FORENSICALLY RELEVANT INSECTS IN HARRIS COUNTY

An Undergraduate Research Scholars Thesis

by

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RESEARCH COMPLIANCE CERTIFICATION

Research activities involving the use of human subjects, vertebrate animals, and/or biohazards must be reviewed and approved by the appropriate Texas A&M University regulatory research committee (i.e., IRB, IACUC, IBC) before the activity can commence. This requirement applies to activities conducted at Texas A&M and to activities conducted at non-Texas A&M facilities or institutions. In both cases, students are responsible for working with the relevant Texas A&M research compliance program to ensure and document that all Texas A&M compliance obligations are met before the study begins.

I, Olivia Crozier, certify that all research compliance requirements related to this Undergraduate Research Scholars thesis have been addressed with my Research Faculty Advisor prior to the collection of any data used in this final thesis submission.

This project did not require approval from the Texas A&M University Research Compliance & Biosafety office.

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ABSTRACT

Forensically Relevant Insects in Harris County

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Forensic entomology is the application of insects and other arthropods in a legal setting. Most commonly, forensic entomologists are asked to perform time of colonization calculations (TOC), which may equate to the post mortem interval (PMI) of the deceased. Those are made from entomological evidence collected or photographed at crime scenes and are calculated based off known insects in the area, developmental data sets for those species, and ambient temperature. This study is designed to equip forensic entomologists in the Harris County area with the most common forensically relevant insects as well as their developmental data sets based off case work done in the area. Data from fifty-two cases in the Harris County area was examined between 2015 and 2020 and the most common forensically relevant insects were determined. The most common order, with presence in every case, was Diptera. The most common families were Calliphoridae and Sarcophagidae. The most common species were *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) and *Chrysomya megacephala* (Fabricus) (Diptera: Calliphoridae). These data can be used in the future by other forensic entomologists as a guide for the most common forensically relevant insect species in Harris

County as well as their development data to aid in TOC calculations and PMI estimations. It also helps show researchers what species do not have developmental data sets available and which species those studies should be performed on.

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1. INTRODUCTION

The application of entomology to forensic science is something that has been around for decades but is just now making many breakthroughs in technology and the media (Benecke 2001, Michaud et al.2015). Popularity and knowledge in forensic entomology is rising, and with it, so is research. Forensic entomology is the application of insects and other arthropods in a legal setting (Byrd & Tomberlin 2019, Catts & Goff 1992). Most commonly, forensic entomologists are asked to perform Time of Colonization calculations (TOC) or to estimate the minimum postmortem interval (mPMI) (Tomberlin et al. 2011, Wells & Lamotte 2017). A TOC is the elapsed time since an insect has colonized a carrion or dead body (Sanford 2015).

In medico-legal cases, this insect is typically a blow fly (Benecke 2001, Byrd & Tomberlin 2019, Greenberg 1991). Blow flies are necrophagous flies that feed on corpses, seek out a cadaver with olfactory senses, and lay their eggs on orifices and openings on a body (Archer & Elgar 2003, Amendt et al. 2004, Greenberg 1985, Zaidi 2017). They are typically the first insect to colonize a corpse, allowing a fairly accurate PMI to be calculated (Reibe & Madea 2010). TOC is calculated based on developmental data that is available for the specific species with factors like temperature and the environment factored in (Villet & Amendt 2011). A higher temperature typically decreases the time it takes for a fly to develop while the opposite is true for lower temperatures (Amendt et al. 2011). With flies having the ability to locate a body minutes after death, TOC can be used to give insight to a potential time of death based on the life stage of the fly (Greenberg 1991, Bugelli et al. 2014). That potential time of death is determined by the postmortem interval, or time since death (Byrd & Tomberlin 2019). If insects are present at the scene, then it can be determined by a forensic entomologist.

