Enhancing forensic entomology applications: identification and ecology

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ABSTRACT

Enhancing forensic entomology applications: identification and ecology

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The purpose of this thesis is to enhance forensic entomology applications through identifications and ecological research with samples collected in collaboration with the OPP and RCMP across Canada. For this, we focus on blow flies (Diptera: Calliphoridae) and present data collected from 2011-2013 from different terrestrial habitats to analyze morphology and species composition. Specifically, these data were used to: 1) enhance and simplify morphological identifications of two commonly caught forensically relevant species; *Phormia regina* and *Protophormia terraenovae*, using their frons-width to head-width ratio as an additional identifying feature where we found distinct measurements between species, and 2) to assess habitat specificity for urban and rural landscapes, and the scale of influence on species composition when comparing urban and rural habitats across all locations surveyed where we found an effect of urban habitat on blow fly species composition. These data help refine current forensic entomology applications by adding to the growing knowledge of distinguishing morphological features, and our understanding of habitat use by Canada's blow fly species which may be used by other researchers or forensic practitioners.

Keywords: Calliphoridae, Canada, Forensic Science, Morphology, Cytochrome Oxidase I, Distribution, Urban, Ecology, Entomology, Forensic Entomology

ACKNOWLEDGEMENTS

"Blow flies are among the most familiar of insects. They are the bright bluebottle and greenbottle flies of our childhood days"

Hall 1948

I am indebted to the wonderful people around me who have offered their kindness throughout this degree. To my committee whose thoughtful suggestions have shaped this thesis, you have all been very supportive in helping me move forward. Dr. Christopher Kyle, I appreciate your dedication to making this work more robust. Thank you for your thoughtful suggestions and your reminders to consider the "why do we care" questions that relate our work to the applications of forensic science that extending beyond "entomologists will find this exciting!" You've made me a better and more focused writer for it. Dr. Dave Beresford, you have a way of creating the most supportive working environment where everyone is just happy to be there and learn about insects! You've taught me so much more than entomology, and as a result, I've grown as a person. Dr. Joanna Freeland, you have always been so kind to me and I am honoured to have you on my committee. Your thoughtful insights and opinions on this work have been invaluable. I am indebted to every member of my committee for all of their valuable time spent with me. Thank you to the CPRC for funding this work, to my reviewers whose thoughtful suggestions refined my work, and to my external examiner and the chair of my defence for sharing their valuable time with me. Thank you to Mary-Lynn Scriver and Linda Cardwell in the ENLS office whose kindness goes above and beyond for every student and to the staff in the MaDGIC department for their time in helping students with GIS questions. I would also like to thank the researchers whose excitement shine through in

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"Nothing great was ever achieved without enthusiasm"

Ralph Waldo Emmerson 1841

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

A note on the format: I have used the reference and citation style of the journal where these chapters were either published or are to be submitted.

CHAPTER 1

General Introduction

PREFACE

In this chapter, I introduce blow flies (Diptera: Calliphoridae), discuss their importance to forensic science, and explore the overarching methods used to conduct this research. In my data chapters, I first describe a new feature to consistently and reliably differentiate two blow fly species morphologically, and secondly, I describe the distribution and abundance of the various species found across Canada in context of blow flow habitat and rural and urban landscapes. This research was facilitated by police officers from Royal Canadian Mounted Police (RCMP) and Ontario Provincial Police (OPP) detachments across Canada who volunteered and deployed our blow fly traps that provided the baseline data for the entire thesis.

DESCRIPTION OF THE STUDY ANIMALS: DIPTERA: CALLIPHORIDAE

The family Calliphoridae is common worldwide and species in this family exhibit a range of appearances and behaviours. Calliphorids can be partially differentiated from other Diptera by their wing venation (Marshall 2011). Features used to distinguish blow flies from other similar looking metallic Diptera are located on the antennae and thorax; specifically, the presence of plumose arista, bristles on the meron and a weak or absent subscutellum (Marshall 2011). Calliphoridae includes 150 genera and 1000 species worldwide (Rognes 1991). Five subfamilies exist in North America: Calliphorinae, Luciliinae, Chrysomyinae, Polleniinae and Melanomyinae. Calliphorinae are larger bodied carrion flies with some cold tolerant species. These flies are distinguished by their non-metallic thorax with a metallic abdomen, sometimes referred to as blue bottle flies.

Luciliinae are generally smaller, warm-loving and colonizers of carrion. They are metallic on both the thorax and abdomen, sometimes referred to as green bottle flies. Chrysomyinae are similar to Luciliinae with their metallic thorax and abdomen, but are distinguished by the presence of setae on the dorsal side of their stem vein. These flies are generally darker and duller, granting them the common name black blow flies. Polleniinae are not metallic in appearance, but have long crinkly golden hairs that cover their thorax and shimmery gold pattern on their abdomen. Unlike carrion-attracted blow flies, members of the Polleniinae subfamily are primarily parasites of worms. Polleniinae are commonly known as cluster flies for their behaviour. Finally, Melanomyinae are another non-metallic subfamily.

The blow flies belonging to Calliphorinae, Luciliinae and Chrysomyinae are the focus in this thesis. These subfamilies that lay their eggs on decomposing animal tissues have been studied extensively to understand their lifecycle. This lifecycle consists of 6 parts: egg, first-instar larvae, second-instar larvae, third-instar larvae, puparium and adult (Wall 1992). The most recent blow fly key in Canada covers Eastern Canada and includes 22 species from the subfamilies Calliphorinae, Luciliinae and Chrysomyinae (Marshall 2011). The second most recent key covers North America and Mexico with 41 species from those subfamilies, with an additional genus not further described (Whitworth 2008). Also in this key, 6 species from the subfamilies Polleniinae and Melanomyinae are included with 3 genera not further described (Whitworth 2008). The first comprehensive key to North American blow flies was produced in 1948 and covers more species (Hall 1948). Each of these keys have merits and provide useful information to distinguish species morphologically, and when considered together they provide the

best coverage of information. For instance, the removal of species from updated keys can mean that those species are overlooked when identifications are made. This can lead to rare species or species outside of their range being misidentified as a more common or expected species. On the other hand, too much reliance on older outdated keys can lead to using features which do not best represent the species. Continued work in refining and finding new features that consistently differentiate between similar species will continue to make keys more robust in the future to avoid misidentifications, which can have important legal consequences in forensic science applications.

HISTORY OF BLOW FLIES IN MEDICINE AND FORENSICS

Historically, blow fly maggots have been used in medicine to clean open wounds, a practice which is still used today (Preuss 2004). Carrion-eating blow fly maggots placed on a living person's necrotic tissue result in the maggots eating the dead cells and leaving the living cells intact (Preuss 2004). Blow flies are also present in art throughout history. Maggots on decomposing bodies were drawn in the piece "the grave of Robert Touse" and "the dance of death" (Benecke 2001). The phrase "fly blown" was coined by William Shakespeare and appears in some of his plays (Benecke 2001). This reference is what eventually gave Caliphorids their common name (Benecke 2001). Blow flies are also known pollinators who transport pollen between flowers while taking a nectar meal. When farmers us blow flies specifically for their pollination abilities, they will often be placed in isolation cages to ensure they stay with the crop (Howlett 2012). Some species can pollinate flowers at temperatures lower than honey bees which can prove useful for crops grown in cooler areas (Howlett 2012). Currently, blow fly research is most widely conducted in a forensic context, where the colonization of a corpse and the development

of larvae are most often studied for their use as a predictor of post mortem interval (PMI) (Catts 1992). This interval represents the time since a person has died, and can accurately correspond to the amount of time that these insects have been developing on the corpse.

Many species of blow flies are predictably attracted to decomposing animal tissues as a breeding substrate and can appear on a corpse within minutes of death (Catts 1992, Anderson 2004), making their development an excellent indicator of PMI (Amendt 2007). By determining the ambient temperature in the area during decomposition along with the development rate of the species of blow fly whose development is in question, the time since the eggs were laid on the corpse can be calculated (Amendt 2007). This calculation estimates the time since colonization occurred which, due to the early arrival time of blow flies post-mortem, can approximate the PMI. For their attraction to necrotic tissue, blow flies have also been used as an indication of child or elder neglect, wounds on a decomposing corpse, or drug use prior to death (Catts 1992). Perhaps, the most important Canadian example of blow flies as evidence comes from R v. Steven Truscott, which resulted in a conviction in 1959, and later an acquittal in 2007. This particular case took into account the developmental rates of the blow flies found on the corpse compared to other carrion colonizing insects, as well as the potential for blow flies to lay eggs at night. Understanding the differences in carrion insect developmental rates was used for estimating the post mortem interval in this case. Developmental rates differ between species, making species determination a key factor in accurate estimations (Amendt 2004).

RESEARCH RATIONALE

Applications of blow flies to forensic work rely on predictability in the types of insects developing on the body and their development times, however there is still much refining to do with this application. The National Academy of Sciences from the United States released a report in 2009 that highlights the need to strengthen forensic science in the courtroom (NAS 2009). Correct species identification is at the core of all blow fly research, as correct identifications are required to infer information about development times and compare literature. Thus, identifications must continue to be strengthened for further work on this subject. Due to the variance of developmental rates, temperature ranges, distribution and behaviour between species, correct identification is essential. When entomology research is applied to evidence in a criminal matter, accurate species identification gains an added importance as an incorrect assessment of the evidence can impact an investigation and trial. Understanding and reporting consistent morphology between species allows for a more diverse set of reliable features for species determination. This set of usable features is added to and refined over time to produce reliable keys to differentiate species.

A genetic analysis of species through the cytochrome oxidase I (COI) mitochondrial region can strengthen the identifications made by morphology in both research and evidence processing (Wells 2008). A thorough understanding of morphological identifications, as they compare to genetic analysis, also provide justification for features used to distinguish species. For instance, morphological features with a range of variance in a species or those which are often found damaged when sampling from natural populations can require genetic testing. With species

identifications confirmed genetically, we can continue to understand the limitations of morphological features when identifying species. Genetic analysis can also provide identifications in a forensic investigation if a specimen is damaged beyond what can be accurately identified morphologically or if immature stages of blow flies cannot be accurately identified or reared to adulthood for identification.

Habitat is a crucial parameter influencing species distribution, yet there remains much uncertainty regarding habitat specificity of different species. Knowing what species to expect in an environment is important for forensic science to help predict PMI (Amendt 2007) and to note unexpected species (Richards 2009). This is especially important given that what a blow fly does away from a carcass is not yet fully understood (Wells 2008). Although much research has been conducted on their actions while seeking and using a carcass for reproduction, a corpse represents an ephemeral resource, existing at random on the landscape for a short period of time. It is important to understand how this fits into the requirements of blow flies, which can differ between species, where some have more specific needs and others are able to breed using less suitable substrates (Norris 1965). For instance, *Lucilia sericata* is reportedly able to breed in vegetable matter (Norris 1965). This species also exhibits a lower daily flight distance, as shown in mark-recapture studies, compared to other blow fly species (Norris 1965). It has long been suggested that human produced waste may encourage the breeding of blow flies by providing a constant breeding substrate, in contrast to the ephemeral carcass resources in rural areas (Norris 1965). A difference in species caught between urban and rural areas has also been noted (Norris 1965, Moretti 2013), including relative abundance studies (Brundage et. al. 2011). As there has been no observed delay in colonization in urban

areas (Anderson and VanLaerhoven 1996, Grassberger and Frank 2004), blow flies are already present in the urban environment rather than traveling to urban areas when suitable substrates become available.

PROJECT OVERVIEW

This work is a collaboration with the OPP and RCMP through a grant from the Canadian Police Research Centre. Our OPP and RCMP collaborators were invaluable partners in this work, allowing us to collect blow flies across the country (FIGURE 1.1.). Officers volunteered their time to deploy and collect blow fly traps for five sampling sessions between 2011 and 2013 (TABLE 1.1.). Each participating officer was sent a package of two bottle traps (FIGURE 1.2.). Most locations received one package per sampling session, but select locations in Ontario received three sets of traps per session. Each trap was deployed for two weeks, with insects collected from the traps at the end of each week. Their contribution to this work was invaluable.

THESIS ORGANIZATION

This thesis has a general introduction, two data chapters and a general discussion. The first data chapter focuses on enhancing morphological identification of two forensically relevant blow fly species, *Phormia regina* and *Protophormia terraenovae*. The second focuses on further understanding blow fly distribution and habitat association across the landscape by comparing species catches in urban and rural areas. In these chapters I explain how these blow flies were collected and what species were captured.

To summarize, this research is driven by two questions that arose from a practical consideration of the needs of forensic entomology: 1) a method for simple and rapid identification to species of field derived specimens, and 2) an understanding of how

applicable species surveys and studies from different parts of North America are to forensic entomological investigations, specifically:

1. Can a limited and robust set of morphological features be used to identify forensically important species? The genesis of this question is a recognition that identification to species is often required for specimens that are not in good condition. We answer this question in an example using the frons-width to head-width ratio to differentiate *P. regina* and *P. terraenovae.*
2. What scale of urban habitat influences blow fly species in urban areas, and is an urban area a distinct habitat across ecozones?

This project began with the second question, and from working with the sampled specimens it quickly became obvious that there is need for a rapid and simple method to identify the more important forensic species, the subject of my first data chapter. Our goal is to strengthen forensic entomology through the above objectives, by adding my research to the understanding of blow fly species.

REFERENCES

2005 North American Land Cover at 250 m spatial resolution [Map]. Natural Resources Canada/Canadian Center for Remote Sensing, United States Geological Survey; Insituto Nacional de Estadística y Geografía (INEGI), Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) and Comisión Nacional Forestal (CONAFOR), 2005.

Anderson GS. Determining time of death using blow fly eggs in the early postmortem interval. International Journal of Legal Medicine 2004;118:240-4.

Anderson GS, VanLaerhoven SL. Initial studies on insect succession on carrion in southwestern British Columbia. J Forensic Sci 1996;41:617-25.

Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall MJR. Best practice in forensic entomology–standards and guidelines. Int J Legal Med 2007;121:90-104.

Amendt J, Krettek R, Zehner R. Forensic entomology. Naturwissenschafter 2004;91:51-65.

ArcMAP [Computer Program] ArcGIS desktop version 10.2. Redlands, CA: ESRI, 2011.

Benecke M. A brief history of forensic entomology. Forensic Sci Int 2001;120:2-14.

Brundage A, Bros S, Honda JY. Seasonal and habitat abundance and distribution of some forensically important blow flies (Diptera: Caliphoridae) in Central California. Forensic Sci Int 2011;212(1-3):115-20.

Catts EP, Goff ML. Forensic entomology in criminal investigations. Annu Rev Entomol 1992;37:253-72.

Grassberger M, Frank C. Study of arthropod succession on pig carrion in a central European urban habitat. J Med Entomol 2004;41(3):511-23.

Hall DG. The blowflies of North America. Lafayette, Indiana: Thomas Say Foundation, 1948.

Howlett BG. Hybrid carrot seed crop pollination by the fly Calliphora vicina (Diptera: Calliphoridae). J Appl Entomology 2012 Sept;136(6):421-30.

Marshall SA, Whitworth T, Roscoe L. Blow flies (Diptera: Calliphoridae) of eastern Canada with a key to Calliphoridae subfamilies and genera of eastern North America, and a key to the eastern Canadian species of Calliphorinae, Luciliinae and Chrysomyiinae. Can J Arthropod Ident 2011;11:1-93.

Moretti TD, Godoy WAC. Spatio-temporal dynamics and preference for type of bait in necrophagous insects, particularly native and introduced blow flies (Diptera: Calliphoridae). J Med Entomol 2013;50(2):415-24.

National Research Council. Strengthening forensic science in the United States: a path forward. Washington: The National Academies Press, 2009.

Norris KR. The binomics of blow flies. Annu Rev Entomol 1965; 10:47-68.

Preuss SF, Stenzel MJ, Esriti A. The successful use of maggots in necrotizing fasciitis of the neck: a case report. Head Neck 2004 Aug;26(8):747-50.

Richards CS, Williams KA, Villet MH. Predicting geographic distribution of seven forensically significant blowfly species (Diptera: Calliphoridae) in South Africa. Afr Entomol 2009;17(2):170-82.

