BLOW FLY (DIPTERA: CALLIPHORIDAE) COMMUNITY STRUCTURE AT A NEW NORTHERN LATITUDE FORENSIC RESEARCH FACILITY AND DURING AQUATIC DECOMPOSITION

By

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ABSTRACT

BLOW FLY (DIPTERA: CALLIPHORIDAE) COMMUNITY STRUCTURE AT A NEW NORTHERN LATITUDE FORENSIC RESEARCH FACILITY AND DURING AQUATIC DECOMPOSITION

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Blow flies (Diptera: Calliphoridae) are necrophagous insects which are attracted to decomposing remains. The presence and life stage of their larvae are an important factors in the estimation of the postmortem interval (PMI) during death investigations. The purpose of this study was to survey the blow fly communities present under two separate circumstances: first, in the pre- and early-operation stages of a new outdoor forensic research facility in Northern Michigan (FROST); and second, in the context of large vertebrate (*Sus scrofa*) decomposition in a freshwater aquatic system in Mid-Michigan. Eight blow fly species were collected at the Forensic Research Outdoor Station (FROST) from May-September with *Phormia regina, Lucilia illustris*, and *Cochliomyia macellaria* composing a cumulative 94.2% of the total collections. Month was a significant factor in the mean relative abundance of these major species. Two blow fly species were collected over the course of approximately one month during aquatic vertebrate decomposition with *Phormia regina* composing 90.5% of the collections. Time was not a significant factor in the mean relative abundance of the species collected, but larval length changes over this period suggested multiple oviposition events. The findings in these two studies provide new and important information which can be utilized by forensic entomologists in future research as well as by forensic science professionals during death investigations and related casework.

To my supportive family and friends who tolerate my dinner conversation topics. I couldn't have done this without you.

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Introduction

Forensic entomology is the study of arthropods in the context of the legal system. There are three recognized primary subfields of forensic entomology, including stored product entomology, urban entomology, and medicocriminal entomology (Rivers and Dahlem 2014). Stored product entomology concerns the presence of arthropods in food and food products and the associated legal repercussions. Urban entomology focuses on the detrimental presence of arthropods in and around human structures, both in terms of their undesirability and their economic impact. Medicocriminal entomology involves the use of arthropod evidence to aid criminal cases, often in association with homicides, other violent crimes or cases of extreme neglect. This subdiscipline can include the use of arthropod evidence to determine the postcolonization interval (PCI) or postmortem interval (PMI) (Goff 1993), to establish the relocation of a corpse (Catts and Goff 1992), to connect a suspect to a crime (Catts and Goff 1992), or even to indicate neglect and abuse in humans and animals (Bonacci et al. 2017, Anderson and Huitson 2004).

Necrophagous insects are the most valuable and commonly used arthropods during the investigation of criminal cases. The four general categories of necrophagous insects are 1) those that feed on decomposing remains but which may or may not breed and develop in them, 2) parasitoids and predators which do not feed on the remains themselves but rather on other necrophagous insects, 3) omnivorous species which feed on both the remains and on other necrophagous insects, and 4) adventitious species which utilize the remains as an extension of their habitat (Rivers and Dahlem 2014). Those necrophagous insects which feed on and utilize human remains for reproduction are the most forensically relevant, as their offspring completely rely on the carcass for growth and development during their larval stages (Rivers and Dahlem 2014). Blow flies (Diptera: Calliphoridae) and other dipteran families (e.g., Sarcophagidae) are within this category of necrophagous insects. While certain beetles (Coleoptera) are also associated with human remains during later stages of decomposition, blow flies and flesh flies are the primary colonizers. The predictability of the immature stages of blow flies and their absolute reliance on the remains as a resource means that the presence of adult and larval blow flies is commonly used to assist in the determination of the PCI, or time since discovery of human remains by arthropods, and PMI, or time since death (Weatherbee et al. 2017, Wells and Lamotte 2009). While there are other proposed methods of estimating PMI, such as the quantification of mRNA degradation (Bauer et al. 2003), tracking chemical biomarkers associated with decomposition such as certain amino acids and neurotransmitters (Vass et al. 2002), and new evidence demonstrating the potential of microbial community profiles (Metcalf et al. 2013, Pechal et al. 2014), the use of forensically relevant insects is far more typical.

Blow Fly Community Composition and Biodiversity

Understanding the species composition of regional blow fly communities is an important factor when estimating a PCI, PMI, or location of death in a homicide case. This is because individual blow fly species often possess unique climatic preferences, resulting in their primary occupation of certain geographic regions or climatic niches in accordance with the season (Norris 1965). These preferences create differential blow fly community compositions depending on the location and time of year in which a corpse is found. For example, the blow fly species *Phormia regina* is often considered a colder-climate species that is abundant in the northern United States and Canada during the summer but which is more abundant in the southern United States during the winter (Byrd and Allen 2001). Some species have even more specific geographic limitations, such as *Cynomya mortuorum*, which is only found near the

Arctic Circle in places such as northern Alaska and the Kola Peninsula of Russia (Whitworth 2006). As an example and in the context of a case, therefore, a hypothetical species which is known to be abundant during autumn in Mexico and the southern United States but which has never been observed during autumn in the northern United States would not be expected at a crime scene in Wisconsin during the month of October. If specimens were to be found in an unexpected context, it may be an indicator that the corpse was relocated from a geographic region that is characterized by a different climate or simply by an established and different biogeographic distribution (Catts and Goff 1992). However, if no or sparse information is available for what is to be "expected" for a certain location at a certain time of year, then there is little one can infer about the typicality of the collected insect specimens. Critical analysis by a forensic entomologist may then be negatively impacted, particularly if they do not have access to updated taxonomic keys for local blow fly species (Sanford 2017).

There are a limited number of studies in which geographic surveys have been conducted specifically to describe regional blow fly community composition. Examples from the United States include surveys from New Jersey (Weidner et al. 2015), California (Brundage et al. 2011), Texas (Sanford 2017), Illinois (Baumgartner 1988), Michigan (Babcock 2018), and Arizona (Deonier 1942), as well as region-specific successional studies from Virginia (Tabor et al. 2004), South Carolina (Payne 1965, Payne 1967, Payne and King 1972), Louisiana (Watson and Carlton 2005), and Ohio (Benbow et al 2013). There have also been geographic surveys conducted internationally, with examples from Canada (Anderson and VanLaerhoven 1996), Australia (Archer 2003), Jamaica (Cranston 2008), Peru (Baumgartner and Greenberg 1985), the Brazilian Amazon (Amat et al. 2016), Spain (Zabala et a. 2014), South Africa (Richards et al. 2009), Malaysia (Heo et al. 2008), Pakistan (Kurahashi and Afzal 2002), and the broader Middle East

(Akbarzadeh et al. 2015). These studies emphasize the importance of understanding the biodiversity of local blow fly species through their unique community composition results. For example, within the state of New Jersey, Weidner et al. (2015) surveyed blow flies at six sites across three regions (north, central, and south New Jersey) during a two year period. The north and central regions had significant temperature differences in the summer months and the north was significantly colder than the central and south regions during the winter months. They determined that there were differences in the dominant species for each season across all three regions and that the relative abundance of three of the most common blow flies were different between each region. An example of these results is the species *Lucilia sericata*, which composed 62.3% of the total flies for the central region, 4.3% for the north region, and 2.8% for the south region during the summer. These intra-state differences are strong indicators of the necessity of conducting local surveys of necrophagous blow flies in order to understand community structure, information which is particularly important for forensic research facilities that use donated human bodies to study decomposition.

Outdoor Forensic Research Facilities

One means of studying necrophagous insects such as blow flies in the context of human decomposition is through outdoor forensic research facilities. Colloquially known as "body farms", outdoor forensic research facilities with a mission to conduct studies on human decomposition and taphonomy for science are rare in the United States and globally. Currently, there are eight operating outdoor forensic research facilities in the United States that involve research on human remains from willed body programs: University of Tennessee at Knoxville, Western Carolina University, Texas State University-San Marcos, Colorado Mesa University, Sam Houston State University, Southern Illinois University, University of South Florida, and Northern Michigan University (Table 1). There is at least one comparable facility outside of the United States, located at the University of Technology in Sydney, Australia, although others may be planned or in their early stages of development. The Forensic Anthropology Center (FAC) at the University of Tennessee at Knoxville was the first of these facilities in the world, having been founded by forensic anthropologist Dr. William M. Bass in 1971. The Forensic Research Outdoor Station (FROST) at Northern Michigan University is the newest facility in the United States with active research and teaching programs involving human bodies with active research that began in July 2018. It is also the first in the world in a northern geographic region.

The opening of FROST is important because it provides a unique opportunity to survey the local blow fly community prior to human decomposition research activities, thus providing a baseline understanding of forensically important organisms in northern Michigan. All other currently operating forensic research facilities are south of the Great Lakes where winters are mild compared to northern latitude regions like Marquette, MI, which is considered a cold climate based on Köppen climate classification (Peet et al. 2007). Additionally, there are no published records of pre-operation or early operation (e.g., first few months of research) surveys of the local necrophagous insect community for all other operating facilities. Furthermore, most of the current operational facilities did not survey the site-specific necrophagous insect communities at all. Subsequently, there is no record documenting community composition changes following inaugural research subjects and serial exposure to human bodies over monthly and seasonal time scales. Without such pre- or early-operation baseline necrophagous insect surveys, it is unknown if serial placement and exposure of multiple human bodies in a single geographic location will impact the abundance, composition or function of the local blow fly community. One study has compared the necrophagous insect populations between swine models and control sites over a 12-day period (Shahid et al. 2003), but that short-term study on insects was conducted after 30 years of operation at the Forensic Anthropology Research Facility in Knoxville, TN and could not be compared to earlier insect data. A second, follow-up study focused only on predatory and parasitic arthropods (Schoenly et al. 2005). Therefore, there is no clear information about whether the long-term, serial placement of bodies in a region affects the local necrophagous insect community over time, and specifically whether the local community composition of blow flies is affected. For example, if long-term, serial body placement were to increase the diversity of the blow fly community in a region, the availability of species with various rates of colonization and decompositional functions may be influenced. It is known that the speed in which adult blow flies arrive at a decomposing body can vary according to species (Byrd and Castner 2009). Therefore, if serial body placement influences the composition of the local blow fly community in a way that favors species with earlier arrival times, decomposition studies may begin to reflect decreased times of colonization. This may then result in data which indicate more immediate dipteran larval development than that which actually occurs for bodies found in routine death investigations within the same state or geographic region. Determining whether serial body placement results in community saturation of primary colonizers and thus creates an altered decomposition environment is dependent on baseline and early operation blow fly surveys on which to make comparisons.

