DETERMINING WHICH TAXONOMIC RESOLUTION PROVIDES GREATEST INSIGHT TO IMPACT OF MASS MORTALITY EVENTS ON EARLY CARRION COLONIZERS.

An Undergraduate Research Scholars Thesis

by

ABBY KIMPTON JONES

Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. Jeffery Tomberlin

May 2018

Major: Forensic Science
Entomology

TABLE OF CONTENTS

ABSTRACT

Determining Taxonomic Resolution provides greater insight to impact of mass morality events on early carrion colonizers

Abby Jones
Department of Entomology
Texas A&M University

Research Advisor: Dr. Jeffery Tomberlin
Department of Entomology
Texas A&M University

Mass mortality events (MME) impact local ecosystems especially in systems where insects play a major role in recycling carrion. Insects may be unable to efficiently recycle the carrion. Selecting the appropriate taxonomic resolution will be advantageous especially when assessing sensitivity of arthropod communities to variably sized MMEs. A collaborative study examining an artificial MME using feral swine (*Sus scrofa Linnaeus*) was conducted at Mississippi State University (MSU). Five different masses of swine carcasses ranging from 24.9 kg to 725.7 kg were placed in a pine forested area, starting in the spring of 2017. Different plots (1 km apart) were created based on density, carrion presence or absence and accessibility by scavengers. Sticky traps were placed among the plots to collect insects and were replaced as needed (between 24 hours and two weeks). Sticky traps were sent to Texas A&M University for identification of insects. This project involves determining the most useful and practical taxonomic level to identify insects using the MME experiment. Based on the different biomass sites, the hypothesis is the diversity and richness of the arthropod community will not vary at each site over time regardless of taxonomic resolution and the best taxonomic resolution is the species level.

ACKNOWLEDGEMENTS

NOMENCLATURE

MME   Mass Mortality Event

MSU   Mississippi State University

TAMU Texas A&M University

FLIES   Forensic Laboratory for Investigative Entomological Sciences (Laboratory)

PMI   Post Mortem Interval

TOC   Time of Colonization

IACUC The Institutional Animal Care and Use Committee

NMDS Non-metric Dimensional Scaling

CHAPTER I

INTRODUCTION

Ecological Community and Diversity

Ecology involves the study of the environment and community interactions within it (Michaud et al. 2015). Ecologists (especially before 1900) have long been interested in environment succession (Tansley 1935), which can be defined as changes in the composition of a community over time (Cain et al. 2008, Michaud et al. 2015). To this day, there are still discussions centered around questions involving Clements v. Gleason in relation to succession (Michaud et al. 2015). Clements initiated the discussion on this topic by pontificating about community structure and how the community behaves in a "superorganism" fashion with succession of the community leads to the environment reaching a stable state/climax over time (Clements 1916). In contrast, Gleason was more flexible with his definitions and believed each individual plays an independent part in the ecosystem and the process of succession (Clements 1916, Gleason 1926, Michaud et al. 2015). An ecosystem can instead have multiple climaxes throughout time and succession can be disrupted by disturbance events. Communities consist of many different food webs, creating different forms of competition (Hutchinson 1959). This kaleidoscope of species interactions can generate a wide variety of community structures and ecological niches resulting in a complex diversity and species richness (Elton 1927).

Species richness is the number of species within an area (Simon et al. 2009). Diversity can also be seen and compared at different sites and carrion biomasses (Mittelbach 2012), and consists of multiple factors, including the variability of different species and differing relative abundances (Magurran 1988). When comparing diversity, boundaries are determined by the

circumstances of the habitats as well as what the ecologist/scientist suggests and determines them to be (Mittelbach 2012). There is already a general species variation in terms of species richness across the globe (Mittelbach 2012) with most species richness occurring at the equator (Mittelbach 2012). There are several theories as to why this may exist. The null model theory suggests a pattern will form, and does form, without the presence of a mechanism (Mittelbach 2012). There is also the theory that evolution simply led to the ecological diversification of species. Historical effects could also be the culprit (Mittelbach 2012). Ice ages can alter the pole climates which can drive extinction or speciation events (Mittelbach 2012). One of the most popular theories is that species diversification is due to climate changes from pole to equator. More species will occur and be more diverse around the equator while that will be restricted around the poles (Mittelbach 2012).

Species diversity can be measured in several ways: alpha diversity is the differences in species richness and diversity within a local habitat, such as within different areas in the thesis research (Mittelbach 2012), and beta diversity, the species richness and differences across two habitats (Mittelbach 2012). Alpha diversity multiplied by Beta diversity reveals Gamma diversity (Mittelbach 2012), which is the species differences and richness over an entire region (such as several different habitats or between our five biomass sites) (Mittelbach 2012). These terms allow for a comparison of diversity across habitats (as well as individual spaces).

Forensic Entomology

The National Research Council of 2009 determined a whole range of the forensic sciences needed to invest more in research. An overhaul of forensic science must be performed to achieve better and more accurate results (Tomberlin et al. 2011). There had been a wide range

of errors involved with criminal investigations and evidence present. Forensic entomology is no exception to the National Research Council ruling.

Forensic entomology is the use of insects in legal investigations (Byrd and Castner 2001). Insects are particularly useful in the case of Post Mortem Interval (PMI) and Time of Colonization (TOC) calculations. PMI is the time that has elapsed since the death (Byrd and Castner 2001) but can vary widely depending on the location, climate, temperature, and circumstances. Different insect species thrive and survive under different temperatures. Reference data can be used to help with these calculations (Byrd and Castner 2001). Development data for many different species of flies has been created. PMI can then be determined using this development data to determine age of the insect. PMI can also be calculated with stages of succession, comparing the insects present (or absent) on a carrion to the assemblage of insects that would be present during that phase of succession. An estimation of the PMI can potentially be determined based on varying factors including temperature, location, and list them (Byrd and Castner 2001). Time of colonization is the time that the insects detected and reached the carrion. It is important to determine when the insects colonized the body as this might provide more information on the deceased, including how and when they died (Tomberlin et al. 2011).

Overall, forensic entomologists were heavily focused on the estimation of the PMI and TOC. There was also an interest in insect succession (Byrd and Castner 2001). Understanding these processes may provide greater insight into the mechanisms regulating ecological phenomena ranging from trophic interactions to nutrient cascades. However, most studies examining this relationship focus on a single or few carcasses in a location at a given time. A

large-scale event may have a larger effect on the environment and arthropod community than smaller events.

To fully understand the concept of insect colonization, the different phases of colonization of carrion must be studied. The decomposition process can be split into two different intervals: the pre-colonization and the post-colonization intervals (Tomberlin et al. 2011), which are separated by the TOC. The first phase after death is the exposure phase, which involves the carrion being accessible to insects (Tomberlin et al. 2011). Then the detection phase, occurs when the insects being to detect the carcass, often due to the presence of volatiles (Tomberlin et al. 2011). The acceptance phase then begins once the first insects locate that carrion and ends when the insects colonize that carrion. Afterwards the consumption phase starts as well as the post-colonization interval (Tomberlin et al. 2011). After the carcass is consumed as much as possible, the insects begin to leave the carrion in the dispersal phase. The post-colonization interval is what the focus of both this forensic entomology and carrion ecology (Tomberlin et al. 2011).

Historically, understanding the community composition in association with vertebrate remains at their time of discovery could be used potentially to estimate a minimum time of death (Tomberlin et al. 2011). Arthropod succession on vertebrate carrion is critical for its recycling.

Calliphoridae

The order of Diptera has approximately 86,000 species, with 1,000 species being in the family Calliphoridae (blow flies) (Byrd and Castner 2001). Calliphoridae is one of several families of Diptera that have forensic importance, the other two being Sarcophagidae (flesh flies) and Muscidae (filth flies) (Byrd and Castner 2001). Blow flies are one of the first groups of insects to arrive on a carrion, and can arrive almost immediately after the death (Byrd and

Castner 2001). *Phormia regina* (Meigen) may arrive soon after death or a little later such as a day or two (Byrd and Castner 2001). *Cochliomyia macelleria* (Fabricius) is another important species that will arrive within the first day of death, but not necessarily immediately (Byrd and Castner 2001). Multiple *Lucilia* species are also seen soon after the death, depending on the species (Byrd and Castner 2001). These species are active in warm weather in places such as Mississippi (Byrd and Castner 2001). A total of twelve different species of blow flies have been recorded in Mississippi as of 1983 (Goddard and Lago 1983). *Phormia regina* is the most common blow fly in Mississippi and is encountered year-round (Goddard and Lago 1983). These insects are particularly associated with decomposing organic matter. *Cochliomyia macelleria* is also very common throughout all of Mississippi and arrives to most types of carrion (Goddard and Lago 1983). Several species of *Lucilia* are also present in Mississippi (Goddard and Lago 1983). Most of these species are either attracted to garage or decaying animal matter. Overall, these are some of the most important species when it comes to carrion feeding in Mississippi.