Rognes K. Blowflies (Diptera: Calliphoridae) of Fennoscandia and Denmark. New York: Brill, 1991.

Wall R, French NP, Morgan KL. Effect of temperature on the development and abundance of the sheep blowfly Lucilia sericata (Diptera: Calliphoridae). Bull Entomol Res 1992;82:125-31.

Wells JD, Stevens JR. Application of DNA-based methods in forensic entomology. Annu Rev Entomol 2008;53:103-20.

Whitworth T. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. Proc Entomol Soc Wash 2006;108(3):689-725.

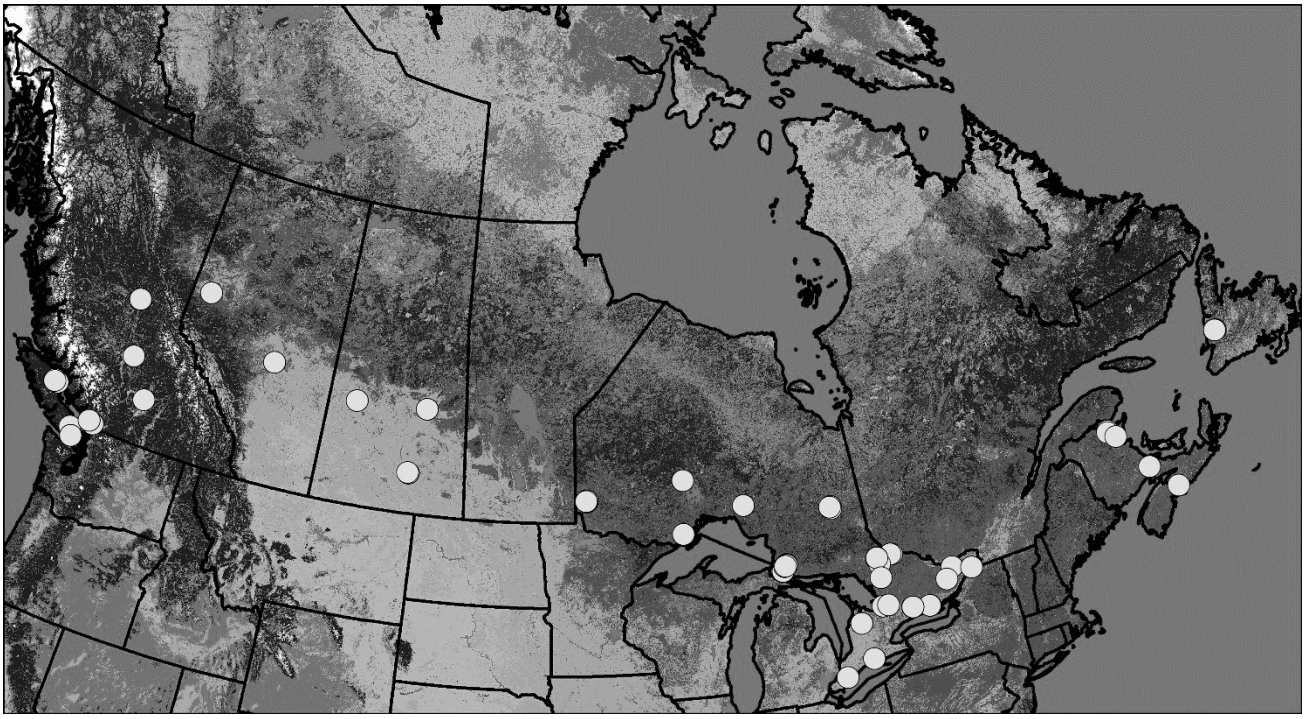


FIGURE 1.1. Trapping locations between 2011 and 2013 (projection: Canadian Lambert Conformal Conic, datum: NAD 1983, produced with ArcMAP 2011, USGS 2005).

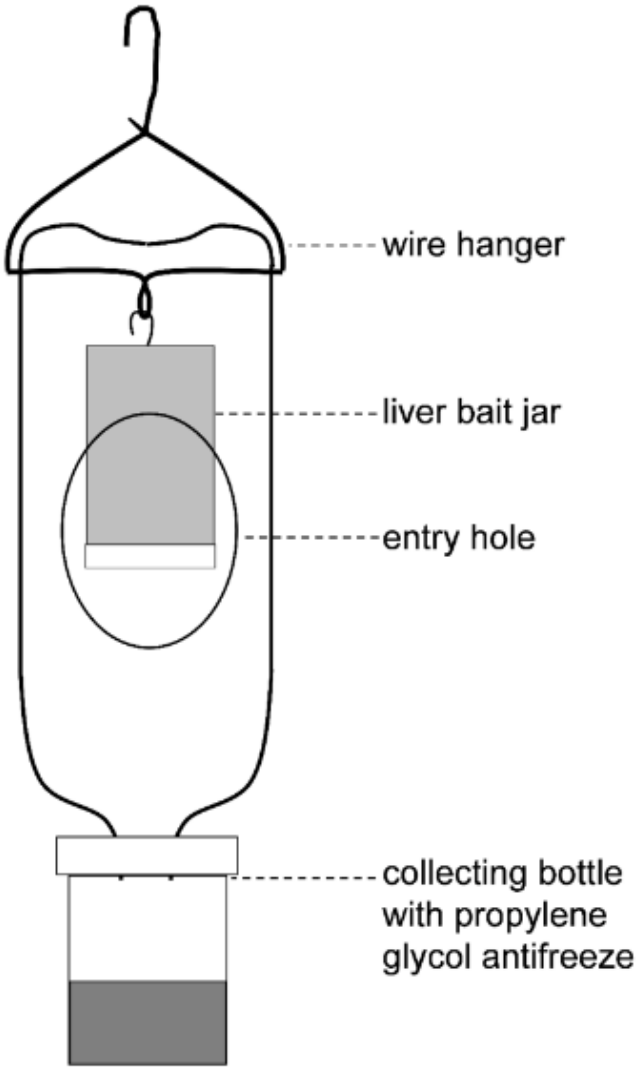


FIGURE 1.2. Baited bottle trap design used to capture insects attracted to decomposing carrion.

TABLE 1.1. List of participating locations and officers

Location	Prov	Contact(s)	2011 Mid-Season	2012 Mid-Season	2013 Early-Season	2013 Mid-Season	2013 Late-Season
Armstrong	ON	Sgt. Hubert Beauclair	X				
Bathurst	NB	Cpl. Louis Bedard Cpl. D. Allen	X		X	X	
Beaver Bank	NS	Cpl. Trevor Penny	X				
Belleville	ON	I/C Larry Hall		X	X	X	X
Campbell River	BC	Sgt. Shelly Massey	X		X	X	X
Chatham	ON	I/C Darren Soucie I/Sgt. Curt Lansens	X		X	X	X
Corner Brook	NL	Cpl. Michael Wyllie	X	X	X	X	X
Edmonton	AB	Crystal Samborski	X	X	X	X	X
Grande Prairie	AB	Holly Allen (FIA)	X	X			
Kamloops	BC	Cst. Marian Oden	X				
Kenora	ON	I/C John Frankcom I/Sgt. Carm McCann	X	X	X	X	X
Long Sault	ON	I/S/Sgt. Gord Lefebvre I/C Robert Lamarche I/C H. Dicaine		X	X	X	X
Manitouwadge	ON	P/Cst. Roland Smith	X	X	X	X	
Mattawa	ON	Cst. Mark Desrochers	X			X	
Moncton	NB	Cpl. Brian Babin	X	X	X	X	
Mount Forest	ON	I/C Jeffrey Myatt I/Sgt. Trevor McLeod	X		X	X	
North Battleford	SK	Cpl. Jon Kachur	X	X	X	X	
North Bay	ON	I/C Dave Lince I/Sgt. Andrew Emmerson I/C Mike Cruickshank	X		X	X	
Orillia	ON	I/C Steven Barnstaple I/Sgt. Dave Vaughan I/C Amy Moore	X		X	X	
Ottawa	ON	Rolanda Lam	X				
Perth	ON	I/C Bernie Graveline I/Sgt. Deanna Walton I/Sgt. Shauna Marshall	X				
Peterborough	ON	I/Sgt. John Aitkenhead I/C Heath Crichton	X		X	X	X
Prince George	BC	Cpl. Sigrid Tveita	X		X	X	X
Regina	SK	Cpl. Wayne Love Cpl. Tommy Thifault	X	X	X	X	
Sault Ste. Marie	ON	I/C William Zorzi	X	X	X		X
South Porcupine	ON	I/Sgt. Dan Ehman	X				

Location	Prov	Contact(s)	2011 Mid-Season	2012 Mid-Season	2013 Early-Season	2013 Mid-Season	2013 Late-Season
Surrey	BC	Lindsay Nowack Cst. Jeffrey Jackson	X	X	X	X	X
Thunder Bay	ON	I/Sgt. Scott Hlady I/C Al MacDonald	X	X	X	X	
Tillsonburg	ON	I/Cst. Chuck McNeil	X	X	X	X	X
Tisdale	SK	Cpl. Erin Neggers	X	X	X		
Victoria	BC	Sgt. Nav Hothi Cpl. Nina Johnson	X	X	X	X	X
Williams Lake	BC	Cst. M Berns	X		X		

CHAPTER 2

Using Frons Width to Differentiate Blow Fly Species (Diptera: Calliphoridae) *Phormia regina* (Meigen) and *Protophormia terraenovae* (Robineau-Desvoidy).

PREFACE

Title: Using frons width to differentiate blow fly species (Diptera: Calliphoridae) *Phormia regina* (Meigen) and *Protophormia terraenovae* (Robineau-Desvoidy).

Authors: Sarah V. Langer, B.Sc.F.S.; Christopher J. Kyle, Ph.D.; and David V. Beresford, Ph.D.

Reference: Langer SV, Kyle CJ, Beresford DV. Using Frons Width to Differentiate Blow Fly Species (Diptera: Calliphoridae) *Phormia regina* (Meigen) and *Protophormia terraenovae* (Robineau-Desvoidy). J Forensic Sci 2016 Mar;62(2):473-5. http://onlinelibrary.wiley.com/doi/10.1111/1556-4029.13281/abstract

Changes from publication version:

The figure and table numbers have been changed to fit with this thesis' format, including the removal of one figure from this chapter (in this thesis, FIGURE 1.2.), to avoid replicating the same figure throughout chapters.

ABSTRACT

Protophormia terraenovae (Robineau-Desvoidy) (Diptera: Calliphoridae) and *Phormia regina* (Meigen) (Diptera: Calliphoridae) are morphologically similar blow fly species commonly used for estimating post mortem intervals. Field collection and storage of adults can result in colour changes, in particular on calypters and palps; often collected specimens show damage such as wing fray or fungal growth. We measured the frons width: total head width ratio using photographs (ImageJ version 1.49) to differentiate these two species. Both sexes were distinguishable to species, with the greatest difference was between males: 12.34% *P. terraenovae* vs 1.62% *P. regina*; less so for females: 40.25% *P. terraenovae*, vs 33.65% *P. regina*. Incorporating this feature into future blow fly keys would help with distinguishing field caught specimens when other features are obstructed

Keywords: forensic science, forensic entomology, Calliphoridae, morphology, cytochrome oxidase I gene, species determination

INTRODUCTION

When blow flies are used for forensic purposes, it is important to ensure the species identifications are correct. Dichotomous identification keys that use morphological traits are based on archived specimens of intact individuals that are preserved in a manner to allow visual access to important distinguishing features. In North America, Hall produced the first modern comprehensive key to Calliphoridae, and included species descriptions with many additional measurements (1). This work was revisited by Whitworth, who provided an updated and easier to use key which, for simplicity, removed some of the additional measurements provided by Hall (2). Recently

an excellent and accessible illustrated key has been produced for eastern North America (3), which excludes western species, as well as many of the additional measurements provided by Hall.

In all of these keys, little is provided about identifying field collections and the concomitant problems that can arise. These include colour changes, in particular the calypters and palps, caused from storage in ethanol, and damage due to age such as wing fray or fungal growth.

From our work with samples stored in 80% ethanol, we have found individuals with their thoracic spiracles obscured by a white fungal growth, making it impossible to use the color of this feature for distinguishing between *Phormia regina* and *Protophormia terraenovae* (e.g., 2, 3). To overcome this problem, we used the frons to head width ratios to distinguish between these two similar, common holarctic (4) species.

Frons and head widths were provided by Hall in his descriptions of each species but not as distinguishing features (1). Subsequent keys (5-8) have also included frons and head width measurements, but have relied on spiracle colour alone for distinguishing *P. regina* from similar species such as *P. terraenovae*. More recent keys use frons and head width, for example for *Tryptocalliphora, Protocalliphora, Calliphora, Chrysomya,* and *Lucilia* (2, 3, 9), but not *Phormia* or *Protophormia* spp. This feature is easy to view and remains unaltered in stored specimens, an important characteristic in legal applications. This feature is not based on colour and is unchanged by fungal growth. Frons width measurements have been highlighted as a useful feature in other studies (2, 10).

METHODS

Blow flies were collected across Canada (TABLE 2.1.) through collaboration with the OPP and RCMP in a nationwide survey using liver baited traps (bottle traps). The traps were based on *Nicrophorus* (Coleoptera: Silphidae) bottle traps (11) and modified for blow flies. Briefly, the trap consists of a 2L inverted soda bottle with a hole cut in the side. A 250 ml plastic collection bottle with decomposing liver (3-10 days at ambient temperature) was placed inside the trap with a perforated lid. Flies attracted to the liver fell down the trap into a 500 ml bottle filled with propylene glycol (non-toxic RV antifreeze). After collection, specimens were stored in 80% ethanol until they could be pinned for identification and measurement.

We measured the frons and head widths of 88 *P. regina* (51 female, 37 male) and 91 *P. terraenovae* (57 female, 34 male). More females were caught due to using baited traps. The frons includes two structures, the frontal vitta is the center-most structure and the fronto-orbital plates are present between the vitta and the eyes (2). The frons width was expressed as a % of head width, calculated as minimum frons width/maximum head width x 100.

Specimens were photographed with a Cannon Rebel T2i EOS 550D using a tripod and Remote Pro. Head widths and frons widths were measured on the digital photographs using the program ImageJ, version 1.49 (Bourne pers. comm). Measurements were taken of the frons width at the narrowest point, and the head width at the widest point (FIGURE 2.1.).

The COI mitochondrial region of DNA was sequenced using specimen's legs, which left important morphological features intact so that the specimens could be

reassessed morphologically. Qiagen extraction protocol was conducted followed by amplification using the insect specific primers UEA1 and UEA10 (12), interpreted using Mega, version 6 (13), and compared to sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (14). Using this region (at a minimum of 200 bases) the resulting sequences returned the same species as our morphological identifications. The identity of 87 *P. regina* and 89 *P. terraenovae* were confirmed.

RESULTS AND DISCUSSION

The mean frons width of *P. terraenovae* females was 40.25% of the total head width (SD = 1.35, range 37.48% to 42.97%), compared to 33.65% for *P. regina* females (SD = 1.89, range 29.75% to 36.83%) (FIGURE 2.2.). In males, the mean for *P. terraenovae* was 12.34% of the total head width (SD = 1.41, range 9.53% to 15.40%) compared to 1.62% for *P. regina* males (SD = 0.69, range 0.85% to 3.27%) (FIGURE 2.2.). In *P. regina* males, the frons is small, the frontal vitta is not continuous up the length of the head, and the measurement was the whole of the fronto-orbital plate. In neither sex did the species measurement ranges overlap.

We found that there was 100% discrimination between the two species for males and females (males: Hotelling's $T^2 = 953$, $F = 469$, d.f. = 1, 70, $p < 0.0001$; females: Hotelling's $T^2 = 489$, $F = 160$, d.f. = 1, 107, $p < 0.0001$; PAST version 2.17c) (15).

Hall (1) reported mean frons to head widths but did not include any variability measurements. He found that the frons/head widths (x 100) of female and male *P. regina* were 31% and 1% (compared to our 33.65% and 1.62%), and *P. terraenovae* were 37% and 13% (compared to our 40.25% and 12.34%), similar to our mean values.

