ADULT BLOW FLY (DIPTERA: CALLIPHORIDAE) COMMUNITY STRUCTURE ACROSS URBAN-RURAL LANDSCAPE CHANGE IN MICHIGAN

By

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ABSTRACT

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Necrophagous insects play an important role in the decomposition of vertebrate carrion in the environment. The well documented colonization, development and succession of blow flies (Diptera: Calliphoridae) on decomposing carcasses makes studying their communities relevant for use in forensic investigations to establish post-colonization intervals. during an investigation. The main objective of this research was to conduct a baseline survey of adult Calliphoridae communities among urban – rural landscape types in the Great Lakes region. It was hypothesized that Calliphoridae communities would vary in composition, diversity and distribution across multiple cities and between two landscape types (urban and rural) in Mid-Michigan. To test how adult blow fly distribution varied with changing landscape conditions in Mid-Michigan, sampling with baited jars and hanging traps were implemented over the summer months of June, July, and August in 2017. To determine how blow communities changed from an urban to rural landscape, seven cities were selected with site locations ranging from high intensity developed areas to cultivated crop fields. Over 97,000 individual flies were captured with a total of 11 Calliphoridae species identified. The adult Calliphoridae communities were primarily structured by landscape type and month of collection, with these two factors having an interactive effect as well. The two most abundant species, *Phormia regina* (Meigen) and *Lucilia sericata* (Meigen), cumulatively comprised 88.5% of the identified adult flies. These finding are important to provide a helpful taxonomic baseline of Calliphoridae species in the Great Lakes region that forensic scientists may potentially use in both future research and case work.

To my amazing wife Jody, for always supporting me in my crazy adventures, and to my awesome kids Ari, Ana, and Ethan for making everyday a new adventure!

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INTRODUCTION

Forensic entomology is where the study of arthropods intersects with the judicial system (Byrd 2002). Crimes can be committed leaving little physical evidence of what took place. The ability to determine how and where that crime was committed can take a team of investigators and forensic scientists. Forensic entomology can be relevant in both criminal and civil litigation (Joseph et al. 2011) and can be broadly broken down into three sub-areas: (i) urban, (ii) stored-product, (iii) medico-legal. Urban forensic entomology is using entomological evidence in a dwelling, commercial and public buildings, garden, or misuse of pesticides for litigation purposes. Where stored-product entomology is the use of arthropods in contamination of food sources and usually includes issues where litigation may be taking place.

The most easily recognized subarea of forensic entomology is medico-legal. It involves using arthropods as evidence to locate bodies, estimate the post-mortem interval (PMI), aid in cause of death determination (Nolte et al. 1992), establish if a body was moved after death (Benecke 1998), and identify criminal suspects (Catts and Goff 1992). Medico-legal cases can also include issues of human or animal abuse, neglect, or poaching (Anderson 1999, Amendt et al. 2004). Investigating a homicide will require a wide range of tools to be used by forensic scientists who are supporting an investigation. When using information like body temperature or livor/rigor mortis to estimate a time of death, accuracy may only be possible for the first few days; however, by using necrophagous insects that time can be extended to weeks or months (Amendt et al. 2004). In recent years, there have been legal cases that used less common taxa used as evidence. Lice (order Phthiraptera) for example, were used to determine if an elderly woman had been neglected before her death (Pilli et al. 2016). While mites (class Arachnida) have been found to be forensically important in all three sub-sets of forensic entomology (OConnor 2009). Forensic entomology needs to be built on a stronger scientific foundation like other physical evidence such as blood stains, hairs, fibers, and fingerprints, and be (Amendt et al. 2007, Tomberlin et al. 2010).

Decomposition

The decomposition of both humans and animals creates a unique and ephemeral environment for a wide range of insects and other arthropods to use (Benbow et al. 2015). There are varying ideologies amongst scientists regarding the stages of decomposition. However, it is most important to understand that the stages of decomposition are simply used to help communicate descriptions of decomposition. Goff (2009) suggested that there are five stages of decomposition which are: (i) fresh, (ii) bloated, (iii) decay, (iv) post decay, and (v) skeletal/remains.

The conditions under which carrion are found can not only impact the stages of decay, but what types of arthropods can be discovered (Galloway et al. 1989). One study in Hawaii found ten orders, twenty-seven families, and forty genera of arthropods reported on domestic swine carcasses over a twenty-day span (Tullis and Goff 1987). During the fresh stage *Creophilus maxillosus* (Linnaeus) (Coleoptera: Staphylinidae) and *Thyreocephalus albertisi* (Fauvel) (Coleoptera: Staphylinidae) were found on the carrion, as well as two Calliphoridae (Diptera) species, *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart)). As decomposition progressed to the bloat stage more Staphylinidae species were observed, and while there was no increase in Diptera species it was the stage where there was the highest number of adults on the carcass. The same trend occurred during the decay stage and post-decay stage where there was no increase in Diptera species while the Staphylinidae increased. Although there was no change in Diptera species on the carrion the increase in Staphylinidae can be associated with the fact that they feed on the larval flies (Payne 1965). A 2001 study in Austria also evaluated succession rates of arthropods on swine carcasses, but provided different results. Fourteen dipteran species were found on pig carrion fitted with human clothes. The clothes became soiled with blood and fluids leaking from the corps, which allowed for larger larval masses resulting in faster decomposition (Grassberger and Frank 2004). In Michigan forty-one swine carcasses were buried at a depth of 60 cm in an agricultural field and both Sarcophagidae and Muscidae species were found to colonize the buried carrion (Pastula and Merritt 2013).

Arthropods associated with decomposition

There are four categories of species that make up the carrion necrophagous community: (i) necrophagous species that feed on the carrion, (ii) predators and parasites of necrophagous insects, (iii) omnivorous species feeding on the carrion and necrophagous insects, and (iv) insects that use the carrion as an extension of their environment, such as spiders (Smith 1986). The two most common insect orders evaluated in forensic investigations are Diptera (flies) and Coleoptera (beetles) (Joseph et al. 2011, Payne 1965). The most common dipteran families found associated with decomposition are Calliphoridae (blow flies), Sarcophagidae (flesh flies), and Muscidae (house flies) (Campobasso et al. 2001).

Entomological evidence is important and useful tool to determine when decomposition began. During the earlier stages of decomposition, the life cycles of Calliphoridae are most beneficial to help estimate a PMI. As remains undergo multiple stages of decomposition, it is more difficult to establish a PMI since the life cycles of insects come to be difficult to interpret (Goff 1993). At this point succession-based PMI becomes a better tool. This method evaluates the changes in arthropod communities that occur throughout the process of decomposition. From the consumers to predators and parasitoids there are a wide range of entomological factors that could influence PMI calculations.

Necrophagous insects and the parasites and predators who prey upon them are two of the most significant groups of arthropods used to establish a PMI (Goff 2009). Since some of these insects have the ability to feed on the skin and flesh of animals within minutes of death (Joseph et al. 2011) it makes them a reliable indicator for PMI within the first few weeks after death (Amendt et al. 2004). The predators who consume the necrophagous insects are also helpful because of their predictable arrival during the various life stages of their prey (Joseph et al. 2011). However, when there are high abundances of predators, parasites, and omnivorous species on carrion it could interfere with PMI calculations. Mello and Aguiar-Coelho (2009) showed in laboratory conditions how parasitoids can alter the development cycle of Calliphoridae and cause the PMI to be miscalculated by weeks. Skin beetles (Coleoptera: Dermestidae) have also been used to estimate PMI since they arrive on carrion in late stages of decay (Souza and Linhares 1997). To establish the amount of time that has passed since death a PMI can be established for a body using factors such as temperature, moisture, pH, and partial pressure of oxygen (Vass 2011).

Using entomological evidence to establish a PMI can be misleading since some insect colonization can be altered based upon the circumstances surrounding a death. Goff (1992) was able to delay the oviposition of Diptera by 2.5 days by wrapping a body tightly in a blanket. Additionally, Pechal et al. (2014) revealed that delaying insect access to carrion changed the insect community structure and decomposition process. Therefore, an alternative way to define the time from insect colonization until discovery as a post-colonization interval (PCI) (Tomberlin et al. 2010, Weatherbee et al. 2017).

Evaluating the distribution of blow fly species within a geographic region can be important particularly when practitioners only have outdated, difficult to access and restricted taxonomic keys (Sanford 2017). Distribution databases of Calliphoridae, Muscidae and Sarcophagidae species could assist law enforcement in determining PCI for a given region. The distribution information could also help identify if a body had been moved from one location to another. For forensic entomology data to be useful within a case it is critical to have a dataset on the species present within the region (Weidner et al. 2017).

There have been numerous studies of forensically important blow fly distribution around the world (Supp. Table S1) In Ankara Province China, it was determined that *Calliphora vomitoria* (Linnaeus) *and Calliphora vicina* (Robineau-Desvoidy) can be used to establish PCI when ambient temperatures are low in spring and autumn (Sabanoğlu and Sert 2010). While forty-eight species of blow flies belonging to eighteen genera were detected across Pakistan (Kurahashi and Afzal 2002). During a two-year span in Peru, 34,389 Calliphoridae were captured and examined with the major two taxa being *Compsomyiops callipes* (Bigot) (38%) and *Phormia regina* (Meigen) 23% (Baumgartner and Greenberg 1985). In South Africa *Chrysomya albiceps* (Wiedemann) and *Chrysomya marginalis* (Robineau-Desvoidy) were the widest spread species while *Chrysomya chloropyga* (Wiedemann) and *Calliphora croceipalpis* (Jaennicke) were found in high altitudes (Richards, Williams, and Villet 2009). A seasonal adult Calliphoridae distribution study in Spain covered 7,000 km^2 and found ten blow fly species with C. vicina (35%) and C. vomitoria (23%) (Zabala et al. 2014). With a wide range of studies, and equally varying results of dominate species it is important to have more regional specific studies to understand local distribution (Weidner et al. 2017).

Calliphoridae in the Midwest

The only Great Lakes study of necrophagous Calliphoridae distribution took place in the spring of 1980 and only focused on urban locations. Nearly 1,200 flies were collected on rat carrion, enhanced with chemicals such as ethyl mercaptan and ammonium carbonate, throughout Chicago, Illinois. Two main abundant species collected were *Lucilia sericata* and *Phormia regina* (Baumgartner 1988); however, this study did not account for how summer month temperatures effect the distribution of these two Calliphoridae species. Previous research has shown that *P. regina* has also been found to be intolerant of warmer temperatures (Byrd and Allen 2001), and therefore abundances could be expected to change during the summer. If the abundances of any one particular species should decrease within a region it could be expected that another species will increase to fill the void within the ecosystem. It has also been shown that Calliphoridae can vary based upon geographic region. For example, *P. regina* have varied in distribution from 29.2% (Weidner et al. 2017), 23% (Brundage et al. 2011), and 17% (Baumgartner 1988) within their respective study areas.

Hypothesis

This study will provide one of the first surveys of Calliphoridae distribution between an urban and rural landscape change for the Great Lakes region. It will also provide a baseline database from 2017 that forensic entomologist in the region will be able to use as a resource. While this study was designed as a descriptive study, based on previous understanding of Calliphoridae from other regions of the USA, a set of hypotheses were developed. The null hypothesis (H_0) is that Calliphoridae communities will be similar and that species will be evenly distributed across an urban to rural landscape change in Mid-Michigan. The alternative hypothesis (H_a) is that Calliphoridae communities and species will vary in distribution across an urban to rural landscape. As *L. sericata* is a synanthrope blow fly (Mariluis et al. 2008, Marshall et al. 2011), it is predicted that this species will be most likely be predominant in more urban landscapes. Previous research showed that *P. regina* is attracted to both manure and carrion

(Marshall et al. 2011), thus it is predicted that this species will be collected at higher abundances in rural areas where these food sources would be more plentiful than in urban areas.

MATERIALS AND METHODS

Study Location

This study was conducted in Mid-Michigan, a region that allowed for evaluating the distribution of carrion flies across urban to rural landscape change. The City of Lansing and the surrounding communities has mixed land use of both industrial and agricultural landscape cover that allowed for development of a sampling array that represented an urban-rural landscape change. The urban locations were predominantly low intensity developed areas, whereas the rural locations were cultivated crops and developed low intensity/open space (Figures 1- 3). Cities in the greater Lansing Capital Area selected for sampling adult Calliphoridae were: Charlotte, DeWitt, Grand Ledge, Mason, Lansing, Perry, and Williamston. The 2010 US Census showed the populations of the cities ranged from 116,020 in Lansing to 2,103 in Perry (Figure 1).

To survey adult Calliphoridae communities at replicate urban-rural land cover types four traps were used at each 1 km (urban) and 10 km (rural) distance from each city center, with the four traps placed in a cardinal direction (Figure 1). The city center was determined as the pin placement within Google Earth (Earth version 7.3.2) when a city was searched. The ruler function within Google Earth was used to establish the urban and rural locations around each city center. Google Earth was used to determine the urban to rural landscape transition, and then was ground-truthed by vehicle to confirm where housing subdivisions ended and agricultural land became predominant. Permission from landowners and businesses was acquired for the placement of traps resulting in some small distance variation from both the urban and rural locations.

Of the seven cities surveyed, Lansing was the only city that did not have rural landscape cover due to the distance needed to establish a rural location from Lansing. Thus, the Lansing 10 km sampling resulted in placement within a surrounding city suburb (e.g., primarily subdivision housing) and was not a representation of a rural location. Additionally, some cities did not allow for a true 10 km distance because the trap would have been within an urban or suburban land use setting. In this situation, a trap was placed at a location halfway between the two urban locations. The placement of the trap was in an area that was as close to a rural setting as possible.

