Insect Faunal Succession and Development of Forensically Important Flies on Deer Carcasses in Southwest Virginia

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University

in partial fulfillment of the requirements for the degree of

Master of Science

In

Entomology

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July 12th, 2012

Blacksburg, VA

Keywords:

Forensic Entomology, Insect Succession, Wildlife Poaching, Postmortem Interval,

Phormia regina, Venison, Pork

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ABSTRACT

Forensic entomology has become synonymous with medico-legal entomology and involves the use of insects in legal and criminal investigations. Insects have been used as evidence in cases of wrongful death of humans and in wildlife poaching cases for many years. The first jail time sentence for wildlife poaching in Manitoba, Canada was awarded after insect evidence was used to create a timeline for the crime. In the interest of advancing the science of forensic entomology, insect faunal succession was studied on four white-tailed deer carcasses in southwest Virginia in the summers of 2009 and 2010. The patterns of insect succession between the summers of 2009 and 2010 were similar at $\alpha = 0.05$. Necrophagous insects arrived in a successional pattern as has been observed on other animal models (*e.g.* pigs) during past studies conducted in southwest Virginia. To further explore the role of wildlife specific variables to forensic entomology, larvae of *Phormia regina,* Meigen, were reared on pork and venison in a laboratory at Virginia Tech. Environmental rearing conditions were 30° C, 75% RH and 14:10 hour light dark cycle. Significant differences in lengths of 3rd instar and combined overall maggot lengths were found for maggots reared on the different meat sources. Mean adult weights and wing lengths of venison-reared flies were significantly greater than those reared on pork at α=0.05.

DEDICATION

I dedicate this work to my loving family who always nurtured my curiosity and helped me to realize my goals.

Acknowledgements

I would like to thank my Advisor, Dr. Richard D. Fell for the invitation to learn and grow as a student in his lab.

Thank you to my graduate advisory committee, Mr. Nelson Lafon, Dr. Kimberly Kreitlow and Dr. Carlyle Brewster. The support and guidance you have provided me has helped to shape this research and this thesis into a worthwhile work of which I am proud.

Dr. David Gerrard and the members of his Meat Science and Muscle Biology Laboratory, Eric England and Sulaiman Matarneh, provided invaluable help and meat sample analysis, thank you.

Scotty Bolling, Kathy Shelor, Sarah Kenley and Sandra Gabbert have helped me throughout my research process and I owe the successful completion of my research project to them because of their support.

Thanks are owed to the community members who volunteered their time, efforts and permission to the acquisitioning of my faunal succession subjects.

Lastly I would like to thank the members of the Department of Entomology at Virginia Tech for their support and friendship. I am proud to call this department home and I look forward to continuing to work here.

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1 Introduction

Forensic entomology has become synonymous with medico-legal entomology and involves the use of insects in legal and criminal investigations. However, the discipline also encompasses legal matters associated with stored products and urban entomology, two other areas of entomology that deal with the conflict between insects and humans (Byrd and Castner 2001, Hall 2001). Insects have been used as evidence in cases of wrongful death of humans and in wildlife poaching cases for many years. The first jail time sentence for wildlife poaching in Manitoba, Canada was awarded after insect evidence was used to create a timeline for the crime (Anderson 1999). Carcass-feeding (necrophagous) insects utilize decaying animals, including humans, so readily that they often arrive at a carcass before other animals, especially humans, detect them. The presence of carrion-attending insects at carcasses, combined with their decomposition-dependent life cycles, ties them to carcasses and their presence or absence can be used as evidence in investigations into death.

Insects arrive at a carcass in a predictable and observable pattern referred to as a faunal succession (Walker 1957, Reed 1958, Payne 1965). The pattern of succession is similar to resource succession patterns observed elsewhere in nature. The first insects to arrive colonize the carcass and utilize the soft tissues that are the first to break down. This group is most often made up of calliphorid and sarcophagid flies (Diptera) and their larvae (maggots) that feed on the soft tissues. After the flies colonize the carcass, predators of the maggots arrive and acquire the nutrients secured by the maggots. As other tissues become exposed or accessible in the decomposition process, additional waves of insects arrive and colonize the carcass (Reed 1958, Payne 1965, Braack 1987).

Given enough time and temperature, insects can consume by an entire carcass, leaving only the bones.

Documentation and manipulation of insect faunal succession has allowed entomologists to establish timelines for succession patterns (Payne 1965, Anderson and VanLaerhoven 1996, Watson 2003, 2005). The insects found on a carcass can be used in concert with environmental factors to estimate the minimum time since insect colonization (Byrd and Castner 2001). This estimation often approximates postmortem interval (time since death) when colonization quickly follows death, and is referred to as a minimum Postmortem Interval (PMI_{MIN}). The keen ability of certain flies to detect a carcass often results in their presence within minutes of death (Catts and Goff 1992). PMI_{MIN} estimates constructed using baseline faunal succession data can be used in scenarios where discovery of the body is well after the initial colonization. Depending on the environment, PMI_{MIN} estimates can be used for long periods of time after death. Studies of the baseline fauna have been conducted in southwest Virginia but have not focused on the remains of wildlife for poaching investigations (Tabor et al. 2005a, Tabor et al. 2005b, Monthei 2009). Additional methods for estimating the PMI_{MIN} utilize finer scales of time and are closely related to the life cycles of the initial colonizing insects (Anderson 1999).

Short-term estimates of PMI_{MIN} rely on finding and measuring the oldest insect to develop on a carcass, with greater accuracy when oviposition occurred shortly after death. Once the oldest insect has been identified, the thermal history of the insect is determined and a growth rate for that insect is developed (Catts and Haskell 1990). Insect growth is regulated by temperature and food availability and is a more reliable indicator of PMI_{MIN} than traditional methods utilized by medical examiners. Measures of livor mortis, algor mortis, rigor mortis and vitreous fluid are not reliable beyond 72 hr after death (Greenberg and Kunich 2002). Insect growth is a more accurate determinant once the environmental conditions are documented from local weather data or crime scene data recorders.

One method of determining the age of live maggots requires the rearing of the maggot to pupation or the adult stage for comparison to data from rearing studies. If the maggot is still feeding when collected, it is necessary to sustain the larvae on a suitable food source. Maggots collected from decomposing meat should ideally be reared on the same material. In investigations of wildlife poaching, short term estimations may involve the collection of maggots from one species of animal and then lab rearing the larvae to adult on another species.

The purpose of this study is to examine the insect faunal succession patterns on white-tailed deer, *Odocoileus virginianus* Rafinesque, in southwest Virginia, and the development of the forensically important black blow fly, *Phormia regina*, Meigen, on different diets. Insect faunal succession was observed on four different carcasses over the summers of 2009 and 2010. The observed species and patterns were compared to past succession studies on domestic pig, *Sus scrofa* L., conducted in the region. Comparative rearing using different diets was conducted with the black blow fly because of its forensic importance and past studies in this region. The effect that the rearing medium has on the development of fly larvae has not been studied, and part of this study was to determine if the potential differences in larval development could affect PMI_{MIN} estimations.