While digital methods were used to obtain the measurements, an ocular micrometer would have been equally suitable.

CONCLUSION

Although arguably measuring the frons and head widths can require more work than using some of the other distinguishing features reported in recent keys, this method provides an additional tool for identifying species when other features are obscured or damaged. The inclusion of this additional measurement would mean less reliance on other features used in the same couplet, and is a feature not susceptible to obstructions found on field collected specimens. The proportional width of the frons remains unaltered even in damaged specimens, as long as the head is included in the specimen. With modern digital photographic methods widely available, we suggest that frons width to head width ratios be incorporated into future keys as a reliable method of distinguishing the two species discussed here.

REFERENCES

1. Hall DG. The blowflies of North America. Lafayette, Indiana: Thomas Say Foundation, 1948.

2. Whitworth T. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. Proc Entomol Soc Wash 2006;108(3):689-725.

3. Marshall SA, Whitworth T, Roscoe L. Blow flies (Diptera: Calliphoridae) of eastern Canada with a key to Calliphoridae subfamilies and genera of eastern North America, and a key to the eastern Canadian species of Calliphorinae, Luciliinae and Chrysomyiinae. Can J Arthropod Ident 2011;11:1-93.

4. Desmyter S, Gosselin M. COI sequence variability between Chrysomyinae of forensic interest. Forensic Sci Int: Genet 2009;3(2):89-95.

5. James MT. The blowflies of California [bulletin]. Bull Calif Insect Surv 1955;4(1):1-34.

6. Hall RD, Townsend LH. The blow flies of Virginia (Diptera: Calliphoridae) [bulletin]. The insects of Virginia 1977;11:1-48.

7. Smith KGV. A manual of forensic entomology. London: Trustees of the British Museum (Natural History), 1986.

8. Rognes K. Blowflies (Diptera: Calliphoridae) of Fennoscandia and Denmark. New York: Brill, 1991.

9. Whitworth T. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of the West Indies and description of a new species of Lucilia Robineau-Desvoidy. Zootaxa 2010;2663:1-35.

10. Sofield RD, Douglas ME, Hansens EJ, Vrijenhoek RC. Diagnosis and detection of cryptic species: the Tabanus nigrovittatus complex (Diptera: Tabanidae) in coastal New Jersey. Ann Entomol Soc Am 1984 Aug;77(5):587-91.

11. LeGros DL, Beresford DV. Areal foraging and sexual dimorphism is burying beetles (Silphidae: Coleoptera) in a central Ontario forest. J Ent Soc Ont 2010;141:3-10.

12. Lunt DH, Zhang DX, Szymura JM, Hewitt GM. The insect cytochrome oxidase gene: evolutionary patterns and conserved primers for phylogenetic studies. Insect Mol Biol 1996;5(3):153-85.

13. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 2013;30(12):2725-9.

14. Altschul SF, Madden TL, Schaffer AA, Zhang J, Zang Z, Miller W et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25(17):3389-402.

15. Hammer Ø, Harper DAT, Ryan PD. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 2001 May;4(1):1-9.

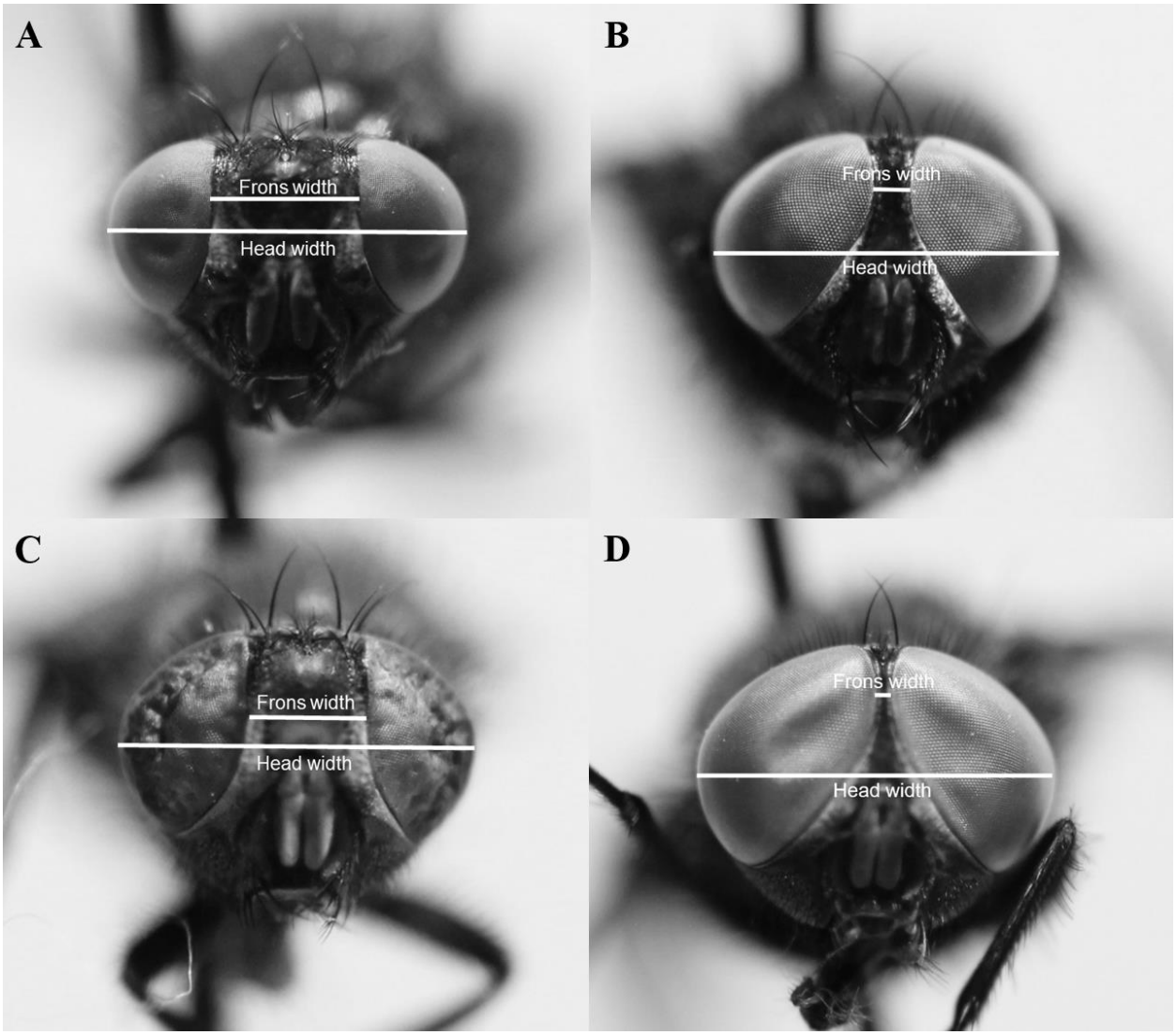


FIGURE 2.1. Location of measurements of the frons width and head width used for identification to species for *Protophormia terraenovae* female (A) and male (B) and *Phormia regina* female (C) and male (D).

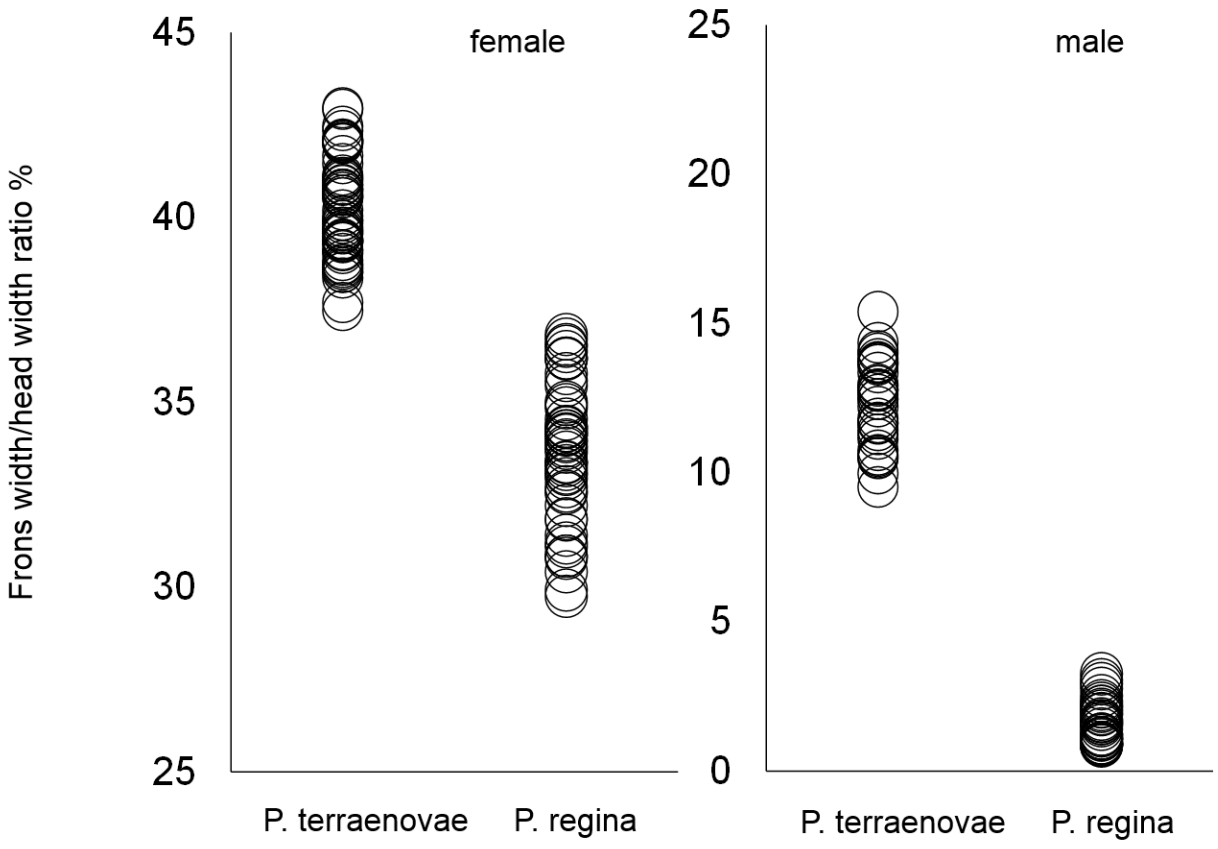


FIGURE 2.2. Distribution of frons width to head width ratios for *Protophormia terraenovae* and *Phormia regina* between females (left) and males (right).

TABLE 2.1. Total catches from each province used for the frons width to head width analysis.

Species	Sex	Province					
		British Columbia	Alberta	Saskatchewan	Ontario	New Brunswick	Newfoundland
Protophormia terraenovae	f	14	2	16	22	1	2
	m	7	1	7	16	2	1
Phormia regina	f	6	9	0	35	0	1
	m	2	6	0	29	0	0

CHAPTER 3

Urban and Rural Spatial Delineations in Blow Fly (Diptera: Calliphoridae) Species across Canada: Implications for Forensic Entomology

PREFACE

Title: **Urban and Rural Spatial Delineations in Blow Fly (Diptera: Calliphoridae) Species across Canada: Implications for Forensic Entomology**

Running Head: Spatial delineations of Canadian blowflies (for publication)

Authors: Sarah V Langer, Christopher J Kyle, Mike Illes, David V Beresford

Status: Formatted for submission to the Journal of Medical Entomology

Changes from publication version:

The figure and table numbers have been changed to fit with this thesis' format, including the removal of one figure from this chapter (in this thesis, FIGURE 1.2.), to avoid replicating the same figure throughout chapters.

ABSTRACT

Habitat selection and scale of selection are key questions in ecology that can have practical implications in forensic entomology where understanding the suite of species expected to be present can influence criminal proceedings. Specifically, blow flies (Diptera: Calliphoridae) are thought to exploit different niches that are associated with anthropogenic influences in rural and urban landscapes that provide different resources for different species. We explored the scale at which resources are tied to species presence and absence, where we sampled 7272 flies from 32 sites across Canada. We used mapping analysis to assess urban and rural landcover classifications, and tested urban and rural differences through abundance comparisons, and statistical measures including a principal component analysis and NMPANOVAs. We take these data to suggest that blow fly species compositions are more similar in urban areas across Canada than they are to their surrounding rural areas. We discuss the importance of this for forensic investigations.

Keywords: blow fly, Calliphoridae, urban, forensic, entomology, habitat selection

INTRODUCTION

The influence of urban, suburban, and rural habitat distinctions have been a focus of study across many taxonomic groups (Picket et al. 2017), including insects. Different species interacting with increasingly urban environments have been compared globally where similarities between urban habitats worldwide have been found (McKinney 2006). This global homogenization work also draws from island biogeography theories were isolated areas such as islands are generally found to have fewer species (Villa 1992). These anthropogenic impacts on species' distributions can be pronounced but varied, with distinctions being made as to how different landscapes influence species presence, density, and dispersion. For example, at one extreme, the bed bug *Cimex lectularius* (L.) has a close association with humans and human settlements, and thus is strongly associated with urban areas (Booth et al. 2015), though in general, few specialist species are found in urban areas (Forman 2016). At the other extreme, host-specific butterfly and moth communities have shown decline due to a loss of host plants (Niell et al. 2007) which has been seen in areas of greatest urban disturbance (McGeoch and Chown 1997). This rural-urban distinction may be less pronounced for species such as blow flies (Diptera: Calliphoridae), where their requirements are not as strictly associated with one habitat type but rather, with their ability to find breeding and feeding substrates that can occur across multiple types of habitat.

The most common forensic application of blow fly research is in estimating the post-mortem interval (PMI), meaning the time since a person died, based on the developmental stage and successional species composition of insects present on the corpse. Blow flies are an early colonizing species, and can arrive within minutes after

death (Catts and Goff 1992, Byrd and Castner 2009). Understanding this timeline of blow fly interactions with a corpse aids in estimating PMI. In a forensic investigation, an understanding of the species developing on the body, their known rates of development and the temperature experienced on or within the body can be used to make this estimation (Catts 1992). In terms of species succession, simply the presence of blow flies on a corpse can narrow the timeline based on their early arrival post-mortem (Catts 1992). The many blowfly species available for corpse colonization can have different developmental rates, making it imperative that unambiguous identifications are obtained for the larvae on the body (Wells 2008). This is especially important for species that are morphologically similar even in their adult stages, while greatly different in their development times. For example, *L. illustris* (Meigen) and *L. caesar* (L.) where only the former has been thoroughly studied (Gontran et al. 2012), or *L. sericata* (Meigen) and *L. cuprina* (Wiedemann) that are known to vary considerably with the former requiring nearly double the development time at 17°C (Stevens et al. 2002) can complicate PMI estimates if incorrectly identified. When the composition of local insects available for colonization changes between landscapes, records of species caught in an area can aid in understanding the species expected to be present on the body, and possibly provide evidence of corpse movement after colonization (Erzinclioglu 1989, Catts and Goff 1992, Grassburger 2004 Anderson 2009, Fremdt and Amendt 2014).

Blow fly species may have preferences between urban and rural habitats from their relative abundance in each area (Brundage et al. 2011). Some authors describe blow flies as being ‘very specific’ in their habitat preference, attributing this to resource partitioning (Anderson 2009). For blow flies seeking resources in urban or rural areas,

one key factor is the availability and preference of breeding substrate (Norris 1965). Areas with food, garbage and other organic materials are known to support pest species (Forman 2016), such as blow flies. Available garbage is closely associated with urbanization and is another factor consistent between urban areas (McKinney 2006). The ability to breed on garbage would be an important consideration for blow fly research in urban areas, but may not be as common a factor in rural areas (Norris 1965). In contrast, carcasses are ephemeral, inconsistent resources more common in rural areas (Towne 2000, Carter et al. 2007, Michaud and Moreau 2009). Carcass colonization by blow flies is not delayed in urban habitats (Anderson and VanLaerhoven 1996, Grassberger and Frank 2004), further supporting the idea that blow flies are already present in urban areas prior to an attractive substance becoming available. On a broad scale, differences between geographical areas can help determine which species are available to colonize carcasses as well as the variables controlling tissue decomposition (Anderson 2009). Systematic studies incorporating baited traps to acquire species data have been suggested to address such habitat associations (Fremdt and Amendt 2014).