Trap Design

The traps used to collect adult Calliphoridae (and other taxa) were built using an inverted cone design, similar to a butterfly trap (Figure 4, Figure 5). Each trap was 76.2 cm in length and 25.4 cm wide; traps were constructed using plywood for the frame and polyester mosquito netting (180 holes per 6.5cm^2) for the walls. In the center of the base, a 15.2 cm hole was cut out of netting and the edges were suspended from the top of the trap using 58.4 cm long rope to make the inverted cone. This trap design allowed flies to enter but not exit the trap at the base. At the base and top of each trap a bead of hot glue was applied to prevent flies from exiting the trap once they had entered. A zipper running the entire height of the trap was used for specimen removal after sample collection. Each trap had a zinc-plated threaded eyehook placed at the top with a 45.7 cm rope used for hanging the trap. At each location a trap was hung from a 1.2 m garden shepherd's hook and two 35.5 cm guide lines were placed at the base of each trap to prevent the trap for moving with the wind (Figure 4). When the trap was placed in the field the zipper was duct taped in the closed position preventing the trap from coming open.

Necrophagous Dipteran Attraction Resource

To attract adult necrophagous insects, bait jars were use at each trap. Bait jars were made using pig liver purchased from Michigan State University's Meat Lab. Frozen liver was purchased in a vacuum sealed bag and allowed to thaw to room temperature before being placed into a jar. Each bait jar consisted of 300 g of pig liver and 100 ml of reverse osmosis water. The jars were sealed using standard Mason jar lids and bands, and placed at an average room temperature of 25^{o}C for 350 accumulated degree hours (ADH), calculated using the methods provided in Vass et al. (2002). Bait was allowed to decompose for a total of seven days as previous research has shown maximum insect activity associated with aged bait between seven to seventeen days (Fisher, Wall, and Ashworth 1998). Each bait was used in the field for an additional seven sampling days. During placement in the field, an individual 1.4 L Mason jar with bait was placed with the bottom of the trap suspended approximately 2-4 cm above the top of the jar; the standard Mason jar lid was removed and replaced with screen to prevent insects from falling into the bait.

Necrophagous Dipteran Collections

Trapping took place during the summer months of June, July and August in 2017. All traps for any given city were placed into the field and collected one day during each month. The traps were left at the field site for four hours each day: placement occurred at the first site at 1000 and all traps were sequentially collected in the order they were placed beginning at 1400. This time frame was chosen since these hours of the day were previously reported to have the highest diurnal Calliphoridae activity in the Midwest (Central Ohio) (Berg and Benbow 2011). It took approximately two hours to place all eight traps within a city. When collecting each trap, the entry hole was spun closed by hand preventing flies from exiting. At the end of each day all traps were transported to the laboratory and placed in a -20°C freezer for no less than twelve hours, to ensure the flies had been euthanized prior to preservation. Flies were placed in 50 ml conical tubes with locality labels that included collection date, city, and trap location (Supp. Table S2) and placed into the -20°C freezer until identification.

Necrophagous Dipteran Subsampling and Identification

Once flies were removed from the freezer they were presorted by visual morphology into Calliphoridae and non-Calliphoridae taxa. Non-Calliphoridae were placed in a 50 ml conical tube, labeled and stored in a -20°C freezer. Calliphoridae were identified to species under a stereomicroscope using the *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* (Marshal et. al. 2011).

Traps returned a broad range of captured number of flies from 25 – 3,011 (Table 1). To facilitate sample processing and insect identification a subsampling study was performed to determine the minimum number of flies per trap that would be needed to represent the Calliphoridae community at each location. Four sites for the Williamston location were used to evaluate blow fly species in the following sample subsets: 100 specimens and 25%, 50% and 100% of total specimens. The total number of flies for each of the four sites were 1332, 1416, 170, and 175. These site totals were selected because they represented the two highest counts, and the two lowest counts.

For each sample, the flies were thawed, placed in a 1.4 L Mason jar and homogenized by hand inversions for 10 seconds. For the first subsampling approach 100 flies were randomly collected from each sample. For the 25%, 50% and 100% subsamples the respective fly number for each percentage was calculated and then individual flies were randomly selected. From this pilot study, it was determined that a count of 100 flies provided an equivalent Calliphoridae species representation as that of the three percentage subsampling levels.

For all of the remaining samples, the following protocol was used: 1) flies were removed from the -20°C freezer and allowed to thaw; 2) after thawing all specimens were placed in a

Mason jar and homogenized by hand using inverted movements for 10 s; and 3) 100 flies were randomly selected and identified using the previously described methods. All data were entered into a table by city, date, and location.

Statistical Analysis

A Permutational Multivariate Analysis of Variance (PERMANOVA) test was used with a Bray-Curtis dissimilarity matrix to test for Calliphoridae community differences among spatial [cities, location (rural/urban)] and temporal (month) scales. This nonparametric, multivariate method has been used in other studies to test for community differences among covariates (Pechal et al. 2018). A nonparametric test is best for testing community data that does have a normal distribution or homogeneity of variance (Caruso and Migliorini 2006, McArdle and Anderson 2001). Community differences were visualized using a Principal Coordinates Analysis (PCoA). In addition, the non-parametric Kruskal-Wallis Rank Sum test was used to compare beta diversity among the urban and rural locations. Tukey's Honest Significant Difference (Tukey) post-hoc test was performed to test for pairwise differences in individual species abundances among cities, locations, and months. To run the Tukey test the relative abundance data were transformed using the arcsine square root transformation. All p-values were considered significant with alpha at or below 0.05. All statistical analysis was done using RStudio 1.1.453 (RStudio Inc., Boston, MA, USA).

RESULTS

Overall Calliphoridae Community

The Calliphoridae communities collected in urban-rural landscape types in Mid-Michigan were composed of eleven species: *Calliphora vomitoria* (L.), *Calliphora vicina* (Robineau-Desvoidy), *Chrysomya rufifacies* (Macquart), *Cochliomyia macellaria* (Fabricius), *Cynomya cadaverina* (Robineau-Desvoidy), *Lucilia illustris* (Meigen), *Lucilia sericata* (Meigen), *Lucilia silvarum* (Meigen), *Lucilia coeruleiviridis* (Macquart), *Protophormia terraenovae* (Robineau-Desvoidy), and *Phormia regina* (Meigen). There were over 97,000 flies captured in June, July and August of 2017 across the sites in Mid-Michigan (Table 1). The city of Grand Ledge yielded the highest number of flies at 18,237 while Mason had the lowest at 13,565. The urban Lansing sampling returned 8,029 flies, but used only half the number of traps since there were no rural locations (Table 1). At the species level, there were four predominant species: *P. regina* was the most abundant (10,800 flies) and accounted for 69.7% of the total Calliphoridae community; the *L. sericata* abundance was second highest at 18.8% (2,913 flies); and *C. macellaria* and *L. illustris* were equally abundant at 4.0% (619 flies) each. The least abundant Calliphoridae was *C. vomitoria,* which only represented 0.08% (13 flies) of the overall community.

For each of the seven cities *P. regina* and *L. sericata* were the two most abundant species, with *P. regina* the most predominate blow fly species at each sampling location and *L. sericata* the second most abundant (Table 2, Supp. Table S3). While *P. regina* was the most dominate overall there was an inverse relationship with *L. sericata* within each city (Table 2). From June to August three species increased in overall abundance each month (*C. macellaria, L. sericata, P. terraenovae*) while *P. regina* decreased (Table 3, Figure 6-8) across all cities. *Lucilia illustris* relative abundances were similar over the study summer (Table 3).

Community Diversity and Structure

Calliphoridae community diversity was highest at rural landscape locations, with rural sites accounting for 66% of the most diverse locations (Supp. Table S4). DeWitt had five of the top ten locations with the highest diversity of all samples at 1.68 (Supp. Table S4). There was one rural location in Grand Ledge sampled in June with no diversity, since *P. regina* was the only identified Calliphoridae species. Of the covariates tested, month ($p < 2e-16$, $F = 51.34$) was the most significant factor influencing the Calliphoridae diversity (Table 4) followed by landscape type ($p = 0.001$, $F = 10.53$), and their interaction ($p = 0.017$, $F = 4.16$). The diversity of each landscape type changed significantly by month with the greatest increase taking place from July to August for rural locations and June to July for urban locations (Figure 9, Supp. Figure F2).

Calliphoridae community was further confirmed to be structured by month ($p = 0.001$, $F = 26.8$), landscape type ($p = 0.001$, $F = 24.20$), and their interaction ($p = 0.001$, $F =4.79$) (Table 5). Although city had a significant influence on community structure ($p = 0.002$, $F = 3.8$) it was the weakest (Table 5) and its interaction with landscape type ($p= 0.161$, $F=1.4$) and month ($p= 0.055$, $F=1.5$) were not significant. For this reason, the subsequent statistical analyses focused on the two factors that had the strongest and interactive effects on the Calliphoridae communities; the effect of city was evaluated and can be found in supplemental material (Supp. Table S5) but is not the focus here. Additionally, subsequent analyses focused on those species with more than 1% of the overall abundance (*C. macellaria*, *L. coeruleiviridis*, *L. illustris*, *L. sericata*, and *P. regina*, and *P. terraenovae*), and how they were affected by month and landscape type (Figure 10), as species with these abundances had enough power for meaningful statistical analyses.

Calliphoridae Population Changes Over Time

Most of the predominant Calliphoridae species populations significantly changed over the 2017 summer at both urban and rural landscape types (Table 6, Figures 6-8, Figures 11-14). From June to August, *C. macellaria, L. sericata, P. terraenovae* relative abundances increased each month while *P. regina* decreased, and *Lucilia illustris* had consistent values over the summer (Table 3, Figures 6-8, Figures 11-14). There were also significant differences for *P. regina*, *L. sericata*, and *C. macellaria* among each of the months (Table 6), with the biggest differences between June and August ($p < 0.0001$) (Table 6). There was a significant increase in abundance from June to August for *L. sericata* and *C. macellaria*, but not *P. regina* (Table 3, Figure 6, Supp. Figure F1). While not as strong, there were significant increases in relative abundance between July and August for *L. sericata* ($p = 0.0002$), *C. macellaria* ($p = 0.0020$) and *L. coeruleiviridis* ($p = 0.0010$). There was no significant difference in *L. illustris* relative abundance amongst the months.

Calliphoridae Populations Related to Landscape Differences

There were significant differences in the mean relative abundances of *P. regina*, *L. sericata*, and *C. macellaria* between urban and rural landscape types (Table 7; Figures 7-8, Figure 11). *Phormia regina* was collected in significantly higher mean proportions ($p = 0.0025$) in rural (76.9% ± 5.45% SE) than urban (63.6% ±5.47% SE) areas (Figure 7), compared to *L. sericata* which made up 27.4% (± 5.2% SE) of the Calliphoridae communities at urban locations and only 8.6% (± 3.2% SE) in rural (Figure 8). The populations of *L. coeruleiviridis* and *L. illustris* were not significantly different between urban and rural locations, likely because of their overall low relative abundances and variance among samples.

Calliphoridae Populations by City

While not the primary focus of the analyses, the Calliphoridae community structure results showed the effect of city was significant but not as important as month and landscape type, there were significant associations among cities and species (Table 5). *Phormia regina* was the most abundant in all of the cities, but only showed a significant decrease between Williamston and Lansing ($p = 0.010$) and between Williamston and Charlotte ($p = 0.029$) with the change between Lansing and Williamston being the greatest (Table 2, Supp. Table S5). *Lucilia sericata* significantly decreased in relative abundance from Charlotte to DeWitt, Grand Ledge, and Williamston. The greatest decrease in relative abundance was between Charlotte and Grand Ledge ($p = 0.001$) (Supp. Table S5), while *P. terraenovae* had the highest abundance within Lansing and showed a significant change with the other six cities (all $p < 0.001$) (Supp. Table S5). The greatest increase of *P. terraenovae* was the change from Lansing to Mason, while the largest decrease in abundance was Lansing to Charlotte. *Lucilia illustris* was found to be the most abundant in DeWitt, which was significantly greater than Grand Ledge, Lansing, and Williamston. The most significant decrease for *L. illustris* was from DeWitt to Charlotte ($p = 0.007$) (Table 2, Supp. Table S5). *Lucilia coeruleiviridis* was most abundant in Grand Ledge and was significantly greater in Grand Ledge than Williamston ($p = 0.015$) (Supp. Table S5). *Cochliomyia macellaria* was captured in all of the cities (Table 2) but did not significantly vary amongst them.

DISCUSSION

The overall goal of this research was to provide an initial database of adult Calliphoridae species in the Mid-Michigan region for potential use in future forensic investigations. The study provided a survey of Calliphoridae community diversity and structure and species population differences between urban and rural landscape types over a summer in the Mid-Michigan region. In addition to Calliphoridae, other Diptera of forensic interest collected were flesh flies (Diptera: Sarcophagidae), rove beetles (Coleoptera: Staphylinidae), and carrion beetles (Coleoptera: Silphidae), but were not the focus of the present study. Similar research has been conducted on the west coast in California where *Lucilia sericata (*Meigen), *Lucilia cuprina* (Wiedemann), *C. vomitoria, and P. regina* were the most common species (Brundage et al. 2011). In New Jersey, *L. sericata, L. coeruleiviridis and P. regina* were the three most predominant species respectively (Weidner et al. 2017). In Ohio, Calliphoridae were found to colonize carrion within an hour of placement, and stopped occupying the carrion in less than an hour after sunset (Berg and Benbow 2013), and limited blow fly colonization was detected during fresh decomposition during the winter months (Benbow et al. 2013). In Illinois, *L. sericata* and *P. regina* were found to be the two predominant species in Chicago (Baumgartner 1988). In addition, research evaluating oviposition during sunrise, sunset, and nighttime in Mid-Michigan only captured seven Calliphoridae species (*Cochliomyia macellaria, Pollenia rudis, L. coeruleiviridis, C. vomitoria, C. vicina, L. sericata, and P. regina)* (Zurawski et al. 2009). By studying the distribution of Calliphoridae within a geographic region resulting information could provide an important resource for investigators when establishing a post-colonization interval, or the time from insect colonization until discovery, for homicide investigations (Anderson 2000).