When there are insects found at a crime scene, traditionally, the crime scene technician will collect physical and photographic insect evidence (Amendt et al. 2011, Gibb & Oseto 2020, Warlen 1995). The evidence will then be sent to a practicing forensic entomologist that is certified by the American Board of Forensic Entomology (ABFE). At the Harris County Institute of Forensic Science, an ANSI National Accreditation Board Forensic Science Testing Laboratories Program (ISO/IEC 17025), when a forensic entomologist receives insect evidence, they first identify it down to the lowest taxonomic level possible given published keys. Fly species identification is based off multiple morphological structures (Tarone & Foran 2010, Wallman 2002). If it is in its larval stages, spiracles, tubercles, and body shape are all used to help determine species morphologically (Amendt et al. 2010). If in its adult stages, genetic markers or unique morphological structures are used to identify species (Whitworth 2019, Chen et al. 2004, Alajmi et al. 2021). Then, developmental data sets are researched for the identified species. These data sets are used to determine the time at which this species develops at a certain temperature (Baque & Amendt 2012). This information is used to determine the TOC (Nabity et al. 2006). Once the TOC is calculated, a case report is created encapsulating all the evidence and information within the case (Kotze et al. 2021, Tomberlin et al. 2012). This report is then sent to another board-certified forensic entomologist for a peer review. This ensures the accuracy of the insect identification and TOC calculation.

Developmental or life cycle studies are studies that are done on the time it takes for a particular species to go through all its life stages at a certain temperature (Byrd & Butler 1997, Hu et al. 2019, Nabity et al. 2006). A species is reared in a lab and the time it takes for the insect to complete its full life cycle, from egg to adult, is recorded. Typically, they are performed at multiple temperatures in each study to show the difference in development rates (Flores et ala.

2014, Byrd & Butler 1997). When calculating a TOC, forensic entomologists work backwards from the latest life stage of the insect evidence that was collected (Bambaradeniya et al. 2017, Brundage 2020). The temperature at the scene where the evidence was collected is compared against developmental data sets to calculate how long that insect has been there. This time is subtracted from the time of evidence collection and a TOC is determined.

Each geographical location has a native insect species (Brundage et al. 2011, Gruner et al. 2007, Weidner et al. 2020.) Some species are more common than others in a certain area (Gaston 1991). As a forensic entomologist, if you are familiar with the most common species in your area, then you can be better equipped to handle cases more efficiently and accurately. This study is designed to equip forensic entomologists in the Harris County area with the most common forensically relevant insects as well as their developmental data sets based off case work done in the area.

2. METHODS

Fifty-two case reports between 2015 and 2020 were evaluated for commonalities among insect species. These cases were all located in the Harris County area and were processed by the Harris County Institute of Forensic Sciences. They each had some sort of insect activity that was either photographed or collected according to the ANSI National Accreditation Board Forensic Science Testing Laboratories Program (ISO/IEC 17025) standards. Each case was evaluated and the insects that were identified in the case report were recorded. The lowest taxonomic identification of each collected insect was recorded. These were considered “observed insects”. In multiple cases, insects were given “tentative identifications”. These insects were not able to be confidently identified by the forensic entomologist and were recorded as “tentative” but were not included in the total number of observations or the statistical analyses run. An ANOVA and Tukey’s post hoc test were run on these data to determine which species were observed significantly. Developmental data sets for the Dipteran species observed in these cases were researched to determine if there are any published and if they are under open access or if there is a paywall. Data sets were searched for on the first two pages of Google Scholar and ProQuest Biological Science Collection database (Regelson & Fain 2006, Van Deursen & Van Dijk 2009).

3. RESULTS

The insect orders identified were Diptera, Lepidoptera, Coleoptera, Dermaptera, Trichoptera, and Hemiptera. Other observed were mites, snails, Isopods, and millipedes. Within Diptera, ten families were identified: Calliphoridae, Chironomidae, Culicidae, Fanniidae, Muscidae, Phoridae, Piophilidae, Sarcophagidae, Stratiomyidae, and Tipulidae. Both Caliphoridae and Sarcophagidae were significantly observed ($p<0.05$). Calliphoridae was observed 284 times, Chrionomidae was observed one time, Culicidae was observed six times, Fanniidae was observed three times, Muscidae was observed 19 times, Phoridae was observed 18 times, Piophilidae was observed five times, Sarcophagidae was observed 57 times, Stratiomyidae was observed six times, and Tipulidae was only observed once within the 400 identified Dipteran observations (Chart 1).