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2 Literature Review

2.1 Introduction to forensic entomology

Forensic entomology is the application of insects in legal and criminal investigations (Byrd and Castner 2001). Legal investigations can benefit from collaboration with an entomologist who is able to interpret entomological evidence in cases involving a variety of factors, such as food contamination, violation of pest control contracts, infant or elderly neglect, ante mortem infestation, or postmortem colonization of humans and wildlife. Medico-legal forensic entomology involves insect evidence in medical and criminal cases of injury or neglect. Forensic entomology has been applied to cases of wrongful death or suspected foul play for decades, with a surge of research in this area in recent years. Insects often discover deceased individuals before humans do and their presence provides useful information to investigators (Catts and Goff 1992, Byrd and Castner 2001). Once initial colonization occurs, insects arrive in an often-predictable pattern according to resource availability, and this predictability allows entomologists to relate the time the carcass has been exposed to the insects that are found (Anderson and VanLaerhoven 1996, Watson 2003, 2005). Insect evidence can be as small as a single egg mass or as prominent as a large maggot mass when a carcass is in the active decay stage (Deonier 1940, Greenberg 1991). At other times, only the dry remains are present and the insect evidence is left behind. Beetles that feed on dried remains or fly puparia may provide clues as to how long the remains have been there. Most research concerns the use of insect evidence in human death cases and often utilizes domestic pigs, *Sus scrofa* L., as human analogues. Using pigs allows investigators and researchers to manipulate the ante and postmortem events surrounding death to simulate situations that human decedents may have encountered. Manipulations and observations provide valuable information about the condition of remains and insects present when the carcass was originally discovered. Forensic entomology provides the justice system with the ability to extrapolate a minimum time since death based on evidence of the period of insect activity.

Until recently, forensic entomology has not been a common tool in legal investigations even though it has been in practice for centuries. In the *Washing Away of Wrongs*, Sung Tzu provides an account where entomological evidence was used to solve a murder in a 13th century Chinese agricultural village. After determining that a farmer had been slashed and killed with a sickle, the investigator ordered the village farmers to present themselves with their sickles. The investigator observed that though all sickles were clean of visible blood, flies landed on just one sickle. Knowing that flies would be attracted to blood and its remnant properties, the investigator confronted the owner of the sickle who then confessed to the murder (Song and McKnight 1981). Later cases involving the life cycles of insects occurred but without great frequency or accuracy. For many years medical examiners and crime scene investigators would remove and discard insects found on and around bodies. Recently, through the continued investigation of the ecology surrounding death and the reliance of law enforcement on scientific findings, forensic entomology has made great progress as an asset to the justice system. In 2009, the National Research Council published a 352 page review of the forensic sciences and called for more objective research that focuses on standardization within the forensic sciences (Community and Council 2009). Recent efforts have been made to comply with the NRC's recommendations to improve the accuracy of field techniques. These efforts have focused on more precise terminology and the exploration of potentially confounding factors associated with the precision of estimating the period of insect activity (Amendt et al. 2004, Tomberlin et al. 2011).

Entomological evidence can support or contradict the interpretations of other types of physical evidence and witness accounts. Blood feeding insects found on a victim can contain DNA from its original host, which can be used as evidence of the suspect's contact with the victim (Lord et al. 1998). The most commonly applied use of insect evidence is through the estimation of the minimum postmortem interval (PMI_{MIN}) (Byrd and Castner 2001). Currently, there is a shift from the terminology of the PMI_{MIN} to the period of insect activity, or PIA, (Amendt et al. 2004, Tomberlin et al. 2011). The PIA ideally includes the initial discovery, mating, and oviposition on a carcass, whereas the PMI_{MIN} estimates the minimum period of time that insects have utilized the carcass. The PIA can be used in cases of antemortem infestation, while the PMI_{MIN} relies on the positive identification of the insects collected and determines the timing of infestation as ante, peri or postmortem (Catts and Goff 1992, Tomberlin et al. 2011). The following chapters will utilize the terminology of the PMI_{MIN} because of its current acceptance in the forensic entomology literature.

Witness accounts and evidence of the victim's last activities can provide a rough timeline, assuming these sources of evidence are accurate. When insect activity is utilized to construct a timeline of victim exposure, the estimation of a PMI_{MIN} based on the insect biology includes a specific confidence interval. The accounts of witnesses and victim activity may be wholly inaccurate and typically have no measureable confidence interval. An estimate of the PMI_{MIN} is generally constructed based on the age of the oldest insect that has carried out its life cycle on the carcass or decedent. Often the first insects to arrive and lay eggs on a carcass are flies (Order: Diptera). Additionally, because insects colonize carcasses in a predictable pattern, the PMI_{MIN} can be based on the composition of insect fauna on the carcass. The natural ability of insects to locate and utilize decaying material, while having temperature dependent growth, is what makes them valuable as evidence in PMI_{MIN} estimation.

Carrion ecology has been well studied, and the community of insects associated with carrion can be divided by the feeding guilds of insects that hold specific niches in the community. Groups of insects other than flies utilize carcasses as food sources and either feed directly upon them or upon the maggots that are utilizing the carcass tissues (Braack 1987). After an initial colonization, the carcass is no longer a resource for a single group of organisms, but becomes a small-scale community ecosystem, or microsere (Walker 1957a). Various resources in this micro ecosystem become available at different times throughout the stages of the carcass' decomposition. Insects utilize the bodily fluids, skin, muscle tissue, hair, hooves, and the contents of the digestive tract and arrive when their preferred diet becomes available (Braack 1987).

The faunal succession pattern at a carcass is observable over time and is often predictable based on local species distributions and weather patterns. The phenomenon of faunal succession on a carcass can serve as one source of insect evidence in forensic entomology. The species of insects found can often indicate a specific stage of succession and, when combined with appropriate weather data, a long term PMI_{MIN} can be estimated. Colonization and resource utilization processes are more rate dependent than decomposition, and insects collected can provide a snapshot of the respective successional stage. In North America, there are five commonly accepted stages of decomposition: fresh, bloat, decay, post-decay and the skeletal, or dry stage (Payne 1965). The decomposition of organic material is subject to a wide variety of exogenous and endogenous factors including sunlight, temperature, humidity, evaporation rates, enteric bacterial content, type of trauma, and toxicological factors of the decedent. Decomposition has many variables to be rate dependent and is not reliable as the sole source for PMI_{MIN} estimation. The growth and development of insects are rate dependent processes that are greatly affected by heat and resource availability (Wigglesworth 1972). Major factors influencing insect growth and development, eg. temperature and relative humidity, are monitored and recorded by the National Oceanographic and Atmospheric Administration throughout the United States. These data can be used to extrapolate the development rates, and in turn the age of the insects at the time of their collection. Extensive, region-specific data sets are limited by the location and abundance of weather stations. Temperature data allow the development rate to be extrapolated using an accumulated degree-day model that constructs a reliable PMI_{MIN} with a given confidence interval.