These species will mate on or around carrion and then oviposit (Byrd and Castner 2001). Temperature and humidity are key for these flies as the eggs cannot hatch under temperatures that are too hot or cold. This impact of temperature can vary depending on the species of the Calliphoridae (Byrd and Castner 2001). Under the optimal temperatures, the eggs will then hatch into the first stadium and continue growing up until the third stadium, they will then pupate, then emerge as adults (Byrd and Castner 2001).

Since this mock mass mortality event (MME) takes place in Mississippi, the Calliphoridae species present in this state need to be analyzed. In 1983, Goddard and Lago identified 15 calliphorid species within eight genera in Mississippi. These genera included *Cochliomyia*, *Phormia*, *Protocalliphora*, *Bufolucili*a, *Phaenicia* (*Lucilia*), *Calliphora*,

Cynomyopsis (*Cynomya*), and *Pollenia* (Goddard and Lago 1983). Several of these species were found in throughout our research that were indeed present in the Mississippi data from several *Lucilia* species (*L. coeruleiviridis, L. cuprina,* and *L. sericata), C. macalleria,* and *P. regina.* Seasonality was also observed for most of the species as done in the study of from Goddard and Lago in 1983.

Mass Mortality Events (MMEs)

Mass mortality events are episodes of high mortality for a population of a given species (Fey et al. 2015). Little information on mass mortality events is available because there are very few opportunities to observe these largely unpredictable events (Fey et al. 2015). Fey et al. (2015) conducted a review on MMEs of multiple taxa, including fish, reptiles, amphibians, invertebrates, birds, and mammals (Fey et al. 2015). The cause of a MME can range from disease, poisoning, pollution, to other stress factors on the environment and population have generally increased in frequency across taxa since 1940 (Fey et al. 2015). MMEs have a wide effect on the entire ecosystem including the animals and environment and can affect other organisms and environments outside of the original ecosystem (Fey et al. 2015). The spread of disease can occur with the effects to the microbes in the soil and further spread that disease to other areas (Pain et al. 2003, Markandya et al. 2008). Mass animal mortalities are one example of a unifying problem that unite ecology and entomology.

One of the other potential issues with MMEs is the huge amount of nutrients being placed into the system (Tomberlin et al. 2017). A system is defined as the whole complex of factors within an environment that connect and may work together (Tansley 1935). This influx of nutrients can change soil composition as large amounts elements such as nitrogen and carbon are added into the earth (Zak 2014). This also influences both vertebrate and invertebrate

communities by altering the ecosystem and changing their food source (Tomberlin et al. 2017). Decomposers become an even more crucial part of the process as they become a large part of the environment and effected other organisms (Tomberlin et al. 2017). Fungi, bacteria, scavengers and necrophagous insects become extremely useful in the recycling of carrion (Tomberlin et al. 2017).

Soil nutrients have a significant effect on the biodiversity of various microbial communities (Novais and Sousa 2017). The only caveat is this involved aquatic carrion and an aquatic mass mortality event rather than terrestrial carcasses. It does observe the terrestrial microbial communities; however, insects are not a factor nor is the experiment under similar conditions to the set-up mass mortality event. It is essential to discover the effects this environmental change will have on the entomological communities.

Taxonomic Resolution

Taxonomic resolution is defined as the most informative level in taxonomy (e.g., species, genus, family) to identify a specimen for explaining ecological phenomena (Marshall et al. 2006). If the taxonomic level is too broad, it will be difficult to see the individual effects. If the taxonomic level is too specific, it may be difficult to discern the whole picture. Taxonomic resolution can help create clarity in understanding this relationship. This taxonomic level must provide the best level of information while conserving resources and time (Marshall et al. 2006). In some instances, researchers have determined genus level identification provide similar information as species level identifications (Marshall et al. 2006, Waite et al. 2004). Even more interesting, enhanced taxonomic resolution from genus to species yielded practically the same results at 92% (Marshall et al. 2006). In the event of some more coarse impacts, genus did reveal a few more differences (Waite et al. 2004). Genus may also be used in richer areas to detect other

impacts that family may not involving factors such as pollution (Waite et al. 2004). In the case of Waite et al. papers work, species were unidentifiable, which is why genus was more commonly used (Waite et al. 2004). However, family was still determined as the best detection factor for the effects of macroinvertebrates in benthic communities (Waite et al. 2004). Overall, family identifications were useful for visualizing community patterns (Hirst 2006). For conservation studies and biodiversity, species level taxonomic level is more accurate and provides more information about the effects on species involved in these biodiversity studies (Fjeldsa 2000; LaFerla et al. 2002) and should be used when monitoring invasive species (Konar and Iken 2009).

Natural disasters and pollution may also have affects that can only be seen at the species level (Olsgard et al. 1998). Family can be an acceptable taxonomic level for identification of macroinvertebrates as shown in a study where family compared to species was only 6% less informative (Marshall et al. 2006). Even with pollution, the resolution between family and species is not great therefore making family the best taxonomic level as well (Olsgard et al. 1998). However, this may depend on the circumstances (Bowman and Bailey 1997). Aquatic environments are also said to have a greater taxonomic diversity, so species level identification may be more appropriate (Bowman and Bailey 1997). However, many of the taxonomic resolution papers discussed involve aquatic rather than terrestrial communities. However, even those studies that focus on terrestrial and carrion ecology may still contain several differences (Chin 2016). In the case of Chin 2016, family was determined as the best taxonomic resolution. However, there was still a marked difference between order and genus. Also, in this case, fewer carrion were used. This was not a mass mortality event level of disturbance (Chin 2016). It is

possible that the level of disturbance a mass mortality event has will alter the taxonomic resolution.

In general, most papers had different objectives which may affect what taxonomic resolution may be the most useful. Data that predicted species and family data had less than a 6% resolution loss was observing quantification and identification of macroinvertebrates (Marshall et al. 2006). Other objectives can also be detected or observed.