Overall, in the Mid-Michigan area blow fly communities were most affected by the month of the summer and landscape type. There was a significant but less strong impact of city especially when compared to landscape type and month. These findings suggest that city is likely confounded with landscape; therefore, is may be that the flies were not responding to a city, but rather to landscape type of a particular city. The null hypothesis of the study was that there would be an even distribution of blow fly species in the Mid-Michigan region; however, the results from the study calls for rejection of the null hypothesis. This is supported by *P. regina* being found at higher abundance rates in rural landscapes, and *C. macellaria* and *L. sericata* in urban landscapes. The Calliphoridae communities were also affected most by the month providing additional evidence that blow fly species do vary by not only landscape, but time as well. For that reason, the alternative hypothesis of Calliphoridae species varying in distribution is accepted. The Tukey tests showed multiple blow fly species were significantly different between both landscape type and between months.

The Calliphoridae community in Mid-Michigan was diverse and there were species dependent changes over time and between landscape types. In this study, *L. sericata* supported previous research that reported higher abundances in urban areas (Mariluis et al. 2008, Marshall et al. 2011), and being a synanthropic species (Mariluis et al. 2008, Marshall et al. 2011). *Phormia regina* was found to be in higher abundance in rural areas, reflecting their preferred resources that include both carrion and manure as suggested by Marshall et al. (2011). *Cochliomyia macellaria* was collected in higher abundances within rural habitats, and this finding could be related to being attracted to similar food sources as *P. regina* (Baumgartner and Greenberg 1985). A unique observation from previous research was the abundance of *L. silvarium* previously found in Grand Haven during a multi-state Calliphoridae survey (Schoof et al. 1956). A study in Grand Haven resulted in anywhere between 1.0 and 2.5% of the flies captured as *L. silvarium* (Schoof et al. 1956). The research in the present study only identified sixteen specimens of *L. silvarium* accounting for on 0.1% of the total fly community abundance and the populations did not change over the summer.

Some of the Calliphoridae populations changed in relative abundance as the summer progressed. The overall relative abundance of *P. regina* was 69.7% for the study and was much higher than reported in previous research in California (23%) (Brundage et al. 2011), and New Jersey (28.6%) (Weidner et al. 2017). The abundance rate of *P. regina* in Mid-Michigan declined throughout the summer which coincided with previous Calliphoridae research in Texas, and Florida. In Texas, the decline of *P. regina* was confirmed by evaluating arthropods from more than 200 forensic entomology cases (Sanford 2017), and in Florida the same trend was seen in a regional survey using swine carcasses (Gruner et al. 2007). The increase in abundance for both *L. sericata* and *C. macellaria* as overall temperatures increased was also reported in previous research (Weidner et al. 2017, Brundage et al. 2011, Goddard and Lago 1985).

Previous research has shown that fluctuation between high and low environmental temperatures, and change in seasons alters adult Calliphoridae activity (Payne 1965, Watson and Carlton 2005, Tabor et al. 2004). For this reason a more in-depth study should be considered for multiple years. This would help identify if the results from this study were affected by the seasonal conditions during the summer of 2017. In Mid-Michigan the average monthly temperature (^{o}C) was for the study was 28.3, 28.9, and 26.1 for June, July and August respectively. Compared to the historic temperatures of June (25.6) and July (27.8) both months were above average while August (26.7) was below the normal. The rainfall (cm) during the summer was higher in June (9.5), and lower in July (6.9) and August (5.1) compared to the historical amounts of 8.8 (June), 7.2 (July), and 8.2 (August) ("Climate-United States-Monthly averages" 2018, Supp. Table S6).

While there were some weak, but significant differences among cities, they are likely reflective in small differences more related to urban to rural landscape type transitions. Since using the term city does not define a particular population size or land cover type there can be difference among them. For instance, the city of Mason has a higher development intensity and population compared to Williamston. Of the four Williamston urban locations three sites were classified as developed open space compared to all four of the Mason urban sites being listed as developed low intensity. The changes of cover class within any region could also have an impact on the Calliphoridae community by creating microhabitats. For example, within a city if there are more developed open spaces the food resources in that area could be different than where the land cover is developed high intensity. For the blow flies within a rural environment they are most likely using trash as a food source. Using the premise that more trash would be found in high intensity areas compared to open space areas it is possible that the blow fly species preferring trash as a food source would be higher in high intensity areas. The low intensity areas for urban locations were about double the number of open spaces but collected nearly three times the number of flies. The same relationship can be seen with the rural locations. The number of cultivated crops collection sites were double the amount developed open space sites and accounted for nearly three times more of the number of flies collected. By using the term city, it helped define a given collection area; however, it is important to recognize that each defined city had its own unique landscape.

This study was affected by several limiting factors. One observation was that the age of the bait may have affected collection yields, as the odor of the bait was qualitatively different between the first time it was used and subsequent sampling events. Although there were flies captured during all trapping dates, if the odor profiles change with bait age that may affect the species that are attracted. Additionally, this was a one summer survey, with flies collected only once per month. To make more broadly applicable conclusions, future studies could increase fly sampling frequency to weekly events over multiple years. This would allow for finer temporal scale evaluation of how Calliphoridae communities change over time and with landscape, both aspects that would be useful for future forensic entomology research.

The dataset established by this research could provide a helpful tool for forensic scientists in both future research and case work. The results show that depending on the time of the year, and landscape in which a body is found the insect evidence may inform about postmortem body movement and if insect colonization was delayed. For example, a hypothetical situation in Mid-Michigan could be where a body was found in an urban landscape type during the month of August, then the expectation would be that there would be a higher abundance of *L. sericata* than *P. regina*. If more than 70% of the Calliphoridae specimens collected were *P. regina* it could point to the possibility that the body was moved from a more rural environment. To some extent this was observed during the fly identification process of the research project. When blindly provided specimens from a random sample the landscape type of the sample could be routinely predicted. While highly qualitative, this observation supports the potential that this database holds promise for future research and importance to forensic investigations; however, additional studies are need that could be designed to test and validate this potential application.

CONCLUSION

In summary, this study provided the first Calliphoridae survey across an urban to rural landscape change for the state of Michigan and the Great Lakes region, including a list of blow fly species and the communities vary over summer months and to urban to rural landscape types. Further, this baseline assessment will help provide a resource as regional temperatures change either seasonally or long term. It will also provide an initial community assessment of the indigenous Calliphoridae species in the event of potential non-indigenous Calliphoridae species invasion into Michigan.

Although this study has provided important baseline survey information that has potential use in future forensic investigations, several questions regarding Calliphoridae dispersion remain. The fact that *P. regina* is found more in rural landscape types has been suggested by previous research as being driven by food source selection, but it is possible that rural areas provide more land cover that keep ambient temperatures cooler. Additionally, it is not known how late into the fall *L. sericata* will continue to increase in relative abundance, or an understanding of how many of the adult Calliphoridae species respond to both acute and chronic changes in temperature. These are new and exciting areas for future research.

There are also a wide range of studies that could use this new baseline adult Calliphoridae survey to develop and test new hypothesis and answer applied research questions related to forensic entomology. While insects have been used in many cases to assist in estimating a postmortem (or post-colonization) interval, one of the driving ideas behind this research was to provide a species list of Calliphoridae that could potentially be used to determine if a body had been moved after death. In this scenario, if a body that is recovered in an urban environment is heavily colonized by a species predominately found in rural landscapes, additional investigation may be warranted. However, a regional baseline understanding of

Calliphoridae diversity and distribution over time and related to landscape was needed. It will also be important to replicate this study not only in Michigan but other areas of the United States as well to provide a broader representation of how these species change by region and by multiple seasons. There is potential that the Great Lakes have prevented some species from entering Michigan by creating a natural barrier, and influencing the states weather patterns. Future studies should also evaluate every month of the year to provide a better understanding of when Calliphoridae species enter diapause. As research continues on the distribution and abundances of Calliphoridae communities more information will become available that can potentially be useful in the forensic sciences.

APPENDICES

APPENDIX A

TABLES

Table 1. **Total overall number of Calliphoridae adult specimens captured during the summer of 2017 for each city location and site in urban and rural locations. NA indicates that there was not a rural location sampled for Lansing, MI. *Traps that experienced a failure during the collection period. ** Sample was lost after collection was completed.**

		June		July		August		Total	
City	Site	Urban	Rural	Urban	Rural	Urban	Rural	Site	City
Charlotte	North	1,010	60	1,190	207	1,018	671	4,156	14,201
	South	432	375	219	784	649	37*	2,496	
	East	485	197	463	2,283	391	1,483	5,302	
	West	235	1,199	449	169	88	107	2,247	
DeWitt	North	410	210	957	844	618	753	3,792	15,740
	South	114	354	325	2,197	615	504	4,109	
	East	430	334	97	857	159	829	2,706	
	West	39*	796	342	1,999	201	1,756	5,133	
Grand Ledge	North	417	37*	1,551	542	1,472	164	4,183	22,237
	South	1,276	857	765	2,292	957	674	6,821	
	East	623	79	1,589	467	706	4,886	8,350	
	West	722	74	386	699	615	387	2,883	
Lansing	North	161	NA	694	NA	3,011	NA	3,866	8,029
	South	416	NA	530	NA	617	NA	1,563	
	East	414	NA	132	NA	782	NA	1,328	
	West	221	NA	230	NA	821	NA	1,272	
Mason	North	153	387	165	240	268	400	1,613	10,723
	South	322	734	428	204	541	0**	2,229	
	East	431	1,801	25	362	612	100	3,331	
	West	1,705	233	326	483	718	85	3,550	
Perry	North	353	27*	1,894	323	141	344	3,082	13,565
	South	51*	574	1,598	1,102	662	225	4,212	
	East	41	148	409	1,092	321	605	2,616	
	West	42	971	407	1,351	441	443	3,655	
Williamston	North	400	170	265	755	438	567	2,595	12,538
	South	278	1,416	286	936	142	476	3,534	
	East	619	175	285	1,122	296	541	3,038	
	West	431	1,332	371	449	456	332	3,371	
Total		**12,231**	**12,540**	**16,378**	**21,759**	**17,756**	**16,369**	**97,033**	

Table 2. **Calliphoridae species mean (SE) percent relative abundance in Mid-Michigan by city over the months of June, July, and August 2017. For Charlotte, Dewitt, Grand Ledge, Perry and Williamston N=24, Lansing N=12, and Mason N=23.**

	Charlotte	DeWitt	Grand Ledge	Lansing	Mason	Perry	Williamston
Calliphora vicina	0.00 (0.00)	0.04 (0.04)	0.00 (0.00)	0.25 (0.18)	0.04 (0.04)	0.38 (0.33)	0.00 (0.00)
Calliphora vomitoria	0.00 (0.00)	0.38 (0.21)	0.00 (0.00)	0.17 (0.17)	0.00 (0.00)	0.08 (0.08)	0.00 (0.00)
Chrysomya rufifacies	0.35 (0.14)	0.63 (0.24)	0.5 (0.28)	0.08 (0.08)	0.47 (0.19)	0.08 (0.06)	0.33 (0.22)
Cochliomyia macellaria	4.18 (1.21)	3.47 (1.06)	5.46 (1.48)	3.17 (1.3)	4.53 (1.33)	2.08 (0.63)	4.67 (1.59)
Cynomya cadaverina	0.00 (0.00)	0.17 (0.10)	0.00 (0.00)	0.25 (0.25)	0.00 (0.00)	0.5 (0.38)	0.04 (0.04)
Lucilia coeruleiviridis	0.96 (0.29)	2.16 (0.59)	2.46 (1.28)	1.33 (0.58)	1.00 (0.25)	2.07 (1.11)	0.21 (0.12)
Lucilia illustris	2.82 (0.81)	7.14 (1.26)	2.03 (0.43)	1.25 (0.43)	3.47 (0.79)	7.62 (1.34)	2.25 (0.43)
Lucilia sericata	30.68 (6.14)	12.99 (3.66)	14.1 (14.08)	28.6 (0.35)	18.91 (4.59)	18.9 (4.25)	12.3 (12.3)
Lucilia silvarum	0.00 (0.00)	0.13 (0.09)	0.04 (0.04)	0.00 (0.00)	0.35 (0.19)	0.13 (0.13)	0.04 (0.040
Phormia regina	61.00 (6.63)	72.9 (4.48)	75.2 (3.68)	55.7 (7.79)	68.5 (5.72)	68.1 (5.01)	79.4 (79.4)
Protophormia terraenovae	0.00 (0.00)	0.04 (0.04)	0.29 (0.11)	9.25 (2.35)	2.74 (2.74)	0.04 (0.04)	0.83 (0.750

Table 3. **The overall monthly mean (SE) relative abundance of Calliphoridae collected in Mid-Michigan over the 2017 summer. In June and July all species were represented by N = 52, while for August it was N = 51.**

	June		July		August	
Species	Urban	Rural	Urban	Rural	Urban	Rural
Calliphora vicina	0.11 (0.08)	0.33 (0.33)	0.04 (0.04)	0.00 (0.00)	0.04 (0.04)	0.04 (0.04)
Calliphora vomitoria	0.21 (0.13)	0.25 (0.18)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.04 (0.04)
Chrysomya rufifacies	0.00 (0.00)	0.00 (0.00)	0.04 (0.04)	0.04 (0.04)	0.92 (0.20)	1.28 (0.36)
Cochliomyia macellaria	0.25 (0.10)	0.17 (0.10)	2.86 (0.63)	5.08 (1.06)	4.11 (0.59)	12.64 (1.90)
Cynomya cadaverina	0.11 (0.11)	0.04 (0.04)	0.08 (0.08)	0.04 (0.04)	0.04 (0.04)	0.52 (0.40)
Lucilia coeruleiviridis	1.09 (0.30)	1.33 (0.70)	0.86 (0.22)	0.38 (0.22)	1.47 (0.35)	3.95 (1.61)
Lucilia illustris	3.44 (0.83)	4.41 (0.94)	3.47 (0.73)	4.21 (1.03)	2.79 (0.51)	6.11 (1.58)
Lucilia sericata	9.27 (1.61)	3.70 (0.84)	27.51 (4.02)	7.38 (2.42)	45.40 (4.26)	15.05 (4.02)
Lucilia silvarum	0.11 (0.08)	0.00 (0.00)	0.14 (0.14)	0.00 (0.00)	0.07 (0.05)	0.30 (0.16)
Phormia regina	84.37 (1.85)	89.56 (1.92)	62.43 (4.57)	82.83 (3.11)	43.89 (3.56)	57.46 (5.62)
Prorophormia terraenovae	1.04 (0.50)	0.21 (0.17)	2.57 (1.12)	0.04 (0.04)	1.29 (0.95)	2.61 (2.47)

Table 4. **Two-way ANOVA statistics that tested Calliphoridae diversity by both month and landscape type during the summer of 2017.**

	Degree of Freedom	Sum of squares	Mean sum of squares	F-value	Pr(>F)
Landscape type	1	0.643	0.642	10.534	0.001 **
Month	2	6.264	3.131	51.337	< 2e-16 ***
Landscape:Month	2	0.508	0.253	4.162	0.017 *
Residuals	149	9.090	0.061		

Table 5. **Permutational multivariate analysis of variance (PERMANOVA) of Calliphoridae species from the cities of: Charlotte, DeWitt, Grand Ledge, Lansing, Mason, Perry, and Williamston in Mid-Michigan during the summer of 2017. Bolded text indicates significant effects**.