2.2 Current research with *Sus scrofa* L.

The PMI_{MIN} is useful in the death investigations of humans but only limited studies have been conducted using human remains. Much of the research that concentrates on the human aspects of forensic entomology uses the remains of domestic pigs (*Sus scrofa* L). Pigs and humans have similar body composition and hair covering. A small pig (22 kg) can serve as an excellent analogue to the human torso (Catts and Goff 1992, Anderson and VanLaerhoven 1996). Utilizing a pig carcass can allow the researcher to manipulate variables or recreate death scenes and then observe how the original conditions may have affected the insect activity on the carcass. Previous pig studies have included exploration of victim toxicology (Goff and Wayne 2000, Tabor et al. 2005b, Monthei 2009), burning of the carcass (Avila and Goff 1998), and concealment by wrapping (Goff 1992). Faunal succession studies utilizing pigs have been ongoing since the 1960s starting with Payne's exhaustive study of the baby pig (1965). Payne documented the stages of decomposition and the succession of arthropods on decomposing pigs (Payne 1965). Later pig studies were used to explore succession patterns in specific regions (Anderson and VanLaerhoven 1996, Tabor et al. 2004, Gill 2005, Tabor et al. 2005a, Chin et al. 2007, Gruner et al. 2007, Matuszewski et al. 2008, 2010). Having used decaying human cadavers for over 20 years at the Anthropology Research Facility in Knoxville, TN, the facility recently used pigs as controls to monitor the effects of decomposing cadavers on the abundance and composition of the carrion-attending arthropod community (Shahid et al. 2003, Schoenly et al. 2005). Research using pig carcasses can be expensive and, in many cases, other animal models have been used to sample the necrophagous insect community in the area of the study (Reed 1958, Payne 1965, Joy et al. 2002, Watson 2003, 2005).

Geographic location can serve as an important external factor in the composition of the colonizing insects based on their various distributions and habits. The population ranges of forensically important species affect their presence or absence at a carcass. Certain species can also exhibit preferences for egg-laying sites and may be attracted to very specific components of the decaying carcass. For example, the latrine fly (*Fannia scalaris* Fabricius) can be found worldwide on fecal material or at carcasses where the gut contents have been exposed (Byrd and Castner 2001). Timing and seasonality of necrophagous arthropods are also particularly important factors, as temperature can directly affect the rate dependent growth and behavior of the insects that are most commonly used in PMI_{MIN} construction. The black blow fly *Phormia regina*, Meigen, is mainly a cool weather species while the sheep blow fly *Lucillia sericata,* Meigen, may be found in the same area during hot summer months. Interspecifc competition can also affect species composition. *Chrysoma rufifacies*, Macquart may outcompete other species of blow fly because of its predacious habits in large maggot masses (Catts and Goff 1992). Other carrion-associated insects are variable in distribution and habits and their presence or absence can affect the ecological interactions between species.

2.3 Role of PMI_{MIN} in Forensic Entomology and Faunal Succession

Forensic entomology examines the relationships of organisms associated with an ephemeral and valuable resource, the carcass. The exposed carcass decays and decomposes by means of different processes, such as autolysis, bacterial activity, and insect colonization (Braack 1987, Byrd and Castner 2001). In natural environments, insects are often the first organisms to arrive at the carcass, playing a significant role in the process of decomposition by removing vast amounts of soft tissues (Reed 1958, Payne 1965, Braack 1987, Byrd and Castner 2010). The process of insect colonization usually begins at the onset of death and exposure. These insects are primarily flies that rapidly respond to the signs of death and decay while searching for a potential food resource or oviposition site. The chemicals emitted by a decaying body can be thought of as semiochemicals, or chemicals that serve as signals. The prefix "semio," stems from the Greek word *simeon*, meaning 'sign' or 'signal' (Agelopoulos et al. 1999). When considering a carrion-attending arthropod's response to a carcass, the signal comes from one species and is received by another; this inter-specific semiochemical is refered to as an allelochemical (Nordlund and Lewis 1976). To be even more exact, something dead may emit chemicals that no longer benefit the emitter but only the receiver. The specific allelochemical involved is termed an apneumone. Apneumone comes from the Greek word *â-pneum,* meaning 'lifeless' or 'breathless' (Nordlund and Lewis 1976).

Upon death, decomposition proceeds when cells begin to break down from the inside out, as various enzymes and chemicals are no longer kept in check by processes that were once active in the living cells (Vass 2001). Self-digestion (autolysis), and the bacterial decomposition of a body produce volatile organic compounds as byproducts, such as putrescine and cadaverine (Vass 2001). When these compounds emanate from a carcass, they serve as major olfactory cues for many pioneer species of carrion-attending insects, such as flies. In most situations, blowflies (Calliphoridae) and flesh-flies (Sarcophagidae) are among the first insects to arrive and will readily oviposit or larviposit, respectively. Upon hatching, the resulting maggots will greatly accelerate decomposition by consuming the soft tissues of body. The feeding action of individual maggots is limited by the absence of mandibles, but is accomplished with the aid of mouthhooks. Without chewing mouthparts, individual maggots cannot break the epidermal layer of carcasses but the formation of large masses facilitate the liquefaction and digestion of soft tissues

Adult females will typically oviposit in or near orifices, wounds or other areas of major trauma. The invasion of these orifices is so regular that there are observable and predictable feeding patterns that provide useful information about tissue damage even after the tissue has been consumed. Often in human investigations, areas of trauma can be identified by examining patterns of insect colonization and soft tissue consumption long after decomposition has obscured the composition of the body surface. Trauma may provide additional areas of entry into the body cavity, thereby disrupting the typical colonization pattern.

Regional studies provide a more precise view of the role that these exogenous variables can play at the death or discovery scene. Faunal succession studies have been conducted on each of the inhabited continents. Apart from initial studies by Fuller (1934) and Bornemisscza (1957) in Australia, most early faunal succession research was conducted in the United States. The initial impetus to study necrophagous insects was Motter's (1898) interest in Megnin's observations of insect activity on cadavers in France during the mid 19th century (Motter 1898). Motter examined 150 exhumed human cadavers from Washington D.C. between 1896 and 1897. Canine remains were also exhumed and examined for insects. Notes were later made on the differences in the carrion-attending species between human and canine cadavers. Fuller (1934) began a study of carrion insects in order to gather information about flies that might cause myiasis in sheep (Fuller 1934). Bornemisscza (1957) later used guinea pigs in Austrailia to further examine succession of arthropods and the soil fauna. In Tennessee, Walker (1957) observed the succession of fauna attracted to different decaying materials. Also in Tennessee, Reed (1958) documented the faunal succession on decomposing dog carcasses. One of the most important studies was that of Payne (1965), in which he explored the various animal model types that may be appropriate for conducting faunal succession studies. Payne also built on the concept of decomposition stages and documented the stages that are still commonly accepted today (Amendt et al. 2004). Initial studies were carried out on the east coast of the United States and later faunal succession studies were undertaken throughout the world.