There is a severe lack of entomological data related to previously established outdoor forensic research facilities even beyond baseline and early operation surveys. In fact, to my knowledge, only one currently operating facility has published detailed community entomological data specific to their site at all. A literature search utilizing Google Scholar and

Table 1. **A summary of anthropological research facilities that use willed human bodies; comparing years of operation, location, and climate zone including average monthly minimum and maximum temperature, precipitation, and snowfall. Climate zone was based on the Köppen climate classification system. Temperature, precipitation, and snowfall values were taken from NOAA representing years 1961-1990, except for the *Australian facility (www.meteorology.com.au/local-climate-history/nsw/yarramundi).**

University	Facility Name	Initial Year of Operation	Climate Zone	Average Monthly Min Temp. (°C)	Average Monthly Max Temp. (°C)	Average Monthly Precipitation Range (mm)	Average Monthly Snowfall Range (cm)
Northern Michigan University	Forensic Research Outdoor Station	2018	Humid Continental	-26.7	31.9	33.3-80.3	0.0-74.9
University of Tennessee Knoxville	University of Tennessee Anthropological Research Facility	1981	Humid Subtropical	-12.4	35.1	63.8-129.0	0.0-6.9
Western Carolina University	Forensic Osteology Research Station	2006	Humid Subtropical	-13.1	32.9	73.9-118.1	0.0-10.4
Texas State University	Forensic Anthropology Research Facility	2008	Humid Subtropical	-2.9	39.4	47.8-112.8	0.0-1.0
Sam Houston State University	Southeast Texas Applied Forensic Science Facility	2009	Humid Subtropical	-2.9	38.0	81.3-150.6	0.0-0.5
Colorado Mesa University	Forensic Investigation Research Station	2012	Semi-Arid	-8.3	34.0	11.7-26.9	0.0-12.4
Southern Illinois University	Complex for Forensic Anthropology Research	2010	Humid Subtropical	-5.0	31.7	77.7-136.4	0.0-10.4
University of South Florida	Facility for Outdoor Research and Training	2017	Humid Subtropical	-7.8	37.2	38.1-185.4	0.0-0.0
*University of Technology	Australian Facility for Taphonomic Experimental Research	2016	Humid Subtropical	3.7	30.1	30.7-116.3	0.0-0.0

PubMed provided only three total relevant results when using search terms such as "entomology", "insect", or "Diptera" along with the name of the individual facilities or affiliated universities. All three relevant hits were from the University of Tennessee – Knoxville, one being a broad but potentially outdated survey from 1983 (Rodriguez and Bass 1983) and two being associated with the saturation hypothesis discussed previously (Shahid et al. 2003, Schoenly et al. 2005). These search results demonstrate a knowledge gap pertaining to entomological communities at outdoor forensic research facilities in general. A characterization of the local blow fly community at FROST could therefore act as a comparison point for any future studies at other established outdoor forensic research facilities.

Statement of Purpose and Hypotheses

The purpose of this study was to provide baseline and early operation community blow fly data for the new forensic research facility at Northern Michigan University, FROST. This purpose was achieved through the accomplishment of two objectives: 1) a late spring to early autumn survey of the blow flies present at FROST and 2) a survey of blow fly species as an early community response to the nearby presence of a novel resource (human donor bodies). Such surveys are important for providing a known community composition to which future entomological studies at FROST can be compared, for providing early information on the impact of human donors on the blow fly community composition, and for providing forensic entomology practitioners in the upper Midwest with blow fly community composition information.

The expectation for the blow fly sampling conducted at FROST in the context of the first objective was to determine changes in 1) the species composition of the blow fly community as the months and seasons progress (from late spring to summer to fall) as well as 2) the total number of individuals collected. Some blow fly species are considered 'warm' or 'cold' weather flies; for example, *Phormia regina* has been considered the most prevalent blow fly in the United States and tends to migrate from the north during the summer to the south during the winter (Byrd and Allen 2001). Based on these studies, I predicted a higher proportion of *P. regina* during the late spring and early summer months than during the later summer and fall, resulting in a community composition shift based on temperature and independent of the presence of donor bodies at FROST. Blow flies in general are not as active in colder temperatures, and so we expected the number of specimens for all collected species would decline from late spring and summer to autumn. Expected species in the Calliphoridae community included *P. regina* and *Lucilia spp.*, particularly *L. sericata*, which are the most commonly collected necrophagous blow flies in North America (Babcock 2018, Anderson and VanLaerhoven 1996). Others included those in the genus *Calliphora* (particularly *C. vomitoria* which is common to rural locations in North America) as well as the secondary screwworm, *Cochliomyia macellaria*, which is another species common to the United States (Rivers and Dahlem 2014). Species that were not expected included *C. rufifacies*, which is invasive and has not yet been reported in the northern part of Michigan (but has been collected in Mid-Michigan by Babcock 2018), or any other species which is more common to very warm, southern climates.

The expectation in the context of the second objective was to determine changes in 1) the species composition of the blow fly community immediately before, on the day of, and several days after the placement of the human donors as well as 2) the total number of individuals collected. As a null hypothesis we expected the relative proportion of each blow fly species and the total number of collected individuals to remain consistent between these dates. The alternative hypothesis would be that the relative proportions of each blow fly species and the total number of collected individuals would not be consistent between the three collection dates surrounding human donor placement. Expected blow fly species were those detailed above.

Materials and Methods

Study Location

This study was conducted at Northern Michigan University's Forensic Research Outdoor Station (FROST) the highest latitude forensic research facility in the world located, in Marquette, MI (46°32'36.74" N -87°23'43.51" W) (Figure 1). FROST is divided into two functional areas: a sally port and the research field. The sally port is approximately 50ft x 50ft and includes an insulated and temperature controlled building for equipment storage and limited on-site temporary sample storage and data analysis. The research field is approximately one acre in area. The sally port and the research field both have 16-foot gates that can be opened to allow vehicles to pass through when necessary. The field is equipped with a site-specific HOBO U30 NRC weather station that monitors air and soil temperature, soil moisture, humidity, solar radiation, sunlight, wind speed and direction, and rainfall data.

Figure 1. **Aerial photograph of the FROST facility, courtesy of Dr. Jane Wankmiller at Northern Michigan University. Circled numbers indicate the approximate positions of blow fly traps 1-7.**

Insect Sampling Methods

The blow fly community at FROST was surveyed monthly during the summer prior to body placement on July 23, 2018 and then directly after, from May through September 2018. One collection was completed per month with the exception of July, in which three collections were completed with one occurring prior to donor body placement and two afterwards (22, 23, and 26 July 2018), as well as August, in which no collections were made. This resulted in a total of four month-specific collections and two additional July collections. The four month-specific collections were intended for the consideration of the first objective, while the two additional July collections were to provide survey information on the day of body placement and several days afterwards.

Seven inverted cone traps as described in Babcock (2018) were used to capture these flying insects (Figure 2). The traps were hung from 1.2 m garden shepherd's hooks and held in place with two 35.5 cm guidelines. The traps were designed to allow flies to enter from the base of the trap but prevent escape. Glass mason jars of decomposed pig liver as described in Babcock (2018) (300 g liver and 100 mL of reverse osmosis water, previously sealed for approximately 350 accumulated degree hours at an average room temperature of 25°C) were placed beneath the base of the traps to attract blow flies. The lids of the jars were replaced with screen during insect collection to prevent flies from entering the bait. The location of the traps was assigned at the initiation of the study with one located near each of the four corners of the facility, one near the center, and two just outside its boundaries. The location of the traps was not altered for the duration of the project. Collected blow fly specimens were stored in 50 mL conical tubes labeled with trap number and date information in a -20 °C freezer prior to their identification. Preserved specimens were thawed and identified under a stereomicroscope using the identification guide *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* (Marshall et. al. 2011).

Figure 2. **Image from Babcock 2018. The trap set-up pictured above was replicated in this study using the same traps, Shepard's hooks, and mason jars of bait.**

Weather Data

Weather information including hourly temperature and rainfall were collected using the site-specific HOBO U30 NRC weather station mentioned previously. These data were occasionally supplemented with weather information from Sawyer County International Airport in Marquette, MI when site-specific data was unavailable. Daily temperature averages and total rainfall were calculated for each day during the study's collection months (May, June, July, and September 2018).

Human Subjects

While human donors were not the direct subjects for this study, they were present during early operation insect community surveys. These were individuals whose bodies were donated through self-donation or through next-of-kin donation to the FROST facility. FROST donors and their next-of-kin were provided with extensive informational packets and were asked to sign forms acknowledging their free and willing donations, as well as their understanding of FROST policies prior to donation. Institutional Review Board (IRB) review is not required for research on human remains. For IRB purposes, they are considered under the same rules as those applied to autopsy specimens and are not considered human subjects research. There was no tissue sampling or removal of tissue. HIPAA is not a consideration after death and so there were no privacy issues associated with insect sampling near bodies.

Statistical Analysis

One-way Analyses of Variance (ANOVAs) were employed to compare differences in mean relative abundance values between major Calliphoridae species throughout time. This test is appropriate for normally distributed data, which was confirmed for each group (month and/or date) within the dataset. One-way ANOVA analysis was done both in terms of comparing the range of monthly collection dates (with only 22 July considered for the month of July) as well as separately for comparison of the three July collection dates (22, 23, and 26 July). Cardinal directions were also compared in terms of the species' mean relative abundance values with four of the seven traps being chosen as representatives of the cardinal directions: trap 5 was identified as North, trap 2 or 3 as South, trap 7 as East, and trap 1 or 6 as West. Tukey's Honest Significant Difference (Tukey) post-hoc testing was performed to test the significance of any resulting pairwise differences. Linear regression analysis was completed for mean relative abundance vs. time for *Phormia regina* and *Lucilia illustris*; the corresponding correlation coefficients were compared using Fisher Z-Transformation. All statistical analysis was done using RStudio 1.1.453 (RStudio Inc., Boston, MA, USA).

Results

Weather Data

Average daily temperatures were calculated for each collection month (Figure 3). The overall monthly averages (°C) were 11.7 for May, 14.8 for June, 20.4 for July, and 15.1 for September. The total daily rainfall (mm) at the FROST facility was also calculated for each collection month from daily totals (Figure 4). These values resulted in total monthly rainfall values (cm) of 3.2 for May, 12.7 for June, 6.8 for July, and 16.3 for September.

