg 1994, Archer 2003, Archer and Elgar 2003, Brundage et al. 2011, Moretti et al. 2011, Horenstein et al. 2012, Benbow et al. 2013, Moretti and Godoy 2013). The fall season was characterized by having more species and higher levels of species evenness, diversity, and niche breadth than spring and summer. The summer blow fly community was characterized by having few species, low evenness, low diversity, and high levels of dominance, particularly by *P. regina*. Community indices did not change over time during the summer, indicating summer communities reflect a "first-come, first-served" scenario, where the number of female flies available for colonization during the first few hours post-mortem largely determines the resultant community structure. Community indices changed over time in spring, with the number of species increasing and the highest α-diversity occurring during the 3rd and 4th quartiles. However, this was accompanied by periods of species dominance, and lower evenness and diversity. Collectively this suggests that when the blow fly community is developing (i.e. 1st quartile), there may be adult and larval interactions in addition to dispersal that are important in determining community structure (Beaver 1977, De Jong 1979, Kuusela and Hanski 1982, Atkinson and Shorrocks 1984). As the community develops and larval interactions diminish as larvae leave the resource in search for a suitable pupation site, the blow fly community begins to lose its structure, which is evident in a decrease in species evenness, diversity and niche breadth.

Heterogeneity in environmental conditions allows variability in microclimatic conditions and differential resource availability, which can influence community dynamics and biodiversity (Simberloff and Wilson 1969, Levins 1979, Sulkava and Huhta 1998). Previous research regarding habitat associations of blow flies is conflicting, with some studies concluding that habitat preferences exist (Smith 1986, Greenberg 1991, Smith and Wall 1997, Ferreira and Barbola 1998, Horenstein et al. 2007, Eberhardt and Elliot 2008, Brundage et al. 2011, Moretti et al. 2011), while other studies have found little or no habitat associations (Macleod and Donnelly 1957, Smeeton et al. 1984, Goddard and Lago 1985, Martinez-Sanchez et al. 2000, Joy et al. 2002, Centeno et al. 2004, Brundage et al. 2011). My study determined that forest and field blow fly communities were similar in community structure: habitat had no effect on species number, evenness, diversity levels or niche breadths. Although habitat associations may be important in structuring other communities (Simberloff and Wilson 1969, Levins 1979, Sulkava and Huhta 1998), it was not a distinguishing factor in blow flies (Chapter 2; see Figure 2.9b), confirming that in southwestern Ontario, association with forests or field habitats is not a driving factor in the coexistence of blow fly species.

Since blow flies are known to travel long distances to reach a resource (Macleod and Donnelly 1957, 1963). Differences in distribution that at first appear to be a result of active habitat choice may, in fact, result from differential dispersal from source populations, coupled with chemotaxis towards the carrion, and culminating in tactile and klinotactic responses that exceed minimum stimulus thresholds suitable for oviposition. This can explain the situation in which fly species that were once considered to be urban residents have eventually been found in rural settings (Smith and Wall 1997, Schnack et

al. 1998, Martinez-Sanchez et al. 2000, Grassberger and Frank 2004, Centeno et al. 2004, Horenstein et al. 2007). More research needs to be conducted regarding minimum and maximum stimulus thresholds of different species of blowflies for the various components of their host search, host acceptance, and oviposition behaviours. Based on my results, distinct habitat preferences that were inferred in previous studies should be treated with caution. In addition, appropriate experimental designs should be employed with stringent controls, independent replications, and proper carcass size, age, and placement between specific habitat types in order to fully evaluate the habitat (or seasonal) influences on blow fly species or any other insects associated with carrion.

This study demonstrated that microclimatic differences existed between test sites. Due to the cumulative nature of ADD calculations, it is imperative that site-specific differences in temperature be accounted for. The common practice of using the nearest weather station data for PMI estimations is insufficient to account for site-specific ADD. This study supports the recent view in the field of forensic entomology that calls for the examination of decomposition and successional data based on ADD (Michaud and Moreau 2009, 2011, 2013, Simmons et al. 2010, Tomberlin et al. 2011a,b, 2012, Archer 2014). The use of ADD provides standardization that reduces variability in successional patterns that is extensive when data are presented on a daily basis, particularly when comparing data from different regions where daily temperatures differ considerably.

This study used manipulative, lab-based experimentation that demonstrated important priority effects in blow flies. These effects varied based on which species of flies were present. Moreover, the occurrence of both positive and negative priority effects can further add to the complexity of species interactions. I conclude that

facilitation occurs between blow flies, specifically that *P. regina* and *C. rufifacies* are positively facilitated in the adult and larval stages by the presence of *L. sericata*. In adult interactions, arrival order influenced colonization behaviour to various degrees. *Lucilia sericata* colonized piglets (*Sus scrofa domesticus* L.) in the expected manner: adult females laid egg masses immediately after death in the mouth, ears, and nose regardless of whether another species was present. However, *L. sericata*, acted as a facilitator species for *P. regina* and *C. rufifacies*, the females of which exhibited delayed colonization and laid eggs in less desirable locations in the absence of other species. However, when presented a carcass with *L. sericata* adults or larvae present, *P. regina* and *C. rufifacies* females laid eggs within three hours of resource exposure in the highly desirable locations of the mouth, ear, nose and often on or near *L. sericata* eggs.