CHAPTER II

METHODS

Field Site

Sites for collecting insects were set up by Mississippi State University (MSU). Feral pigs (*Sus scrofa*) were killed under a federal removal program and given to MSU. This study, however, did not require IACUC approval. The pigs were later thawed before placement. Five different sites were created in a mixed pine forest in Mississippi. Each site was one kilometer apart for the next adjacent site. The individual plots within the site were 15 m in circumference and at least 100 meters away from the next plot. Each site had a varying biomass to simulate a mock mass mortality event. The sites each contained one of the following amounts of pigs: 55 kg, 130 kg, 400 kg, 800 kg, 1600 kg. Sites are numbered 1 through 5 respectively. Within each site was six different areas containing different plots. Treatments (nutrient additive group, control group (no pigs), or pig carcass group) were randomized within plots, and plots were either opened or closed with fencing to prevent scavengers and birds from accessing the pigs.
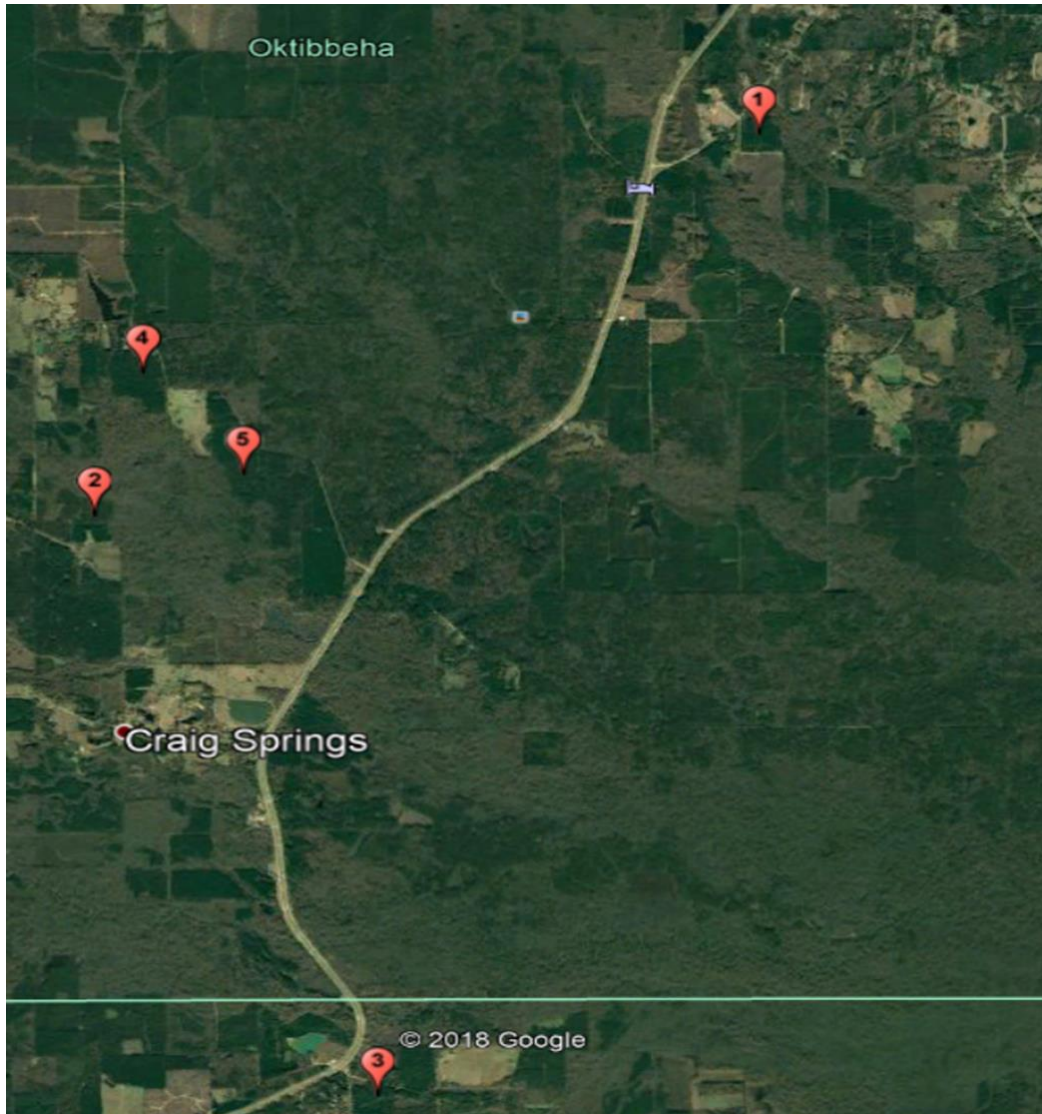
Figure 1. This map shows the location of the five different sites in Mississippi as taken from Google Earth.
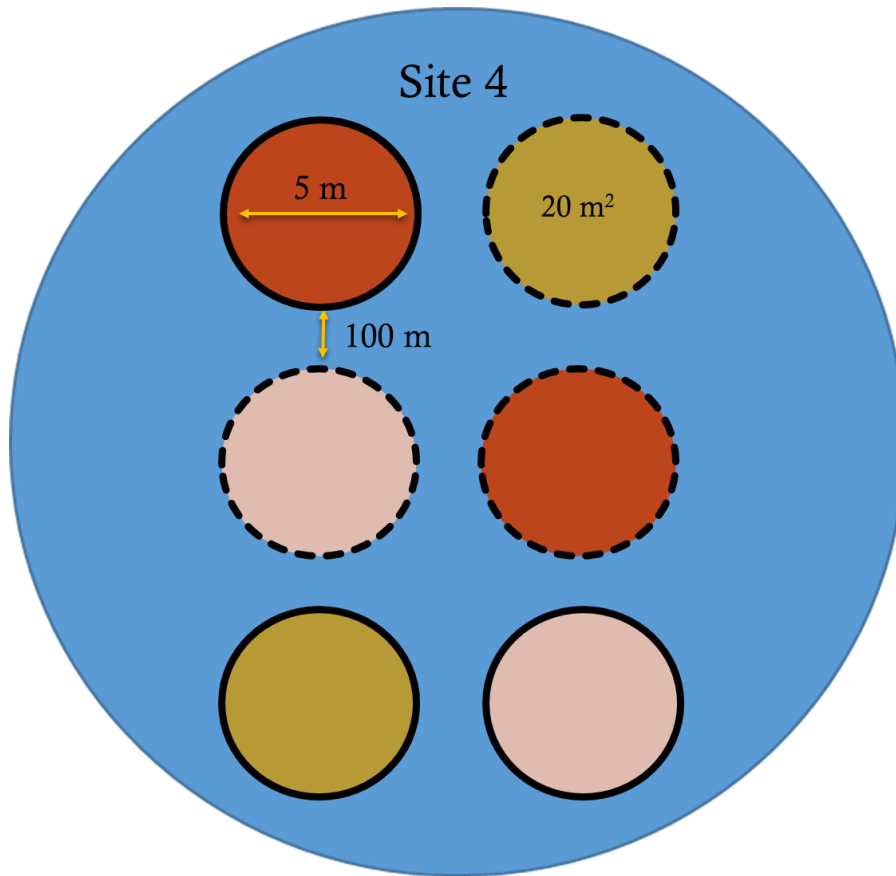
Figure 2. An example of one of the sites (out of five total) by Mississippi State University to demonstrate the different treatments used. The three different colors represent the three different treatments: nutrient additive, control group, and pig carrion. The solid lines around circle indicate a closed plot while the dashed lines indicated an open plot.

Chicken wire cages were made to be 1 m by 15 m in circumference and placed in the closed plots to protect from scavengers. To monitor any vertebrates that did show up, trail cameras were used. Pigs were then laid side by side and multiple sticky traps were placed in each area. These sticky traps were then collected and placed either in large plastic bags or in boxes according to the date range. The traps were collected when they were full or after two weeks. These traps were then sent to Texas A&M University, where they were identified.

Texas A&M University Identification

      The sticky traps were processed by Texas A&M University. The identifications made were only for forensically-important blow flies such as *C. macelleria, P. regina,* and various *Lucilia* sp. using Whitworth (2006) key.

Statistical Analysis

      Community ecology with R programming will be used for statisitical analysis. Typical packages used for this include ellipse, ecodist, Biodiversity R, and vegan (community ecology package). Traditionally, a variety of different statistical analyses have been performed for taxonomic resolution. These include PERMANOVA, , NMDS, Analysis of Covariance, Mantel's matrix, Chi-square, Shannon Index, and more (Marshall et al. 2006, Waite et al. 2004, Bailey and Bowman 1997). The used statistics vary based on the objective used with taxonomic resolution. In this case of this project, Permutational Multivariate Analysis of Variance (PermANOVA) will first be used to assess the combinations of variables for the dataset (Anderson and Walsh 2013). This will be followed by Bray-Curtis Dissmilarity, which will be performed to obtain the Non-Metric Multidimensional Scaling (NMDS). Bray-Curtis is a type (indicate p value) of ordination that will help when clarifying the relationships that will be used in NMDS (Gauch Jr. 1973). NMDS will compare distances between the genus and species (taxonomic levels) across time period, site, and treatment by clustering the data (Kenkel and Orlocs 1986). NMDS has been used before in cases of carrion ecology (Pechal et al. 2015). This observed insect community structure and decomposition over time with nonparametric dimensional analysis (Pechal et al. 2015). Finally, species indicator analysis was used to identify the most important out of the variables that contribute to the taxonomic resolution among plots (Pechal et al. 2015).

CHAPTER III

RESULTS

Approximately 275 of the traps had Calliphoridae species present. There were three genera identified: *Lucilia*, *Cochliomyia*, and *Phormia*. Five species were identified including *Phormia regina, Cochliomyia macelleria, Lucilia coeruleiviridis, Lucilia cuprina,* and *Lucilia sericata.* However, there were only 22 total *Lucilia cuprina* identified and 3 *Lucilia sericata.*

Figure 1: This shows the resulting data of the PermANOVA test. The bold indicates a **significant relationship between multiple treatments or treatment and variable. Italicized indicates approaching significance.**

Factor	F	*P*	R^2
Date	13.2146	**0.001**	0.23172
N (Total)		12	
Biomass	12.8622	**0.001**	0.08201
N (Total)		5	
Treatment	19.0920	**0.001**	0.06087
N (Total)		3	
Fencing	7.5777	**0.001**	0.02416
N (Total)		3	
Date:Biomass	3.2548	**0.001**	0.15565
N (Total)		31	
Date:Treatment	3.6032	**0.001**	0.05744
N (Total)		11	

Biomass:Treatment **N (Total)**	1.3361	0.189 7	0.01278
Date:Fencing **N (Total)**	1.1058	0.303 11	0.01763
Biomass:Fencing **N (Total)**	3.6022	**0.001** 5	0.02297
Treatment:Fencing **N (Total)**	1.6952	0.130 3	0.00540
Date:Biomass:Treatment **N (Total)**	2.5871	*0.067* 2	0.00412
Date:Biomass:Fencing **N (Total)**	1.2336	0.143 22	0.04130
Date:Treatment:Fencing **N (Total)**	1.0292	0.410 3	0.00328
Biomass:Treatment:Fencing **N (Total)**	0.5338	0.771 3	0.00170
Total **N**	--	**--** 283	1.0000
---	**--**	**--**	**--**

Significant interactions of date and biomass as well as date and treatment were determined for calliphorid abundance (figure 1). There was some noted significance across three treatments which are date, biomass, and treatment

Bray-Curtis dissimilarity index was used to conduct Non-Metric Multidimensional

Scaling (NMDS). The stress treatment indicates that our data are explained through 3 dimensions

(appendix 1). NMDS was then tested for all variables including date, biomass, treatment, and

fencing.

