2015

The effect of rainfall on blowfly (Calliphoridae) activity and decomposition on recently deposited animal remains

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

THE EFFECT OF RAINFALL ON BLOWFLY (CALLIPHORIDAE) ACTIVITY AND DECOMPOSITION ON RECENTLY DEPOSITED ANIMAL REMAINS

By

ELIZABETH VAN HOVEN MCLEOD

B.A., University of Vermont, 2012

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2015

Approved by

First Reader ____

James T. Pokines, Ph.D., D-ABFA
Assistant Professor of Anatomy and Neurobiology
Program in Forensic Anthropology

Second Reader ____

Peter Cummings, M.Sc., M.D.
Forensic Pathologist and Staff Medical Examiner
Massachusetts Office of the Chief Medical Examiner

Third Reader ____

Ian Dadour, Ph.D.
Director of the Centre for Forensic Sciences
University of Western Australia

DEDICATION

This research is dedicated to my husband, Ryan McLeod, to my family, Richard Lednicky, Andrea Van Hoven, and Samantha Lednicky, and to Jimmy K. My husband and my family have shown the overwhelming emotional, financial, and spiritual support that has made this exciting journey possible. Without the work of Jimmy K., I would not be here to have these opportunities. Thank you.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my major professor, Dr. James Pokines, for his constant presence and input while deep in research and writing. He has always been available for my endless emails, questions, edits, and curiosities. Thank you for sharing your unique interest in all things relating to death and taphonomy.

I would also like to thank my second reader, Dr. Peter Cummings, for helping me remember the end game. He motivates me to follow my dreams of becoming a forensic pathologist. I must also thank my third reader, Dr. Ian Dadour, and his colleague Dr. Paola Magni, for their expertise in forensic entomology. I appreciate that they shared their vast knowledge of the insects that visit the dead and aided in insect identifications. I never knew how fascinating a simple fly could be.

I am also grateful for the support of my friends and family who have put up with my stressful demeanor while I not only completed my research and writing for this thesis, but also became a wife and made major decisions about my future. Their faith in my abilities and constant love has provided me with the strength and drive to push forward in the face of adversity. Because of this, I am dedicated to pursuing my dreams, whatever they may turn out to be.

THE EFFECT OF RAINFALL ON BLOWFLY (CALLIPHORIDAE) ACTIVITY AND DECOMPOSITION ON RECENTLY DEPOSITED ANIMAL REMAINS

ELIZABETH VAN HOVEN MCLEOD

ABSTRACT

The accurate estimation of the postmortem interval (PMI) is one of the most important determinations in a forensic investigation of decomposing human remains. Forensic entomology has gained popularity in death investigation due to its reliability and precision in the estimation of the minimum postmortem interval (mPMI). Forensically significant insects are mainly necrophagous species, which feed only on decomposing animal matter, and the most common necrophagous insects are the blowflies (Diptera: Calliphoridae). Estimations of the mPMI by entomological methods are made using the known developmental rates of various species of blowfly and via the successional patterns of the carrion insect community in a given region. It is generally assumed that blowflies oviposit quickly after death, so in many cases this time may equate to the time since death. The precision of mPMI estimations based on the developmental rates of blowflies often relies on this assumption.

Rainfall may effect decomposition by inhibiting access of insects to the cadaver or carcass for oviposition. The current study investigated the effects of rainfall on blowfly activity, behavior, and overall decomposition of decaying animal material in an outdoor environment in the northeastern United States, conducted at the Boston University Outdoor Research Facility (ORF). It was hypothesized that natural rainfall, typically light to moderate in the geographic area of study, will disturb initial blowfly activity by acting as a physical barrier, diminishing access to the remains, and creating a delay in colonization and subsequent larval development. This hypothesized delay would result in an underestimation of the mPMI by entomological methods when rainfall has occurred. Also examined were several questions about the nocturnal behavior of blowflies and their activity in heavy rain.

In the experimental trial 12 pig (*Sus scrofa*) heads were exposed under normal conditions (N; no rain controls), and 15 pig heads were exposed under rainy conditions (R; rain treatment), split into uncovered (N, n=6; R, n=5), covered (NC, n=5; RC, n=5), and covered partially (RCP, n=5) treatments. Additionally, there were three pig heads used in a preliminary trial and three pig heads exposed in an active rain trial. Generally, the results show that while a negative correlation exists between the amount of rainfall experienced and the coded number of flies observed, the light to moderate rainfall typical of many rainy days in the northeastern Unites States will not totally inhibit blowfly activity or disturb established maggot masses. A one-way analysis of variance (ANOVA) determined that there was no statistically significant difference ($p > 0.05$) between the N, NC, R, RC, and RCP treatments in the number of days it took to reach the advanced decomposition stage. While constant, heavy rainfall may inhibit blowfly activity; the results suggest that the irregularity of natural rainfall would rarely produce the conditions necessary for this to make a significant impact estimation of the PMI by entomological methods, although further studies are needed to confirm this conclusion.

The results show a positive correlation between solar radiation and the coded number of flies observed. Time of day as a function of the coded number of flies observed during the first 48 hours of exposure forms a bimodal bell curve, confirming that blowflies are diurnal in their natural environment. Additionally, evidence of scavenging by turkey vulture (*Cathartes aura*) and some unknown animal(s) was observed. The results of this study illustrate the complicated, multivariable nature of the process of decomposition. This study provides preliminary data on the effect of rainfall on blowfly activity and overall decomposition, while future studies will be required to determine the effects of the duration and the intensity of rainfall.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADDs .... Accumulated Degree Days

ANOVA .... Analysis of Variance

ARF .... Anthropological Research Facility, Knoxville, TN

DEET .... N,N-Diethyl-meta-toluamide

FDA .... Food and Drug Administration

mPMI .... Minimum Postmortem Interval

ORF .... Outdoor Research Facility, Holliston, MA

PMI .... Postmortem Interval

TBS .... Total Body Score

CHAPTER 1: INTRODUCTION

The accurate estimation of the postmortem interval (PMI) is one of the most important determinations for effective forensic investigation of decomposing human remains (Mann *et al.* 1990; Micozzi 1991; Rodriguez and Bass 1983; Vass *et al.* 1994; Voss *et al.* 2011). The accurate estimation of the PMI is valuable because it can narrow down the possible pool of decedents by reducing the number of missing persons from a particular time period, exclude possible assailants from that time period, and corroborate witness testimony (Megyesi *et al.* 2005). Forensic analysis of the PMI generally concerns shorter time frames than other scientific disciplines that also deal with human remains, such as bioarchaeology or paleontology. The forensic anthropologist or forensic pathologist is usually tasked with determining the PMI on the scale of hours to days, in the case of recently deceased individuals, or to decades, in the case of war crimes (Pokines 2014).

Under generalized conditions, a dead body will proceed through a series of initial soft tissues changes, loss of the soft tissues, and dispersal of the remaining hard tissues during the process of decomposition (Mann *et al.* 1990; Micozzi 1991; Pokines 2014). As the time interval from death to discovery and analysis increases, the number of methods that can provide precise estimations of the PMI decrease due to these above changes. This is in part due to an increase in the number of variables that can affect the decomposition rate as the time period that the body is exposed to the environment increases. These environmental variables can work to increase or decrease decompositional change, and include, but are not limited to, temperature, access to the body by insects, burial and depth of burial, indoor/outdoor environment, access to the body by carnivores/rodents, trauma, humidity/aridity, other weather variables, body size and mass, embalming, presence of clothing, type of surface the body is placed on, and soil pH (Mann *et al.* 1990).

Traditionally, forensic pathologists use gross observations of the soft tissues, such as rigor mortis or livor mortis, to estimate the PMI. However, these are highly subjective, qualitative processes that rely on the experience of the observer, can be confounded by the environment type in which a body is exposed, and are limited to the first 72 hours after death (Amendt *et al.* 2011; Megyesi *et al.* 2005). Other taphonomic indicators are useful once the remains are fully skeletonized and include subaerial weathering, plant growth, soil staining, and cortical erosion. However, these indicators generally give insight into the latter end of the forensically significant PMI, because these processes can take years or even decades to alter osseous remains, and thus, become available for analysis (Behrensmeyer 1978; Gordon and Buikstra 1981; Pokines 2014; White and Hannus 1983).

Forensic Entomology

Forensic entomology is often utilized in death investigation due to its reliability and precision in the estimation of the minimum PMI (mPMI) for time periods of hours to months since death (known as "the gold standard"; Marks *et al.* 2009). The mPMI is the shortest amount of time that a body could have been exposed and result in the degree of insect colonization that is observed (Amendt *et al.* 2011; Baqué and Amendt 2013; Mahat *et al.* 2009). Through using the known timing of colonization, developmental rates, and biology of various species of insects associated with decaying materials, accurate, mPMI estimates can be made (Catts and Haskell 1990; Gaudry and Dourel 2013; Magni *et al.* 2013). In this way the amount of time that has elapsed since initial colonization of the remains can be deduced. This information is then used to gauge the window of time in which the death must have occurred and an estimate of the precolonization time.

Forensically significant insects are the necrophagous, or carrion, species, which feed only on decomposing animal matter (Campobasso *et al.* 2001). Estimations of the mPMI by entomological methods are generally made in two ways. For the first method, the developmental stage of the oldest immature carrion insects found in association with the remains is determined and calculated into real time using the known developmental rates and temperature data. The second method is performed by reviewing the successional patterns of the carrion insect community for a given region and determining the time period when a certain insects are observed (Baqué and Amendt 2013; Michaud and Moreau 2009; Voss *et al.* 2011). The mPMI estimation developed using the developmental stage of immature carrion insects are most useful in the earlier stages of decomposition (days to weeks), while carrion insect succession patterns are useful in the more advanced stages of decomposition (weeks to months) (Baqué and Amendt 2013).

Researchers have described the succession of carrion insects in various regions and their importance in cadaver decomposition in great detail (Archer 2003a, 2003b; Carvalho *et al.* 2000; Catts and Haskell 1990; Centeno *et al.* 2002; Michaud and Moreau 2009, 2011; Tabor *et al.* 2004; Voss *et al.* 2011). Because each taxon of necrophagous insect follows a predictable pattern of colonization and development that is temperature dependent (Amendt *et al.* 2007), they are commonly used to estimate the mPMI in conjunction with weather and temperature data. The sequence of insect colonization is also related to the decomposition stage of the cadaver. This has been researched in different geographic areas and climate types, and under different extrinsic conditions (Anderson 2011; Campobasso *et al.* 2001; Carvalho *et al.* 2007; Centeno *et al.* 2002; Richards and Goff 1997; Voss *et al.* 2008, 2011).

Diptera (true flies) are the predominant necrophagous Order of insects and are found at almost all decomposition events due to their inherent mobility of flight (Voss *et al.* 2011; Yang and Shiao 2012). In carrion insect succession patterns, Diptera are the most widely represented Order, followed by Coleoptera (beetles) (Campobasso *et al.* 2001; Centeno *et al.* 2002; Magni *et al.* 2013; Reibe and Madea 2010; Yang and Shiao 2012; Zanetti *et al.* 2014). Specifically, blowflies (Diptera: Calliphoridae) and their larvae have the greatest effect on the decomposition of cadavers because of their ability to rapidly reduce the soft tissues (Anderson 2011; Archer 2004; Catts and Haskell 1990; Shelomi *et al.* 2012). Blowflies have been observed colonizing a body outdoors almost immediately after exposure, within minutes after death (Amendt *et al.* 2011; Mahat *et al.* 2009). They prefer natural orifices for oviposition sites such as the eyes, nose, mouth, and ears, and in the latter end of the fresh stage of decomposition, the genitalia (Cross and Simmons 2010; Rodriguez and Bass 1983).

It is generally assumed that blowflies oviposit so quickly after death that time since oviposition is the equivalent of the time since death. This assumption provides the theoretical basis of entomological estimations of the mPMI (Magni *et al.* 2013). However, the actual time since death could differ from the time since oviposition that is calculated by mPMI estimations for a number of reasons (Tomberlin *et al.* 2011).

Since the accuracy of the entomologically estimated PMI relies on these baseline assumptions, this potential time gap has prompted research investigating the factors that could affect blowfly oviposition, and thus the overall decomposition of a cadaver or nonhuman carcass (Baldridge *et al.* 2006; Voss *et al.* 2011).

Oviposition

The ability to oviposit is dependent on access to the decomposing tissue by an adult female blowfly. Access to the cadaver or carcass can be restricted physically, environmentally, or chemically, and due to the environmental impact at deposition (Archer 2004; Goff 1992; Higgs and Pokines 2014; Magni *et al.* 2013; Mahat *et al.* 2009; Mann *et al.* 1990; Shelomi *et al.* 2012). Goff (1992) reported on the discovery of a corpse that had been tightly wrapped in two blankets for disposal by the perpetrator. A two and a half day delay in oviposition was observed due to the blankets physically reducing access to the body. Mann *et al.* (1990) similarly found that cadavers buried more than 1-2 feet below the surface of the ground decomposed slower than their surface-deposited counterparts. While it was suggested this was partially due to the decreased temperatures underground, this was also associated to a reduction in the ability to access the corpse by necrophagous insects for oviposition and subsequent larval colonization. In the same way, aquatic environments show a decrease in decomposition rates due to the lower numbers of aquatic necrophagous fauna when compared to the terrestrial necrophagous fauna that are available to advance the processes of decomposition (Higgs and Pokines 2014; Magni *et al.* 2013). The presence of rainfall, bright sunlight, and toxic substances have also been shown to influence initial oviposition by "restricting" access to the remains (Archer 2004; Mahat *et al.* 2009; Shelomi *et al.* 2012).

Rainfall

The effect of rainfall on decomposition, specifically as an inhibitor to insects accessing a cadaver or carcass for oviposition, has received little attention (Archer 2004; Mahat *et al.* 2009; Reibe and Madea 2010). When rainfall has been discussed, most studies have made anecdotal statements about the effect of the rain without providing any experimentally derived data. For example, Rodriguez and Bass (1983) stated that moderate to heavy rainfall does not affect larval activity once the maggot mass is established but will inhibit initial oviposition, without providing experimental data that substantiates these statements. Some have noted that rainfall occurred during their field studies but made no other statements about the effect of the rainfall on their research (Anderson 2011; Centeno *et al.* 2002; Tabor *et al.* 2004). The few who have directly noted the effect of rainfall report contradictory results.

Reibe and Madea (2010) observed that adult blowfly activity was inhibited during heavy rainfall, while Archer's (2004) study suggested that rainfall actually increases the decomposition rate by aiding in removal of the decomposing tissues.

Due to the paucity of information regarding the impact of rainfall on decomposition, the current study investigated the effect of rainfall on blowfly activity, behavior, and overall decomposition of decaying animal material in an outdoor environment in the northeastern United States. It was hypothesized that natural rainfall will disturb initial blowfly activity by acting as a type of physical barrier, diminishing access to the decomposing remains and creating a delay in colonization and subsequent development. This hypothesized delay could result in an underestimation of the mPMI by entomological methods. The presence or absence of rainfall during the first two days of exposure, and the presence or absence of a cover to prevent the direct mechanical effect of rainfall and other weather variables were examined. However, it is unclear if this delay will affect the overall decomposition rate as assessed by the Total Body Score (TBS; Megyesi *et al.* 2005). Additional observations are reported on the nocturnal behavior of blowflies and their activity/behavior in heavy rain as a guide for future studies. The data collected will provide reference material for the northeastern United States detailing the effect of rainfall on the behavior of blowflies. This will be useful in providing meaningful estimates of the mPMI by entomological methods in cases where rainfall has occurred shortly after body deposition.

CHAPTER 2: PREVIOUS RESEARCH

The study of entomology, understanding the life cycle and biology of various species of insect, has wide ranging and economically important applications, from agriculture to forensics. French entomologist Jean Pierre Mègnin is considered the forefather of Western forensic entomology. In his 1894 publication, *Fauna of Cadavers*, he reported a pattern in the arrival and departure times for various necrophagous insects as they colonized cadavers, which he observed to be associated with the stage of decomposition. This "succession" was described as a process by which various species of insect colonized, reproduced, utilized, and left the cadaver. Although Mègnin (1894) described this insect succession as a series of discrete units occurring independently, it is now known that insect succession occurs on a continuum with overlapping colonization periods (Byrd and Castner 2009). Mègnin's (1894) work highlighted the forensic utility of entomology and has stimulated ongoing research in this discipline.

Insect succession patterns can vary depending on a multitude of factors. Some species of necrophagous insect are only present in particular climates, during certain seasons, in particular geographic area, or certain locations, such as urban or rural (Archer 2003a, 2003b; Centeno *et al.* 2002; Michaud and Moreau 2009; Voss *et al.* 2011). As a result, research has been conducted aiming to describe carrion insect succession patterns in a wide variety of locales, geographic areas, and seasons (Carvalho *et al.* 2000; Grassberger *et al.* 2003; Richards and Goff 1997; Wallman 2001). Additionally, other factors that are known to influence decomposition rate also influence necrophagous insect activity. These can include, but are not limited to, temperature, indoor versus outdoor locations, time of day, presence of toxins, and weather patterns (Campobasso *et al.* 2001; Centeno *et al.* 2002; Reibe and Madea 2010; Shean *et al.* 1993; Voss *et al.* 2011; Voss *et al.* 2008).

Decomposition Stage

Decomposition stage and insect succession are correlated and the stages of decomposition have been thoroughly defined by many researchers (Archer 2004; Michaud and Moreau 2009, 2011). These definitions differ in the number of stages that are described and the degree of detail for each stage. Payne (1965) defined five stages of decomposition: the fresh stage (no decomposition or swelling); the bloated stage (bloating of the abdomen with purging of body fluids, ending as the corpse deflates); the active decay stage (occurs with liquefaction and disintegration of the corpse, high insect activity and odor); the advanced decay stage (most of the soft tissues have disappeared and odor begins to fade); and the remains stage (only bones, hair, and desiccated flesh remain, with no odor). Rodriguez and Bass (1983) combine the 'active decay' stage and 'advanced decay' stage into a single 'decay' stage, simplifying the process down to four stages. Generally, it is agreed that decomposition takes place in four overlapping, sequential phases: fresh, early decomposition/bloat, late decomposition, and dry/skeletonization (Archer 2004; Campobasso *et al.* 2001; Damann and Carter 2014; Megyesi *et al.* 2005; Payne 1965; Meyer *et al.* 2013; Rodriguez and Bass 1983; Vass 2011).

The current study will follow the previous research by Megyesi *et al.* (2005) in description and categorization of decomposition stage. This system defines four decomposition stages: fresh, early decomposition, late decomposition, and skeletonization, and provides further, more detailed, categories within each stage that are associated with an alphanumeric score. This system requires independent assessment of three distinct areas of a complete human body: the head and neck, including cervical vertebrae; the trunk, including thorax, pectoral girdle, abdomen, and pelvis; and the limbs, including the hands and feet. Each area is assessed and assigned the appropriate alphanumeric score that also refers to a numeric point value. Then, the numeric point values for each area of the body are summed to provide the TBS. For the current research, only pig heads were used as a proxy for human remains; thus, only the alphanumeric scoring system and associated numeric point values representative of the TBS specific for the head and neck were utilized (Megyesi *et al.* 2005).

Insect Succession

Decomposition stage has been correlated with insect succession patterns (Anderson 2011; Campobasso *et al.* 2001; Carvalho *et al.* 2007; Centeno *et al.* 2002; Richards and Goff 1997; Voss *et al.* 2008, 2011). Rodriguez and Bass (1983) described a general schematic of insect succession on human remains as it relates to decomposition stage observed in Tennessee. During the fresh stage, blowflies and muscid flies (Diptera: Muscidae) were the primary taxa attracted to a corpse. Their basic activities were feeding and ovipositing. Fly activity continued until the dry stage. At the onset of the early decomposition/bloated stage, carrion beetles (Coleoptera: Silphidae), followed by rove beetles (Coleoptera: Staphylinidae) and clown beetles (Coleoptera: Histeridae) were observed. They fed on both the fly larvae and the decomposing cadaver. Flesh flies (Diptera: Sarcophagidae) also joined the succession at this stage, feeding and ovipositing. During the late decomposition stage, sap beetles (Coleoptera: Nitidulidae) were noted, and through this stage there was a decline in all other types of insects until just sap and rove beetles remained. The dry stage brought dermestid (Coleoptera: Dermestidae), checkered (Coleoptera: Cleridae), and lamellicorn

(Coleoptera: Scarabaeidae) beetles, which were observed feeding on the dry tissues, hair, and fungus that remained on the cadavers (Rodriguez and Bass 1983).

This general schematic of fly and beetle succession is supported by other research (Centeno *et al.* 2002; Magni *et al.* 2013; Michaud and Moreau 2009, 2011; Richards and Goff 1997; Voss *et al.* 2011, 2008). Centeno *et al.* (2002) reported on faunal associations with decomposition stage and the length of the PMI in Argentina using pig (*Sus scrofa*) carcasses. These researchers found that the fresh stage of decomposition was characterized by the presence of adult blowflies, blowfly eggs, and 1st instar blowfly larvae. During the bloated stage, blowfly larvae progressed through their 2nd instar and 3rd instar phases, and were joined by adult carrion beetles. The active decay stage showed an abundance of 3rd instar blowfly larvae, and both adult and larval carrion beetles. In the advanced decay stage blowfly larvae had reached the post-feeding stage and migrated from the carcasses, leaving behind carrion beetle larvae. By the remains stage, beetles were the most prominent group present, although no family was specifically associated with this stage (Centeno *et al.* 2002).

Similarly, in a study conducted in Western Australia also using pig carcasses, Voss *et al.* (2011) found that calliphorid taxa typically colonized the carcasses during either the fresh or bloat stages of decomposition, while sarcophagid taxa arrived toward the end of the bloat or beginning of the wet decay stages. Coleoptera taxa, while present as early as day 2 of their study, increased in abundance during the wet decay and dry remains stages and were the only taxa present in the late dry remains stage (Voss *et al.* 2011). Insect succession studies that were conducted by Anderson (2011) in Edmonton, Alberta, and by Carvalho *et al.* (2000) in southeastern Brazil, reported comparable results.

The Porcine Model

Many studies have employed pig carcasses as an alternative to human remains to explore various research questions involving traumatic injury and decomposition (Archer 2004; Baumer *et al.* 2010; Jordana *et al.* 2013; Michaud and Moreau 2009, 2011; Powell *et al.* 2012; Powell *et al.* 2013; Reibe and Madea 2010; Voss *et al.* 2011). Significantly, pigs can be used to model decomposition in humans under many different conditions and are generally accepted as the preferred substitute for human decomposition in research (Schoenly *et al.* 2007). Additionally, pigs are readily available for scientific research and conform to the ethical concerns implicit in decomposition studies. Human cadavers and pig carcasses of certain weight classes decompose in a consistent manner because of common bacterial fauna in the gut and very similar skin structures. Domestic pigs are also consistent with humans, because they are mostly hairless, omnivorous, and have similar internal anatomy, fat distribution, physical and chemical blood characteristics, respiratory tracts, bones, and muscles (Jordana *et al.* 2013). A pig of approximately 22 kg is currently considered the best model to simulate the decomposition of an average human torso (Michaud and Moreau 2009). In the current study, pig heads, including a variable number of cervical vertebrae, were used as a proxy for human decomposition due to availability, ease of transport and storage, and budgeting concerns.

Temperature

Temperature is known to have the greatest effect on the decomposition rate of a cadaver (Archer 2004; Campobasso *et al.* 2001; Mann *et al.* 1990; Meyer *et al.* 2013; Vass 2011). The intrinsic processes of decomposition, autolysis and putrefaction, are positively correlated with temperature; as temperatures decrease, these processes decrease until they virtually cease (Micozzi 1986). Although studies have suggested that this temperature is 0°C, none provide experimental evidence to support this claim (Megyesi *et al.* 2005; Micozzi 1991; Vass *et al.* 1992). Similarly, temperature has an effect on many extrinsic factors of decomposition including insect activity. The growth and development of blowfly larvae are largely governed by the ambient temperature, with the rate of development increasing as the temperature increases from the biologically determined developmental minimum temperature to the optimum developmental temperature for a given species (Amendt *et al.* 2011).

The biologically determined developmental minimum temperature is often generalized to be about 10°C but is variable by species. This represents the minimum temperature for oviposition to occur and larvae to hatch (Meyer *et al.* 2013). However, large established maggot masses will usually survive below this temperature because of the heat produced by friction within the writhing larval masses and the protection of the body cavities (Amendt *et al.* 2011). Likewise, above the optimum developmental temperature, insects and their larvae will quickly overheat and die. Under ideal conditions in a temperate zone, e.g. warm to hot weather, it is estimated to take approximately 2-4 weeks for a body to reach the skeletonized stage of decomposition as a result of both the intrinsic processes (putrefaction and autolysis) and the necrophagous insect activity (Centeno *et al.* 2002; Mann *et al.* 1990; Megyesi *et al.* 2005).

It is most difficult to determine accurately the PMI during months where temperatures fluctuate between warm and cold due to temperature's large impact on autolysis, putrefaction, and insect development (Archer 2004; Mann *et al.* 1990). The determination of Accumulated Degree Days (ADDs) has been used to normalize the processes of decomposition as it relates to temperature (Meyer *et al.* 2013) and to estimate the PMI (Megyesi *et al.* 2005). ADDs represent the heat units available to propel the biological processes described above, including bacterial growth and larvae development. As previously discussed, ADDs are calculated by summing all average daily temperatures above 0°C for a given period of time (Megyesi *et al.* 2005). Megyesi *et al.* (2005) used ADDs in conjunction with an assessment of the TBS to develop a regression formula to predict the PMI. In this study the authors found that by assessing the TBS of a cadaver, the regression formula can be used to determine the ADDs necessary to reach that degree of decomposition. The investigator can then use this information with temperature data to calculate backwards from the time of discovery when the TBS was assessed, to the estimated time of death (Megyesi *et al.* 2005).

Environmental

Other environmental factors can also impact the rate of decomposition. In order to colonize the remains, the insects must have physical access, and there are many environmental factors that can act as a barrier to colonizing insects (Voss *et al.* 2008). The presence of clothing, wrapping, covering, shelter, burial, and placement within an indoor environment are a few barriers that are physical in the literal sense. However, exposure to toxic substances, placement in aquatic environments, and extreme weather has also been shown to provide a physical barrier in the sense that insects would be restricted from accessing the body due to these variables. This restriction can greatly affect the timing and types of insects that colonize a carcass (Anderson 2011; Centeno *et al.* 2002; Galloway *et al.* 1989; Mahat *et al.* 2009; Reibe and Madea 2010; Shelomi *et al.* 2012; Voss *et al.* 2011).

To investigate the effects of clothing on decomposition and insect activity, Voss *et al.* (2011) dressed their experimental sample of pig carcasses in a T-shirt and elastic waistband shorts. When compared to the controls it was observed that the presence of clothing prolonged the wet decay stage by preventing desiccation, although clothing did not affect the timing of insect arrival and colonization (Voss *et al.* 2011). Kelly *et al.* (2009) observed decomposition and insect colonization of pig carcasses that were clothed only, clothed and wrapped, wrapped only, and without clothing or wrapping. Both the clothed and wrapped carcasses supported larger larval masses and stayed moist for longer periods of time. However, it was also observed that oviposition occurred simultaneously on all carcasses despite the presence or absence of clothing or wrappings (Kelley *et al.* 2009).

Arid environments, such as in the southwestern United States, can result in delayed decomposition. In a retrospective study of 189 cases provided by the Pima County Medical Examiners Office, Galloway *et al.* (1989) found that the arid outdoor environment in southern Arizona often caused mummification of the body. This in turn inhibited insect activity and slowed the decomposition rate. There is also a general pattern of decreased decomposition in aquatic environments when compared to terrestrial environments (Higgs and Pokines 2014). This is because of the decreased temperatures underwater and diminished access to the body by the forensically significant necrophagous fauna discussed above (Higgs and Pokines 2014; Magni *et al.* 2013; Westling 2012). There does not exist an aquatic faunal equivalent of blowflies and their larvae that can rapidly decompose the soft tissues with the relatively little dispersal (Higgs and Pokines 2014).

Anderson (2011) observed decomposition and insect succession in pigs deposited in indoor and outdoor locations. They found that there was a five-day delay in colonization of the indoor carcasses in comparison to the outdoor carcasses. The indoor carcasses were colonized by fewer species of carrion insect, had smaller larval masses, and had a delay in overall decomposition (Anderson 2011). In a similar study, by comparing the number of egg batches simultaneously deposited on pig carcasses in each location, Reibe and Madea (2010) investigated whether indoor or outdoor deposition sites affected blowfly oviposition. They found that the number of egg batches on the outdoor pig carcasses was significantly higher than those observed on the indoor pig carcasses. The number of egg batches was highly correlated to the location (indoor versus outdoor) and to the duration of exposure, but not to temperature or length of day both indoors and outdoors. Although insect development is highly influenced by temperature, it probably did not play a role in this study, because the temperatures remained moderate (15°C to 25°C) throughout the study in both locations, e.g. within the developmental limits of most necrophagous species of insect (Reibe and Madea 2010).

Shelomi *et al.* (2012) investigated whether the insect repellant N,N-Diethyl-meta-toluamide (DEET) affected the overall decomposition of pig carcasses, and the associated blowfly activity, oviposition, and subsequent development of larvae. The experimental group was comprised of previously frozen pig carcasses sprayed with DEET, and these were compared to unsprayed control pigs for differences in blowfly behavior, colonization level and timing, and larvae development. It was found that the DEET sprayed pigs experienced significant delays in blowfly visitation, oviposition, development of larvae, and overall decomposition, as well as a reduction in the volume of larvae and the individual size of the larvae that developed on the remains (Shelomi *et al.* 2012).

Nocturnal Activity

Blowflies are considered to be diurnal insects. It is reported that they are only active during the daytime hours when temperatures are high enough to stimulate their activities (George *et al.* 2013). However, the results of research regarding nocturnal oviposition have been somewhat contradictory. Yang and Shiao (2012) reported that laboratory flies were active under dark conditions. It should be noted these authors created artificial nighttime conditions by turning off the lights and did not make their observation under actual nocturnal conditions. In a study conducted by George *et al.* (2013), it was shown that blowfly colonization in an outdoor setting began shortly after sunrise and continued until sunset, with peaks occurring in the mid-morning, midday, and in the hours preceding sunset. In an additional study, wild blowflies were captured, taken into a laboratory, and supplied with colonization baits during nighttime hours. Despite their assumed diurnal biology, colonization of the baits occurred on four of five nights. It is surmised by the authors that blowflies trapped in a confined, warm laboratory environment are more stimulated to oviposit than those in a natural outdoor environment (George *et al.* 2013).

In another study specific to nocturnal oviposition, performed in central Texas, Baldridge *et al.* (2006) observed that fly activity on mice and pig carcasses ceased within 50 minutes post-sunset and did not resume until after 0600 h. These results were found with both the presence and absence of artificial outdoor lighting (Baldridge *et al.* 2006). While the results of these studies are somewhat contradictory, the majority of evidence supports the claim that blowflies in a natural outdoor environment will behave diurnally and do not oviposit nocturnally (Haskell *et al.* 1997; Tessmer *et al.* 1995; Spencer 2002). This aspect of blowfly biology could result in mPMI discrepancies because of a delay in oviposition equal to the length of the night.

Additional Variables

There are many other processes that contribute to the overall decay of a cadaver. It has recently been shown that penetrative trauma, such as gunshot wounds or sharp force injury, play little to no role in increasing or decreasing decomposition rate. Cross and Simmons (2010) found that the presence of penetrating trauma to pig carcasses did not decrease the time it took to reach the skeletonization stage of decomposition, as was previously thought (Mann *et al.* 1990). In fact, Diptera were preferentially attracted to the natural orifices of the body, the eyes, ears, nose, and mouth, over the trauma sites (Cross 2007;

Cross and Simmons 2010). Similarly, in a study conducted at the Boston University Outdoor Research Facility (ORF), Smith (2014) found that while the pattern of decomposition differed between subjects with penetrative trauma and those with none, overall decomposition rate was the same.

Other preferences that may influence oviposition behaviors, and thus play a role in decomposition, have been investigated. These include spatial aggregation, predator and prey relationships, and oviposition and developmental differences between field and laboratory populations (Fenton *et al.* 1999; Fiene *et al.* 2014; Gião and Godoy 2007; Pitts and Wall 2004; Yang and Shiao 2012). Yang and Shiao (2012) and Gião and Godoy (2007) found that larval predator-prey relationships influenced where adult female blowflies oviposited. For example, adult females of *Chrysomya megacephala* avoided ovipositing on media already colonized by the predacious larvae of *Chrysomya rufifacies* (Yang and Shiao 2012).

Fenton *et al.* (1999) and Fiene *et al.* (2014) both investigated the factors influencing spatial aggregation preferences for oviposition. Fenton *et al.* (1999) found that in ovine cutaneous myiasis, blowflies were preferentially attracted to sheep that had been previously colonized and Fiene *et al.* (2014) studied the utilization of limited resources, such as carrion in forested areas. The study performed by Pitts and Wall (2004) highlights the differences between field and laboratory populations of

Lucilia sericata and emphasizes that care should be taken when comparing life-history parameters deduced from artificial habitats to insects in their natural environments.

Geographic and temporal climate variation should also be taken into consideration when investigating the processes of decomposition and insect colonization (Voss *et al.* 2011). The University of Tennessee, Knoxville, Anthropological Research Facility (ARF) is known for forensic research utilizing donated human remains, yet most of Tennessee has a humid, subtropical climate, while some areas are more temperate due to heightened elevation, with hot summers and mild to cool winters in most regions. The environment at ARF is not consistent with all locations where a forensically significant decomposition event may occur, although many highly referenced decomposition studies have been performed at this site. The geographic areas and associated climates in which decomposition studies have been undertaken are clustered near similar research facilities, and include, but are not limited to, the climates of Tennessee (Damann *et al.* 2012; Mann *et al.* 1990; Rodriguez and Bass 1983; Schoenly *et al.* 2007), Arizona (Galloway *et al.* 1989), Nebraska (Meyer *et al.* 2013), and Massachusetts (Barretta 2012; Decota 2011; Smith 2014; Tandy 2011) within the United States; Edmonton, Alberta, Canada (Anderson 2011); South Africa (Kelly *et al.* 2009); Western

Australia (Voss *et al.* 2011); Brazil (Carvalho *et al.* 2000); and Argentina (Centeno *et al.* 2002).

The ORF, Holliston, Massachusetts

The current study was conducted at the ORF in Holliston, MA. This facility is specially designed for outdoor decomposition studies and other forensic research. While many researchers have utilized this space for a variety of forensic studies (Brouchoud 2014; Moore 2014; Pendray 2015; Ricketts 2013; Smith 2014; Tandy 2011; Westling 2012), the research conducted by Barretta (2012) and Decota (2011) are relevant to the current study. Decota (2011) performed an outdoor study investigating the rate of decomposition for pig carcasses at the ORF. The results of this study suggest that decomposition in this area proceeds similarly to other areas where decomposition studies have been conducted passing through the same stages and time periods produced by Bass (1997). This study provides descriptive data of the observation of six pig carcasses over a 30-day period (Decota 2011).

Most relevant to the current study is the research conducted at the ORF by Barretta (2012). This study documented entomological specimens collected both from pig carcasses within the decomposition enclosure and from the surrounding ORF property. Two classes of arthropods were collected, Insects and Arachnids, representing six Insect

Orders and one Arachnid Order. Sixty-nine percent of the total specimens collected were represented by dipteran species. The most common dipteran species documented were *Phormia regina* (Meigen), followed by *Lucilia* spp., *Oxysarcodexia* spp., and several species of sarcophid and muscid flies. Additionally, 15.6% of the total specimens were represented by coleopteran species including *Creophilus maxillosus* (Linnaeus) and *Necrophila americana* (Linnaeus). The additional orders of insect collected include Hymenoptera, Odonata, Lepidoptera, and Orthoptera (Barretta 2012).

Rainfall

Very few studies have been conducted with the intent of specifically examining the effect of rainfall on decomposition and necrophagous insect activity. Additionally, due to the variety of methodological approaches used by these authors, the description of the influence of rainfall varies greatly between studies (Archer 2004; Mahat *et al.* 2009; Reibe and Madea 2010). Archer (2004) examined the pattern of decomposition in neonatal pigs over a two-year entomological and decomposition study. Sets of neonatal pig carcasses were placed in large scavenger-proof mesh cages outdoors for each season (spring, summer, fall, and winter) over a two-year period. Carcass appearance and mass loss were recorded until the latter became confounded by bone loss through the mesh cages. It was found that after 5-7 days, mass loss positively correlated with cumulative rainfall in the preceding days. It was also noted that the number of weeks taken to reach the end of the decay stage was negatively correlated with cumulative rainfall in the first 20 days after exposure, i.e., increased cumulative rainfall increased mass loss of the carcass and decreased the length of time to reach the end of the decay stage (Archer 2004). This study showed that rainfall aids in washing away decomposed tissues, increasing mass loss over time and accelerating overall decomposition. However, neonatal pig carcasses cannot be compared to typical human remains due to weight concerns and the effect of rainfall on blowfly oviposition was not investigated in this study.

Mahat *et al.* (2009) investigated the influence of rainfall and malathion, an organophosphorus compound commonly utilized in suicidal poisoning, on the oviposition and development of blowflies in Malaysia. Malaysia is a humid, tropical country with rainy and less rainy months of the year, where the effect of natural rainfall on decomposition could be easily investigated. Malathion generally is used as an insecticide or rodent poison but is reported to contribute to 9.1% of drug-related fatalities, mostly suicides, in Malaysia. The authors of this study reported that the heavy rainfall experienced during the months of November, December, and January delayed blowfly oviposition by 1-2 days, while the presence of malathion, administered orally to induce death in the rabbits (*Oryctolagus cuniculus*) utilized in this study, also produced a delay in oviposition of 1-3 days with the delay increasing with increasing concentrations of malathion (Mahat *et al.* 2009). The presence of malathion also stunted the development of the blowfly larvae, resulting in smaller 3rd instar larvae and causing a longer period of pupation than in the control samples (Mahat *et al.* 2009). This result is consistent with the smaller larvae discovered developing on the DEET-treated carcasses of the experiment by Shelomi *et al.* (2012).

The study conducted by Reibe and Madea (2009) investigating the timing of oviposition in remains deposited indoors versus outdoors, while not directly investigating the effect of rainfall, did record observations about rainfall and reported data on the amount of rainfall experienced by their outdoor pig carcasses. The researchers reported that rainfall did not prevent, but may in some cases have delayed, blowfly oviposition. They observed that the blowflies either waited for the rain to cease, delaying access, or would simply access the carcasses by crawling toward them in the rain from a sheltered area. However, in this study, the carcasses were protected from the effects of direct rainfall by the eaves of a shed, and it did not rain at all during several of the experimental runs (Reibe and Medea 2010).

Decomposition is highly dependent on differences in geographic location, climate, season, habitat, physical state of the remains, weather exposure, and decomposition environment, whether natural or artificial (Voss *et al.* 2011). Although many variables that affect human decomposition have been studied, there are still many environmental variables, natural and artificial, and climate types that may affect decomposition, insect activity, and estimation of the PMI. As the previously reported literature highlights, there is still a paucity of data investigating the influence of rainfall on decomposition and necrophagous insect activity/behavior.

This research represents a preliminary study that used natural rainfall as the primary independent variable, with decomposition rate and blowfly activity as the dependent factors that were observed. It was hypothesized that natural rainfall will act as an inhibitor to blowfly activity and possible result in a decreased rate of decomposition due to decreased blowfly activity. The objectives of this research were to determine the effects of natural rainfall on blowfly activity and decomposition, make observations regarding the nocturnal oviposition behavior of blowflies, record additional species of arthropod that are observed at the decomposition sites, and make supplementary scavenging observations.

CHAPTER 3: MATERIALS AND METHODS

The current study was conducted at the ORF in Holliston, Massachusetts (Figure 3.1). The ORF consists of 32-forested and semi-forested acres of land. The new growth forest is comprised of conifer and deciduous trees. There are several marshy, artificial ponds with connecting streams that had previously been used as cranberry bogs and several grassy clearings. The current study was conducted in a fenced-in, grassy clearing adjacent to a research building. This area was chosen for its large size, approximately 27.4 m by 53.3 m, which allowed the pig heads to receive equal amounts of sun exposure and to be placed in identical environments in terms of vegetation, soil type, and weather. The area was cleared of large brush and mowed before the beginning of the study, leaving only low grasses and other vegetation. This area is contained by a high chain-link and barbwire fence, and will be referenced to as the "decomposition enclosure" (Figure 3.2).