Figure 3. **Average daily temperature (°C) at the FROST facility (Chapter I) for each collection month ± standard error. Red arrows indicate major monthly collection dates. Red * indicate additional July collection dates.**

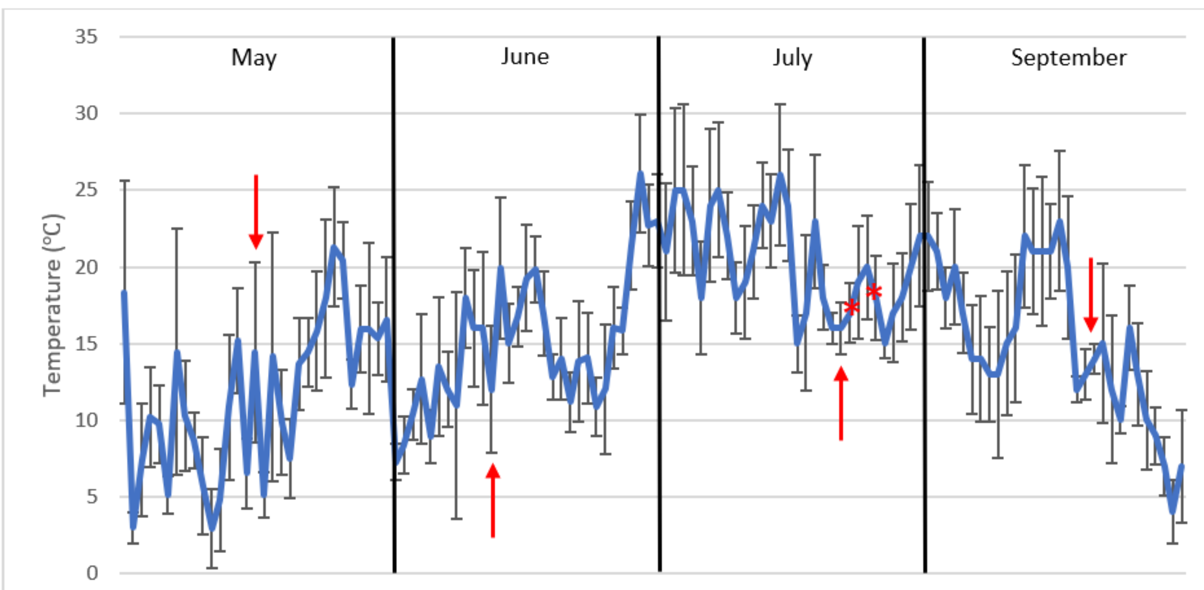
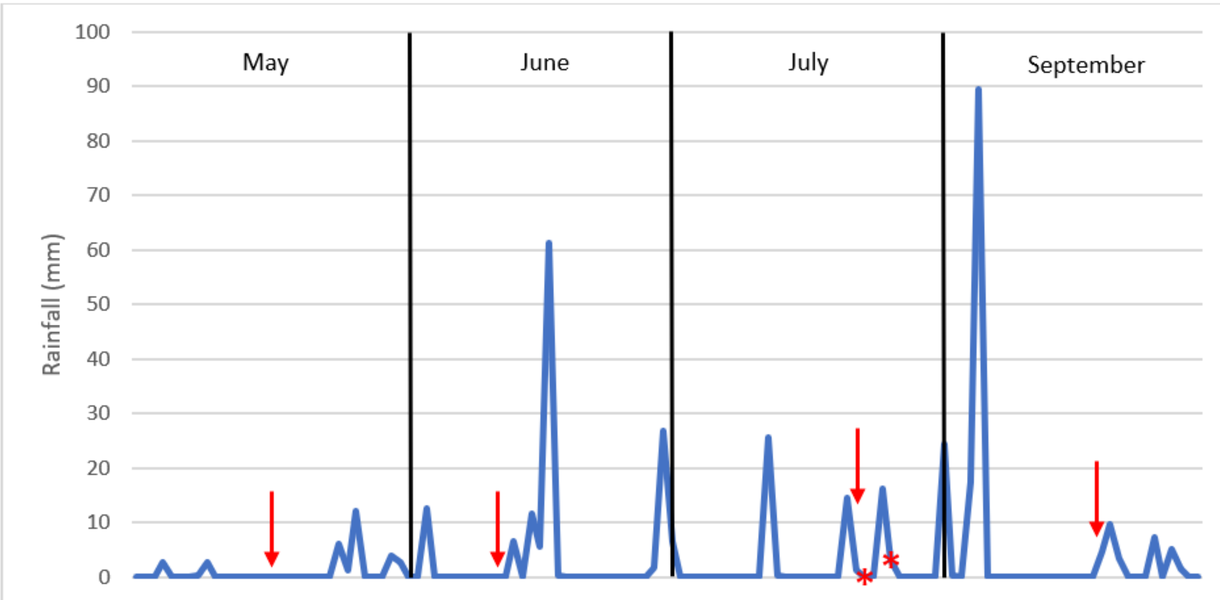


Figure 4. **Total daily rainfall (mm) at the FROST facility (Chapter I) for each collection month. Red arrows indicate major monthly collection dates. Red * indicate additional July collection dates.**



Calliphoridae Community Composition

Eight species of blow flies (Diptera: Calliphoridae) were collected at the FROST facility from May through September 2018: *Phormia regina* (Meigen), *Lucilia illustris* (Meigen), *L. sericata* (Meigen), *L. silvarum* (Meigen), *Calliphora vomitoria* (L.), *C. vicinia* (Robineau-Desvoidy), *Cynomya cadaverina* (Robineau-Desvoidy), and *Cochliomyia macellaria* (Fabricius). A total of 3,311 flies were collected for the first research objective using the July 22 collection point (Table 2). The greatest number of flies was collected in June (2,522 or 76.2%), followed by 22 July (578 or 17.5%), and May (187 or 5.6%), with the fewest were collected in September (24 or 0.7%). The most abundant Calliphoridae species were *P. regina*, *L. illustris*, and *C. macellaria*. The total number of *P. regina* collected was 2,033 (61.4% of the Calliphoridae community), the total number of *L. illustris* collected was 898 (27.1%), and the total number of

C. macellaria collected was 187 (5.6%). The least abundant species collected was *C. vicinia*, with only 2 total individuals (< 0.1%). While *P. regina* rapidly decreased in abundance after its highest numbers in June, *L. illustris* numbers increased and *C. macellaria* spiked in abundance from June to July (Figure 5, Supp. Figure 1).

An additional 653 blow flies were collected on 23 July and 26 July for the second research objective, resulting in a total of 1,231 blow flies collected for the month of July (Table 3). The greatest number of flies was collected on 22 July (578 or 47.0%), the day prior to the placement of donor bodies, the next greatest on 23 July (547 or 44.4%), the day of donor body placement, and the fewest on 26 July (106 or 8.6%), several days after the placement of donor bodies. *L. illustris* made up the largest proportion of these collected blow flies at 45.3% (558), *C. macellaria* the next largest at 32.0% (394), and *P. regina* the next largest at 15.4% (190). The least abundant was *C. cadaverina* at 0.6% (7) with the exception of two species which were not collected in July at all; these were *C. vomitoria* and *C. vicinia*.

Table 2. **Total number of adult Calliphoridae specimens collected in May, June, July (22), and September 2018.**

	Month				
Species	**May**	**June**	**July (22)**	**September**	**Total**
P. regina	155	1788	89	1	**2033**
L. illustris	3	600	281	14	**898**
L. sericata	2	2	39	8	**51**
L. silvarum	0	11	6	0	**17**
C. cadaverina	21	30	4	0	**55**
C. vomitoria	4	63	0	1	**68**
C. macellaria	0	28	159	0	**187**
C. vicinia	2	0	0	0	**2**
Total	**187**	**2522**	**578**	**24**	**3311**

Figure 5. **Mean percent relative abundance of *Phormia regina*, *Lucilia illustris*, and *Cochliomyia macellaria* over time. Note: only the first collection date in July (22) is considered. Error bars represent standard error.**

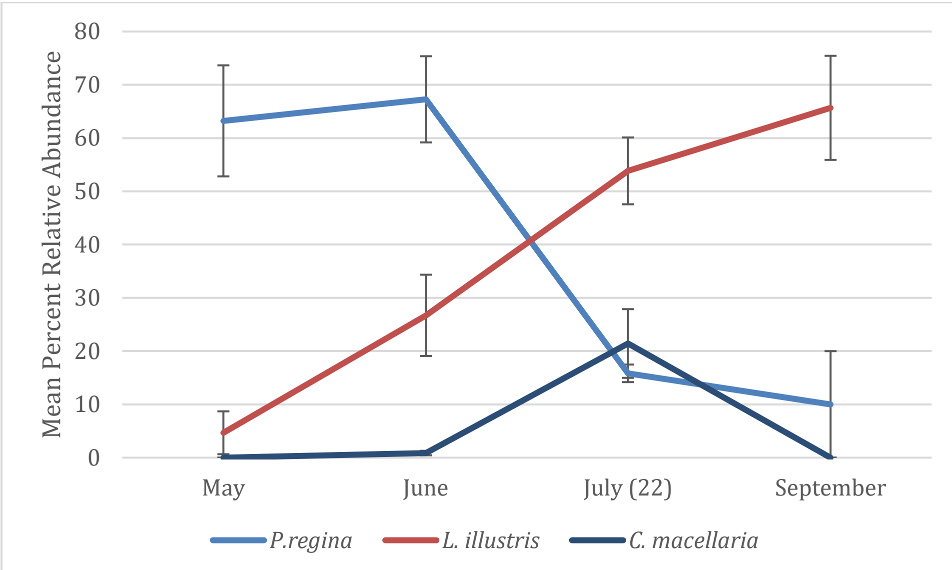


Table 3. **Total number of adult Calliphoridae specimens collected in July 2018 (22, 23, and 26 July). Donors were placed at FROST on 23 July.**

Species	**22 July**	**23 July**	**26 July**	**Total**
P. regina	89	97	4	**190**
L. illustris	281	192	85	**558**
L. sericata	39	24	6	**69**
L. silvarum	6	2	5	**13**
C. cadaverina	4	2	1	**7**
C. vomitoria	0	0	0	**0**
C. macellaria	159	230	5	**394**
C. vicinia	0	0	0	**0**
Total	**578**	**547**	**106**	**1231**

Calliphoridae Populations Over Time

Time was a significant factor in the mean relative abundances of the three most abundant species: *P. regina*, *L. illustris*, and *C. macellaria*. The mean relative abundance of each of these species significantly changed over the study period (F=17.190, p<0.001; F=6.406, p=0.002; and F=10.720, p<0.001, respectively) with changes to *P. regina* being the most significant (Table 4, Supp. Table 4). *P. regina* had a consistent relative abundance from May to June and from 22 July to September; however, the populations significantly decreased between June and September with (p<0.001) (Table 5). The opposite trend was found for *L. illustris*, from May to June and 22 July to September but with an increase in between and a significant increase from May to 22 July (p=0.003). The linear regression correlation coefficients for each of these species (r=-0.936 and r=0.910 respectively) are significantly different (z=2.28, p=0.020). *C. macellaria* spiked in mean relative abundance in July but made up a very small proportion earlier in the summer and into September. The largest, most significant differences for this species were from June to 22 July and from 22 July to September (p<0.001) (Table 5).

Table 4. **One-way ANOVA results for the comparison of mean relative abundance values for *Phormia regina*, *Lucilia illustris*, and *Cochliomyia macellaria* by month. Note: only the first collection date in July (22) is considered.**

Species		**Degrees of Freedom**	**Sum of Squares**	**Mean Sum of Squares**	**F-Value**	**Pr(>F)**
P. regina	Month	3	2.056	0.685	17.190	3.57e-06
	Residuals	24	0.957	0.040		
L. illustris	Month	3	1.028	0.343	6.406	2.42e-03
	Residuals	24	1.284	0.054		
C. macellaria	Month	3	0.235	0.078	10.720	1.17e-04
	Residuals	24	0.176	0.007		

Table 5. **Tukey's Honest Significant Difference test comparing monthly differences in mean relative abundance of the three major Calliphoridae species (*Phormia regina*, *Lucilia illustris*, and *Cochliomyia macellaria*) collected from May-September 2018. The date used for the month of July was 22 July. Only significant pairwise comparisons with p values of less than 0.05 are listed.**

Species	**Month**	**Diff**	**Lower**	**Upper**	**P-adj**
Phormia regina	May-Jul	0.474	0.180	0.768	9.275e-04
Phormia regina	Jun-Jul	0.514	0.220	0.809	3.597e-04
Phormia regina	Sep-May	-0.561	-0.855	-0.266	1.211e-04
Phormia regina	Sep-Jun	-0.601	-0.896	-0.307	4.730e-05
Lucilia illustris	May-Jul	-0.492	-0.833	-0.151	2.940e-03
Lucilia illustris	Sep-May	0.422	0.081	0.763	0.011
Cochliomyia macellaria	May-Jul	-2.056e-01	-0.332	-0.080	8.024e-04
Cochliomyia macellaria	Jun-Jul	-2.144e-01	-0.340	-0.088	4.982e-04
Cochliomyia macellaria	Sep-July	-2.144e-01	-0.340	-0.088	4.982e-04

Calliphoridae Populations in Response to Novel Resources

When comparing individual July collection dates (22 July, 23 July, and 26 July), *P. regina* maintained a steady relative abundance while the mean relative abundance of *L. illustris* and *C. macellaria* significantly changed ($F=8.047$, $p=0.003$ and $F=10.470$, $p=0.001$ respectively) (Table 6). In the case of both *L. illustris* and *C. macellaria*, these differences took place between 23 July and 26 July (Table 7). However, for *L. illustris* this difference occurred as an increase in mean relative abundance while for *C. macellaria* the difference occurred as a decrease in mean relative abundance.