Figure 2: Letters indicate what they mean in terms of date ranges in figure 3.

a	6/24 to 7/05
b	07/05 to 07/07
c	07/07 to 07/09
d	07/07 to 07/12
e	07/12 to 07/19
f	07/18 to 7/19
g	07/19 to 07/20
h	07/28 to 08/02
i	08/02 to 08/09
j	08/19 to 08/27
k	08/27 to 09/06

Figure 3: Clusters of the different date ranges in two dimensions (x and y axis) to

determine community response.
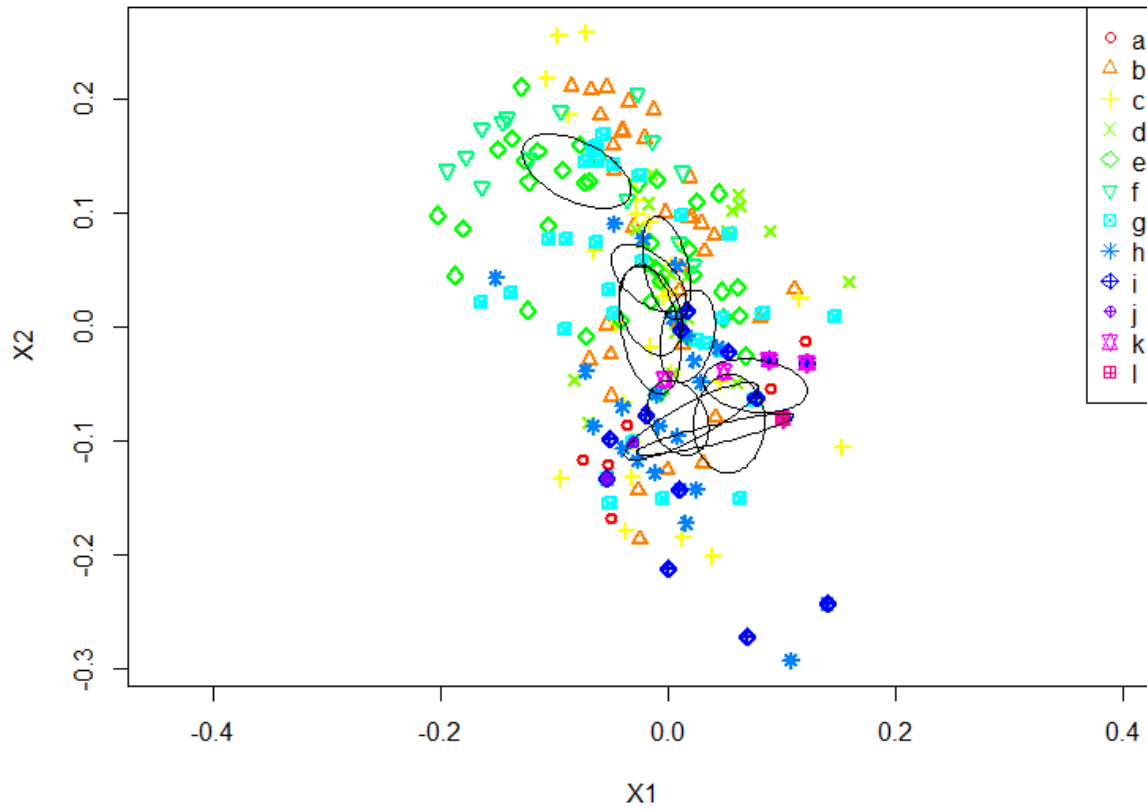
Figure 4: Clusters of different fencings in two dimensions (x and y axis) to determine community response.
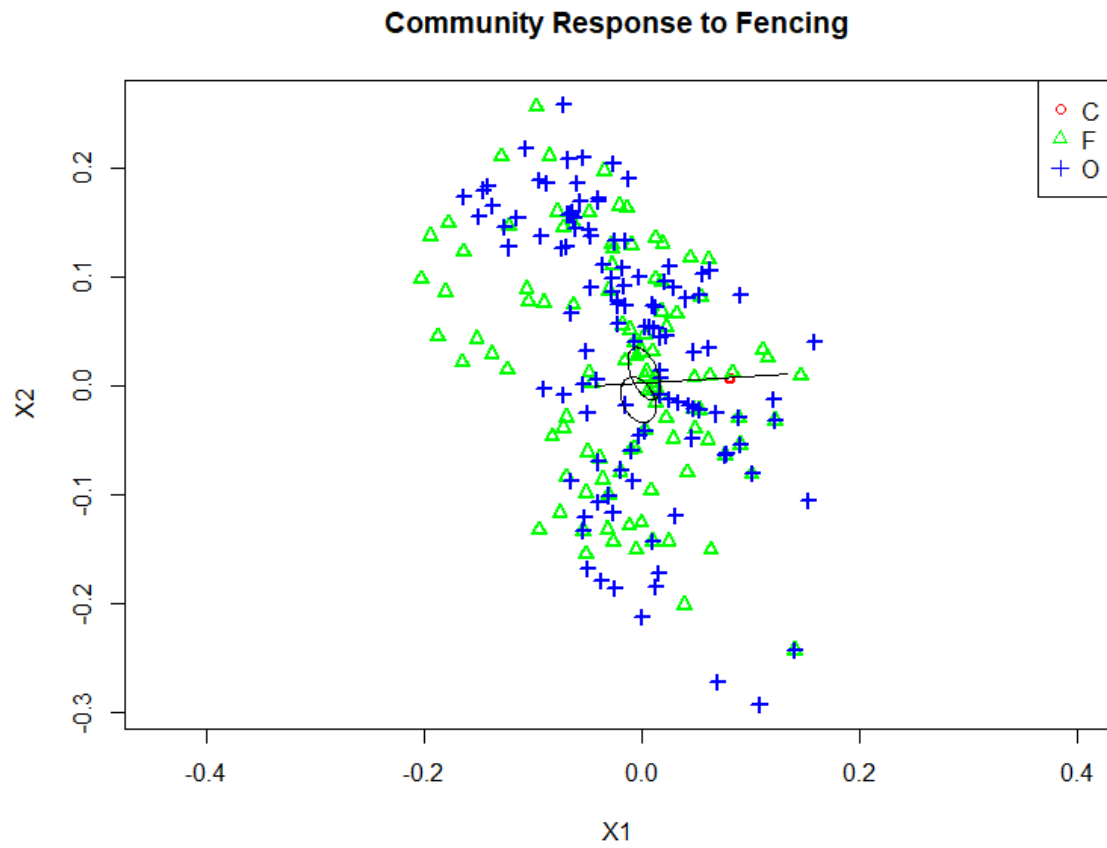
Figure 5: Clusters of different biomasses in two dimensions (x and y axis) to determine community response.