In the current study, pig heads, including a variable number of cervical vertebrae, were used as a proxy for human decomposition. Pig heads were utilized due to availability, ease of transport and storage, and budgeting concerns. Pig heads also offer the preferred oviposition sites, the natural orifices (Cross and Simmons 2010), and were low cost compared with a whole body pig. Thirty-three frozen pig heads were

Figure 3.1. Map of the ORF, located in Holliston, MA. Buildings are in yellow, forested areas are in green, marshes and ponds are in blue, and property boundaries are in red. Reproduced from Schofield Brothers 2010.

Figure 3.2. ORF decomposition enclosure.

obtained for research purposes from a commercial retailer (Taurus Meats Co., Boston, MA). Butchery of the pig heads had left a large open wound on the posterior aspect of the head, bisecting the cervical vertebral column and soft tissues, including the esophagus, trachea, and neck musculature. The eyes had been removed. The pig heads were received wrapped in blue plastic and sealed in cardboard boxing, with three pig heads per box. This packaging ensured that the pig heads had not been exposed to oviposition prior to their deposition outdoors for the research.

The pig heads were transported to the ORF and stored in several large chest freezers at -8° C until the research began. Two to three days prior to the onset of each portion of the study the required amount of pig heads were removed from the freezers and thawed in refrigeration at 4° C, continuously protected from all sources of oviposition. Just before outdoor deposition, the pig heads were transferred to large black plastic bags, sealed to prevent insect access, and placed at room temperature (approximately 22° C) for several hours in order to equalize their temperature with the temperature of the environment.

High-resolution photographs were taken using a digital camera throughout the study. Pictures of the pig heads and their natural orifices were taken to record the presence or absence of eggs oviposited by blowflies, larval hatching/larvae presence, other insect activity, and decomposition changes. A weather station located at the ORF collected data on temperature (° C), rainfall (mm), and solar radiation (W/m^2) throughout the entire study at 30-minute intervals. Solar radiation (W/m^2) is a quantitative measure of the amount of sunshine reaching the earth's surface and represents the amount of solar electromagnetic energy per area. Decomposition change was recorded according to the scoring system described by Megyesi *et al.* (2005), using only the alphanumeric scoring system specific for the head and neck (Table 3.1). Pig heads were described as reaching the advanced decomposition stage when they had reached a Megyesi *et al.* (2005) alphanumeric score of at least C1.

Table 3.1. Categories and stages of decomposition for the head and neck. Adapted from Megyesi *et al.* (2005) Table 2, pp. 4.	
Description	TBS
A. Fresh	
1. Fresh, no discoloration.	1
B. Early decomposition	
1. Pink-white appearance with skin slippage and some hair loss.	2
2. Gray to green discoloration: some flesh still relatively fresh.	3
3. Discoloration and/or brownish shades particularly at edges, drying of nose, ears and lips.	4
4. Purging of decompositional fluids out of eyes, ears, nose, mouth, some bloating of face and neck may be present.	5
5. Brown to black discoloration of flesh.	6
C. Advanced decomposition	
1. Caving in of the flesh and tissues of eyes and throat.	7
2. Moist decomposition with bone exposure less than one half that of the area being scored.	8
3. Mummification with bone exposure less than one half that of the area being scored.	9
D. Skeletonization	
1. Bone exposure of more than half of the area being scored with greasy substances and decomposed tissues.	10
2. Bone exposure of more than half the area being scored with desiccated or mummified tissue.	11
3. Bones largely dry, but retaining some grease.	12
4. Dry bone.	13

Insect Collection Procedures

To collect information about the species of blowflies (and other arthropods) that were active in the decomposition enclosure during the time of study, specimens were collected or photographed for identification. For both the preliminary trial and experimental trial, ten adult blowflies were collected during their peak activity times. When blowfly activity was maximized, usually around day two or day three post deposition, agricultural grade sticky traps (*Stiky Strips Insect Traps*, Olson Products, Medina, OH) were placed within 60 cm of the pig heads to collect passively the blowflies visiting the carcasses. Ten adult blowflies were removed from the sticky traps using baby oil, then killed and preserved in 80% ethyl alcohol. The preserved blowflies were later dried and pinned for identification.

As other species of arthropods were observed over the course of the study, one to two specimens were collected by hand with forceps, then killed and preserved in 80% ethyl alcohol for identification. Some arthropods were not collected, but identified from photographs. All other arthropods, not including the preserved blowflies, were reported as present or absent when observed in association with the carcasses, although in interesting cases additional notes were recorded. Adult blowflies were identified based on published entomological keys for North America (Whitworth 2006), a forensic insect guide (Castner and Byrd 2000), and with the aid of forensic entomologists Dr. Ian Dadour and Dr. Paola Magni (University of Western Australia). This information, while not an exhaustive entomological study, adds to previous research investigating the species of arthropods that are prevalent at the ORF during the summer months (Barretta 2011).

Preliminary Trial

The preliminary trial was designed to familiarize the author with the processes of decomposition and blowfly activity more generally. Observations were also made in reference to nocturnal behavior and oviposition to determine if nighttime observations were necessary during the experimental trial. On June 14, 2014, two pig heads were placed during the daytime, at approximately 1500 h, for observation. The pig heads were placed directly in the ground, neck wound down and snout up (Figure 3.3). One pig head was left uncovered and one pig head was covered with a large black plastic slotted basket, placed upside down over the pig head, to provide shade and shelter from weather variables (Figure 3.4). One pig head was also placed, in an identical fashion, uncovered, after dark (approximately 2100 h).

Observation of each pig head in the preliminary trial occurred every 15 minutes for three hours after deposition to record blowfly activity, and the exact number of blowflies observed at the moment of observation was recorded. The preliminary trial pig heads were subsequently observed on days 1-5, 8, 12, and 14. Notes were recorded in reference to decomposition change using the Megyesi *et al.* (2005)

scoring system, the arthropods visiting and colonizing the remains, and any additional variables that were observed. Data collection and research methods/observation-timing were developed from this information for use during the experimental trial (Appendix A).

Figure 3.3. Preliminary trial. Preliminary 1, uncovered, example of placement and condition of pig heads at beginning of research.

Figure 3.4. Preliminary trial. Preliminary 2, covered, example of cover provided for covered groups throughout the research.

Experimental Trial

The experimental trial proceeded with two treatments, each in two replicates. The control treatment was exposed under normal conditions (N), and the rain treatment was exposed under rainy conditions (R). Each treatment was split into uncovered pig heads (N and R) and covered pig heads (NC and RC). The rain treatment also included a group of partially covered pig heads (RCP). Cover was accomplished as previously described and the cover was removed on day three of exposure for the RCP group. Local weather data from the closest weather station, the National Weather Service Station at Norwood Memorial Airport (Lat. N

42.19, Long. W 71.17), approximately 30 km from the ORF, was used to determine the start date of each treatment.

The control treatment proceeded in two replicates that began when rain was *not expected* for at least two consecutive days at the beginning of the treatment. The rain treatment also proceeded in two replicates that each began when rain was *expected* for at least two consecutive days from the beginning of the treatment. Rainfall is described as light, moderate, or heavy: light rainfall is generally less than 2.5 mm per hour, moderate is between 2.5 and 7.5 mm per hour, and heavy rainfall is more than 7.5 mm per hour (U.S. Department of Commerce, 2011). Although it is normal for rainfall to vary in the region of study, the rain treatment was aimed to begin during when at least moderate rainfall was expected.

Control Treatment (N) Details

The control treatment began when rainfall was *not expected* for at least two consecutive days. Sets of pig heads were randomly designated to N or NC. The N pig heads were set out in the decomposition enclosure, positioned exactly as described in the preliminary trial, uncovered and approximately 5 m apart. The NC pig heads were set out in the same manner, but each NC pig head was covered as previously described. A total of six pig heads were exposed under N conditions and a total of six pig heads were exposed under NC conditions. The trial was conducted in two replicates: the first began June 27, 2014, and the second began July 18, 2014, over a total time of 27 and 28 days, respectively.

Rain Treatment (R) Details

The rain treatment began when rainfall was *expected* for the first two days at the beginning of the experiment. Sets of pig heads were randomly designated to R, RC, or RCP. The R pig heads were set out in the decomposition enclosure, positioned exactly as described in the preliminary trial, uncovered and approximately 5 m apart. The RC pig heads were set out in the same manner, but each RC pig head was covered as previously described. The RCP pig heads were set out in the same manner as the RC pig heads, covered, then uncovered on day three (after the rain had ceased) for the remainder of the experiment. A total of five pig heads were exposed under R conditions, a total of five pig heads were exposed under RC conditions, and a total of five pig heads were exposed under RCP conditions. The trial was conducted in two replicates: the first began on July 14, 2014, and the second began on August 12, 2014, over a total time of 30 and 48 days, respectively.

Time of Day

All pig heads were deposited in the decomposition enclosure within one hour of 1200 h, except for the first replicate of the control trial. This replicate was deposited at 0500 h to make additional observations about the initiation of blowfly activity after sunrise. All pig heads were observed every two hours for the first 48 hours between the times of 0800 h and 1800 h. Observation continued once per day for approximately one week, and weekly thereafter until the termination of each replicate as described above.

Appendix A is a reproduction of the data collection sheets that were utilized throughout the experimental trial. These data collection sheets were used to record all observations. The approximate number of blowflies visiting each carcass was recorded. This number was later transcribed as a coded number for graphing purposes. The coding system uses a numeral scale to represent the approximate number of flies observed in association with the carcasses; with “0” corresponding to zero flies observed, “1” corresponding to <10 flies observed, “2” corresponding to 10-30 flies observed, “3” corresponding to 30-50 flies observed, and “4” corresponding to >50 flies, all observed in association with the decomposing pig heads. This coding system was used for defining the estimated number of flies observed during the experimental and active rainfall trial in the Results section below. Also recorded was the presence of eggs and sites eggs were observed. When larvae hatched, this was recorded with location of larvae, size of masses, and the approximate size of larvae observed (1-2 mm, 3-4 mm, or 5+ mm). All additional arthropod activity and any other pertinent observations were also recorded.

Active Rainfall Trial

The primary difficulty in obtaining relevant data during the experimental trial was predicting the time and amount of natural rain that would fall. The author found that the occurrence of natural rainfall is variable and unpredictable. In attempting to control for all other variables, making the presence of rainfall the only difference between control and rain treatments, several experimental guidelines that were established may have caused some methodological errors. For example, to control for differences in blowfly activity as the result of the time of day the remains were deposited within one hour of 1200 h for both replicates of the rain treatment and the second replicate of the control treatment. This was done on a day rain was expected from the weather forecast for the rain treatment (Weather.com 2014). However, at that time rain was not actively falling and did not for several hours after deposition. Although eventually substantial rainfall occurred and continued for approximately two days intermittently, meeting the study conditions, the author observed that blowfly activity during the short time between deposition and the start of rainfall resulted in successful oviposition. Thus, at the onset of rainfall, egg masses that were protected within the mouth, throat, and snout seemed to be unaffected by any light or moderate rain.

In order to observe blowfly activity on remains that are deposited while it is actively raining, an active rain trial, designated AR, was conducted. The active rain trial began on day two (August 13, 2014) of the second replicate of the experimental trial, because constant, moderate to heavy rainfall was actively occurring at that time. A set of three pig heads was deposited in the decomposition enclosure, positioned exactly as described in the preliminary trial, uncovered and approximately 5 m apart. These pig heads were placed at 1000 h directly during active rainfall. Observations occurred every two hours for 24 hours between the times of 0800 h and 1800 h. Observation continued once per day for approximately one week, and weekly thereafter until termination of all trials on September 15, 2014. Data were also collected during the active rainfall trial as described in Appendix A.

CHAPTER 4: RESULTS

The data described below details the observations recorded during the course of the field research and the statistical analysis performed on a subset of the data that were collected.

Preliminary Trial Results

During the preliminary trial, the exact number of blowflies that were observed visiting preliminary pigs 1 – 3 at 15-minute intervals was recorded. Table 4.1 shows the exact number of flies observed at each time interval.

Table 4.1. Preliminary trial. The exact number of blowflies visiting preliminary 1 – 3 at 15 minute intervals during the first three hours of the PMI.

PMI (min.)	Prelim. 1 (1500 h) Uncovered, day	Prelim. 2 (1500 h) Covered, day	Prelim. 3 (2100 h) Uncovered, night
15	1	1	0
30	0	0	0
45	0	0	0
60	2	0	0
75	0	0	0
90	0	0	0
105	0	0	0
120	5	2	0
135	5	1	0
150	4	3	0
165	2	2	0
180	3	1	0

Table 4.2 reports the Megyesi *et al.* (2005) decomposition scores for the three pig heads utilized in the preliminary trial over the two-week period of observation. In addition to the blowflies observed in association with the pig heads of the preliminary trial, ants (Formicidae) were also observed feeding on the decomposing tissues.

Table 4.2. Preliminary trial. Megyesi *et al.* (2005) score for the pig heads deposited during daytime and nighttime hours during the first two weeks of the PMI.

PMI (days)	Prelim. 1 (1500 h) Uncovered, day	Prelim. 2 (1500 h) Covered, day	Prelim. 3 (2100 h) Uncovered, night
1	A1	A1	A1
2	B1	B1	B1
3	B3	B1	B3
4	B3	B2	B3
5	B4	B3	B4
8	C1	C2	C1
12	C3	C2	C3
14	C3	C3	C3

Insect Identification

The flies that were collected during the preliminary trial and the experimental trial were identified to family, genus, and species where possible. The number of flies collected and identified for each taxon is reported in Table 4.3. Other arthropods that were observed or collected over the course of the research were also classified as specifically as possible by taxa and are reported as present or absent (Table 4.3).

Table 4.3. Arthropod identification. The number of identified flies caught on sticky paper and preserved is reported. Other identified arthropods are reported as present (P) or absent (A). Calliphorid species are bolded.

Arthropod	Preliminary 1-3	Normal 1-3	Normal 4-6	Rain 1-3	Rain 4-5
Phormia regina	8	4	5	5	7
***Lucilia* spp.**	1	3	2	4	3
Sarcophagidae	1	1	–	1	–
Muscidae	–	2	2	–	–
Piophilidae	P	P	P	P	P
Necrophila americana	A	A	P	P	P
Creophilus maxillosus	A	A	P	P	P
Nicrophorus spp.	A	A	A	A	P
Formicidae (ants)	P	P	P	P	P
Vespidae (wasps)	A	A	P	A	P
Gryllidae (crickets)	A	A	P	A	P
Araneae (spiders)	A	A	P	A	A

Experimental Trial

During both the control and rain treatments, the coded number of flies observed in association with the pig heads as a function of time of day and correlated with solar radiation exposure was plotted. In the experimental trial, the coded number of flies observed as a function of the amount of rainfall in mm that had fallen since the last observation is also reported. Additionally, the degree of decomposition plotted against ADDs, average number of days for each group to reach the advanced decomposition stage, and the Megyesi *et al.* (2005) scores have also been graphed.

Time of Day

Figures 4.1 – 4.5 report the coded number of adult flies observed in association with the pig heads as a function of time of day for the first 48 hours after deposition for each treatment and condition. Each pig head is represented by a different colored bar on the bar graphs and shows the change in the coded number of flies observed over a time period of two days.

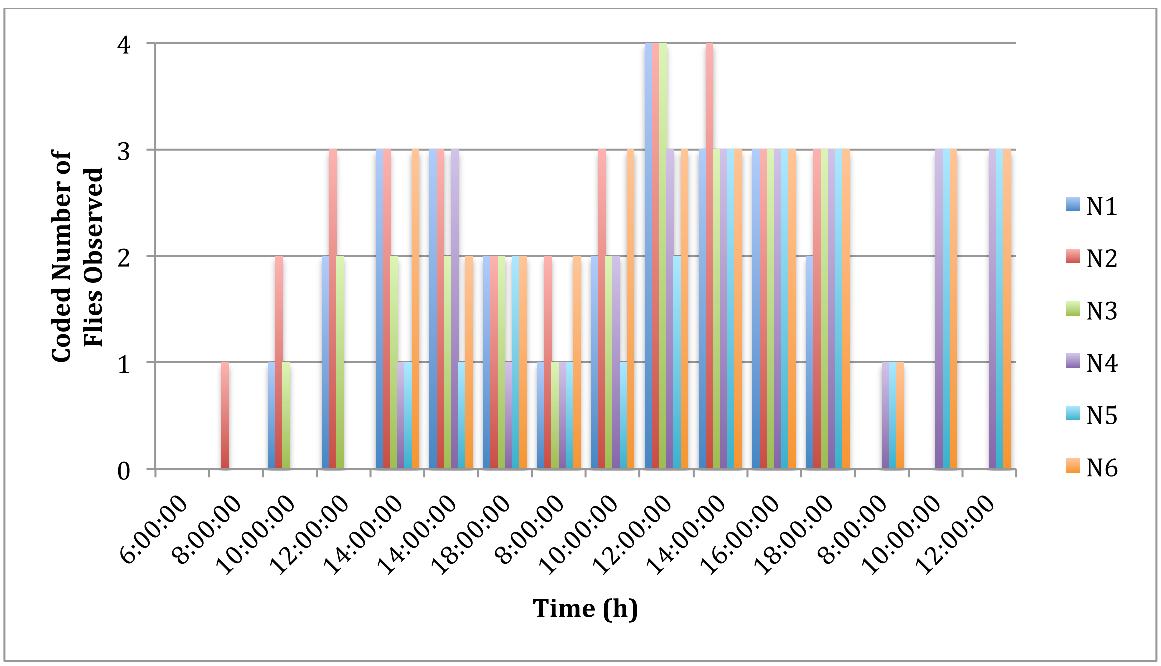

Figure 4.1. The coded number of flies observed by time of day (h), normal conditions, uncovered (N).

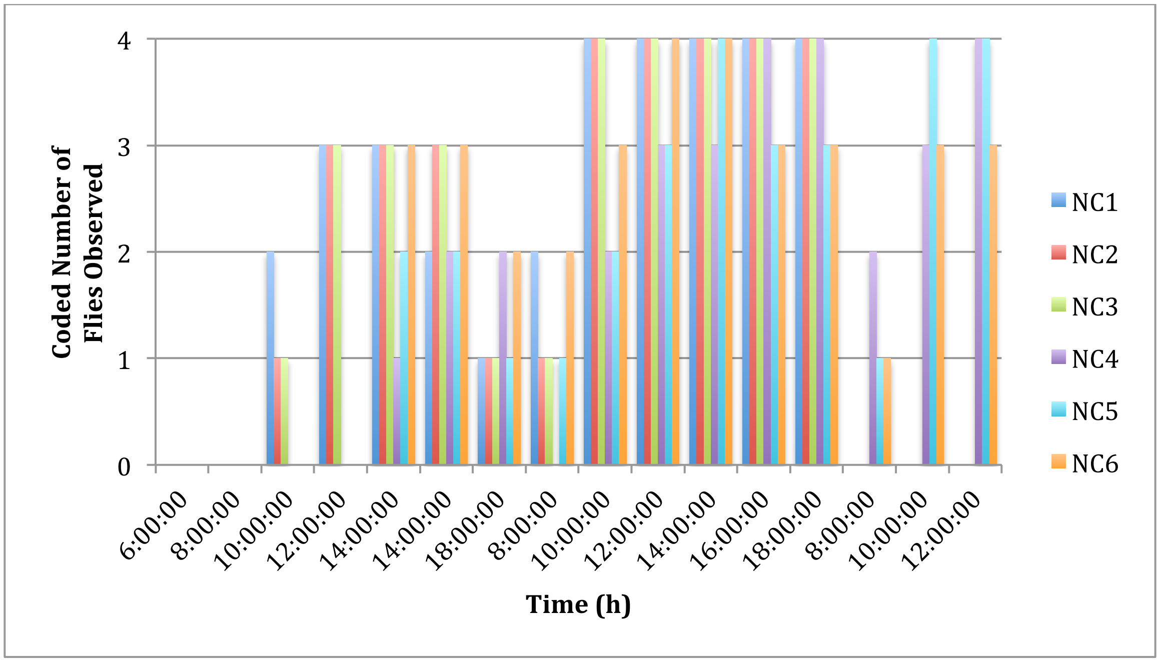

Figure 4.2. The coded number of flies observed by time of day (h), normal conditions, covered (NC).

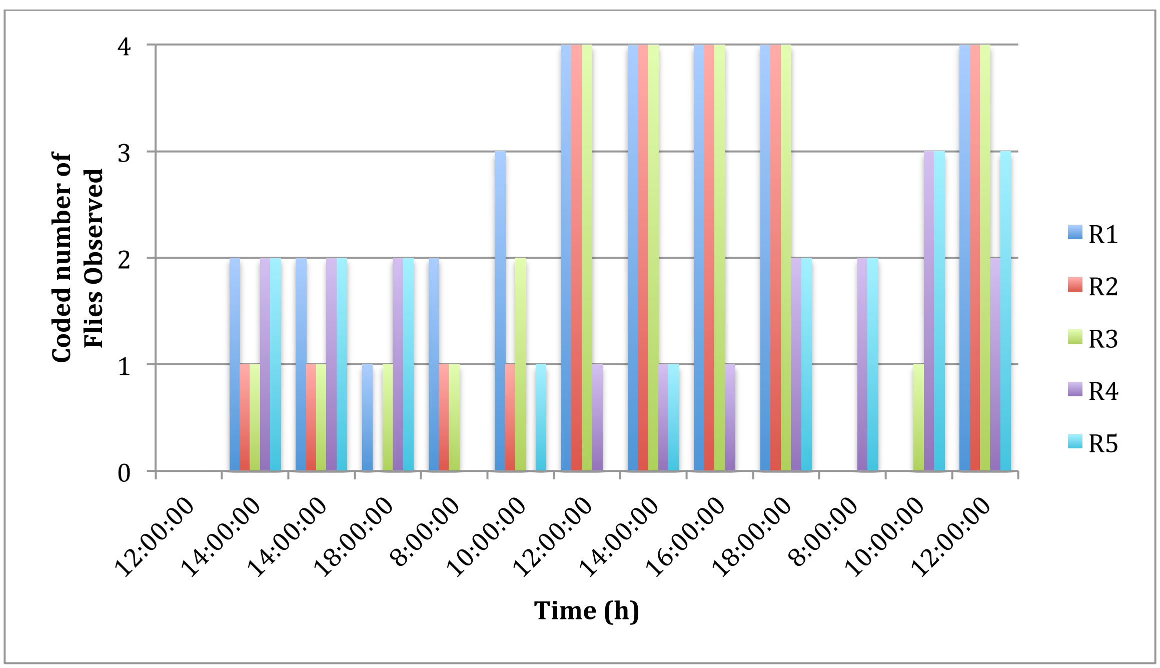

Figure 4.3. The coded number of flies observed by time of day (h), rainy conditions, uncovered (R).

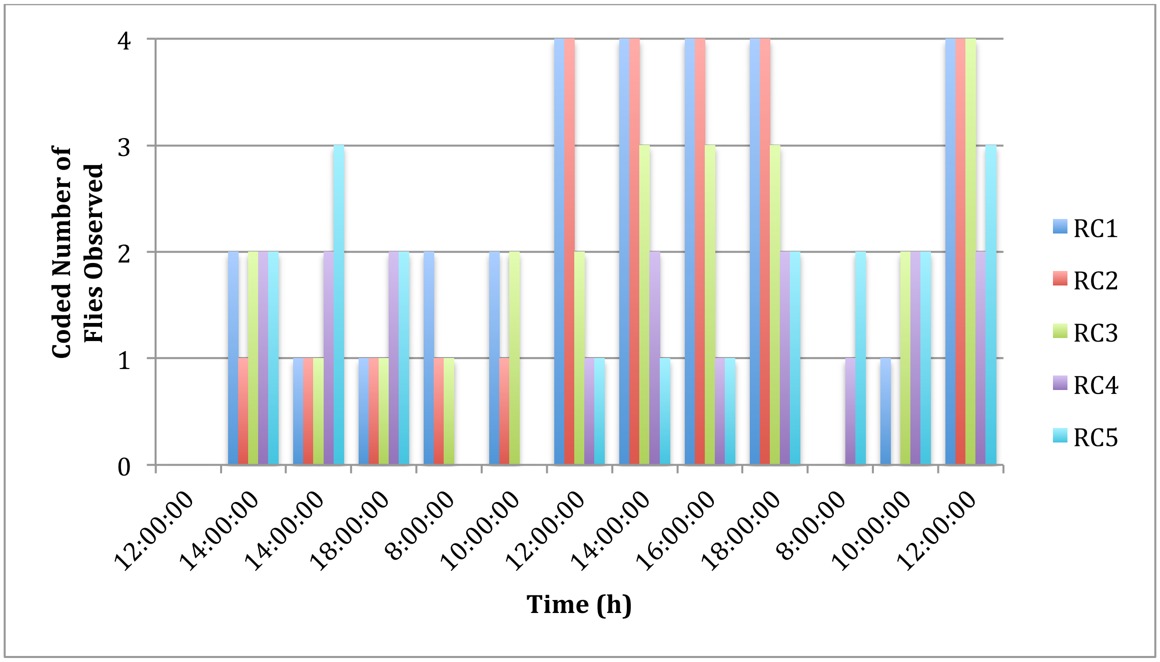

Figure 4.4. The coded number of flies observed by time of day (h), rainy conditions, covered (RC).

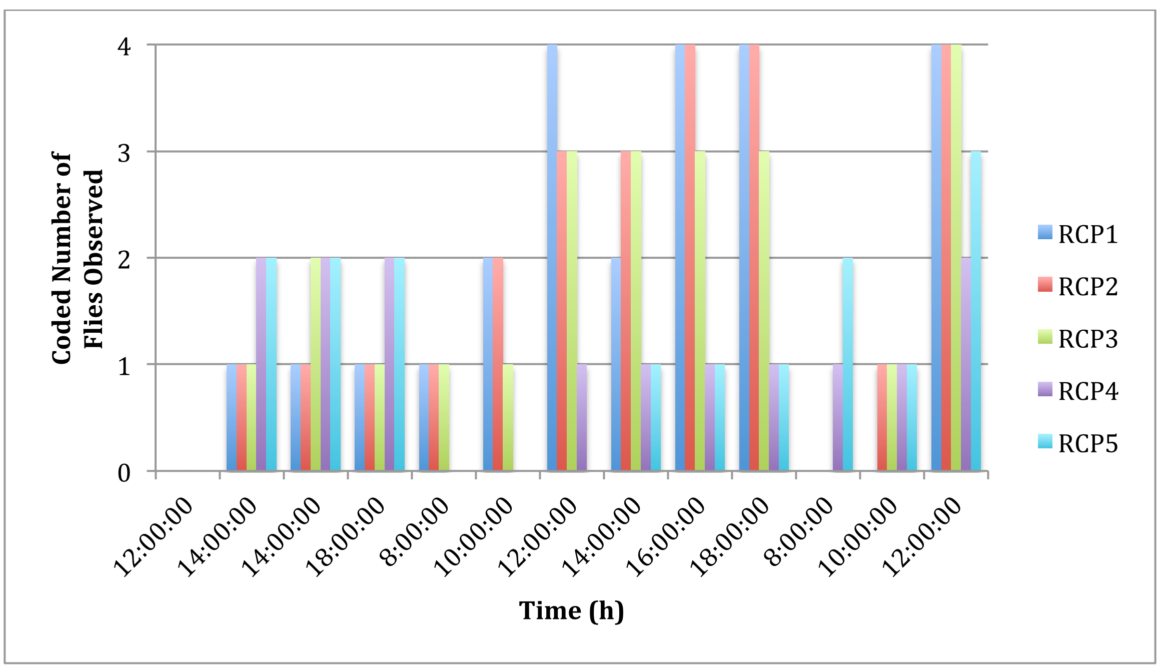

Figure 4.5. The coded number of flies observed by time of day (h), rainy conditions, covered partially (RCP).

Solar Radiation

The amount of solar radiation (W/m^2) that the pig heads were exposed to at the time of observation was plotted against the coded number of flies observed at that time. The results show a positive correlation between solar radiation and the approximate number of flies observed. Appendix B shows all solar radiation data as a function of the coded number of flies observed.

Rainfall

No rainfall occurred during the first 48 hours after deposition for both replicates of the control treatment. The rainfall accumulation during the first 48 hours after deposition for both replicates of the rain treatment is reported in Figure 4.6. Rainfall did not begin during replicate one of the rain treatment until 5 hours post deposition and for 16.5 hours post deposition for replicate two of the rain treatment.

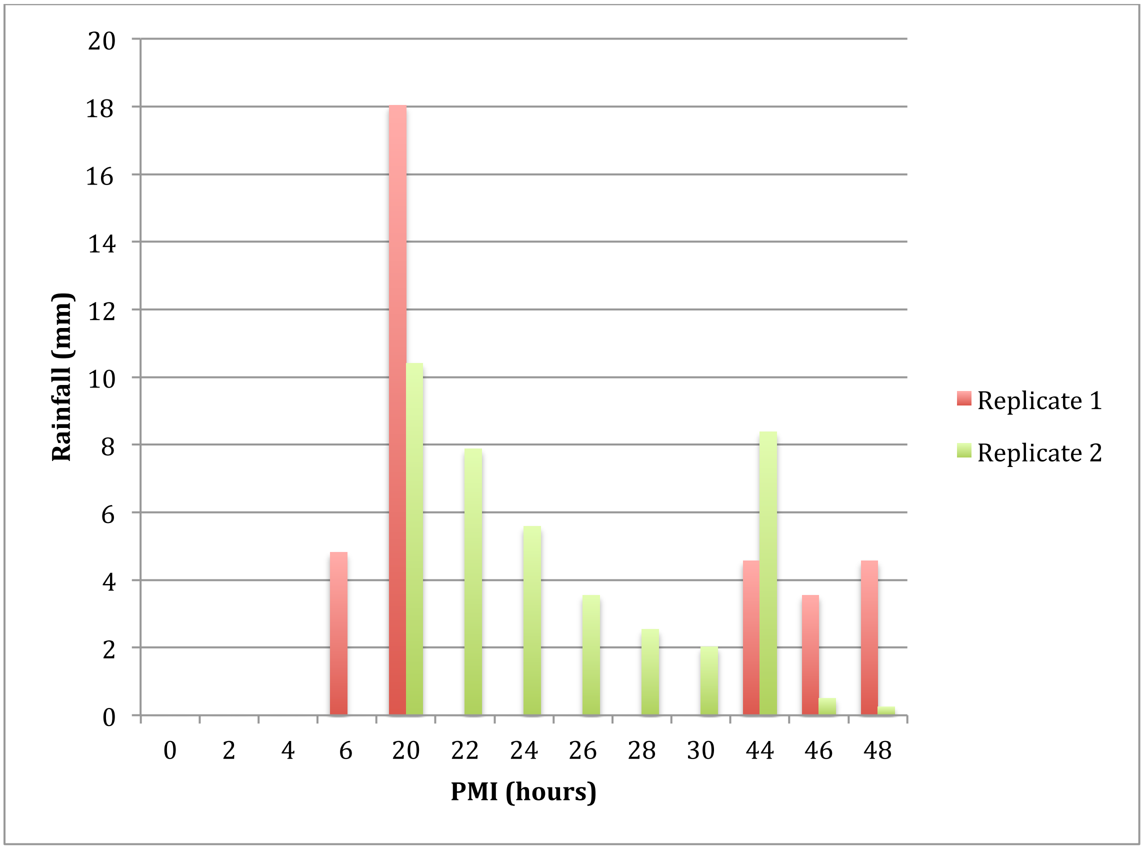

Figure 4.6. Rainfall accumulation (mm) during the first 48 hours after deposition experienced by the pig heads in both replicates of the rain treatment.

For the two replicates of the rain treatment, the amount of rain in mm that had fallen since the last observation was plotted against the coded number of flies observed (Appendix C). The results show a negative correlation between rainfall (mm) and the coded number of flies observed. Table 4.4 reports the R^2 values of these correlations for each pig head in the rain treatment.

Table 4.4. R^2 values of the correlations between rainfall (mm) and the coded number of flies observed.

Pig Head	R^2 Value
R1	0.05226
R2	0.07088
R3	0.08444
R4	0.27487
R5	0.20195
RC1	0.02097
RC2	0.06572
RC3	0.05591
RC4	0.41759
RC5	0.30563
RCP1	0.06196
RCP2	0.06814
RCP3	0.05341
RCP4	0.39285
RCP5	0.23519

Decomposition

Figure 4.7 shows the degree of decomposition, as reported by the TBS as a function of the ADDs for all treatments and conditions. The x-axis shows the ADDs, which allows for comparison between replicates and treatments by normalizing the temperature data. The y-axis shows the TBS, which also normalizes the degree of decomposition for comparison purposes. Figures 4.8 – 4.12 show the changes in the Megyesi *et al.* (2005) alphanumeric decomposition scores over the PMI in days for each treatment and condition of the study. Table 3.1 in the Methods section gives the description of the alphanumeric scoring system (Megyesi *et al.* 2005). If a pig head had passed through several decomposition stages between observations, only the most advanced stage is reported in Figures 4.8 – 4.12. Figure 4.13 is an example of the decomposition changes with associated Megyesi *et al.* (2005) alphanumeric score, as utilized in the current study.

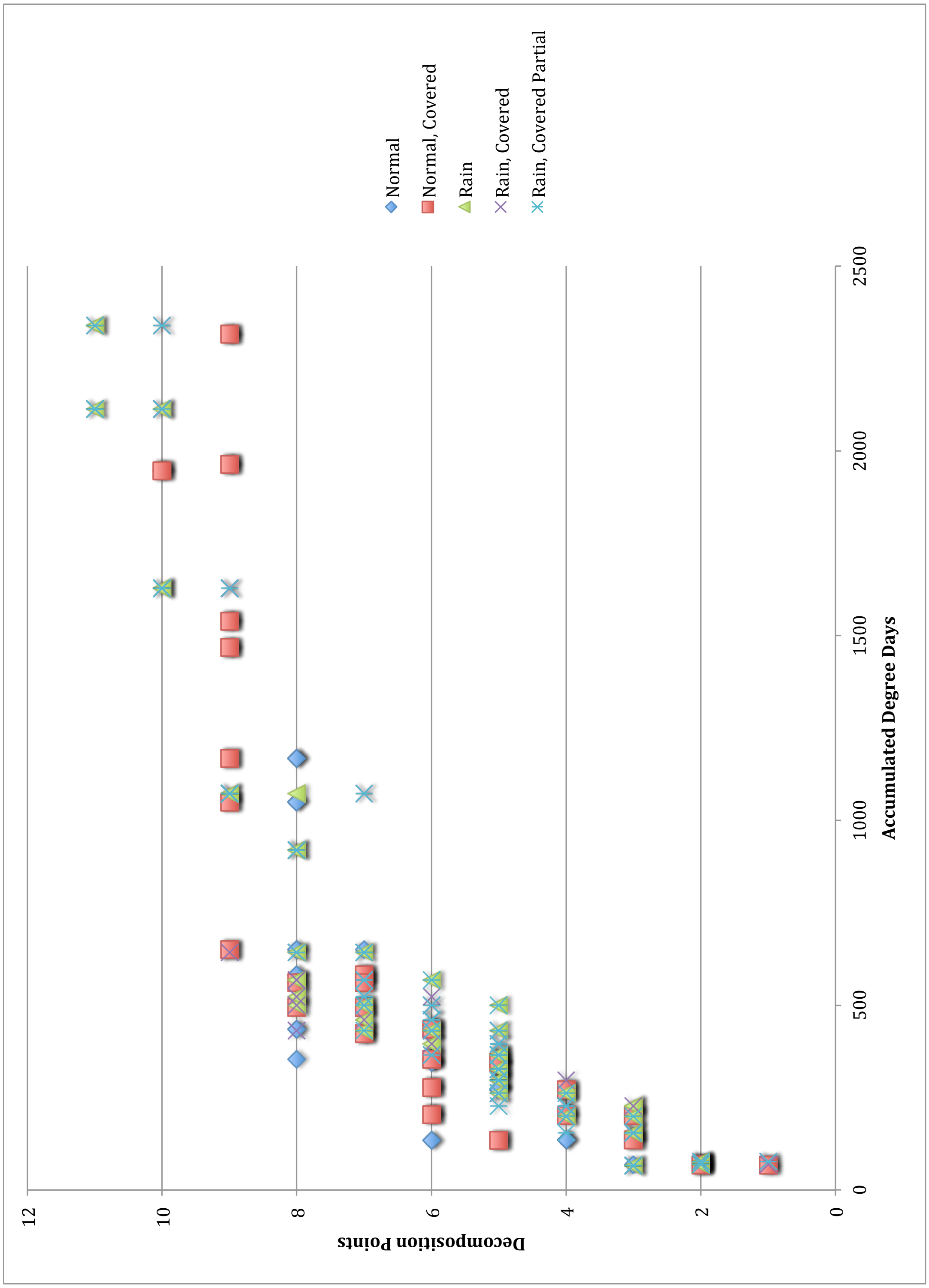

Figure 4.7. Degree of decomposition reported in TBS points (Megyesi *et al.* 2005) by ADDs for control and rain treatments.

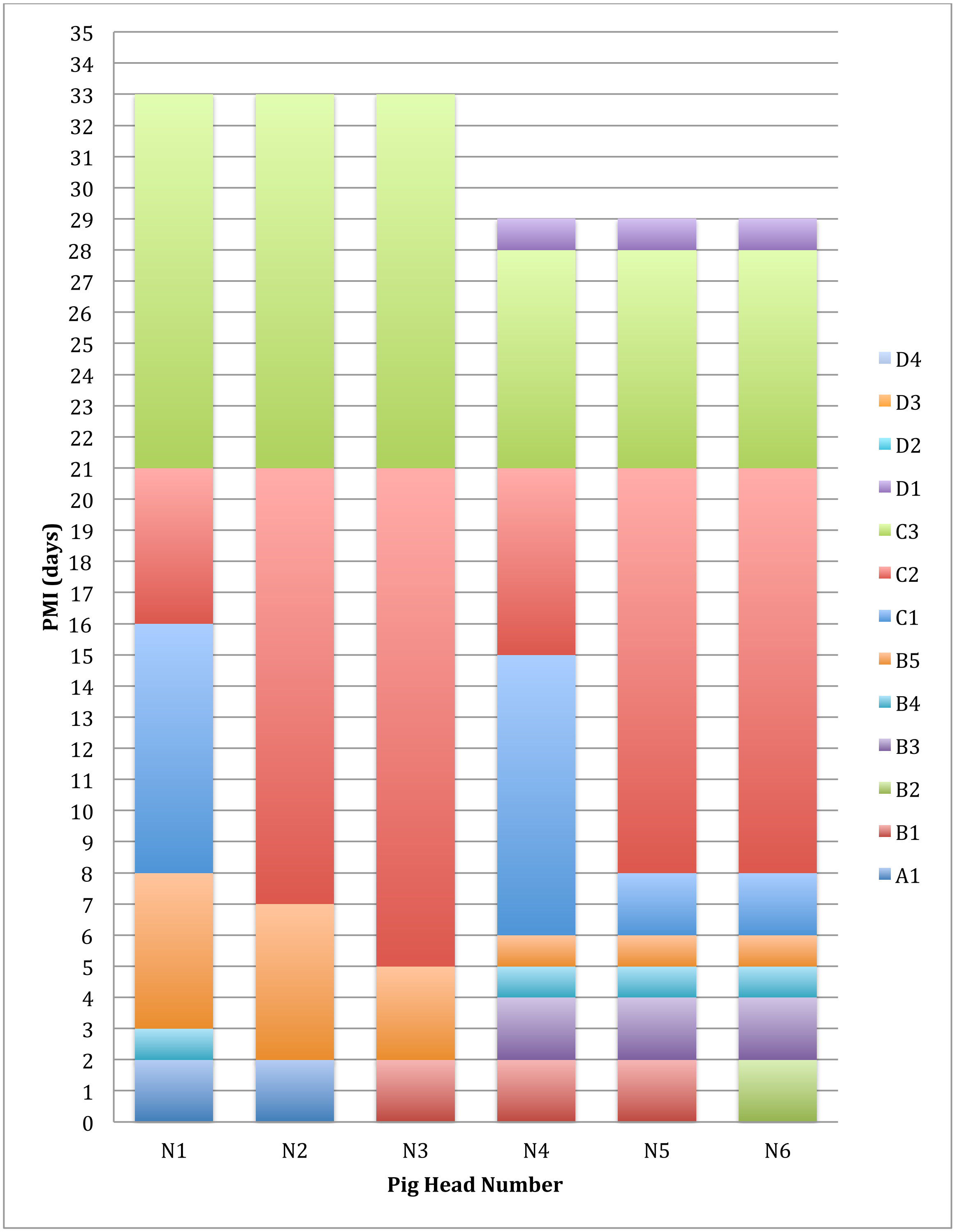

Figure 4.8. Megyesi *et al.* (2005) alphanumeric decomposition score reported for the PMI in days (N).