	Degree of freedom	Sum of squares	Mean sum of squares	F. Model	R^2	P - value
Landscape	**1**	**1.2448**	**1.24479**	**24.2034**	**0.09299**	**0.001*****
City	**6**	**1.18**	**0.19666**	**3.8239**	**0.08815**	**0.002****
Month	**2**	**2.7648**	**1.3824**	**26.8791**	**0.20654**	**0.001*****
Landscape:City	5	0.3707	0.07414	1.4416	0.02769	0.161
Landscape:Month	**2**	**0.4924**	**0.24618**	**4.7866**	**0.03678**	**0.001*****
City:Month	12	0.9465	0.07887	1.5336	0.0707	0.055
Landscape:City:Month	10	0.4215	0.04215	0.8196	0.03149	0.701
Residuals	116	5.9659	0.05143		0.44566	
Total	154	13.3866			1	

Table 6. **Tukey's Honest Significant Difference test of the four major Calliphoridae species (*C. macellaria, L. sericata, L.illustris, P. regina)* collected in Mid-Michigan and compared among months of the 2017 summer. Only pairwise comparisons that were found significant with alpha at or below 0.05 are given.**

Species	Month	Diff	Lower	Upper	P-adj
Lucilia sericata	JUL-AUG	-0.1630	-0.2576	-0.0684	0.0002
Lucilia sericata	JUN-AUG	-0.3669	-0.4616	-0.2723	< 0.0001
Lucilia sericata	JUN-JUL	-0.2039	-0.2981	-0.1098	< 0.0001
Phormia regina	JUL-AUG	0.2472	0.1362	0.3582	< 0.0001
Phormia regina	JUN-AUG	0.4259	0.3149	0.5368	< 0.0001
Phormia regina	JUN-JUL	0.1787	0.0682	0.2891	0.0006
Cochliomyia macellaria	JUL-AUG	-0.0715	-0.1201	-0.0229	0.0020
Cochliomyia macellaria	JUN-AUG	-0.2122	-0.2608	-0.1636	< 0.0001
Cochliomyia macellaria	JUN-JUL	-0.1407	-0.1891	-0.0924	< 0.0001
Lucilia coeruleiviridis	JUL-AUG	-0.0623	-0.1024	-0.0221	0.0010

Table 7. **Tukey's Honest Significant Difference test of the predominant Calliphoridae species collected in Mid-Michigan and compared between landscape types during the summer of 2017. Only pairwise comparisons that were found significant with alpha at or below 0.05 are given.**

Species	Landscape	Diff	Lower	Upper	P-adj
Cochliomyia macellaria	Urban - Rural	-0.0669	-0.1001	-0.0338	0.0001
Phormia regina	Urban - Rural	-0.1180	-0.1937	-0.0422	0.0025
Lucilia sericata	Urban - Rural	0.2664	0.2018	0.3310	< 0.0001

Supp. Table S1. **A summary of previous adult blow fly studies assed by location, length of study, bait type, and landscape type.**

Citation	Location	Number of Months	Carrion Source	Landscape Type
Baumgartner 1988	Illinois	3	Rats	Urban
Baumgartner and Greenberg 1985	Peru	36	Fish, liver and pigs	Rural
Berg and Benbow 2011	Ohio	5 (over 2 years)	Pig carcasses	Rural
Brundage et al. 2011	California	24	Beef liver	Urban, rural, riparian
Grassberger and Frank 2004	Austria	7	Pig carcasses	Urban
Gruner et al. 2007	Florida	24	Pig carcasses	Rural
Pastula and Merritt 2013	Michigan	3	Pig carcasses	Rural
Payne 1965	South Carolina	6 (over 2 years)	Pig carcasses	Rural
Sabanoğlu and Sert 2010	China	12	Pig carcasses	Rural
Schoof et al. 1956	New York, Michigan, Kansas, Arizona	5	Chicken and fish	Urban
Tullis and Goff 1987	Hawaii	4	Pig carcasses	Rural
Weidner et al. 2017	New Jersey	12	Pig carcasses	Urban
Zabala et al. 2014	Western Europe	12	Pig liver	Urban, rural
Zurawski et al. 2009	Michigan	6 (over 2 years)	Pig carcasses	Rural

Supp. Table S2. **Urban to Rural Landscape type research sites, land use, and GPS locations from the summer of 2017.**

City	Location	Cover Class	North Heading (42º)	West Heading (084º)
Charlotte	East Urban	Developed, Low Intensity	33.738	49.630
Charlotte	East Rural	Cultivated Crops	34.055	42.598
Charlotte	North Urban	Developed, Low Intensity	34.394	50.170
Charlotte	North Rural	Developed, Open Space	40.184	51.412
Charlotte	South Urban	Developed, Medium Intensity	33.305	50.133
Charlotte	South Rural	Developed, Open Space	27.406	50.753
Charlotte	East Urban	Developed, Low Intensity	33.738	49.630
Charlotte	East Rural	Cultivated Crops	34.055	42.598
Charlotte	North Urban	Developed, Low Intensity	34.394	50.170
Charlotte	North Rural	Developed, Open Space	40.184	51.412
Charlotte	South Urban	Developed, Medium Intensity	33.305	50.133
DeWitt	North Rural	Cultivated Crops	56.666	34.080
DeWitt	South Urban	Developed, Open Space	46.439	34.139
DeWitt	South Rural	Developed, Open Space	47.061	33.702
DeWitt	West Urban	Developed, Open Space	50.578	34.952
DeWitt	West Rural	Cultivated Crops	50.511	44.184
Grand Ledge	East Urban	Developed, Open Space	45.127	44.077
Grand Ledge	East Rural	Developed, Low Intensity	45.076	37.829
Grand Ledge	North Urban	Developed, Low Intensity	45.754	44.824
Grand Ledge	North Rural	Cultivated Crops	51.001	45.473
Grand Ledge	South Urban	Developed, Open Space	44.743	44.747
Grand Ledge	South Rural	Cultivated Crops	41.301	44.665
Grand Ledge	West Urban	Developed, Low Intensity	45.198	45.369
Grand Ledge	West Rural	Cultivated Crops	45.051	51.920
Lansing	East Urban	Developed, High Intensity	43.995	32.557
Lansing	North Urban	Developed, Medium Intensity	44.400	33.281
Lansing	South Urban	Developed, High Intensity	43.618	33.320
Lansing	West Urban	Developed, Low Intensity	43.890	33.947
Mason	East Urban	Developed, Low Intensity	34.718	25.785
Mason	East Rural	Hay/Pasture	34.589	18.032
Mason	North Urban	Developed, Low Intensity	35.286	26.528
Mason	North Rural	Developed, Low Intensity	40.174	27.400
Mason	South Urban	Developed, Low Intensity	34.292	26.643
Mason	South Rural	Developed, Low Intensity	28.820	27.012
Mason	West Urban	Developed, Low Intensity	34.779	27.350
Mason	West Rural	Deciduous Forest	34.828	34.939
Perry	East Rural	Cultivated Crops	49.546	12.787
Perry	East Urban	Developed, Low Intensity	50.305	05.178
Perry	North Rural	Developed, Open Space	50.060	13.241
Perry	North Urban	Developed, Medium Intensity	55.279	13.888
Perry	South Urban	Developed, Low Intensity	49.118	13.116
Perry	South Rural	Cultivated Crops	43.481	12.764

Supp. Table S3. **Calliphoridae species in Mid-Michigan expressed as relative abundance by location during the summer months of June, July, and August in 2017.**

Location	Species	Mean (%)	SE (%)
North Rural (N=18)	*Phormia regina*	80.52	4.26
	Cochliomyia macellaria	6.89	1.97
	Lucilia sericata	6.37	1.61
	Lucilia illustris	3.98	1.58
	Chrysomya rufifacies	0.61	0.36
	Lucilia coeruleiviridis	0.57	0.32
	Calliphora vicina	0.44	0.44
	Cynomya cadaverina	0.17	0.12
	Lucilia silvarum	0.17	0.12
	Protophormia terraenovae	0.17	0.12
	Calliphora vomitoria	0.11	0.11
South Rural (N=17)	*Phormia regina*	80.18	5.94
	Lucilia sericata	7.66	4.15
	Cochliomyia macellaria	7.42	2.35
	Lucilia illustris	3.30	0.85
	Lucilia coeruleiviridis	1.22	0.06
	Calliphora vomitoria	0.06	0.06
	Chrysomya rufifacies	0.06	0.06
	Protophormia terraenovae	0.06	0.06
	Calliphora vicina	0.00	0.00
	Cynomya cadaverina	0.00	0.00
	Lucilia silvarum	0.00	0.00
East Rural (N=18)	*Phormia regina*	72.29	6.28
	Lucilia sericata	9.54	3.47
	Lucilia illustris	6.56	1.56
	Cochliomyia macellaria	4.28	1.50
	Protophormia terraenovae	3.44	3.16
	Lucilia coeruleiviridis	2.94	1.74
	Chrysomya rufifacies	0.33	0.20
	Calliphora vomitoria	0.22	0.22
	Lucilia silvarum	0.22	0.17
	Cynomya cadaverina	0.11	0.08
	Calliphora vicina	0.06	0.06
West Rural N=18	*Phormia regina*	74.74	5.33
	Lucilia sericata	10.85	3.60
	Lucilia illustris	5.60	1.36

Supp. Table S3. (cont'd)

West Rural N=18	*Cochliomyia macellaria*	4.97	1.62
	Lucilia coeruleiviridis	2.65	1.46
	Chrysomya rufifacies	0.69	0.33
	Cynomya cadaverina	0.50	0.50
	Calliphora vicina	0.00	0.00
	Calliphora vomitoria	0.00	0.00
	Lucilia silvarum	0.00	0.00
	Prorophormia terraenovae	0.00	0.00
North Urban N=18	*Phormia regina*	63.59	4.91
	Lucilia sericata	26.81	4.74
	Lucilia illustris	3.03	0.44
	Prorophormia terraenovae	2.86	1.64
	Cochliomyia macellaria	2.00	0.61
	Lucilia coeruleiviridis	1.33	0.34
	Chrysomya rufifacies	0.24	0.12
	Lucilia silvarum	0.10	0.10
	Calliphora vomitoria	0.05	0.05
	Calliphora vicina	0.00	0.00
	Cynomya cadaverina	0.00	0.00
South Urban N=21	*Phormia regina*	64.98	5.48
	Lucilia sericata	27.38	5.58
	Lucilia illustris	3.41	1.00
	Cochliomyia macellaria	2.43	0.60
	Lucilia coeruleiviridis	0.71	0.30
	Prorophormia terraenovae	0.62	0.36
	Chrysomya rufifacies	0.24	0.14
	Calliphora vicina	0.14	0.10
	Calliphora vomitoria	0.10	0.10
	Cynomya cadaverina	0.00	0.00
	Lucilia silvarum	0.00	0.00
East Urban N=21	*Phormia regina*	62.60	5.09
	Lucilia sericata	27.35	5.14
	Lucilia illustris	3.10	0.89
	Cochliomyia macellaria	2.57	0.65
	Prorophormia terraenovae	2.00	1.01
	Lucilia coeruleiviridis	1.25	0.35
	Chrysomya rufifacies	0.49	0.24
	Lucilia silvarum	0.29	0.20

Supp. Table S3. (cont'd)

East Urban N=21	*Cynomya cadaverina*	0.15	0.11
	Calliphora vomitoria	0.14	0.14
	Calliphora vicina	0.05	0.05
West Urban N=21	*Phormia regina*	63.10	6.42
	Lucilia sericata	28.02	5.46
	Lucilia illustris	3.40	0.83
	Cochliomyia macellaria	2.63	0.84
	Lucilia coeruleiviridis	1.27	0.37
	Prorophormia terraenovae	1.05	0.67
	Chrysomya rufifacies	0.30	0.14
	Cynomya cadaverina	0.14	0.14
	Calliphora vicina	0.05	0.05
	Lucilia silvarum	0.05	0.05
	Calliphora vomitoria	0.00	0.00