Across Canada, several studies have aimed to document decomposition and blow fly species presence within a province (e.g., Anderson 1996, VanLaerhoven 1999, Anderson 2002, LeBlanc 2002, Simpson 2002, Gill 2005, Hobischak 2006, Sharanowski 2008, Anderson 2009). Ecologically based distinctions such as Canadian ecozones have also been used to define sampling areas (Sharanowski 2008) which includes generalized biological and geological distinctions (Natural Resources Canada 2016). Specific to British Columbia, biogeoclimactic zone classifications are available and have been used to compare differences in decomposition and blow fly species (Anderson 1996,

VanLaerhoven 1999). Further, many studies have described the blow fly species found in an area as an aid in forensic investigations (e.g., VanLaerhoven and Anderson 1999, Anderson et al. 2002, LeBlanc and Strongman 2002, Simpson and Strongman 2002, Archer and Elgar 2003, Archer 2003, Grassberger and Frank 2004, Tabor et al. 2005, Gill 2005, Hobischak et al. 2006, Gruner et al. 2007, Sharanowski et al. 2008, Matuszewski et al. 2010, Brundage et al. 2011, Nakano and Honda 2015, Weidner et al. 2015). These studies often stress that such data have limited applicability outside of the particular geographical area, and that further geographically specific studies are needed (Anderson 2009). Such inventories can direct investigators to focus on the species that are expected to be there and highlight a need for further inquiry when unexpected species are found (Archer and Elgar 2003, Richards et al. 2009, Brundage et al. 2011). For a forensic investigator attending a death scene, this understanding of expected species is a valuable tool for both processing the evidence and evaluating its value in understanding the crime.

A challenge in forensic entomology and the aforementioned studies is how urban and rural areas are defined, as definitions vary with the individual studies. Here, we have classified our areas using landcover mapping data available through the United States Geological Survey (USGS), which allowed us to standardize our perception of urban areas across Canada using landcover data (NRCan/CCRS and USGS. 2005). We suggest that this method of classifying areas eliminates the variability of our overall perception of an area, but it is important to highlight that any classification can be further complicated by the fact that what we perceive as urban and rural may not be at the same scale as a blow flies' perception. One important study in aimed to assess blow fly succession in an urban environment and found some species generally associated with rural areas

(Grassberger and Frank 2004). However, from their description, the extent of the urban green space near the carcass could have also played a role in the presence of these species. Urban ecology has highlighted green spaces as variables to species dispersal (Forman 2016).

The focus of this study is to assess blow fly species assemblages in urban areas, which is one critical variable used in many succession studies. We aim to determine whether blow fly communities are different between urban and rural habitats across Canada when using standardized urban classifications. We wanted to know the scale at which an urban habitat has an effect on the community of blow fly species in an urban area, and whether urban areas act as a distinct habitat across different ecozones (Natural Resources Canada 2016). We reasoned that the blow fly species in an urban area are some combination of two origins: 1) those blow fly species from the surrounding ecozone or rural areas (*H*) that can persist in an urban area, and 2) the blow fly species that the urban habitat contributes (*U*). Our hypotheses are presented in the following models. The general model can be expressed as:

$$D_{urban} = a*H + b*U \quad (1)$$

in which D_{urban} is a measure of species diversity in a particular urban area; *H* is the set of species found in the surrounding rural or ecozone habitat with *a* governing the urban effect on the ecozone species; *U* is the set of species that thrive in urban habitats with *b* governing the contribution of the urban species to the diversity of a particular urban area. The relative importance of *H* and *U* led us to two hypotheses:

1) blow flies in urban areas are those that are found in the surrounding rural area or ecozone that are capable of living in an urban habitat, that is

$$D_{urban} = a*H, \qquad 0 < a < 1, b = 0 \tag{2}$$

2) blow flies in urban areas are those that are associated with urban habitats as distinct habitats, irrespective of the surrounding rural area or ecozone, that is

$$D_{urban} = b*U, \qquad a = 0, 0 < b < 1 \tag{3}$$

Generally, the predictions derived from these hypotheses are based on whether the collection sites tend to group together by ecozone (H1) or urban and rural habitat type (H2). Specifically, under hypothesis 1, a PCA using individual species should cluster into ecozones that include urban and rural sites, whereas from hypothesis 2 sites should cluster into urban and rural groups. These data have the potential to assist and refine current applications of forensic entomology, most notably predictions of species assemblages, assessment of corpse movement, and PMI based on expected species in the habitat where a colonized corpse is found.

MATERIALS AND METHODS

Insect Sampling and Trap Design

Insects were trapped by volunteer Ontario Provincial Police (OPP) and Royal Canadian Mounted Police (RCMP) officers across Canada between 2011 and 2013 using baited bottle traps that followed the design of Langer et al. (2016). The catch bottle contained 100 millilitres of non-toxic plumbing antifreeze as a killing solution. Raw beef liver was used as bait as it attracts forensically important species of blow flies (Brundage et al. 2011) searching for carrion as a breeding substrate (Hayes et al. 1999). Traps were set out across Canada between the date ranges in TABLE 3.1. Each year we sampled during mid-season to assess the blow flies present in the warmest summer period.

Seasons were defined by mailout periods, as samples could not be deployed and collected on the same dates by our volunteers.

For our analysis we used 2011, 2012, and the second mailout of 2013 as replicates, due to their dates spanning approximately the same season. The first mailout of 2013 was used as an early-season data set and third mailout of 2013 as a late-season data set. Participating officers were mailed instructions and the components for two traps. Both traps were deployed by each officer at a recommended height of around 2 m, and at a recommended distance of 50 m apart in a site chosen by the officer. Insects were collected from both traps at the end of the first and second week, preserved in the non-toxic antifreeze in the catch bottle and returned in the mail.

Insect Curation and Identification

Returned samples were processed immediately upon receipt, and all insects were transferred to 80% ethanol. Blow flies of forensic relevance in the subfamilies Lucillinae, Calliphorinae and Chrysomyinae were pinned, then identified using the keys of Marshall et al. (2011), Whitworth (2006) and Hall (1948). Genetic validation was preformed on a subset of our samples using the COI mitochondrial region. All pinned specimens were archived in the Trent University insect collection.

Mapping

ArcMAP (ESRI 2011) was used to objectively categorize the urban distinction. All trapping locations were projected in NAD1983 on a map from the North American Land Change Monitoring System in ArcGIS, which was based on 19 categories of landcover data at a resolution of 250m (NRCan/CCRS and USGS 2005). We combined all rural and wilderness landcover categories (1-16 and 19) to represent the rural category

in further analyses, while category 17, urban, and 18, water, were kept separate (TABLE A.1.). Zones were created around each GPS point with radii of 250 m, 500 m, 1 km, 5 km, 10 km, 15 km, 20 km and 25 km, and the percent landcover types were determined. The largest radius of 25 km was chosen to approximate the longest distances of blow fly dispersal from mark-recapture studies reported by Norris (1965). Water was then excluded to calculate percent landcover with urban and rural values only. To group sample locations for comparison in further analyses, locations with urban landcover equal to or greater than 90% were categorized as urban, 0% urban were categorized as rural, and between 0% and 90% were categorized as intermediate. These definitions of urban, rural and intermediate were used for all further analyses.

Statistical Analysis

The program PAST (Hammer et al. 2001) was used to assess significance of variance between species assemblages through several tests: non-parametric multivariate analysis of variance (NPMANOVA) using Bray-Curtis as the similarity measure, a rarefaction analysis, and a Principal Component Analysis (PCA). Trap catch numbers of each species were normalized using a logarithmic transformation (ln(1+N)). Bray-Curtis was chosen as the similarity measure as it allows abundance-based species comparisons to be made on rank rather than numbers, and thus is not sensitive to collection differences between samples.

A two-way NPMANOVA analysis assessed the sites using two factors, first the category of ecozone and second, whether a site was urban or rural. Under H1 the ecozone was expected to have the greater influence on the blow fly community similarity (H in the model, equation 2), whereas under H2, if a site is urban or not will have the greater

influence on the blow fly community similarity at the various collection sites (U in the model, equation 3). For this, we used an NPMANOVA.

RESULTS

We caught 7,272 individuals representing 14 species over 3 years of sampling (TABLE 3.2.). The most commonly caught species was *Phormia regina* (Meigen), 3,377 specimens (46%). In all participating provinces except Newfoundland and Saskatchewan, the two most common species overall, *P regina* (Meigen) and *C. cadaverina* Robineau-Desvoidy, were also the most common within that province (TABLE A.2a-c.). In Saskatchewan, however, *P. regina* (Meigen) was the third most common, and in Newfoundland, the most common species were *C. vomitoria* (L.) and *L. illustris* (Meigen).

Urban Effect on Blow Fly Species

An NPMANOVA was performed using the Bray-Curtis similarity measure on species counts from mid-season data of 2011-2013 (TABLE 3.3.) using 77 sets of traps and species data from all 14 species. These data were used to compare the three categories of landcover derived from ArcGIS (urban, intermediate and rural) at each radius to determine the scale showing most significant effect of landcover using our species data. Radii over 5 km did not produce urban areas over 90% or rural areas where urban values were 0%, and thus were not compared. The greatest effect of urban land cover was found using a 500 m radius when comparing urban and rural areas as well as urban and intermediate areas ($F = 3.63$, $p < 0.01$) (TABLE 3.2).

In mid-season (FIGURE 3.1.a), all 14 species were present in areas in the rural category. Two species, *C. stelviana* (Brauer & Bergenstamm) and *L. silvarum* (Meigen)

were not present in the intermediate category, leaving 12 species present. These species are also absent from the urban category, along with *C. livida* Hall, *L. coeruleiviridis* Macquart, *C. montana* (Shannon) and *C. lowei* Enderlein leaving 8 species present. *P. regina* (Meigen) had the greatest contribution to the dataset in rural and intermediate settings; however, *C. cadaverina* Robineau-Desvoidy was the most common species in urban settings.

In early-season (FIGURE 3.1.b), the 9 most abundant species overall were present. These 9 species were found in both rural and intermediate categories. Four species, *P. regina* (Meigen), *P. terraenovae* (Robineau-Desvoidy), *C. vomitoria* (L.) and *L. illustris* (Meigen), were not found in early-season urban areas.

In late season (FIGURE 3.1.c), 10 species were captured overall, with some differences in species between late- and early-season catches. All 10 species were found in both rural and intermediate areas. The only species found in urban areas was *C. vicina* Robineau-Desvoidy; however, only one location in this sampling session was classified as urban. Between early- and late-season catches, *C. livida* Hall was found in early-season and not late-season, whereas *L. coeruleiviridis* Macquart and *C. loewi* Enderlein were found in late-season and not early-season.

To address whether the decrease of species in urban areas relative to rural and intermediate could be explained simply by sample size, we performed a rarefaction analysis (FIGURE 3.2.a-b) on all mid-season samples. The rural curve with the greatest number of species began to plateau within the limits of our sampling effort, suggesting that more samples would not necessarily gain more species. The intermediate category plateaus much earlier. The urban samples did not reach this plateau; however, the shape

of the curve lies much lower than the others, suggesting that it would not reach the number of species obtained in intermediate and rural categories as samples increased.

Urban Effect Considering Ecozone

Species data for each season were tested by ecozone (TABLE A.3a-c.). A PCA was used to show how each sampling location clustered when species data were compared using our largest single dataset: the 2011 mid-season (FIGURE 3.3.a-c). The same distribution of points is shown in both FIGURE 3.3.a and b, first categorized by urban, intermediate and rural categories (FIGURE 3.3.a) then by the seven ecozone categories: Pacific Maritime, Montane Cordillera, Boreal Plains, Prairies, Boreal Shield, Mixwood Plains and Atlantic Maritime (FIGURE 3.3.b). PC1 explained 53.11% of the variance, whereas PC2 explained 11.98% of variance. Urban points formed a cluster tighter than intermediate and rural, and were found only on the negative side of PC1. In comparison, intermediate points cover a larger area on PC2, and covering both sides of PC1. Rural points have the largest distribution, still moving further to the positive side of PC1 and covering the largest area of PC2. When ecozones are compared, some zones fall more to one side or another of each component, such as Montane Cordillera on the negative side of PC2. Species loadings all appear on the positive side of PC1, opposite of the urban classified locations (FIGURE 3.3.c).

Finally, a 2-way NPMANOVA was performed using Bray-Curtis similarity measure to determine whether a greater effect on the data could be explained by urban and rural categories, or by ecozone categories at the 500m radius (TABLE 3.4.). Significance was seen for mid-season data with urban and rural categories in 2011 ($F = 0.9499$, d.f. = 1, $p < 0.05$) and 2013 ($F = 0.9750$, d.f. = 1, $p < 0.05$). Though ecozone

categories showed significant differences in the 2013 mid-season data ($F = 0.4476$, d.f. = 1, $p < 0.05$), there were no differences between categories in the 2011 mid-season data ($F = 0.0953$, d.f. = 1, $p > 0.05$)., The early-season 2013 species differences were significant between urban and rural categories ($F = 0.3710$, d.f. = 1, $p < 0.05$) whereas 2013's late-season data showed significance differences in species between ecozone categories ($F = 0.1063$, d.f. = 1, $p > 0.05$). The interaction for this test was not significant ($p>0.05$) for any of the sample sets.

DISCUSSION

Our data revealed differences in the blow fly communities between urban and rural categories across Canada. We found that urban areas have an effect on blow fly communities, even when considering ecozone (TABLE 3.4.); however, no one species was found exclusively in urban areas (FIGURE 3.1.a-c). From the model in equation 1, we reasoned that blow fly communities in urban areas were determined by some combination of the rural habitat (*H*) and urban influence (*U*); we devised two hypotheses suggesting that either urban blow flies are from the surrounding rural area or ecozone and are capable of living in an urban habitat (hypothesis 1, equation 2), or that blow flies in urban areas are those that are associated with urban habitats as distinct habitats, irrespective of the surrounding rural area or ecozone (hypothesis 2, equation 3).

Overall, our results show that *H* and *U* are both important when understanding the blow fly community in urban areas. In support of hypothesis 1 (equation 2), we found that urban areas did not harbour unique species (FIGURE 3.1.a-c). From this, we suggest that the species present in an urban area are not the result of an impoverished rural landscape as equation 3 implies. Therefore, a difference is found between urban and rural

blow fly communities, is not because of urban areas having a distinct community impervious to adjacent rural habitats.

We also found support for equation 3, where urban areas appear to be distinct, irrespective of the surrounding rural area. First, the shift in most common species catches within urban suggests that the blow flies are not just rural species capable of living in urban areas. For example, in our mid-season data, species abundance shifted between rural and urban areas where *C. cadaverina* Robineau-Desvoidy becomes the most common species in urban areas, but still with a strong presence in rural landscapes (FIGURE 3.1.a). Second, our PCA showed a tighter cluster of urban locations entirely on the negative side of PC1, with less similarity within intermediate locations or rural locations (FIGURE 3.3.a). Third, our two-way NPMANOVA (TABLE 3.4.) showed significant differences between urban and rural in 3 of 4 datasets, and ecozones in 2 of 4 datasets. This supports our hypotheses that blow flies in urban areas are those that are associated with urban habitats rather than specific to the surrounding rural area or ecozone. The effect of urban landcover was strongest (TABLE 3.3.) when landcover was assessed at a radius of 500m from the trapping site. The results of the intermediate category presented an interesting point of discussion as it highlights the continuum of area classifications when calling areas either urban or rural. Across urban and rural gradients, others have also found species assembled into distinct communities rather than random combinations as areas become more urban or rural (McKinney 2006). Regional differences in species abundances were also seen between provinces (TABLE A.2a-c.).

Here, we discuss the species patterns shown in our dataset between urban, intermediate and rural habitats, rather than by habitat, to highlight the relative abundances

and differences between habitat compared to the literature. The 14 species caught in our study (TABLE 3.2.) are discussed below with reference to literature comparing arrival times and habitat types.