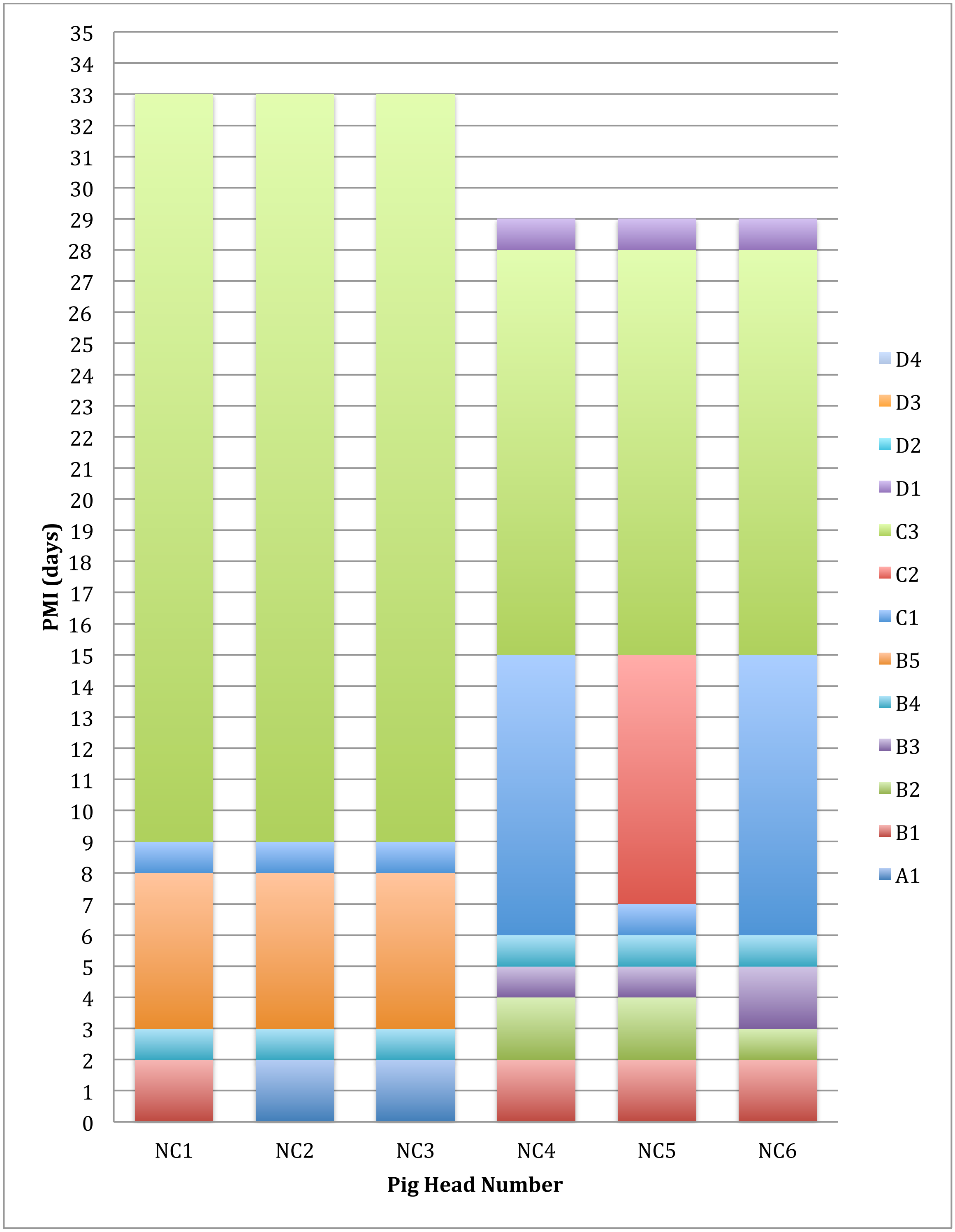

Figure 4.9. Megyesi *et al.* (2005) alphanumeric decomposition score reported for the PMI in days (NC).

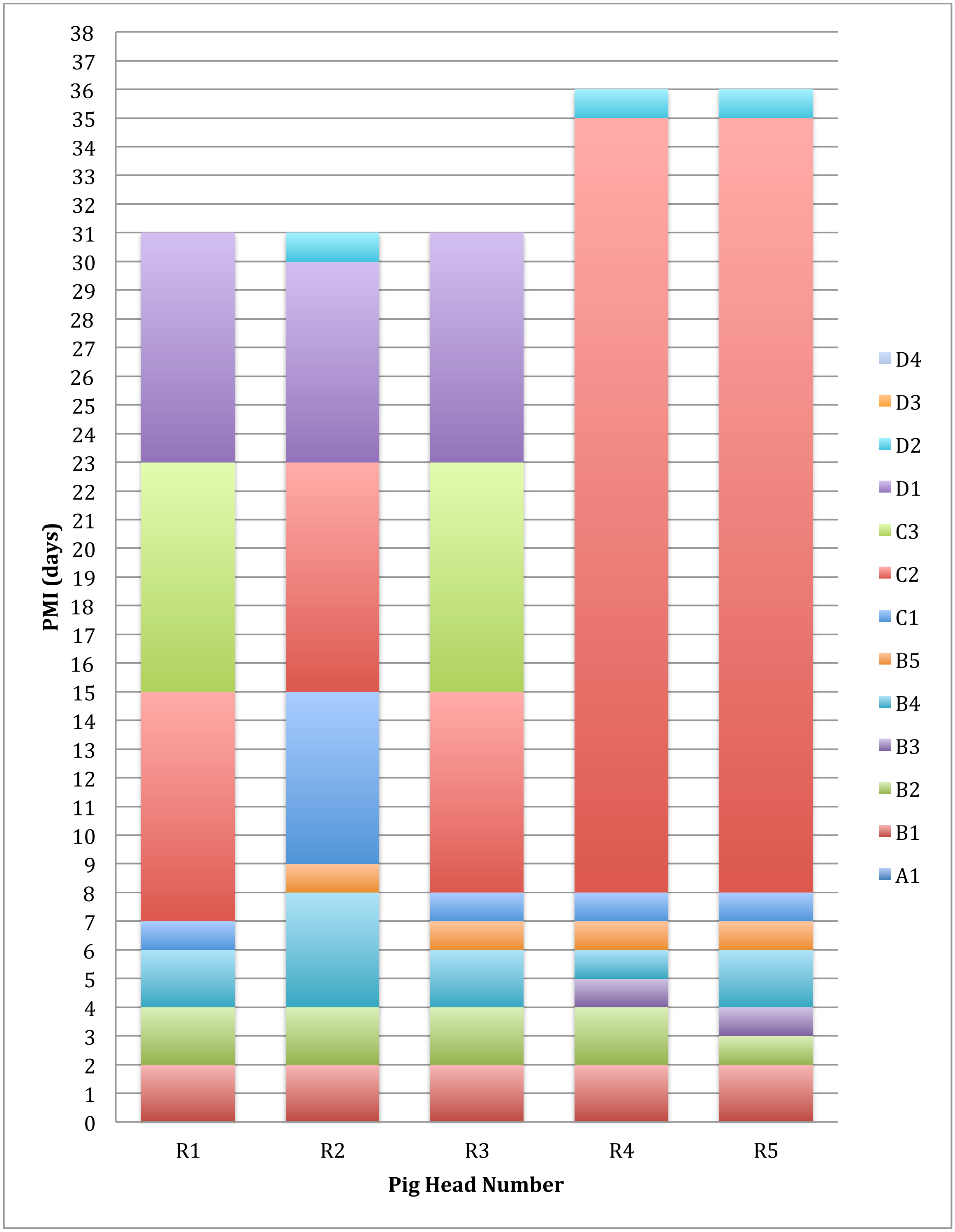

Figure 4.10. Megyesi *et al.* (2005) alphanumeric decomposition score reported for the PMI in days (R).

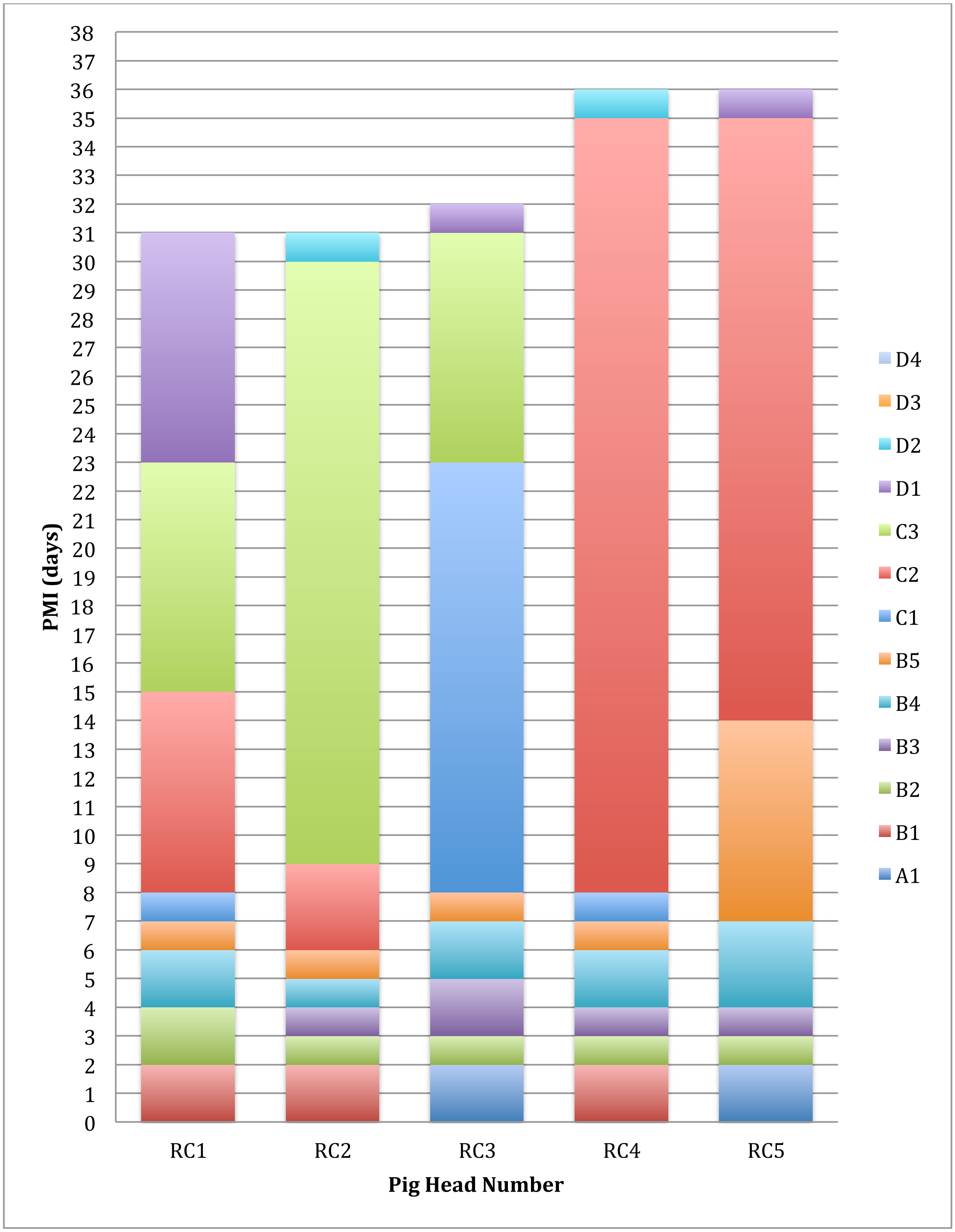

Figure 4.11. Megyesi *et al.* (2005) alphanumeric decomposition score reported for the PMI in days (RC).

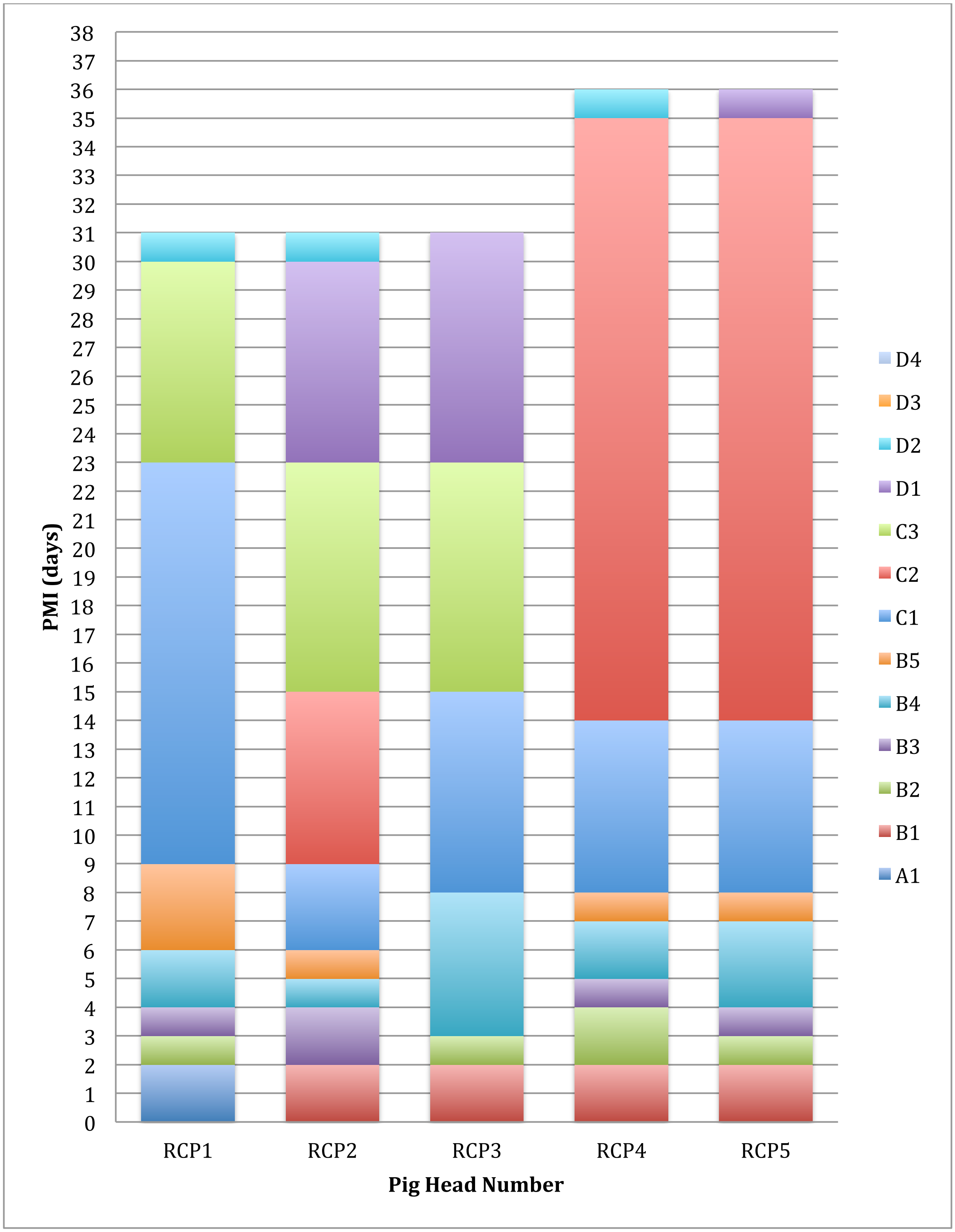

Figure 4.12. Megyesi *et al.* (2005) alphanumeric decomposition score reported for the PMI in days (RCP).

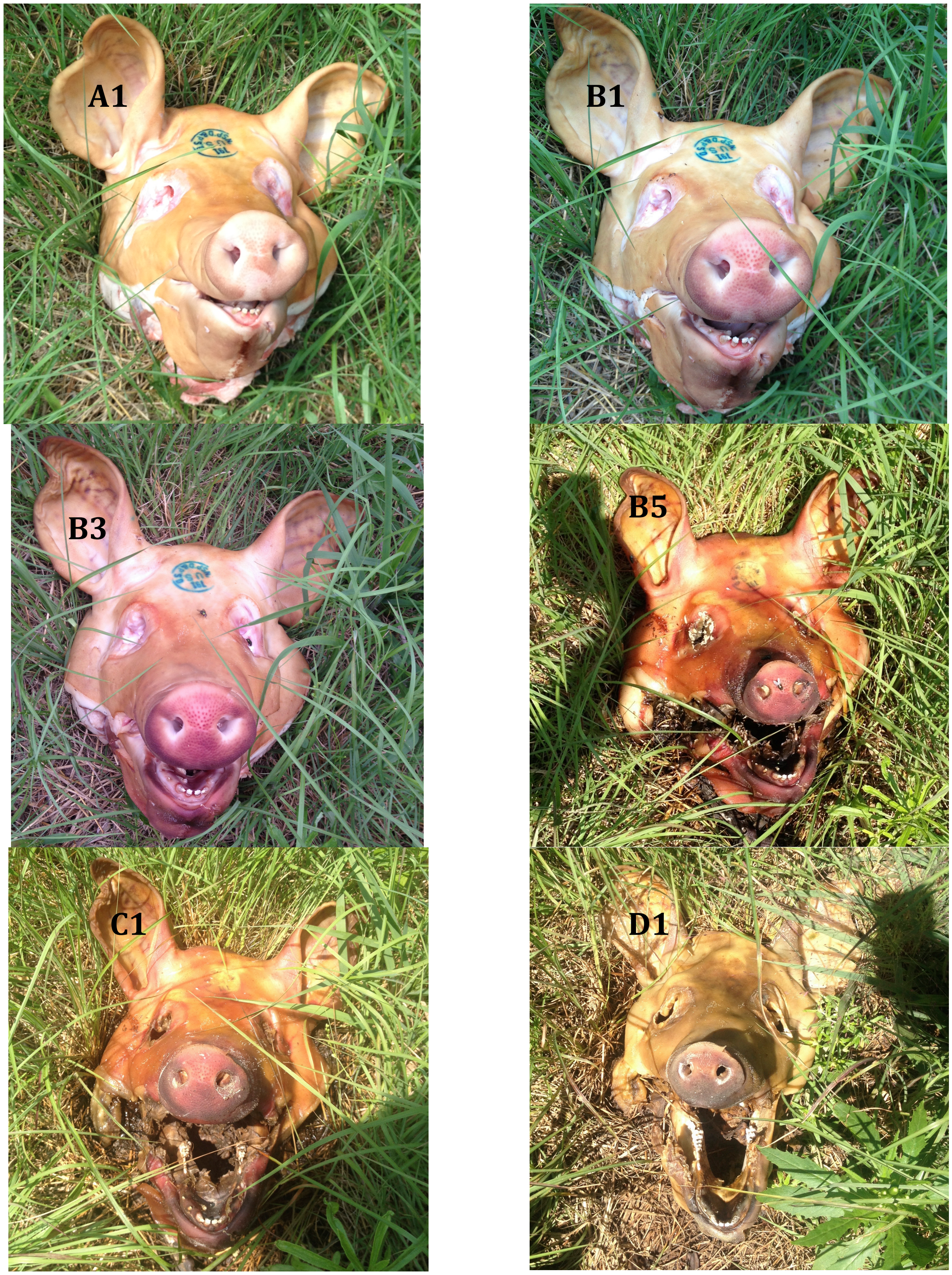

Figure 4.13. Example of the decomposition changes observed during the current study with associated Megyesi *et al.* (2005) alphanumeric score.

The average number of days to reach the advanced decomposition stage for each condition in the control and rain treatments is reported in Figure 4.14 with standard deviations. A one-way analysis of variance (ANOVA) was used to determine if there were significant differences in the number of days to reach the advanced decomposition stage, and the results indicate there were no significant differences between treatments and/or conditions ($p > 0.05$).

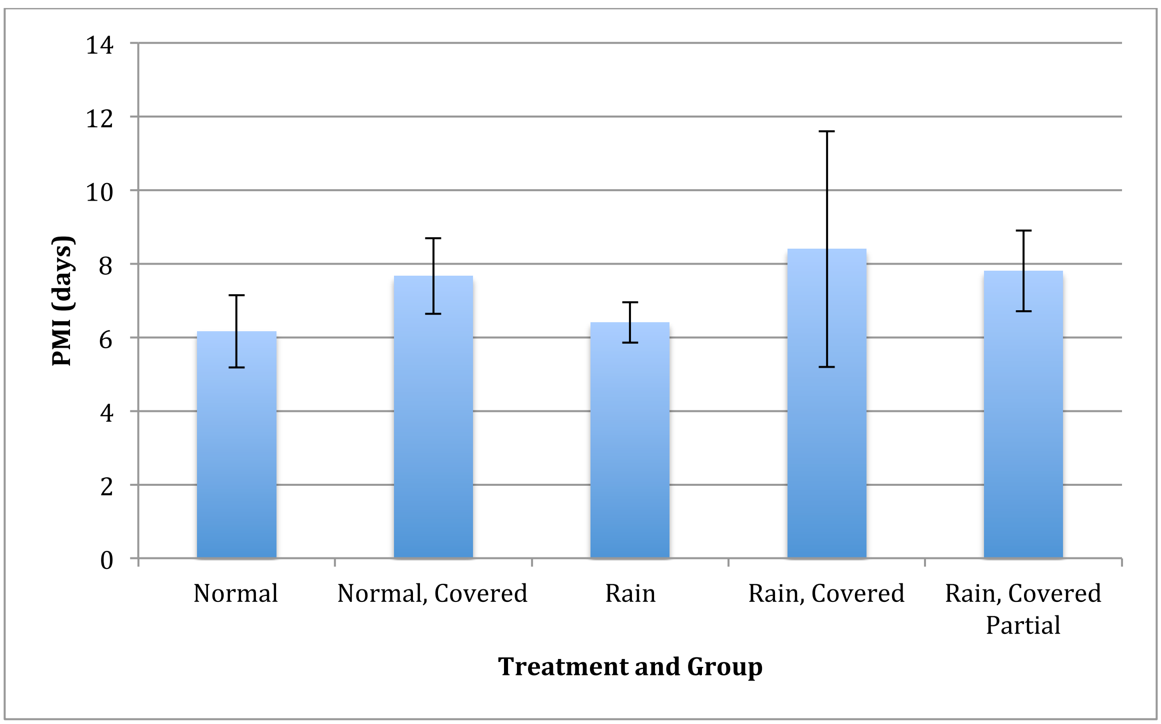

Figure 4.14. Average number of days for each treatment and condition to reach the advanced decomposition stage.

Active Rainfall Trial

The amount of rain (mm) that had fallen since the last observation was plotted against the coded number of flies observed for the active rainfall trial. The results show a negative correlation between rainfall (mm) and the coded number of flies observed (Figures 4.15 - 4.17).

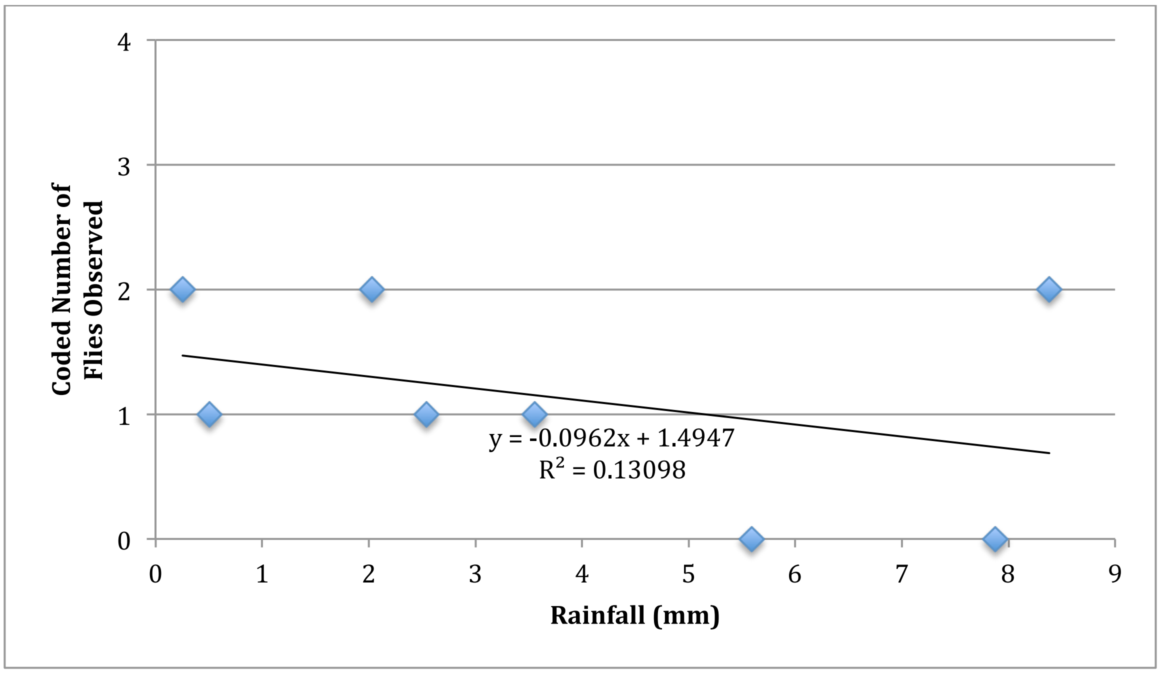

Figure 4.15. The amount of rainfall since the previous observation and the coded number of flies observed (AR1).

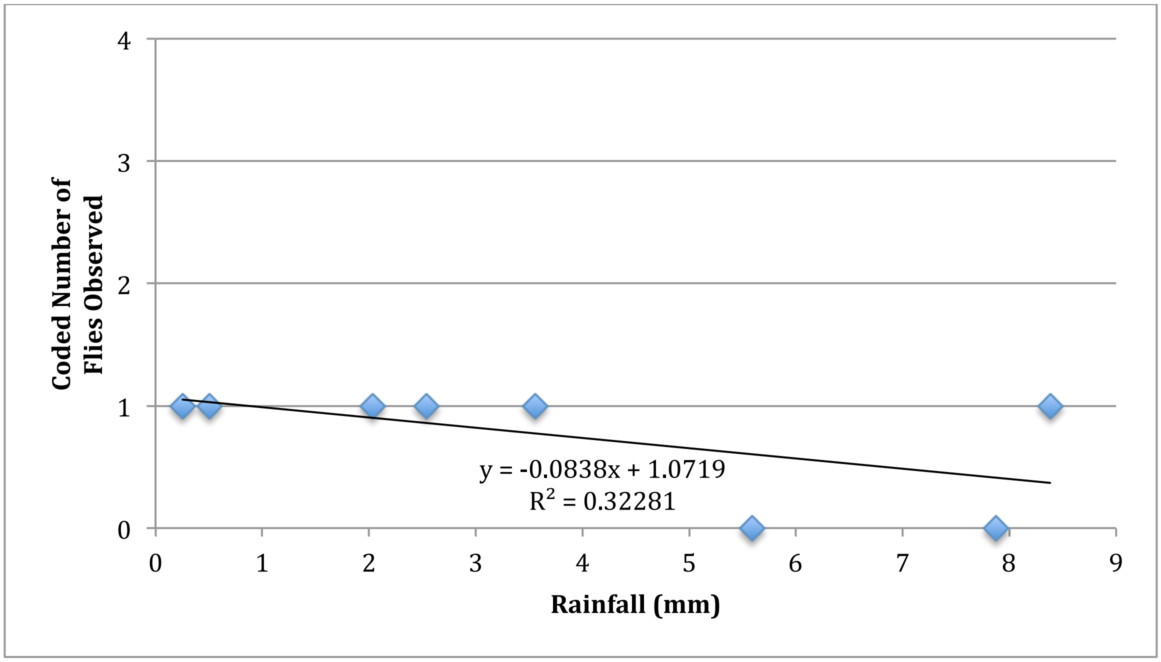

Figure 4.16. The amount of rainfall since the previous observation and the coded number of flies observed (AR2).

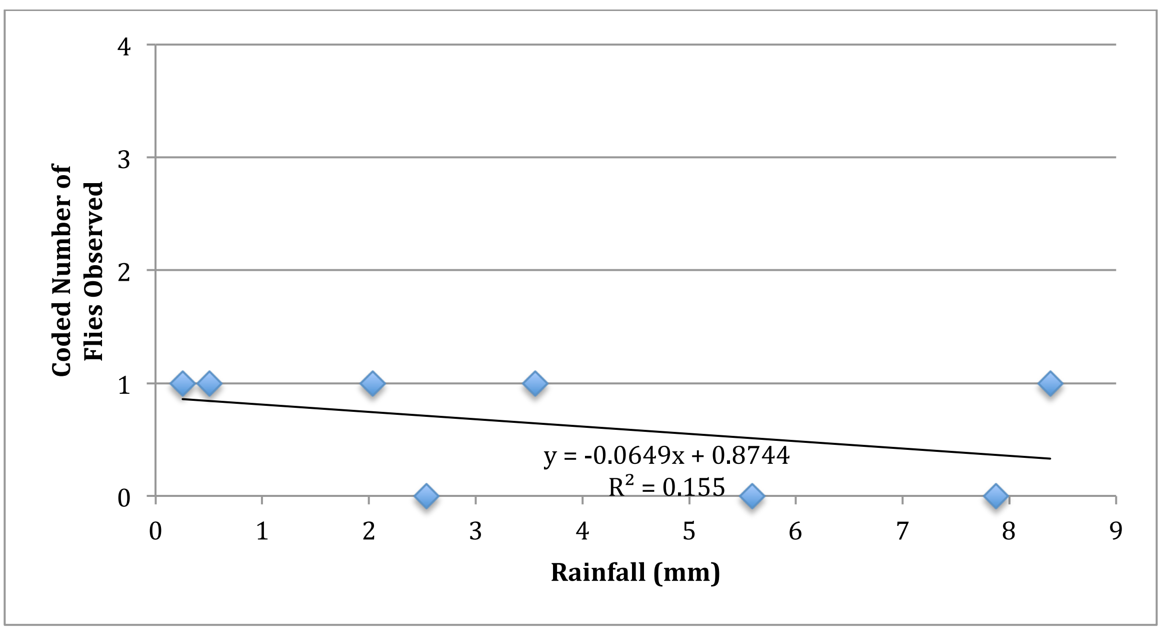

Figure 4.17. The amount of rainfall since the previous observation and the coded number of flies observed (AR3).

Additional Observations

In addition to the results of the preliminary, experimental, and active rainfall trials, there were several additional observations. Appendix D shows all field notes that were recorded for the experimental and active rainfall trials. Please refer to Appendix D for detailed report of the additional observations.

Inter-Trial Observations

The data collected during the experimental trial and the active rainfall trial have been combined to compare for inter-trial differences in the intensity of the rainfall experienced. The average amount of time for the first flies to arrive and the average amount of time for the first egg batches to be observed on the carcasses is reported in Table 4.5.

Table 4.5. Average time (hours) for the first flies to arrive and the first egg batches to be observed on the carcasses.

Section and Group	Mean and SD of first fly arrival (hours)	Mean and SD of first egg batch (hours)
Normal, uncovered (N)	2.7 ± 1.03	5.2 ± 1.79
Normal, covered (NC)	3.0 ± 1.09	5.0 ± 1.09
Rainy, uncovered (R)	2.0 ± 0	2.0 ± 0
Rainy, covered (RC)	2.0 ± 0	2.0 ± 0
Rainy, covered partially (RCP)	2.0 ± 0	2.8 ± 1.09
Active rain (AR)	4.0 ± 0	6.7 ± 1.15

Additional Insect Observations

During the first replicate of the rain treatment, hairy rove beetles were observed in high numbers on most of the pig heads. In fact, on several pig heads, masses of these beetles were observed feeding on the fly eggs and larvae in such high numbers that larval activity was almost halted for a period of time before the flies eventually re-colonized the carcasses. As shown in Appendix D, larvae were first observed hatched from the egg masses on RC2 at 22 hours postmortem and quickly grew into small masses (Figure 4.18). By 28 hours postmortem, over ten hairy rove beetles were observed feeding on the larvae and by 48 hours postmortem, over twenty hairy rove beetles had diminished the population of larvae to almost non-existent (Figure 4.19). On day four postmortem, the population of hairy rove beetles had fallen and new blowfly egg masses were observed as re-colonization occurred (Figure 4.20). These observations highlight how predator-prey relationships can affect colonization of a carcass and the resulting carrion community that is observed.

Figure 4.18. First larvae observed hatched on RC2 at 1000 h (22 hours postmortem) on day two of study.

Figure 4.19. RC2 at 1200 h (48 hours postmortem) on day three of study, over twenty hairy rove beetles have diminished fly larvae population.

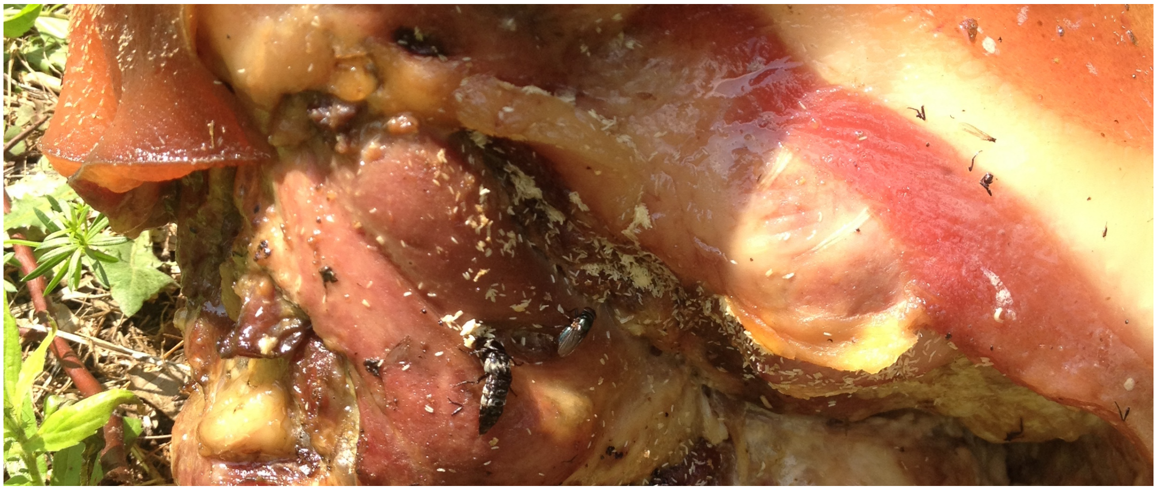

Figure 4.20. Reduced numbers of hairy rove beetles and new blowfly eggs laid on RC2 on day four of the study.

It was observed that larvae tended to distribute across the moistened skin in the RC and RCP groups, often covering the face, than in the NC group (Appendix D). Additionally, on day 8 during replicate one of the control treatment, a small amount of rain gathered in the handles of the overturned plastic basket. This "moat" of water surrounding pig heads (NC1-3) served to drown the post-feeding larvae migrating from the carcasses that day. The handles were cut off all baskets used in the remainder of the study.

As suggested by previous research, certain species of insects observed in association with specific stages of decomposition (Anderson 2011; Campobasso *et al.* 2001; Carvalho *et al.* 2007; Centeno *et al.* 2002; Richards and Goff 1997; Voss *et al.* 2008, 2011). A general pattern can be seen in the Appendix D field notes. The calliphorid species, adults and larvae, are found during the fresh, early decomposition, and late decomposition stages. Sarcophagid and muscid taxa were collected during the same decomposition stages. The American carrion beetle and hairy rove beetle generally arrived after calliphorid larvae had hatched, often feeding on the larvae themselves. Advantageous species, arthropods not specifically necrophagous but who will use the resources and shelter of a decomposing carcass, did not seem particularly associated with a specific decomposition stage, but rather utilized the carcasses throughout the study. Finally, piophilid taxa were identified only in after the advanced decomposition stage was reached (Appendix D).

Blowfly Eggs and Rainfall

The rainfall experienced during both replicates of the rain treatment did not affect pre-established egg masses and subsequent hatching of larvae. For example, as reported in Appendix D, pig head R1 was placed in the decomposition enclosure at 1200 h. When observation occurred at 1400h and 1600 h, several egg masses had already been oviposited within the nostrils and mouth. Rainfall began at 1700 h, and by 1800 h, the egg masses were wet, yet the majority of eggs were protected from the direct effect of the rainfall and from drowning deep within the natural cavities. It was later observed that although some of the exposed eggs were drowned, a large enough number survived for larval masses to become well established (Appendix D).

Turkey Vulture Scavenging

On day 8 of the preliminary trial, Preliminary 3 was found to have moved approximately one meter from its original position. A blackened area, soaked with decomposition fluid, marked the original site of deposition, and the decomposing tongue muscle was missing from the carcass (Figure 4.21). On day 12 of the preliminary trial, bird droppings were observed in association with the carcass leading the author to surmise a scavenging bird was responsible for the disturbance (Figure 4.22). During the control treatment replicates, pig head N1 was moved approximately 1.5 m, N2 30 cm, N3 1.5 m, and N6 60 cm; and during the rain treatment replicates, RCP4 was moved 60 cm and AR3 moved 60 cm on one occasion and over 4 m two days later, all with associated bird dropping and feathers. On day 5 of the active rainfall trial, a turkey vulture (*Cathartes aura*) was observed on AR3.

Figure 4.21. Preliminary 3 on day three of preliminary trial. Note movement from original deposition position marked by black stain of decomposition fluids.

Figure 4.22. Preliminary 3. Note the white bird droppings across the snout of the pig head.

Additional Scavenging Observations

In addition to scavenging by a turkey vulture, several other disturbances not linked to insect activity were noted. On day 6 of the second replicate of the rain treatment, R4 was observed to have moved approximately 60 cm and exhibited heavy soil disturbance (Figure 4.23). On the same day, AR1 and AR2 also exhibited a large amount of excavated soil and disturbed vegetation next to the carcasses (Figure 5.). A high chain-link fence with barbed wire protects the decomposition enclosure, and it is unknown what type of scavenging animal could have produced these disturbances. Possibilities include the raccoon (*Procyon lotor*), fisher (*Martes pennant*), red fox (*Vulpes vulpes*), and several other small to medium-bodied omnivorous and carnivorous animals.

Figure 4.23. Disturbance of R4, note the original deposition site marked by black decomposition fluid and soil disturbance.

Figure 4.24. Freshly excavated soil above AR1, note the loose nature of the soil and disturbed vegetation.

CHAPTER 5: DISCUSSION

The current study hypothesized that natural rainfall would disturb initial blowfly activity by acting as a type of physical barrier, creating a delay in initial colonization and subsequent development. Rain was expected to be a variable that would influence adult blowfly activity and larval development, but the results suggest otherwise. Generally, the results show that the light to moderate rainfall typical of rainy days in the northeastern Unites States will not inhibit fly activity or disturb established maggot masses. The active rainfall trial suggests constant, heavy rainfall was shown to inhibit blowfly activity, but more research is needed to understand if the typical conditions of natural rainfall would make a significant impact on the estimation of the PMI by entomological methods. Additionally, as one of very few studies to address the issue of natural rainfall, some methodological errors need to be addressed to confirm the suggestions made by this study and further research is needed to enhance the results of this study, specifically with a larger sample size for statistical analysis.

Diurnal Behavior

The observation made in the preliminary trial, confirm that in the geographic area of study, blowflies behave diurnally as previously suggested (Amendt *et al.* 2008; Baldridge *et al.* 2006; George *et al.* 2013; Tessmer *et al.* 1995). Flies were observed visiting the two pig heads deposited during daytime hours (1500 h) within 15 minutes of deposition. However, for the pig head deposited during nighttime hours (2100 h), no flies were observed visiting the carcass for the first three hours of observation (Table 4.1). However, by 1300 h on day two of the preliminary study, all pig heads were observed to have 10-30 blowflies in association with the carcasses, appearing to engage in feeding and mating, although no eggs were yet observed. Despite the six-hour difference in the PMI, and the differences in initial blowfly activity between the daytime deposited pig heads and the nighttime deposited pig head, all pig heads in the preliminary trial reached the advanced decomposition stage by day 8 of the trial (Table 4.2).