Table 6. **One-way ANOVA results for the comparison of mean relative abundance values for *Phormia regina*, *Lucilia illustris*, and *Cochliomyia macellaria* by July date (22, 23, and 26 July).**

Species		Degrees of Freedom	Sum of Squares	Mean Sum of Squares	F-Value	Pr(>F)
P. regina	Date	2	0.044	0.022	3.456	0.054
	Residuals	18	0.116	0.006		
L. illustris	Date	2	0.407	0.204	8.047	3.19e-03
	Residuals	18	0.456	0.025		
C. macellaria	Date	2	0.325	0.162	10.470	9.64e-04
	Residuals	18	0.279	0.016		

Table 7. **Tukey's Honest Significant Difference test comparing differences in mean relative abundance of the three major Calliphoridae species (*Phormia regina*, *Lucilia illustris*, and *Cochliomyia macellaria*) across July 2018 collection dates (22, 23, and 26 July). Only significant pairwise comparisons with p values of less than 0.05 are listed.**

Species	Date	Diff	Lower	Upper	P-adj
Lucilia illustris	Jul 23-Jul 26	-0.340	-0.557	-0.123	2.319e-03
Cochliomyia macellaria	Jul 23-Jul 26	0.304	0.134	0.474	6.652e-04

Calliphoridae Populations by Cardinal Direction

Considering the seven baited traps in terms of cardinal direction did not reveal any significant differences in mean relative abundance between the three major species: *P. regina*, *L. illustris*, and *C. macellaria*. One-way ANOVA testing resulted in p-values >0.05 (p=0.567 for *P. regina*, p=0.380 for *L. illustris*, and p=0.777 for *C. macellaria*).

Discussion

This study was intended to provide survey information on Calliphoridae collected from late spring to early autumn at the FROST facility in Northern Michigan as well as in the context of introducing human donor bodies as a novel resource. These surveys, completed monthly from May-September with additional collections in July surrounding donor placement, were intended to identify the species of blow flies present at the FROST facility, their relative numbers, and any changes to this community over a summer and into autumn. This knowledge was obtained for use in future forensic investigations in the region as well as future decomposition or entomological studies at the FROST facility. Other than the Calliphoridae collected during this survey, small numbers of flesh flies (Diptera: Sarcophagidae) and a single carrion beetle (Coleoptera: Silphidae) were also trapped. These species were not the focus of this study. Similar studies focusing on the collection and identification of blow flies have been completed in the past. These include a recent study in Mid-Michigan in which blow flies were collected using the same trapping system as this study in both urban and rural areas during the summer of 2017 (Babcock 2018). The most common blow flies collected were *Phormia regina* (Meigen), *Lucilia sericata* (Meigen), *Lucilia illustris* (Meigen), and *Cochliomyia macellaria* (Fabricius). A recent study in New Jersey found *L. sericata*, *P. regina*, and *Lucilia coeruleiviridis* (Macquart) to be the three most common species (Weidner et al. 2017), while another in Central California found *Compsomyiops callipes* (Bigot), *P. regina*, and *Calliphora vomitoria* (Linnaeus) to be the most common (Brundage et al. 2011). In Ohio, *P. regina* and *L. coeruleiviridis* were the two most common Calliphoridae species collected during the summer (Benbow et al. 2013) and in Illinois the two most common species were *P. regina* and *L. sericata* (Baumgartner 1988). In Southwestern British Columbia, Canada, *L. illustris* and *P. regina* were collected during the summer (Anderson and VanLaerhoven 1996), and in Indiana *P. regina*, *C. macellaria*, and *L.*

coeruleiviridis were identified (Weatherbee 2016). There are no published pre- or early-operation surveys of necrophagous insects, including Calliphoridae, for any established outdoor forensic research facility. The results of these studies and the lack of surveys for outdoor forensic research facilities suggest the importance of conducting location-specific surveys which capture the diversity of different regions across the United States and internationally.

Eight total Calliphoridae species were collected in this study: *Phormia regina* (Meigen), *Lucilia illustris* (Meigen), *Lucilia sericata* (Meigen), *Lucilia silvarum* (Meigen), *Calliphora vomitoria* (L.), *Calliphora vicinia* (Robineau-Desvoidy), *Cynomya cadaverina* (Robineau-Desvoidy), and *Cochliomyia macellaria* (Fabricius). Perhaps unsurprisingly, this range of species is most similar—although slightly narrower—to that collected in Mid-Michigan by Babcock (2018). Of the eight species collected in this study, all were collected in the Mid-Michigan study; species not collected in this study but which were found in Mid-Michigan include *L. coeruleiviridis*, *Protophormia terraenovae* (Robineau-Desvoidy), and *Chrysomya rufifacies* (Macquart). The absence of the invasive species *C. rufifacies* is of particular note as this implies that it may not have reached Northern Michigan yet. The three most prevalent species in this study, *P. regina*, *L. illustris*, and *C. macellaria*, somewhat align with the most prevalent species in the Mid-Michigan study. In both this study and Babcock (2018), *P. regina* was the most prevalent species across the entire collection period (61.4% vs. 69.7%). These percentages are more similar to each other than to the numbers collected in California (23.0%) and Ohio (28.6%) (Brundage et al. 2011, Weidner et al. 2017). However, while *L. sericata* was the second most common species collected in Mid-Michigan at 18.8%, this species made up only 1.5% of the total Calliphoridae collected under the first objective of this study. Instead, the second most common species was *L. illustris* at 27.1%, a species which composed only 4.0% of the blow flies collected in Mid-Michigan. The third most common species in this study was *C. macellaria* at 5.6% of the total population, which is similar to the 4.0% composition in the Mid-Michigan study.

Month was a significant factor in the relative mean abundance of the three major Calliphoridae species collected. The mean relative abundance of *P. regina* was similar from late spring (May) to early summer (June) and from mid-summer (July) to early autumn (September), but significantly decreased in between with an overall drop from 63.2 ± 10.4% of the total population in May to 10.0 ± 10.0% in September. This decrease is consistent with what was observed in Mid-Michigan (Babcock 2018) as well as in studies in Florida and Texas (Sanford 2017, Gruner et al. 2007). While the relative mean abundance of *P. regina* decreased over time, however, the relative mean abundance of *L. illustris* significantly increased with an overall rise from 4.7 ± 4.0% of the total population in May to a maximum of 65.7 ± 9.8% in September (when in consideration of the one-a-month collection dates completed under the first objective of this study). These numbers indicate that *L. illustris* becomes more active as summer progresses into autumn as compared to *P. regina*, which is more active in the beginning of the summer and decreases thereafter. This is a relationship that was observed in Mid-Michigan as well (Babcock 2018). The mean relative abundance of *C. macellaria* did not follow either of these trends; instead, the abundance spiked in July at 21.4 ± 6.5% of the total population but composed a much smaller mean relative abundance before and after this date (0.9 ± 0.4% and 7.8 ± 3.0% respectively). This spike occurred during the warmest collection month in the survey. *C. macellaria* increasing in mean relative abundance during high temperatures has been witnessed in previous studies (Babcock 2018, Weidner et al. 2017, Brundage et al. 2011).

It is uncertain whether the introduction of human donor bodies as a novel resource had an important or significant effect on the mean relative abundance of the Calliphoridae species at FROST. The collections on the day prior to donor placement (22 July), the day of donor placement (23 July), and several days after donor placement (26 July) all resulted in consistent mean relative abundances of *P. regina*. However, the mean relative abundance for *L. illustris* increased significantly from 23 July to 26 July and the mean relative abundance decreased significantly for *C. macellaria* between these same dates. These trends—of *L. illustris* increasing past the first July collection and *C. macellaria* decreasing past the first July collection—were observed under the first objective from July to September and are generally consistent with other studies (Babcock 2018, Weidner et al. 2017, Brundage et al. 2011). Therefore, these changes cannot necessarily be linked with the introduction of the human donors as a novel resource. In terms of overall numbers, the total flies collected on 22 July and 23 July were highly similar while approximately 80% fewer blow flies were collected on 26 July. The reason for this shift is uncertain but may be due to the increase in rainfall experienced during this final July collection date as compared to the points previous. It began to rain approximately halfway through the designated trapping time for 26 July and continued for the entire time thereafter, potentially prohibiting the flight of blow flies or their recognition of the bait. It is known that rain delays oviposition on a carrion resource (Mahat et al. 2009) and so this may extend to attraction to a resource, although further research would be required to confirm this suggestion.

Calliphoridae activity depends on temperature and season according to previous research (Byrd and Allen 2001, Payne 1965, Tabor et al. 2004, Watson and Carlton 2005). Regardless of differences in relative species composition, it was expected that the total number of blow flies collected would decrease as the weather became colder in early autumn with the largest collections occurring during the warmer summer months. This expectation was generally observed; the total number of collected blow flies was highest for the month of June (76.2% of total individuals collected), second highest for July (17.5%), third highest in May (5.6%), and lowest in September (0.7%). The average temperature (℃) was warmest for the collection month of July at 20.4, second warmest in September at 15.1, third warmest in June at 14.8, and coldest in May at 11.7. Rain during the September collection window may have been responsible for the low number of blow flies collected during this period despite September being warmer than June on average. These 2018 temperature values were generally higher than or consistent with the averages for the previous year with the exception of September, as 2017 averages (℃) for Marquette, MI were 8.1 in May, 14.9 in June, 17.6 in July, and 16.8 in September; historical averages in this city are 10.6, 15.5, 19.2, and 15.2 respectively ("Climate-United States-Monthly averages" 2019). The total rainfall (cm) at the FROST facility was highest for September at 16.3, second highest for June at 12.7, third highest for July at 6.8, and lowest in May at 3.2. The 2017 rainfall totals (cm) for Marquette, MI were 10.0 in May, 12.2 in June, 5.1 in July, and 5.8 in September; historical averages for this city are 6.4, 6.8, 7.2, and 8.0 respectively ("Climate-United States-Monthly averages" 2019). These annual and historical variations suggest the necessity of conducting multi-year studies in order to identify any annual changes in Calliphoridae numbers and their relative species compositions in this location.

There were multiple limitations to this study. A primary one was the inability to trap in the month of August, leaving a gap in what would otherwise be a complete survey of late spring to early autumn. A collection date during August would have allowed for the finer assessment of observed trends. Related is the fact that this is a pre-/early-operation study which, while critically important to have completed upon the opening of FROST, would only increase in meaningfulness upon the completion of additional years of trapping. Adding to this dataset would allow for the long-term tracking of the local Calliphoridae community and provide the opportunity to learn more about the potential effects of a steady supply of human donors to this same community. It would likely be even more informative if the number of collections were to increase to several times per month instead of once per month. Additional collections, in the context of expanding this work to a multi-year survey of the Calliphoridae community at FROST, would be highly beneficial to the facility itself as well as to the broader forensic science community.