Supp. Table S4. **Shannon Diversity Index of adult Calliphoridae distributed amongst seven cities in Mid-Michigan during the summer of 2017.**

City	Month	Landscape Type	Shannon Diversity
DeWitt	AUG	Rural	1.6755
DeWitt	AUG	Rural	1.4415
Perry	AUG	Rural	1.4041
Mason	JUL	Urban	1.3971
DeWitt	AUG	Rural	1.3274
Charlotte	AUG	Rural	1.2671
DeWitt	AUG	Urban	1.2365
Lansing	AUG	Urban	1.2112
DeWitt	AUG	Urban	1.1891
Grand Ledge	AUG	Urban	1.1759
Lansing	JUL	Urban	1.1625
Mason	AUG	Rural	1.1517
Charlotte	JUL	Rural	1.1269
Grand Ledge	AUG	Urban	1.1243
Perry	JUN	Rural	1.1234
Lansing	JUL	Urban	1.1198
Mason	AUG	Rural	1.1070
Mason	JUL	Urban	1.0994
Perry	AUG	Rural	1.0835
Mason	AUG	Urban	1.0755
DeWitt	AUG	Urban	1.0689
Charlotte	AUG	Urban	1.0496
Williamston	AUG	Urban	1.0421
Lansing	AUG	Urban	1.0312
Williamston	JUL	Urban	1.0016
Perry	AUG	Rural	1.0011
Grand Ledge	AUG	Rural	1.0008
DeWitt	AUG	Rural	0.9830
Mason	JUL	Urban	0.9779
Mason	AUG	Urban	0.9705
Perry	AUG	Urban	0.9657
Lansing	AUG	Urban	0.9651
Lansing	JUL	Urban	0.9606
Mason	AUG	Urban	0.9428
Grand Ledge	AUG	Urban	0.9424

Supp. Table S4. (cont'd)

Mason	AUG	Urban	0.9345
Williamston	JUL	Urban	0.9327
Lansing	JUN	Urban	0.9323
Lansing	JUN	Urban	0.9193
Charlotte	AUG	Urban	0.9139
Williamston	AUG	Rural	0.9135
Grand Ledge	JUN	Urban	0.8997
Perry	JUN	Rural	0.8985
Charlotte	AUG	Rural	0.8951
Lansing	AUG	Urban	0.8854
Charlotte	JUL	Urban	0.8831
Perry	AUG	Rural	0.8761
Williamston	AUG	Rural	0.8574
Grand Ledge	AUG	Urban	0.8540
Mason	JUL	Urban	0.8513
Perry	JUL	Urban	0.8426
Mason	JUL	Rural	0.8325
Grand Ledge	AUG	Rural	0.8321
Perry	JUL	Rural	0.8252
Williamston	AUG	Urban	0.8195
Williamston	AUG	Urban	0.8191
Grand Ledge	AUG	Rural	0.8134
Charlotte	JUL	Urban	0.8130
Charlotte	AUG	Urban	0.8130
Perry	JUL	Rural	0.8055
Williamston	AUG	Urban	0.8033
Williamston	JUN	Urban	0.7950
Perry	JUL	Urban	0.7906
Grand Ledge	JUL	Urban	0.7896
Grand Ledge	AUG	Rural	0.7868
Perry	AUG	Urban	0.7822
Perry	JUL	Rural	0.7734
Grand Ledge	JUL	Urban	0.7700
Charlotte	AUG	Rural	0.7684
Grand Ledge	JUL	Urban	0.7563
Williamston	JUL	Urban	0.7543
Lansing	JUL	Urban	0.7515

Supp. Table S4. (cont'd)

DeWitt	AUG	Urban	0.7479
Perry	AUG	Urban	0.7432
Charlotte	JUN	Urban	0.7368
Grand Ledge	JUL	Urban	0.7144
Perry	JUN	Urban	0.7054
DeWitt	JUN	Rural	0.6880
Perry	AUG	Urban	0.6860
Perry	JUN	Urban	0.6491
DeWitt	JUL	Rural	0.6400
Charlotte	JUN	Urban	0.6398
DeWitt	JUL	Urban	0.6350
Charlotte	JUN	Rural	0.6307
DeWitt	JUL	Urban	0.6294
Mason	AUG	Rural	0.6291
Mason	JUN	Urban	0.6256
DeWitt	JUN	Urban	0.6244
DeWitt	JUL	Urban	0.6219
Williamston	JUL	Rural	0.6117
Grand Ledge	JUN	Urban	0.6066
Charlotte	JUL	Rural	0.5925
Charlotte	JUL	Urban	0.5917
DeWitt	JUN	Urban	0.5875
Mason	JUN	Rural	0.5837
Williamston	JUL	Urban	0.5654
Grand Ledge	JUN	Urban	0.5651
DeWitt	JUL	Rural	0.5560
Williamston	JUL	Rural	0.5542
DeWitt	JUN	Rural	0.5506
Williamston	JUL	Rural	0.5498
Grand Ledge	JUL	Rural	0.5486
Grand Ledge	JUL	Rural	0.5455
Williamston	AUG	Rural	0.5430
DeWitt	JUN	Urban	0.5299
Williamston	JUN	Urban	0.5138
Charlotte	AUG	Rural	0.5112
Mason	JUL	Rural	0.5067
Mason	JUL	Rural	0.5042

Supp. Table S4. (cont'd)

Grand Ledge	JUN	Rural	0.5040
Perry	JUL	Urban	0.5000
Lansing	JUN	Urban	0.4941
Charlotte	AUG	Urban	0.4896
Charlotte	JUN	Rural	0.4887
Perry	JUN	Urban	0.4744
Charlotte	JUN	Rural	0.4717
DeWitt	JUL	Rural	0.4664
Perry	JUL	Urban	0.4531
Charlotte	JUL	Urban	0.4422
Grand Ledge	JUL	Rural	0.4280
DeWitt	JUL	Urban	0.4216
Charlotte	JUN	Urban	0.4210
Mason	JUN	Urban	0.4210
Perry	JUN	Urban	0.4196
Grand Ledge	JUL	Rural	0.4149
DeWitt	JUN	Urban	0.4119
Mason	JUN	Urban	0.4053
Charlotte	JUL	Rural	0.3862
Perry	JUN	Rural	0.3746
DeWitt	JUN	Rural	0.3576
Mason	JUN	Rural	0.3567
Williamston	JUN	Rural	0.3508
Williamston	JUL	Rural	0.3094
Grand Ledge	JUN	Urban	0.2929
Lansing	JUN	Urban	0.2877
Perry	JUN	Rural	0.2877
Perry	JUL	Rural	0.2790
DeWitt	JUN	Rural	0.2540
Mason	JUN	Rural	0.2322
Williamston	JUN	Rural	0.2270
Williamston	AUG	Rural	0.2235
Grand Ledge	JUN	Rural	0.2103
Williamston	JUN	Urban	0.2095
DeWitt	JUL	Rural	0.2095
Charlotte	JUN	Rural	0.1904
Mason	JUL	Rural	0.1904

Supp. Table S4. (cont'd)

Williamston	JUN	Urban	0.1679
Charlotte	JUL	Rural	0.1677
Mason	JUN	Rural	0.1119
Grand Ledge	JUN	Rural	0.1119
Charlotte	JUN	Urban	0.0980
Williamston	JUN	Rural	0.0980
Mason	JUN	Urban	0.0560
Williamston	JUN	Rural	0.0560
Grand Ledge	JUN	Rural	0.0000

Supp. Table S5. **Tukey's Honest Significant Difference test for pairwise comparisons of the predominant Calliphoridae species evaluated by city collected in Mid-Michigan during the summer of 2017. Only results are displayed where p-values were considered significant with alpha at or below 0.05.**

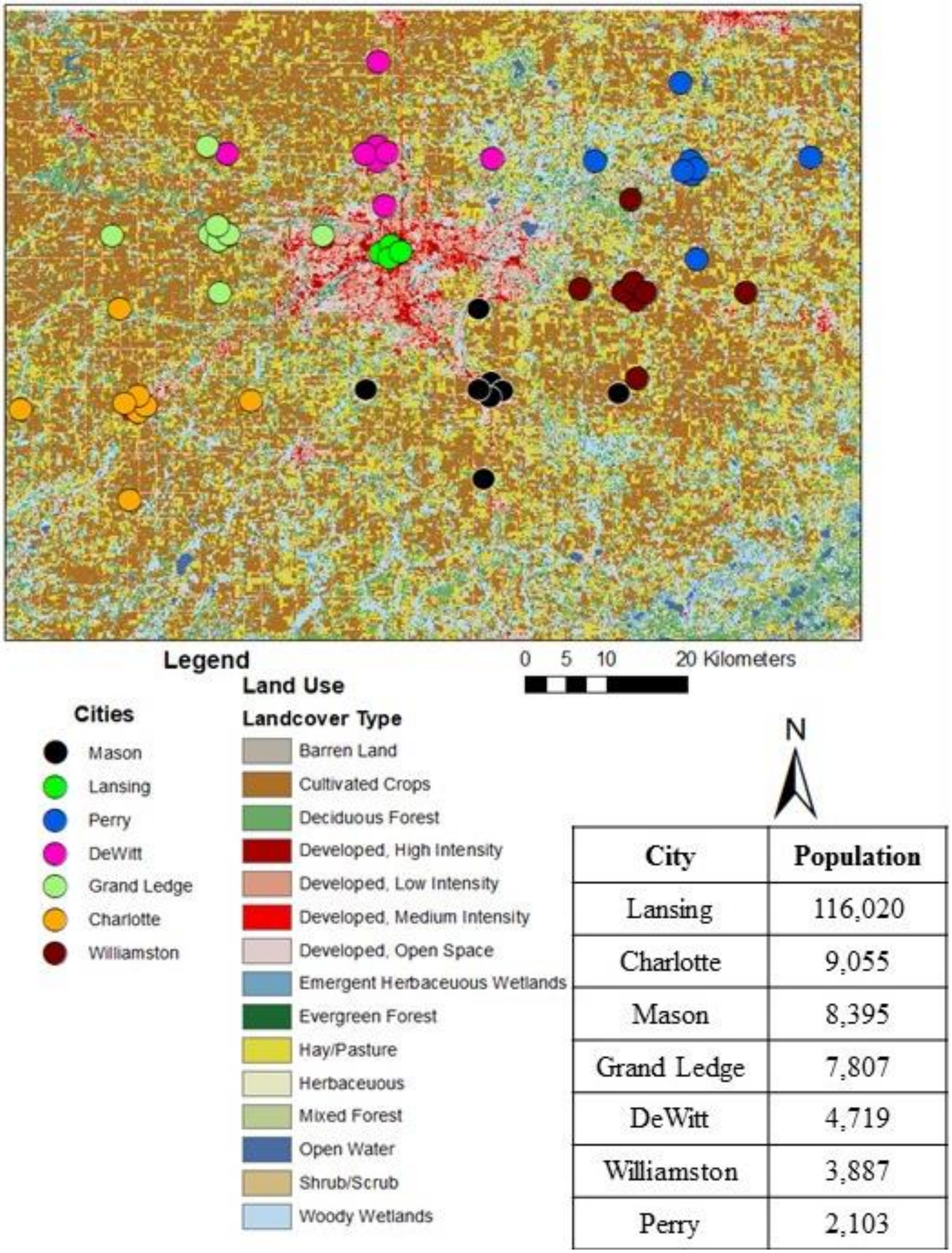
Species	**City**	**Diff**	**Lower**	**Upper**	**P-adj**
Phormia regina	Williamston-Lansing	0.2971	0.0455	0.5487	0.0100
Phormia regina	Williamston-Charlotte	0.2188	0.0134	0.4242	0.0289
Lucilia coeruleiviridis	Williamston-Grand Ledge	-0.0848	-0.1591	-0.0105	0.0146
Lucilia illustris	DeWitt-Charlotte	0.1102	0.0197	0.2008	0.0069
Lucilia illustris	Grand Ledge-DeWitt	-0.1213	-0.2119	-0.0308	0.0019
Lucilia illustris	Lansing-DeWitt	-0.1376	-0.2485	-0.0267	0.0055
Lucilia illustris	Williamston-DeWitt	-0.0977	-0.1883	-0.0072	0.0255
Lucilia illustris	Perry-Grand Ledge	0.1009	0.0104	0.1915	0.0185
Lucilia illustris	Perry-Lansing	0.1172	0.0063	0.2281	0.0310
Lucilia sericata	DeWitt-Charlotte	-0.2367	-0.4119	-0.0615	0.0017
Lucilia sericata	Grand Ledge-Charlotte	-0.2420	-0.4172	-0.0668	0.0012
Lucilia sericata	Williamston-Charlotte	-0.2389	-0.4140	-0.0637	0.0015
Protophormia terraenovae	Lansing- Charlotte	2.7794 e-1	0.1858	0.3700	< 0.0001
Protophormia terraenovae	Lansing-DeWitt	2.7376 e-1	0.1817	0.3658	< 0.0001
Protophormia terraenovae	Lansing- Grand Ledge	2.5116 e-1	0.1591	0.3432	< 0.0001
Protophormia terraenovae	Mason-Lansing	-2.2581 e-1	-0.3186	-0.1331	< 0.0001
Protophormia terraenovae	Perry-Lansing	-2.7377	-0.3186	-0.1817	< 0.0001
Protophormia terraenovae	Williamston-Lansing	-2.5133	-0.3434	-0.1593	< 0.0001

Supp. Table S6. **Average daily temperatures in degree Celsius for the Mid-Michigan area during the summer of 2017. Temperature data was recorded using the Capital City Weather Station at the Capitol Regional Airport (LAN), Lansing, MI 48906.**