In Kenya, Coe (1978) first explored the community of insects that colonized African elephant (*Loxodonta spp.*) carcasses after a devastating drought. Braack (1987) later conducted succession studies using impalas (*Aepyceros melampus*) in South Africa. Later studies used varying animal models that appear to have been chosen based on their availability. Shi et al. (2009) utilized dead rabbits to observe carrion-associated arthropods in China. Rabbits have also been used in studies in Egypt (Tantawi et al. 1996), Spain (Arnaldos et al. 2001), France (Bourel et al. 1999), Colorado (De Jong and Chadwick 1999), and California (Denno and Cothran 1976). Archer and Elgar (2003) used pigs and baited traps to observe carcass-colonizing arthropods in Australia. In Norway, faunistic studies were conducted using roe deer (*Capreolus capreolus*), and focused on the diversity of coleopterans present on the carcass (Melis et al. 2004). Extensive studies using pig carrion have been undertaken in Poland (Matuszewski et al. 2008, 2009, 2010) and rat carrion studies were conducted in Venezuela to observe regional faunal succession patterns (Velasquez 2008).

Recent reviews of human centered research can be found in Tabor et al. (2005) and Monthei (2009). These studies were conducted in Blacksburg, VA, in the Mid-Atlantic region of the US. In Tabor et al. (2005), the effects of ante-mortem ethanol ingestion were examined, while Monthei (2009) explored the effects of various drugs in a post-mortem exploration of entomotoxicology. These studies sought to isolate the specific challenges of estimating the PMI by manipulating diet and environmental conditions to better understand human cases without the use of cadavers. Elsewhere in the region, Joy et al. (2006) examined the role of shade in the temperature regime of a death scene, utilizing raccoon (*Procyon lotor* L.) carrion. Rodriquez and Bass (1983) have utilized the decay facility at the University of Tennessee to explore the role of insects in the decay of human cadavers.

2.4 Development and Laboratory Rearing of the black Blowfly *Phormia regina* Meigen

The black blow fly, *Phormia regina* Meigen, has been recognized as being important in forensic entomology. The species is common throughout the northeastern United States and is active until the midsummer months when temperatures peak, and in the southern United States the species is active when temperatures are cooler (Byrd and Allen 2001, Byrd and Castner 2010). Because this species has been prevalent in many postmortem interval estimations, the biology of the species and development of the larvae have been the focus of laboratory research and observation for over 50 years (Kamal 1958). Rearing studies of *P. regina* have investigated the effects that temperature, light conditions, geographic population differences, and entomotoxicological substances may have on the development of larvae (Kamal 1958, Greenberg 1991, Cyr 1993, Anderson 2000, Byrd and Allen 2001, Tabor 2004, Nabity et al. 2006, Nabity 2007, Monthei 2009). The development rates of *P. regina* are often utilized to estimate a PMI. Variables that affect insect development may in turn affect the subsequent PMI_{MIN} estimate.

Early studies of blow fly development sought to define minimum and maximum development temperatures (Kamal 1958, Anderson 2000). Basic assumptions of insect growth presume that growth is linear given adequate temperature and food

(Wigglesworth 1972). In the calculation of degree-days, it is assumed that development and growth will cease below a specific, observed temperature and progress above that temperature. When temperature exceeds the upper threshold, development will be limited and the rate of growth will be negatively affected. To calculate the accumulated degree-days, the base temperature (below which growth does not occur) is subtracted from the actual temperature at a given time and the resulting degrees are counted as periods of growth for the given time period (Wigglesworth 1972). Because temperature is often recorded as an hourly average, the initial calculation unit of time is in hours and can be converted to days if necessary. In the field, the average hourly temperature is used and multiplied by the number of hours to yield accumulated degree-days. Thermal summation has been used for agricultural pests for quite some time, but the accumulation of degree-days was first used in forensics in 1985 (Greenberg and Kunich 2002).

There are major assumptions and limitations to the process of calculating the accumulation of degree-days that, if not taken into account, can skew the accuracy of the timeline that is later applied to the PMI_{MIN}, or can affect the overall precision of the PMI_{MIN}. Temperatures are rarely constant in natural environments but rearing at constant temperatures is one manner of isolating other factors in larval development. Rearing at cyclic temperatures is utilized in the re-creation of field conditions, but can have limiting effects on insect growth in the early larval stages. Older instars of maggots produce metabolic heat by their feeding activity. Large aggregations of maggots create a maggot mass effect that causes deviation from environmental temperatures in both the laboratory and the field (Deonier 1940, Greenberg 1991, Greenberg and Kunich 2002, Monthei 2009). Limitations and challenges to constructing the PMI_{MIN} using the accumulation of degree-days are often the focus of research in faunal succession and Dipteran development studies.

Temperature is one of the major factors affecting both the colonization of a carcass and the subsequent development of the carrion-attending insects present. Accurate determination of the thermal history at the death or discovery scene can have a significant effect on the PMI_{MIN} estimate (Greenberg and Kunich 2002). To account for the differences between the reference temperature data (available from proximate weather stations) and the on-site temperatures, data loggers can be left at the scene for a period of days after discovery to record the difference for later correction in the calculation of the PMI_{MIN}. Often researchers will employ data loggers at experimental sites to provide greater accuracy in their data (Tabor et al. 2005a, Monthei 2009). Knowledge or precise estimation of the time of oviposition and adult eclosion is key for the Accumulated Degree-day (ADD) estimation method of PMI_{MIN} (Greenberg and Kunich 2002). Physical barriers can prevent or delay oviposition and insect colonization of a carcass, especially if the corpse is wrapped, or if exposed but indoors (Goff 1992, Reibe and Madea 2010). Condition of the carcass can affect colonization; for example, cases where the body has been burned have been associated with delayed oviposition (Avila and Goff 1998). The manner or type of trauma can also affect the oviposition and succession pattern of insects on a carcass. In addition, weather and temperature may delay initial colonization because insects are poikilotherms and the ambient temperature might be below the minimum temperature necessary to allow flight.

2.5 Previous animal and wildlife research in forensic entomology

The ecology surrounding death has been studied as a micro community ecosystem that revolves around the changing condition of a carcass (Walker 1957a, Reed 1958, Payne 1965). Research in region-specific surveys may be one of the only ways that surveys of necrophagous communities are conducted in remote areas of the world. Through surveying, many different climates, locales, and host carcasses have been observed. From these observations, basic ecological trends have emerged in the understanding of the ecology of carrion. Primarily, research has focused on vertebrate carrion and included a range of carcass sizes (Coe 1978, Tomberlin and Adler 1998). As indicated earlier, the phenomenon of faunal succession has been observed in a variety of animal models (Walker 1957b, Reed 1958, Payne 1965, Denno and Cothran 1976, Coe 1978, Anderson 1999, Bourel et al. 1999, De Jong and Chadwick 1999, Joy et al. 2002, Watson 2003, Anderson and Huitson 2004, Melis et al. 2004, Tabor et al. 2004, Joy et al. 2006, Gruner et al. 2007). Depending on the body composition and the climatic conditions, the pattern of succession may vary with different host carcasses. Variation in the colonization and succession patterns of different species of carrion may be due to the variety of exterior surfaces of wildlife; for example, the dense fur of a black bear may impact colonization differently than the scaly skin of the alligator (Watson 2003, 2005). The variation in colonization and insect succession of carcasses provides room for forensic entomology research in the field of wildlife forensics.