P. regina (Meigen), our most commonly caught species, was found in high numbers in both urban and rural environments (FIGURE 3.1.a-c) which agrees with the literature (Anderson and VanLaerhoven 1996, Tabor et al. 2005, Gill 2005, Sharanowski et al. 2008). Though there was a decrease in counts in urban areas relative to intermediate and rural, this species still represented a large portion of urban catches (e.g., 40.96% of rural, 54.81% of intermediate, 13.83% of urban mid-season catches). In the literature, *P. regina* (Meigen) is considered a dominant species for several studies that concentrated on small, local areas (Tabor et al. 2005, Gill 2005, Sharanowski et al. 2008). This species remained the most abundant species in a Manitoba study for both sunlit and shaded carrion in the summer, fall and spring (Gill 2005). *P. regina* (Meigen) has no significant preference between habitat and season together (Brundage et al. 2011), and is reported as common in both urban and rural areas (Anderson and VanLaerhoven 1996) as well as sunlit and shaded areas (Anderson et al. 2002, Gill 2005), though others have found this species to be highly dependent on season (Benbow et al. 2013). For instance, this species was common in the summer months in urban and rural areas in Nova Scotia (Simpson 2002), but was not commonly found in similar studies in the fall (LeBlanc and Strongman 2002, Michaud and Moreau 2009). In a Saskatchewan study, this species was found on sunlit areas (Sharanowski et al. 2008). *P. regina* (Meigen) is considered a "summer active species" in South Carolina (Tomberlin and Adler 1998), though others consider it to be a cold weather species (Byrd and Castner 2009); however, this may be

attributed to geographical area, where Canadian summers can be cooler. For instance, this species was found in the winter of a Texas study (Mohr and Tomberlin 2014). We found *P. regina* (Meigen) in rural and intermediate areas as well in our early-season (72.88% of rural, 57.86% of intermediate catches) and late-season (16.99% of rural and 14.99% of intermediate catches) data. Some report this species to arrive a day or two after death (Anderson 2009) during later PMI stages (Benbow et al. 2013, Mohr and Tomberlin 2014). Studies from British Columbia (Anderson and VanLaerhoven 1996), Alberta (Anderson et al. 2002), Manitoba (Gill 2005) and Ontario (Anderson 2009) found that adults showed interest in the remains shortly after death. With *P. regina* (Meigen) representing a high proportion of species in our dataset within each habitat and season, our data agree with the previous reports that this is an abundant species.

C. cadaverina Robineau-Desvoidy was our second most common species overall, with strong numbers in rural areas (e.g., 21.40% of rural mid-season catches); however, this species showed the greatest numbers overall for urban areas for our mid- and early-season data (61.01% of urban mid-season and 71.23% of urban early-season catches). In previous studies, this species was found in cooler weather, during the spring and fall months, and can overwinter in houses (Byrd and Castner 2009). A rural Manitoba study found this species exclusively in the spring (Gill 2005) and it was one of the most abundant species in shaded carrion in a spring Saskatchewan study (Sharanowski et al. 2008). This was the only species found on both urban and rural placed pigs in a Nova Scotia study, and was also commonly captured by sweep net in urban wooded areas (Simpson and Strongman 2002) *C. cadaverina* Robineau-Desvoidy is attracted to feces, in addition to carrion in later stages of decomposition (Byrd and Castner 2009). In our

study, this species showed strong numbers in both urban and rural areas, which is not unusual from the literature, but the shift in this species outnumbering *P. regina* (Meigen) in our mid-season urban samples (*C. cadaverina* Robineau-Desvoidy representing 61.01% versus *P. regina* (Meigen) representing 13.83%) is not mirrored in the literature.

P. terraenovae Robineau-Desvoidy was found in each area, but in greatest numbers from the intermediate area (0.94% of rural, 19.35% of intermediate, 3.77% of urban mid-season catches). This species has been described as the northern-counterpart to *P. regina* (Meigen). It is found in cool climates and can be found in the early spring in Canada (Byrd and Castner 2009). Other authors note this species as common in the spring and in warmer areas (Anderson et al. 2002). *P. terraenovae* (Robineau-Desvoidy) was one of the most abundant species in both sunlit and shaded carrion in a Saskatchewan study of carcasses in an agricultural prairie area (Sharanowski et al. 2008). This species was also very abundant on many of the carcasses in Alberta where pigs were placed in a forested and agricultural areas (Anderson et al. 2002, Hobischak et al. 2006). A Manitoba study found this species in the summer, fall and spring months in a rural area, but in low numbers (Gill 2005). Many larvae of this species were collected from carcasses in a forested area in British Columbia (VanLaerhoven and Anderson 1999). We also found this species represented in our early-season collections (1.96% of rural, 15.71% of intermediate catches) and late-season collections (0.65% of rural, 0.49% of intermediate catches) but this species was found only in urban areas in our mid-season samples. A gap in our knowledge of this species in urban areas is seen in the literature. Our work suggests that this species is more likely to be found in urban areas in warmer parts of the year; however more work would be needed to test this further.

C. vomitoria (L.) was found in rural, intermediate and urban areas in our mid-season data, though decreased in number as areas became more urban (9.72% of rural, 2.68% of intermediate, 1.26% of urban catches). In early- and late-season sampling, this species was absent from urban areas. This species is reported as a rural and shade species in the spring and summer months by many authors (Smith 1986, Anderson and VanLaerhoven 1996, LeBlanc and Strongman 2002, Schroeder et al. 2003, Hwang and Turner 2005), although this may not hold true in all cases. One study reports a large number of this species in an urban backyard, and notes that this species can no longer be considered to indicate the area in which the body was colonized (Grassberger and Frank 2004), though the urban area included in this study had a large green space and therefore may not be urban to a blow fly. *C. vomitoria* (L.) is also known to prefer large carcasses, which are produced in a rural environment (Norris 1965) and are less abundant in urban areas (LeBlanc and Strongman 2002). One study from Nova Scotia showed that individuals of this species were predominantly collected in a park setting within an urban area (LeBlanc and Strongman 2002). Other sources suggest this species prefers shaded wooded areas but can also be found in suburban areas (Byrd and Castner 2009). A rural Manitoba study notes this species in the fall only (Gill 2005). We found a large proportion of this species in intermediate areas in late season (9.15% of rural, 39.07% of intermediate, 0% of urban catches). Our findings correspond to the literature in that this species showed strong late-season numbers, and was more commonly captured in rural areas, though not exclusive to the rural habitat.

L. illustris (Meigen) was found in rural and intermediate areas in high numbers, but decreased in urban areas in our mid-season samples (6.91% of rural, 8.72% of

intermediate, 1.26% of urban mid-season catches) and was absent from urban in our early- and late-season samples. This is described as very common, often found in open woodlands in the summer (Byrd and Castner 2009). A rural Manitoba study noted this species arrives shortly after death, then to returns several days later in the summer, spring and fall in both shaded and sunlit areas (Gill 2005). This species was also commonly collected from both urban and rural areas in Nova Scotia (Simpson 2002). This species occurred within minutes of death for research conducted in British Columbia however in England, was delayed for 2-3 days (Anderson 2009). Other authors have noted their quick arrival on remains (Byrd and Castner 2009). One Saskatchewan successional study noted this species as uncommon when surveyed from an agricultural prairie area (Sharanowski et al. 2008). This species was our 5th most common species overall, which seems reasonable given its reported "very common" and "uncommon" descriptions. Our finding of this species in both urban and rural areas corresponds to Simpson's (2002) work.

C. terraenovae Macquart was found in rural and intermediate areas, decreasing in urban areas (8.76% of rural, 0.86% of intermediate, 0.63% of urban mid-season catches) and not present in late-season urban areas, which agrees with previous studies. In one California study, this species was found in cooler shaded areas with lower numbers at other sites including urban sites (Nakano and Honda 2015). This species has also been found in to favour fish carcasses (Meehan and Seminet-Reneau 2005), though can be found in fish and non-fish baited traps (Nakano and Honda 2015).

C. vicina Robineau-Desvoidy was found in rural, intermediate and urban areas with a spike in urban catches (e.g., 4.66% of rural, 3.04% of intermediate, 6.92% of

urban mid-season catches). This species is reported as primarily urban caught (Smith 1986, LeBlanc and Strongman 2002, Hwang and Turner 2005, Byrd and Castner 2009, Pinilla et al. 2012). This species prefers cooler weather (Byrd and Castner 2009, Gruner et al. 2007, Fremdt and Amendt 2014) and is considered a winter species in the southern United States (Gruner et al. 2007), a spring and fall species in temperate zones, and a summer species in subpolar regions (Byrd and Castner 2009). We caught this species in all areas in our early-season (3.76% of rural, 3.67% of intermediate, 17.81% of urban catches) and late-season (6.54% of rural, 5.90% of intermediate, 100% of urban catches) samples. This was the only species found in late-season urban however only one location was assigned urban from the late-season data. In some studies *C. vicina* is found throughout the year (Schroeder et al. 2003). Interestingly, in a Manitoba study, this species was found exclusively on the sunlit carrion in the summer, and on shaded carrion in the spring (Gill 2005). This species arrived on day 8 of shaded carrion in Saskatchewan (Sharanowski et al. 2008) and on day 24 on sunlit carrion in the summer in Manitoba (Gill 2005). Though this species showed similar proportions within habitats in our work, the higher proportion of this species in urban catches compared to its proportion in rural catches agrees with reported literature.

L. sericata (Meigen) was found in rural, intermediate and urban areas, and made up a large proportion of urban catches (e.g., 3.79% of rural, 3.00% of intermediate, 11.32% of urban catches), but was not found in urban areas in our late-season data. In the literature, this species is said to be found in mainly urban areas (Anderson and VanLaerhoven 1996, LeBlanc and Strongman 2002, Schroder 2003, Brundage et al. 2011, Fremdt and Amendt 2014). *L. sericata* (Meigen) has been noted in many studies as

warmth-loving and often preferring sunlit carrion (Anderson et al. 2002, Schroder 2003, Gill 2005). This species does not have a strict preference for carrion (Byrd and Castner 2009), and has been observed laying eggs and developing on garbage (Brundage et al. 2011), even garbage consisting entirely of vegetable matter (Norris 1965), which may add to the evidence that they are well suited to inhabit an urban area. Additionally, although historically having a Holarctic range, this species is now found in a variety of areas (Byrd and Castner 2009). This species is very common in western Canada, and can be found worldwide (Byrd and Castner 2009). Others have found few of this species in their spring studies, with more presence in the summer (Sharanowski et al. 2008). This species made up a larger proportion of total catch in our mid-season catches than it did in our early-season (0.65% of rural, 1.62% of intermediate, 4.11% of urban catches) or late-season (2.62% of rural, 0.98% of intermediate, 0% of urban catches). Interestingly, we did find this species in early-season, though higher proportions of this species were seen in the mid-season data which aligns with the above literature. An increased proportion was seen in urban areas in our mid-season and early-season data which also aligns with others work above.

The final six species in our dataset, each with catches less than 100 individuals, were all found in rural areas. Of these, *L. silvarum* (Meigen) and *C. stelviana* (Brauer & Bergenstamm) were exclusively found in rural areas, with the rest showing a decrease in numbers for intermediate habitats except for *C. loewi* Enderlein which had higher numbers in that category (FIGURE 3.1.a). None of these species were found in our urban samples. *C. livida* Hall was found during the coldest days of the year in rural Florida (Gruner et al. 2007) and was said to be an indicator of rural areas in spring in a New

Jersey study (Weidner et al. 2015). *L. coeruleiviridis* Macquart is attracted to almost all decaying animal material and has been described as one of the most common species in the fresh stage of decomposition (Byrd and Castner 2009). In an Ohio study, this species was shown to be dependent on season, with a high presence in summer and autumn during the fresh stage of decomposition (Benbow et al. 2013). *C. montana* (Shannon) is found in North America at lower elevations, east of the Rocky Mountains. Sparse data exists for this species. *C. loewi* Enderlein is said to be a rural species (Hwang and Turner 2005). *L. silvarum* (Meigen) is most frequently collected west of the Rocky Mountains, and during the summer (Byrd and Castner 2009). In one Saskatchewan study, this species was found in low numbers and only on shaded carrion (Sharanowski et al. 2008). This species is also known to parasitize amphibians, and remains active in the evening in low light (Byrd and Castner 2009). This species can be caught in higher numbers when fish-baited traps are used (Nakano and Honda 2015). Finally, *C. stelviana* (Brauer & Bergenstamm) is a Holarctic species found in both urban and rural environments (Hwang and Turner 2005, Malewski et al. 2010). The presence of these species in our rural data aligns with the literature above.

Though the majority of research comparing urban and rural habitats do not specify how their areas were determined to be urban or rural, these studies often agree on which species can be predominately found in each area. Studies suggesting urban or rural preferences between blow fly species use categories in addition where each species was caught. For instance, between seasons where others have found that habitat is a variable in the rate of decomposition only in the spring (Sharanowski et al. 2008). While previous studies examined blow fly distribution from localized regions, our study examined

comprehensive blow fly distribution on a Canada-wide scale to find that urban areas have a greater effect on blow fly community structure than the surrounding rural areas, and provides information about the scale that this effect is seen. Our observations show that urban areas are more similar to one another than they are to rural areas when habitat is considered at a 500m radius. This effect is seen even when ecozones are considered. This relationship suggests that species data from urban areas may be comparable to other urban areas when local species surveys are not available. Though urban areas influence the community structure of blow flies, classifying individual species as either urban or rural may not tell the whole story. This highlights the importance of using urban and rural species classifications as rough estimates of expected finds rather than exclusive categories when assessing the species developing on a body, as well as future research continuing to compare urban and rural areas across large geographical areas. Additionally, through our data, urban and rural differences affect the community structure and relative abundances of species captured. As we found that all urban caught species were also found in rural areas, a body moved from an urban to rural habitat post-colonization may not be apparent; the body would not show species indicative of urban colonization. With the ever-growing pressure to enhance the rigor of forensic applications, it is important to acknowledge limitations of our assumptions and when investigative finds can be used as evidence or little more than suggest possible investigative leads. Continued research contributions on blow fly habitat preferences will be needed to enhance forensic entomological applications, their accuracy and expectations.

REFERENCES

Anderson, G. S. 2009. Factors that influence insect succession on carrion. *In* J.H. Byrd and J.L. Castner (eds). Forensic entomology: the utility of arthropods in legal investigations, 2nd ed. CRC Press, Boca Raton FL.

Anderson, G. S., and S. L. VanLaerhoven. 1996. Initial studies on insect succession on carrion in southwestern British Columbia. J Forensic Sci. 41: 617-625.

Anderson, G. S., N. R. Hobischak, O. Beattie, and C. Samborski. 2002. Insect succession on carrion in the Edmonton, Alberta region of Canada. Technical report TR-04-2002, Canadian Police Research Centre, Ottawa, Ontario.

Archer, M. S. 2003. Annual variation in arrival and departure times of carrion insects at carcasses: implications for succession studies in forensic entomology. Aust J Zool. 51:569–576.

Archer, M. S., and M. A. Elgar. 2003. Yearly activity patterns in southern Victoria (Australia) of seasonally active carrion insects. Forensic Sci Int. 132: 173-176.

Benbow, M. E., A. J. Lewis, J. K. Tomberlin, and J. L. Pechal. 2013. Seasonal necrophagous insect community assembly during vertebrate carrion decomposition. J Med Entomol. 50(2): 440-450.

Booth, W., O. Balvín, E. L. Vargo, J. Vilímová, and C. Schal. 2015. Host association drives genetic divergence in the bed bug, Cimex lectularius. Mol Eco. 24:980-992.

Brundage, A., S. Bros, and J. Y. Honda. 2011. Seasonal and habitat abundance and distribution of some forensically important blow flies (Diptera: Caliphoridae) in Central California. Forensic Sci Int. 212(1-3):115-120.

Byrd, J. H., and J. L. 2009. Castner. Insects of forensic importance. *In* J.H. Byrd JH and J.L. Castner (eds.), Forensic entomology: the utility of arthropods in legal investigations, 2nd ed. CRC Press, Boca Raton FL.