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

APPENDIX B

FIGURES



City	Population
Lansing	116,020
Charlotte	9,055
Mason	8,395
Grand Ledge	7,807
DeWitt	4,719
Williamston	3,887
Perry	2,103

Figure 1. **Urban and Rural research site locations and surrounding land cover types in Mid-Michigan. City populations as indicated by the 2010 United States Census Bureau. Land use types defined by using Geographic Information System (GIS).**

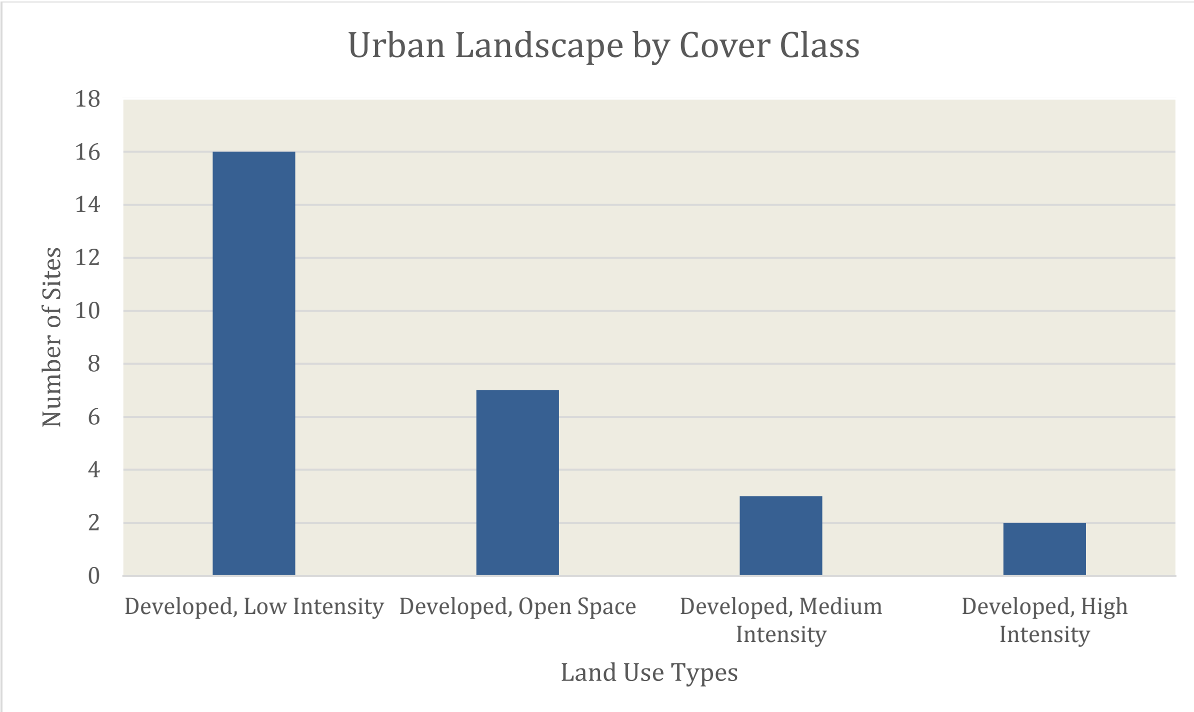


Figure 2. **Total land use types for urban locations in Charlotte, DeWitt, Grand Ledge, Lansing, Mason, Perry, and Williamston. Land use types defined by using Geographic Information System (GIS).**

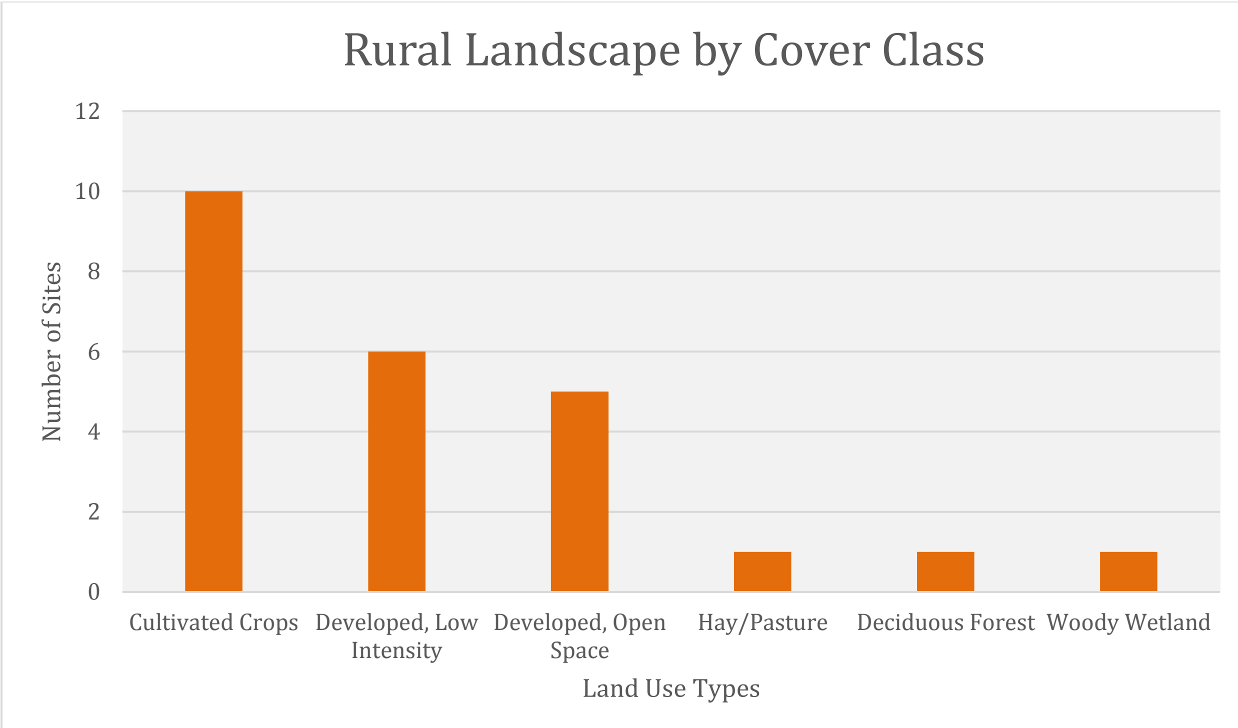


Figure 3. **Total land use types for rural locations in Charlotte, DeWitt, Grand Ledge, Lansing, Mason, Perry, and Williamston. Land use types defined by using Geographic Information System (GIS).**

Figure 4. **Williamston South urban trap placement with Shepard's hook, bait jar, and guide lines. 15JUN2017 (278 flies captured in this trapping event).**

Figure 5. **Williamston South rural trap after placement in field for 4 hours. Photo taken 15JUN2017 (1,416 flies captured in this trapping event).**

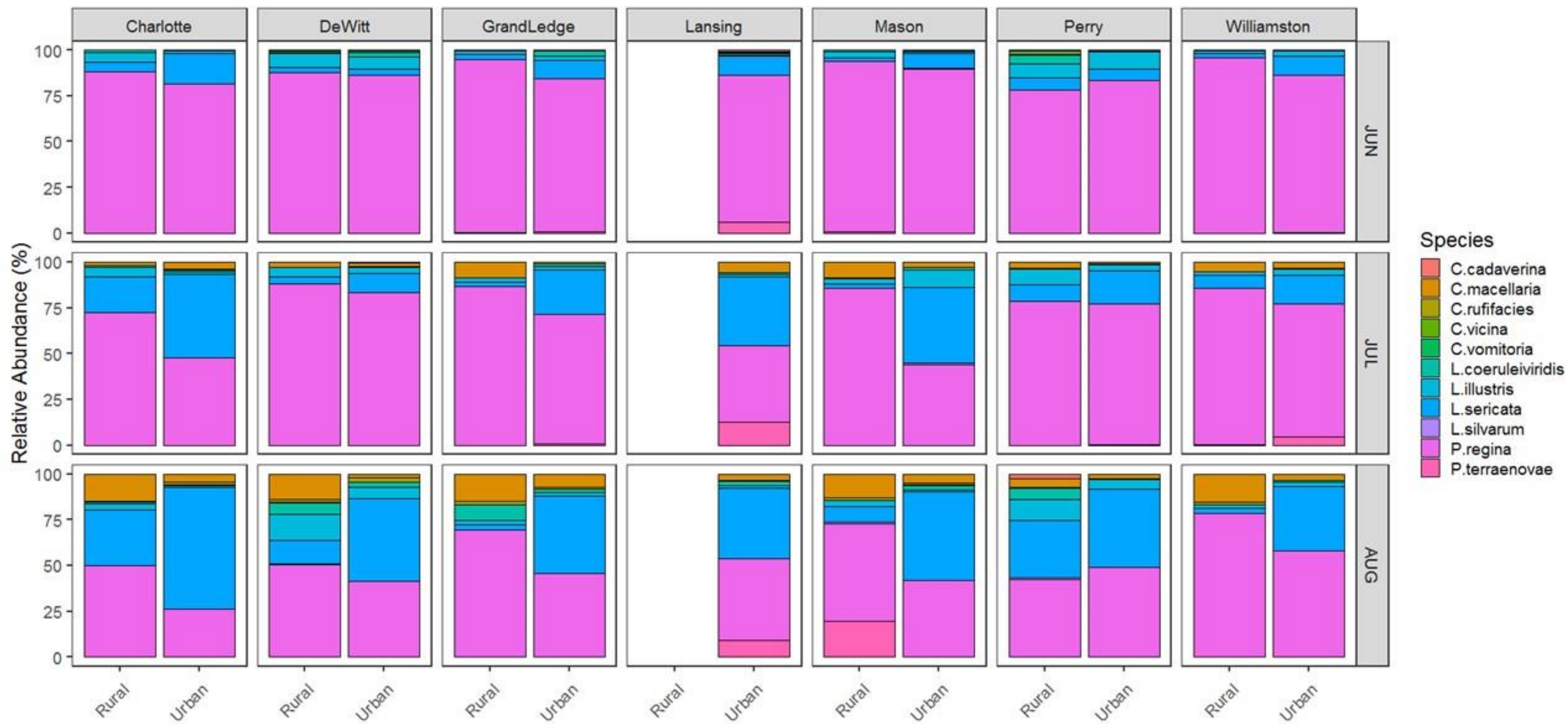


Figure 6. **Percent relative abundance of Calliphoridae species for urban and rural landscape types, city and month over the 2017 in Mid-Michigan.**

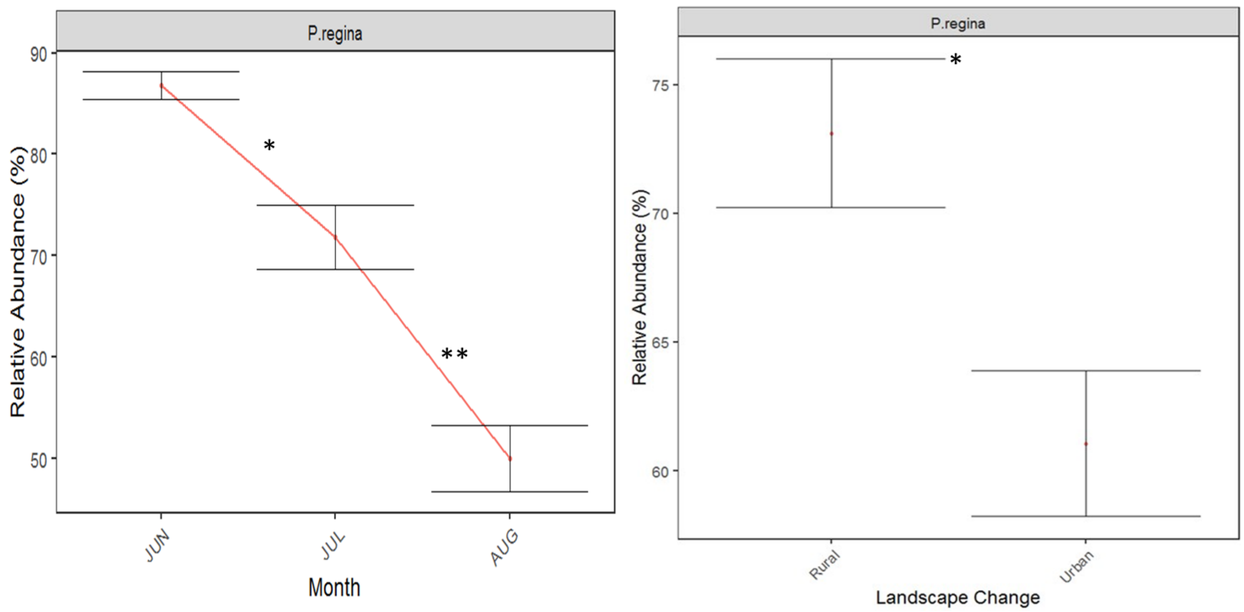


Figure 7. ***Phormia regina*** **mean (SE) relative abundance by month with trend line (A) and landscape type (B) during the summer of 2017. Tukey's Honest Significant Different Test: (A) *Adjusted P-value <0.0020 **Adjusted P-value <0.0001 (B) *Adjusted P-value= 0.0030**