Influence of Time of Day and Solar Radiation

Figures 4.1 – 4.5 show the relationship between time of day (h) and the coded number of flies observed. Generally, the pattern of a bimodal bell curve is seen over the 48-hour period of observation with the peaks corresponding to approximately midday. These results suggest that blowflies take up to 2-4 hours to be attracted to a carcass once it is deposited outdoors, then increase in numbers peaking midday, followed by a decrease in activity as night falls, only to begin again the following morning, also confirming the diurnal behavior of blowflies. By day two of the trials, increasing decomposition appears to increase the blowfly activity until in some cases, a decrease in numbers was not as pronounced as dusk approached.

A change in solar radiation occurs as the sun moves across the sky during the course of the day, peaking midday when the sun is the closest to earth, with the amount of solar radiation positively correlating to temperature (Bristow and Campbell 1984). Thus, the positive correlation between the coded number of flies observed in association with the carcasses and the amount of solar radiation being experienced at the time of observation that is reported in the results was expected due to the diurnal behavior of flies. These findings are consistent with the results reported by George *et al.* (2005). These authors noted that blowfly colonization in an outdoor environment begins slightly after sunrise and continues until sunset, with peaks in the midmorning, midday, and just before sunset (George *et al.* 2005).

Influence of Rainfall on Blowfly Activity

The results show that the amount of rainfall experienced by each pig head in the rain treatment of the experimental trial, and in the active rainfall trial, is negatively correlated with the coded number of flies observed in association with the carcasses, i.e. as more rain accumulated, fewer flies were observed visiting the carcasses. Table 4.4 shows the R^2 values representing the degree of correlation between the coded number of flies observed and rainfall. The pig heads of replicate one of the rain treatment (R1-3, RC1-3, and RCP1-3; average R^2 = 0.05930) experienced a weaker correlation than the pig heads of replicate two (R4-5, RC4-5, and RCP4-5; average R^2 = 0.30468). Pig heads RC4 (R^2 = 0.41759) and RCP4 (R^2 = 0.39285) experienced the greatest correlation between amount of rainfall and the number of flies observed despite the fact that both pig heads were covered, offering protection from the direct mechanical effects of rain. This result is consistent with the hypothesis that moderate to heavy rainfall will inhibit fly activity by acting as a physical barrier. Fewer flies were able physically to access the pig heads during moderate to heavy rainfall even when cover was provided for them. These results are similar to results reported by Mahat *et al.* (2009) and the observations reported by Reibe and Madea (2009).

The large difference in correlation values between the two replicates of the rain treatment is likely due to differences in the pattern of rainfall between replicates. Figure 4.6 shows the pattern of rainfall over the first 48 hours after deposition for both replicates. As previously mentioned, due to reliance on the natural pattern of rainfall, rainfall did not begin immediately after deposition for either replicate, but rain did fall intermittently for two days post deposition, meeting the conditions outlined in the methods section. Once it began to rain, replicate one initially received more rainfall in the first 20 hours than replicate two, but replicate two experienced more continuous rainfall over the entire two days. This result suggests that the presence of continuous rainfall over the first 48 hours inhibits blowfly activity to a greater degree than shorter bursts of heavier rainfall.

The temperate climate and coastal geography of the northeastern United States are known to produce irregular rainfall patterns (Huntington *et al.* 2009). While northern New England can receive over 250 cm of precipitation yearly (including rain and snow), southern New England can receive less than 100 cm. Additionally, rainfall in this region typically is considered light to moderate, only reaching heavy for short periods of time or during unique weather events such as hurricanes in the summer or nor'easters during winter months (Huntington *et al.* 2009). The results of this study highlight how the unpredictability and variation of natural rainfall can produce inconsistent results dependent on a variety of factors such as the length of time it is raining, the amount of rainfall that accumulates, and the rate or intensity of the rainfall.

Effect of Rainfall on Decomposition

The results of the experimental trial suggest that the presence of rainfall does not affect overall decomposition rate. By reporting the ADDs instead of PMI in days, as a function of TBS we can account for the minor differences in temperature experienced by each control treatment and rain treatment replicate. Figure 4.7 shows that all trials, treatments, and replicates experienced very similar decomposition rates when normalized by the ADDs.

Figures 4.8 – 4.12 show the changes in the decomposition scores (Megyesi *et al.* 2005) over the PMI in days. It can be seen that all pig heads reached the advanced decomposition stage (represented by evaluation of at least stage C1) between day 5 and day 9 of the PMI. Figure 4.14 shows the average number of days each group within the control and rain treatments took to reach the advanced decomposition stage. Statistical analysis of these results show no significant difference between the average number of days to reach the advanced decomposition stage for all treatments and conditions, suggesting that all pig heads decomposed at approximately the same rate.

Active Rainfall Trial

Although rainfall had been predicted by the weather forecast (Weather.com 2014) for the day that each rain treatment of the experimental trial was started, there was a time gap between the deposition of the pig heads and the beginning of rainfall, 5 hours for replicate one and 16.5 hours for replicate two (Figure 4.5). Blowflies were so quickly attracted to the carcasses that all the rain treatment pig heads were colonized before the rainfall began. Then, once the rainfall began, while reducing the number of adult blowflies that were actively visiting the carcasses, it had little to no effect on the egg masses already present and the hatching of larvae (Appendix D).

Figures 4.15 – 4.17 show the amount of rainfall since the previous observation as a function of the coded number of flies observed for each pig head in the active rainfall trial (AR1 – AR3). It can be seen that no more than 10-30 (code = 2) flies were observed during the first 48 hours after deposition while rain was actively falling, while with no rain or during light to moderate rain over 50 flies (code = 4) were quickly attracted to the carcasses. A negative correlation is observed between the coded number of flies observed and amount of rainfall in active rainfall trial. The degree of correlation (average R^2 = 0.20293) for the active rain trial is also similar to the degree of correlation for replicate two of the rain treatment of the experimental trial (average R^2 = 0.30468). This degree of correlation is likely lower due to the overall fewer number of flies observed visiting the pig head in the active rainfall trial.

Oviposition and Larval Hatching

Data were collected identifying the average initial time of oviposition and the average initial observation of larvae for the experimental trial and active rainfall trial (Table 4.6). These results show that for the both the control and rain treatments of the experimental trial, the mean time for flies to arrive at the carcasses was very similar, between two and three hours, while the flies were not observed until four hours post-deposition in the active rainfall trial. However, for the observation of the first egg batches, a longer period before any egg batches were observed was found during the control treatment (means: N, 5.2 ± 1.79 hours; NC, 5.0 ± 1.09 hours) than the experimental treatment (means: R, 2.0 ± 0 hours; RC, 2.0 ± 0 hours; RCP, 2.8 ± 1.09 hours). This result was not expected and it is suspected that these results are skewed by methodological errors.

Observing adult flies visiting the carcasses, landing on the carcasses, and moving around the carcasses is a straightforward observation and the author was able to assess the approximate number of flies engaging in these activities. However, the first replicate of the control trial was the first experience the author had with observation of oviposition and identifying the presence of egg batches. During this replicate, it was observed that the adult flies crawled deep into the nostrils, oral cavity, and ears of the pig heads, presumably to oviposit

(Figure 5.1). The heads were not manipulated to check for the presence of egg batches. However, investigator disturbance has been found to have no significant impact on overall decomposition, insect succession patterns, or insect community composition (De Jong and Hoback 2006; De Jong *et al.* 2011). Thus, during both the second replicate of the control treatment and both replicates of the rain treatment, the author manipulated the carcasses to identify the presence or absence of the majority of egg batches. Removing the first replicate of the control section from the data set results in the average time for observation of the first egg batches in the control treatment to become similar to the average time of observation in the rain treatment (means: N, 4.0 ± 0 hours; NC, 4.0 ± 0 hours). The results suggest that there are no differences in the time of fly arrival or the time of oviposition between the control and experimental treatments.

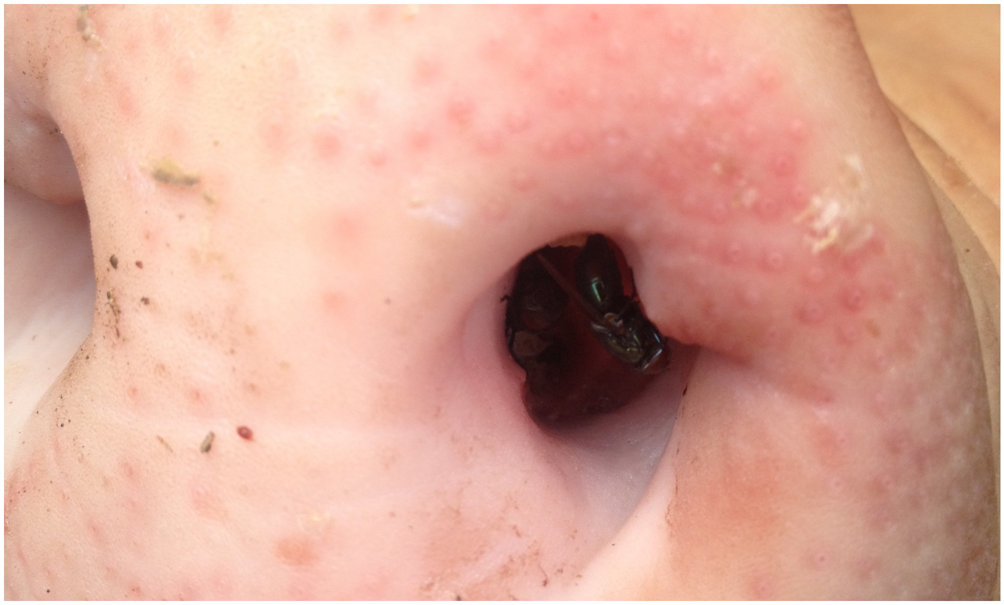

Figure 5.1. Adult fly crawling deep into the nostril of N2, presumably to oviposit.

Blowfly and Other Insect Activity

Two calliphorid species were identified in association with the pig heads, *Phormia regina* and *Lucilia* spp. (Table 4.3). Several other Dipteran families were also collected or observed and include Sarcophagidae, Muscidae, and Piophilidae. While the calliphorid, sarcophagid, and muscid species were all collected via sticky traps during the day two or three of each treatment and replicate, the piophilids were observed only during the advanced decomposition stage in both the adult and larval forms (Appendix D). The observation of these types of taxa are all consistent with other insect succession studies from both other geographic locations and the ORF (Barretta 2012;

Centeno *et al.* 2002; Magni *et al.* 2013; Michaud and Moreau 2009, 2011; Richards and Goff 1997; Rodriguez and Bass 1983; Voss *et al.* 2011, 2008).

In addition to the Dipteran species that were observed in association with the carcasses, several other common carrion insects and advantageous arthropods were identified (Table 4.3). These include the American carrion beetle (*N. americana*), the hairy rove beetle (*C. maxillosus*), beetles of the genus *Nicrophorus*, and several species of ant (Formicidae; Figure 5.2), wasp (Vespidae; Figure 5.3), cricket (Gryllidae; Figure 5.4), and spider (Araneae; Figure 5.5). In particular, the American carrion beetle and hairy rove beetle were observed on the majority of the pig heads, ranging in numbers from a single beetle to more than twenty (Appendix D). These beetles were observed mating, ovipositing, and feeding on both the decomposing tissues and fly larvae. These observations are confirmed by studies also performed at the ORF by Barretta (2012) and Decota (2011).

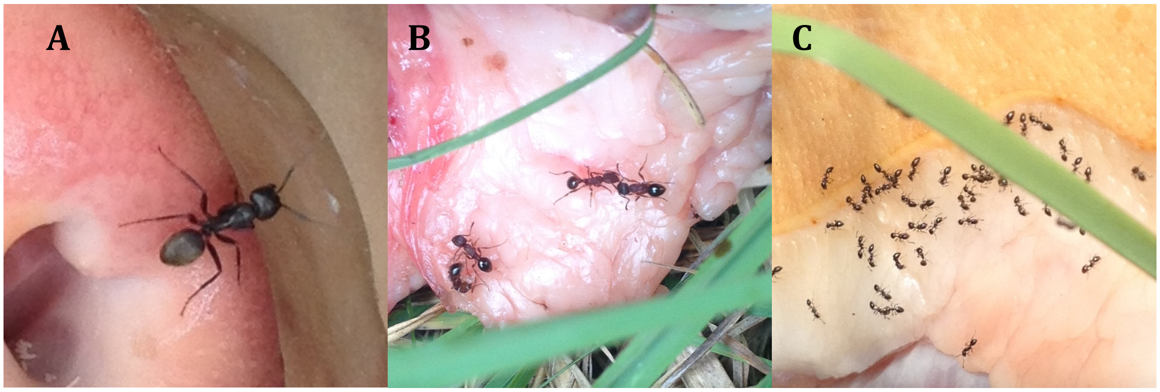

Figure 5.2. Various species of ants (Formicidae) observed on (A) NC6, (B) NC5, and (C) N5.

Figure 5.3. Wasps (Vespidae) observed on NC6.

Figure 5.4. Cricket (Gryllidae) observed on N5.

Figure 5.5. Spider (Araneae) observed on N4.

Predator-Prey Relationships and Spatial Aggregation

The observations described in Appendix C highlight the complicated nature of insect activity, succession patterns, and insect impact on decomposition. As previously discussed, larval predator-prey relationships can influence where adult female blowflies oviposit (Gião and Godoy 2007; Yang and Shiao 2012). This behavior can lead to variation in the species of insects that colonize similar carcasses in identical environments as female blowflies may avoid carcasses previously colonized by another species. Especially in the research context, where many identical carcasses are available to choose from, this may result in inconsistent results within and between replicates (Fiene *et al.* 2014).

Fiene *et al.* (2014) noted that most studies have reported significant variations in successional patterns, particularly among replicate carcasses. The impact of the hairy rove beetles on the blowfly larvae colony of RC2 is an example of the variation seen in the current study. No hairy rove beetles were observed on other pig heads within the same replicate, and fly colonization occurred continuously (Appendix D). Barretta (2012) reported similar hairy rove beetle behavior at the ORF, suggesting that inter-species relationships can play a role in the carrion insect community that is observed. Thus, it is suggested that the traditional temporally based successional paradigm does not fully explain all of the ecological factors influencing insect colonization. The addition of a spatially based successional paradigm has been proposed to help explain this variation (Fiene *et al.* 2014). Spatial aggregation is based on the idea that intraspecific aggregation occurs on patchy resources. In other words, when resources are typically limited and isolated (i.e., patchy), there is high variance in the density of different species of insects. Insects tend to prefer co-colonizing patches already colonized by the same species (Fiene *et al.* 2014). Spatial aggregation helps explain the variation seen between carcasses within the same replicate and between replicates.

Turkey Vulture Scavenging

Due to the high, chain-link fencing surrounding the decomposition enclosure, little to no animal activity was expected. However, this fencing did not protect the pig heads from arboreal scavengers. The turkey vulture is easy distinguishable from other birds by its bright red, featherless head, dark plumage, and large size (Pokines and Baker 2014). Turkey vultures are common to the northeastern United States during the summer months and often inhabit forested areas (Bent 1961). The turkey vulture is known to possess a well-developed olfactory system that allows them to locate carrion over long distances, making them accomplished scavengers. The damage to the pig heads as a result of turkey vulture scavenging highlights the complex, interplay between species that affects overall decomposition. Figure 5.6 shows how turkey vultures feed, stripping the flesh away from the bone in a process called *flensing* (Pokines and Baker 2014). Further studies are currently being designed at the ORF to investigate the role of birds of prey in decomposition.

Figure 5.6. Active rain study pig head AR3. Note how the flesh has been stripped from the mandible by the turkey vulture.

Study Materials

The pig heads utilized for the current study were obtained from a commercial retailer. The Food and Drug Association (FDA) has special requirements for the handling and sanitization of meat meant for human consumption. Sanitization procedures are meant to prevent the growth of pathogenic microorganisms and prevent the spread of foodborne illnesses. While these procedures vary between production facilities, and the particular method employed on the pig heads used in the current study is unknown, sanitization methods can include treatment with chlorine, ozone, chlorine dioxide, trisodium phosphate, hydrogen peroxide, peracetic acid, and flash steaming (Howard and Gonzalez 2001).

The pig heads used in this study were also received frozen. Freezing will kill many of the intrinsic bacteria responsible for the processes of putrefaction. Additionally, the use of pig heads meant that most of the bacterial fauna of the gut, generally responsible for putrefaction were absent from the decomposition environment. These concerns have been investigated by Micozzi (1985) who studied the effect of freezing and thawing on the decomposition of baby pigs. He found that previously frozen pigs predominately showed decay ("outside-in" manner), while freshly killed pigs predominately showed putrefaction ("inside-out" manner). While this allowed for insects more easily to invade the tissues of the previously frozen pigs, it was not found to affect insect succession or overall decomposition rate (Micozzi 1985). For the current study, all the pig heads had been sanitized and frozen in an identical manner, allowing for observation of the relative differences between study treatments.

Another concern involving the study materials was the plastic slotted baskets used as cover throughout the study. These baskets protected the pig heads from the direct mechanical effect of rainfall, but did not fully prevent the pig heads from becoming wet. Water was able to freely drip in between the slots and moisten the flesh of the covered pig heads within the experimental section. This moisture may have influenced the ability of larvae to move more freely around the carcasses (Appendix D).

Decomposition in an outdoor environment has been shown to be a multi-variable process with many overlaying factors that may even create differential observations between carcasses within the same replicate and under the same conditions. The results of this study suggest that rainfall plays little to no role in inhibiting insect activity or affecting overall decomposition. This study has provided both preliminary data for the effect of rainfall on blowfly activity and decomposition, and a multitude of observational data. Several directions for future studies to both confirm the current study and in response to additional questions introduced by this study will be suggested in the conclusions.

CHAPTER 6: CONCLUSIONS

Concerning the effect of rainfall on blowfly activity and overall decomposition, several important conclusions are suggested. This study has provided preliminary evidence suggesting that the light to moderate rainfall typical of rainy days in the northeastern Unites States will not inhibit blowfly activity or disturb established maggot masses. Thus, light to moderate rainfall will most likely not effect the estimation of the PMI by entomological methods. While heavy rainfall may inhibit blowfly activity, if heavy enough over a long enough time period, the irregularity of natural rainfall in the northeastern United States would rarely produce these conditions. These issues have also provided some suggestions for directions of future studies.

Future Studies

Concerning the diurnal behavior of blowflies, this research has suggested their activity to be connected to the time of day and more specifically, the amount of solar radiation that is occurring. While the author observed one pig head (Preliminary 3) placed after sunset for three hours during the preliminary trial and saw no blowfly activity, a larger sample size in necessary to confirm these findings. Additionally, although no blowflies were observed after sunset for the three hours post deposition, it was observed that on day two of the experimental trial the estimated number of blowflies did not reduce as much as sunset approached, probably due to the increased decomposition of the carcasses. Future research is needed to observe what happens after sunset on these types of previously established carcasses.

Additionally, more research is needed examining the effect of different intensities and durations of rainfall. Relying on natural rainfall was found to have many inherent difficulties. The experimental trial results suggested that the presence of continuous rainfall over the first 48 hours inhibits blowfly activity to a greater degree that short bursts of heavier rainfall. The active rainfall trial also confirmed this result. These results should direct future studies to parse out the effects of intensity and duration of rainfall. It may be useful to begin future studies during a time of year, such as the spring, when rainfall occurs more often and heavier. However, this would also affect the species of blowfly that are observed due to seasonality (Archer 2003a, 2003b; Centeno *et al.* 2002; Michaud and Moreau 2009; Voss *et al.* 2011). Additionally, more generally, a larger sample size is needed to increase the statistical strength of the results.

Several methodological errors were found to be a possible source of error within the current study and should be avoided in future research. These include the impact of meat sanitization processes and the use of frozen carcasses. Although not available for the current study, it is possible to obtain unsanitized, freshly killed carcasses for future studies from local agricultural sources. The concerns for using frozen carcasses can also be avoided in this way. Another methodological concern involved the plastic slotted baskets uses for cover. If future research questions wish to provide complete cover from rainfall, another method of cover should be employed. Also, for further research in which post-feeding larvae, pupae, or teneral flies are of interest, it would be important to avoid issues similar to the "moat" created in the handles of the overturned plastic baskets.

Forensic entomologists may be interested extending the current research to answer more specific questions about the species of blowflies that are colonizing the carcasses. With a proper entomological methodology, it may be possible to detect variation in the species of blowfly that are active on rainy versus non-rainy days. For example, certain species of blowfly are known to prefer shady environments (Mahat *et al.* 2009). The nature of rainstorms creates a temporarily shaded environment via cloud cover. These and other species-specific questions may direct future studies.

The results of this study illustrate how complicated and multivariable the process of decomposition can be. A vast number of variables can increase or decrease decompositional change. In the current study, along with the main variable of rainfall, temperature, access to the body by insects, spatial aggregation, time of day, solar radiation, covering, and scavenging by turkey vultures all were observed to play a role in decomposition and blowfly activity. Of significant importance is the difficulty in pinpointing the effect of a specific variable on decomposition in an outdoor field study and controlling for one variable in the dozens at play. Specific to this study was the difficulty in predicting the occurrence and amount of rainfall during the rain treatment. Further studies with an increased amount of samples and replicates would help parse out these issues.

While it was hypothesized that natural rainfall would disturb initial blowfly activity by acting as a type of physical barrier and create a delay in colonization and subsequent development, the preliminary results suggest otherwise. Generally, the results show that the light to moderate rainfall typical of rainy days in the northeastern Unites States will not inhibit blowfly activity or disturb established maggot masses. While heavy rainfall may inhibit blowfly activity, it is thought that the irregularity of natural rainfall may only rarely produce the conditions necessary for this to make a significant impact estimation of the PMI by entomological methods. More specifically, the preliminary results of this study showed no statistically significant differences between the experimental trial treatments and the active rainfall trial in the average amount of time to reach the advanced decomposition stage. All pig heads decomposed at a similar rate.

APPENDIX A

Data Collection Sheet – McLeod Thesis Summer 2014

Conditions & Sample Number:						
Date	Time (h)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005) Decomp.	Other Insects Observed/Notes

APPENDIX B

Appendix B reports the graphical representation of the amount of solar radiation (W/m^2) the pig heads were exposed to plotted against the coded number of flies observed. These graphs generally show a positive correlation of solar radiation and the coded number of flies observed across all groups in the experimental trial.

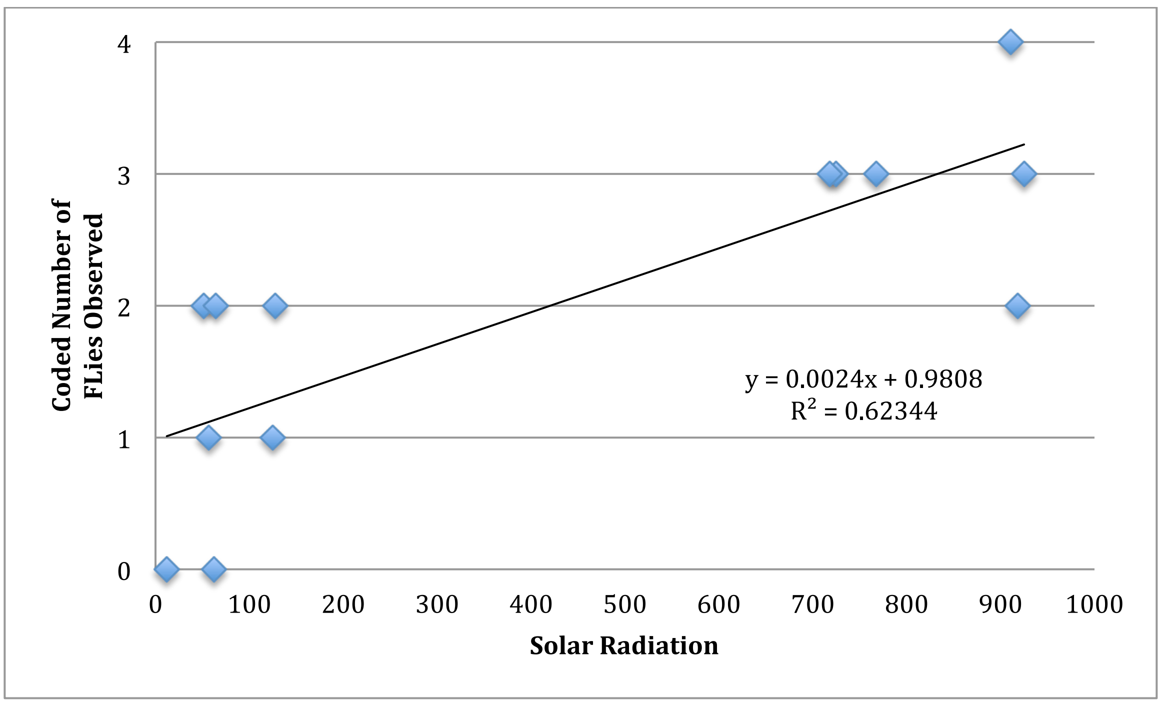

Figure B.1. Normal conditions, uncovered (N1).

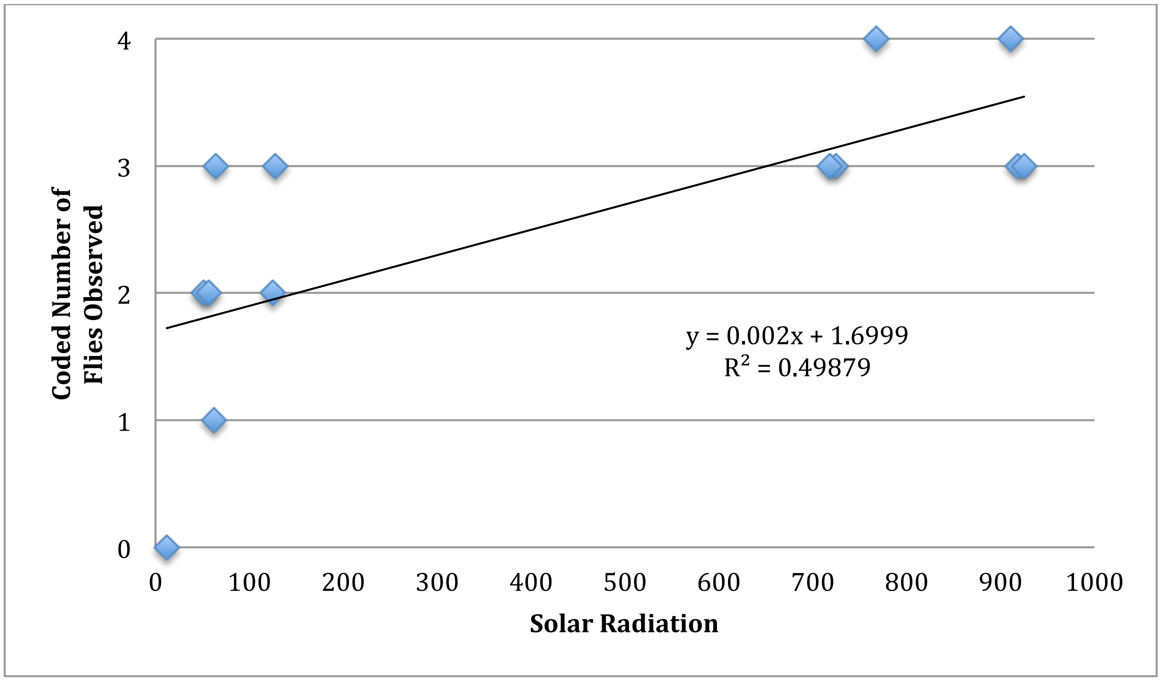

Figure B.2. Normal conditions, uncovered (N2).

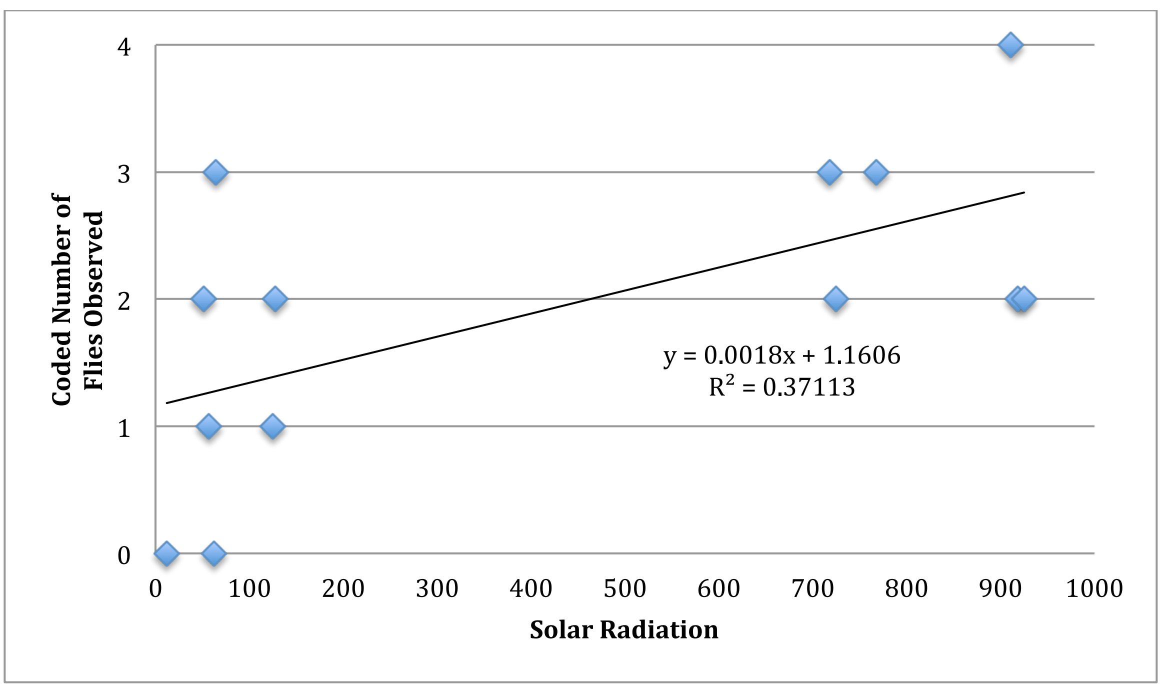

Figure B.3. Normal conditions, uncovered (N3).

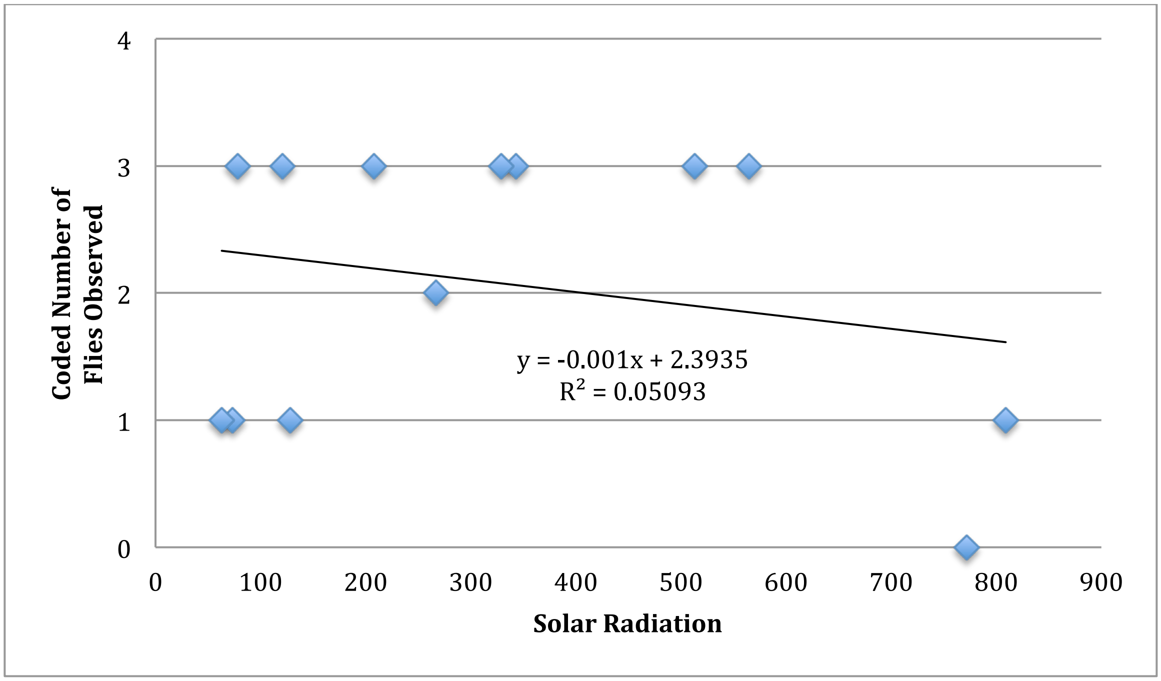

Figure B.4. Normal conditions, uncovered (N4).

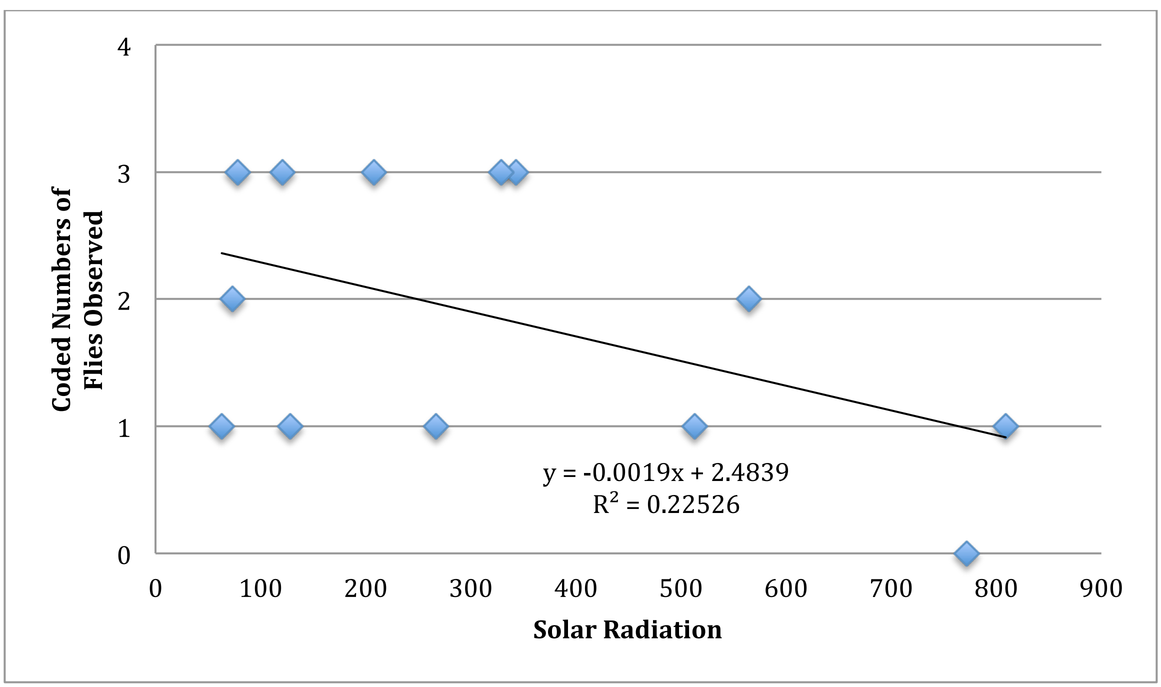

Figure B.5. Normal conditions, uncovered (N5).

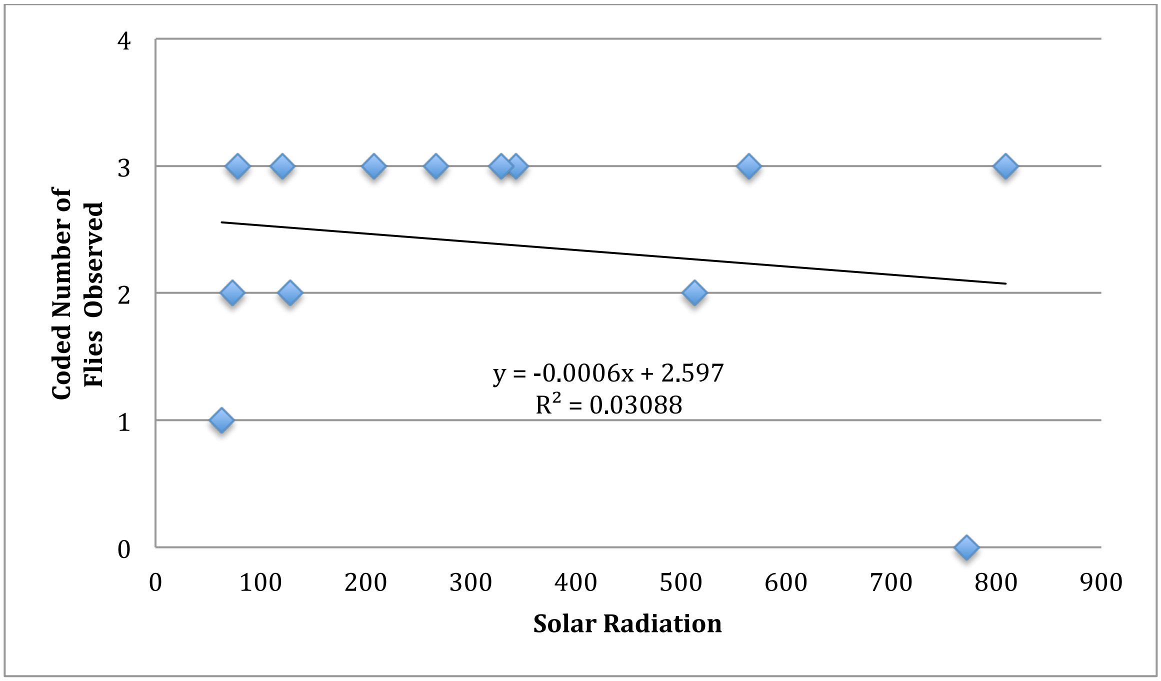

Figure B.6. Normal conditions, uncovered (N6).

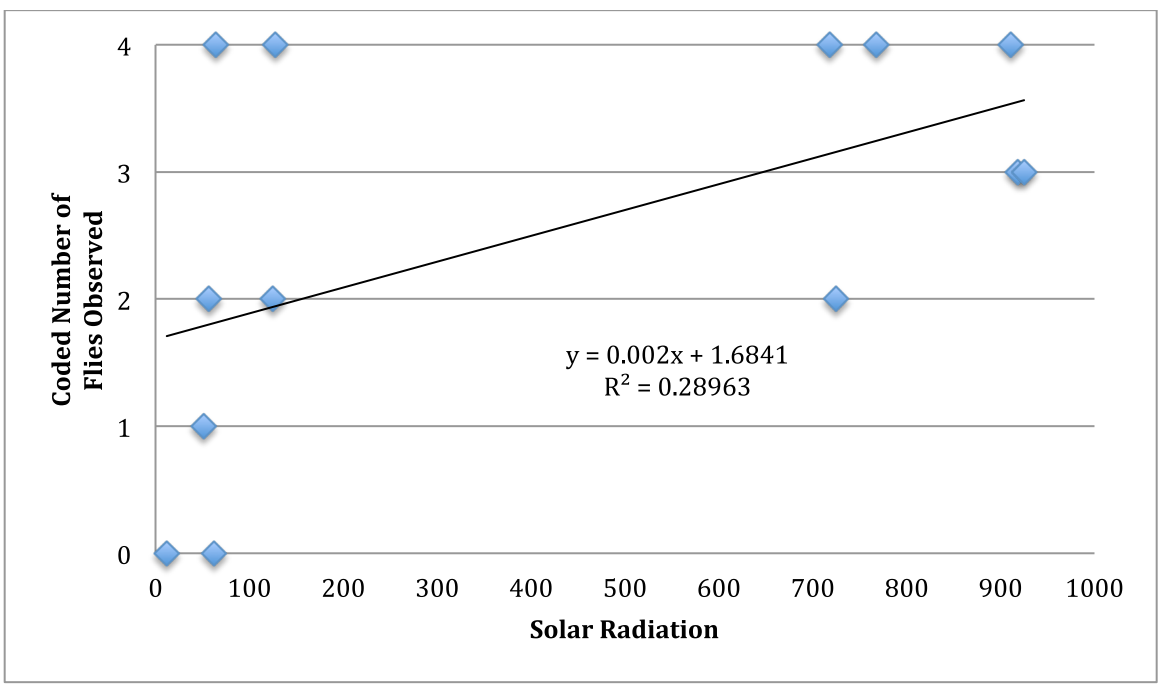

Figure B.7. Normal conditions, covered (NC1).