With respect to larval interactions, *P.regina* and *C. rufifacies* larvae that developed in the presence of *L. sericata* were larger, had better survival and had higher adult fitness, with higher fecundity suggested by larger size (e.g., tibia, thorax and wing sizes). These effects were pronounced when *P. regina* was second in arrival order following *L. sericata*. Conversely, *L. sericata* suffered negative priority effects from the presence of *P. regina* by being smaller and consequently having lower adult fitness, and having a higher mortality rate. Higher mortality also occurred in the presence of *C. rufifacies*, however, the negative effects exerted on *L. sericata* by *C. rufifacies* could be overcome if the two species colonized simultaneously. In these treatments, although larvae experienced a higher mortality rate, any surviving individuals exhibited higher adult fitness, indicating that predation effects are limited and larvae may benefit from a reduction in intra-specific competition. *Chrysomya rufifacies* was not affected by arrival

order, with larval and pupal mortality rates remaining low and adult fitness levels being high in all treatments when *L. sericata* was present. However, when *C. rufifacies* was alone it experienced high levels of mortality and a reduction in adult fitness, indicating again that it benefits from the presence of an additional species. *Chrysomya rufifacies* is known to become a facultative predator during the second and third instar stages of larval development (Wells 1991, Wells and Greenberg 1992, Baumgartner 1993, Shiao and Yeh 2008, Flores 2013), during which it can feed on the resource itself (i.e. carcass tissue) as well as on other dipteran larvae. The presence of *L. sericata* provided developing *C. rufifacies* larvae with an additional nutritious food source, which presumably resulted in the positive effects seen in this study.

However, *P. regina* experienced positive effects due to facilitation, which was confirmed through further experimentation. Wash experiments determined that *P. regina* larvae that were exposed to sterile and non-sterile aqueous washes from actively feeding *L. sericata* larvae exhibited the same trends seen in the assembly experiments when in presence of *L. sericata*. These larvae had lower larval mortality rates and higher adult fitness and spent more time feeding upon the resource, with larvae not leaving the resource during the 2nd instar stage. Since this trend was observed with both sterile and non-sterile washes and not in the control or water sham treatments, it is likely that a protein or other compound(s) from *L. sericata* larvae must have facilitated the feeding and breakdown of the resource. Though this chemical influence may be bacterial in nature, these effects do not result from changes in the bacterial fauna that is associated with *L. sericata* as the sterile wash treatments yielded the greatest increase in fly fitness. It would be interesting to determine if this chemical effect is derived from *L. sericata*

larvae directly or from bacteria associated with *L. sericata*. *Phormia regina* had higher adult fitness in sterile wash treatments, indicating that the presence of bacteria may lead to resource competition between bacteria and larvae, which has been demonstrated in other carrion insects (Rozen et al. 2008, Ahmad et al. 2006, Burkepile et al. 2006, Mumcuoglu et al. 2001). This hypothesis would also hold true if the compound isolated from *L. sericata* wash was, in fact, antimicrobial, and acted to reduce or eliminate bacterial competition with *P. regina*. Further analysis of this compound would provide insight into the true mechanism that underlies this facilitory effect.

The facilitation between the native species examined in this study could also explain the high level of dominance of *P. regina* in the blow fly community, especially during spring and summer trials (see Chapter 2). This would also support the conclusion that other non-competition mechanisms are important, with the success of *P. regina* in the community relying on exploitation of other species within the sarcosaprophytic guild. This facilitory effect could be more or less pronounced in the presence of additional species, which would extend beyond the specific interactions with *L. sericata* that were quantified in this study. If these additional interactions were also positive, then I hypothesize that the presence of multiple facilitory species would lead to a further increase in larval survival of *P. regina*, while other species would experience negative priority effects such as higher larval mortality and a resultant decrease in abundance levels within the community dependent upon arrival order. Conversely, there could be additional inhibitory species present within the blow fly community, however negative interactions were not identified with the three species used in my study.