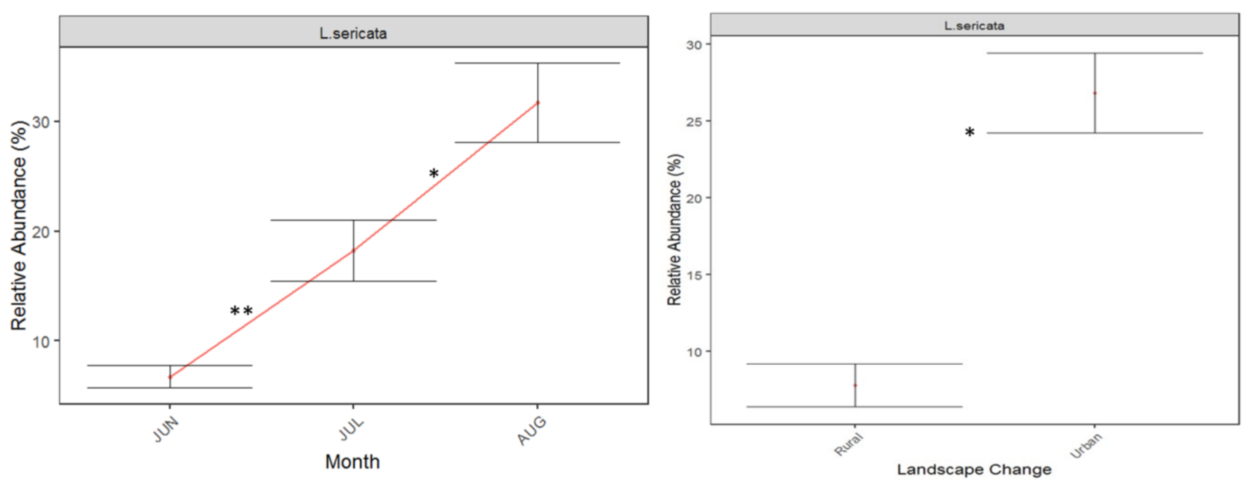


Figure 8. ***Lucilia sericata*** **mean (SE) relative abundance by month with trend line (A) and landscape type (B) during the summer of 2017. Tukey's Honest Significant Different Test: (A) *Adjusted P-value <0.0020 **Adjusted P-value <0.0001 (B) *Adjusted P-value <0.0001**

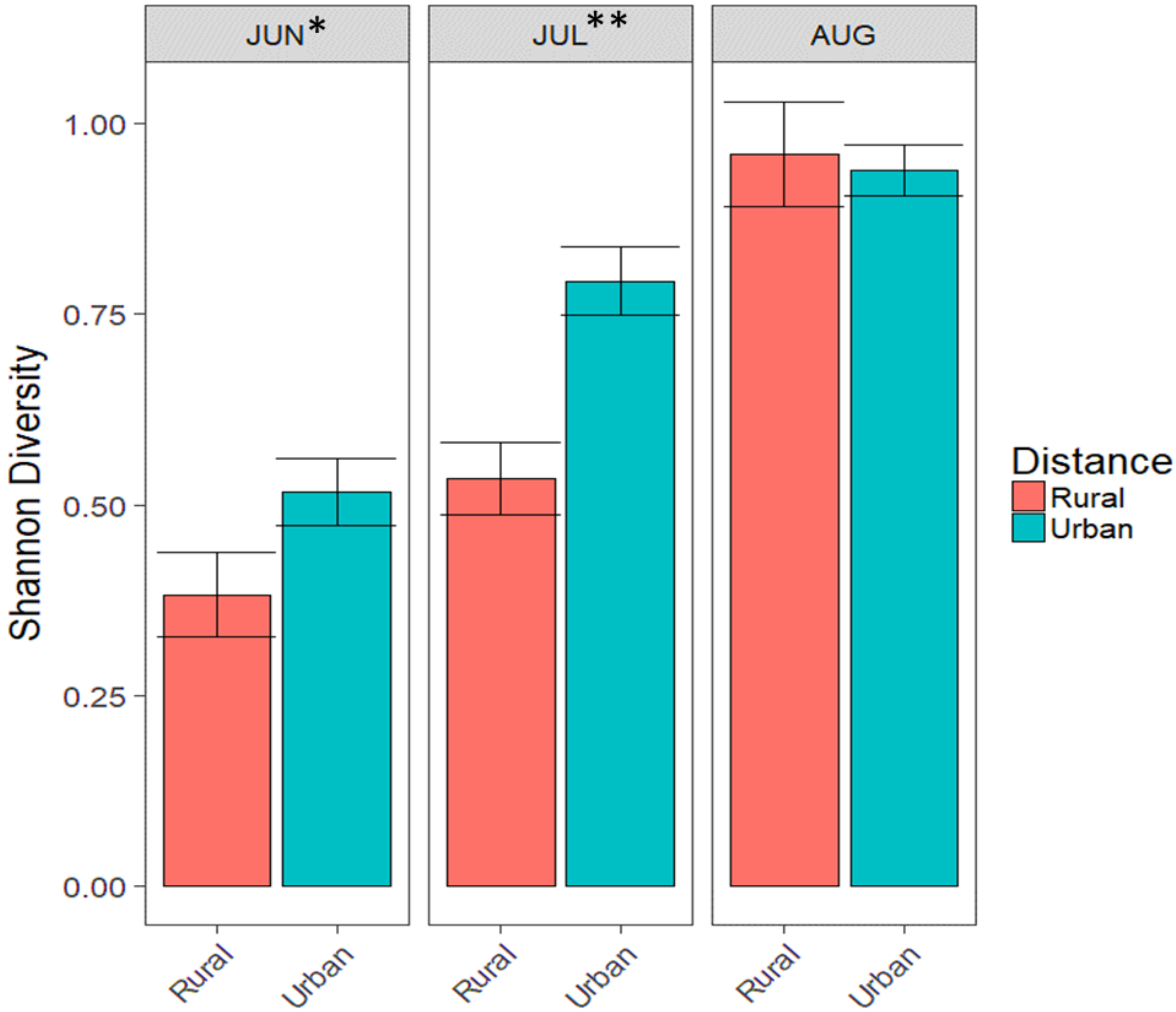


Figure 9**. Shannon diversity of Calliphoridae species indicated by landscape type and month in Mid-Michigan during the summer of 2017. Kruskal Wallis Rank Sum test: p-value < 0.030* p-value < 0.001****

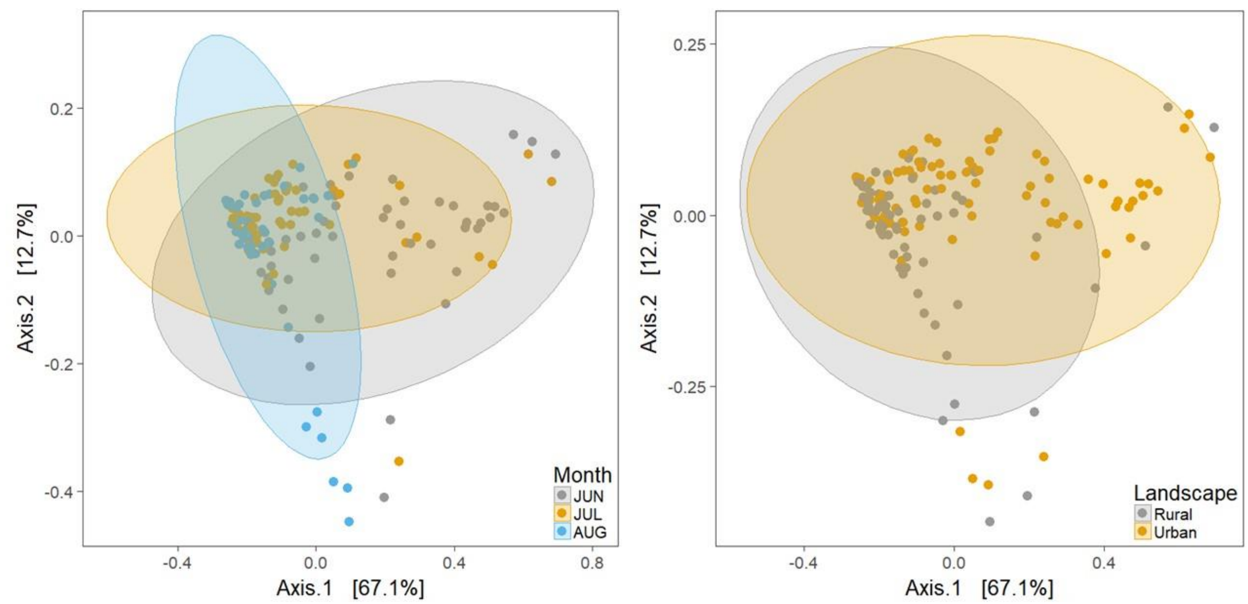


Figure 10. **Principal Coordinates Analysis (PCoA) ordination of relative abundance distribution by month (A) and landscape type (b) during the summer of 2017. The ellipses represent 95% confidence intervals for the mean of each group. See PERMANOVA results for statistical tests of these factors.**

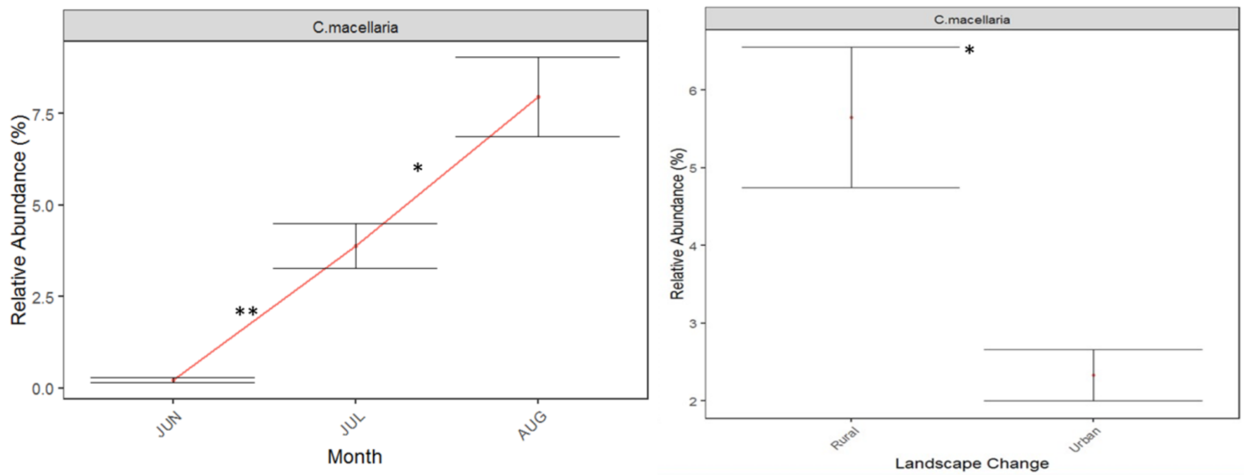


Figure 11. ***Cochliomyia macellaria*** **mean (SE) relative abundance by month with trend line (A) and landscape type (B) during the summer of 2017. Tukey's Honest Significant Different Test: (A) * Adjusted P-value <0.0020 **Adjusted P-value <0.0001 (B) *Adjusted P-value <0.0030**

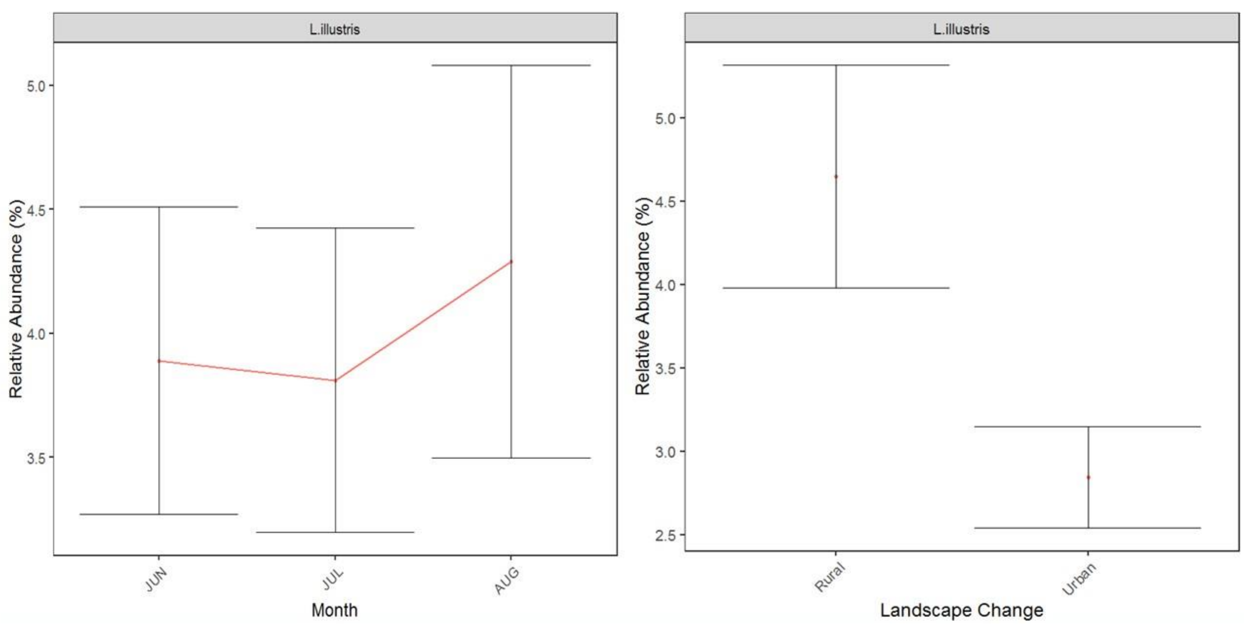


Figure 12**. *Lucilia illustris* mean (SE) relative abundance by month with trend line (A) and landscape type (B) during the summer of 2017.**