This work provides important information regarding the biodiversity of blow flies in Northern Michigan and at the new cold weather outdoor forensic science facility, FROST. It is now known that a similar range of Calliphoridae species can be found in Northern Michigan as compared to Mid-Michigan, with some key regional differences. These differences include a narrower range of species and a higher mean relative abundance of *L. illustris* as compared to *L. sericata*. Information such as this is useful to future researchers at FROST, whether they're directly studying the necrophagous insects on the site or simply observe them during the course of taphonomic studies. In addition, understanding which species are present in this region and how their relative proportions change over the course of the late spring, summer, and autumn may be useful to local investigators. For example, in a hypothetical situation where a wrapped body is located in Northern Michigan during September but the dead larvae found on the corpse are primarily *P. regina*, it may suggest that the person had originally been murdered in the late spring or early summer and was then frozen for several months before being disposed of.

Location-specific surveys of blow fly diversity such as this one contribute to the broader understanding of Calliphoridae populations in these regions and how they may change over time.

Conclusion

This study provided the first survey of Calliphoridae species present in Northern Michigan, and the first baseline survey of any necrophagous insect at a newly established outdoor forensic research facility. It was also only the second of its kind in the entire state of Michigan and the Great Lakes region. The findings of this study include a list of blow fly species collected in the area, the relative abundances of these species, and how some of these abundances change over time during the late spring, summer, and early autumn months. This survey information, combined with new knowledge regarding changes to this Calliphoridae population over time, will be useful to any professional forensic scientists or investigators who consider entomological information in their death investigation cases. It will also benefit future studies at the FROST facility which either focus on the local necrophagous insects themselves or on general human decomposition at northern latitudes.

Despite this new and valuable information, further research would do well to expand upon the findings of this study. Surveys of region-specific Calliphoridae populations are always needed. The work that has been done here in Northern Michigan can be replicated anywhere which does not have information regarding the species present near that area. Although it is technically too late for any other operational outdoor forensic science facility to conduct a pre- and/or early-operation entomological survey such as this one, it is not too late for these facilities to begin cataloguing the species present at their sites and tracking any changes to these populations over time. Furthermore, continued research at the FROST facility itself with the inclusion of additional collection points throughout the year as well as repeating the procedure annually would be highly beneficial. This information would expand upon the observations in this study to create a larger picture of the Northern Michigan Calliphoridae community and any changes it experiences over time.

CHAPTER II:

BLOW FLY COLONIZATION OF AQUATIC VERTEBRATE DECOMPOSITION

Introduction

One of the major goals of any death investigation is to determine the postmortem interval (PMI), or time since death, of the decedent. Most forensic decomposition studies and methods of estimating PMI are based upon the assumption that the remains were found in a terrestrial location, such as a home residence or the forest floor (Merritt and Wallace 2009). Research which employs forensic entomology in terrestrial locations is particularly well known and often involves the analysis of larval blow fly (Diptera: Calliphoridae) development (Catts and Goff 1992). Human remains are not always found on dry land, however, and may also be found in freshwater or marine environments such as lakes, rivers, or oceans (Anderson 2008). While drowning victims are an example of this, so are homicide victims whose remains are dumped in a body of water in an attempt to dispose of the evidence (Dix 1987). In these cases experts may attempt to estimate a postmortem submergence interval (PMSI), or time since the corpse was submerged in water, *in lieu* of a PMI. Studies on estimating the PMSI for remains found in an aquatic environment are much fewer in number than those for estimating a PMI in terrestrial environments, specifically in regard to forensic entomology (Haefner et al. 2004, Haskell et al. 1989).

Current State of PMSI Estimation Using Entomological Evidence

There are several published studies that have described the utility of insects in estimating the PMSI for carrion decomposing in freshwater. Relevant insects can include those which are traditionally observed in terrestrial forensic entomology studies, such as blow flies (Diptera: Calliphoridae), as well as aquatic invertebrates, such as caddisflies (Trichoptera) and water striders (Hemiptera: Gerridae) (Hobischak and Anderson 2002). Blow flies do not colonize completely submerged bodies but can colonize exposed remains in shallow water or on those which float to the surface during some stage of the decomposition process (Mann et al. 1990).

Floating is particularly characteristic during early decomposition stages (Rodriguez 1997) and so in addition to the ability of mid-to-late stage blow fly pupae to survive for up to 4 days underwater (Singh and Greenberg 1994), it may be possible to use blow fly evidence in the calculation of a minimum PMSI even if remains begin to sink. However, while particularly applicable to cases that include partial exposure due to their extremely rapid arrival time to carrion (Rivers and Dahlem 2014), the utility of blow fly evidence in an aquatic context becomes more limited when the remains are completely submerged. These are the circumstances in which aquatic invertebrates may be important. Aquatic invertebrates come with their own set of complications as there are no specialized necrophagous aquatic invertebrates that exploit carrion as a resource (Merritt and Wallace 2009, Fenoglio et al. 2014). Instead, they may facultatively utilize it as a food source or as protection and anchorage depending on factors such as the position of the body, the flow and depth of the water, the season, and the temperature (Haskell et al. 1989).

There are few studies which specifically address terrestrial insect and aquatic invertebrate succession of carrion in a freshwater aquatic environment. Hobischak and Anderson (2002) completed perhaps the most extensive experiment by placing pig (*Sus scrofa domesticus*) carcasses in ponds and streams in British Columbia, Canada and allowing them to decompose for one year. They found a predictable succession of terrestrial and aquatic invertebrates including the early appearance of blow fly and caddisfly larvae. With this information they were able to create a successional database for use in casework. However, they could not determine whether patterns were season-specific and did not find any one species that could be used to estimate PMSI on its own. Therefore, they argued that the combination of the total species present, their relative numbers, and the decompositional stage of the remains must be used to determine the

PMSI. Barrios and Wolff (2011) completed a similar but smaller-scale study in the Colombian Andes in which they observed invertebrate succession on pig carcasses in stream and artificial lake environments over the course of two months. They identified over 11,000 invertebrates associated with the carcasses in the lake and over 7,000 in the stream, both aquatic and terrestrial, in which blow flies were a clear indicator of the early floating stage. They did not attempt to calculate PMSI based on the presence of any one species and also noted that specific ecological categories of aquatic invertebrates (including shredders, collectors, and predators) could not be significantly associated with particular stages of decomposition.

Decomposition studies on trout carrion in freshwater river systems have also offered insight into the aquatic invertebrate colonization and succession in aquatic ecosystems. Fenoglio et al. (2005) tracked the decomposition of rainbow trout in a North Italian stream and identified nearly 7,000 invertebrates belonging to 58 taxa which colonized the carcasses. The most abundant taxa included the Chironomidae (29%) (Diptera) and Ephemerellidae (27%) (Ephemeroptera) as well as *Habrophlebia* sp. of the Leptophlebiidae (10%) (Ephemeroptera). Carcasses were sampled for approximately one month beginning in May with some changes in community composition being observed over time where early colonizer taxa, such as Chironomidae and *Dugesia* sp., were replaced by late colonizers, such as Polycentropodidae and Leptoceridae. In a later study, Fenoglio et al. (2010) also reported nearly 2,000 invertebrates belonging to 42 taxa on fish carcasses, with mean total abundance increasing over 48 days of decomposition in February. They also found that Chironomini (Diptera) were the most dominant at ~61% of all invertebrates. Euholognatha stoneflies (Plecoptera) (*Protonemura* sp., *Leuctra* sp., and *Nemoura* sp.) as well as Limnephilidae caddisflies (Trichoptera) were the next most prevalent taxa (~22% combined and ~3%, respectively).

These studies as a whole, despite diverging in whether a predictable successional pattern was observed or not, suggest the following: that blow flies are clearly associated with partially exposed remains and so have at least some forensic value, and that while aquatic invertebrates are more complicated they should not be ignored in aquatic death investigations. This potential utility of aquatic invertebrates was emphasized by Wallace et al. (2008) who successfully used the known development timing of caddisfly larvae to assist in the estimation of a PMSI range that was consistent with when the decedent disappeared.

Gaps in Knowledge

Current research involved in PMSI estimation for remains found in freshwater aquatic environments is limited. In particular there is a lack of survey and successional studies for a variety of geographic locations and seasons. Invertebrate presence and succession may differ between areas, meaning that the successional database created by Hobischak and Anderson (2002) in British Colombia may not be relevant to a drowning case in southern Florida. The same is true for seasonality. While Hobischak and Anderson (2002) attempted to address this factor, they did not come to any concrete conclusions. An additional example by Tomberlin and Adler (1998) directly compared the decomposition and insect succession of rat (*Rattus rattus*) carrion both on land and in freshwater during two seasons in South Carolina. They found differences in both decomposition rate and species composition during the winter, but they did not use a natural or human-made body of water and instead used small tubs of freshwater that were placed in a field. These would have been devoid of any natural aquatic invertebrate communities. Rats are also much smaller and have more hair than humans or swine proxies and so do not closely represent human decomposition.

Statement of Purpose and Hypothesis

The purpose of this study was to provide blow fly community and succession data within the context of aquatic decomposition using swine models (n=3). This objective was designed in an effort to provide new information on the blow fly colonization of decomposing remains in a freshwater aquatic environment, with the goal of assisting investigators in knowing which species are expected at an aquatic scene during a death investigation in a mid-Michigan geographic area during the summer. We predicted similar blow fly community composition between the three replicate carcasses, with minimal variation in species throughout time during the decomposition process. It was possible that we would observe different blow fly species in particular arriving to the carrion sources at different times in a successional pattern. If this were to occur, a successional pattern would potentially produce multiple oviposition events which could be demonstrated by the observation of separate larval masses developing over time with different primary species being associated with each. Multiple oviposition events (and subsequent differences in the distribution of third instar larvae of particular species, with third instars being those primarily collected by law enforcement and CSI teams) could potentially lead to differences in the calculation of the PMSI depending on which larvae were used. Overall, expected blow fly species in the community include those commonly found in the Midwest USA such as *P. regina* and *Lucilia spp.*as well as *Calliphora* and *Cochliomyia macellaria* as detailed in Chapter 1.

Materials and Methods

Sampling Location

The sampling for this study was conducted at the Michigan State University Entomology Research Farm, a remote plot of land administered by the Department of Entomology and located approximately 0.5 km east of Collins Road in East Lansing, Michigan. The land is composed of cornfields and wooded areas with a small, unnamed pond offset from the road. This pond is approximately three meters in depth at the center and provided the body of water necessary for the collection of insects (Diptera and aquatic invertebrates) associated with aquatic decomposition. The site was inaccessible to the public and was removed from the areas in which other Michigan State research groups conduct their work.