Recent research in forensic entomology has crossed over to explore the use of insect evidence in wildlife and veterinary forensic cases, where the practices and concepts concerned with human death may be applied to animal death (Anderson 1999, Watson 2003, 2005, Merck 2007). In the case of two black bear cubs (*Ursus americanus* L.) that had been killed and disemboweled for their gall bladders, Anderson (1999) analyzed the insects found on the carcasses to determine a PMI_{MIN}. Blow fly eggs collected from the cub carcasses had not hatched and the remaining time to egg eclosion was used to construct the PMI_{MIN}. The PMI_{MIN} estimate was based on the eggs of *Phormia regina* Meigen and *Phaenicia sericata* Meigen, and matched the time frame of the known activities of the poaching suspects. DNA evidence also tied one suspect to the scene and, when combined with the insect based PMI_{MIN}, led to the first jail term for poaching in Manitoba, Canada. Watson and Carlton (2003, 2005) focused research on the establishment of reference data for the investigation of wildlife deaths that are suspected to be illegal. The research utilized Louisiana black bear (*Ursus americana luteolus* Grifith), white-tailed deer (*Odocoileus virginianus* Rafinesque), American alligator (*Alligator mississippiensis* Daudin), and the domestic pig. The study of the faunal succession of these four species provided baseline data on which carrion-attending arthropods can be expected, and when they can be expected to arrive for each species in the study area. Watson and Carlton (2003, 2005) also looked at the differences between the seasons, conducting research in the spring, fall and winter months. These studies were not manipulative but focused on the ecology surrounding the specific carrion species and are similar to studies done in humans and with human analogues. The ecological focus of early carrion community research reinforces the application of human concepts to animal models, upon which most foundations of forensic entomology are based.

2.6 Poaching in southwest Virginia

The Commonwealth of Virginia holds wildlife in public trust and, as a consequence, the taking of game or wildlife species without the permission of the Commonwealth is a Class 3 misdemeanor (Code of Virginia 29.1-335). Poaching is the taking of wildlife without permission (including licensure from the governing authority), out of season, or in excess of the limits as put forth every year by the Virginia Department of Game and Inland Fisheries (VDGIF) in hunting and trapping regulations. If poaching is committed on private or public lands without permission to access such lands, trespassing charges may be involved as well. Trespassing in Virginia is a Class 1 misdemeanor that can be punishable with up to 12 months of jail time (Code of Virginia 18.2-11). Poaching of white-tailed deer is common in the two mountain regions in Virginia, with over 1,400 cases reported during the last 5 years by surveyed Conservation Police Officers (VDGIF, unpublished data). The survey responses did not indicate the use of insect evidence in their investigations. The officers more often relied on measures of algor mortis, rigor mortis, and conditions of the eye that are not typically reliable beyond 72 hours after death (Greenberg and Kunich 2002). Research specific to white-tailed deer and forensic entomology may add to the resources available to the VDGIF for use in investigations of illegally harvested wildlife. Both species-specific and region-specific studies can add to the body of knowledge surrounding deer poaching in the mountain regions of Virginia. This thesis explores insects and their potential role as evidence in the observed decomposition of four white-tailed deer in southwest Virginia.

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3 Insect Faunal Succession on White-tailed Deer Carcasses in Southwest Virginia

3.1 ABSTRACT

Insect faunal succession was studied on four white-tailed deer carcasses in southwest Virginia in the summers of 2009 and 2010. Deer were obtained immediately after being harvested. Larval and adult insect sampling began the day after death and continued for 12 additional days. The patterns of insect succession between the summers of 2009 and 2010 were similar at $\alpha = 0.05$. Necrophagous insects arrived in a successional pattern as has been observed on other animal models (*e.g.* pigs) during past studies conducted in southwest Virginia. The dominant dipteran was *Phormia regina* (Calliphoridae), while the dominant coleopterans were *Necrophila americana* (Silphidae) and *Euspilotus assimilis* (Histeridae). Analysis of species occurrence compared necrophagous insect species present in this study to those observed during past studies with pigs in the same area. The occurrence of individual insect species varied between the deer study and pig studies. Our data indicate that the determination of PMI_{MIN} based on necrophagous insect succession patterns in wildlife poaching should be done with carcass specific (e.g. deer) baseline succession data.

3.2 INTRODUCTION

Forensic entomology enables crime investigators to utilize insects as evidence in cases concerning unknown time of death of humans, or in cases involving neglect of infants or the elderly (Catts and Haskell 1990, Byrd and Castner 2010). The use of forensic entomology techniques has also been helpful in cases involving wildlife (Watson 2003, 2005), such as in cases of poaching (Anderson 1999). The natural ability of insects to locate and utilize a carcass often allows them to be the first to discover a recently exposed carcass. However, insect discovery of a carcass can be affected by temperature, geography, the condition of the body, and the extent to which the carcass has been exposed to the elements. Many of these variables need to be taken into account when using insects as evidence in PMI_{MIN} determinations.

The greatest value that insects provide to an investigation is as a determinant of the time that has elapsed since the carcass was first exposed. The time elapsed since exposure can be used to determine the minimum postmortem interval, PMI_{MIN}. The short-term PMI_{MIN} can be estimated by using the age of the oldest insects that are developing or have completed development on a carcass. Anderson (1999) used Calliphorid eggs found on disemboweled black bear cubs (*Ursus americana* L.) by measuring the amount of time it took them to eclose after collection and extrapolating how much time had passed since oviposition. Long-term PMI_{MIN} determination, on the other hand, may use the naturally occurring patterns of insect faunal succession as a point of comparison for the insects collected at the time of discovery of the carcass.

Insect succession is a natural phenomenon whereby insect species discover and colonize a carcass at a predictable rate and pattern according to access, resource availability, and climatic conditions. The phenomenon has been observed on all of the inhabited continents and documented for over 100 years (Motter 1898, Braack 1987, Catts and Goff 1992, Bourel et al. 1999, Melis et al. 2004, Amendt et al. 2007, Shi et al. 2009). Long-term PMI_{MIN} determination relies on the presence or absence of a species on a carcass when compared with patterns observed in the past. The work of Watson et al. (2003, 2005) investigated the patterns of insect succession on Louisiana black bear

(*Ursus americana luteolus* Grifith), white-tailed deer (*Odocoileus virginianus* Rafinesque), American alligator (*Alligator mississippiensis* Daudin), and the domestic pig (*Sus scrofa* L.) to record potential differences in the patterns and to create a baseline of data for the host species in Louisiana.