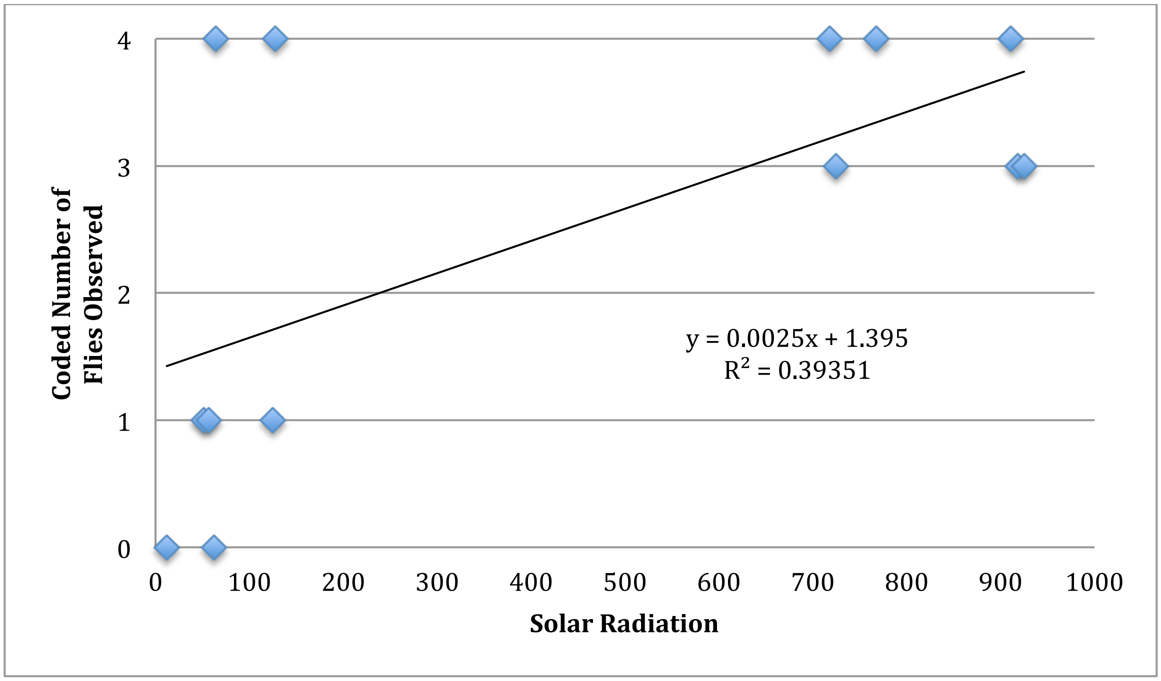

Figure B.8. Normal conditions, covered (NC2).

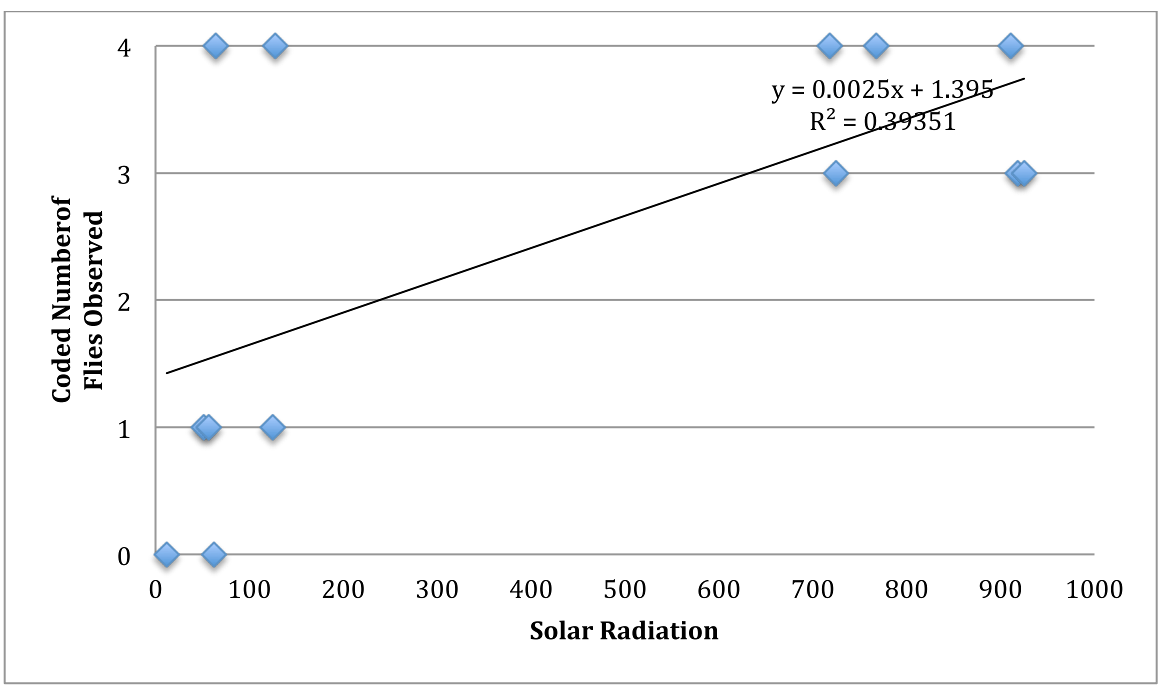

Figure B.9. Normal conditions, covered (NC3).

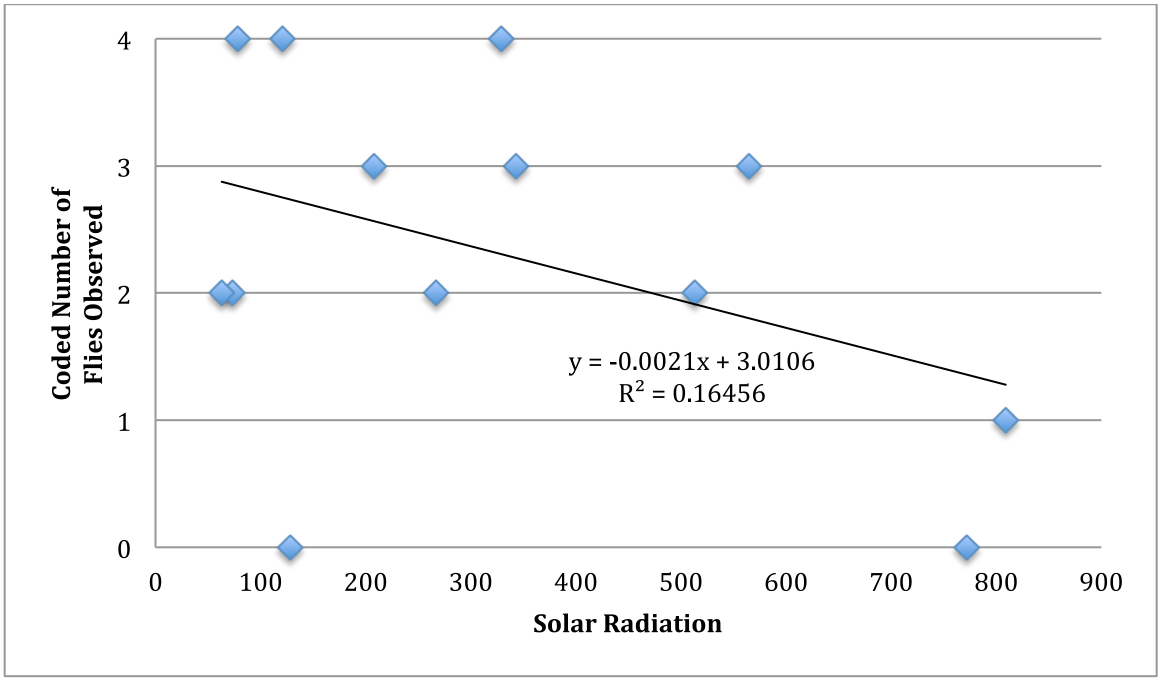

Figure B.10. Normal conditions, covered (NC4).

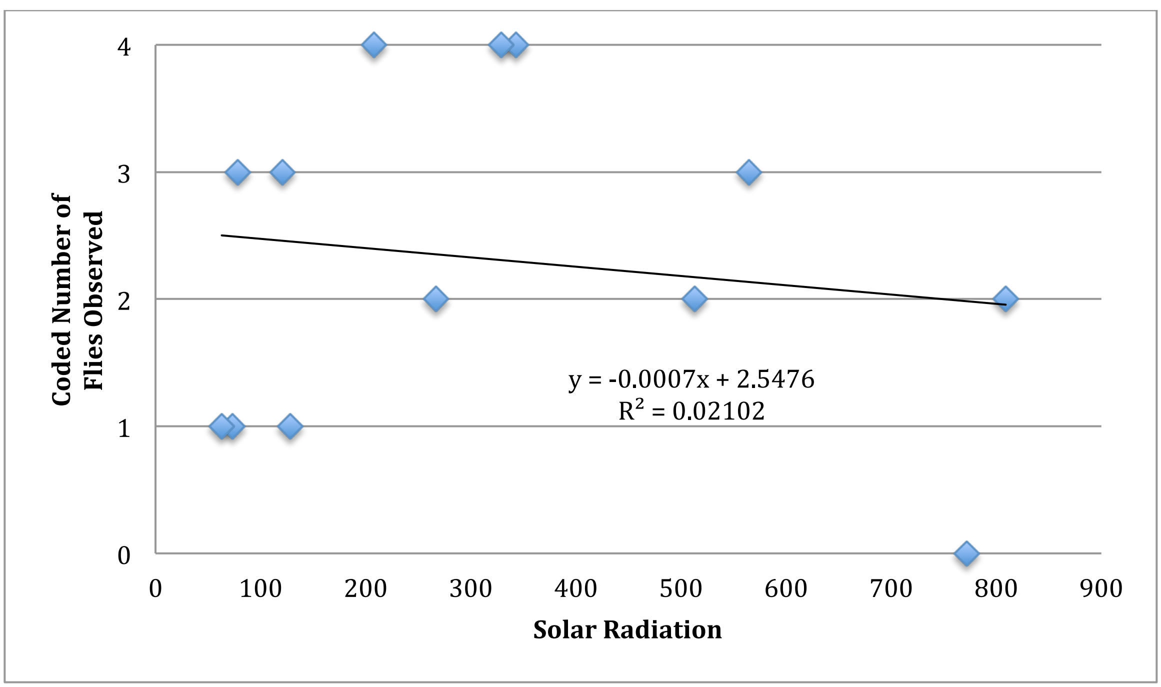

Figure B.11. Normal conditions, covered (NC5).

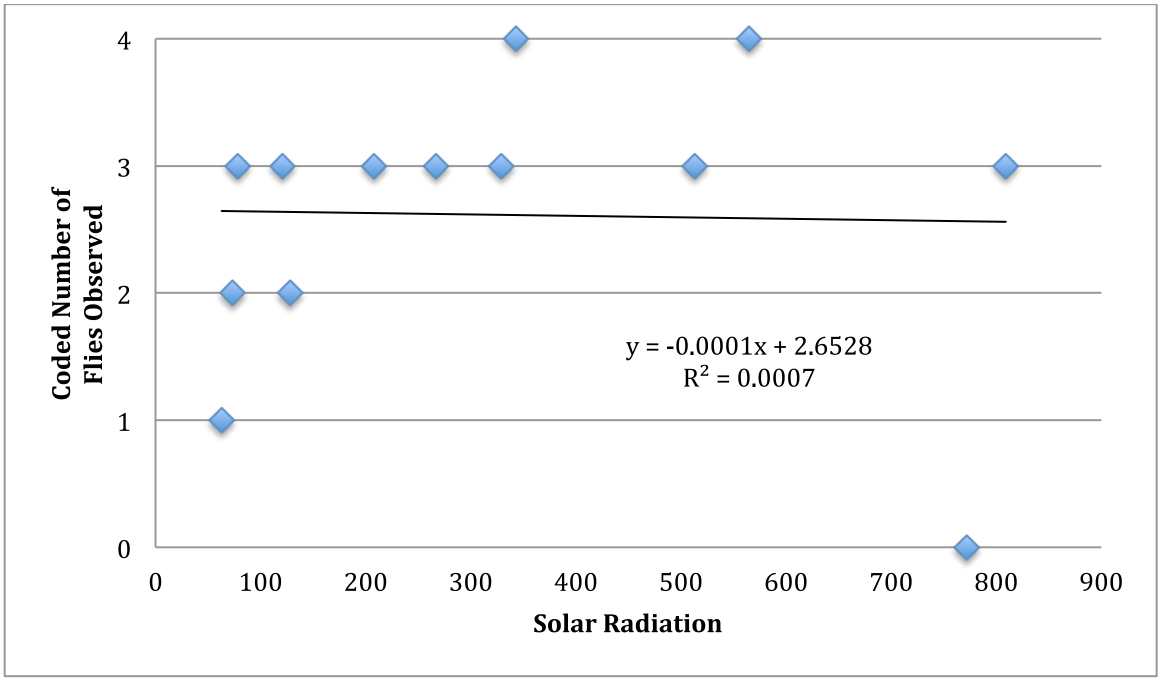

Figure B.12. Normal conditions, covered (NC6).

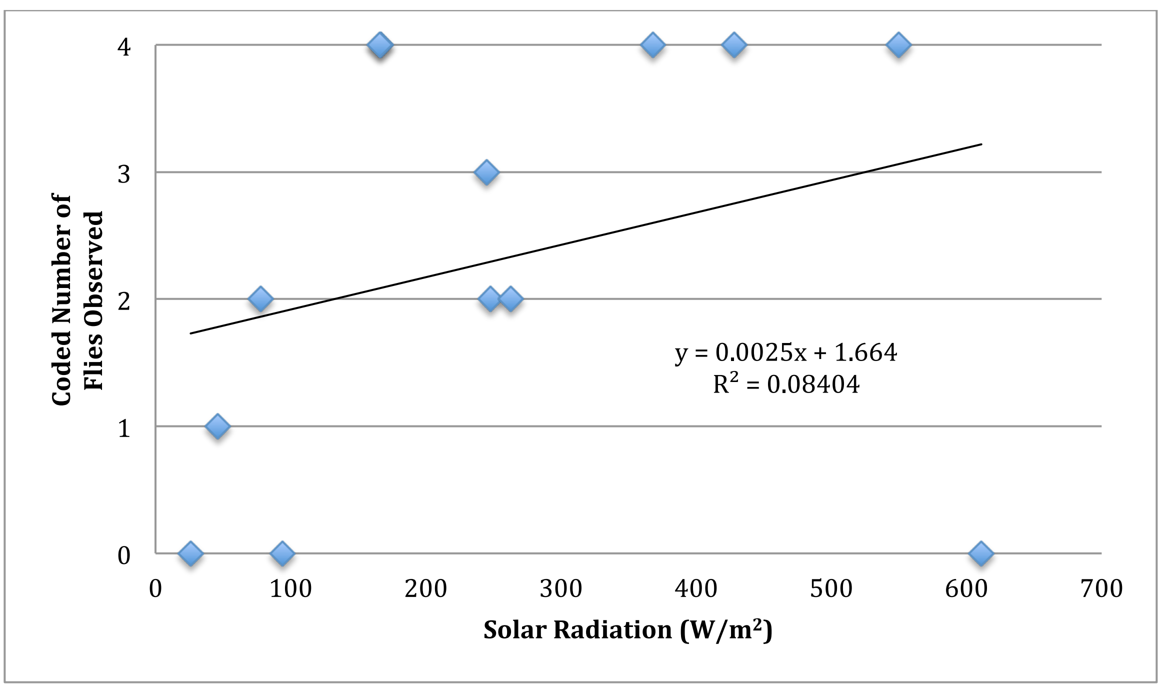

Figure B.13. Rainy conditions, uncovered (R1).

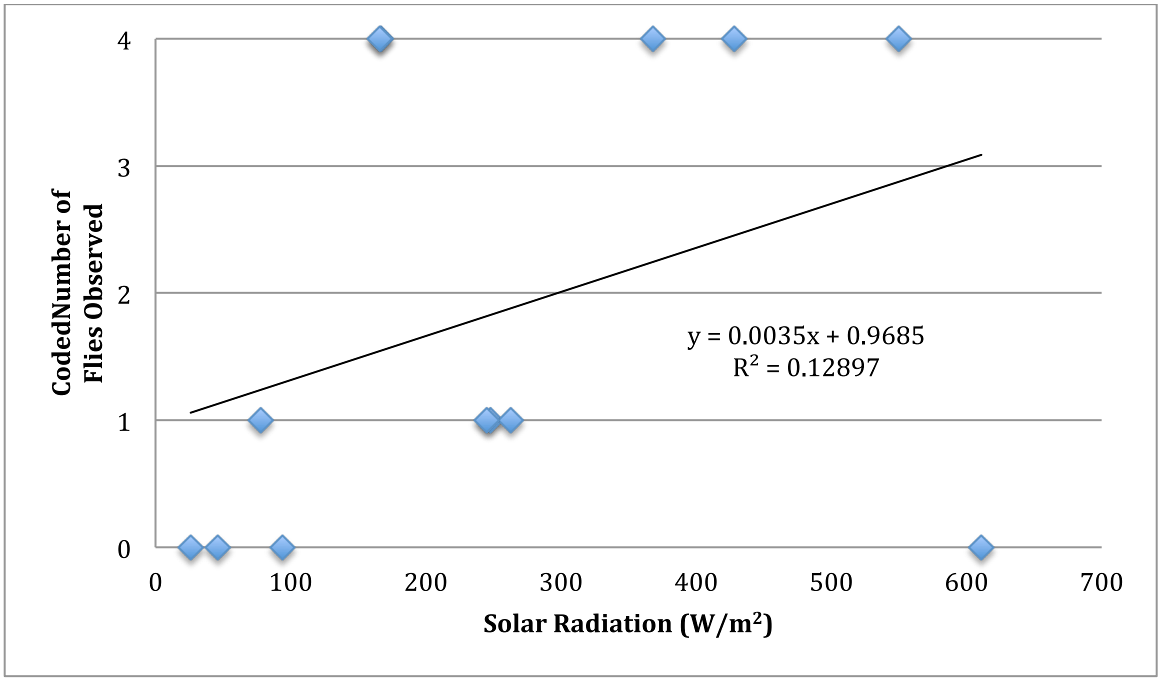

Figure B.14. Rainy conditions, uncovered (R2).

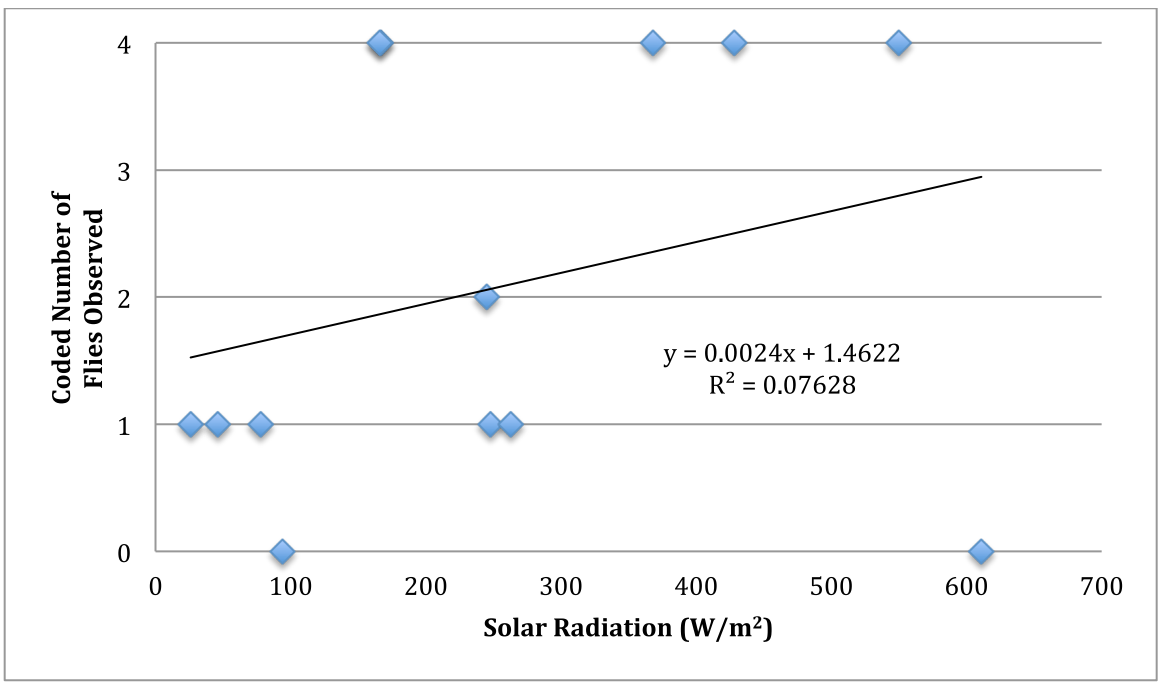

Figure B.15. Rainy conditions, uncovered (R3).

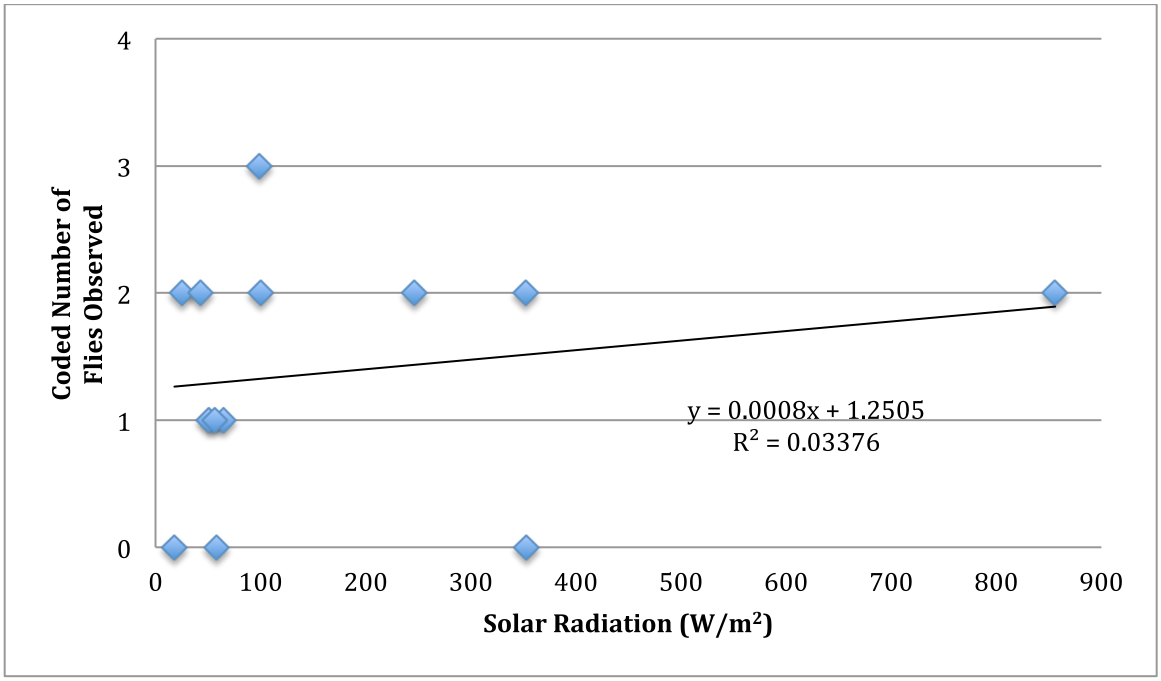

Figure B.16. Rainy conditions, uncovered (R4).

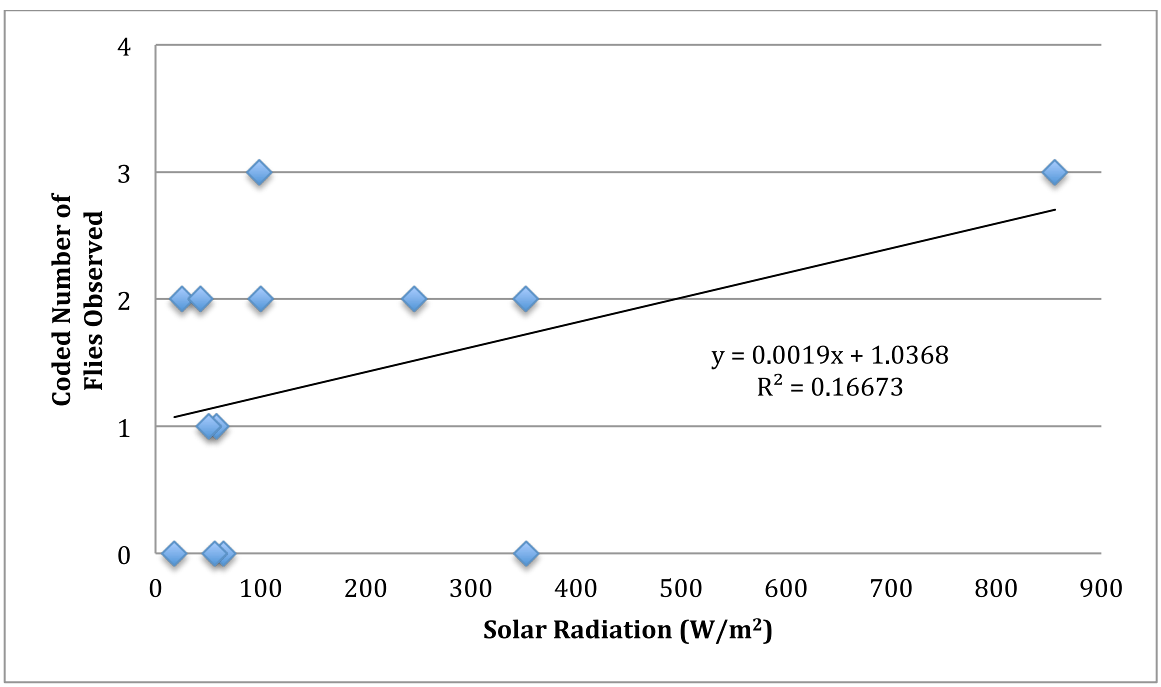

Figure B.17. Rainy conditions, uncovered (R5).

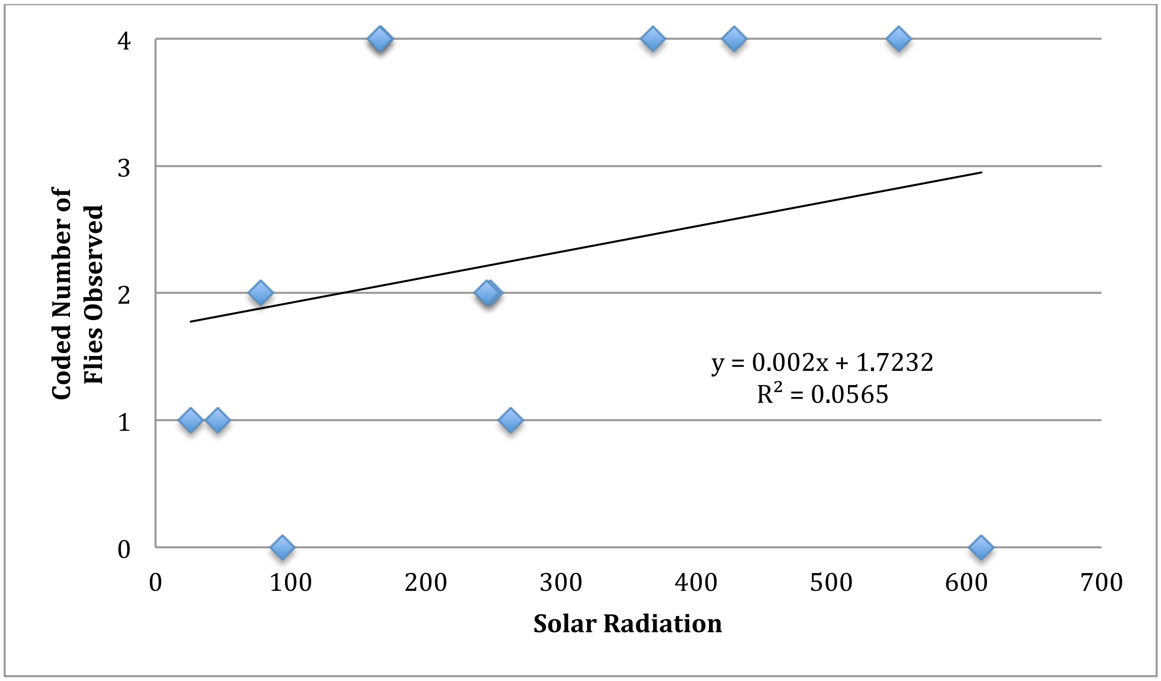

Figure B.18. Rainy conditions, covered (RC1).

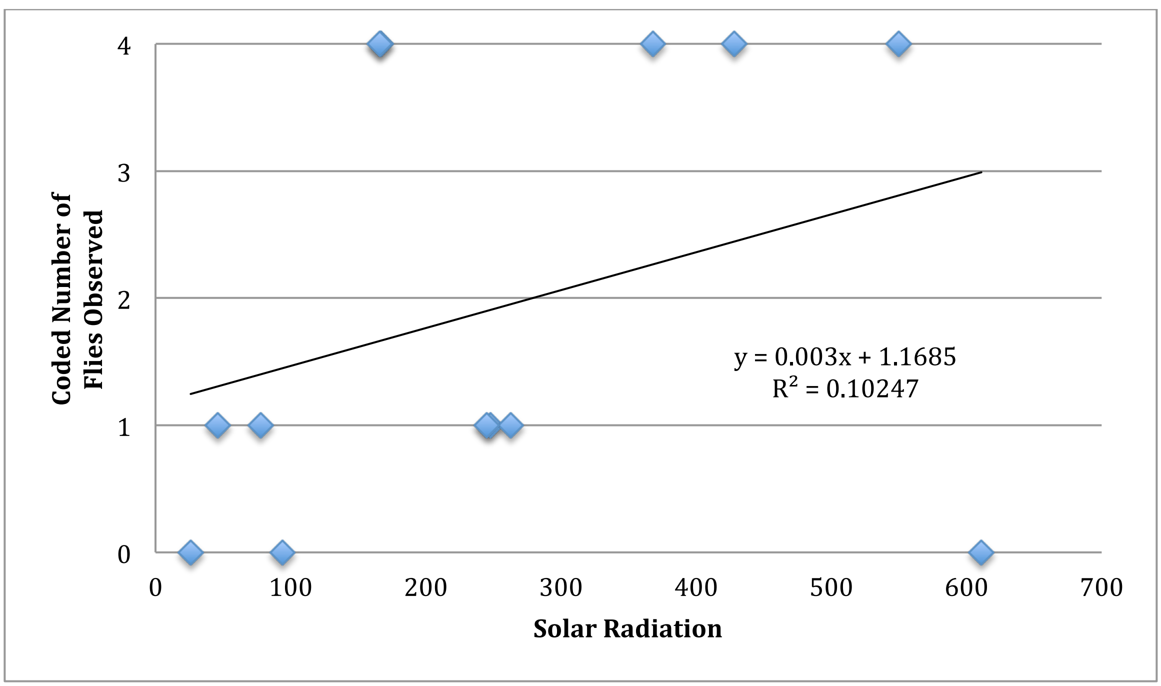

Figure B.19. Rainy conditions, covered (RC2).

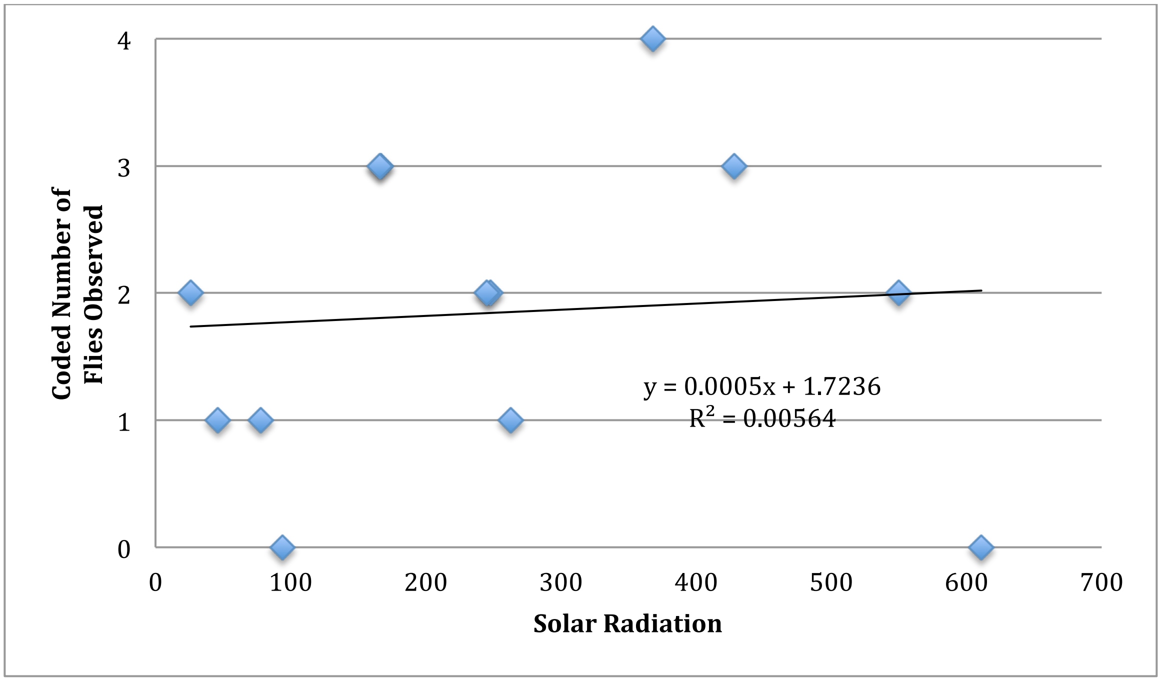

Figure B.20. Rainy conditions, covered (RC3).

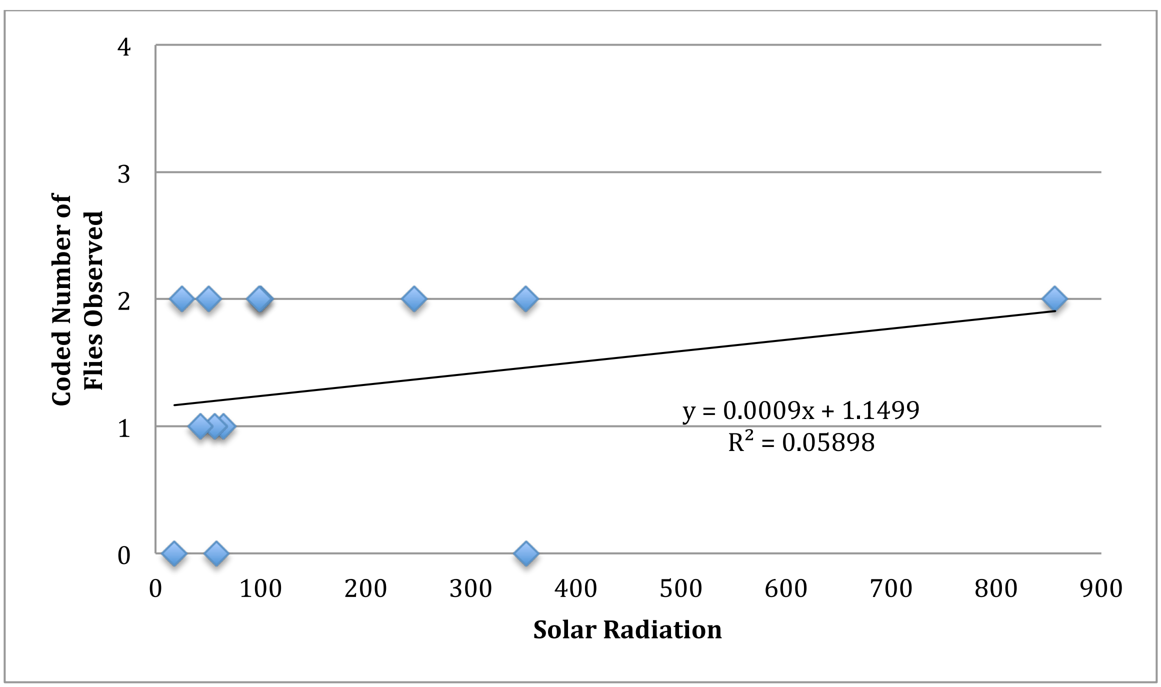

Figure B.21. Rainy conditions, covered (RC4).

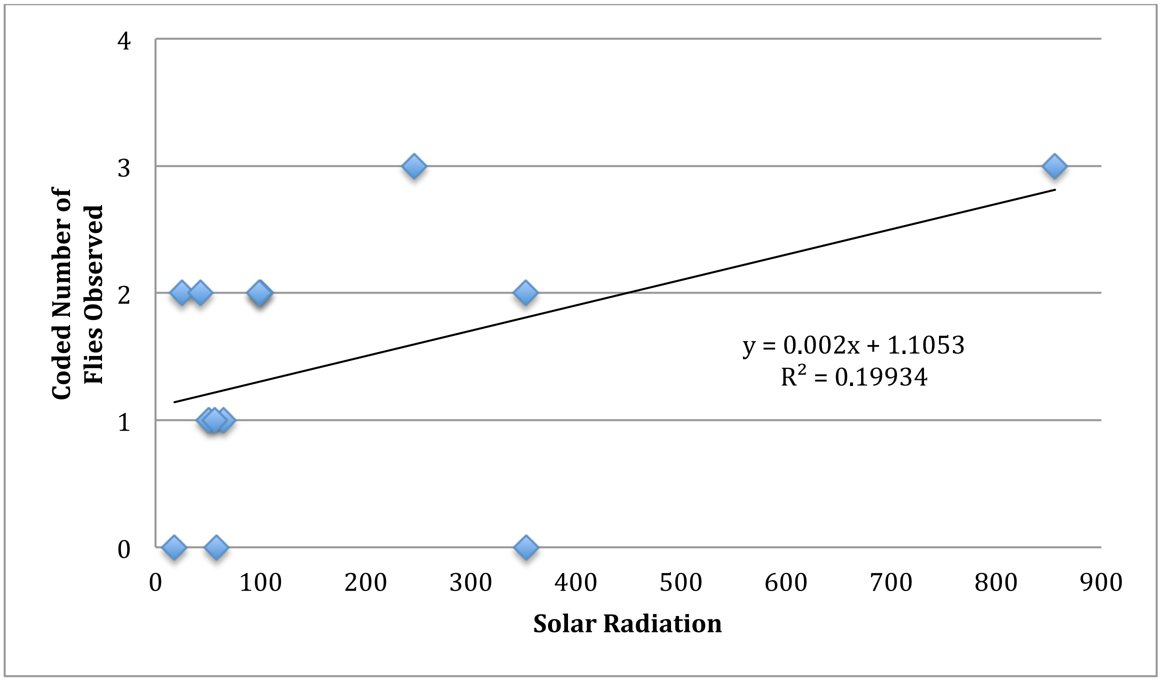

Figure B.22. Rainy conditions, covered (RC5).