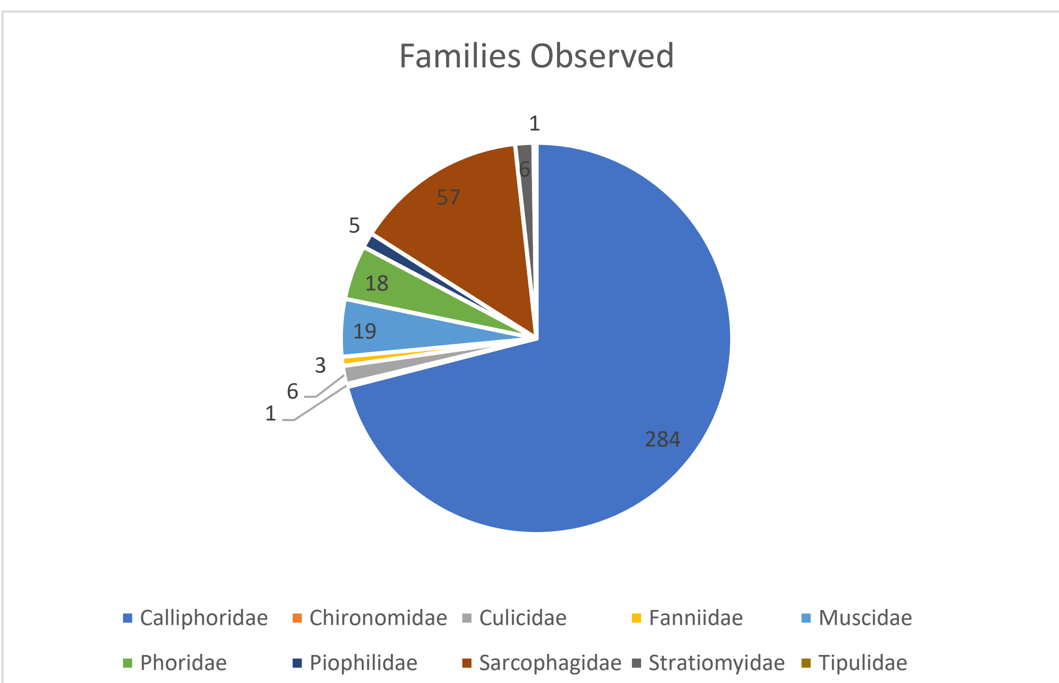

Chart 1: The number of times each Dipteran family was observed.

Two-hundred forty Dipterans were identified to their species level. *Chrysomya megacephala* and *Chrysomya ruffifacies* were both observed significantly more times than the other species ($p<0.05$). *Aedes albopictus* (Skuse)(Diptera: Culicidae) was observed four times, *Blaesoxipha plinthopyga* (Wiedemann)(Diptera: Sarcophagidae) was observed two times, *Calliphora coloradensis* (Hough)(Diptera: Calliphoridae) was observed one time, *Chrysomya megacephala* was observed 49 times, *Chrysomya rufifacies* was observed 111 times, *Cochliomyia macellaria* (Fabricus)(Diptera: Calliphoridae) was observed 31 times, *Fannia canicularis* (Linnaeus)(Diptera: Fanniidae) was observed three times, *Hermetia illucens* (Linnaeus)(Diptera: Stratiomyidae) was observed six times, *Lucilia cuprina* (Wieddemann)(Diptera: Calliphoridae) was observed two times, *Lucilia eximia* (Wiedemann)(Diptera: Calliphoridae) was observed three times, *Megaselia scalaris* (Loew)(Diptera: Phoridae) was observed 12 times, *Phormia regina* (Meigen)(Diptera: Calliphoridae) was observed 10 times, *Piophila casei* (Linnaeus)(Diptera: Piophilidae) was observed one time, *Stomoxys calcitrans* (Linnaeus)(Diptera: Muscidae) was observed two times, and *Synthesiomia nudiseta* (Wulp)(Diptera: Muscidae) was observed three times (see Chart 2).