Community Response to Biomass

Figure 6: Representative data from Multiple Response Permutation Procedure Analysis performed after NMDS for the biomass data, indicating the different amounts and significance.
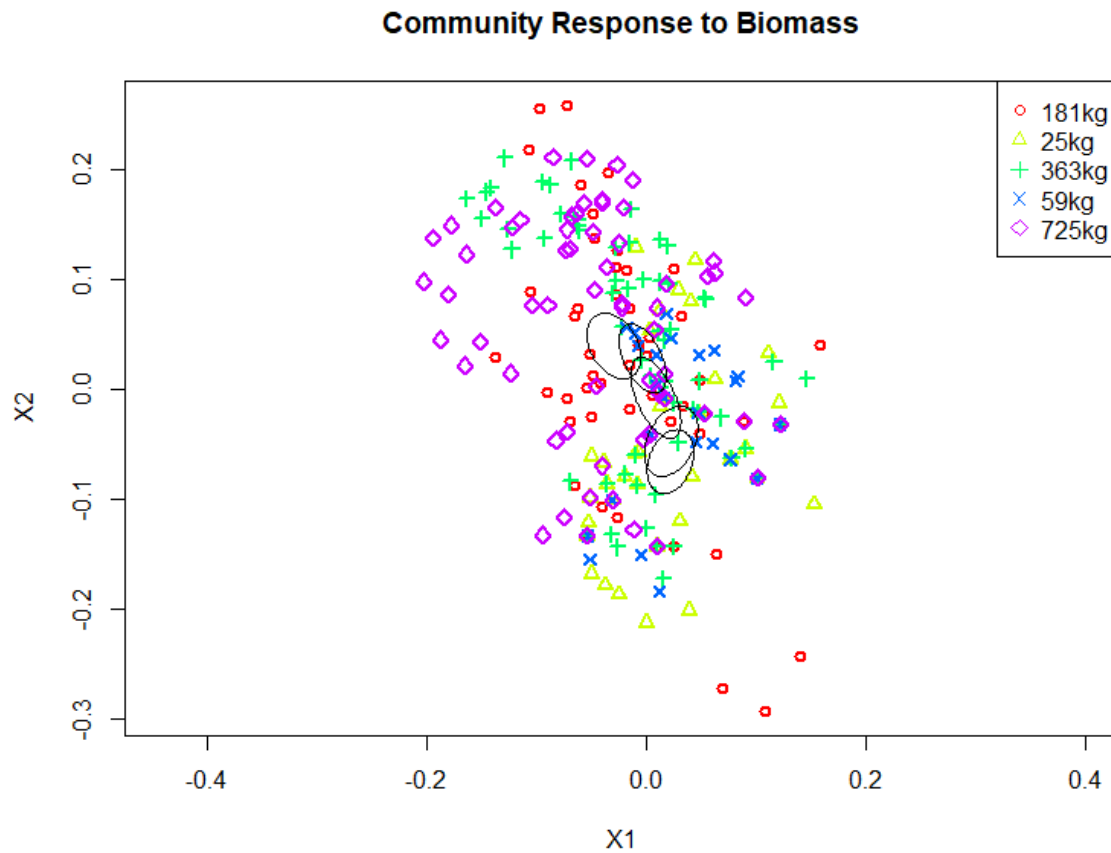
```
Class means and counts:

        ab      bc      cd     de      ef
delta 0.1628 0.1521 0.205 0.1982 0.1983
n       46      32     62     71      72
```

This shows how different or similar the biomass are with the Calliphoridae species present at each site. Zero or a number close to zero indicates the species in the biomass are similar. As noted, most of these biomasses all had similar, not very diverse species.

Figure 7: Clusters of different treatments in two dimensions (x and y axis) to determine community response.

Community Response to Treatment
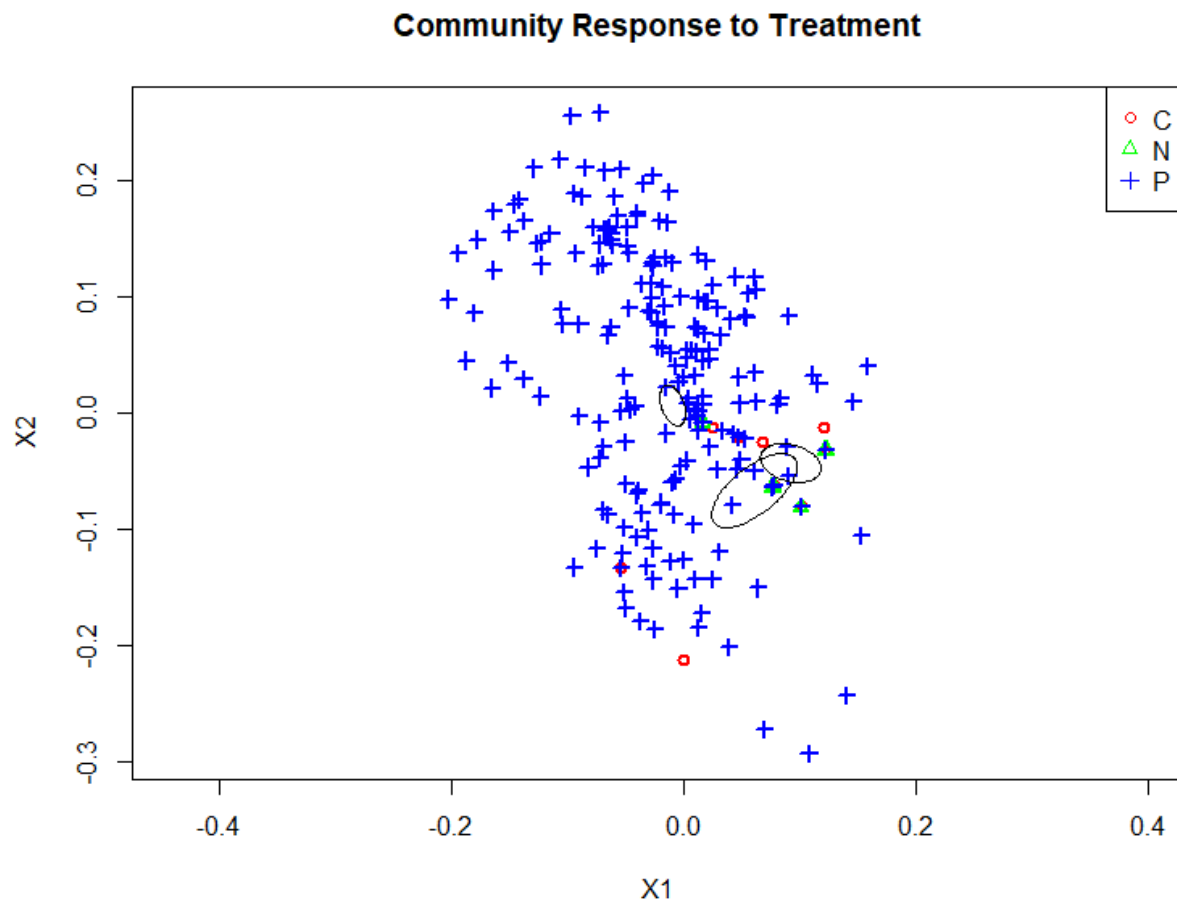
Figure 8: MRPP results indicating amount per treatment (N) and significance. The low numbers indicate similar species groups. C is the control group, N is nutrient additive, and P is the pig carrion.

```
        C       N       P
delta 0.1444  0.1091  0.198
n       18      13     252
```

NMDS, it appears that in most instances, the species were all relatively similar. This would provide data for genus level being an accurate taxonomic resolution.

Figure 9: Indicator species analysis, which shows what variables are important and used for determining significance (as noted in the p value) specifically for treatment types.

```
$indval
          C           F           O
lcoer 0.3424325 0.3371390 0.3204285
lcupr 0.3388025 0.3320492 0.3291483
lseri 0.3344466 0.3311069 0.3344466
pregi 0.3100332 0.3175633 0.3724035
cmace 0.1869707 0.3855809 0.4274484
lsp   0.3453396 0.3347396 0.3199208
csp   0.1869707 0.3855809 0.4274484
psp   0.3100332 0.3175633 0.3724035
call  0.3476361 0.3106890 0.3416749

$maxcls
lcoer lcupr lseri pregi cmace   lsp   csp   psp  call
    1     1     1     3     3     1     3     3     1

$indcls
     lcoer      lcupr      lseri      pregi      cmace       lsp
 0.3424325  0.3388025  0.3344466  0.3724035  0.4274484  0.3453396
       csp        psp       call
 0.4274484  0.3724035  0.3476361

$pval
lcoer lcupr lseri pregi cmace   lsp   csp   psp  call
0.971 1.000 1.000 0.527 0.119 0.954 0.113 0.508 0.893

$error
[1] 0

attr(,"class")
[1] "indval"
```

Indicator Species Analysis was run to determine what variables had a significant effect. All treatments were insignificant representatives of the community structure. However, the family level of Calliphoridae did have significant effects, suggesting that family represents community patterns best.