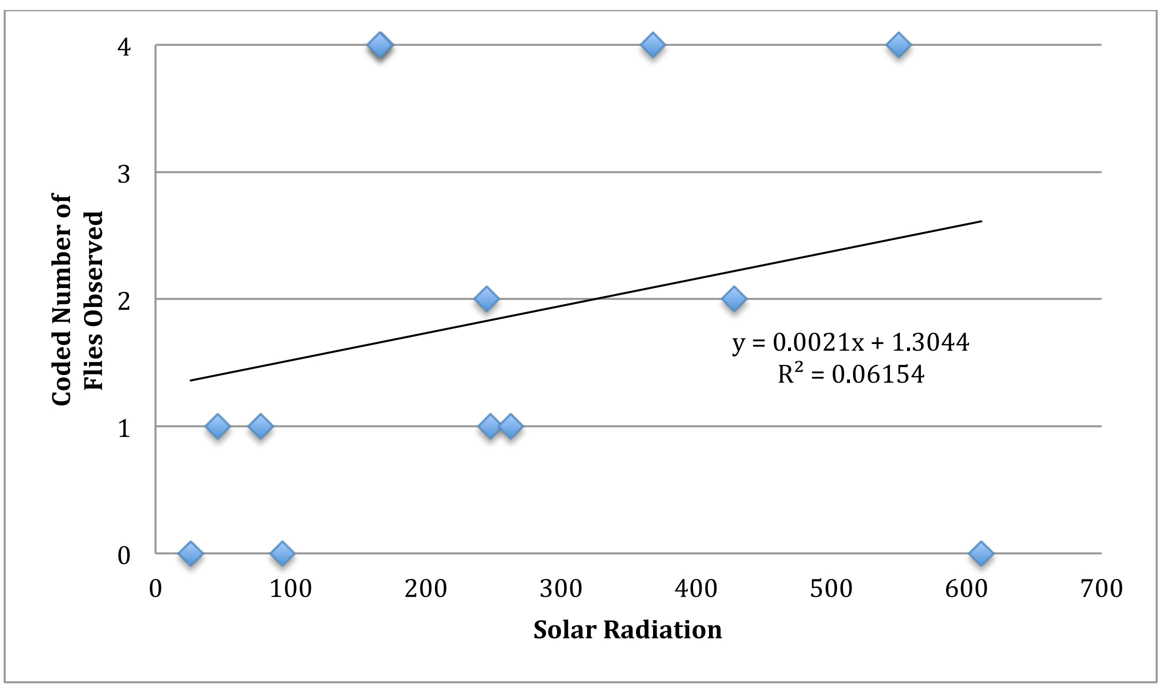

Figure B.23. Rainy conditions, covered partially (RCP1).

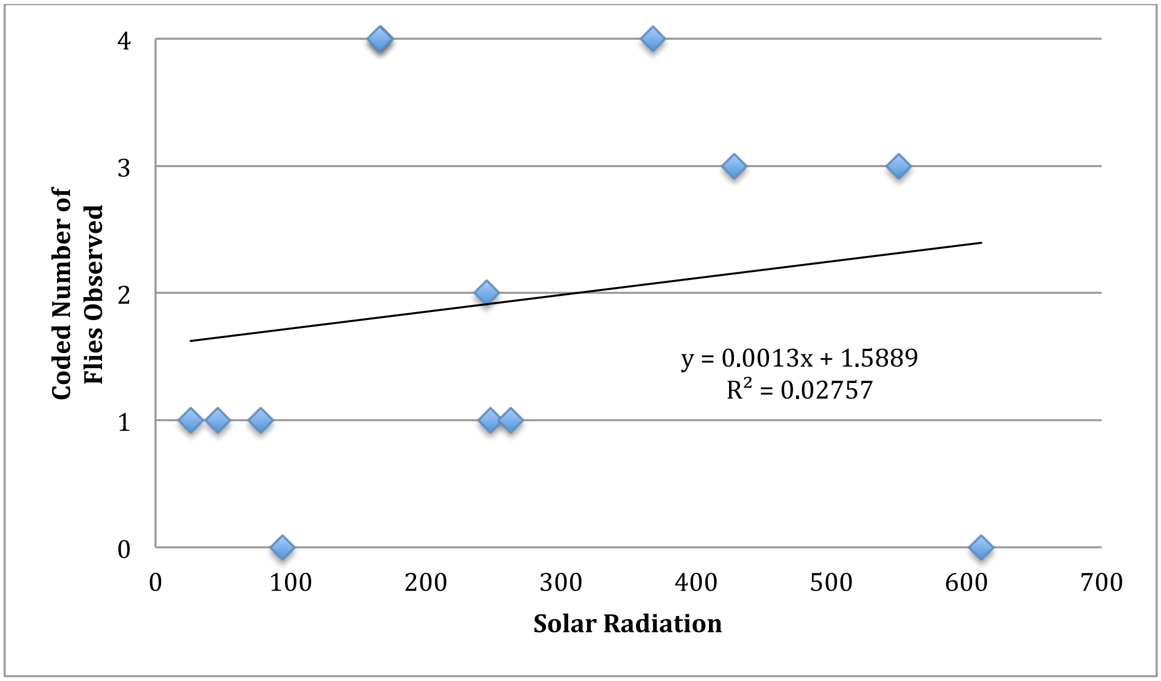

Figure B.24. Rainy conditions, covered partially (RCP2).

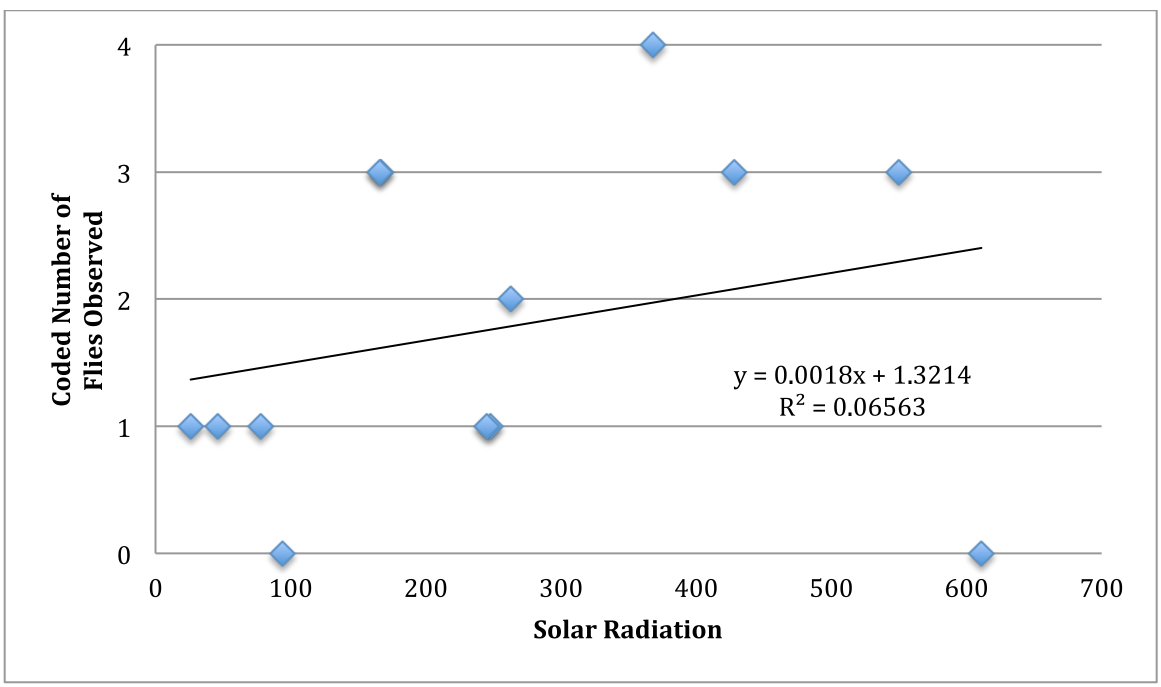

Figure B.25. Rainy conditions, covered partially (RCP3).

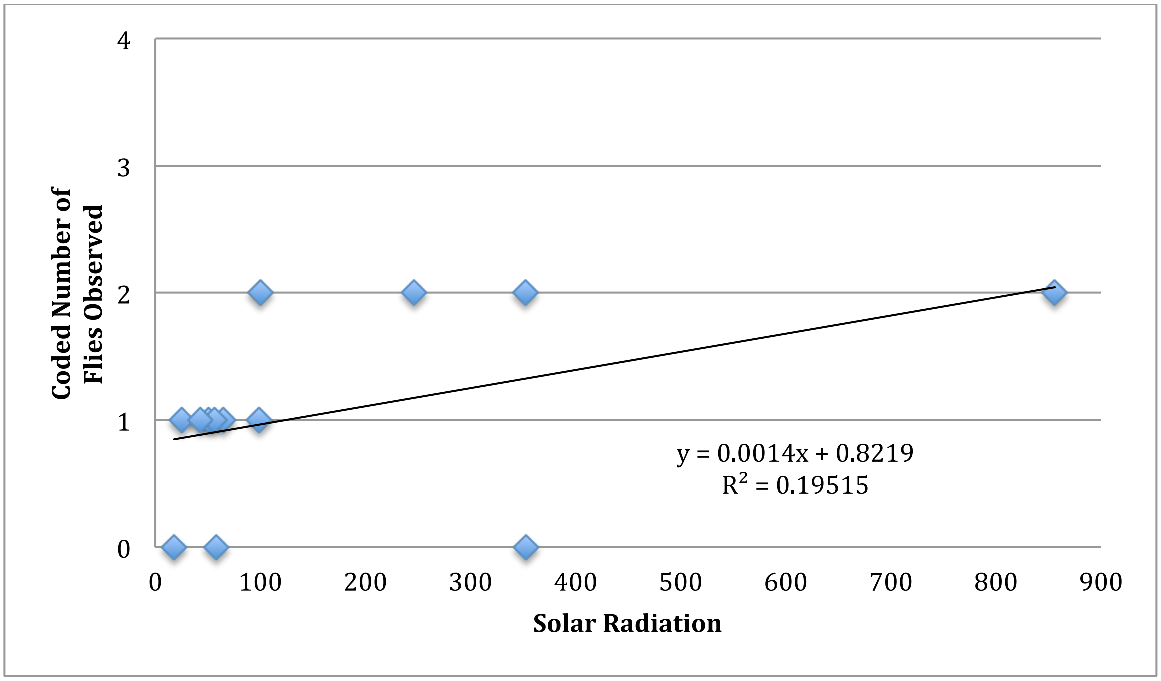

Figure B.26. Rainy conditions, covered partially (RCP4).

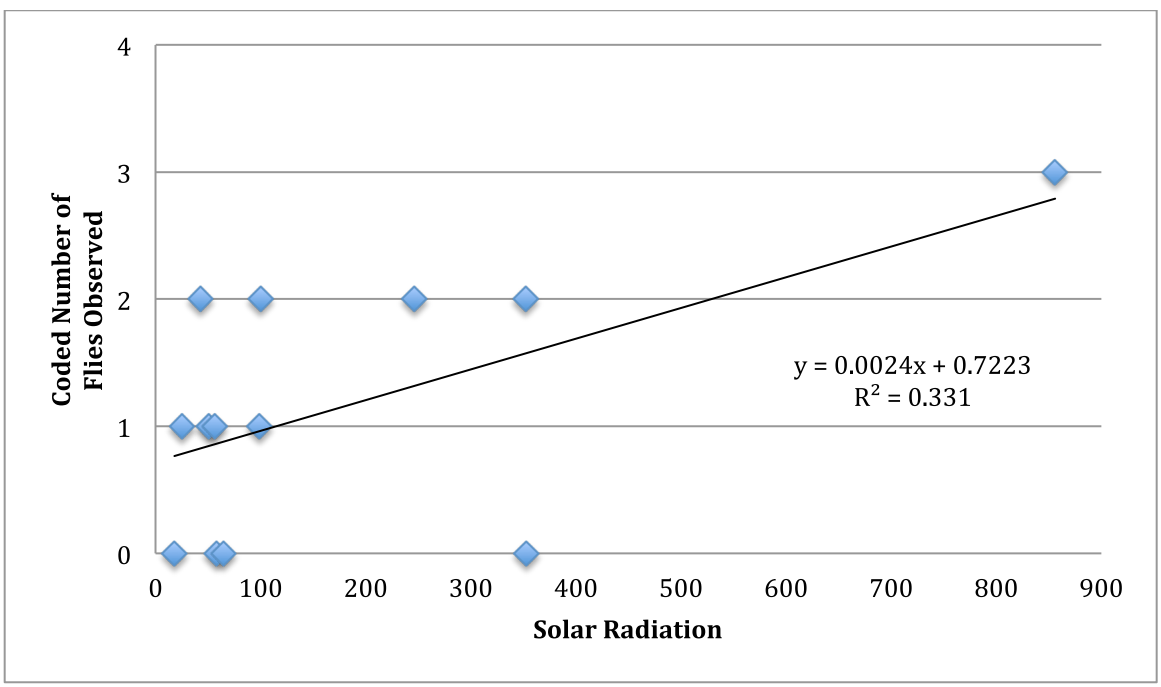

Figure B.27. Rainy conditions, covered partially (RCP5).

APPENDIX C

Appendix C reports the graphical representation of the amount of rain (mm) that had fallen since the last observation plotted against the coded number of flies observed. These graphs all show a negative correlation of rainfall and the coded number of flies observed across all groups in the experimental treatment.

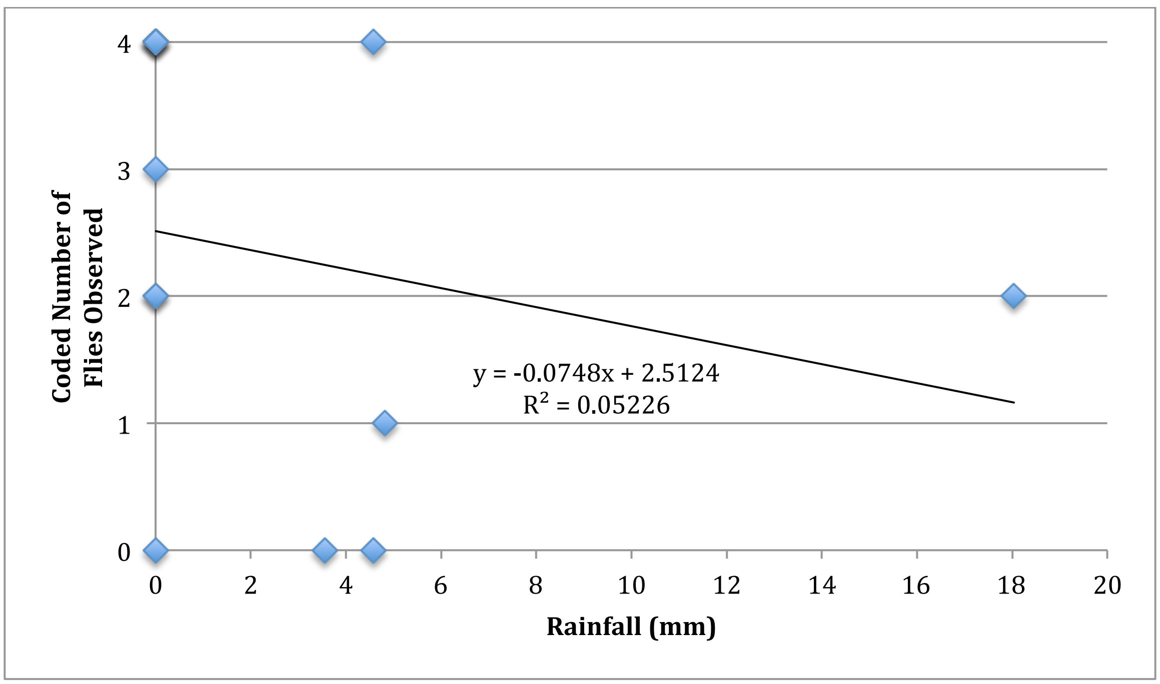

Figure C.1. Rainy conditions, uncovered (R1).

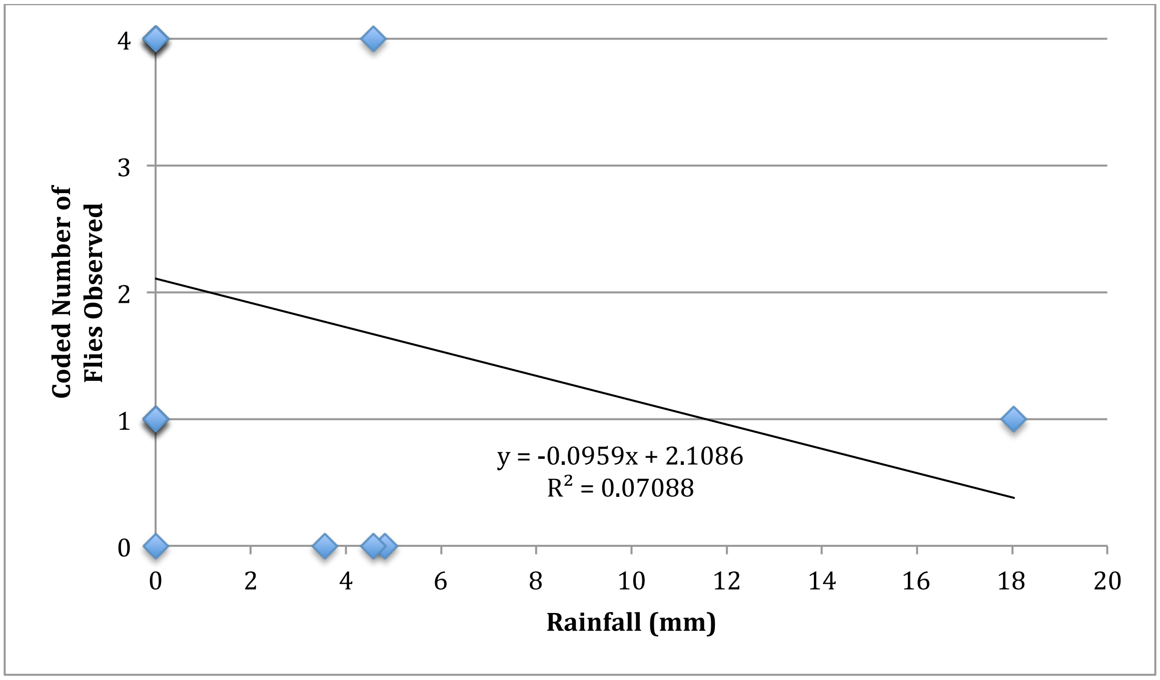

Figure C.2. Rainy conditions, uncovered (R2).

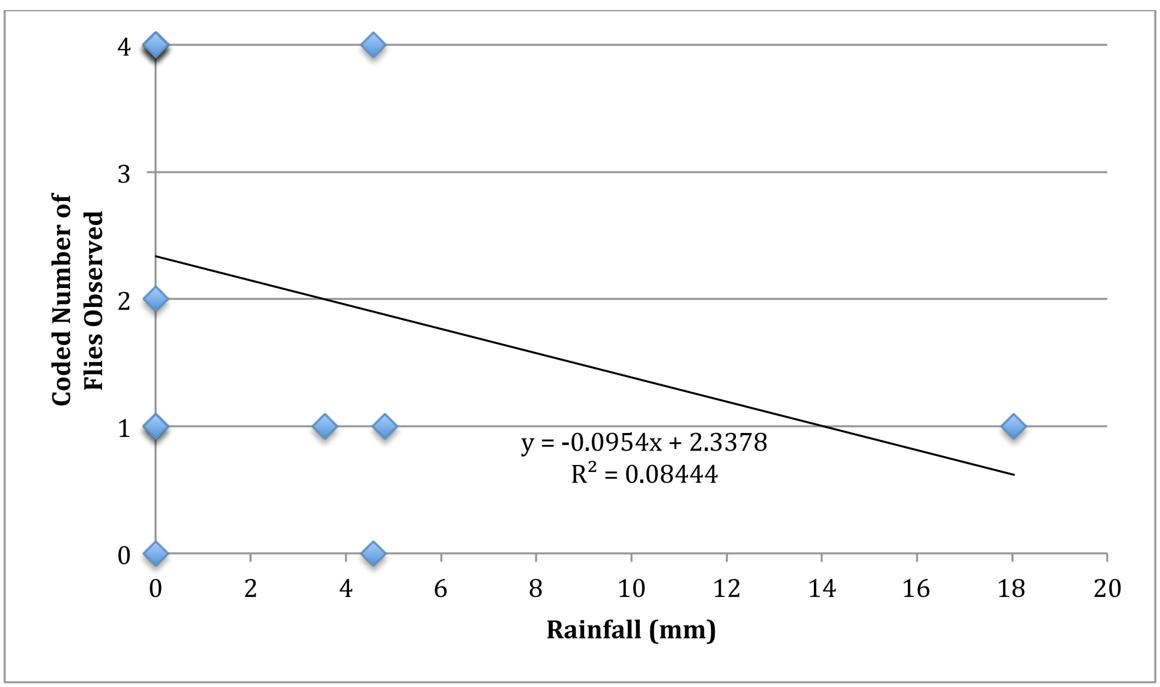

Figure C.3. Rainy conditions, uncovered (R3).

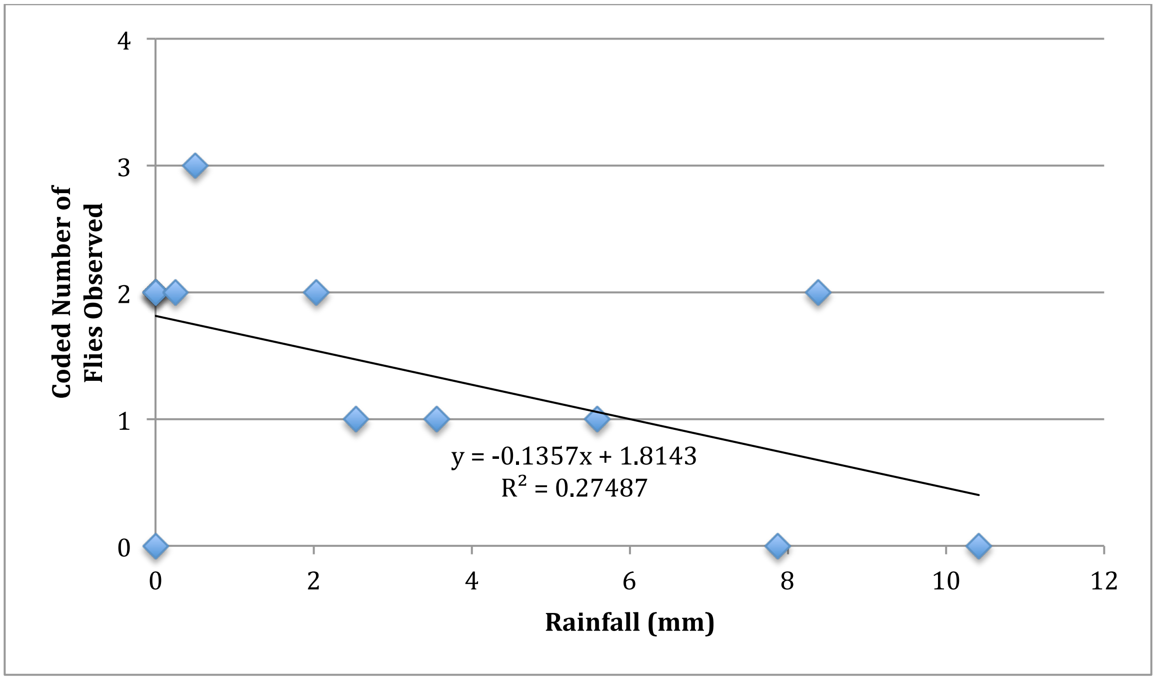

Figure C.4. Rainy conditions, uncovered (R4).

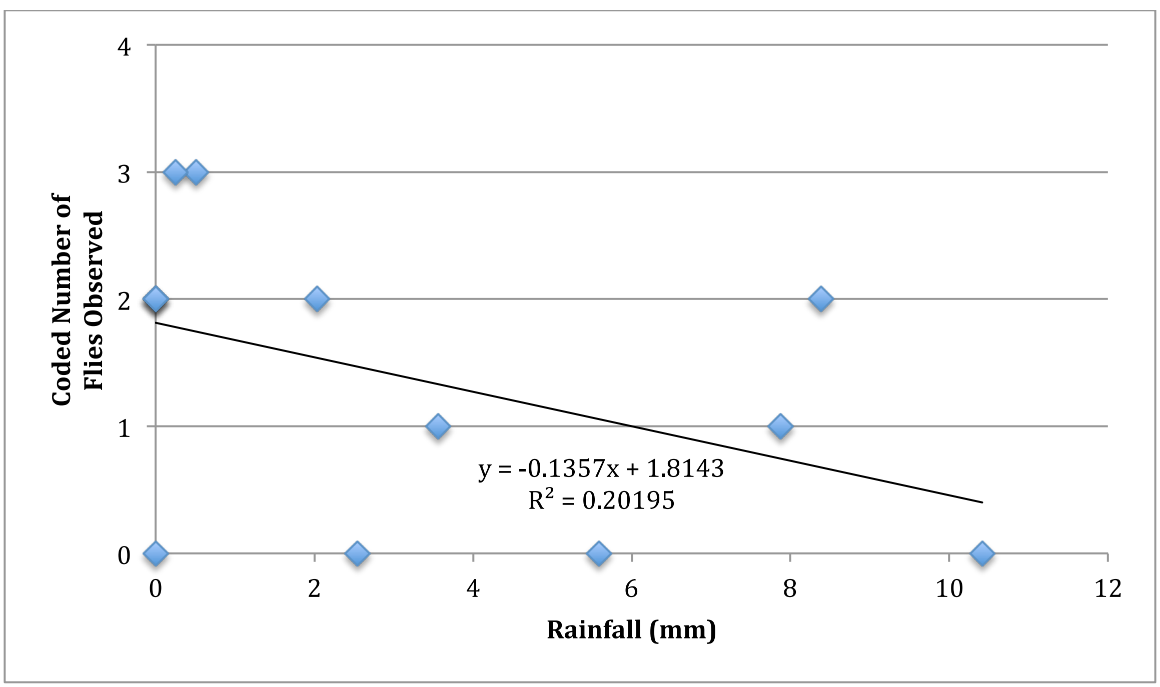

Figure C.5. Rainy conditions, uncovered (R5).

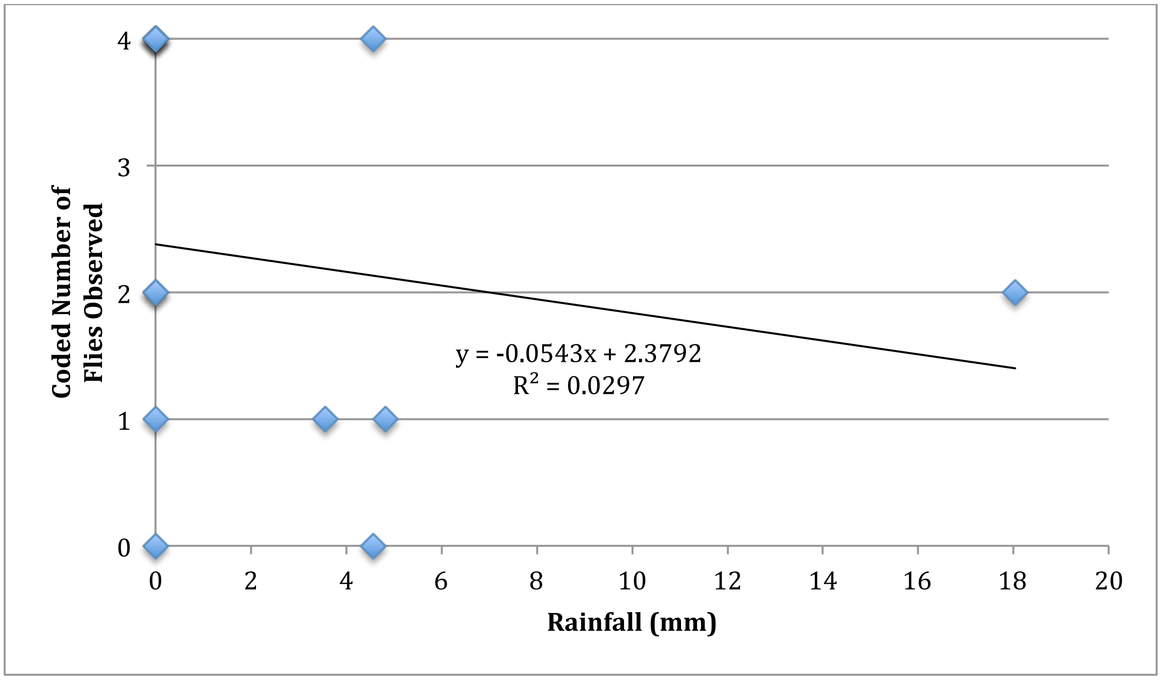

Figure C.6. Rainy conditions, covered (RC1).

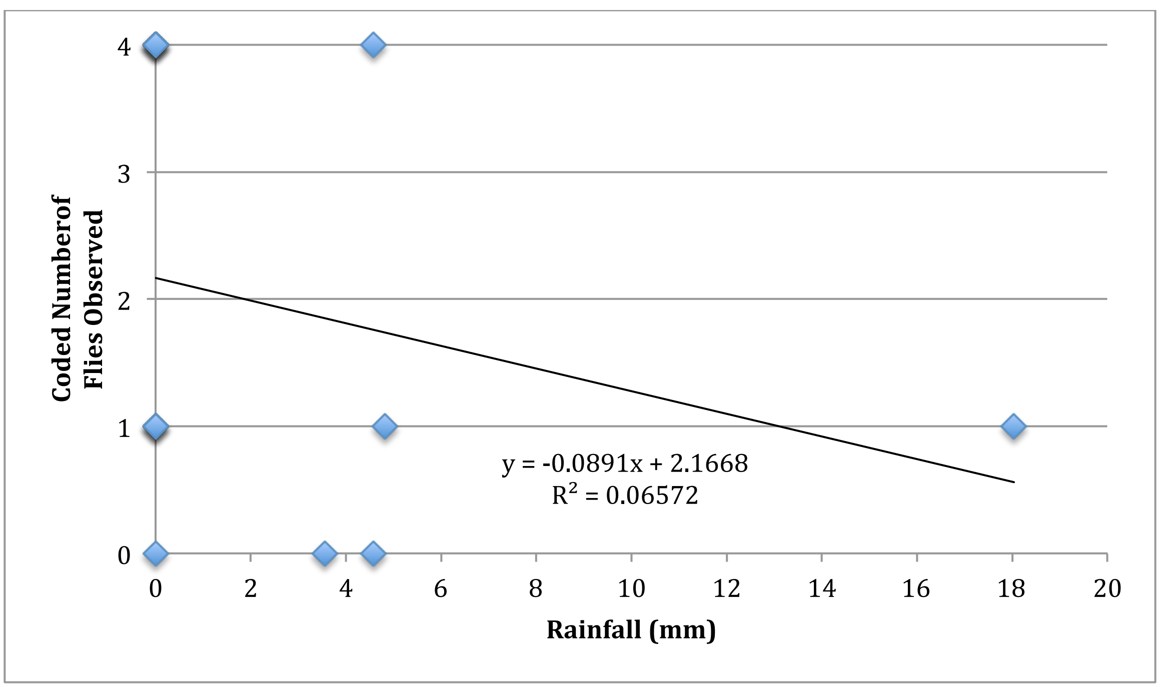

Figure C.7. Rainy conditions, covered (RC2).

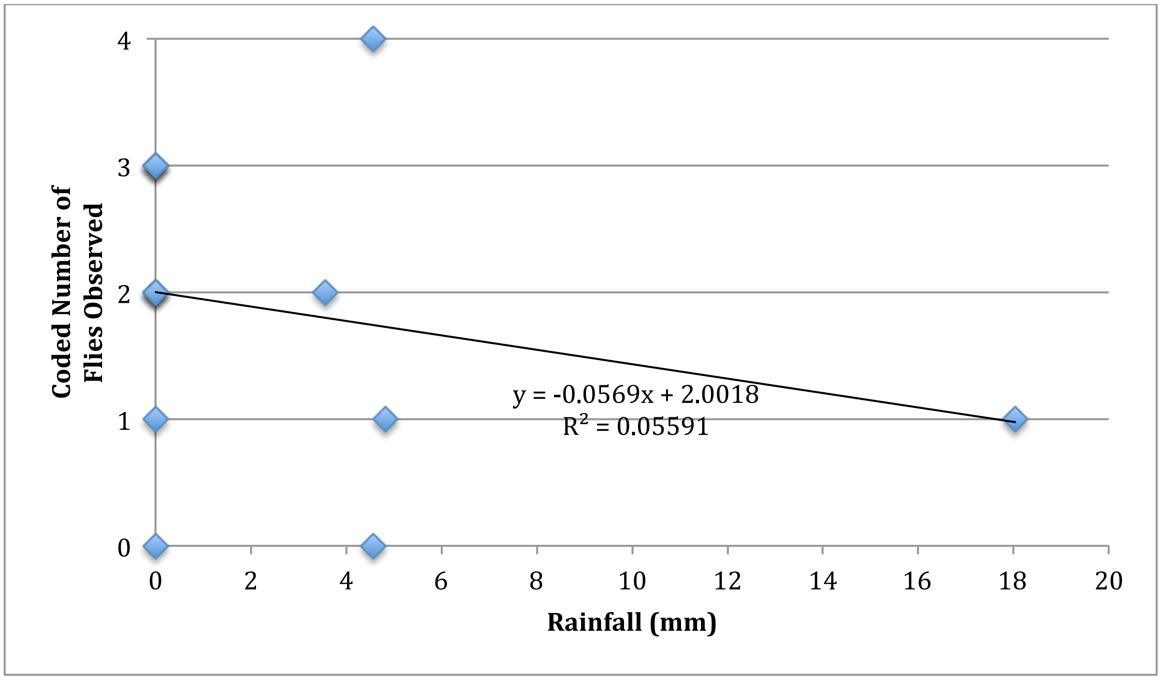

Figure C.8. Rainy conditions, covered (RC3).

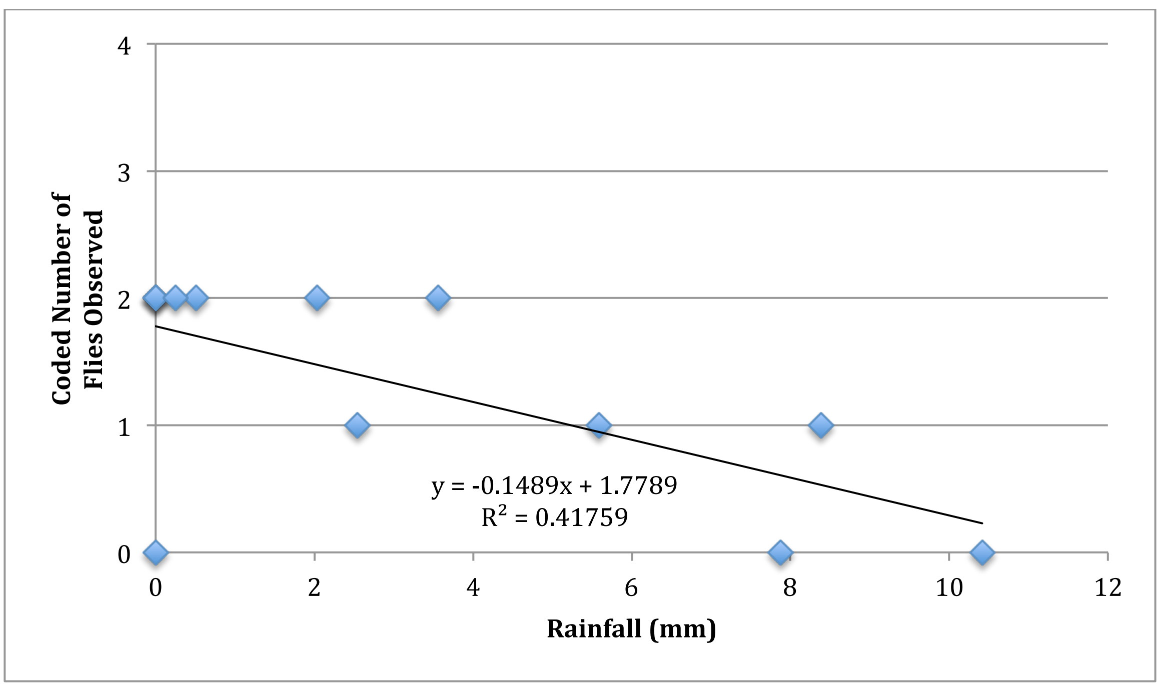

Figure C.9. Rainy conditions, covered (RC4).

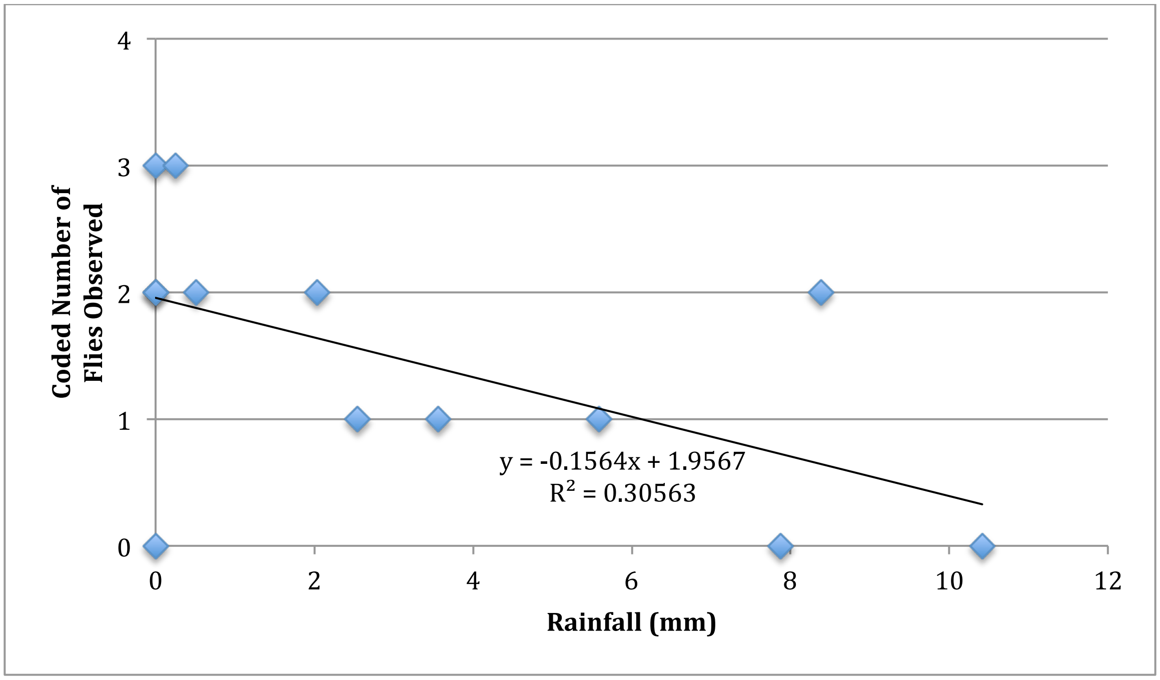

Figure C.10. Rainy conditions, covered (RC5).

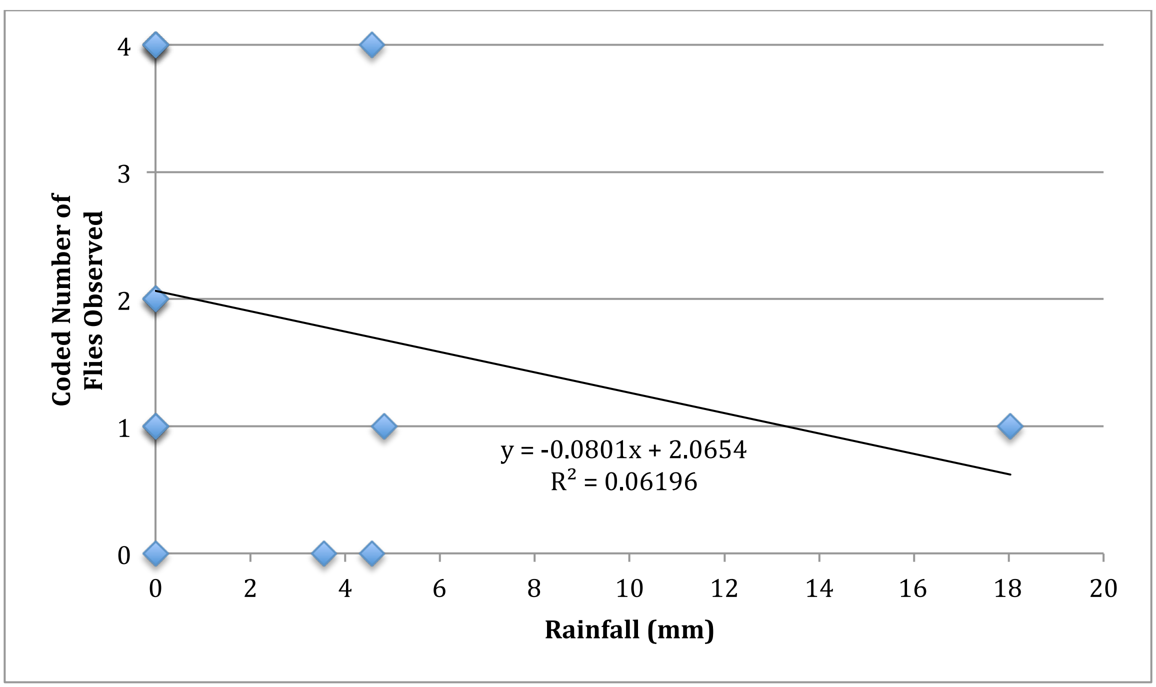

Figure C.11. Rainy conditions, covered partially (RCP1).