Carrion Source

The source of carrion for this study was the domestic pig, *Sus scrofa domesticus*. Three replicate adult pigs weighing approximately 45 kg were sourced from the MSU Swine Facility and euthanized using barbiturates by a certified veterinarian (MSU IACUC approved: AUF #: 07/18-091-00). They were received immediately after euthanization and transported to the sampling location within 10 minutes for placement into the Entomology Research Farm pond. Each pig was dressed in the same set of clothing (black tank top with a white logo and jean shorts) so as to best simulate the aquatic decomposition of a clothed corpse. They were secured at least 20 m apart in water approximately 1 m deep using nylon rope attached to stakes and left to decompose with observations, photographs and insect sampling (upon the condition of their presence) occurring approximately every other day for a period of three weeks during July and August 2018. Stages of aquatic decay were determined as according to Benbow et al. 2015b. A

HOBO® (Onset Computer Corporation) temperature logger was secured to each replicate carcass to record the temperature of the water nearby at 15 min intervals.

Insect Sampling Methods

Larval blow flies of at least the second instar were collected when present approximately every other day for a period of three weeks during the decomposition of the replicate pig carcasses. Adult blow flies were collected using sweep nets when possible. Approximately 10% or less (for very large masses) of the total visible dipteran larval community (when present) was collected both for preservation (in molecular grade ethanol) in the case of larval identification and for rearing to adulthood to confirm larval IDs. Collected larvae were usually among the largest of the individuals present. Adult and larval blow flies were identified under a stereomicroscope using the identification guides *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* (Marshall et. al. 2011), and *Fly Larvae: Key to some species of public health importance* (Stojanovich et. al 1962).

Statistical Analysis

A normal data distribution was confirmed prior to additional testing. One-way Analyses of Variance (ANOVAs) models were employed to compare mean relative abundance values of major Calliphoridae species over time and among replicates, lengths of larvae over time, and lengths of larvae among replicate pigs. All statistical analysis was done using RStudio 1.1.453 (RStudio Inc., Boston, MA, USA).

Results

Weather Data

Water temperatures were recorded near each of the three replicate pig carcasses at the study site (Supp. Table 5, Figure 6). A mean temperature of 26℃ was recorded for each replicate over the entire study period. The mean air temperature in the shade at the study location was 23℃. The low temperature was 20℃, and the high was 28℃ (Supp. Table 5, Figure 7). These values closely align with those from the nearest airport, Capitol Region International Airport in Lansing, MI (approximately 8 km away from the study location), where the mean temperature for this time period was 23℃. The low temperature was 20℃, and the high was 29℃.

Figure 6. **Average daily temperature (℃) ± standard error of the water directly near each replicate pig (Chapter II) over the course of the collection period.**

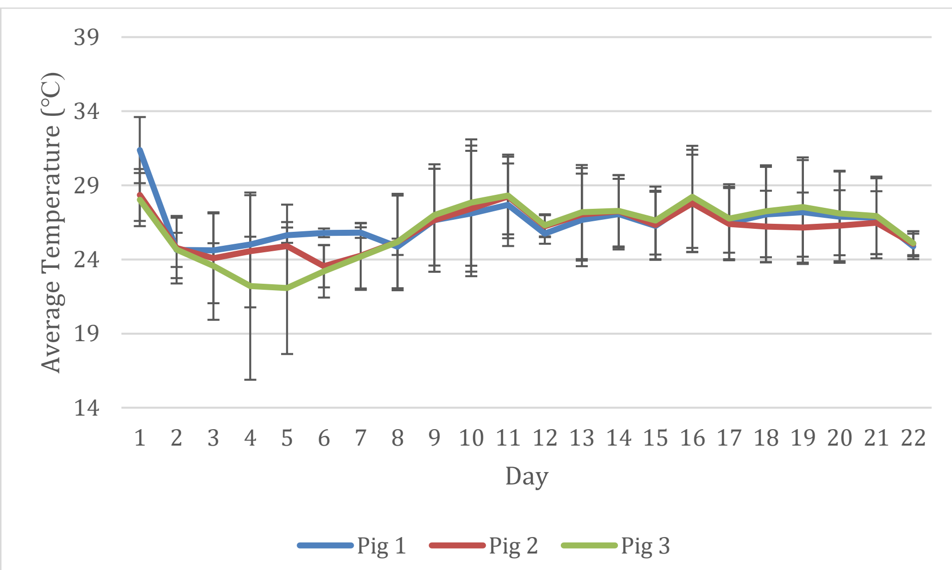
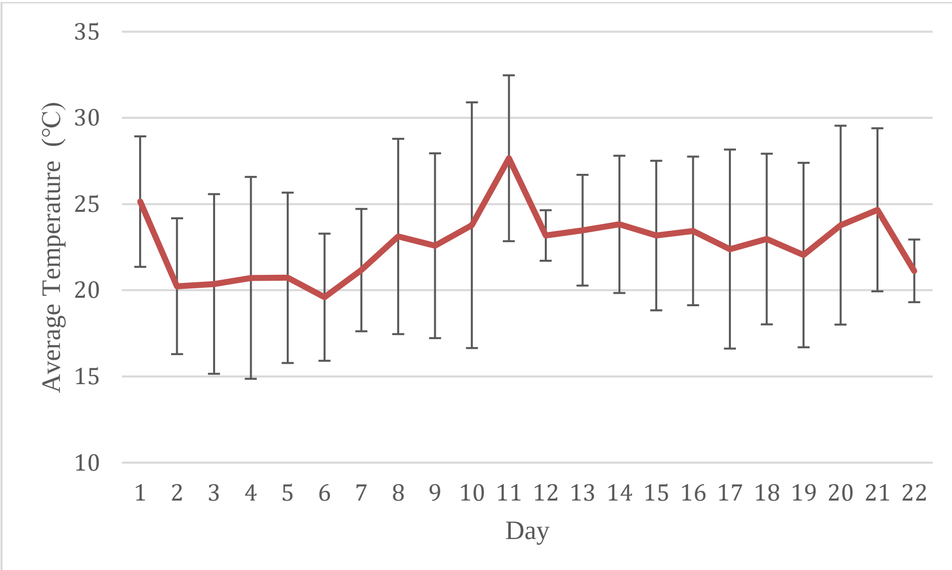


Figure 7. **Average air temperature in °C (± one standard deviation) for the study period at a shaded area immediately adjacent to the study location.**



Calliphoridae Community Composition

Two species of larval Calliphoridae were collected over the course of carcass decomposition (Table 8, Figure 8): *Phormia regina* (Meigen) and *Cochliomyia macellaria* (Fabricius). *Phormia regina* was the major species representing 90.5% of the community, while *Cochliomyia macellaria* made up only 9.5% (Table 9). Larval Calliphoridae of at least the second instar were first observed and collected on 3 August 2018 (Day 9) and were last collected on 16 August 2018 (Day 22). The greatest number of Calliphoridae larvae were collected on 5 August 2018 (94 or 33.1%) with the next greatest number collected on 3 August 2018 (83 or 29.2%); both dates occurred during the Floating Decay stage (Table 8). The number of collected larvae decreased following 5 August with only 5 total individuals (1.8%) collected on 16 August 2018 (Advanced Floating Decay stage). There were no significant differences in the mean relative abundance of either collected species (*Phormia regina* and *Cochliomyia macellaria*) among replicates or over time (Figure 9, Supp. Table 6).

Table 8. **Decomposition stages of Pigs 1-3 as they align with the day and date ranges of the experiment.**

Decomposition Stage	Day Range	Date Range
Submerged Fresh	1-3	7/26/18 – 7/28/18
Early Floating	3-5	7/28/18 – 7/30/18
Floating Decay	5-13	7/30/18 – 8/7/18
Advanced Floating Decay	13-22	8/7/18 – 8/16/18

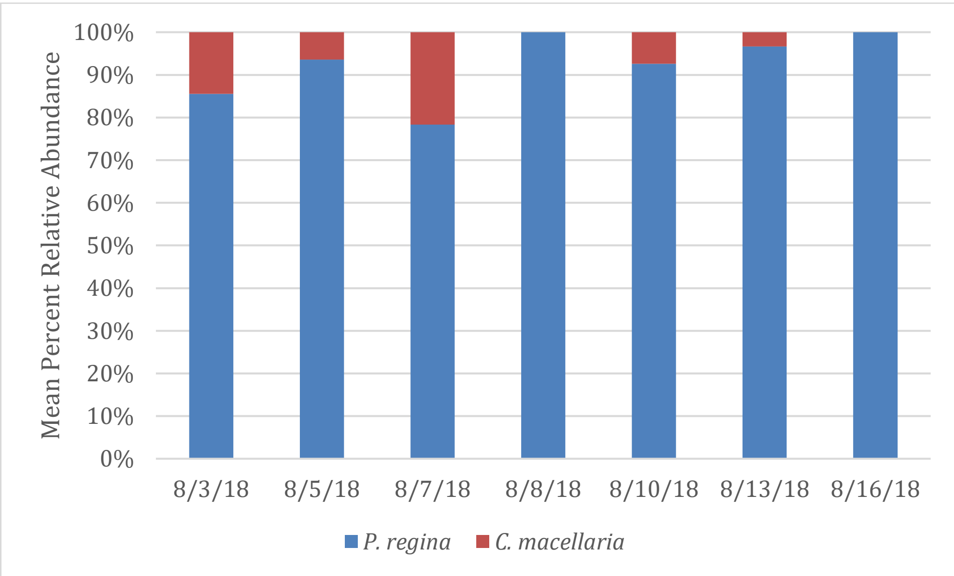
Figure 8. **Representational images of the decomposition states of Pigs 1-3 (A. Submerged Fresh, B. Early Floating, C. Floating Decay, D. Advanced Decay).**

Submerged Fresh | Early Floating | Floating Decay | Advanced Floating Decay

Table 9. **Total numbers of *Phormia regina* and *Cochliomyia macellaria* larvae collected from Pigs 1-3 in August 2018.**

	Date							
Species	**8/3/18**	**8/5/18**	**8/7/18**	**8/8/18**	**8/10/18**	**8/13/18**	**8/16/18**	**Total**
P. regina	71	88	22	29	25	17	5	**257**
C. macellaria	12	6	6	0	2	1	0	**27**
Total	**83**	**94**	**28**	**29**	**27**	**18**	**5**	**284**

Figure 9. **Mean percent relative abundance of *Phormia regina* and *Cochliomyia macellaria* larvae collected from Pigs 1-3 in August 2018. For N values, refer to Table 9.**