Priority effects have been demonstrated in carrion communities previously

(Beaver 1977, Hanski and Kuusela 1977, Shorrocks and Bingley 1994, Bruno et al. 2003). The priority effects detected in my study suggested that the mechanisms governing these interactions could differ between species, as seen with *P. regina* and *C. rufifacies*, and also within a species, as seen in *C. rufifacies* adult and larval interactions. Differences in arrival order can lead to differences in the timing and location of oviposition events, mortality and survival during larval development and adult fitness. Larval interactions and the mechanisms that govern them can have profound influences on individuals (i.e. survival, dispersal, reproduction), populations (i.e. population dynamics, stability, future recruitment), and overall community structure (Fuller 1934, Denno and Cothran 1975, Hassell 1975, Allen and Hunt 2001, Boggs and Freeman 2005). Thus, larval interactions cannot be ignored when seeking to understand the ecology of communities. Moreover, priority effects can trickle down through the community to cause widespread changes in community patterns and in the coexistence of species over large temporal or spatial scales (Connell and Slatyer 1977, Hanski and Kuusela 1977, Atkinson and Shorrocks 1981, Bruno et al. 2003). Therefore, it is important to examine a community at multiple levels, both spatially and temporally, and to extend studies within and between guilds in the carrion insect community in order to determine mechanisms of assembly within the community as a whole.

As summarized above, my studies allowed me to evaluate the relative importance of spatial and temporal partitioning in structuring the blow fly community. Season played a dominant role in determining community structure (i.e., there was temporal partitioning among species), while habitat played little or no role in the blow fly community (i.e., spatial partitioning was not detected). The examination of adult and

larval interactions between three blow fly species was important in elucidating the substantial role of priority effects within the blow fly community. Examination of the carrion community over a large (Chapter 2) and fine (Chapters 3 and 4) spatial and temporal scales was important in demonstrating the complexity of interactions between species at various life stages and between blow fly populations.

Further work should incorporate additional species and extend the temporal scale used, specifically to address pre- and post- larval developmental effects. Results from adult experiments (Chapter 3) indicated that blow flies may alter their colonization behaviour in response to the presence of an additional species, and that these changes in behaviour differ between native and non-native species. These findings combined with results from larval experiments (Chapter 4) indicate that these changes may be the result of blow fly females maximizing offspring fitness, particularly in the interactions between native species. In non-native interactions, *L. sericata* did not alter adult colonization behaviour due to the presence of *C. rufifacies*, however, *L. sericata* larvae could increase their chances of persistence within communities with non-native species by having an increased adult size despite high levels of larval predation. Also, *L. sericata* experienced less predation when it colonized at the same time as *C. rufifacies*. Given prolonged exposure to *C. rufifacies*, will co-evolution result in changes in adult egg-laying strategy of native species that will reduce the negative impacts exerted by this, and other, invasive species? The blow fly system provides an opportunity to compare the diverse array of positive and negative interactions, to study the consequences of priority effects present between blow flies, and to study the interactions and mechanisms for coexistence between native and non-native species.

A common view in the field of ecology is that community assemblages result from a hierarchy of interacting factors that change in importance over temporal as well as spatial scales (HilleRisLambers et al 2012). There is a fundamental belief among some authors that mechanisms operating at the individual level, or small spatial/temporal scale, can have profound effects on mechanisms that operate on the community level, or over a large spatial/temporal scale (Connor and Simberloff 1979, Drake 1991, Levin et al. 2001, HilleRisLambers et al. 2012). Due to the complexity in community assemblages, ecologists have begun to turn to small scale, manipulative experimental approaches to disentangle the factors that contribute to community assembly (Gilbert and Owen 1990, Drake 1991, Farrell 1991, Levin et al. 2001, HilleRisLambers et al. 2012). This series of studies has highlighted the complexity of the carrion insect community, as well as its consistency in assemblages, specifically over different habitats and over a large regional spatial scale. However, interactions between individual species can strongly influence the assembly of species within the carrion community. Mechanisms in addition to competition, such as facilitation and inhibition, should be incorporated into theoretical and empirical approaches (McCook 1994, Bruno et al. 2003, Alonso et al. 2006, McGill et al. 2006, Thompson and Townsend 2006, HilleRisLambers et al. 2012). The carrion insect community has long been recommended as an important tool for investigating many ecological processes that extend well beyond its applications in forensic entomology. It is becoming a model ecosystem for the field of ecology as a whole.