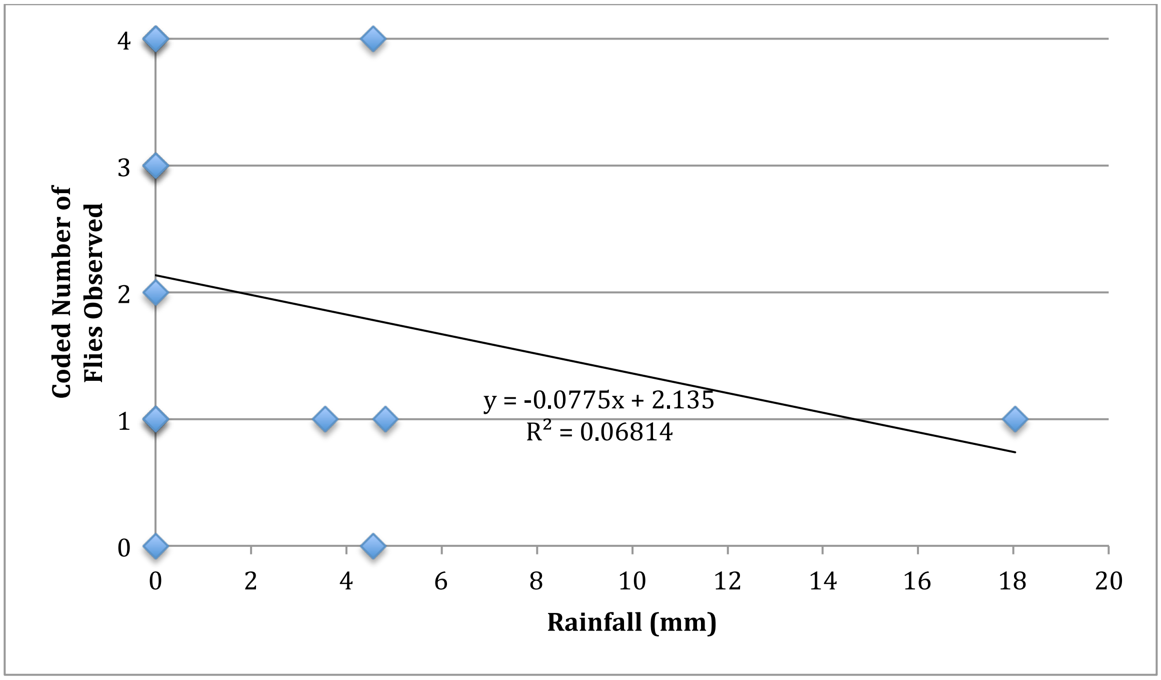

Figure C.12. Rainy conditions, covered partially (RCP2).

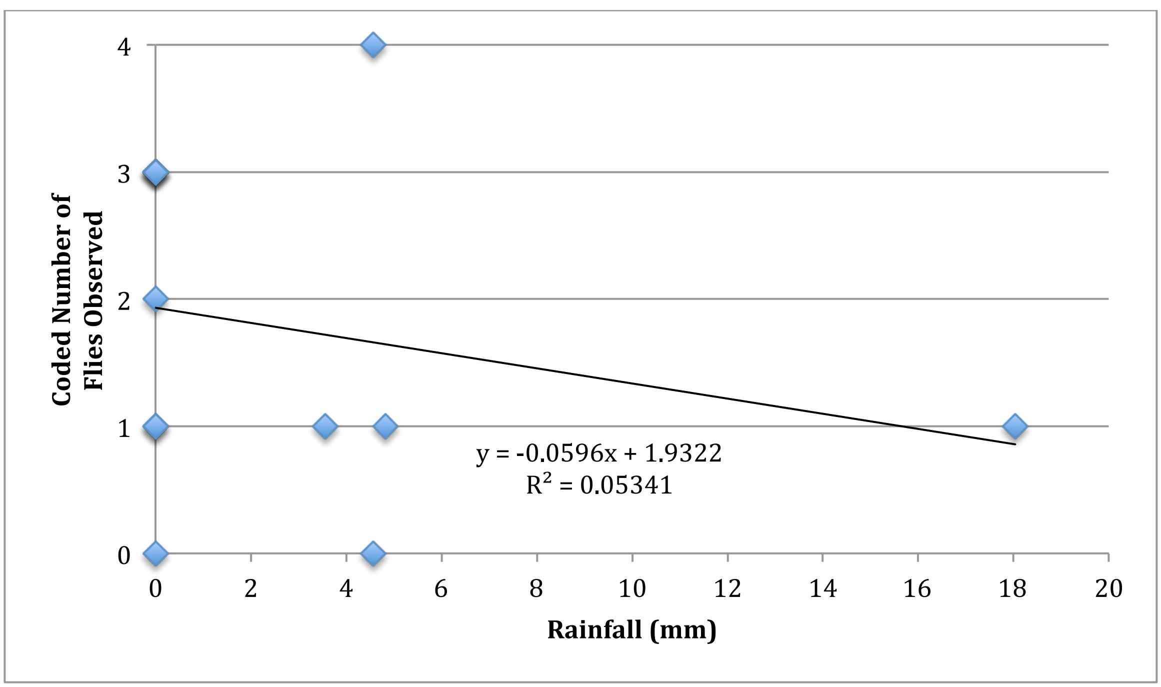

Figure C.13. Rainy conditions, covered partially (RCP3).

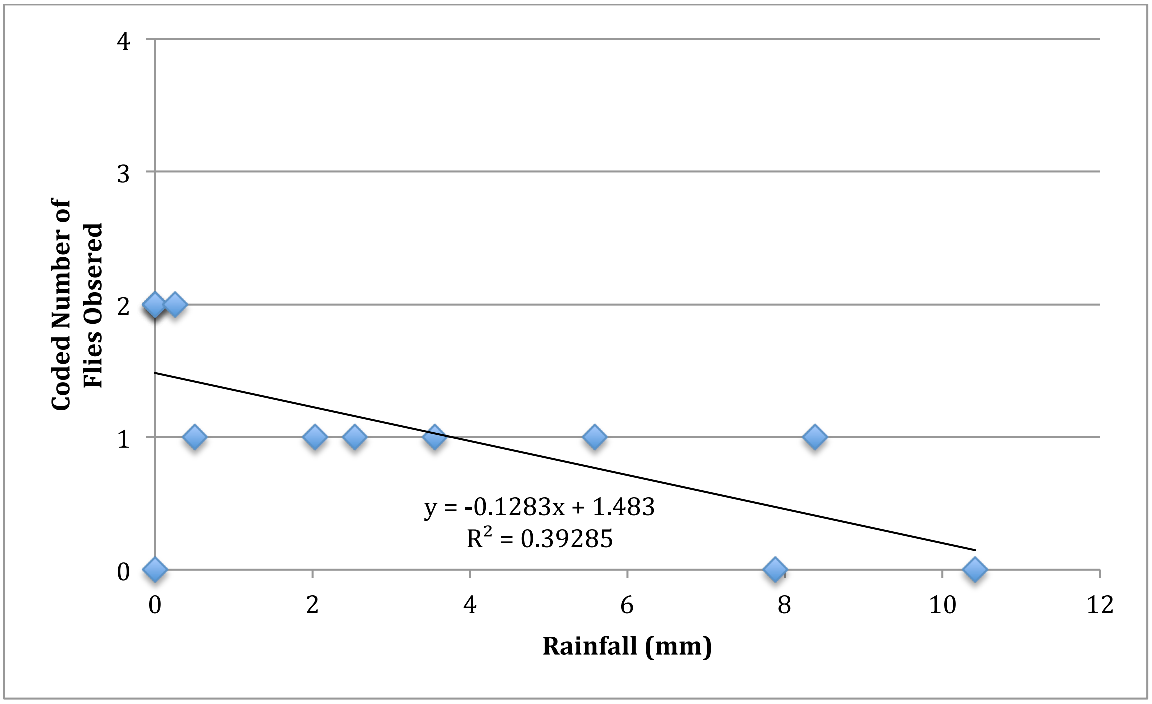

Figure C.14. Rainy conditions, covered partially (RCP4).

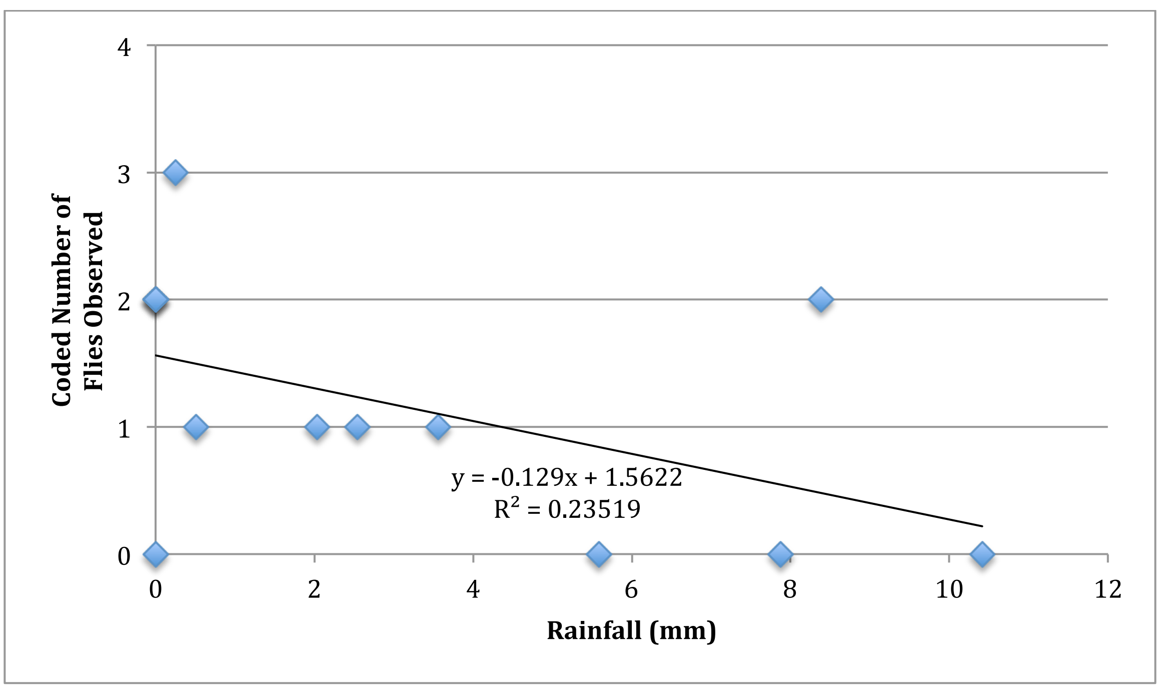

Figure C.15. Rainy conditions, covered partially (RCP5).

APPENDIX D

Appendix D reports the field notes recorded for each pig head in the study. Most significantly, additional observations not directly related to the study hypothesis were recorded and may be useful in directing future studies. Tables D.1 – D.30 represent the field notes have been transcribed from the handwritten field notes recorded for each pig head over the entire study period from June 27, 2014 through September 15, 2014. According to date and for the first two days the PMI in hours, the approximate number of blowflies observed visiting each carcass is reported. The presence of eggs is recorded by noting the sites eggs that were observed. When larvae hatched, this was recorded with the approximate size of larvae observed (1-2 mm, 3-4 mm, or 5+ mm) and if masses were forming. The Megyesi *et al.* (2005) alphanumeric decomposition score was also recorded. All additional arthropod activity, scavenging activity, and any other pertinent observations were recorded in the “other” section.

Table D.1. Field notes, normal, uncovered (N1).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
6/27/2014	0	0	0	0	A1	No rain, sun
6/27/2014	2	0	0	0	A1	No rain, sun
6/27/2014	4	10-	0	0	A1	No rain, sun, ants
6/27/2014	6	10+	0	0	A1	No rain, sun, ants
6/27/2014	8	30+	Nostril	0	A1	No rain, sun, ants
6/27/2014	10	20+	Nostrils, tongue	0	A1	No rain, sun, ants
6/27/2014	12	10+		0	A1	No rain, sun, lots of ants, in shade
6/28/2014	26	10-	Throat	0	B1	No rain, sun
6/28/2014	28	20+		1-2mm	B1	No rain, sun
6/28/2014	30	50+	Eye	1-2mm	B3	No rain, sun
6/28/2014	32	30+	Ears	1-2mm	B4	No rain, sun
6/28/2014	34	30+		1-2mm	B4	No rain, sun
6/28/2014	36	20+		3-5mm, masses	B4	No rain, sun
6/29/2014		50+		3-5mm, masses	B5	No rain, sun
6/30/2014		50+	All hatched	3-5mm, masses	B5	No rain, sun, head moved position slightly
7/1/2014		20+		3-5mm, masses	B5	No rain, sun, lots of bloat, moved approximately 1.5 m away
7/2/2014		10-		3-5mm, masses	B5	No rain, sun, bird poop
7/4/2014		0		6+mm, masses	C1	Heavy rain, dry skin moistened
7/5/2014		10-		6+mm, masses	C1	No rain, sun
7/12/2014		0		Few	C2	No rain, sun
7/17/2014		10-			C3	No rain, sun
7/23/2014		0			C3	No rain, sun
7/28/2014		0			C3	No rain, sun, saw turkey vulture, probably responsible for movement of carcass

Table D.2. Field notes, normal, uncovered (N2).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
6/27/2014	0	0	0	0	A1	No rain, sun
6/27/2014	2	1	0	0	A1	No rain, sun, ants
6/27/2014	4	10+	0	0	A1	No rain, sun, ants
6/27/2014	6	30+	Nostril	0	A1	No rain, sun, ants
6/27/2014	8	30+		0	A1	No rain, sun, ants
6/27/2014	10	30+	Tongue	0	A1	No rain, sun, ants
6/27/2014	12	10+	Base of tongue	0	A1	No rain, sun, lots of ants, in shade
6/28/2014	26	20+	Nostrils	0	B1	No rain, sun
6/28/2014	28	30+		0	B1	No rain, sun, in shade
6/28/2014	30	50+		0	B3	No rain, sun
6/28/2014	32	50+	Eyes	1-2mm	B4	No rain, sun
6/28/2014	34	30+		1-2mm	B4	No rain, sun
6/28/2014	36	30+	Roof of mouth	1-2mm	B5	No rain, sun, in shade
6/29/2014		50+		3-5mm, masses	B5	No rain, sun
6/30/2014		50+	Hatched	3-5mm, masses	B5	No rain, sun
7/1/2014		20+		6+mm, masses	B5	No rain, sun, head moved approximately 1 foot
7/2/2014		10-		Dying down	C2	No rain, sun
7/4/2014		0		6+mm, masses	C2	Heavy rain
7/5/2014		10+		Few	C2	No rain, sun
7/12/2014		0		0	C2	No rain, sun, mold
7/17/2014		10-		0	C3	No rain, sun
7/23/2014		0		0	C3	No rain, sun
7/28/2014		0		0	C3	

Table D.3. Field notes, normal, uncovered (N3).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
6/27/2014	0	0	0	0	A1	No rain, sun
6/27/2014	2	0	0	0	A1	No rain, sun
6/27/2014	4	10-	0	0	A1	No rain, sun, bee
6/27/2014	6	10+	0	0	A1	No rain, sun
6/27/2014	8	20+	0	0	A1	No rain, sun
6/27/2014	10	20+	Tongue	0	B1	No rain, sun
6/27/2014	12	20+	Base of tongue	0	B1	No rain, sun, in shade
6/28/2014	26	10-	Throat	0	B1	No rain, sun
6/28/2014	28	20+		1-2mm	B2	No rain, sun, in shade
6/28/2014	30	50+		1-2mm	B4	No rain, sun
6/28/2014	32	30+	Eyes	1-2mm	B4	No rain, sun
6/28/2014	34	30+		1-2mm	B4	No rain, sun
6/28/2014	36	30+	Side of face	3-5mm, masses	B5	No rain, sun
6/29/2014		50+	Ears	3-5mm, masses	B5	No rain, sun
6/30/2014		50+	Hatched	3-5mm, masses	B5	No rain, sun
7/1/2014		30+		6+mm, masses	C2	No rain, sun, some movement, a chunk of flesh detached
7/2/2014		10-		Died down	C2	No rain, sun, pupae in ear
7/4/2014		0		6+mm, masses	C2	Heavy rain, dry skin moistened
7/5/2014		10+		Few	C2	No rain, sun
7/12/2014		0			C2	No rain, sun
7/17/2014		10-			C3	No rain, sun
7/23/2014		0			C3	No rain, sun
7/28/2014		0			C3	No rain, sun

Table D.4. Field notes, normal, uncovered (N4).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/18/2014	0	0	0	0	A1	No rain, sun
7/18/2014	2	10-	0	0	A1	No rain, sun
7/18/2014	4	30+	One nostril	0	A1	No rain, sun, bee, ants, large green dragonfly
7/18/2014	6	10-	Lip	0	B1	No rain, cloudy, wasp
7/19/2014	20	10-		0	B2	No rain, partly cloudy, wasp, ant, spider
7/19/2014	22	20+	Under neck, mouth	0	B2	No rain, cloudy, ants, spider, wasp
7/19/2014	24	30+	Throat	0	B2	No rain, cloudy
7/19/2014	26	40+	Teeth, roof of mouth	1-2mm	B3	No rain, cloudy, bee
7/19/2014	28	40+	Eye	1-2mm	B3	No rain, cloudy
7/19/2014	30	40+		1-2mm	B3	No rain, cloudy, wasp
7/20/2014	44	10-		3-5mm, masses	B3	No rain, cloudy
7/20/2014	46	40+	Mouth	3-5mm, masses	B3	No rain, cloudy, bee
7/20/2014	48	40+		3-5mm, masses	B3	No rain, partly cloudy, wasp
7/21/2014		50+		6+mm, masses	B4	No rain, sun
7/22/2014		10+		6+mm, masses	B5	No rain, sun, carrion beetle, wasp
7/23/2014		10-		6+mm, masses	C1	No rain, sun, dragonfly
7/24/2014		10-		Few	C1	No rain, cloudy
7/25/2014		10-		Maggot migration	C1	No rain, sun
8/1/2014		0			C2	Moderate rain
8/7/2014		0			C3	No rain, sun
8/14/2014		10-			D1	No rain, sun, crickets, small brown beetles and jumping larvae

Table D.5. Field notes, normal, uncovered (N5).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/18/2014	0	0	0	0	A1	No rain, sun
7/18/2014	2	10-	0	0	A1	No rain, sun
7/18/2014	4	10-	Both nostrils	0	A1	No rain, sun
7/18/2014	6	10^{+}	Lip	0	B1	No rain, cloudy, ants
7/19/2014	20	10-		0	B1	No rain, partly cloudy
7/19/2014	22	10-		0	B1	No rain, cloudy, crickets, ants
7/19/2014	24	20^{+}		1-2mm	B2	No rain, cloudy, carrion beetles, ants
7/19/2014	26	30^{+}	Under skin flaps	1-2mm	B3	No rain, cloudy, bee
7/19/2014	28	30^{+}		1-2mm	B3	No rain, cloudy, wasp
7/19/2014	30	40^{+}		1-2mm	B3	No rain, cloudy
7/20/2014	44	10-		3-5mm, masses	B3	No rain, cloudy
7/20/2014	46	40^{+}	Ground	3-5mm, masses	B3	No rain, cloudy
7/20/2014	48	40^{+}	Chin	3-5mm, masses	B3	No rain, partly cloudy, wasp
7/21/2014		50^{+}		6^{+}mm, masses	B4	No rain, sun
7/22/2014		10^{+}		6^{+}mm, masses	B5	No rain, sun
7/23/2014		10-		6^{+}mm, masses	C1	No rain, sun
7/24/2014		10-		Few	C1	No rain, cloudy, small black beetles (clown), rove beetle, crickets
7/25/2014		0			C2	No rain, sun
8/1/2014		0			C2	Moderate rain
8/7/2014		0			C3	No rain, sun
8/14/2014		0			D1	No rain, sun, small brown beetles and jumping larvae

Table D.6. Field notes, normal, uncovered (N6).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/18/2014	0	0	0	0	A1	No rain, sun
7/18/2014	2	30^+	0	0	A1	No rain, sun
7/18/2014	4	20^+	Lip	0	A1	No rain, sun
7/18/2014	6	10^+	Roof of mouth	0	B2	No rain, cloudy, orange carrion beetle, wasp, bee, ants carrying eggs
7/19/2014	20	20^+	Mouth	0	B2	No rain, partly cloudy
7/19/2014	22	30^+	Throat	0	B2	No rain, cloudy, carrion beetle
7/19/2014	24	40^+		1-2mm	B3	No rain, cloudy, carrion beetle
7/19/2014	26	40^+	Lips	1-2mm	B3	No rain, cloudy, carrion beetle
7/19/2014	28	40^+		1-2mm	B3	No rain, cloudy, 5^+ carrion beetles
7/19/2014	30	40^+		1-2mm	B3	No rain, cloudy, wasps, carrion beetle
7/20/2014	44	10-		1-2mm	B3	No rain, cloudy
7/20/2014	46	30^+		3-5mm masses	B3	No rain, cloudy, wasps, lots ants
7/20/2014	48	40^+	Under chin	3-5mm masses	B3	No rain, partly cloudy
7/21/2014		20^+	Lip	6^+mm, masses	B4	No rain, sun, in shade, wasp
7/22/2014		10-		All over face	B5	No rain, sun, in shade, rove beetle
7/23/2014		10-		6^+mm, masses	C1	No rain, sun, in shade
7/24/2014		0		Maggot migration	C1	No rain, cloudy
7/25/2014		0		Few	C2	No rain, sun
8/1/2014		0			C2	Moderate rain
8/7/2014		0			C3	No rain, sun, head moved approx. 2 feet
8/14/2014		0			D1	No rain, sun, small brown beetles and jumping larvae

Table D.7. Field notes, normal, uncovered (NC1).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
6/27/2014	0	0	0	0	A1	No rain, sun
6/27/2014	2	0	0	0	A1	No rain, sun
6/27/2014	4	10+	0	0	A1	No rain, sun, ants
6/27/2014	6	30+	Nostril	0	A1	No rain, sun, ants
6/27/2014	8	30+	Both nostrils	0	A1	No rain, sun, ants
6/27/2014	10	20+		0	B1	No rain, sun, in shade
6/27/2014	12	10-		0	B1	No rain, sun
6/28/2014	26	10+	Large amount in nostrils	0	B1	No rain, sun, ants
6/28/2014	28	50+		0	B1	No rain, sun
6/28/2014	30	50+		0	B3	No rain, sun
6/28/2014	32	50+	Back of tongue	0	B4	No rain, sun
6/28/2014	34	50+		0	B4	No rain, sun, in shade
6/28/2014	36	50+	Eyes	1-2mm	B4	No rain, sun
6/29/2014		50+	Ears	1-2mm	B5	No rain, sun
6/30/2014		50+	Hatched	3-5mm, masses	B5	No rain, sun
7/1/2014		30+		3-5mm, masses	B5	No rain, sun, bird poop, maggots seem to crawl freely over surface more than uncovered
7/2/2014		10-		6+mm, masses	B5	No rain, sun
7/4/2014		0		6+mm, masses	C1	Heavy rain, many maggots drowned in edges of laundry baskets
7/5/2014		10-		Few	C3	No rain, sun
7/12/2014		0			C3	No rain, sun
7/17/2014		10-			C3	No rain, sun
7/23/2014		0			C3	No rain, sun
7/28/2014		0			C3	No rain, sun

Table D.8. Field notes, normal, uncovered (NC2).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
6/27/2014	0	0	0	0	A1	No rain, sun
6/27/2014	2	0	0	0	A1	No rain, sun, in shade
6/27/2014	4	10-	0	0	A1	No rain, sun, ants
6/27/2014	6	30+	One nostril	0	A1	No rain, sun, ants
6/27/2014	8	30+	Both nostrils	0	A1	No rain, sun, ants
6/27/2014	10	30+	Base of tongue	0	A1	No rain, sun, ants, in shade
6/27/2014	12	10-		0	A1	No rain, sun, in shade
6/28/2014	26	10-	Nostrils, tongue	0	B1	No rain, sun
6/28/2014	28	50+		0	B1	No rain, sun
6/28/2014	30	50+	Eye	0	B3	No rain, sun
6/28/2014	32	50+	Under tongue	1-2mm	B4	No rain, sun
6/28/2014	34	50+		1-2mm	B4	No rain, sun
6/28/2014	36	50+	Eyes	1-2mm	B4	No rain, sun, in shade
6/29/2014		50+		1-2mm	B5	No rain, sun
6/30/2014		50+	Hatched	3-5mm, masses	B5	No rain, sun
7/1/2014		30+		3-5mm, masses	B5	No rain, sun
7/2/2014		10-		6+mm, masses	B5	No rain, sun
7/4/2014		0		6+mm, masses	C1	Heavy rain, many maggots drowned in edges of laundry baskets
7/5/2014		10-		Few	C3	No rain, sun
7/12/2014		0		0	C3	No rain, sun
7/17/2014		10-		0	C3	No rain, sun
7/23/2014		0		0	C3	No rain, sun
7/28/2014		0		0	C3	No rain, sun

Table D.9. Field notes, normal, uncovered (NC3).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
6/27/2014	0	0	0	0	A1	No rain, sun
6/27/2014	2	0	0	0	A1	No rain, sun
6/27/2014	4	10-	0	0	A1	No rain, sun, ants
6/27/2014	6	30+	Nostril	0	A1	No rain, sun, ants
6/27/2014	8	30+	Both nostril	0	A1	No rain, sun, ants
6/27/2014	10	30+	Base of tongue	0	A1	No rain, sun, ants
6/27/2014	12	10-		0	A1	No rain, sun, in shade
6/28/2014	26	10-	More in nostrils	0	B1	No rain, sun
6/28/2014	28	50+		0	B1	No rain, sun
6/28/2014	30	50+	One eye	0	B3	No rain, sun
6/28/2014	32	50+	Under tongue	1-2mm	B4	No rain, sun
6/28/2014	34	50+		1-2mm	B4	No rain, sun
6/28/2014	36	50+	Eyes	1-2mm	B4	No rain, sun, in shade
6/29/2014		50+		1-2mm	B5	No rain, sun
6/30/2014		50+	Hatched	3-5mm, masses	B5	No rain, sun
7/1/2014		30+		3-5mm, masses	B5	No rain, sun
7/2/2014		10-		6+mm, masses	B5	No rain, sun
7/4/2014		0		6+mm, masses	C1	Heavy rain, many maggots drowned in edges of laundry baskets
7/5/2014		10-		Few	C3	No rain, sun
7/12/2014		0			C3	No rain, sun
7/17/2014		10-			C3	No rain, sun
7/28/2014		0			C3	No rain, sun
7/28/2014		0			C3	No rain, sun

Table D.10. Field notes, normal, uncovered (NC4).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/18/2014	0	0	0	0	A1	No rain, sun
7/18/2014	2	10-	0	0	A1	No rain, sun
7/18/2014	4	10+	Both nostrils	0	A1	No rain, sun
7/18/2014	6	20+	Lips, mouth	0	B1	No rain, cloudy
7/19/2014	20	0		0	B1	No rain, partly cloudy, cricket
7/19/2014	22	20+	Eye, throat	0	B1	No rain, cloudy, bee
7/19/2014	24	30+	Under throat	1-2mm	B1	No rain, cloudy
7/19/2014	26	40+	Eyes	1-2mm	B2	No rain, cloudy
7/19/2014	28	50+	Roof of mouth	1-2mm	B2	No rain, cloudy
7/19/2014	30	50+	Lips	1-2mm	B2	No rain, cloudy, carrion beetle
7/20/2014	44	10+		3-5mm, masses	B2	No rain, cloudy
7/20/2014	46	40+		3-5mm, masses	B2	No rain, cloudy
7/20/2014	48	50+	Under chin	3-5mm, masses	B2	No rain, partly cloudy
7/21/2014		50+	Lips	6+mm, masses	B3	No rain, sun
7/22/2014		20+		6+mm, masses	B4	No rain, sun, carrion beetle, rove beetle
7/23/2014		10-		6+mm, masses	C1	No rain, sun
7/24/2014		0		Few	C1	No rain, cloudy, rove beetle, small flies
7/25/2014		10-		Few	C1	No rain, sun
8/1/2014		10-			C3	Moderate rain
8/7/2014		0			C3	No rain, sun
8/14/2014		10-			D1	No rain, sun, small brown beetle and jumping maggots

Table D.11. Field notes, normal, uncovered (NC5).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/18/2014	0	0	0	0	A1	No rain, sun
7/18/2014	2	20^{+}	0	0	A1	No rain, sun
7/18/2014	4	10^{+}	Nostrils	0	A1	No rain, sun
7/18/2014	6	10-	Lip	0	B1	No rain, cloudy
7/19/2014	20	10-		0	B1	No rain, partly cloudy, ants
7/19/2014	22	20^{+}	Mouth, lips	0	B1	No rain, cloudy
7/19/2014	24	30^{+}		0	B1	No rain, cloudy, ants
7/19/2014	26	50^{+}	Mouth full	0	B1	No rain, cloudy, ants
7/19/2014	28	40^{+}		1-2mm	B2	No rain, cloudy, ants
7/19/2014	30	40^{+}		1-2mm	B2	No rain, cloudy, ants
7/20/2014	44	10-		3-5mm, masses	B2	No rain, cloudy, ants
7/20/2014	46	50^{+}		3-5mm, masses	B2	No rain, cloudy, ants
7/20/2014	48	50^{+}	Under chin	3-5mm, masses	B2	No rain, partly cloudy, spider
7/21/2014		50^{+}	Lip	6^{+}mm, masses	B3	No rain, sun
7/22/2014		20^{+}		6^{+}mm, masses	B4	No rain, sun, rove beetle
7/23/2014		10-		6^{+}mm, masses	C1	No rain, sun
7/24/2014		10-		Few	C2	No rain, cloudy, 10^{+} rove beetles, little flies
7/25/2014		0		Few	C2	No rain, sun
8/1/2014		0			C3	Moderate rain, ant nest around cover edges
8/7/2014		0			C3	No rain, sun, ant nest, cricket
8/14/2014		0			D1	No rain, sun, ant nest, small brown beetles

Table D.12. Field notes, normal, uncovered (NC6).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/18/2014	0	0	0	0	A1	No rain, sun
7/18/2014	2	30+	0	0	A1	No rain, sun
7/18/2014	4	30+	Throat, lip	0	A1	No rain, sun, bee, ant
7/18/2014	6	10+	Nostril	0	B1	No rain, cloudy, bee, ant
7/19/2014	20	10+		0	B1	No rain, partly cloudy, bee, ant
7/19/2014	22	30+	Under throat	0	B1	No rain, cloudy, bee, ant
7/19/2014	24	50+		0	B2	No rain, cloudy, big ants taking eggs
7/19/2014	26	50+	Both eyes	0	B2	No rain, cloudy, big ants
7/19/2014	28	40+		1-2mm	B2	No rain, cloudy, big ants
7/19/2014	30	40+		1-2mm	B2	No rain, cloudy, big ants
7/20/2014	44	10-	Neck	1-2mm	B3	No rain, cloudy
7/20/2014	46	30+		3-5mm, masses	B3	No rain, cloudy, wasps, big ants
7/20/2014	48	40+	Roof of mouth	3-5mm, masses	B3	No rain, partly cloudy, big ants
7/21/2014		50+	Lip	6+mm, masses	B3	No rain, sun, in shade
7/22/2014		10+		6+mm, masses	B4	No rain, sun, in shade
7/23/2014		10-		6+mm, masses	C1	No rain, sun, in shade
7/24/2014		0		Maggot migration	C1	No rain, cloudy
7/25/2014		0		Maggot migration	C1	No rain, sun
8/1/2014		10-			C3	Moderate rain
8/7/2014		0			C3	No rain, sun
8/14/2014		0			D1	No rain, sun, small brown beetles and jumping larvae

Table D.13. Field notes, normal, uncovered (R1).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10+	Both nostrils	0	A1	No rain, cloudy
7/14/2014	4	10+	Lip	0	B1	No rain, cloudy, ants
7/14/2014	6	2	Wet masses	0	B1	Heavy rain
7/15/2014	20	10+	Dispersed	0	B1	No rain, cloudy
7/15/2014	22	30+	Mouth	0	B1	No rain, partly cloudy
7/15/2014	24	50+	Throat, ear	0	B1	No rain, partly cloudy
7/15/2014	26	50+	Ears, lip	1-2mm	B2	No rain, partly cloudy, carrion beetle, ants
7/15/2014	28	50+	Ground	1-2mm	B2	Moderate rain, carrion beetle
7/15/2014	30	50+		1-2mm	B2	No rain, cloudy, rove beetle
7/16/2014	44	0		3-5mm, masses	B2	No rain, cloudy
7/16/2014	46	0	Wet masses	3-5mm, masses	B2	Moderate rain, carrion beetle
7/16/2014	48	50+		3-5mm, masses	B2	No rain, cloudy, carrion beetle, ants
7/17/2014		50+		3-5mm, masses	B4	No rain, partly cloudy, 15+ rove beetles
7/18/2014		30+		6+mm, masses	B4	No rain, sun, 5+ rove beetles
7/19/2014		10+		6+mm, masses	C1	No rain, partly cloudy
7/20/2014		10-		Few	C2	No rain, cloudy, rove beetle
7/21/2014		10-		Maggot migration	C2	No rain, sun, in shade
7/22/2014		0			C2	No rain, sun, in shade
7/28/2014		10-			C3	No rain, partly cloudy, small flies
8/5/2014		0			D1	No rain, sun, in shade, small flies
8/12/2014		0			D1	No rain, partly cloudy, small flies, head removed from study area, small brown beetles and jumping larvae (Piophilidae)

Table D.14. Field notes, normal, uncovered (R2).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10+	Nostril, lip	0	A1	No rain, cloudy
7/14/2014	4	10+	Nostrils	0	B1	No rain, cloudy
7/14/2014	6	0	Wet masses	0	B1	Heavy rain
7/15/2014	20	10+	Dispersed	0	B1	No rain, cloudy
7/15/2014	22	20+	Mouth, top of nose	1-2mm	B1	No rain, partly cloudy
7/15/2014	24	50+	Throat	1-2mm	B1	No rain, partly cloudy
7/15/2014	26	50+	Ears, ground	1-2mm	B1	No rain, partly cloudy
7/15/2014	28	50+		1-2mm	B1	Moderate rain, rove beetles eating eggs/maggots
7/15/2014	30	50+		3-5mm, masses	B2	No rain, cloudy, 15+ rove beetle
7/16/2014	44	0	None visible	3-5mm, masses	B2	No rain, cloudy
7/16/2014	46	0		3-5mm, masses	B2	Moderate rain, 10+ rove beetle
7/16/2014	48	50+		3-5mm, masses	B2	No rain, cloudy, 10+ rove beetles
7/17/2014		40+		3-5mm, masses	B4	No rain, partly cloudy
7/18/2014		30+	New in mouth	6+mm, masses	B4	No rain, sun
7/19/2014		40+	Mouth	6+mm, masses	B4	No rain, partly cloudy
7/20/2014		30+	Nose	6+mm, masses	B4	No rain, cloudy
7/21/2014		10-		6+mm, masses	B5	No rain, sun
7/22/2014		10-		6+mm, masses	C1	No rain, sun
7/28/2014		10-		Maggot migration	C1	No rain, sun
8/5/2014		10-		Few	C2	No rain, partly cloudy, adipocere
8/12/2014		0			D1	No rain, sun, small flies

Table D.15. Field notes, normal, uncovered (R3).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10-	Both nostril, lip	0	A1	No rain, cloudy
7/14/2014	4	10-	Side	0	B1	No rain, cloudy
7/14/2014	6	10-	Wet masses	0	B1	Heavy rain
7/15/2014	20	10-		1-2mm	B1	No rain, cloudy
7/15/2014	22	20+	New eggs at throat	1-2mm, one 8mm fat maggot	B1	No rain, partly cloudy
7/15/2014	24	50+		1-2mm	B1	No rain, partly cloudy, carrion beetle
7/15/2014	26	50+		1-2mm	B2	No rain, partly cloudy, carrion beetle
7/15/2014	28	50+		1-2mm	B2	Moderate rain, carrion beetle
7/15/2014	30	50+		3-5mm, masses	B2	No rain, cloudy, carrion beetle
7/16/2014	44	0	None visible	3-5mm, masses	B2	No rain, cloudy
7/16/2014	46	10-		3-5mm, masses	B2	Moderate rain, carrion beetle
7/16/2014	48	50+		3-5mm, masses	B2	No rain, cloudy, carrion beetle (black orange)
7/17/2014		20+		6+mm, masses	B4	No rain, partly cloudy, 10+ rove beetles eating maggots
7/18/2014		10+		6+mm, masses	B4	No rain, sun, 5+ rove beetles
7/19/2014		10+		6+mm, masses	B5	No rain, partly cloudy, beetle with orange
7/20/2014		10+		Few	C1	No rain, cloudy, rove beetle
7/21/2014		10-		Maggot migration	C2	No rain, sun, in shade
7/22/2014		10-			C2	No rain, sun, in shade
7/28/2014		10-			C3	No rain, partly cloudy, small flies
8/5/2014		0			D1	No rain, sun, in shade, small flies
8/12/2014		1			D1	No rain, partly cloudy, small flies, small brown beetles and jumping larvae (Piophilidae)

Table D.16. Field notes, normal, uncovered (R4).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/12/2014	0	0	0	0	A1	No rain, partly cloudy
8/12/2014	2	20+	Nostril	0	A1	No rain, partly cloudy, yellow jackets, black/white wasp
8/12/2014	4	20+	Lip	0	B1	No rain, partly cloudy, yellow jackets
8/12/2014	6	20+		0	B1	No rain, partly cloudy, yellow jackets
8/13/2014	20	0	Very wet, many eggs washed away	0	B1	Heavy rain
8/13/2014	22	0		0	B1	Moderate rain
8/13/2014	24	10-		0	B2	Heavy rain
8/13/2014	26	10-	Mouth	1-2mm	B2	Moderate rain, cricket
8/13/2014	28	10-		1-2mm	B2	Heavy rain
8/13/2014	30	10+		1-2mm	B2	Moderate rain
8/14/2014	44	20+		1-2mm	B2	No rain, sun, in shade, yellow jacket
8/14/2014	46	30+	Ear	1-2mm	B2	No rain, sun, in shade
8/14/2014	48	20+		1-2mm	B2	No rain, sun, in shade, black/white wasp
8/15/2014		40+	Eye	3-5mm, masses	B3	No rain, cloudy, rove and carrion beetles
8/16/2014		50+		3-5mm, masses	B4	No rain, sun, rove and carrion beetles
8/17/2014		50+		6+mm	B5	No rain, partly cloudy, rove and carrion beetles, head has been moved ~60cm and soil has been excavated nearby
8/18/2014		30+		Few 6+mm	C1	No rain, partly cloudy, 20+ rove beetles
8/19/2014		30+		Few 6+mm	C2	No rain, sun, rove beetles
8/25/2014		10-		New activity in ear	C2	No rain, sun, in shade, small flies
9/17/2014		0			D2	No rain, sun, small brown beetles and jumping maggots

Table D.17. Field notes, normal, uncovered (R5).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/12/2014	0	0	0	0	A1	No rain, partly cloudy
8/12/2014	2	20+	Nostrils	0	A1	No rain, partly cloudy, yellow jacket
8/12/2014	4	20+	Mouth, under throat	0	B1	No rain, partly cloudy, yellow jacket, crickets
8/12/2014	6	20+		0	B1	No rain, partly cloudy, yellow jacket
8/13/2014	20	0	Very wet, many eggs washed away	0	B1	Heavy rain
8/13/2014	22	10-		0	B1	Moderate rain, yellow jacket
8/13/2014	24	0		0	B2	Heavy rain, yellow jacket
8/13/2014	26	10-	Mouth	1-2mm	B2	Moderate rain, yellow jacket
8/13/2014	28	0		1-2mm	B2	Heavy rain
8/13/2014	30	20+	Eye, ear	1-2mm	B2	Moderate rain
8/14/2014	44	20+	Under throat	1-2mm	B3	No rain, sun, rove beetles, ants
8/14/2014	46	40+		1-2mm	B3	No rain, sun
8/14/2014	48	40+		1-2mm	B3	No rain, sun
8/15/2014		50+		3-5mm, masses	B4	No rain, cloudy
8/16/2014		30+		3-5mm, masses	B4	No rain, sun, rove beetles, carrion beetles
8/17/2014		40+		6+mm, masses	B5	No rain, partly cloudy
8/18/2014		40+		6+mm, masses	C1	No rain, partly cloudy, post-feeding maggots dispersing
8/19/2014		20+		Few	C2	No rain, sun, post-feeding maggots dispersing
8/25/2014		10-			C2	No rain, sun, small flies
9/17/2014		0			D2	No rain, sun, small brown beetles and jumping maggots