Figure 10: General Indicator species analysis, which shows what variables are important and used for determining significance (as noted in the p value).

```
$indcls
      lcoer       lcupr       lseri       pregi       cmace
 0.11354154  0.08518642  0.08362211  0.12757625  0.14702069
        lsp         csp         psp        call
 0.11666221  0.14702069  0.12757625  0.18614027

$pval
lcoer lcupr lseri pregi cmace   lsp   csp   psp  call
0.046 0.643 1.000 0.013 0.006 0.045 0.004 0.012 0.001
```
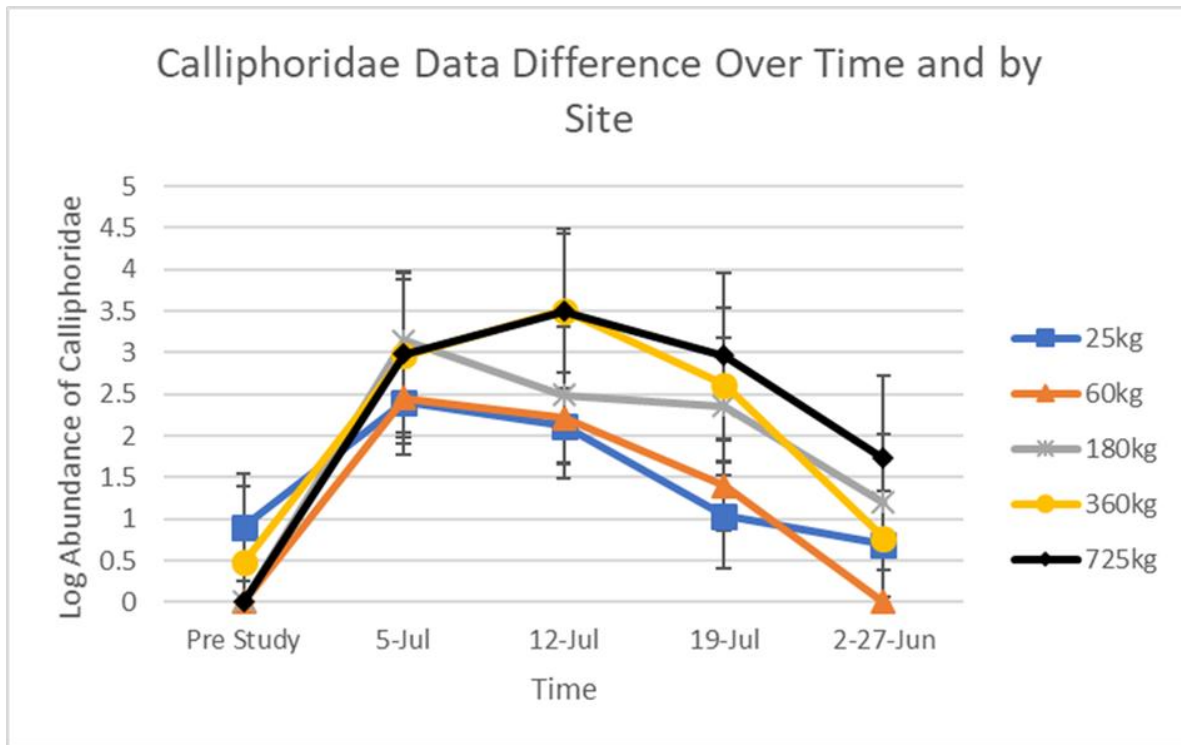
Abundance was clearly, as predicted, higher where pig carrion was located. There was some presence of Calliphoridae in the other two treatment areas, but this was not significant.

Figure 11: The change in abundance data over time per biomass. Each biomass is indicated by the line and shows the change over time.

The highest presence of Calliphoridae occurred within the first couple of days after the presence of carrion. The larger biomasses may have had such a high increase in Calliphoridae abundance that it may be generations for the population to stabilize. The populations are at such a carrying capacity (K) that time will be needed for the population to fall back under K. The last date recorded here was June 27th while the start date of pig carrion placement was July 5th.

CHAPTER IV

DISCUSSION

Family level appears to be the most significant taxonomic level and resolution. However, the significance may have been due to the testing only involving one family. Excluding family, genus was the most significant level. Multiple interaction effects could have influenced these results (figure 1). Overall, the Calliphoridae abundance followed the same general pattern for all biomasses with some abundance variation (figure 11).

Ecology involves studies of organisms and their interactions within the environment (Michaud et al. 2015). An important aspect of ecology is observing species richness. This can also play a role when observing forensic entomology and insect colonization (Byrd and Castner 2001). One such forensically important Diptera family is Calliphoridae, which often lays its eggs and consumes decomposing material (Byrd and Castner 2001). Mass mortality events (MME) have high amounts of carrion, which therefore may attract Calliphorids. These events occur when an abnormally large group of animals die in an area (Fey et al. 2015). The three most prevalent species of Calliphoridae to arrive to the created MME were *Lucilia coeruleiviridis, Phormia regina,* and *Cochliomyia macelleria.* It is possible taxonomic resolution can help understand more about the Diptera involved. Taxonomic resolution helps understand what taxonomic level will provide enough evidence for the given objective. In this case, genus and species taxonomic levels were analyzed.

PermANOVA was used to analyze and compare relationships of the treatment types to the resulting variables (Anderson et al. 2013). It is important to understand how interactions occur across different levels of analysis (Andersson et al. 2014). There were several interactions

27

noted between two factors including biomass and fencing, biomass and date, and date and treatment (figure 1). The two factors simultaneously drive the taxa abundance. In the first instance, the different biomasses may vary and/or be similar depending on the fencing type. In other words, both of these factors work together to drive abundance. The abundance may be driven by both due to the potential creation of microclimates (Perillo et al. 2015, Payne 1965). It is also notable that vertebrates that arrive to carrion may also affect insects (Turner et al. 2017). Some vertebrates may include large scavengers such as vultures, which were noted at the open scenes. It is possible that, with the varying biomass, these vertebrates were attracted to some biomasses over others. This would then correlate with vertebrates attempting to consume those carrion. Depending on the vertebrate, it is possible some vertebrates are attracted to large carrion. Fencing is often used to protect carrion (Payne 1965, Lopes de Carvalho and Linhares 2001) from vertebrate scavengers. There are fewer experiments observing decomposition and insects that do not include some fencing.

Another potential explanation is the unequal sample effort (Anderson 2001). Some traps from different locations were destroyed or damaged either by the weather or large animals. Therefore, some sites had more samples than others creating an unequal sampling size. PermANOVA can often be affected by unequal sampling and misinterpret significant relationships (Anderson 2001). One example can be seen in figure 8, where there is an inordinate amount pig carrion samples that contain Calliphoridae, but very few Calliphorids present within the other two treatments. Only traps that contained Calliphorids were sampled so as to not skew the results. Therefore, there were far more pig carrion treatments that had Calliphoridae samples than any other treatment type. If we were to repeat this experiment, it would be wise to have more samples. The lack of sample size is also because sites were not replicated. Each site was a

different amount of biomass and each area within those were individual as well. The sticky traps that contained the samples were noted as pseudo replicates (Okansen 2001, Hurlbert 1984). These issues may have caused the PermANOVA results to be concluded as inaccurate. The unequal sampling or too few samples could have skewed the PermANOVA results as each section has to have an equal amount of sample for the results to be accurate (Anderson 2001).

Date and treatment as well as date and biomass were two other interactions. Control and pig treatment plots vary widely in the soil nutrients. Control groups do not alter the soil from its initial composition as nothing is added to the pig carrion. Treatment groups with pig carrion would have altered the soil and environment due to the addition of nutrients (Barton et al. 2012). Therefore, the potential differences in the factors can both have a simultaneous effect on the taxa abundance.

As for the biomass and date, the carrion size may have had multiple effects. Smaller carrion and biomasses actual decompose faster, influencing the date and time (Payne 1965, Lane 1975). Biomass size can also affect the size and present Calliphoridae species on the carcass (Davies 2002). As a result, abundance of the different species is also affected (Davies 2002).

These two interactions can help explain the one three-way interaction noted in figure 1 as having partial significance. The three-way partial interaction was between date, biomass, and treatment. Unfortunately, there is no way to graph these three into a single graph; there is just a known interaction effect. There are too many factors and variables involved. There are many possibilities as to why a semi-significant interaction could have been noted. One example is if another animal dies nearby, the event could be affected in some way. More research needs to be done to determine if these factors are due to outside forces.

Each of the four treatment variables were tested using NMDS. While there may have been an abundance change, as noted in figures 3, there was no noted dissimilarity of species or genera within the family. The same results occurred when observing the community response to fencing in figure 4. There are two possibilities for this inconsistency. One may be that, as previously noted, the PermANOVA is incorrect due to lack of equal samples. Another possibility is that there may be a correlation, but there are too many complicated or overlapping clusters in the ordination plot. For the most part, the NMDS plots were biologically irrelevant.

However, biomass did have dissimilarity results and clusters. Multiple Response Permutation Procedure (MRPP) could be used for this analysis. Based on the low p values, the biomasses all had a relatively similar species richness. Species richness indicates the variety of species within an area. As for species evenness, the appropriation of species among the different sites is similar (Smith and Wilson 1996). The low p values also indicate that species dissimilarity is low, referring back to species evenness. Species evenness is the appropriation of different species across the community (Smith and Wilson 1996). Species are therefore similar across biomass sites. The diversity of species and genera is low in many areas, depending on the season and weather (Matuszewski et al. 2008, Lopes de Carvahlo and Linhares 2001). Therefore, it is possible for this NMDS plot to hold some accuracy.