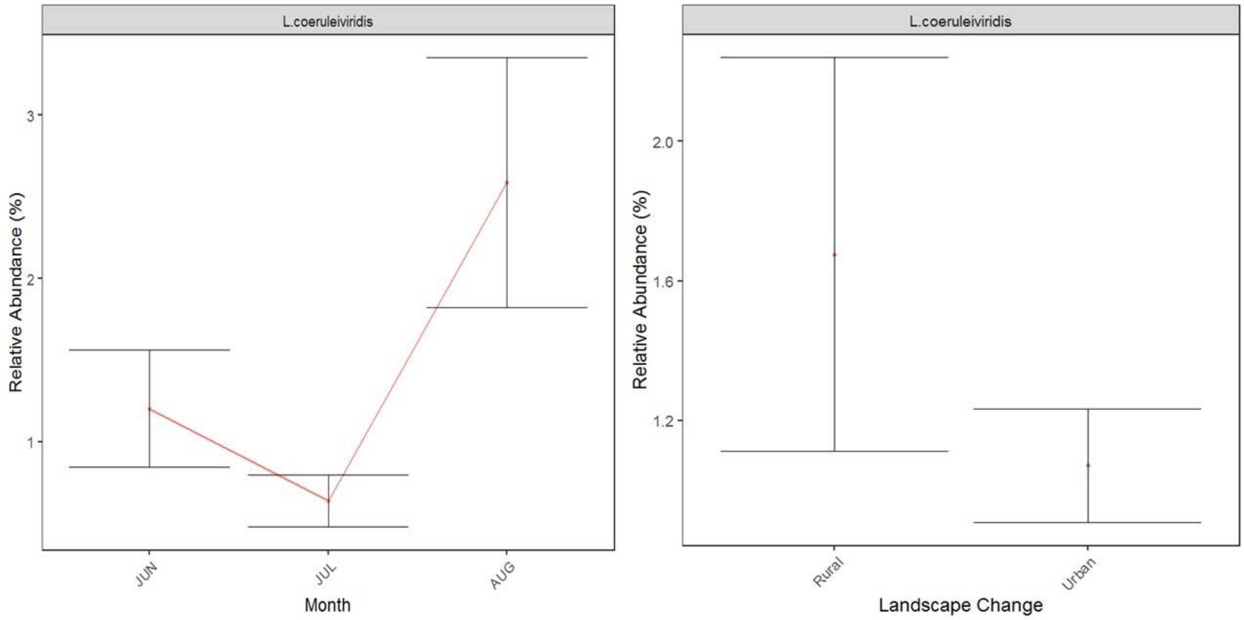


Figure 13. ***Lucilia coeruleiviridis* mean (SE) relative abundance by month with trend line (A) and landscape type (B) during the summer of 2017.**

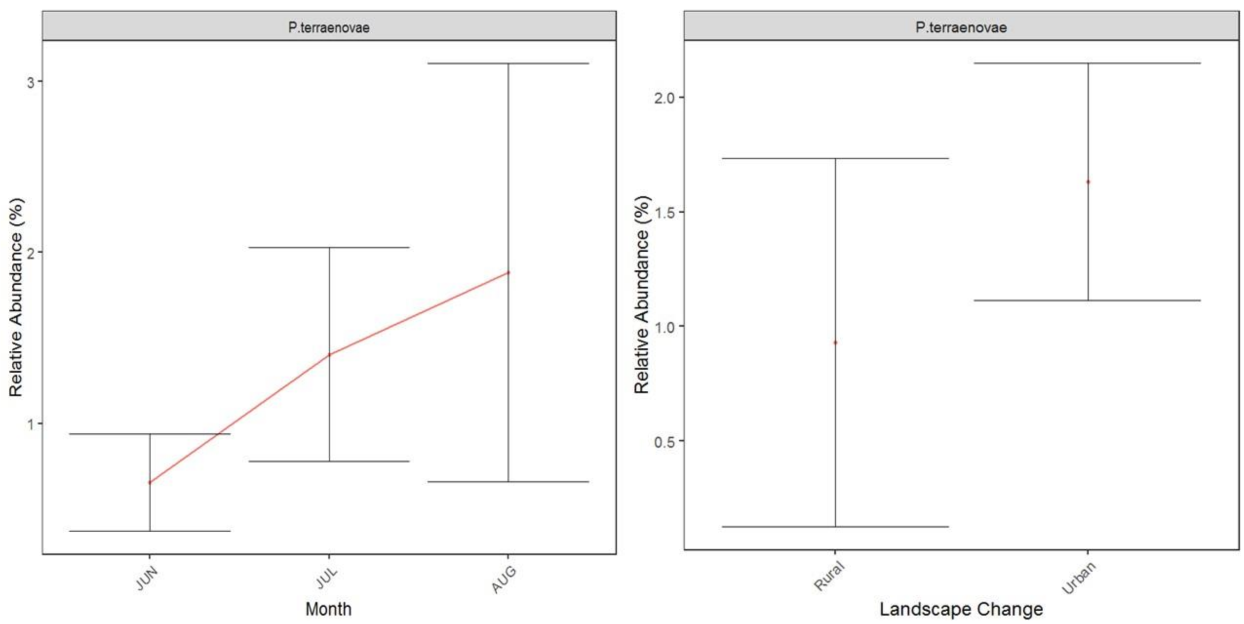
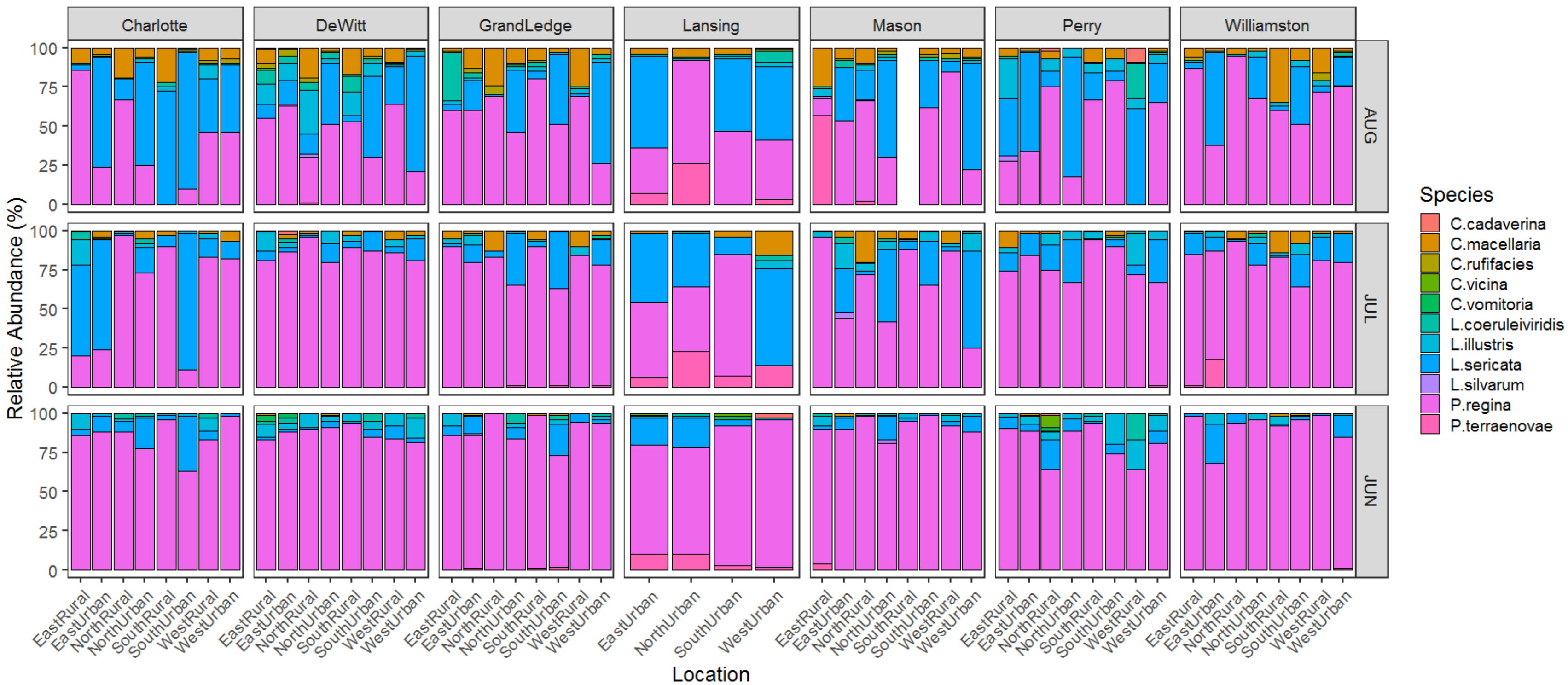
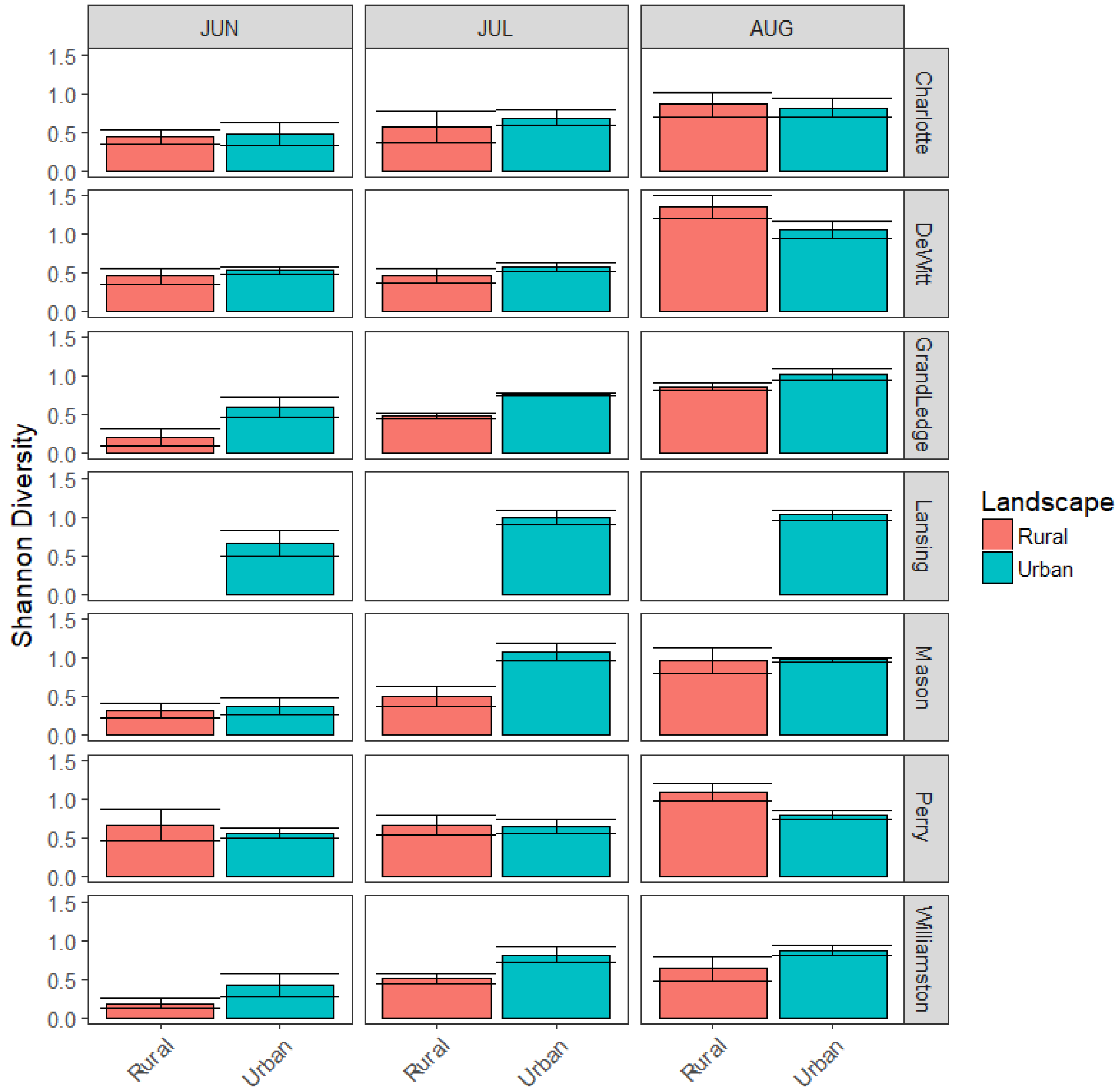


Figure 14**. *Protophormia terraenovae* mean (SE) relative abundance by month with trend line (A) and landscape type (B) during the summer of 2017.**



Supp. Figure F1. **Percent of Calliphoridae species by abundance for urban and rural trapping locations and trap placement site (Cardinal direction) distinguished by city, month and location during 2017 in Mid-Michigan.**



Supp. Figure F2**. Shannon diversity of Calliphoridae species indicated by landscape change, month and city in Mid-Michigan during the summer of 2017.**

APPENDIX C

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those species or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preserved specimens.

Voucher Number: 2018-03

Author and Title of thesis:

Nicholas J. Babcock

Adult blow fly community structure across urban to rural landscape change in Michigan.

Museum(s) where deposited:
Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Specimens:

Family	Genus-Species	Life Stage	Quantity	Preservation
Calliphoridae	*Calliphora vomitoria*	adult	1M/1F	pinned
Calliphoridae	*Calliphora vicina*	adult	1M/1F	pinned
Calliphoridae	*Chrysomya rufifacies*	adult	1M/1F	pinned
Calliphoridae	*Cochliomyia macellaria*	adult	1M/1F	pinned
Calliphoridae	*Cynomya cadaverina*	adult	1M/1F	pinned
Calliphoridae	*Lucilia illustris*	adult	1M/1F	pinned
Calliphoridae	*Lucilia sericata*	adult	1M/1F	pinned
Calliphoridae	*Lucilia silvarum*	adult	1M/1F	pinned
Calliphoridae	*Lucilia coeruleiviridis*	adult	1M/1F	pinned
Calliphoridae	*Protophormia terraenovae*	adult	1M/1F	pinned
Calliphoridae	*Phormia regina*	adult	1M/1F	pinned

REFERENCES

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