Carter, D. O., D. Yellowlees, and M. Tibbett. 2007. Cadaver decomposition in terrestrial ecosystems. Naturwissenschaften. 94:12–24.

Catts, E. P., and M. L. Goff. 1992. Forensic entomology in criminal investigations. Annu Rev Entomol. 37:253-272.

Erzinclioglu, Z. 1989. Entomology and the forensic scientist: how insects can solve crimes. J Biol Ed. 23:300-302.

(ESRI) Environmental Systems Research Institute. 2011. ArcGIS desktop version 10.2. USDA, Redlands, CA.

Forman, R. T. T. 2016. Urban ecology principals: are urban ecology and natural area ecology really different? Landscape Ecol. 31:1653-1662.

Fremdt, H., and J. Amendt. 2014. Species composition of forensically important blow flies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) through space and time. Forensic Sci Int. 236:1-9.

Gill, G. J. 2005. Decompositional and arthropod succession on above ground pig carrion in rural Manitoba. Technical report TR-06-2005, Canadian Police Research Centre, Ottawa, Ontario.

Gontran, S., K. Jordaens, Y. Braet, and S. Desmyter. Why is the molecular identification of the forensically important blowfly species Lucilia caesar and L. illustris (family Calliphoridae) so problematic? Forensic Sci Int. 223:153-159.

Grassberger, M., and C. Frank. 2004. Study of arthropod succession on pig carrion in a central European urban habitat. J Med Entomol. 41(3):511-523.

Gruner, S. V., D. H. Slone, and J. L. Capinera. 2007. Forensically important Calliphoridae (Diptera) associated with pig carrion in rural north-central Florida. J Med Ent. 44(3):509-515.

Hall, D. G. 1948. The blowflies of North America. Thomas Say Foundation: Lafayette, Indiana: USA.

Hammer, Ø., D. A. T. Harper, and P. D. Ryan. 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron. 4(1):1-9.

Hayes, E. J., R. Wall, and K. E. Smith. 1999. Mortality rate, reproductive output, and trap response bias in populations of the blow fly Lucilia sericata. Ecol Entomol. 24(3):300-307.

Hobischak, N. R., S. L. VanLaerhoven, and G. S. Anderson. 2006. Successional patterns of diversity in insect fauna on carrion in sun and shade in the boreal forest region of Canada, near Edmonton, Alberta. Can Entomol. 138:76-83.

Hwang, C., and B. D. Turner. 2005. Spatial and temporal variability of necrophagous Diptera from urban to rural areas. Med Vet Entomol. 19:379–391.

Langer, S. V., C. J. Kyle, and D. V. Beresford. 2016. Using frons width to differentiate blow fly species (Diptera: Calliphoridae) *Phormia regina* (Meigen) and *Protophormia terraenovae* (Robineau-Desvoidy). J Forensic Sci. 62(2):473-475.

LeBlanc, H. N., and D. B. Strongman. 2002. Carrion insects associated with small pig carcasses during fall in Nova Scotia. Can Soc Forensic Sci J. 35(3):145-152.

Malewski, T., A. Draber-Mońko, J. Pomorski, M. Loś, and W. Bogdanowicz. 2010. Identification of forensically important blowfly species (Diptera: Calliphoridae) by high resolution melting PCR analysis. Int J Legal Med. 124:277-285.

Marshall, S. A., T. Whitworth, and L. Roscoe. 2011. Blow flies (Diptera: Calliphoridae) of eastern Canada with a key to Calliphoridae subfamilies and genera of eastern North America, and a key to the eastern Canadian species of Calliphorinae, Luciliinae and Chrysomyiinae. Can J Arthropod Ident. 11:1-93.

Matuszewski, S., D. Bajerlein, S. Konwerski, and K. Szpila. 2010. Insect succession and carrion decomposition in selected forests of central Europe. Part 1: pattern and rate of decomposition. Forensic Sci Int. 194:85-93.

McGeoch, M. A., and S. L. Chown. 1997. Impact of urbanization on a gall-inhabiting Lepidoptera assemblage: the importance of reserves in urban areas. Biodivers Conserv. 6:979-993.

McKinney, M. L. 2006. Urbanization as a major cause of biotic homogenization. Biol Conserv. 127(3):247-260.

Meehan, E. P., and E. E. Seminet-Reneau. 2005. Bear predation on pacific salmon facilitates colonization of carcasses by fly maggots. Am Midl Nat. 153:142-151.

Michaud, J. P., and G. Moreau. 2009. Predicting the visitation of carcasses by carrion-related insects under different rates of degree-day accumulation. Forensic Sci Int. 185:78-83.

Mohr, R. M., and J. K. Tomberlin. 2014. Environmental factors affecting early carcass attendance by four species of blow flies (Diptera: Calliphoridae) in Texas. J Med Ent. 51(3):702-708.

Nakano, A., and J. Honda. 2015. Use of DNA sequences to identify forensically important fly species and their distribution in the costal region of Central California. Forensic Sci Int. 253:1-13.

Natural Resources Canada. 2016. Forest classification. www.nrcan.gc.ca/forests/measuring-reporting/classification/13179

Niell, R. S., P. F. Brussard, and D. D. Murphy. 2007. Butterfly community composition and oak woodland vegetation response to rural residential development. Landsc Urban Plan. 81:235-245.

Norris, K. R. 1965. The binomics of blow flies. Annu Rev Entomol. 10:47-68.

(NRCan/CCRS, USGS) Natural Resources Canada/Canadian Center for Remote Sensing, United States Geological Survey; Insituto Nacional de Estadística y Geografía (INEGI), Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) and Comisión Nacional Forestal (CONAFOR). 2005. 2005 North American Land Cover at 250 m spatial resolution. NRCan/CCRS and USGS: CAN and USA.

Pearman, P. B., A. Guisan, O. Broennimann, and C. F. Randin. 2008. Niche dynamics in space and time. Cell. 23(3):149-158.

Pickett, S. T. A., M. L. Cadenasso, E. j. Rosi-Marshall, K. T. Belt, P. M. Groffman, J. M. Grove, E. G. Irwin, S. S. Kaushal, S. L. LaDeau, C. H. Nilon, C. M. Swan, P. S. Warren. 2017. Dynamic heterogeneity: a framework to promote ecological integration and hypothesis generation in urban systems. Urban Ecosyst. 20:1-14.

Pinilla Beltran, Y. T., N. A. Segura, and F. J. Bello. 2012. Synanthropy of Calliphoridae and Sarcophagidae (Diptera) in Bogota, Columbia. Neotrop Entomol. 41:237-242.

Pulliam, H. R. 2000. On the relationship between niche and distribution. Ecol Letters. 3: 349-361.

Richards, C. S., K. A. Williams, and M. H. Villet. 2009. Predicting geographic distribution of seven forensically significant blowfly species (Diptera: Calliphoridae) in South Africa. Afr Entomol. 17(2):170-182.

Schroeder, H, H. Klotzbach, and K. Puschel. 2003. Insects' colonization of human corpses in warm and cold season. Legal Med. 5:372-374.

Sharanowski, B. J., E. G. Walker, and G. S. Anderson. 2008. Insect succession and decomposition patterns on shaded and sunlit carrion in Saskatchewan in three different seasons. For Sci Int. 179:219-240.

Simpson, G., and D. B. Strongman. 2002. Carrion insects on pig carcasses at a rural and urban site in Nova Scotia. Can Soc Forensic Sci J. 35(3):123-143.

Smith, K. G. V. 1986. A manual of forensic entomology. British Museum (Natural History), London, and Cornell University Press, Ithaca, NY.

Stevens, J. R., R. Wall, J. D. Wells. Paraphyly in Hawaiian hybrid blowfly populations and the evolutionary history of anthropophilic species. Insect Mol Bio. 11(2):141-148.

Tabor, K., R. Fell, and C. Brewster. 2005. Insect fauna visiting carrion in southwest Virginia. Forensic Sci Int. 150:73-80.

Tomberlin, J. K., and P. H. Adler. 1998. Seasonal colonization and decomposition of rat carrion in water and on land in an open field in South Carolina. J Med Entomol. 35:704-709.

Towne, E. G. 2000. Prairie vegetation and soil nutrient responses to ungulate carcasses. Oecologia. 122:232–239.

VanLaerhoven, S. L., and G. S. Anderson. 1999. Insect succession on buried carrion in two biogeoclimatic zones of British Columbia. J Forensic Sci. 44: 31-41.

Villa, F., Rossi, O., and F. Sartore. 1992. Understanding the role of chronic environmental disturbance in the context of island biogeographic theory. Environ Manage. 16(5):653-666.

Weidner, L. M., D. E. Jennings, J. K. Tomberlin, and G. C. Hamilton. 2015. Seasonal and geographic variation in biodiversity of forensically important blow flies (Diptera: Calliphoridae) in New Jersey, USA. J Med Ent. 52(5):937-946.

Wells, J. D., J. R. Stevens. 2008. Application of DNA-based methods in forensic entomology. Annu Rev Entomol. 53:103-120.

Whitworth, T. 2006. Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. Proc Entomol Soc Wash. 108(3):689-725.

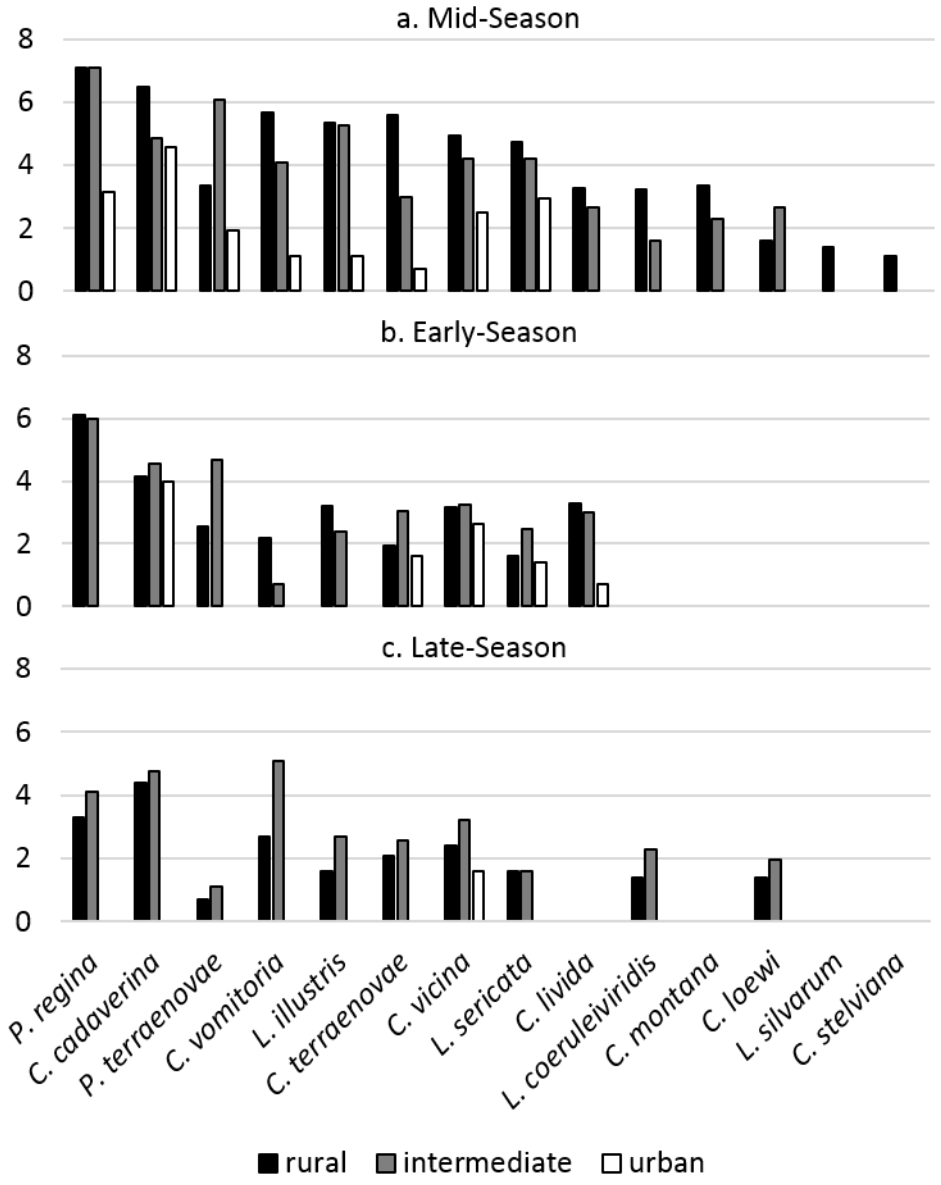


FIGURE 3.1. a-c Log scaled counts for each species collected in rural, intermediate, and urban environments within a 500 m radius for a. mid-season b. early-season and c. late-season catches.

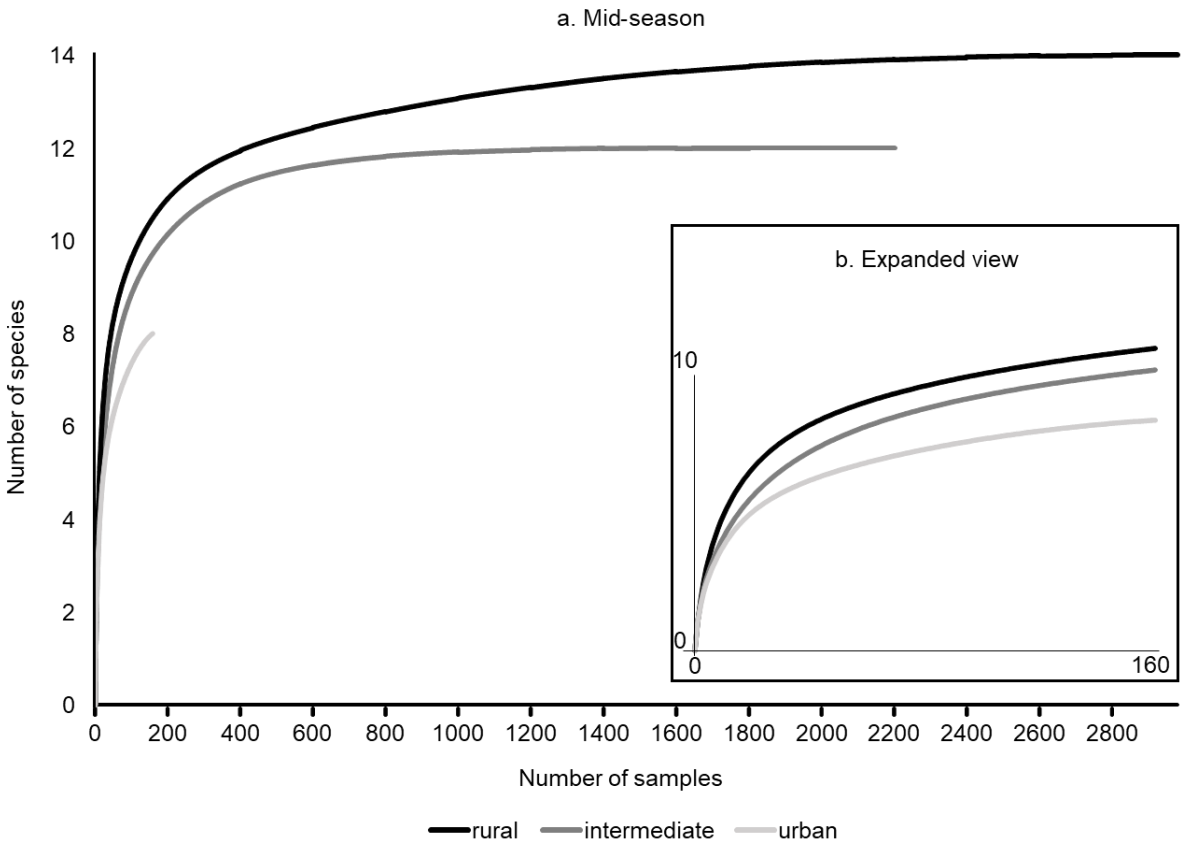


FIGURE 3.2. a-b Rarefaction of blow fly species collected in mid-season (a) in each category of rural, intermediate and urban with an inset (b) showing the slopes from 0-160 samples