Treatment studies with NMDS and MRPP also gave related results. Across each treatment, there were similar results like the biomass analysis. Species were relatively similar across all the treatments. In terms of abundance, there were more Calliphoridae present in the pig carrion treatments, which may have affected the results. The Calliphorids in the nutrient additive and control regions were far fewer than the pig carrion. Even so, according to figure 10, species and genus have similar p values in direct relation to each other. This may be because some

30

genera only had one species such as the Cochliomyia genera only containing the species *Cochliomyia macelleria.* Even so, this plot was difficult to interpret.

The final R programming analysis was performed with Indicator Species Analysis (ISA). As stated, the analysis was done using all four treatment types including date, biomass, treatment, and fencing. However, all of these had relatively similar results. Indicator species analysis observes the different variables, and which one has the highest and most important effect (Hill 1975). In the case of comparing across genus and species for dates, genus had the lowest p value overall (except for Calliphoridae), and therefore was the most significant. Calliphoridae was statistically significant (p=0.001), indicating that family best explains changes within the community. Some approached significant results for date. However, there were almost no significant results across the treatments for any of the groups, as seen in figure 9. The genus, however, was still the lowest when observing treatments, followed by family and then species. Once again, the lack of Calliphoridae evidence in general from the nutrient additive and control areas may have caused these results. The uneven trap amounts also most likely affected why none of the groups were significant. Therefore, there is also a better estimate of different taxa significance.

Family was not one of the taxonomic resolution levels that was being tested in this experiment. While it may have had the most significant effect on taxa abundance as according to the ISA, all the data was under one family, making this result expected. In most papers (Marshall et al. 2006, Fjeldsa 2000, LaFerla et al. 2002, Konar and Iken 2009), a large community of multiple families are observed. Family level identifications have often been used for larger and more stable communities (Marshall et al. 2006). However, other papers observing specific communities such as necrophagous communities can still require a family level taxonomic

31

resolution (Chin 2016). It would be worthwhile for future studies to look at other Diptera families. It would also be useful to observe solely forensic important Dipteran families, or all Dipteran associated with mass mortality events. Family level could then be analyzed to determine if identifying down to genus is not necessary.

Since family level provided significance, it was used to create an abundance graph (figure 11). A general trend can be observed from all five biomasses present. This trend is also seen in normal insect succession where carrion insects follow a normal type pattern (Benbow et al. 2016). Even if biomass may alter the taxa and abundance of Calliphoridae, all the sites still follow the basic trend of necrophagous insect succession.

Referring to the objectives, taxonomic resolution will provide the most detail and how this will be affected by the different variables and differences. Unfortunately, the PermANOVA was not fully accurate in showing what treatment groups had significance, however, we were able to determine that this data did not have stress amounts that were too high to analyze. Three dimensions is a good point to focus on as it can be analyzed.

These results can indicate a few things for taxonomic resolution and mass mortality events. None of the plots created from NMDS were clean or clear. Therefore, it was difficult to determine how the treatment variables clustered together dimensionally. There was no distinct line of the difference between the different created groups of variables. These plots may have been partly affected by the inaccuracies seen in the PermANOVA. However, there may still be several reasons that biomass and treatment provided results. It could be an inaccuracy, or it could be that these two treatments best clustered resulting variables. Treatment had an effect on the Calliphoridae abundance as there were clearly more Calliphorids at the pig carrion site versus the others. Calliphoridae abundance was also influenced by biomass as the more carrion, the more

Calliphorids (Pechal et al. 2014). It was difficult to discern whether or not there was a significant difference between the different biomasses and their abundances (figure 11) as indicated by the large error bars. Overall, the larger biomasses did have a higher abundance of Calliphoridae, as to be expected.

Species Indicator Analysis shows that either genus or even family would be the best taxonomic resolution level. Family level was not being tested in this instance, however, as noted in the ISA for date, family level was significant. However, it may be because there was only one family tested so it would have significance. Even so, it is possible to look at family, but there are also limitations. In this instance, it depends on the question at hand. As previously mentioned, biodiversity, nature conservation, and monitoring invasive species all need species level taxonomic resolution (Fjeldsa 2000; LaFerla et al. 2002, Konar and Iken 2009).

The reasoning behind the hypothesis was that mass mortality events may be similar to monitoring invasive species. Initially, it was believed that this work would have similar results to those when monitoring disturbance events (Chin 2016). However, as can be analyzed from the multiple amounts of statistical and abundance data, genus level appears to be a normal taxonomic resolution identification. Implications for this research could help in future research studies involving mass mortality events. While, in many instances, Calliphoridae must be identified down to species for death investigations (Byrd and Castner 2001), the taxonomic resolution information from this work can be especially useful for future studies. Identify down to genus helps save time, energy, and money that is required for identifications.

Mass mortality events have become of great interest recently as noted in journals such as the National Geographic. There is currently little known about why mass mortality events occur or what the effects of mass mortality events are (Fey et al. 2015). The implications of this work

could help better understand mass mortality events and how to analyze them. Numerous studies that do not contain work with PMI or TOC could benefit from the results presented.

However, there are also ways to improve the work. As previously stated, limitations could be the cause of a lack of replications with different biomasses. The study design could be improved in other ways as well. For example, more samples could be placed to further the accuracy of the work.

Further limitations could include the scope of taxonomic resolution. Only the Calliphoridae family was observed for necrophagous effects. Improvements could be made by extending the field of scope to all the other necrophagous insects including the families Muscidae, Sarcophagidae and others (Byrd and Castner 2001). It would also be beneficial to extend future studies to include all families under the order Diptera, not just necrophagous insects. More beneficial information and a wider scale of samples may provide more information.

Future work could also focus on other insects present in mass mortality events that may otherwise be absent. Even the field of forensically significant insects could be expanded upon. Other work could be expanded upon from information in the research. For example, as previously noted, fencing may have a simultaneous effect with biomass on taxa abundance. It would be an interesting point to observe fencing and what effects, if any, invertebrate and vertebrate competition have (Beasley et al. 2012). This knowledge is unclear from this research alone. There are many ways to further the research ideas and knowledge that has come from this experiment.

Overall, it is important to understand that genus is the most significant and beneficial taxonomic resolution level to identify Calliphorids for mass mortality events (MME). Money,

time, and resources can be saved by only identifying down to the genus level. Future work with mass mortality events could benefit from the correct identification with taxonomic resolution. However, in the future work could be improved with more samples and replicates. Research could also be expanded by identifying more insects involved in mass mortality events. In the case of these necrophagous insects, genus provides the appropriate level of information.

CHAPTER V

CONCLUSION

Overall, the species and genus diversity across the different biomasses, dates, fencing types, and treatments were all relatively similar. There was only a total of three different genera and five different species. *Lucilia cuprina* and *Lucilia sericata,* however, were not very common. In most instances, there was one species per each genera. This made the species diversity and richness very low within the family level.

Statistical analysis with R further provided information. There were also a few other treatments that related significantly with effects on each other. However, this was noted as a potential error, although there are some ways they could relate to each other. NMDS plots, where the biomass and treatment groups had significant clusters of Calliphoridae abundance. As for the Indicator Species Analysis, the family and genus levels were noted as the most significant.

Future experiments with mass mortality events can benefit from the information that resulted from this research. Rangers investigating poaching but who do not have access to professional entomologists could also benefit and use these results. There are many uses for using the correct taxonomic resolution. In conclusion, when comparing the statistics and abundance, genus level out of species and genus appear to be the best for taxonomic resolution when it comes to analyzing mass mortality events.

REFERENCES

Adriana Novais, C. P., Ronaldo Sousa 2017. Effects of invasive aquatic carrion on soil chemistry and terrestrial microbial communities. *Biol. Invasions* 19(8): 2491-2502.

Anderson, M. J. 2001. A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* 26: 32–46.

Anderson, M. J. and D. C. I. Walsh 2013. PERMANOVA, ANOSIM, and the Mantel test in the face of heterogeneous dispersions: What null hypothesis are you testing? *Ecol. Monogr.* 83(4): 557-574.

Andersson, U., A. Cuervo-Cazurra, B. B. Nielsen. 2014. From the Editors: Explaining interaction effects within and across levels of analysis. *J. Int. Bus. Stud.* 45(9): 1063-1071.

Barton, P. S., S. A. Cunningham, D. B. Lindenmayer, and A. D. Manning. 2013. The role of carrion in maintaining biodiversity and ecological processes in terrestrial ecosystems. *Oecologia* 171(4): 761-772.

Beasley, J. C., Z. H. Olson, and T. L. Devault. 2012. Carrion cycling in food webs: comparisons among terrestrial and marine ecosystems. *Okios* 121(7): 1021-1026.