The identification of insects and the corresponding date information allow for the construction of occurrence matrices where the presence or absence of the insect is mapped over time. The occurrence matrix is used to extract binary data values for the presence (noted as a 1) or the absence (noted as a 0) of an insect at the carcass at one date. When presented as a figure showing insect presence over time, the occurrence matrix can provide a clear picture of the patterns of colonization and succession of the insects (Anderson and VanLaerhoven 1996, Watson 2003, Tabor et al. 2004, Tabor et al. 2005a, Monthei 2009).

Past studies in southwest Virginia have compared occurrence matrices of insect faunal succession and analyzed their similarity using an index of similarity or a coefficient of resemblance (Tabor 2004, Tabor et al. 2005a, 2005b, Monthei 2009). The first analysis of carrion succession in southwest Virginia was carried out with the Jaccard similarity index that used the matches of presence between two matrices, the occurrence of one species at one carcass, and its subsequent absence at another carcass. The initial studies by Tabor et al. (2004) provided a comprehensive list of the species that should be present in the study area in southwest Virginia under normal circumstances. The comprehensive list gathered over two years provided a baseline for comparison of other studies over multiple years. Tabor et al. (2004) found that there were no differences in the faunal succession on pig carcasses between multiple spring studies. The comparison of their summer studies to each other yielded the same result of no difference. In studies by Monthei (2009) simple matching coefficients were used to compare the faunal succession patterns of different years of oxycodone-treated and untreated pigs. Monthei (2009) found that there were no differences in the succession patterns of insects regardless of treatment of the pigs. The analysis used by Monthei (2009) incorporated a list of species known to be present in the area with a simple matching coefficient analysis. The procedure takes into account negative matches where neither of the two occurrence matrices being compared have a record of species that were found previously.

The purpose of this study was to document the succession of necrophagous insects on white-tailed deer carcasses in southwest Virginia during the summer. We hypothesized that the patterns of succession would be similar for each of two summer studies and that these patterns would be similar to those observed on pig carcasses from past studies in the area.

3.3 METHODS

3.3.1 Study Sites and Methods

Studies of insect faunal succession on white tailed deer carcasses were carried out in Blacksburg, Virginia (37.24° N, 80.43° W). The area is approximately 640 meters above sea level and receives on average 50.8 cm of rainfall and 103.9 cm of snowfall annually, with a maximum temperature of 37.7 °C and minimum of 5 °C in the summer (National Weather Service). Blacksburg is located in Montgomery County, a county that is comprised of 102,276 hectares, 60% of which is forested, including 7% in the Jefferson National Forest (Virginia Department of Forestry).

The studies of the succession of insect fauna were conducted during the periods June 28–July 13, 2009 and June 29–July 13, 2010 at Moore Farm and Kentland Farm, respectively. Each farm is owned and operated by Virginia Tech as agricultural research and production facilities. White-tailed deer were obtained from the Virginia Department of Game and Inland Fisheries in collaboration with landowners that had significant crop damage from grazing deer. The deer were harvested at the behest of the landowner and by VDGIF permitting; possession was immediately transferred to the Virginia Tech Department of Entomology so that the carcasses could be moved to the field site locations for use in this study. Carcasses were moved minutes after death and transported by covered truck to prevent colonization by insects until placement at the study locations. Carcasses were placed in a fallow field edge in 2009 and in and adjacent to corn fields in 2010. Conservation police officers often find abandoned poached deer carcasses in similar situations in southwest Virginia (C. Mullins, VDGIF, personal communication). The first deer was harvested on June 27, 2009 at 0935 hrs and put out at the Moore Farm field site by 1245 hrs the same day. The second deer was harvested June 29, 2009 at 2020 hrs and placed out at Moore Farm at 2350 hrs that night. In 2010, carcasses A and B were harvested at 1845 hrs and 1905 hrs July 1, 2010, respectively. Both carcasses were placed at Kentland farm by 2400 hrs on the day of harvest. Carcasses were covered by 92 x 92 x 153 cm predator exclusion cages made of a steel tubing frame and covered by 1.27 cm mesh hardware cloth (Figure 1) as described by Tabor (2004). The cages had an open bottom and hinged front door to allow access during sampling. In 2009, pitfall traps were installed approximately 15 cm from the carcass, one behind the posterior end and a second below the edge of the thoracic cavity. Pitfall traps consisted of countersunk plastic cups containing a dish-soap and water solution that prevented escape from the traps (Tabor 2004, Monthei 2009). In the second year, traps were placed outside of the cages and the number of traps was doubled to ensure a more inclusive survey of the ground moving insects that arrived at or departed from the carcasses. Second-year traps were placed at 90-degree angles to the carcass from the anterior, posterior, ventral and dorsal sides. Four-channel HOBO® data loggers (Onset Computer Corporation, Bourne, MA) were installed to record ambient temperature from specific sites in and around the carcasses. In 2009 and 2010, probes were placed at 91.44 cm above the carcass to measure the ambient temperature inside of each cage.

Insect samples from each carcass were collected daily for 13 days between 1200–1500 hrs in the summer of 2009 and between 1100–1300 hrs during the summer of 2010. Sampling consisted of 10 sweeps above the carcass with an aerial sweep net, collection of pitfall traps, adult beetle sampling, and collection of live fly larvae. Collected adults were killed in plastic one-gallon resealable bags, containing a single brown paper towel dampened with ethyl acetate, and pinned for later identification. Collected fly larvae were reared on ground pork loin at 30°C under a 14:10 light dark cycle in rearing containers for later adult identification (Tabor et al. 2004, Monthei 2009). Specimens were identified to genus or species with the use of taxonomic keys. Final determinations were made by comparisons to voucher specimens of verified adults collected by Tabor et al. (2004).

3.3.2 Data and Statistical Analysis

The analysis of the data closely followed that used by Tabor et al. (2004), but with slight variations. The data collected from the two deer carcasses from each year were combined to develop a diagram of succession of insect taxa and an occurrence matrix. For each occurrence matrix, we assessed the variability (mean ± SD) in the number of taxa among sampling intervals and in their frequency of daily occurrence. Unlike Tabor et al. (2004) who used the Jaccard Similarity Index, we instead derived Simple Matching Similarity coefficients to compare the similarity of one sampling interval to the next (Schoenly 1992, Krebs 1999). The simple matching coefficient was calculated as: (Krebs)

$$S_{SM} = \frac{a + d}{a + b + c + d}$$

(1)

where S_{SM} is the Simple Matching Coefficient, a is the number of matching observations (species) found in both intervals A and B, b is the number of species found in interval A and not B, c is the number of species found in interval B and not A, and d is the number of species not found in interval A or B that has been compiled from similar past studies on pig carcasses in the area (Tabor et al. 2005a, Monthei 2009).