Table D.18. Field notes, normal, uncovered (RC1).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10+	One nostril	0	A1	No rain, cloudy
7/14/2014	4	10-	Both nostrils, lip	0	A1	No rain, cloudy
7/14/2014	6	10-		0	B1	Heavy rain
7/15/2014	20	10+		1-2mm	B1	No rain, cloudy
7/15/2014	22	20+	Ears, eyes, side of nose	1-2mm	B1	No rain, partly cloudy
7/15/2014	24	50+	Throat	1-2mm	B2	No rain, partly cloudy
7/15/2014	26	50+	Ground, sides	1-2mm	B2	No rain, partly cloudy
7/15/2014	28	50+		1-2mm	B2	Moderate rain, carrion beetle
7/15/2014	30	50+	Throat	3-5mm, masses	B2	No rain, cloudy
7/16/2014	44	0		3-5mm, masses	B3	No rain, cloudy
7/16/2014	46	10-	Wet eggs	3-5mm, masses	B3	Moderate rain, carrion beetle
7/16/2014	48	50+	Face	3-5mm, masses	B3	No rain, cloudy, carrion beetle, rove beetle
7/17/2014		50+		3-5mm, masses	B4	No rain, partly cloudy, 20+ rove beetles
7/18/2014		30+		6+mm, masses	B5	No rain, sun, 2-3 rove beetles
7/19/2014		10-		6+mm, masses	C2	No rain, partly cloudy, rove beetle
7/20/2014		10-		6+mm, masses	C2	No rain, cloudy, small flies
7/21/2014		0		Few	C2	No rain, sun
7/22/2014		0		Few	C3	No rain, sun
7/28/2014		10-			C3	No rain, partly cloudy, small flies
8/5/2014		0			C3	No rain, sun, in shade, small flies
8/12/2014		0			D2	No rain, partly cloudy, small flies, small brown beetles and jumping larvae (Piophilidae)

Table D.19. Field notes, normal, uncovered (RC2).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10-	One nostril	0	A1	No rain, cloudy
7/14/2014	4	10-	Both nostril	0	B1	No rain, cloudy, ants
7/14/2014	6	10-		0	B1	Heavy rain
7/15/2014	20	10-		0	B1	No rain, cloudy
7/15/2014	22	10-	Face	1-2mm	B1	No rain, partly cloudy, rove beetles
7/15/2014	24	50+	Eye	1-2mm	B1	No rain, partly cloudy, red mites
7/15/2014	26	50+		1-2mm	B1	No rain, partly cloudy
7/15/2014	28	50+	Mouth	1-2mm	B1	Moderate rain, 10+ rove beetles eating eggs/larvae
7/15/2014	30	50+	Eyes	Few	B1	No rain, cloudy, 10+ rove beetles eating eggs/larvae
7/16/2014	44	0	None	Few	B3	No rain, cloudy
7/16/2014	46	0		Few	B3	Moderate rain, 15+ rove beetle
7/16/2014	48	50+		Few	B3	No rain, cloudy, 20+ rove beetles
7/17/2014		20+		Few	B4	No rain, partly cloudy, 20+ rove beetles
7/18/2014		40+	New masses on neck	3-5mm, masses	B5	No rain, sun, 5+ rove beetles
7/19/2014		50+		3-5mm, masses	B5	No rain, partly cloudy, 5+ rove beetles
7/20/2014		50+		6+mm, masses	B5	No rain, cloudy, rove beetle
7/21/2014		30+		6+mm, masses	B5	No rain, sun
7/22/2014		10+		6+mm, masses	C1	No rain, sun
7/28/2014		10-		6+mm, masses	C1	No rain, partly cloudy, small flies
8/5/2014		0			C3	No rain, sun, crickets, small brown beetles
8/12/2014		0			D1	No rain, partly cloudy, small flies, one rove beetle, medium brown beetle, small brown beetles and jumping larvae (Piophilidae)

Table D.20. Field notes, rain, covered (RC3).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10+	One nostril	0	A1	No rain, cloudy
7/14/2014	4	10-	Bridge of nose	0	A1	No rain, cloudy
7/14/2014	6	10-		0	A1	Heavy rain
7/15/2014	20	10-	Ears, mouth	0	B1	No rain, cloudy
7/15/2014	22	10+		1-2mm	B1	No rain, partly cloudy
7/15/2014	24	20+	Nostril, eye, ear	1-2mm	B1	No rain, partly cloudy
7/15/2014	26	30+	Cheek, ground	1-2mm	B2	No rain, partly cloudy, carrion beetle
7/15/2014	28	40+		1-2mm	B2	Moderate rain
7/15/2014	30	30+		3-5mm, masses	B2	No rain, cloudy, rove beetle
7/16/2014	44	0		3-5mm, masses	B3	No rain, cloudy
7/16/2014	46	10+	Wet eggs	3-5mm, masses	B3	Moderate rain, carrion beetle
7/16/2014	48	50+	Mouth	3-5mm, masses	B3	No rain, cloudy, one rove and one carrion beetle
7/17/2014		10+		3-5mm, masses	B3	No rain, partly cloudy, 20+ rove beetles
7/18/2014		10-		Few	B4	No rain, sun, 15+ rove beetles
7/19/2014		10+		Few	B4	No rain, partly cloudy, rove beetles
7/20/2014		30+		6+mm	B5	No rain, cloudy, rove beetle
7/21/2014		10+		Few	C1	No rain, sun, in shade
7/22/2014		10+		Few	C1	No rain, sun, in shade
7/28/2014		10-		Few	C1	No rain, partly cloudy, some adipocere
8/5/2014		0			C3	No rain, sun, in shade, rove beetle
8/12/2014		0			D1	No rain, partly cloudy, small flies, small brown beetles and jumping larvae (Piophilidae)

Table D.21. Field notes, normal, uncovered (RC4).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/12/2014	0	0	0	0	A1	No rain, partly cloudy
8/12/2014	2	20+	Nostril	0	A1	No rain, partly cloudy, yellow jacket
8/12/2014	4	20+	Mouth, lip	0	B1	No rain, partly cloudy, yellow jacket
8/12/2014	6	20+		0	B1	No rain, partly cloudy, yellow jacket
8/13/2014	20	0		0	B1	Heavy rain
8/13/2014	22	0		0	B1	Moderate rain, rove beetle
8/13/2014	24	10-	Cheeks	0	B1	Heavy rain
8/13/2014	26	10+	Mouth	1-2mm	B2	Moderate rain, yellow jacket, ants
8/13/2014	28	10-		1-2mm	B2	Heavy rain, ants
8/13/2014	30	10+		1-2mm	B2	Moderate rain
8/14/2014	44	10-		1-2mm	B2	No rain, sun, in shade
8/14/2014	46	20+		1-2mm	B2	No rain, sun, in shade
8/14/2014	48	20+	Face	1-2mm	B2	No rain, sun, in shade
8/15/2014		50+		3-5mm, masses	B3	No rain, cloudy, sexton beetle or gold-necked carrion beetle
8/16/2014		30+		3-5mm, masses	B4	No rain, sun, rove beetle, sexton or gold-necked carrion
8/17/2014		40+		3-5mm, masses	B4	No rain, partly cloudy, lots of rove and carrion beetles
8/18/2014		30+		6+mm, masses	C1	No rain, partly cloudy, rove beetle
8/19/2014		30+		6+mm, masses	C1	No rain, sun, rove beetles
8/25/2014		10-		All sizes all over face	C2	No rain, sun, shade, maggots everywhere
9/17/2014		0			D1	No rain, sun, small brown beetles and jumping maggots

Table D.22. Field notes, normal, uncovered (RC5).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/12/2014	0	0	0	0	A1	No rain, partly cloudy
8/12/2014	2	10+	Nostrils, top of nose	0	A1	No rain, partly cloudy, yellow jacket
8/12/2014	4	30+	Lip	0	A1	No rain, partly cloudy, yellow jacket, ants
8/12/2014	6	20+	Top of nose	0	A1	No rain, partly cloudy, yellow jacket, ants
8/13/2014	20	0		0	B1	Heavy rain
8/13/2014	22	0		0	B1	Moderate rain, ants
8/13/2014	24	10-	Mouth	0	B1	Heavy rain, yellow jacket
8/13/2014	26	10-		1-2mm	B1	Moderate rain, yellow jacket
8/13/2014	28	10-	Face	1-2mm	B2	Heavy rain, yellow jacket
8/13/2014	30	10+	Mouth	1-2mm	B2	Moderate rain
8/14/2014	44	10+	Nose	1-2mm	B2	No rain, sun, in shade
8/14/2014	46	10+		1-2mm	B2	No rain, sun, in shade, ants
8/14/2014	48	30+	Mouth	1-2mm	B3	No rain, sun
8/15/2014		50+		3-5mm, masses	B4	No rain, cloudy, rove beetle, small flies
8/16/2014		30+		3-5mm, masses	B4	No rain, sun, rove beetle
8/17/2014		50+		6+mm, masses	B4	No rain, partly cloudy, rove beetle
8/18/2014		30+		6+mm, few	B5	No rain, partly cloudy, rove beetle
8/19/2014		20+		6+mm, masses	B5	No rain, sun
8/25/2014		10-		Few	C2	No rain, sun, small flies
9/17/2014		0			D1	No rain, sun, small brown beetle and jumping maggots

Table D.23. Field notes, rain, covered partially (RCP1).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10-	0	0	A1	No rain, cloudy
7/14/2014	4	10-	Nostril, mouth	0	A1	No rain, cloudy, ants
7/14/2014	6	10-		0	A1	Heavy rain
7/15/2014	20	10-		0	B1	No rain, cloudy
7/15/2014	22	10+	Eye, bridge of nose	1-2mm	B1	No rain, partly cloudy, rove beetles
7/15/2014	24	50+	Face	1-2mm	B1	No rain, partly cloudy, rove beetles
7/15/2014	26	20+	Face	1-2mm	B2	No rain, partly cloudy, rove and carrion beetles eating eggs/larvae
7/15/2014	28	50+	All over face	1-2mm	B2	Moderate rain, rove beetle
7/15/2014	30	50+	Eggs gone	Few	B2	No rain, cloudy, 20+ rove beetles eating
7/16/2014	44	0	Eggs gone	Few	B3	No rain, cloudy, 20+ rove beetles eating
7/16/2014	46	0		Few	B3	Moderate rain, rove beetles
7/16/2014	48	50+		0	B3	No rain, cloudy, 15+ rove beetles
7/17/2014		20+		Few 6+mm	B4	No rain, partly cloudy, 10+ rove beetles, UNCOVER
7/18/2014		30+	New lay in mouth	1-2mm	B4	No rain, sun, 5+ rove beetles
7/19/2014		30+	Mouth	3-5mm, masses	B5	No rain, partly cloudy
7/20/2014		30+	Ears	6+mm, masses	B5	No rain, cloudy, rove beetle
7/21/2014		10+		6+mm, masses	B5	No rain, sun, in shade
7/22/2014		10-		6+mm, masses	C1	No rain, sun, in shade
7/28/2014		0		Migration	C1	No rain, sun, in shade
8/5/2014		10-			C1	No rain, partly cloudy, small flies
8/12/2014		0			C3	No rain, sun, in shade, small flies

Table D.24. Field notes, rain, covered partially (RCP2).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10-	0	0	A1	No rain, cloudy
7/14/2014	4	10-	One nostril, lip	0	A1	No rain, cloudy, ants
7/14/2014	6	10-		0	B1	Heavy rain
7/15/2014	20	10-		0	B1	No rain, cloudy
7/15/2014	22	20+	Face, nostrils	1-2mm	B1	No rain, partly cloudy, rove beetles
7/15/2014	24	40+	Side of face	1-2mm	B1	No rain, partly cloudy
7/15/2014	26	40+	Lip	1-2mm	B1	No rain, partly cloudy, carrion beetle, ants
7/15/2014	28	50+	Throat	1-2mm	B1	Moderate rain, carrion beetle
7/15/2014	30	50+	Mouth full	1-2mm	B3	No rain, cloudy, rove beetle
7/16/2014	44	0		1-2mm	B3	No rain, cloudy
7/16/2014	46	10-		3-5mm, masses	B3	Moderate rain
7/16/2014	48	50+	Mouth	3-5mm, masses	B3	No rain, cloudy, rove beetles
7/17/2014		50+	None visible	3-5mm, masses	B4	No rain, partly cloudy, 20+ rove beetles, UNCOVER
7/18/2014		20+		6+mm, masses	B5	No rain, sun,
7/19/2014		40+		6+mm, masses	C1	No rain, partly cloudy
7/20/2014		30+	Ear	6+mm, masses	C1	No rain, cloudy
7/21/2014		10-		Few	C1	No rain, sun, in shade
7/22/2014		10-		Few	C2	No rain, sun, in shade
7/28/2014		10-			C3	No rain, partly cloudy, small flies, small brown beetles
8/5/2014		0			D1	No rain, sun, in shade, small flies
8/12/2014		0			D2	No rain, partly cloudy, small flies, small brown beetles and jumping larvae (Piophilidae)

Table D.25. Field notes, rain, covered partially (RCP3).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
7/14/2014	0	0	0	0	A1	No rain, partly cloudy
7/14/2014	2	10-	Nostril	0	A1	No rain, cloudy
7/14/2014	4	10^{+}	Lip	0	B1	No rain, cloudy, ants
7/14/2014	6	10-		0	B1	Heavy rain
7/15/2014	20	10-		0	B1	No rain, cloudy
7/15/2014	22	10-	Top of nose	0	B1	No rain, partly cloudy
7/15/2014	24	30^{+}	Face, mouth, throat	1-2mm	B1	No rain, partly cloudy
7/15/2014	26	30^{+}	Forehead, eye	1-2mm	B2	No rain, partly cloudy, carrion beetle, ants
7/15/2014	28	40^{+}	Ground	1-2mm	B2	Moderate rain, carrion beetle
7/15/2014	30	40^{+}		1-2mm	B2	No rain, cloudy, rove beetle
7/16/2014	44	0		1-2mm	B3	No rain, cloudy
7/16/2014	46	10-	Face	1-2mm	B3	Moderate rain, carrion beetle
7/16/2014	48	50^{+}		3-5mm, masses	B4	No rain, cloudy, carrion beetle, ants
7/17/2014		50^{+}		3-5mm, masses	B4	No rain, partly cloudy, 15^{+} rove beetles, UNCOVER
7/18/2014		50^{+}		3-5mm, masses	B4	No rain, sun, 5^{+} rove beetles
7/19/2014		50^{+}		6^{+}mm, masses	B4	No rain, partly cloudy
7/20/2014		30^{+}		6^{+}mm, masses	B4	No rain, cloudy, rove beetle
7/21/2014		20^{+}		6^{+}mm, masses	C1	No rain, sun, in shade
7/22/2014		10-		Few	C1	No rain, sun, in shade
7/28/2014		10-		Few	C3	No rain, partly cloudy, small flies
8/5/2014		0			D1	No rain, sun, in shade, small flies
8/12/2014		0			D1	No rain, partly cloudy, small flies, small brown beetles and jumping larvae (Piophilidae)

Table D.26. Field notes, normal, uncovered (RCP4).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/12/2014	0	0	0	0	A1	No rain, partly cloudy
8/12/2014	2	10+	Nostrils, lip	0	A1	No rain, partly cloudy, yellow jacket
8/12/2014	4	20+	Mouth	0	B1	No rain, partly cloudy, yellow jacket, ants
8/12/2014	6	20+		0	B1	No rain, partly cloudy, yellow jacket, ants
8/13/2014	20	0		0	B1	Heavy rain, ants, rove beetle
8/13/2014	22	0		0	B1	Moderate rain, ants
8/13/2014	24	10-	Cheeks	0	B1	Heavy rain, yellow jacket
8/13/2014	26	10-	All over face	1-2mm	B2	Moderate rain, ants, rove beetle
8/13/2014	28	10-		1-2mm	B2	Heavy rain, 2+ rove beetle, ants
8/13/2014	30	10-		1-2mm	B2	Moderate rain, rove beetle, ants, yellow jacket
8/14/2014	44	10-	0	1-2mm	B2	No rain, sun, in shade, ants
8/14/2014	46	10-	Ear	1-2mm	B2	No rain, sun, in shade
8/14/2014	48	10+		1-2mm	B2	No rain, sun, in shade, removed basket
8/15/2014		40+		3-5mm, masses	B3	No rain, cloudy, rove and carrion beetle, something has excavated the soil next to head, UNCOVER
8/16/2014		30+		3-5mm, masses	B4	No rain, sun, rove and carrion beetle
8/17/2014		50+		6+mm, masses	B4	No rain, partly cloudy, rove and carrion beetle
8/18/2014		30+		6+mm, masses	B5	No rain, partly cloudy
8/19/2014		30+		6+mm, masses	C1	No rain, sun, ants
8/25/2014		10-		All sizes all over face	C2	No rain, sun, small file, ants
9/17/2014		0			D2	No rain, sun, small brown beetles, head moved about 2 feet

Table D.27. Field notes, normal, uncovered (RCP5).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/12/2014	0	0	0	0	A1	No rain, partly cloudy
8/12/2014	2	10+	Nostrils	0	A1	No rain, partly cloudy, yellow jacket
8/12/2014	4	20+		0	B1	No rain, partly cloudy, yellow jacket, ants
8/12/2014	6	20+		0	B1	No rain, partly cloudy, yellow jacket, ants
8/13/2014	20	0		0	B1	Heavy rain, yellow jacket
8/13/2014	22	0		0	B1	Moderate rain, yellow jacket
8/13/2014	24	0		0	B1	Heavy rain
8/13/2014	26	10-	Mouth	1-2mm	B1	Moderate rain, yellow jacket
8/13/2014	28	10-		1-2mm	B1	Heavy rain, yellow jacket
8/13/2014	30	10-	Lip	1-2mm	B2	Moderate rain
8/14/2014	44	10+		1-2mm	B2	No rain, sun, in shade
8/14/2014	46	10-		1-2mm, all over face	B2	No rain, sun, in shade
8/14/2014	48	30+	Outer nose	1-2mm	B3	No rain, sun, removed basket
8/15/2014		50+	Mouth	3-5mm, masses	B4	No rain, cloudy, carrion beetle, UNCOVER
8/16/2014		30+		3-5mm, masses	B4	No rain, sun
8/17/2014		50+		3-5mm, masses	B4	No rain, partly cloudy
8/18/2014		30+		6+mm, masses	B5	No rain, partly cloudy
8/19/2014		30+		6+mm, masses	C1	No rain, sun
8/25/2014		10-		6+mm, few	C2	No rain, sun, small flies
9/17/2014		0			D1	No rain, sun, small brown beetles and jumping maggots underneath

Table D.28. Field notes, active rainfall (AR1).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/13/2014	0	0	0	0	A1	Moderate rain
8/13/2014	2	0	0	0	A1	Heavy rain, yellow jacket
8/13/2014	4	10-	0	0	B1	Moderate rain, yellow jacket, cricket
8/13/2014	6	10-	0	0	B1	Heavy rain, yellow jacket
8/13/2014	8	10+	Mouth	0	B1	Moderate rain, yellow jacket
8/14/2014	22	10+		0	B1	No rain, sun, yellow jacket
8/14/2014	24	10-		0	B1	No rain, sun, in shade, yellow jacket
8/14/2014	26	20+	Nostril	0	B2	No rain, sun, yellow jacket
8/15/2014	50	20+	Ground, cheeks	1-2mm	B3	No rain, cloudy, yellow jacket, carrion beetle
8/16/2014		10+		1-2mm	B3	No rain, sun
8/17/2014		50+		3-5mm, masses	B4	No rain, partly cloudy, rove and carrion beetle, black/white wasp, soil is excavated around the carcass
8/18/2014		40+		3-5mm, masses	B4	No rain, partly cloudy, 15+ rove beetles and carrion beetles
8/19/2014		30+		6+ mm, masses	B5	No rain, sun, 5+ rove beetles, small flies
8/25/2014		0	0		C2	No rain, partly cloudy, small flies
9/17/2014		0			D2	No rain, sun, small brown beetles and jumping maggots underneath

Table D.29. Field notes, active rainfall (AR2).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/13/2014	0	0	0	0	A1	Moderate rain
8/13/2014	2	0	0	0	A1	Heavy rain
8/13/2014	4	10-	0	0	B1	Moderate rain
8/13/2014	6	10-	Mouth	0	B1	Heavy rain
8/13/2014	8	10-		0	B1	Moderate rain, yellow jacket
8/14/2014	22	10-		0	B1	No rain, sun, yellow jacket
8/14/2014	24	10-	Nostril	0	B1	No rain, sun, in shade, yellow jacket
8/14/2014	26	10-		0	B1	No rain, sun, in shade, yellow jacket
8/15/2014	50	20+	Mouth	1-2mm	B2	No rain, cloudy
8/16/2014		20+		1-2mm	B3	No rain, sun, 5+ yellow jackets
8/17/2014		50+	Eyes	3-5mm, masses	B3	No rain, partly cloudy, rove and carrion beetles
8/18/2014		40+		3-5mm, masses	B4	No rain, partly cloudy, rove and carrion beetles
8/19/2014		30+		6+mm, masses	B5	No rain, sun, 15+ rove beets, carrion beetles
8/25/2014		10-		Few, ear	C2	No rain, partly cloudy, small flies
9/17/2014		0			D1	No rain, sun, small brown beetles underneath

Table D.30. Field notes, active rainfall (AR3).

Date	PMI (hours)	Flies Observed	Eggs Observed	Larvae Observed	Megyesi *et al.* (2005)	Other
8/13/2014	0	0	0	0	A1	Moderate rain
8/13/2014	2	0	0	0	A1	Heavy rain
8/13/2014	4	10-	0	0	B1	Moderate rain
8/13/2014	6	0	Mouth	0	B1	Heavy rain
8/13/2014	8	10-		0	B1	Moderate rain, yellow jacket, ants
8/14/2014	22	10-		0	B1	No rain, sun, yellow jackets
8/14/2014	24	10-	Nostrils	0	B1	No rain, sun, in shade, yellow jackets
8/14/2014	26	10-		0	B2	No rain, sun, in shade, yellow jackets
8/15/2014	50	20+	Eye	1-2mm	B2	No rain, cloudy
8/16/2014		30+	Removed (see other)	3-5mm, few	B3	No rain, sun, Turkey vulture seen on the carcass, head moved ~60cm, chunks of tissue flensed from jaw bone and eye sockets, this activity has dislodged insect activity
8/17/2014		50+	Mouth	3-5mm, few	B4	No rain, partly cloudy, tons of new fly activity, head moved slightly
8/18/2014		40+		3-5mm, few	B5	No rain, partly cloudy, rove beetles, carcass moved ~3m, feathers collected
8/19/2014		20+		6+mm, few	C1	No rain, sun, head slightly moved
8/25/2014		10-			C2	No rain, partly cloudy, small flies
9/17/2014		0			D2	No rain, sun, small brown beetles under

REFERENCES

Amendt J., C.P. Campobasso, E. Gaudry, C. Reiter, H.N. LeBlanc, and M.J.R. Hall. (2007) Best practice in forensic entomology – Standards and guidelines. *International Journal of Legal Medicine* 121:90-104.

Amendt, J., C.S. Richards, C.P. Campobasso, R. Zehner, and M.J.R. Hall. (2011) Forensic entomology: Applications and limitations. *Forensic Science Medicine and Pathology* 7:379-392.

Anderson, G.S. (2011) Comparison of decomposition rates and faunal colonization of carrion in indoor and outdoor environments. *Journal of Forensic Sciences* 56:136–142.

Archer, M.S. (2003a) Annual variation in arrival and departure times of carrion insects at carcasses: Implications for successional studies in forensic entomology. *Australian Journal of Zoology* 51:569-576.

Archer, M.S. (2003b) Yearly activity patterns in southern Victoria (Australia) of seasonally active carrion insects. *Forensic Science International* 132:173-176.

Archer, M.S. (2004) Rainfall and temperature effects on the decomposition rate of exposed neonatal remains. *Science & Justice* 44:35–41.

Baldridge, R.S., S.G. Wallace, and R. Kirkpatrick. (2006) Investigation of nocturnal oviposition by necrophilous flies in central Texas. *Journal of Forensic Sciences* 51:125-126.

Baqué, M. and J. Amendt. (2013) Strengthen forensic entomology in court - the need for data exploration and the validation of a generalized additive mixed model. *International Journal of Legal Medicine* 127:213-223.

Barretta, M. (2012) *Normal and Taphonomic Arthropod Population Survey in Holliston, Massachusetts.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

Bass, W. (1997) Outdoor decomposition rates in Tennessee. *In:* Haglund, W. & M. Sorg, eds. *Forensic Taphonomy: The Postmortem Fate of Human Remains.* Boca Raton, FL: CRC Press. 181-186.

Baumer, T.G., N.V. Passalacqua, B.J. Powell, W.N. Newberry, T.W. Fenton, and R.C. Haut. (2010) Age-dependent fracture characteristics of rigid and compliant surface impacts on the infant skull: A porcine model. *Journal of Forensic Sciences* 55:993–997.

Behrensmeyer, A.K. (1978) Taphonomic and ecological information from bone weathering. *Paleobiology* 4:150-162.

Bent, A.C. (1961) *Life Histories of North American Birds of Prey*, vol. 9. Dover Publications. pp. 12-27.

Brouchoud, J.E. (2014) *Identification of Saw Marks on Burned Bones.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

Bristow, K.L. and G.S. Campbell. (1984) On the relationship between incoming solar radiation and daily maximum and minimum temperature. *Agricultural and Forest Meteorology* 31:159-166.

Byrd, J.H. and J.L. Castner. (2009) *Forensic Entomology: The Utility of Arthropods in Legal Investigations.* Boca Raton, FL: CRC Press.

Campobasso, C.P., G. Di Vella, and F. Introna. (2001) Factors affecting decomposition and Diptera colonization. *Forensic Science International* 120:18–27.

Carvalho, L.M.L., P.J. Thyssen, A.X. Linhares, and F.A.B. Palhares. (2000) A checklist of arthropods associated with pig carrion and human corpses in Southeastern Brazil. *Memórias do Instituto Oswaldo Cruz* 95:135–138.

Castner, J.L. and J.H. Byrd. (2000) *Forensic Insect Identification Cards.* Gainesville, FL: Feline Press.

Catts, E.P. and N.H. Haskell. (1990) *Entomology & Death: A Procedural Guide*. Clemson, SC: Joyce’s Print Shop, Inc.

Centeno, N., M. Maldonado, and A. Oliva. (2002) Seasonal patterns of arthropods occurring on sheltered and unsheltered pig carcasses in Buenos Aires Province (Argentina). *Forensic Science International* 126:63–70.

Cross P. (2007) *The Influence of Penetrative Trauma on the Rate of Decomposition*. (Master's Thesis) School of Forensic and Investigative Sciences, University of Central Lancashire, Preston, UK.

Cross, P. and T. Simmons (2010) The influence of penetrative trauma on the rate of decomposition. *Journal of Forensic Sciences* 55:295–301.

Damann, F.E. and D.O. Carter. (2014) Human decomposition ecology and postmortem microbiology. *In:* Pokines, J.T. & S.A. Symes, eds. *Manual of Forensic Taphonomy*. Boca Raton, FL: CRC Press. 37-49.

Damann, F.E., A. Tanittaisong, and D.O. Carter. (2012) Potential carcass enrichment of the University of Tennessee Anthropology Research Facility: A baseline survey of edaphic features. *Forensic Science International* 222:4–10.

Decota, K.J. (2011) *Taphonomy and Decomposition in a Massachusetts environment.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

De Jong, G.D. and W.W. Hoback. (2006) Effect of investigator disturbance in experimental forensic entomology: succession and community composition. *Medical and Veterinary Entomology* 20:248-258.

De Jong, G.D., W.W. Hoback, and L.G. Higley. (2011) Effect of investigator disturbance in experimental forensic entomology: Carcass biomass loss and temperature. *Journal of Forensic Sciences* 56:143-149.

Fenton, A., R. Wall, and N.P. French. (1999) Oviposition aggregation by the blowfly *Lucilia cuprina*. *Medical and Veterinary Entomology* 13:453–456.

Fiene, J.G., G.A. Sword, S.L. VanLaerhoven, and A.M. Tarone. (2014) The role of spatial aggregation in forensic entomology. *Journal of Medical Entomology* 51:1-9.

Galloway, A., W.H. Birkby, A.M. Jones, T.E. Henry, and B.O. Parks. (1989) Decay rates of human remains in an arid environment. *Journal of Forensic Sciences* 34:607–616.

Gaudry, E. and L. Dourel. (2013) Forensic entomology: Implementing quality assurance for expertise work. *International Journal of Legal Medicine* 127:1031-1037.

George, K.A., M.S. Archer, and T. Toop. (2013) Nocturnal colonization behavior of blowflies (Diptera: Calliphoridae) in southeastern Australia. *Journal of Forensic Sciences* 58:112-116.

Gião, J Z. and W.A.C. Godoy. (2007) Ovipositional behavior in predator and prey blowflies. *Journal of Insect Behavior* 20:77–86.

Goff, M.L. (1992) Problems in estimation of postmortem interval resulting from wrapping of the corpse: A case study from Hawaii. *Journal of Agricultural Entomology* 9:237–243.

Gordon, C.G. and J.E. Buikstra. (1981) Soil pH, bone preservation, and sampling bias at mortuary sites. *American Antiquity* 46:566-571.

Grassberger, M., E. Friedrich, and C. Reiter. (2003) The blowfly *Chrysomya albiceps* (Wiedemann) (Diptera: Calliphoridae) as a new forensic indicator in central Europe. *International Journal of Legal Medicine* 117:75–81.

Haskell N.H., R.D. Hall, V.K. Cervenka, and M.A. Clark. (1997) On the body: Insect's life stage presence and their postmortem artifacts. *In:* Haglund, W. & M. Sorg, eds. *Forensic Taphonomy: The Postmortem Fate of Human Remains.* Boca Raton, FL: CRC Press. 415-448.

Higgs, N.D. and J.T. Pokines. (2014) Marine environmental alterations to bone. *In:* Pokines, J.T. & S.A. Symes, eds. *Manual of Forensic Taphonomy*. Boca Raton, FL: CRC Press. 143-179.

Howard, L.R. and A.R. Gonzalez. (2001) Food safety and produce operations: What is the future? *Hortiscience* 36:33-39.

Huntington, T.G., A.D. Richardson, K.J. McGuire, and K. Hayhoe. (2009) Climate and hydrological changes in the northeastern United States: recent trends and implications for forested and aquatic ecosystems. *Canadian Journal of Forest Research* 39:199-212.

Jordana, F., J. Colat-Parros, and M. Benezech. (2013) Diagnosis of skull fractures according to postmortem interval: An experimental approach in a porcine model. *Journal of Forensic Sciences* 58:S156–S162.

Kelly, J.A., T.C. van der Linde, and G.S. Anderson. (2009) The influence of clothing and wrapping on carcass decomposition and arthropod succession during the warmer seasons in central South Africa. *Journal of Forensic Sciences* 54:1105-1112.

Magni, P.A., M. Borrini, and I.R. Dadour. (2013) Human remains found in two wells: a forensic entomology perspective. *Forensic Science Medicine and Pathology* 9:413–417.

Magni, P.A., C. Pérez-Bañón, M. Borrini, and I.R. Dadour. (2013) *Syritta pipiens* (Diptera: Syrphidae): A new species associated with human cadavers. *Forensic Science International* 231:e19–e23.

Mahat, N.A., Z. Zafarina, and P.T. Jayaprakash. (2009) Influence of rain and malathion on the oviposition and development of blowflies (Diptera: Calliphoridae) infesting rabbit carcasses in Kelantan, Malaysia. *Forensic Science International* 192:19–28.

Mann, R.W., W.M. Bass, and L. Meadows. (1990) Time since death and decomposition of the human body: Variables and observations in case and experimental field studies. *Journal of Forensic Sciences* 35:103–111.

Marks, M.K., J.C. Love, and I.R. Dadour. (2009) Taphonomy and time: estimating the postmortem interval. *In:* Steadman, D.W., ed. *Hard Evidence: Case Studies in Forensic Anthropology, 2nd edn.* Upper Saddle River, NJ: Prentice Hall. 165-78.

Megnin, J.P. (1894) *La Fauna de Cadavers (Fauna of Cadavers): Application de l'Entomologie a la Medicine Legale, Encyclopedie Scientifique des Aid-Memoire*. Paris: G. Masson.

Megyesi, M.S., S.P. Nawrocki, and N.H. Haskell. (2005) Using accumulated degree-days to estimate the postmortem interval from decomposed human remains. *Journal of Forensic Sciences* 50:618–626.

Meyer, J., B. Anderson, and D.O. Carter. (2013) Seasonal variation of carcass decomposition and gravesoil chemistry in a cold (Dfa) climate. *Journal of Forensic Sciences* 58:1175–1182.

Michaud J-P. and G. Moreau. (2009) Predicting the visitation of carcasses by carrion-related insects under different rates of degree-day accumulation. *Forensic Science International* 185:78-83.

Michaud J-P. and G. Moreau. (2011) A statistical approach based on accumulated degree-days to predict decomposition-related processes in forensic studies. *Journal of Forensic Sciences* 56:229-232.

Micozzi, M.S. (1991) *Postmortem Change in Human and Animal Remains.* Springfield, IL: Charles C Thomas.

Micozzi, M.S. (1986) Experimental study of postmortem change under field conditions: Effects of freezing, thawing, and mechanical injury. *Journal of Forensic Sciences* 31:953-961.

Moore, G. (2014) *Correlation Between Chainsaw Type and Tool Marks in Sectioned Bone.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

Payne, J.A. (1965) A summer carrion study of the baby pig, *Sus scrofa* Linnaeus. *Ecology* 46:592-602.

Pendray, J. (2015) *Differentiation and Reconstruction of Heat Related and Blunt Force Trauma Fractures.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

Pitts, K.M. and R. Wall. (2004) Adult mortality and oviposition rates in field and captive populations of the blowfly *Lucilia sericata. Ecological Entomology* 29:727–734.

Pokines, J.T. (2014) Collection of macroscopic osseous taphonomic data and the recognition of taphonomic suites of characteristics. *In:* Pokines, J.T. & S.A. Symes, eds. *Manual of Forensic Taphonomy*. Boca Raton, FL: CRC Press. 1-17.

Pokines, J.T. and S.E. Baker. (2014) Avian taphonomy. *In:* Pokines, J.T. & S.A. Symes, eds. *Manual of Forensic Taphonomy*. Boca Raton, FL: CRC Press. 427-446.

Powell, B.J., N.V. Passalacqua, T.G. Baumer, T.W. Fenton, and R.C. Haut. (2012) Fracture patterns on the infant porcine skull following severe blunt impact. *Journal of Forensic Sciences* 57:312–317.

Powell, B.J., N.V. Passalacqua, T.W. Fenton, and R.C. Haut. (2013) Fracture characteristics of entrapped head impacts versus controlled head drops in infant porcine specimens. *Journal of Forensic Sciences* 58:678–683.

Reibe, S. and B. Madea. (2010) How promptly do blowflies colonise fresh carcasses? A study comparing indoor with outdoor locations. *Forensic Science International* 195:52–57.

Richards, E.N. and M.L. Goff. (1997) Arthropod succession on exposed carrion in three contrasting tropical habitats on Hawaii Island, Hawaii. *Journal of Medical Entomology* 34:328–339.

Ricketts, D. (2013) *Scavenging Effects and Scattered Patterns on Porcine Carcasses in Eastern Massachusetts.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

Rodriguez, W.C. and W.M. Bass. (1983) Insect activity and its relationship to decay rates of human cadavers in east Tennessee. *Journal of Forensic Sciences* 28:423–432

Schoenly, K.G., N.H. Haskell, R.D. Hall, and J.R. Gbur. (2007) Comparative performance and complementarity of four sampling methods and Arthropod preference tests from human and porcine remains at the Forensic Anthropology Center in Knoxville, Tennessee. *Journal of Medical Entomology* 44:881–894.

Shean, B.S., L. Messinger, and M. Papworth. (1993) Observations of differential decomposition on sun exposed v. shaded pig carrion in coastal Washington State. *Journal of Forensic Sciences* 38:938–949.

Shelomi, M., L.M. Matern, J.M. Dinstell, D.W. Harris, and R.B. Kimsey. (2012) DEET (N,N-Diethyl-meta-toluamide) induced delay of blowfly landing and oviposition rates on treated pig carrion (Sus scrofa L.). *Journal of Forensic Sciences* 57:1507–1511.

Smith, A.C. (2014) The effects of sharp-force thoracic trauma on the rate and pattern of decomposition. *Journal of Forensic Sciences* 59:319-326.

Spencer, J. (2002) *The nocturnal oviposition behavior of blowflies in the southwest of Britain during the months of August and September.* (Doctoral Dissertation) Bournemouth University, Dorset, UK.

Tabor, K.I., C.C. Brewster, and R.D. Fell. (2004) Analysis of the successional patterns of insects on carrion in southwest Virginia. *Entomological Society of America* 41:785-795.

Tandy, C.B. (2011) *Time Line of Decomposition of Porcine Bone Marrow.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

Tessmer, J.W., C.L. Meek, and V.L. Wright. (1995) Circadian patterns of oviposition by necrophilous flies (Diptera: Calliphoridae) in southern Louisiana. *Southwestern Entomologist* 20:439-445.

Tomberlin, J.K., R. Mohr, M.E. Benbow, A.M. Tarone, and S. VanLaerhoven. (2011) A roadmap for bridging basic and applied research in forensic entomology. *Annual Review of Entomology* 56:401-421.

U.S. Department of Commerce. (2011) National Weather Service: Heavy Rainfall Definitions. Retrieved November 23, 2013, from http://www.srh.noaa.gov/tsa/dsp/element.php?element=HEAVY_RAIN

Vass, A.A. (2011). The elusive universal post-mortem interval formula. *Forensic Science International* 204:34–40.

Vass, A.A., W.M. Bass, J. Wolt, J. Foss, and J. Ammons. (1992) Time since death determinations of human cadavers using soil solution. *Journal of Forensic Sciences* 37:1236-1253.

Voss, S.C., D.F. Cook, and I.R. Dadour. (2011) Decomposition and insect succession of clothed and unclothed carcasses in western Australia. *Forensic Science International* 211:67–75.

Voss, S.C., S.L. Forbes, and I.R. Dadour. (2008) Decomposition and insect succession on cadavers inside a vehicle environment. *Forensic Science, Medicine, and Pathology* 4:22–32.

Wallman, J.F. (2001) A key to the adults of species of blowflies in southern Australia known or suspected to breed in carrion. *Medical and Veterinary Entomology* 15:433–437.

Westling, L. (2012) *Aquatic Decomposition: An Examination of Factors Surrounding Freshwater Decomposition in Eastern Massachusetts.* (Master's Thesis) Boston University School of Medicine, Boston, MA.

White, E.M. and L.A. Hannus. (1983) Chemical weathering of bone in archaeological soils. *American Antiquity* 48:316-322.

Whitworth, T. (2006) Keys to the genera and species of blow flies (Diptera: Calliphoridae) of America north of Mexico. *Proceedings of the Entomological Society of Washington* 108:689–725.

Yang, S-T. and S-F. Shiao. (2012) Oviposition preferences of two forensically Important blow fly species, *Chrysomya megacephala* and *C. rufifacies* (Diptera: Calliphoridae), and implications for postmortem interval estimation. *Journal of Medical Entomology* 49:424–435.

Zanetti N.I., E.C. Visciallrelli, and N.D. Centeno. (2014) Taphonomic marks on pig tissue due to cadaveric Coleoptera activity under controlled conditions. *Journal of Forensic Sciences* 59:997-1001.

[redacted]

[redacted]

[redacted]

[redacted]