Benbow, M.E., J.K. Tomberlin, and A.M. Tarone. 2016. Introduction to Carrion Ecology, Evolution, and Their Applications. In: *Carrion Ecology, Evolution and Their Applications.* Benbow, M.E., J.K. Tomberlin, and A.M. Tarone (eds.) pp. 3-11. Boca Raton, CRC Press.

Bowman, M., and R. Bailey. 1997. Does taxonomic resolution affect the multivariate description of the structure of freshwater benthic macroinvertebrate communities? *Can J. Fisheries Aq. Sci.* 54: 1802–1807.

Bray J. R. & Curtis J. T. 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.* 27: 325 – 49.

Byrd, J. H., and Castner, J.L. 2001. Full Book. Forensic Entomology: The Utility of Arthropods in Legal Investigations. CRC Press, Boca Raton, FL.

Cain M. L., Bowman W. D., Hacker S. D. 2008. Ecology, Sunderland (Massachusetts): Sinauer Associates.

Chin, H.C. 2016. Sensitivity of Arthropod and Microbial Communities Associated with Vertebrate Carrion in Response to Delayed Blow Fly Access: Implication for Carrion Ecology and Forensic Entomology. Doctoral dissertation, Texas A & M University. (Thesis – not yet peer reviewed)

Davies, L. 2002. Seasonal and spatial changes in blowfly production from small and large carcasses at Durham in lowland northeast England. *Med. Vet. Entomol.* 13(3): 245-251.

Elton, C.S. 1927. Animal Ecology. Reprint 2001. Chicago, IL, University of Chicago Press.

Fey, S. B., A. M. Siepielskic, S. Nussled, K. Cervantes-Yoshidad, J. L. Hwand, E. Huberd, M. J. Fey, A. Catenazzie, and S. M. Carlsond. 2015. Recent shifts in the occurrence, cause, and magnitude of animal mass mortality events. *PNAS*. 112: 1083-1088.

Fjeldsa, J. 2000. The relevance of systematics in choosing priority areas for global conservation. *Environ. Conserv.* 27(1): 67-75.

Gauch, H. G. 1973. The Relationship Between Sample Similarity and Ecological Distance. *Ecol.* 54(3): 618-622.

Goddard, J. and P. K. Lago. 1983. An annotated list of the Calliphoridae of Mississippi. *J. Ga. Entomol. Soc.* 18: 481-484.

Hill, M. O., R. G. H. Bunce, and M. W. Shaw. 1975. Indicator Species Analysis, A Divisive Polythetic Method of Classification, and its Application to a Survey of Native Pinewoods in Scotland. *J. Ecol.* 63(2): 597-613.

Hirst, A. J. 2006. Influence of taxonomic resolution on multivariate analyses of arthropod and macroalgal reef assemblages. *Mar. Ecol. Prog. Ser.* 324: 83-93.

Hurlbert, S. H. 1984. Pseudoreplication and the Design of Ecological Field Experiments. *Ecol. Monogr.* 54(2): 187-211.

Hutchinson, G. E. 1959. Homage to Santa Rosalia or Why Are There So Many Kinds of Animals? *Am. Nat.* 93(870): 145-159.

Kenkel, N. C., and L. Orlóci 1986. Applying Metric and Nonmetric Multidimensional Scaling to Ecological Studies: Some New Results. *Ecol.* 67(4): 919-928.

Konar, B. and K. Iken 2009. Influence of taxonomic resolution and morphological functional groups in multivariate analyses of macroalgal assemblages. *Phycol.* 48(1): 24-31.

La Ferla, B., J. Taplin, D. Ockwell, and J. C. Lovett. 2002. Continental scale patterns of biodiversity: can higher taxa accurately predict African plant distributions*? Bot. J. Linn. Soc.* 138(2): 225-235.

Lane, R. P. 1975. An investigation into Blowfly (Diptera: Calliphoridae) succession on corpses. *J. Nat. Hist.,* 9: 581-588.

Lopes de Carvalho L.M., and A. X. Linhares. 2001. Seasonality of Insect Succession and Pig Carcass Decomposition in a Natural Forest Area in Southeastern Brazil. *J. Forensic Sci.* 46(3):604–608.

Magurran, Anne E. 1988. Ecological Diversity and Its Measurement, Springer Netherlands.

Markandya, A., T. Taylor, A. Longo, M. N. Murty, S. Murty, and K. Dhavala. 2008. Counting the cost of vulture decline—An appraisal of the human health and other benefits of vultures in India. *Ecol. Econ.* 67(2): 194-204.

Marshall, J. C., A. L. Steward, and B. D. Harch. 2006. Taxonomic Resolution and Quantification of Freshwater Macroinvertebrate Samples from an Australian Dryland River: The Benefits and Costs of Using Species Abundance Data. *Hydrobiol.* 572: 171–194.

Matuszewski, S., D. Bajerlein, S. Konwerski, and K. Szpila. 2008. An initial study of insect succession and carrion decomposition in various forest habitats of Central Europe. *Forensic Sci. Int.* 180(2-3): 61-69.

Michaud, J.-P., K. G. Schoenly, and G. Moreau. 2015. Rewriting ecological succession history: did carrion ecologists get there first? *Q Rev Biol.* 90: 45–66.

Mittelbach, G. G. 2012. Community Ecology, Oxford University Press.

Oksanen, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudo-issue? *Oikos* 94: 27–38.

Olsgard, F., P. Somerfield, and M. Carr. 1998. Relationships between taxonomic resolution, macrobenthic community patterns and disturbance. *Mar. Ecol. Prog. Ser.* 172: 25–36.

Pain, D., Cunningham, A., Donald, P., Duckworth, J., Houston, D., Katzner, T., Timmins, R. 2003. Causes and Effects of Temporospatial Declines of Gyps Vultures in Asia. *Conserv. Biol.*, 17(3), 661-671.

Payne, J. A. 1965. A summer study of the baby pig Sus scrofa Linnaeus. *Ecol.,* 46.

Pechal, J. L., M. E. Benbow, T. L. Crippen, A. M. Tarone, and J. K. Tomberlin 2014. Delayed insect access alters carrion decomposition and necrophagous insect community assembly. *Ecosphere* 5(4): 1-21.

Perillo, A. C., C. J. Kucharik, T. D. Meehan, S. P. Serbin, A. Singh, P. A. Townsend, K. S. Whitney, and C. Gratton. 2015. Use of insect exclusion cages in soybean creates an altered microclimate and differential crop response. *Agri. Forest Meteorol.* 208: 50-61.

Simon, M. F., Grether, R., de Queiroz, L. P., Skema, C., Pennington, R. T., & Hughes, C. E. 2009. Recent assembly of the Cerrado, a neotropical plant diversity hotspot, by in situ evolution of adaptations to fire. *Proc. Natl. Acad. Sci. U.S.A.*, 106(48), 20359–20364.

Smith B., J. B. Wilson. 1996. A consumer's guide to evenness indices. *Oikos* 76: 70–82.

Tansley, A. G. 1935. The use and abuse of vegetational concepts and terms. *Ecol.* 16: 284–307.

Tomberlin, J. K., B. T. Barton, M. A. Lashley, and H. R. Jordan. 2017. Mass mortality events and the role of necrophagous invertebrates. *Curr. Op. Ins. Sci.* 23: 7–12.

Tomberlin, J. K., M. E. Benbow, A. M. Tarone, and R. M. Mohr. 2011. Basic research in evolution and ecology enhances forensics. *Trends Ecol. Evol*. 26: 53–55.

Tomberlin, J., R. Mohr, M. Benbow, A. Tarone, and S. Vanlaerhoven. 2011. A Roadmap for Bridging Basic and Applied Research in Forensic Entomology. *Ann. Rev. Entomol.* 56: 401–421.

Turner, K. L., E. F. Abernethy, L. M. Conner, O. E. Rhodes Jr., and J. C. Beasley. 2017. Abiotic and biotic factors modulate carrion fate and vertebrate scavenging communities. *Ecol.* 98(9): 2413-2424.

Waite, I. R., A. T. Herlihy, D. P. Larsen, N. S. Urquhart, and D. J. Klemm. 2004. The effects of macroinvertebrate taxonomic resolution in large landscape bioassessments: an example from the Mid-Atlantic Highlands, U.S.A. *Freshwater Biol.* 49: 474–489.

Zak, Donald R 2014. Ecosystem Succession and Nutrient Retention: Vitousek and Reiners' Hypothesis. *Bull. of Ecolog. Soc. of Am.* 95(3): 234-237.

APPENDIX

Figure 1: The stress data showing the number of dimensions for the NMDS data sets.