We followed the techniques used in Tabor et al. (2004) and calculated a mean similarity between sampling intervals (S_{gmean}) for the sampling interval comparisons of insect taxa from each occurrence matrix throughout all intervals using the simple matching coefficient. To derive a measure of precision, we constructed matrices of similarity and compared these using a permutation analysis on the corresponding Pearson correlation coefficients (Cheverud et al. 1989, Manly 2007). Overall similarity was recalculated using the permutation, which was carried out by removing each interval sequentially, making a new observation (K_{obs}) and replacing the interval. This operation was carried out 1000 times and used for hypothesis testing with a null hypothesis that the summer studies had no similarity in their patterns of succession (Tabor et al. 2004).

The composition of necrophagous insect species present at deer carcasses was compared with those observed by Tabor et al. (2004) and Monthei (2009) on pig carcasses. The data from the previous studies on pigs and the deer study at the same locations were transformed into presence/absence values and the simple matching coefficient (Equation 1) was used to determine the degree of similarity in the composition of taxa among the three studies.

3.4 RESULTS

The average recorded ambient temperature and ranges, for each carcass from each year, are reported in Table 1. The succession diagrams for insect taxa on carcasses of white tail deer for summer 2009 and 2010 are presented in Figure 2. Twenty eight and 26 insect taxa were observed in 2009 and 2010, respectively. The taxa that arrived first during the summer of 2009 consisted of members of the family Calliphoridae (Diptera) and Silphidae (Coleoptera). The first species to arrive in the summer of 2010 were members of the Calliphoridae and Piophildae (Diptera) and the Silphidae (Coleoptera).

Five families of Diptera were collected on the carcasses in 2009; four families were collected in 2010. In 2009, the families of Diptera collected were Calliphoridae, Muscidae, Sepsidae, Piophilidae and the Sphaeroceridae; no Muscidae were collected in 2010. The Calliphorids collected were *Phormia regina* (Meigen), *Lucilia coeruleviridis* (Macquart), *Cochliomyia macellaria* (F.) and *Protophormia terranovae* (Robineau-Desvoidy). A few adult Sarcophagidae were collected but were rare and not present in the daily larvae samples and as such have been omitted.

The Piophilidae that were collected included *Stearibia nigriceps* (Meigen), *Prochyliza xanthostoma* (Walker), and *Piophila casei* (L.). The Sepsidae were represented by the genus *Sepsis*, *Meroplius stercorarius* (Robineau-Desvoidy), and *Nemapoda nitidula* (Fallén). *Lotphila atra* (Meigen) was the only member of the Sphaeroceridae collected. Similarly, *Muscina assimilis* (Fallén) was the only representative of the Muscidae family. Species of beetles that were found at 10 or more sample intervals for the two study years included *Creophilus maxillosus* (L.) (Staphylinidae), *Oiceoptoma noveboracense* (Forster), *Necrodes surinamensis* (F.), *Necrophila americana* (L.) (Silphidae), *Necrobia violacea* (L.) (Cleridae), and *Euspilotus assimilis* (Paykull) (Histeridae).

Mean pairwise similiarities for insect taxa succession patterns in the occurrence matrices are presented in Fig. 3. Jackknife estimates of overall similarities and the precision of these estimates (S_{gmean} ± 95% CL) for each successional patterns were 0.8582 ± 0.0736 (summer 2009), 0.8462 ± 0.0363 (summer 2010). The permutation analysis rejected the null hypothesis of no similarity in the succession pattern of taxa on carcasses of white tail deer for the summer of 2009 and the summer of 2010 (K_{obs} = 0.997; P = 0.001).

The results also showed a 49% and 56% similarity between the composition of necrophagous insect species observed on white tail deer and those recorded by Tabor et al. (2004) and Monthei (2009) on pig carcasses, respectively. An 80% similarity was found in the composition of insect species collected on deer carcasses in 2009 and 2010.

3.5 DISCUSSION

Many insects and arthropods will visit a carcass, but only those that rely on the carcass to complete some part of their life cycle are considered necrophagous. The guild of necrophagous insects also includes closely associated predators of carrion-feeding insects such as carrion beetles and staphylinids. This study focused on the relationship that carrion-attending insects have with white-tailed deer carcasses in southwest Virginia for the first 13 days after death during the summer.

The patterns of insect succession and the species present on the carcasses of white tail deer during the summers of 2009 and 2010 were similar. The predictable patterns of succession would allow researchers to estimate the PMI_{MIN} of a decedent based on the carrion-feeding insect species found at a carcass (Catts and Haskell 1990, Catts and Goff 1992, Byrd and Castner 2001). The application of this technique to wildlife forensics is supported by the findings of this study as well as the studies conducted in Louisiana by Watson et al. (2003, 2005).

The deer carcasses were visited by 28 different insect taxa in summer 2009 and 2010. Previous succession studies conducted on pig carcasses in the same region reported 47 taxa (Tabor et al. 2004) and 26 taxa (Monthei 2009) during the summer period. Other studies have reported as many as 522 species of insects in South Carolina on pig (Payne 1965). Anderson (2001) attributes variation of local succession patterns to differences in local climate and species distributions, as well as the season of the year when observations were made. Carcass size may also affect decomposition rate and successional patterns (Watson and Carlton 2003).

Dipterans were among the first taxa to arrive at each carcass. Calliphorids were followed by sepsids and piophilids, as well as predaceous beetles (Coleoptera), predominantly silphids. The calliphorid, *Phormia regina* was present 12 out of 13 sampling intervals and 10 of 13 intervals in the 2009 and 2010 study periods, respectively. Daily larval sampling revealed that only two species of calliphorids colonized the carcasses. *Phormia regina* was the dominant calliphorid and has been dominant, or co-dominant, on pig studies from the region (Tabor et al. 2004, Monthei 2009); *Cochliomyia macellaria* was also present.

Beetles were present during the first sampling interval in 2009 and by the second sampling period in 2010. The early occurrence of beetles on deer is unlike the patterns observed by Reed (1958) in Tennessee, but similar to the patterns observed in recent studies in southwest Virginia (Tabor 2004, Monthei 2009). The dominant beetle in 2009 was *Euspilotus assimilis* (Histeridae), which was present for 9 out of 13 sampling intervals, followed by *Necrodes surinamensis* and *Necrophila americana* (Silphidae). In 2010, *Necrophila americana* was the dominant species and was observed for 12 out of the 13 sampling intervals.

Other studies in the mid Atlantic, southeastern region include those by Reed (1958) with dog carcasses in Tennessee, and Joy et al. (2002, 2006) with raccoon and later pig carcasses in West Virginia. The lower numbers of taxa in the current study compared to those observed by Reed (1958) may be attributed to the large number of carcasses and study sites (59 dogs, over 9 sites) used in his study compared to the 4 deer carcasses in this study. Additional differences may in part be the animal model used in each study. Watson and Carlton (2003) found that outside of Baton Rouge, Louisiana, 70 taxa were present on deer carcasses whereas 64 taxa were found on pig carcasses during concurrent succession studies.