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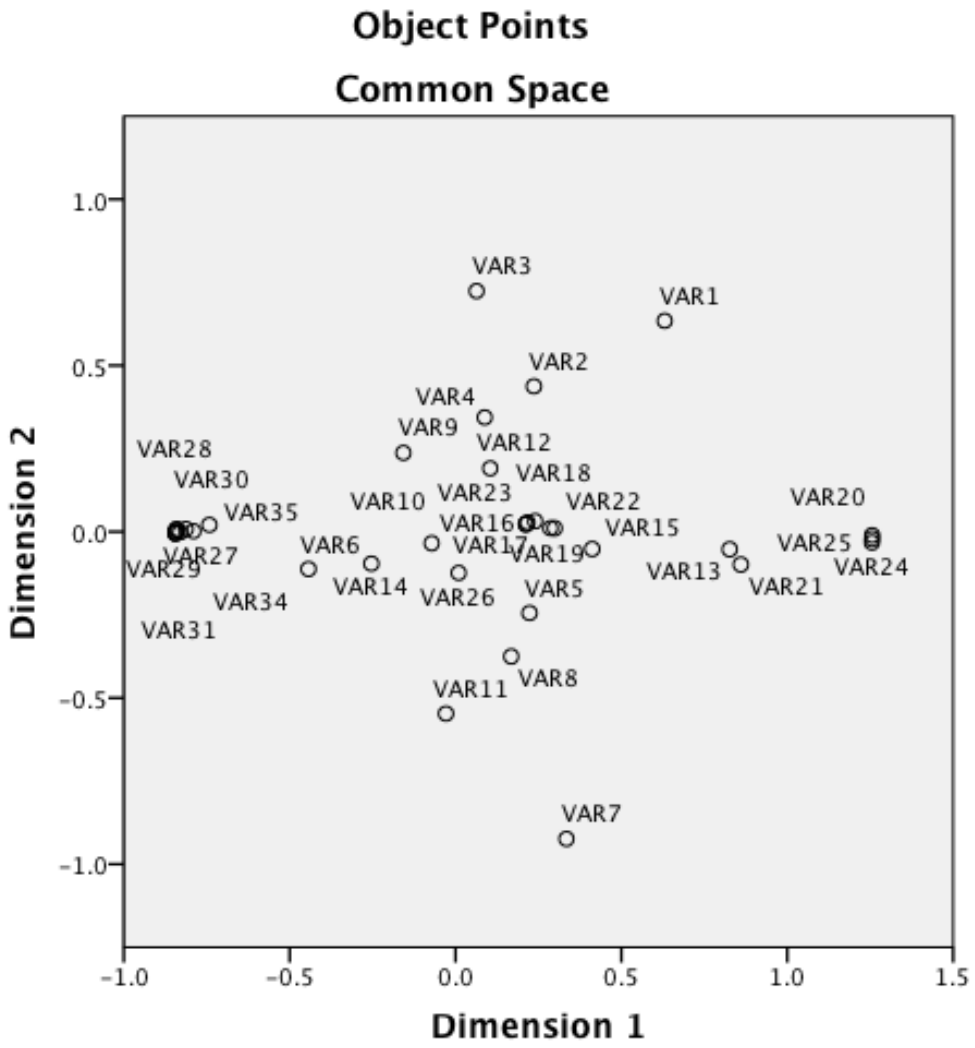
Appendix A

Non-Metric Dimensional Scaling (NMDS) plots for pig sites for blow fly species composition for each treatment condition. Each numbered point on the plot corresponds to the following treatments. Stress measures are outlined after each plot.

TEST SITE	SEASON	HABITAT	PIG
A	fall	forest	1
B	fall	forest	2
C	fall	forest	3
D	fall	forest	4
E	fall	forest	5
F	fall	forest	6
A	fall	field	7
B	fall	field	8
C	fall	field	9
D	fall	field	10
E	fall	field	11
F	fall	field	12
A	spring	forest	13
B	spring	forest	14
C	spring	forest	15
D	spring	forest	16
E	spring	forest	17
F	spring	forest	18
A	spring	field	19
B	spring	field	20
C	spring	field	21
D	spring	field	22
E	spring	field	23
F	spring	field	24
A	summer	forest	25
B	summer	forest	26
C	summer	forest	27
D	summer	forest	28
E	summer	forest	29
F	summer	forest	30
A	summer	field	31
B	summer	field	32
C	summer	field	33
D	summer	field	34
E	summer	field	35
F	summer	field	36