FIGURE 3.3.a

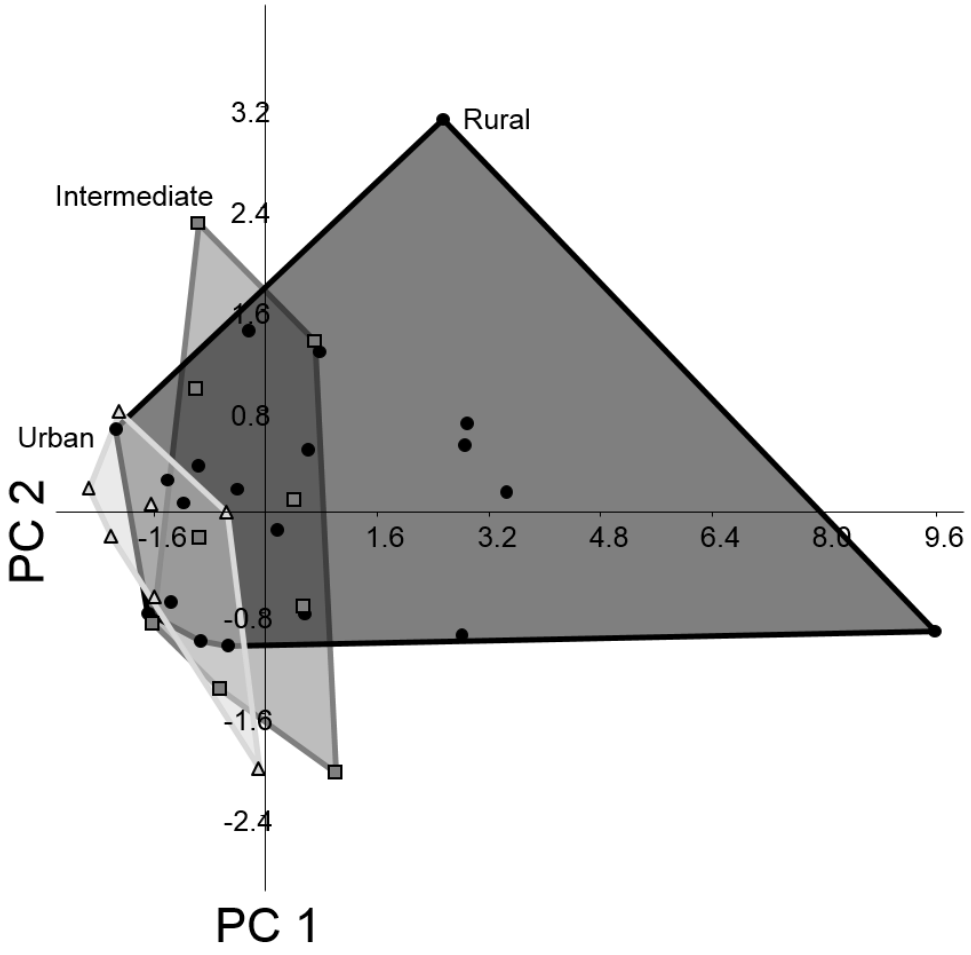
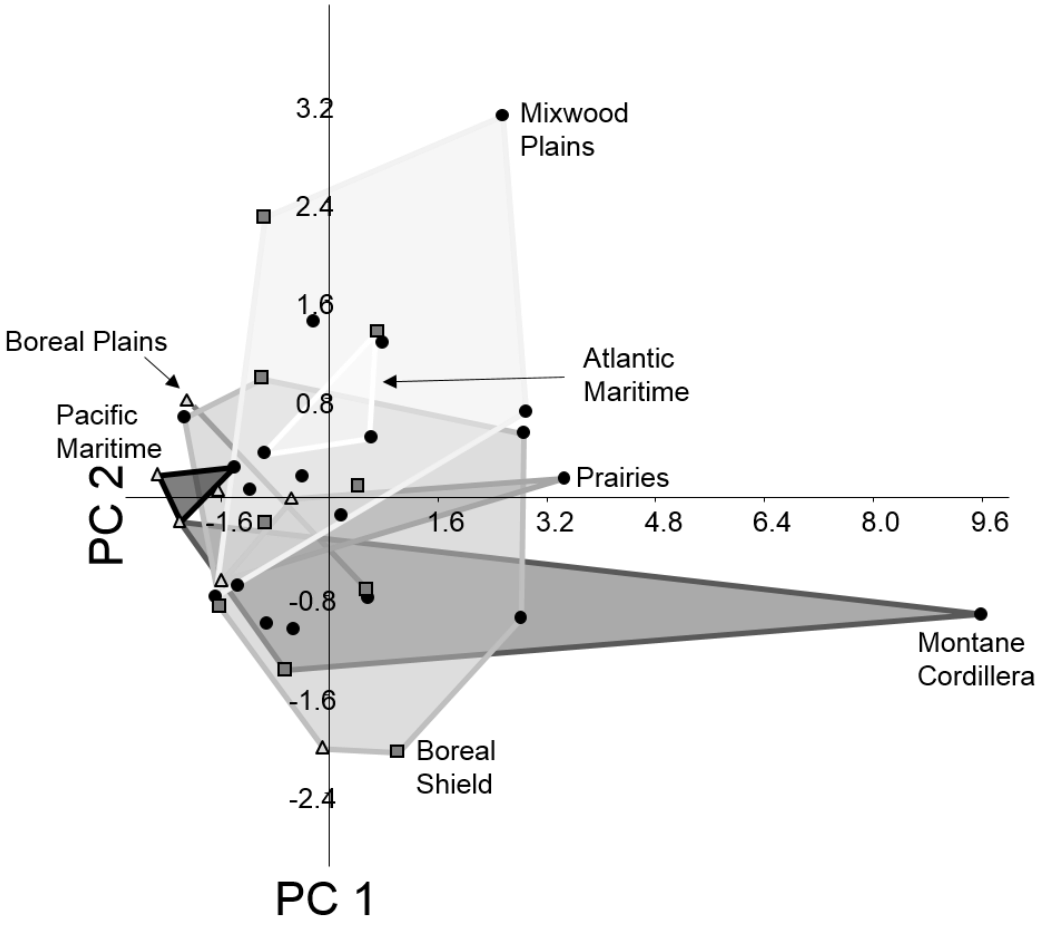

Rural
Intermediate
Urban
3.2
2.4
1.6
0.8
-0.8
-1.6
-2.4
-1.6
1.6
3.2
4.8
6.4
8.0
9.6
PC 2
PC 1

FIGURE 3.3.b


3.2
2.4
1.6
0.8
-0.8
-1.6
-2.4
-1.6
1.6
3.2
4.8
6.4
8.0
9.6
PC 2
PC 1
Mixwood Plains
Boreal Plains
Atlantic Maritime
Pacific Maritime
Prairies
Montane Cordillera
Boreal Shield

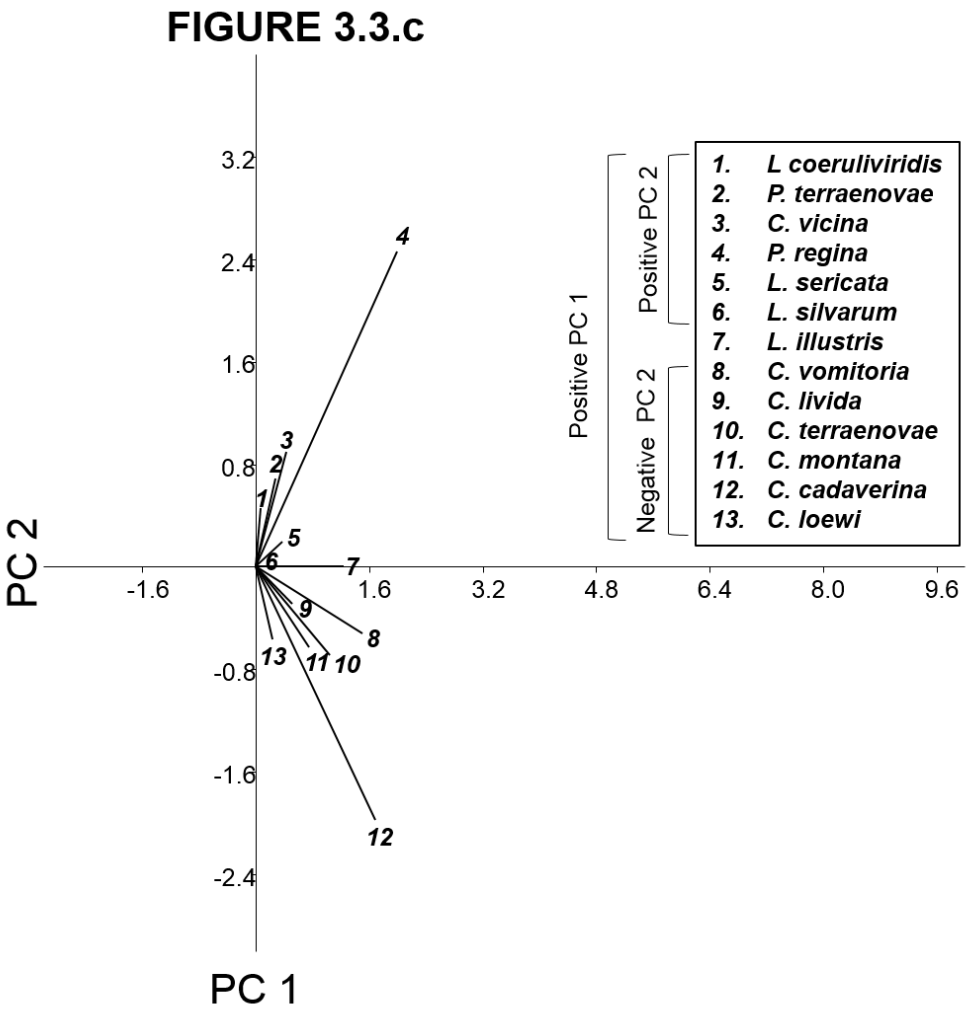


FIGURE 3.3. a-c: PCAs using 2011 mid-season data where black circles represent rural, dark grey squares represent intermediate and light grey triangles represent urban. Polygons representing outer points on each group first categorized by urban value (a). Then, categorized by ecozone value (b) where black through to the lightest grey represents from generalized West to East; Pacific Maritime, Montane Cordillera, Boreal Plains, Prairies, Boreal Shield, Mixwood Plains and Atlantic Maritime. A third component of this PCA is then presented (c) where the biplot of species loadings are shown for their correlation to each component.

TABLE 3.1. Date ranges for each sampling session

Sampling Periods	Date Ranges	Traps Deployed
2011	30 August to 30 October	30
2012	18 July to 30 October	16
2013	4 May to 29 July	24
2013	30 July to 14 October	22
2013	15 August to 5 November	13

TABLE 3.2. Species totals from all sampling periods and their relative percentages in our dataset

Species	Count	%
Phormia regina (Meigen)	3377	46
Cynomya cadaverina Robineau-Desvoidy	1268	17
Protophormia terraenovae (Robineau-Desvoidy)	582	8
Calliphora vomitoria (L.)	533	7
Lucilia illustris (Meigen)	452	6
Calliphora terraenovae Macquart	330	5
Calliphora vicina Robineau-Desvoidy	316	4
Lucilia sericata (Meigen)	223	3
Calliphora livida Hall	84	1
Lucilia coeruleiviridis Macquart	40	0.6
Calliphora montana (Shannon)	36	0.5
Calliphora loewi Enderlein	26	0.4
Lucilia silvarum (Meigen)	3	0.04
Calliphora stelviana (Brauer & Bergenstamm)	2	0.03
Total	7272	100%

TABLE 3.3. p values for NPMANOVA results using 2011-2013 mid-season species data by area caught. Values with an asterisk are significant (Bonferoni corrected alpha 0.05 = 0.0125 (0.05/4 tests)). F value presents a ratio of variances, where P the same presents the p-value when all of our three variables are considered.

	Paired tests *p*-values				
Scale	Urban vs rural	Urban vs intermediate	Rural vs intermediate	F value	P the same
250m	0.0002*	0.1084	0.5594	3.47	0.0003*
500m	0.0001*	0.0011*	0.5717	3.63	0.0002*
1 km	0.0041*	0.0519	0.0675	2.41	0.0004*
5 km	0.0284	0.1677	0.0272	2.095	0.0147

TABLE 3.4. Ecozone and urban category effects on species catches using a 2-way NPMANOVA with Bray-Curtis similarity measure. F values presents a ratio of variances, where interaction presents the p-value for similarity in both data sets.

	Urban value			Ecozone value			Interaction
Sample set	*p*-value	*F*	*d.f.*	*p*-value	*F*	*d.f.*	*p*-value
2011 mid	0.0026	0.9499	1	0.0953	0.3665	6	0.5031
2013 mid	0.0016	0.9750	1	0.0124	0.4476	4	0.0807
2013 early	0.0356	0.3710	1	0.0562	0.2406	5	0.9007
2013 late	0.0643	0.1063	1	0.0429	0.1115	3	0.8785

CHAPTER 4

General Discussion

PREFACE

In this thesis, we have presented two aspects of forensic entomology that add to our growing understanding of this science and its application in a crime scene context. First, we do this by adding to our knowledge of identifying features to assist with this essential first step in forensic entomological applications (Chapter 2). We used the feature of frons width to head with ratio for two blow fly species commonly caught in forensic investigations; *Phormia regina* and *Protophormia terraenovae*. These species are often caught in ecological sampling with their main differentiating feature obscured. We found that this additional feature allowed for differentiation between these species, and recommend its use in the future to complement previously implemented features, namely the colour of the anterior spiracle. Second, we examined Canada-wide sampling data to further understand the differences in blow fly communities in urban and rural habitats, using landcover analysis from the United States Geological Survey (USGS 2005) to classify areas, and to compare species abundances between these areas. We found that blow fly communities differ between urban and rural habitats, even across ecozones.

COLLABORATIVE SAMPLING

Through the diverse fields of forensic science, academic researchers seldom have a direct link to law enforcement. This disconnect, although reasonable given job commitments, has been highlighted as a deficiency in forensic science. The National Academy of Sciences reported that these disciplines can vary greatly, each containing

their own practices (NAS 2009). This disconnect is also seen between the broad categories of science and law enforcement. Although much of the evidence processing is performed by investigating officers, entomological evidence is often collected by officers but examined by trained entomologists (Amendt 2007). Integrating lab-based research and police involvement would allow for a collaborative effort between disciplines for the same outcome: the further understanding and application of forensic evidence research.

In our sampling, we enlisted the help of volunteer OPP (Ontario) and RCMP (outside of Ontario) officers in 7 provinces. Although during this time our contacts changed at many locations, most were willing to take on the task and assist us in data collection. Some officers went above and beyond by providing us excellent detail of study site, day-to-day weather observations, photographs of the trapping locations and home contact information should we need additional information. I would especially like to highlight the dedication to the project from I/C William Zorzi of Sault Ste Marie, who made an extra effort to put the three sets of traps sent to him in different types of locations quite far apart. Officers that I have talked to since the project have commented first on the terrible smell of the traps, but despite that, it was an overall enjoyable experience. An exciting and unexpected aspect to this project was the personal notes from the officers. These notes often offered a window into the exciting events in their lives. One officer apologised for the late deployment of the traps because they were focused on passing their FIS qualification – which they noted they did! Other officers were eager to help more, offering their addresses so that we could look up the trapping location specifically, or offering other means of contact to obtain more site information. Overall,

the sheets we sent with the traps to be filled out with dates and site information were extraordinarily detailed and made our initial assessment of the areas quite easy.

Due to our project being volunteer-based, and due to the shipment of traps taking longer to reach some locations, the dates that the traps were set out varied more than we had anticipated. This resulted in wider and sometimes overlapping trapping windows for each mailout period. We do, however suggest that despite this variability in trapping days, that the overall goal of sampling within periods of similar weather and temperatures were met. Additionally, regional differences in weather and temperature would also affect the traps if they were all deployed on the same schedule.