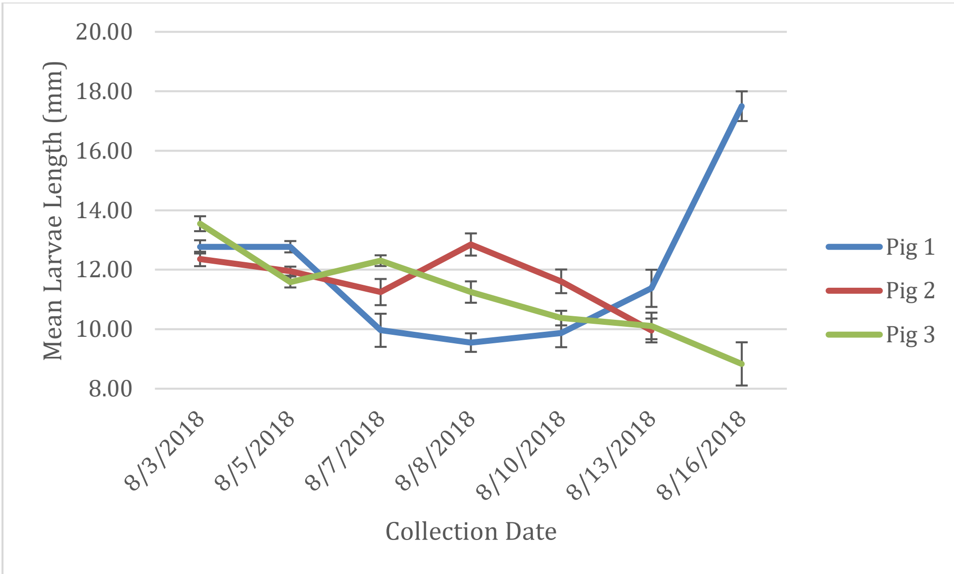


Larvae Lengths Over Time

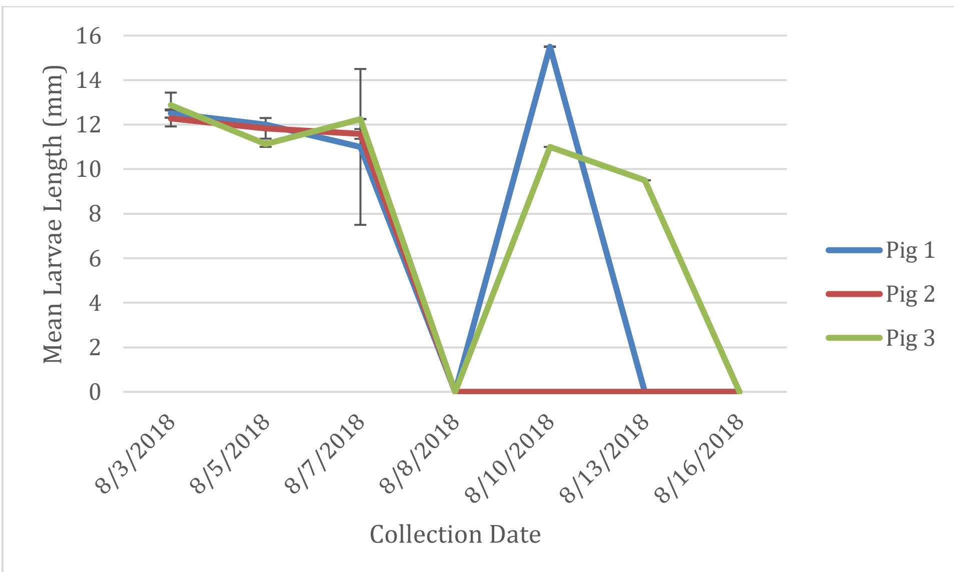
There were no significant differences in the mean length of *Phormia regina* or *Cochliomyia macellaria* larvae among pigs or over time (Supp. Table 7, Supp. Table 8). However, the length data do reveal subtle trends – particularly for *Phormia regina*, as the number of *Cochliomyia macellaria* collected was consistently either far lower than *Phormia regina* or entirely absent across all collection periods. Both species of larvae collected on 3 August 2018 were in the average range of 12-14 mm (for all three pigs) but did not steadily increase thereafter (Figure 10). Instead, for *Phormia regina* the average larval length for Pig 2 and Pig 3 generally decreased with an average larval length at the last day of collection that was smaller than the average larval length at the first day of collection. The average *Phormia regina* larval length for Pig 1 fluctuated more than the other replicates, with an initial decline in length which then reversed directions on 10 August. The maximum average larval length of *Phormia regina* larvae for Pig 1 occurred on 16 August, for Pig 2 on 8 August, and for Pig 3 on 3 August. For *Cochliomyia macellaria*, the average larval length decreased slightly for Pigs 1 and 2 and was relatively consistent for Pig 3 between 3 August and 7 August (Figure 10b). The average length then fluctuated strongly for each species after 7 August, perhaps due to low numbers of collected larvae. The maximum length for Pigs 2 and 3 occurred on 3 August and for Pig 1 on 10 August.

Figure 10. **Mean *Phormia regina* (A) and *Cochliomyia macellaria* (B) larvae length in mm (± SE of the mean) collected from Pigs 1-3 in August 2018. No *C. macellaria* were collected beyond 7 August 2018 for Pig 1 and Pig 2, and no *C. macellaria* were collected on 8 August 2018 for Pig 3.**

A.



B.



Discussion

The purpose of this study was to conduct a survey of the Calliphoridae community attracted to decomposing carrion in a freshwater pond system and to observe any successional patterns of those species. This community structure and successional information would benefit investigators who work death scenes in Mid-Michigan in which an individual has been found in a freshwater aquatic system similar to that described here. It would also benefit land-based geographic survey studies in Michigan, in that the species collected from carrion decomposing in an aquatic environment can be compared to those collected from similar studies on dry land. Other than the Calliphoridae larvae collected during this study, several aquatic invertebrate species were found on or near the swine carrion: aquatic dragonflies (Odonata: Aeshnidae), crayfish (Crustacea), and giant water bugs (Hemiptera: Belostomatidae). These invertebrates were not the focus of this study but confirm that Calliphoridae are not the only taxon attracted to decomposing remains in aquatic environments. Previous Calliphoridae survey work such as this study is limited for aquatic environments. Hobischak and Anderson 2002 catalogued only one species of Calliphoridae during their aquatic swine decomposition study in Canada, *Calliphora vomitoria* (L.), which was collected during what they classified as the Bloat stage. In the Columbian Andes four species of Calliphoridae were collected in a study on swine decomposing in two freshwater systems: *Compsomyiops verena* (Walker), *Calliphora nigribasis* (Macquart), *Sarconesiopsis magellanica* (Le Guillou), and *Chrysomya albiceps* (Wiedemann) (Barrios and Wolf 2011). Each of these species were collected primarily during the stage the authors termed Floating Decay, with small numbers collected during the Early Floating stage as well. There are far more land-based studies than aquatic-based, many of which were previously discussed in Chapter 1 of this thesis.

Two total Calliphoridae species were collected during this study: *Phormia regina* (Meigen) and *Cochliomyia macellaria* (Fabricius). Larvae were collected during Floating Decay and Advanced Floating Decay stages of decomposition. These stages roughly align with those in which Hobischak and Anderson 2002 and Barrios and Wolf 2011 collected Calliphoridae. However, the two identified species do not align with those collected in these two studies. This is not surprising considering the regional differences between Canada, the Columbian Andes, and Mid-Michigan. The relatively low number of collected Calliphoridae species does appear consistent however (Hobischak and Anderson 2002, in particular, only identified one Calliphoridae species). *P. regina* and *C. macellaria* were two of the most common species collected during the same summer in the land-based survey described in Chapter 1 of this dissertation, and were also two of the most commonly collected in a 2017 land-based survey conducted in Mid-Michigan (Babcock 2018). *P. regina* larvae composed 90.5% of the total larvae collected in this study and the mean relative abundance of this species did not significantly change throughout the study period or between replicate pigs. *C. macellaria* larvae composed the remaining 9.5% of the total larvae collected and its mean relative abundance also did not significantly change throughout the study period or between replicates. Therefore, no successional pattern of Calliphoridae species was observed. This is not surprising considering the short time window of collection and the consistent water temperatures observed near each replicate carcass.

The mean length of the *P. regina* and *C. macellaria* larvae collected over the course of this study did not change significantly over time or between replicate pigs. So comparatively few *C. macellaria* were collected that it was difficult to determine any apparent (if insignificant) trends. The greater number of collected *P. regina* larvae did allow for this, however, and it was observed that the average larval length for two of the replicate pigs (Pig 2 and Pig 3) generally decreased over time with a lower average on the last day of collection as compared to the first. The exception to this general decrease are small spikes in measured larval length on 7 August 2018 for Pig 3 and 8 August 2018 for Pig 2. The average larval length fluctuated for Pig 1 and was higher on the last day of collection as compared to the first. These trends indicate that there may have been two oviposition events for Pigs 2 and 3 during the course of the study. While an increase in average length over time would theoretically imply a single oviposition event followed by typical larval growth, a decrease in average length over time paired with small spikes of increased length suggests the presence of more than one cohort. Multiple oviposition events may lead to challenges when calculating a PMSI as the estimated time may change based upon which oviposition event the larvae collected from the crime scene originate from. Further research is necessarily to confirm this explanation of these observations while keeping in mind that no significant changes in length were recorded.

There were several limitations to this experiment. The first of these is the narrow timeframe. Repeating this experiment over the course of several summer months would have been ideal in order to form a larger picture of Calliphoridae species attracted to the carrion and identify any temporal changes. Temperature changes may have influenced the number of species present as well as their mean relative abundance, as witnessed during land-based studies conducted over multiple months. Additionally, this study was conducted in a single freshwater system, a small pond, with no comparison system. The Calliphoridae species and their relative mean abundance may depend on the type of system in which the carrion is placed. For example, simultaneously conducting the experiment in a river or larger standing body of water in the Mid-Michigan area may have produced different results. Any differences would be important to investigators working a scene in which remains were discovered in a body of water considerably different from the one used in this experiment.

This study provides important information regarding which Calliphoridae species are attracted to carrion decomposing in a freshwater system in Mid-Michigan during the summer. It is now known that common species observed during land-based studies in Northern and Mid-Michigan are also observed during cases in which the attractive resource is located in freshwater, although the number of expected species appears to be less in these cases. This work also suggests that multiple oviposition events may occur within a short window of time. This information is useful for both forensic scientists and investigators, as entomologists who estimate the PMSI of remains would need to consider the error that may result from calculating a value based upon larvae which originate from a later oviposition event. Future research which explores the collection of Calliphoridae larvae from carrion in a freshwater aquatic system during additional months of the year would be beneficial to understanding any temporal changes. The scope of this study did not include the calculation of multiple PSMI values based upon larvae collected at differing dates. However, more in-depth work needs to be conducted in order to understand whether multiple oviposition events truly do occur in similar cases and, if so, the repercussions that multiple events may have on the estimation of the PMSI. Future work might address this problem by conducting blind PMSI estimations for several larvae collection points throughout the decomposition of the carrion.

Conclusion

This study provided the first information on the Calliphoridae species attracted to decomposing vertebrate carrion in a freshwater system in the state of Michigan. The larvae of two blow fly species were collected during the Floating Decay and Advanced Floating Decay stages of decomposition for *Sus scrofa* (domesticated pig) and the relative proportions of these species did not indicate a successional pattern. The findings of this study also imply that multiple oviposition events may occur on the same carrion, but further research is required to support this suggestion and make a stronger conclusion. The information gathered by this study will be of use to professional forensic scientists and investigators who handle cases which include remains found in a freshwater system in the Mid-Michigan area during the summer. It also benefits researchers who are conducting aquatic or land-based studies in other regions, as it provides comparative information.

Further research is required to expand upon the initial information obtained during this study. This would include repeating the experiment during additional months, both in the summer and during other times of year, as well as between years. The species present in this study may change during other times of the year or annually, and this expansion of work would help to address these possibilities. It is also necessary to expand upon the information obtained in this study regarding larval length and the possibility of multiple oviposition events. Repeating this experiment with the intention of calculating PMSI, ideally blindly, multiple times using larvae collected over the course of decomposition would be highly informative. The concern regarding possible multiple oviposition events is that PMSI estimates may significantly change depending on which cohort larvae were collected from. Understanding this issue (or debunking it) would assist forensic entomologists and investigators in their both their aquatic and land-based death investigations.