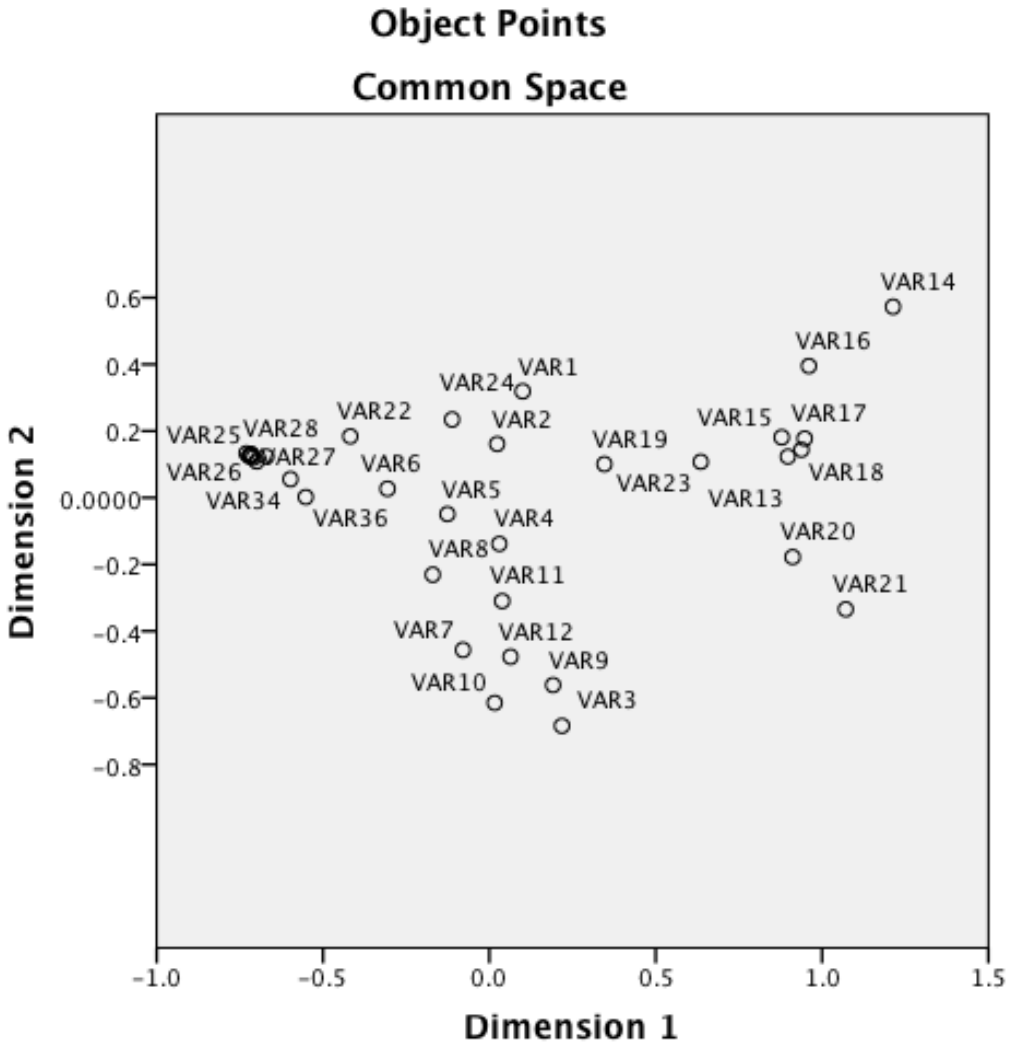
Blow Fly Community Composition 0-50 ADD



Measures

Stress and Fit Measures	
Normalized Raw Stress	0.01875
Stress-I	.13692a
Stress-II	.26368a
S-Stress	.02797b
Dispersion Accounted For (D.A.F.)	0.98125
Tucker's Coefficient of Congruence	0.99058
PROXSCAL minimizes Normalized Raw Stress.	
a Optimal scaling factor = 1.019.	
b Optimal scaling factor = 1.001.	

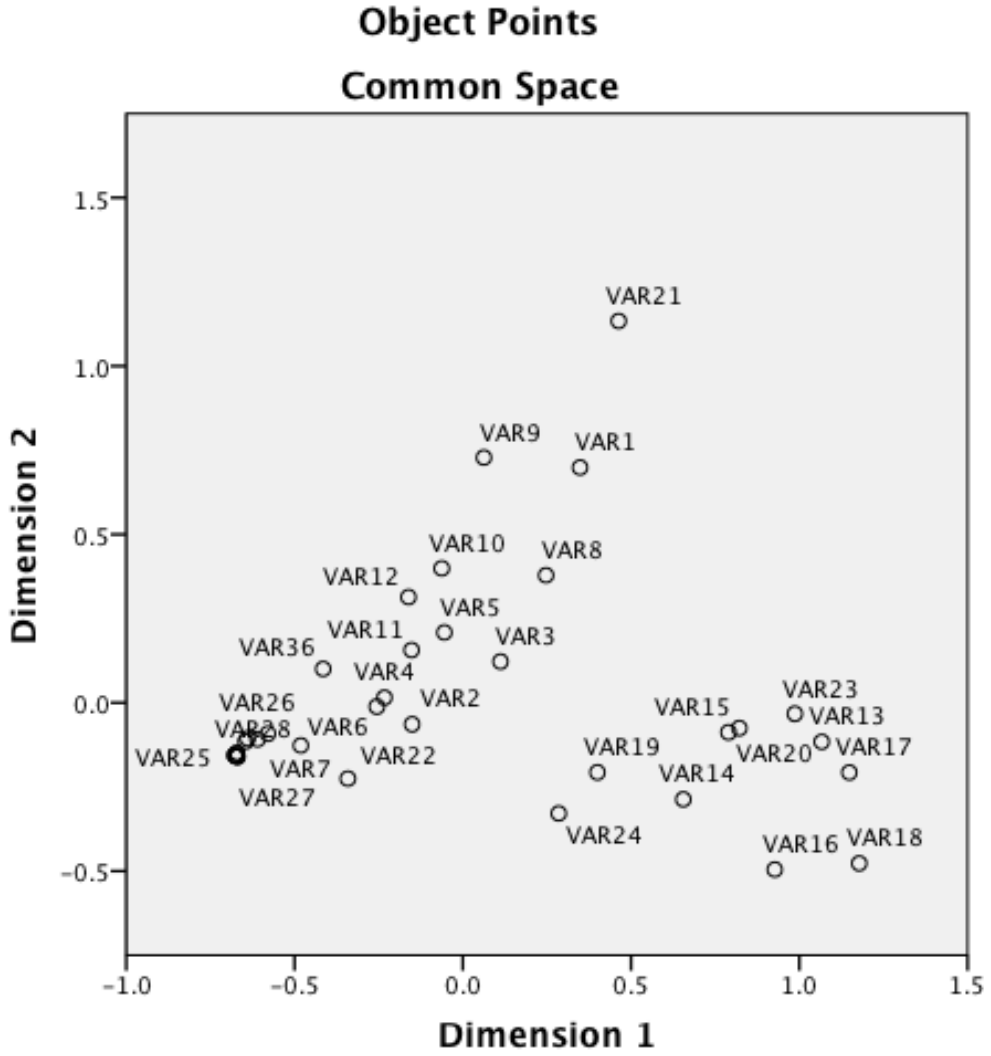
Blow Fly Community Composition 50-100 ADD