MORPHOLOGY IDENTIFICATIONS

The features used to distinguish blow fly species are refined over time and organized into dichotomous keys to provide a detailed but streamlined process for identification. Additional to updated key publications, articles like ours allow for an additional feature to be presented for identification work. As more information is learned from these animals, additional features can be described and current features can be re-assessed making morphological identifications more refined and robust, decreasing the chances of misidentification. Avoiding misidentifications is especially important in forensic science where post mortem interval estimation can influence the outcome of a criminal matter.

URBAN AS A QUANTIFIABLE VARIABLE

Most research aiming to determine a difference in blow fly communities between urban and rural environments, or simply those that document the insect succession in one environment, often use qualitative methods of determining the type of environment in

which the study takes place. These studies are useful to gather information about blow fly abundance; however, our study refined this by using a quantitative landcover analysis consistent across Canada to categorize urban and rural areas. This analysis removed the subjective assessments of each area at limited scales, and the possible inconsistencies between researchers across the country when urban and rural distinctions are made.

CONTRIBUTION TO FORENSIC SCIENCE

Blow flies remain an important organism to understand in relation to a death investigation. However, much work is still needed to fill gaps in our knowledge of how these animals interact with their environment, and how best we can apply our understanding their biology to answering questions about a crime. The importance of understanding the parameters of a crime begs for the need for as many insightful tools as possible available to the investigators, as each crime scene presents a new challenge to find the most informative evidence. As with any scientific discipline, we move forward by a combined effort of researchers each adding detailed research to the growing understanding of the science and its applications. In forensic science, while the backbone of PMI estimation continues to be refined, detailed research such as ours is required. Our work to help differentiate two common blow fly species will assist in minimizing misidentifications and uncertainties. Our Canada-wide assessment of urban and rural blow fly communities will move us towards a greater understanding of blow fly habitat preferences, and the potential to use non-local urban data to predict local urban populations. Together, continued research in entomology moves us towards better understanding and more robust applications in forensic science.

REFERENCES

2005 North American Land Cover at 250 m spatial resolution [Map]. Natural Resources Canada/Canadian Center for Remote Sensing, United States Geological Survey; Insituto Nacional de Estadística y Geografía (INEGI), Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO) and Comisión Nacional Forestal (CONAFOR), 2005.

Amendt J, Campobasso CP, Gaudry E, Reiter C, LeBlanc HN, Hall MJR. Best practice in forensic entomology–standards and guidelines. Int J Legal Med 2007;121:90-104.

National Research Council. Strengthening forensic science in the United States: a path forward. Washington: The National Academies Press, 2009.

APPENDIX

TABLE A.1. Original 19 layer names from the North American Land Change Monitoring System map at a resolution of 250 m used in landcover analyses to create pooled categories of rural, urban and water.

Layer Number	Layer Name	Pooled Categories	Categories encountered in this study
1	Temperate or sub-polar needleleaf forest	Rural	Y
2	Sub-polar taiga needleleaf forest	Rural	N
3	Tropical or sub-tropical broadleaf evergreen forest	Rural	N
4	Tropical or sub-tropical broadleaf deciduous forest	Rural	N
5	Temperate or sub-polar broadleaf deciduous forest	Rural	Y
6	Mixed forest	Rural	Y
7	Tropical or sub-tropical shrubland	Rural	N
8	Temperate or sub-polar shrubland	Rural	Y
9	Tropical or sub-tropical grassland	Rural	N
10	Temperate or sub-polar grassland	Rural	Y
11	Sub-polar or polar shrubland-lichen-moss	Rural	N
12	Sub-polar or polar grassland-lichen-moss	Rural	N
13	Sub-polar or polar barren-lichen-moss	Rural	N
14	Wetland	Rural	Y
15	Cropland	Rural	Y
16	Barren land	Rural	Y
17	Urban	Urban	Y
18	Water	Water	Y
19	Snow and Ice	Rural	N

TABLE A.2. Species totals in mid-season (a), early-season (b) and late-season (c) sessions organized to show relative abundances within each province (B.C. British Columbia, Alta. Alberta, Sask. Saskatchewan, Ont. Ontario, N.B. New Brunswick, N.S. Nova Scotia, N.L. Newfoundland and Labrador). Cell colour from darkest to lightest indicates the most to least abundant species in each province.

a. Mid-season percentage of each species per Province (%)

Species	B.C.	Alta.	Sask.	Ont.	N.B.	N.S.	N.L.	Species Total	Overall percentage
Phormia regina (Meigen)	*35.4*	*48.2*	*20.1*	*52.2*	*54.5*	*60*	*19.4*	2450	*45.86*
Cynomya cadaverina Robineau-Desvoidy	*18.7*	*30.2*	*50*	*13.3*	*9.9*	*10*	*9.5*	862	*16.14*
Protophormia terraenovae (Robineau-Desvoidy)	*0.2*	*4.7*	*5.6*	*13.4*	*2.3*	*3.3*	*1.2*	460	*8.61*
Calliphora vomitoria (L.)	*15.6*	*3.3*	*2.8*	*2.8*	*11.3*	*10*	*12.3*	351	*6.57*
Lucilia illustris (Meigen)	*3.8*	*3.7*	*7.6*	*7.1*	*5.2*	*0*	*37.2*	400	*7.49*
Calliphora terraenovae Macquart	*21*	*1*	*0*	*0.3*	*1.9*	*0*	*3.6*	281	*5.26*
Calliphora vicina Robineau-Desvoidy	*1.7*	*3.7*	*2.1*	*3.9*	*8.9*	*3.3*	*15*	217	*4.06*
Lucilia sericata (Meigen)	*1.2*	*2*	*8.3*	*4.9*	*2.8*	*3.3*	*0.8*	197	*3.69*
Calliphora livida Hall	*0.3*	*0.7*	*2.1*	*0.6*	*2.8*	*3.3*	*1.2*	38	*0.71*
Lucilia coeruleiviridis Macquart	*0*	*0*	*0*	*0.9*	*0*	*0*	*0*	28	*0.52*
Calliphora montana (Shannon)	*1.6*	*2.7*	*0*	*0.3*	*0*	*0*	*0*	36	*0.67*
Calliphora loewi Enderlein	*0.5*	*0*	*0*	*0.3*	*0.5*	*6.7*	*0*	17	*0.32*
Lucilia silvarum (Meigen)	*0*	*0*	*0*	*0.1*	*0*	*0*	*0*	3	*0.06*
Calliphora stelviana (Brauer & Bergenstamm)	*0*	*0*	*1.4*	*0*	*0*	*0*	*0*	2	*0.04*
Province total	1217	301	144	3184	213	30	253	5342	

b. Early-season percentage of each species per Province (%)

Species	B.C.	Alta.	Sask.	Ont.	N.B.	N.S.	N.L.	Species Total	Overall percentage
Phormia regina (Meigen)	*78.7*	*0*	*10*	*64.8*	*36*	*0*	*0*	840	*61.49*
Cynomya cadaverina Robineau-Desvoidy	*2.1*	*100*	*36.4*	*15.8*	*24*	*0*	*100*	209	*15.3*
Protophormia terraenovae (Robineau-Desvoidy)	*8*	*0*	*44.3*	*3.4*	*12*	*0*	*0*	119	*8.71*
Calliphora vomitoria (L.)	*0*	*0*	*0*	*1*	*0*	*0*	*0*	9	*0.66*
Lucilia illustris (Meigen)	*1*	*0*	*0.7*	*3.3*	*0*	*0*	*0*	34	*2.49*
Calliphora terraenovae Macquart	*4.2*	*0*	*6.4*	*0.8*	*8*	*0*	*0*	30	*2.2*
Calliphora vicina Robineau-Desvoidy	*5.2*	*0*	*0*	*5*	*0*	*0*	*0*	61	*4.47*
Lucilia sericata (Meigen)	*0.3*	*0*	*0*	*1.9*	*0*	*0*	*0*	18	*1.32*
Calliphora livida Hall	*0.3*	*0*	*2.1*	*4.1*	*20*	*0*	*0*	46	*3.37*
Lucilia coeruleiviridis Macquart	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora montana (Shannon)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora loewi Enderlein	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Lucilia silvarum (Meigen)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora stelviana (Brauer & Bergenstamm)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Province total	287	1	140	912	25	0	1	1366	

c. Late-season percentage of each species per Province (%)

Species	B.C.	Alta.	Sask.	Ont.	N.B.	N.S.	N.L.	Species Total	Overall percentage
Phormia regina (Meigen)	*13.6*	*42.9*	*0*	*20.9*	*0*	*0*	*1*	87	*15.37*
Cynomya cadaverina Robineau-Desvoidy	*13.6*	*28.6*	*0*	*53.2*	*0*	*0*	*11*	197	*34.81*
Protophormia terraenovae (Robineau-Desvoidy)	*0*	*0*	*0*	*1*	*0*	*0*	*0*	3	*0.53*
Calliphora vomitoria (L.)	*33.9*	*8.6*	*0*	*7.3*	*0*	*0*	*75*	173	*30.57*
Lucilia illustris (Meigen)	*3.4*	*0*	*0*	*4.7*	*0*	*0*	*1*	18	*3.18*
Calliphora terraenovae Macquart	*10.2*	*11.4*	*0*	*1.3*	*0*	*0*	*3*	19	*3.36*
Calliphora vicina Robineau-Desvoidy	*11.9*	*8.6*	*0*	*4.7*	*0*	*0*	*8*	38	*6.71*
Lucilia sericata (Meigen)	*5.1*	*0*	*0*	*2*	*0*	*0*	*1*	10	*1.77*
Calliphora livida Hall	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Lucilia coeruleiviridis Macquart	*0*	*0*	*0*	*4*	*0*	*0*	*0*	12	*2.12*
Calliphora montana (Shannon)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora loewi Enderlein	*8.5*	*0*	*0*	*1*	*0*	*0*	*1*	9	*1.59*
Lucilia silvarum (Meigen)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora stelviana (Brauer & Bergenstamm)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Province total	59	35	0	301	0	0	171	566	

TABLE A.3. Species totals in mid-season (a), early-season (b) and late-season (c) sessions organized to show relative abundances within each ecozone. Cell colour from darkest to lightest indicates most to least abundant species in each ecozone (P.M. Pacific Maritime, M.C. Montane Cordillera, B.P. Boreal Plains, P. Prairies, B.S. Boreal Shield, M.P. Mixwood Plains and A.M. Atlantic Maritime

a. Mid-season percentage of each species per Ecozone (%)

Species	P.M.	M.C.	B.P.	P.	B.S.	M.P.	A.M.	Species Total	Overall percentage
Phormia regina (Meigen)	*16.4*	*36.3*	*15*	*44.4*	*35.2*	*59.4*	*55.1*	2450	*45.86*
Cynomya cadaverina Robineau-Desvoidy	*10.9*	*19*	*51.3*	*33.4*	*28.3*	*2.9*	*9.9*	862	*16.14*
Protophormia terraenovae (Robineau-Desvoidy)	*1.8*	*0.2*	*10*	*3.8*	*0.8*	*20.2*	*2.5*	460	*8.61*
Calliphora vomitoria (L.)	*7.3*	*16*	*3.8*	*3*	*7.5*	*0.9*	*11.1*	351	*6.57*
Lucilia illustris (Meigen)	*3.6*	*3.8*	*11.3*	*3.6*	*13.9*	*6.3*	*4.5*	400	*7.49*
Calliphora terraenovae Macquart	*3.6*	*21.8*	*0*	*0.8*	*1.4*	*0*	*1.6*	281	*5.26*
Calliphora vicina Robineau-Desvoidy	*38.2*	*0*	*3.8*	*3*	*5.4*	*4.3*	*8.2*	217	*4.06*
Lucilia sericata (Meigen)	*18.2*	*0.4*	*2.5*	*4.4*	*5.3*	*4.1*	*2.9*	197	*3.69*
Calliphora livida Hall	*0*	*0.3*	*2.5*	*0.8*	*0.9*	*0.4*	*2.9*	38	*0.71*
Lucilia coeruleiviridis Macquart	*0*	*0*	*0*	*0*	*0.1*	*1.3*	*0*	28	*0.52*
Calliphora montana (Shannon)	*0*	*1.6*	*0*	*2.2*	*0.7*	*0*	*0*	36	*0.67*
Calliphora loewi Enderlein	*0*	*0.5*	*0*	*0*	*0.6*	*0*	*1.2*	17	*0.32*
Lucilia silvarum (Meigen)	*0*	*0*	*0*	*0*	*0.1*	*0.1*	*0*	3	*0.06*
Calliphora stelviana (Brauer & Bergenstamm)	*0*	*0*	*0*	*0.5*	*0*	*0*	*0*	2	*0.04*
Ecozone total	55	1162	80	365	1369	2068	243	5342	

b. Early-season percentage of each species per Ecozone (%)

Species	P.M.	M.C.	B.P.	P.	B.S.	M.P.	A.M.	Species Total	Overall percentage
Phormia regina (Meigen)	*53.3*	*85.5*	*13.3*	*0*	*71.2*	*0*	*36*	840	*61.49*
Cynomya cadaverina Robineau-Desvoidy	*3.3*	*1.8*	*15.2*	*100*	*16.1*	*0*	*24*	209	*15.3*
Protophormia terraenovae (Robineau-Desvoidy)	*10*	*7.5*	*59*	*0*	*2.1*	*0*	*12*	119	*8.71*
Calliphora vomitoria (L.)	*0*	*0*	*0*	*0*	*0.9*	*0*	*0*	9	*0.66*
Lucilia illustris (Meigen)	*0*	*1.3*	*1*	*0*	*1.5*	*0*	*0*	34	*2.49*
Calliphora terraenovae Macquart	*8.3*	*3.1*	*8.6*	*0*	*0.9*	*0*	*8*	30	*2.2*
Calliphora vicina Robineau-Desvoidy	*25*	*0*	*0*	*0*	*3.3*	*0*	*0*	61	*4.47*
Lucilia sericata (Meigen)	*0*	*0.4*	*0*	*0*	*1.6*	*0*	*0*	18	*1.32*
Calliphora livida Hall	*0*	*0.4*	*2.9*	*0*	*2.5*	*0*	*20*	46	*3.37*
Lucilia coeruleiviridis Macquart	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora montana (Shannon)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora loewi Enderlein	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Lucilia silvarum (Meigen)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora stelviana (Brauer & Bergenstamm)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Ecozone total	60	227	105	36	812	101	25	1366	

c. Late-season percentage of each species per Ecozone (%)

Species	P.M.	M.C.	B.P.	P.	B.S.	M.P.	A.M.	Species Total	Overall percentage
Phormia regina (Meigen)	*20*	*10.8*	*0*	*42.9*	*0.6*	*21.1*	*0*	87	*15.43*
Cynomya cadaverina Robineau-Desvoidy	*5*	*18.9*	*0*	*28.6*	*11.6*	*53.2*	*0*	197	*34.93*
Protophormia terraenovae (Robineau-Desvoidy)	*0*	*0*	*0*	*0*	*0*	*1*	*0*	3	*0.53*
Calliphora vomitoria (L.)	*25*	*40.5*	*0*	*8.6*	*74.6*	*7*	*0*	173	*30.67*
Lucilia illustris (Meigen)	*10*	*0*	*0*	*0*	*1.2*	*4.7*	*0*	18	*3.19*
Calliphora terraenovae Macquart	*10*	*10.8*	*0*	*11.4*	*2.9*	*1.3*	*0*	19	*3.37*
Calliphora vicina Robineau-Desvoidy	*20*	*8.1*	*0*	*8.6*	*8.1*	*4.7*	*0*	38	*6.74*
Lucilia sericata (Meigen)	*5*	*0*	*0*	*0*	*0.6*	*2*	*0*	10	*1.77*
Calliphora livida Hall	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Lucilia coeruleiviridis Macquart	*0*	*0*	*0*	*0*	*0*	*4*	*0*	12	*2.13*
Calliphora montana (Shannon)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora loewi Enderlein	*5*	*10.8*	*0*	*0*	*1*	*1*	*0*	9	*1.6*
Lucilia silvarum (Meigen)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Calliphora stelviana (Brauer & Bergenstamm)	*0*	*0*	*0*	*0*	*0*	*0*	*0*	0	*0*
Ecozone total	20	37	0	35	173	299	0	566	