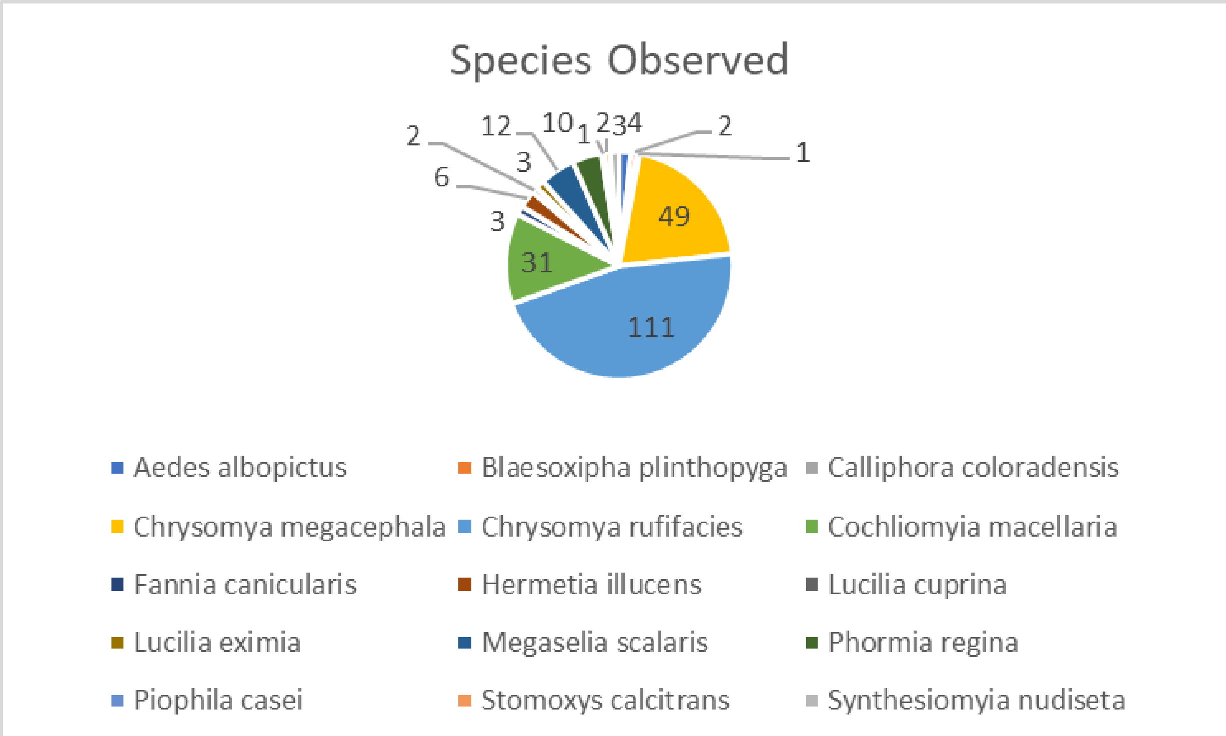

Chart 2: The number of times each Species in Diptera was seen.

Coleoptera was the second most observed order with 19 observations. Eleven were identified as Dermestidae, two were Histeridae, two were Nitidulidae, and one was Staphylinidae. Of the eleven identified as Dermestidae, eight were identified as Dermestes maculatus. Three Hemipterans were identified. One was identified as Cimex lectularis, one was identified as Cimicidae, and one was tentatively identified as *Euborellia annulipes*. Two mites were identified as Acari. Three Isopods were identified as Isopoda. One Dermaptera, one Lepidoptera, and one Trichoptera were identified. One Chilopoda was identified as Polydesmida. Statistical analyses were not run on these findings, as they were beyond the scope of the experiment.

Listed below are the developmental data sets for the observed Dipteran species and their accessibility:

Table 1: Developmental data sets for the observed Dipteran species and their accessibility

Species	**Citation**	**Database: open access or paywall**
Aedes albopictus	Delatte et al. 2009	Open access
Blaesoxipha plinthopyga	Denlinger et al. 1988 El-Hefnawy 2020	Open access Open access
Chrysomya megacephala	Gabre et al. 2005 Gruner et al. 2016 Wells & Kurahashi 1994 Yang et al. 2016 Zhang et al. 2018	Open access Open access Open access Open access Open access
Chrysomya rufifacies	Byrd & Butler 1997 Flores et al. 2014 Hu et al. 2019	Paywall Open access Paywall
Cochliomyia macellaria	Boatright & Tomberlin 2010 Byrd & Butler 1996	Paywall Paywall
Fannia canicularis	Meyer & Mullens 1988 Grzywacz 2019	Paywall Open access
Hermetia illucens	Harnden & Tomberlin 2016 Holmes et al. 2012 Nguyen et al. 2013	Open access Open access Open access

Lucilia cuprina	Dallwitz 1984 Day & Wallman 2006 Kotze et al. 2015	Open access Open access Open access
Lucilia eximia	Ramos-Pastrana 2017	Open access
Megaselia scalaris	Greenberg & Wells 1998 Thomas et al. 2016 Trumble & Pienkowski 1979 Zuha et al. 2012	Paywall Paywall Open access Paywall
Phormia regina	Byrd & Allen 2001 Nabity et al. 2006 Nunez-Vazquez 2013	Open access Paywall Paywall
Piophila casei	Russo et al. 2006	Open access
Stomoxys calcitrans	Gilles et al. 2005 Kunz et al. 1977 Lysyk 1998	Open access Paywall Paywall
Synthesiomyia nudiseta	Velasquez et al. 2012	Open access

4. CONCLUSION

The results of this study show that Dipterans were the most common forensically relevant insect, with Calliphoridae and Sarcophagidae being the two most common families. *Chrysomya rufifacies* and *Chrysomya megacephala* were the two most common Dipteran species.