APPENDICES

APPENDIX A

Supplemental Tables

Supp. Table 1. **Average daily temperature (°C) at the FROST facility (Chapter I) for each collection month. Bolded dates signify dates of collection. *FROST-specific data not available; average daily temperature from Sawyer International Airport, Marquette, MI.**

	May	**June**	**July**	**Sept.**
1	18	7	23	22
2	3	8	21	21
3	7	10	25	18
4	10	13	25	20
5	10	9	23	17
6	5	14	18	14
7	14	12*	24	14
8	10	11*	25	13
9	9	18*	22	13
10	6	16*	18	15
11	3	16*	19	16
12	5	**12***	21	22
13	11	20	24	21
14	15	15	23	21
15	7	17	26	21
16	**14**	19	24	23
17	5	20	15	20
18	14	17	17	12
19	10	13	23	**13**
20	8	14	18	14
21	14	11	16	15
22	14	14	**16**	12
23	16	14	**17**	10
24	18	11	19	16
25	21	12	20	13
26	20	16	**18**	10
27	12	16	15	9
28	16	21	17	7
29	16	26	18	4
30	15	23	20	7
31	17		22	

Supp. Table 2. **Total daily rainfall (mm) at the FROST facility (Chapter I) for each collection month. Bolded dates signify dates of collection. *FROST-specific data not available; total daily rainfall from Sawyer International Airport, Marquette, MI.**

	May	**June**	**July**	**Sept.**
1	0.0	0.0	6.6	24.4
2	0.0	0.0	0.0	0.2
3	0.0	12.6	0.0	0.0
4	2.6	0.0	0.0	17.4
5	0.0	0.0	0.0	89.4
6	0.0	0.0	0.0	0.0
7	0.0	0.0*	0.0	0.0
8	0.4	0.0*	0.0	0.0
9	2.8	0.0*	0.0	0.0
10	0.0	0.0*	0.0	0.0
11	0.0	0.0*	0.0	0.0
12	0.0	**0.0***	25.6	0.0
13	0.0	6.6	0.4	0.0
14	0.0	0.0	0.0	0.0
15	0.0	11.6	0.0	0.0
16	**0.0**	5.6	0.0	0.0
17	0.0	61.2	0.0	0.0
18	0.0	0.4	0.0	0.0
19	0.0	0.0	0.0	**4.6**
20	0.0	0.0	0.0	9.8
21	0.0	0.0	14.6	3.4
22	0.0	0.0	**1.2**	0.0
23	0.0	0.0	**0.0**	0.0
24	6.0	0.0	0.0	0.0
25	1.2	0.0	16.2	7.4
26	12.0	0.0	**3.2**	0.0
27	0.0	0.0	0.0	5.0
28	0.0	0.0	0.0	1.4
29	0.0	1.8	0.0	0.0
30	4.0	26.8	0.0	0.0
31	2.6		0.0	

Supp. Table 3. **Total number of adult Calliphoridae specimens collected for Chapter I in May, June, July (22, 23, and 26 July), and September 2018.**

	Month				
Species	**May**	**June**	**July (all)**	**September**	**Total**
P. regina	155	1788	190	1	**2134**
L. illustris	3	600	558	14	**1175**
L. sericata	2	2	69	8	**81**
L. silvarum	0	11	13	0	**24**
C. cadaverina	21	30	7	0	**58**
C. vomitoria	4	63	0	1	**68**
C. macellaria	0	28	394	0	**422**
C. vicinia	2	0	0	0	**2**
Total	**187**	**2522**	**1231**	**24**	**3964**

Supp. Table 4. **Percent mean relative abundance values for all collected Calliphoridae species by date (± standard error) for Chapter I. N values for each species are varied and can be found in Table 3 and Supp. Table 3.**

Species	**5/16/18**	**6/12/18**	**7/22/18**	**7/23/18**	**7/26/18**	**9/19/18**
Phormia regina	63.23 (10.42)	67.27 (8.09)	15.83 (1.65)	14.70 (4.12)	5.56 (2.80)	10.00 (10.00)
Lucilia illustris	4.68 (4.03)	26.72 (7.63)	53.84 (6.27)	39.28 (4.97)	73.37 (6.66)	65.67 (9.77)
Lucilia sericata	0.48 (0.34)	0.10 (0.08)	7.50 (2.46)	6.62 (3.13)	8.00 (2.92)	19.33 (11.85)
Lucilia silvarum	0.00 (0.00)	0.68 (0.36)	0.98 (0.40)	0.49 (0.41)	4.37 (2.52)	0.00 (0.00)
Cochliomyia macellaria	0.00 (0.00)	0.87 (0.41)	21.44 (6.45)	38.22 (3.96)	7.80 (3.03)	0.00 (0.00)
Cynomya cadaverina	28.56 (8.54)	1.71 (0.48)	0.41 (0.21)	0.59 (0.47)	0.89 (0.89)	0.00 (0.00)
Calliphora vomitoria	1.85 (1.26)	2.65 (0.30)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	5.00 (5.00)
Calliphora vicinia	1.19 (1.19)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)

Supp. Table 5. **Average daily air temperature in °C (± one standard deviation) of the shaded shore near the study site. Average daily water temperatures in °C (± one standard deviation) near each replicate pig.**

Day	Air Temperature	Pig 1	Pig 2	Pig 3	Mean of Pigs
1	25 (4)	31 (2)	28 (2)	28 (2)	29 (2)
2	20 (4)	25 (1)	25 (2)	25 (2)	25 (2)
3	20 (5)	25 (0)	24 (3)	24 (4)	24 (3)
4	21 (6)	25 (1)	25 (4)	22 (6)	24 (4)
5	21 (5)	26 (1)	25 (3)	22 (4)	24 (3)
6	20 (4)	26 (0)	24 (1)	23 (2)	24 (1)
7	21 (4)	26 (0)	24 (2)	24 (2)	25 (2)
8	23 (6)	25 (1)	25 (3)	25 (3)	25 (3)
9	23 (5)	27 (3)	27 (3)	27 (3)	27 (3)
10	24 (7)	27 (4)	27 (4)	28 (4)	27 (4)
11	28 (5)	28 (3)	28 (3)	28 (3)	28 (3)
12	23 (1)	26 (1)	26 (1)	26 (1)	26 (1)
13	23 (3)	27 (3)	27 (3)	27 (3)	27 (3)
14	24 (4)	27 (2)	27 (2)	27 (2)	27 (2)
15	23 (4)	26 (2)	26 (2)	27 (2)	26 (2)
16	23 (4)	28 (3)	28 (3)	28 (3)	28 (3)
17	22 (6)	26 (2)	26 (2)	27 (2)	27 (2)
18	23 (5)	27 (3)	26 (2)	27 (3)	27 (3)
19	22 (5)	27 (4)	26 (2)	28 (3)	27 (3)
20	24 (6)	27 (3)	26 (2)	27 (3)	27 (3)
21	25 (5)	27 (3)	26 (2)	27 (3)	27 (2)
22	21 (2)	25 (1)	25 (1)	25 (1)	25 (1)

Supp. Table 6. **Mean percent relative abundance of *Phormia regina* and *Cochliomyia macellaria* larvae collected from Pigs 1-3 in August 2018 (Chapter II). For N values, refer to Table 9.**

Species	**8/3/18**	**8/5/18**	**8/7/18**	**8/8/18**	**8/10/18**	**8/13/18**	**8/16/18**
Phormia regina	85.58 (7.40)	93.57 (1.96)	78.33 (6.01)	100.00 (0.00)	92.59 (3.70)	96.67 (3.33)	100.00 (0.00)
Cochliomyia macellaria	14.42 (7.40)	6.43 (1.96)	21.67 (6.01)	0.00 (0.00)	7.41 (3.70)	3.33 (3.33)	0.00 (0.00)

Supp. Table 7. **Mean length of *Phormia regina* larvae in mm (± SE of the mean) collected from Pigs 1-3 in August 2018 (Chapter II). For N values, refer to Table 9.**

Pig	**8/3/18**	**8/5/18**	**8/7/18**	**8/8/18**	**8/10/18**	**8/13/18**	**8/16/18**
1	12.77 (0.22)	12.77 (0.19)	9.96 (0.56)	9.55 (0.31)	9.88 (0.48)	11.38 (0.63)	17.50 (0.50)
2	12.36 (0.24)	11.95 (0.15)	11.25 (0.44)	12.85 (0.37)	11.61 (0.40)	9.96 (0.40)	-
3	13.55 (0.25)	11.59 (0.19)	12.31 (0.18)	11.25 (0.36)	10.38 (0.25)	10.11 (0.45)	8.83 (0.73)

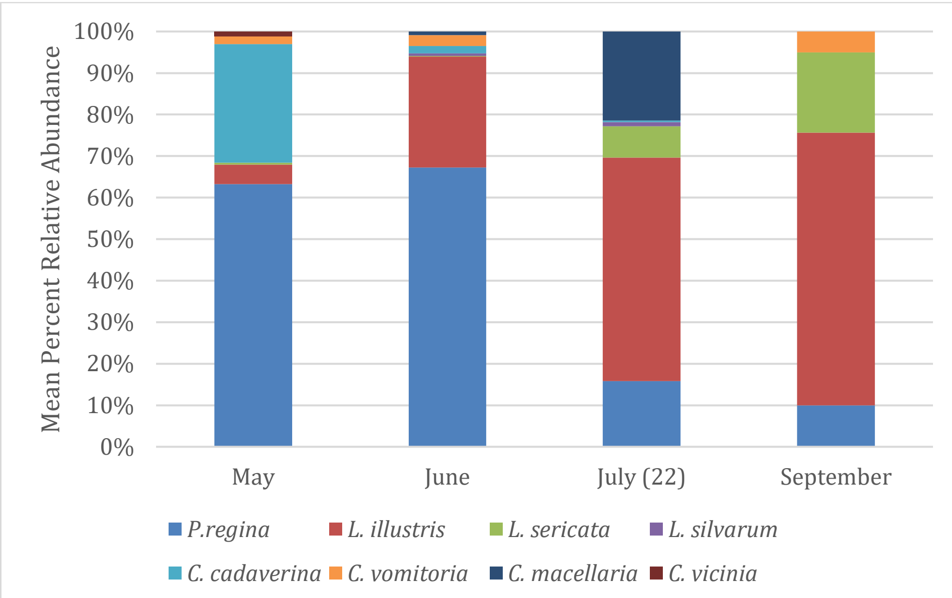
Supp. Table 8. **Mean length of *Cochliomyia macellaria* larvae in mm (± SE of the mean) collected from Pigs 1-3 in August 2018. For N values, refer to Table 9.**

Pig	**8/3/18**	**8/5/18**	**8/7/18**	**8/8/18**	**8/10/18**	**8/13/18**	**8/16/18**
1	12.50 (0.18)	12.00 (0.00)	11.00 (3.50)	-	15.50 (0.00)	-	-
2	12.28 (0.36)	11.83 (0.46)	11.58 (0.22)	-	-	-	-
3	12.88 (0.56)	11.13 (0.13)	12.25 (0.00)	-	11.00 (0.00)	9.50 (0.00)	-

APPENDIX B

Supplemental Figures

Supp. Figure 1. **Mean percent relative abundance of *Phormia regina*, *Lucilia illustris*, and *Cochliomyia macellaria* over time (Chapter I). Note: only the first collection date in July (22 July) is considered.**



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