Measures

Stress and Fit Measures	
Normalized Raw Stress	0.01073
Stress-I	.10357a
Stress-II	.19833a
S-Stress	.01398b
Dispersion Accounted For (D.A.F.)	0.98927
Tucker's Coefficient of Congruence	0.99462
PROXSCAL minimizes Normalized Raw Stress.	
a Optimal scaling factor = 1.011.	
b Optimal scaling factor = 1.002.	

Blow Fly Community Composition 100-150 ADD

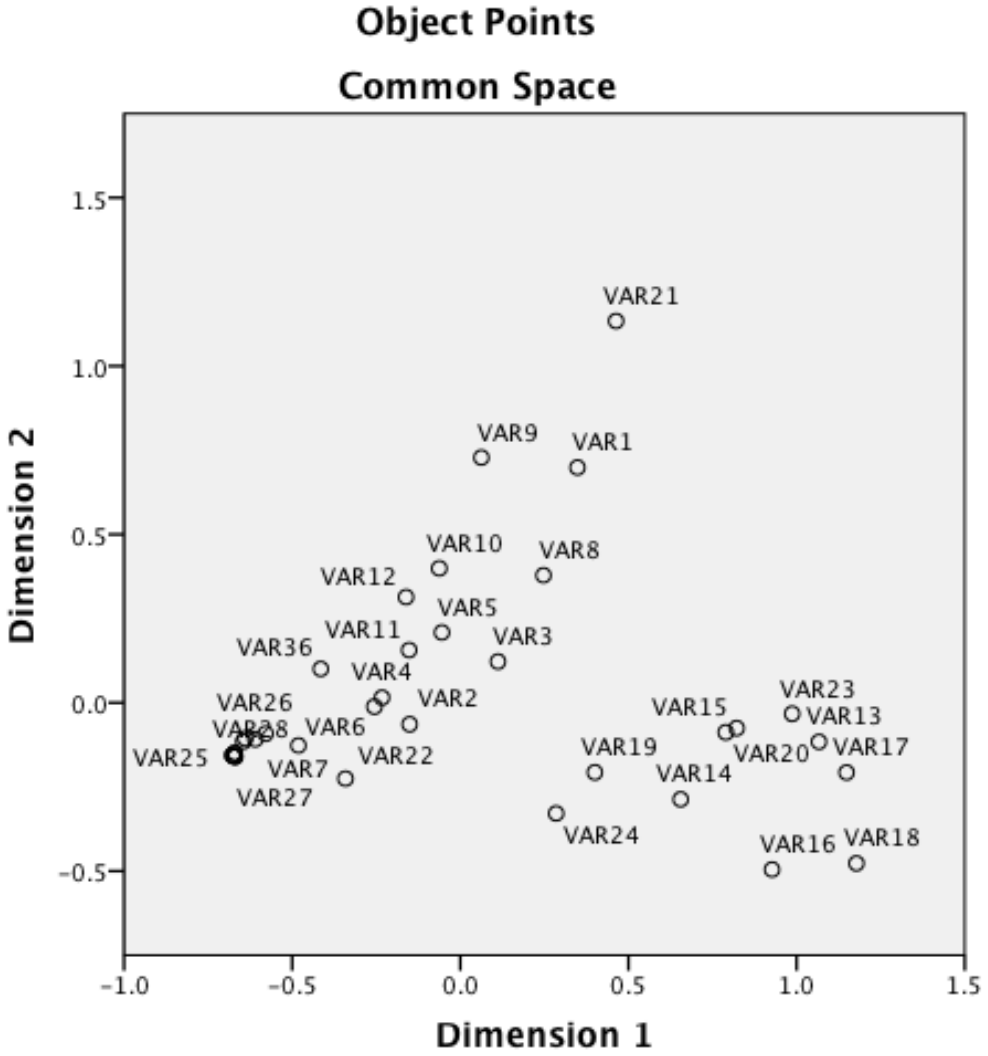


Measures

Stress and Fit Measures	
Normalized Raw Stress	0.00931
Stress-I	.09648a
Stress-II	.18224a
S-Stress	.01448b
Dispersion Accounted For (D.A.F.)	0.99069
Tucker's Coefficient of Congruence	0.99533
PROXSCAL minimizes Normalized Raw Stress.	
a Optimal scaling factor = 1.019.	
b Optimal scaling factor = 1.000.	