Chrysomya rufifacies, also known as the hairy maggot blowfly, is commonly seen in the southern United States (Martin et al. 1996). It is an invasive species, originating in Asia and introduced into the Americas in 1978 (Baumgartner 1993). Its first instar larvae are necrophagous, while its second and third instar larvae have been known to prey on other larvae when needed (Wells & Greenberg 1992). It can be a vector of pathogens and food born illnesses and has been known to perform myiasis in livestock and humans (Sukontason et al. 2005). With it being one of the most common insect species, it has medicolegal importance, as it can be used to calculate TOC (Baumgartner 1993).

Chrysomya megacephala, also known as the oriental latrine fly, is native to Africa and has made its way up to the southern United States (Badenhorst & Villet 2018). It is a dominant colonizer of decaying matter, getting their first before other species prey on them and run them away (Sukontason et al. 2005). Because of this, they are used to calculate TOC. There are also reported cases of C. megacephala associated with human myiasis (Sukontason et al. 2005). They breed on rotting food, human feces, or carrion and can be a public health concern, due to their comfort around humans and their waste (Wells 1991). They can be vectors of diseases and pathogens and spread bacteria to humans through contaminating their food (Badenhorst & Villet 2018).

Aedes albopictus are commonly known by their ability to vector certain diseases such as dengue and heartworm (Gratz 2004). *Blaesoxipha plinthopyga* are known to be associated with human myiasis, yet little information about this species is known, and should be researched further (Wells & Smith 2013). *Calliphora coloradensis* are common in the colder climates of Colorado and are commonly found on human remains there. It is unusual that it was found in the very warm climate of Texas. *Cochliomyia macellaria*, the secondary screwworm, are common on decomposition scenes in Texas and have even been used in maggot debridement therapy, a way in which doctors use the maggot's behavior of feeding on human tissue for healing purposes (Masiero et al. 2020, Owings et al. 2014). *Fannia canicularis* are known as the lesser or little house fly and are attracted to urine and feces and can be used to calculate TOC when those are present in a death or abuse scene (Benecke & Lessig 2001, Wang et al. 2007). *Hermetia illucens*, commonly known as the black soldier fly, are commonly found in tropical areas and its larvae is being used as a cheap alternative for livestock feed (Harnden & Tomberlin 2016, Turchetto & Vanin 2004, Wang & Shelomi 2017). *Lucilia cuprina* are known to be one of the first flies to colonize carrion, making it useful in calculating TOCs (Bansode et al. 2016). *Lucilia eximia* are native to Brazil and has been associated with human myiasis (Sanford et al. 2014). *Megaselia scalaris* are in the family Phoridae and are seen in cases where the temperature is typically low (Reibe & Madea 2010). *Phormia regina* are known as the black blow fly and are commonly seen in secondary myiasis in livestock (Byrd & Allen 2001). *Piophila casei* are common in cooler temperatures and can vector diseases and perform myiasis (Martin-Vega 2011). *Stomoxys calcitrans*, or the stable fly, are known to be a nuisance and vector of diseases to livestock (Bishopp 1913, Greenberg 1985). *Synthesiomyia nudiseta* are a secondary agent of myiasis, a vector of diseases, and have predatory habits (Ivorra et al. 2021).

No developmental data sets are currently published for *Calliphora coloradensis*. *Cochliomyia macellaria* did not have an open access developmental data set. The lack of research and access to available research affects crime solving due to the lack of useful evidence or ability to analyze that evidence. Insect evidence is apparent at the crime scene, but investigators cannot use it to perform TOCs. This could be avoided, and all insect evidence could be used efficiently if developmental data studies were performed and made available.

Studies like these should be performed in all areas since forensically relevant insects differ across geography. If each crime lab had access to the forensically relevant insects in their county, then developmental data sets could be gathered, and insect evidence could be analyzed more efficiently and accurately.

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