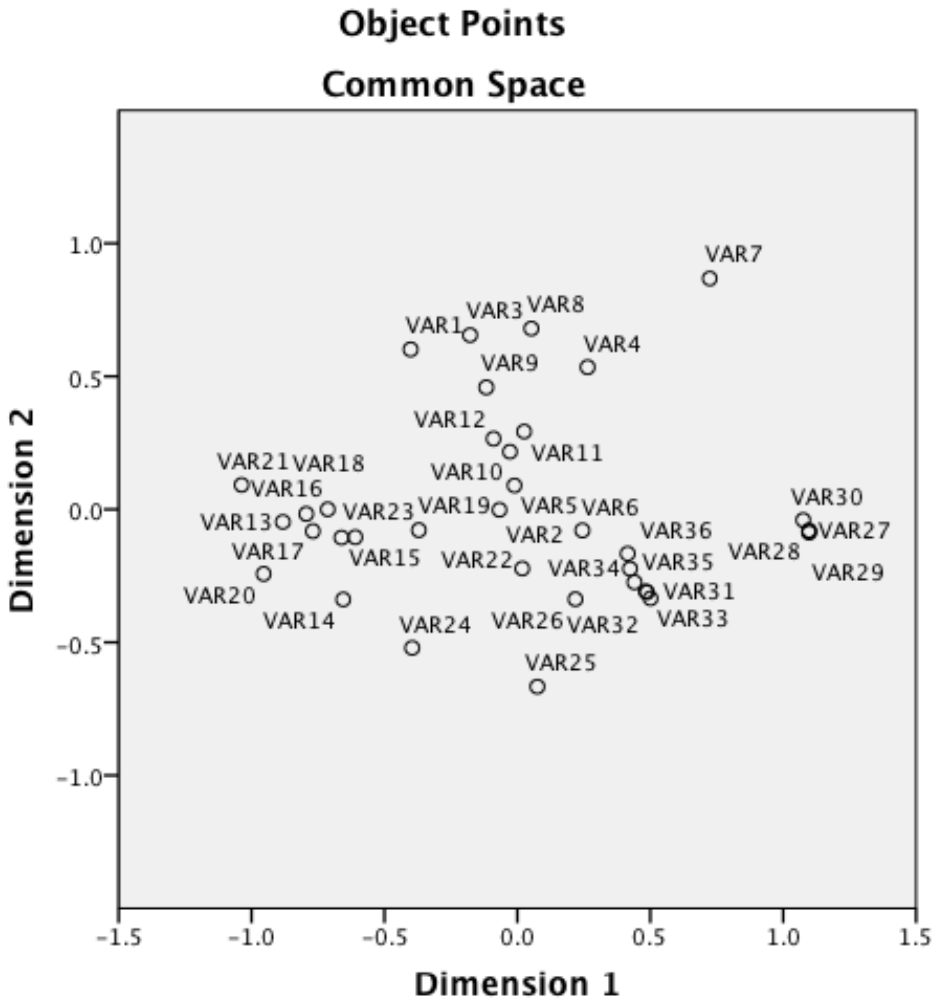
Blow Fly Community Composition 150+ ADD

Measures



Stress and Fit Measures	
Normalized Raw Stress	0.00037
Stress-I	.01911a
Stress-II	.02255a
S-Stress	.00025b
Dispersion Accounted For (D.A.F.)	0.99963
Tucker's Coefficient of Congruence	0.99982
PROXSCAL minimizes Normalized Raw Stress.	
a Optimal scaling factor = 1.000.	
b Optimal scaling factor = 1.000.	

Overall Blow Fly Community Composition



Measures

Stress and Fit Measures	
Normalized Raw Stress	0.01204
Stress-I	.10972a
Stress-II	.23546a
S-Stress	.01730b
Dispersion Accounted For (D.A.F.)	0.98796
Tucker's Coefficient of Congruence	0.99396
PROXSCAL minimizes Normalized Raw Stress.	
a Optimal scaling factor = 1.012.	
b Optimal scaling factor = 1.001.	

Appendix B

Life history and developmental characteristics for each of the three blow fly species used to examine priority effects in this study: *Lucilia sericata*, *Phormia regina*, and *Chrysomya rufifacies*.

Lucilia sericata (Meigen) (Diptera: Calliphoridae)

Lucilia sericata is a green bottle fly that is cosmopolitan in its distribution (Hall 1948, Wall et al. 2002). Adult females generally oviposit four to six days post-emergence (Mackerras 1933, Wall et al. 2002, Pitts and Wall 2004). At each oviposition event, a gravid female lays an average of 225 eggs (Mackerras 1933, Wall 1993, Hayes et al. 1999, Cruickshank and Wall 2002a, Pitts and Wall 2004). Wild adult flies typically have one oviposition event in their lifetime, depositing their full egg load at once, usually in a single egg mass (Greenberg 1991, Pitts and Wall 2004), although wild caged adults have been shown to have multiple oviposition events over one lifetime (Davies 2006). Adults are present during the spring, summer and fall (see Chapter 2), and individuals can overwinter in both the larval and pupal stages (Davies 1929, Mackerras 1933, Green 1951). It is typically found ovipositing within the first 24 hrs of decomposition on freshly killed animals (Fuller 1934, Hall and Doisy 1993, Watson and Carlton 2005, Michaud and Moreau 2009), however, there are some reports of delayed colonization occurring after the first 24 hrs of decomposition (Eberhardt and Elliot 2008). The lower developmental threshold is 9°C for larvae and 11°C for eggs in females (Wall et al. 1992). At 22°C, *L. sericata* requires a mean of 23 ± 1.61 hrs for egg hatching, 179 ± 47.4 hrs for the larval stage, and143 ± 58.63 hrs for the pupal stage with a minimum of 4140 accumulated degree hours (ADH) above 9°C to successfully complete development to the adult stage (Greenberg 1991).

Phormia regina (Meigen) (Diptera: Calliphoridae)

Phormia regina is a black bottle fly that is Holarctic in its distribution (Hall 1948, Byrd and Castner 2001). Adult females generally oviposit six to seven days post emergence (Crystal 1983). Adults are present during spring, summer and fall (see Chapter 2), and individuals can overwinter in the adult and pupal stages (Byrd and Castner 2001). Females colonize fresh carrion within the first 24 hrs postmortem (Greenberg 1991), however, other studies oviposition is delayed until after the first 24 hrs (Illingworth 1927, Watson and Carlton 2005, Gruner et al. 2007, Michaud and Moreau 2009). The lower developmental threshold was determined to be 4.2°C by Greenberg (1991) in Chicago populations. At 22°C, *P. regina* takes a mean of 20 ± 1.2 hrs for egg hatching, 200 ± 51.5 hrs for the larval development, and 116.5 ± 40.8 hrs for the pupal stage, with a minimum of 4038 accumulated degree hours (ADH) to fully complete development to the adult stage (Greenberg 1991).

Chrysomya rufifacies (Macquart) (Diptera: Calliphoridae)

Chrysomya rufifacies is a screwworm fly. It originates from Australia and Asia, however, because of its general association with humans and urban areas, it is presumed to have been introduced into Central America with humans or through transportation of goods (Baumgartner 1993). Since that time, it has increased its geographic range throughout the US and into Southern Ontario (Rosati and VanLaerhoven 2007). *Chrysomya rufifacies* cannot overwinter in more northern climates; however, by dispersing northwards during the growing season it does play a prominent role in the carrion community around the Great Lakes (Rosati and VanLaerhoven 2007). It can

overwinter in the pupal stage during mild winters in warmer climates (Mackerras 1933), enabling populations to become established early in the spring in the southern U.S. An adult female lays between 187-368 eggs, with a mean of 200-210 eggs per batch, with unisexual progeny within each batch (Mackerras 1933, Wilton 1954, Ullerich 1984, Baumgartner 1993). The lower developmental thresholds are 9°C for successful egg hatching, 15°C for larval and pupal development (Wilton 1954, O'Flynn 1983, Byrd and Butler 1997) and 13°C for adult flight (Baumgartner 1993). The upper developmental threshold is 40°C (Waterhouse 1947). This species is considered to be dependent upon previous colonization by an additional species (Fuller 1934, O'Flynn and Moorehouse 1979, Palmer 1980, Goff 2000, Watson and Carlton 2005). It is debatable whether this species has a delay in colonization (Byrd and Butler 1997, Byrd and Castner 2001, Lang et al. 2006, Gruner et al. 2007, Eberhardt and Elliot 2008, Yang and Shiao 2012). At 21°C, Byrd and Butler (1997) determined that *C. rufifacies* takes a minimum of 20 hrs for egg hatching, 148 hrs for the larval stage, and 128 hrs for the pupal stage, while Greenberg (1991) determined *C. rufifacies* required a mean of 4428 ADH (above 10°C) to successfully develop to adult.

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