Jackknife values of overall similarity were higher than those found in similar studies. Carrion succession-derived Jackknife values from the analysis of Schoenly (1992) and Tabor et al. (2004) were in the range of 0.20–0.50 whereas in this study, the values were 0.86 (±0.07) for 2009 and 0.85 (±0.04) for 2010. The differences found here may in part be due to the limited amount of taxa present that could make the turnover (dissimilarity of taxa from one interval to the next) appear lower than past studies and in turn yield a higher similarity of taxa from one day to the next.

The comparisons of insect species occurrence on deer carcasses to pig carcasses from past studies conducted at the same sites are unique. Tests of the similarity of the two pig studies to this study show that the variation between the two carcass species may be enough to limit the potential use of past pig studies as baseline data in wildlife poaching investigations. In the case of the 49% similarity to Monthei's 2009 fetal pigs and Tabor et al's 2004 data , the overall variation suggests that pig models would not produce suitable baseline data in wildlife investigations. Although the general patterns of decomposition and insect succession are ecologically similar, the statistical differences found here require a more extensive study of carcass specific succession to ensure a more accurate determination of succession based $PMI_{MIN.}$

Insect faunal succession can be used to estimate PMI_{MIN} and requires that baseline data be used to reference where the insects collected from a carcass fit into the succession pattern (Tabor et al. 2004). However, there are variations in this succession depending on local insect species distributions, seasonal differences, carcass type, size, injury level, and it's exposure to the elements. The patterns observed in this study from one year to the next were similar and, depending on insect species ranges, could serve as baseline data on insect faunal succession on white-tailed deer during the summer in southwest Virginia. This study also provides additional data on insect faunal succession for the region as the most recent observation of succession patterns of insects on decaying carcasses. Other variables that have been observed in poaching cases, disembowelment, limb removal, and burial, have not been explored here and warrant additional studies for the use of forensic entomology in wildlife forensics.

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Figure 3.1: Deer enclosed in steel cage covered with wire mesh to exclude predators. Insect faunal succession studies were conducted on white-tailed deer in southwest Virginia.

Table 3.1: Ambient temperature averages and ranges measured 91.44 cm above the carcass for four different deer carcasses enclosed in steel cages covered with wire mesh from insect faunal succession studies conducted on white-tailed deer in southwest Virginia.

Carcass ID	Average Temperature, °C	Temperature Range, °C
A 2009	20.47	9.82 – 34.85
B 2009	19.52	10.21 – 32.76
A 2010	24.47	8.23 – 41.52
B 2010	24.52	8.23 – 41.52

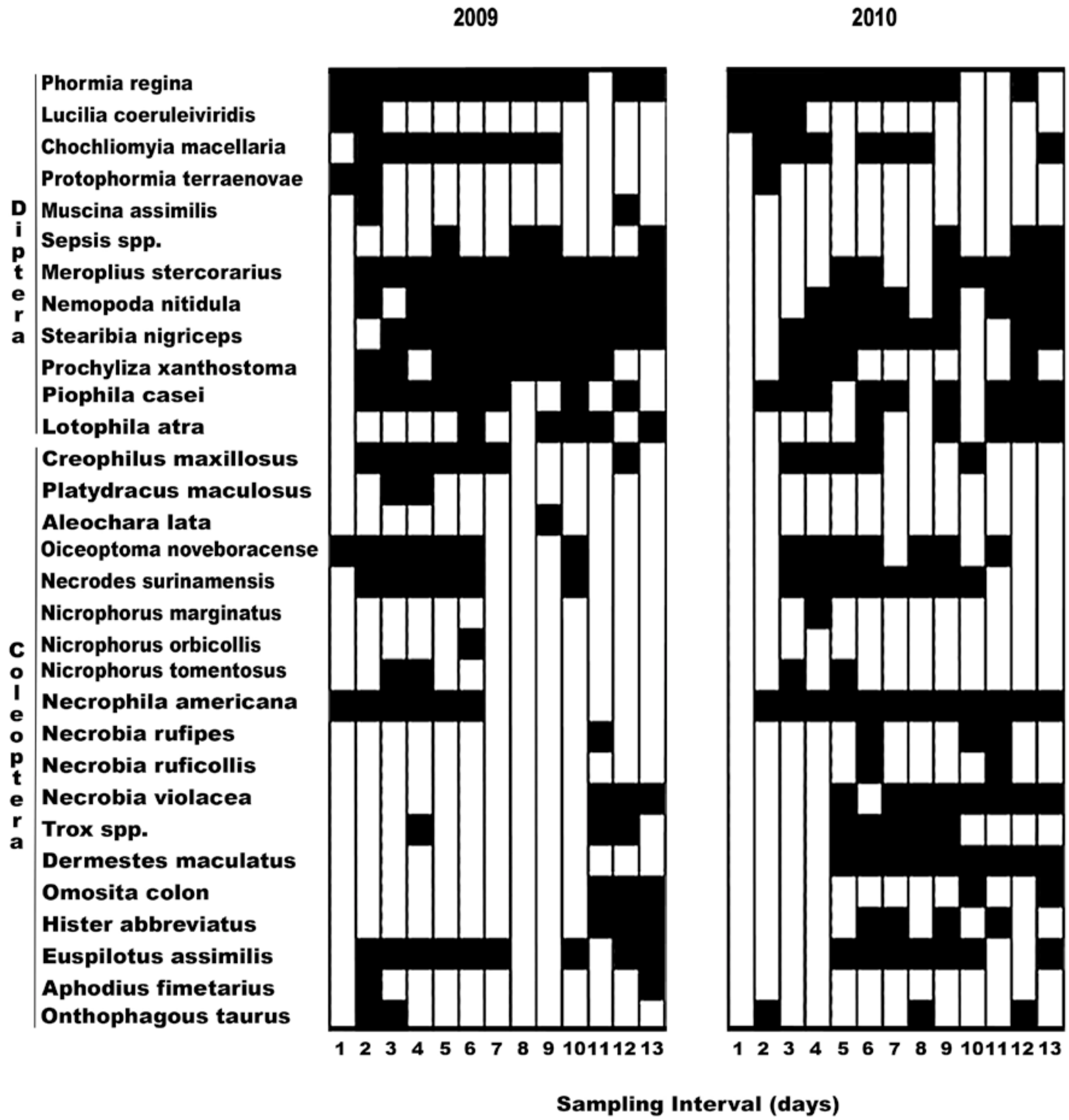

Figure 3.2: Occurrence matrices of necrophagous insect species succession on 4 white-tailed deer carcasses in the summers of 2009 and 2010 in southwest Virginia, black cells indicate at least one specimen was observed during that sampling interval. Species occurrences were pooled for both carcasses during each summer and are shown in a single matrix.

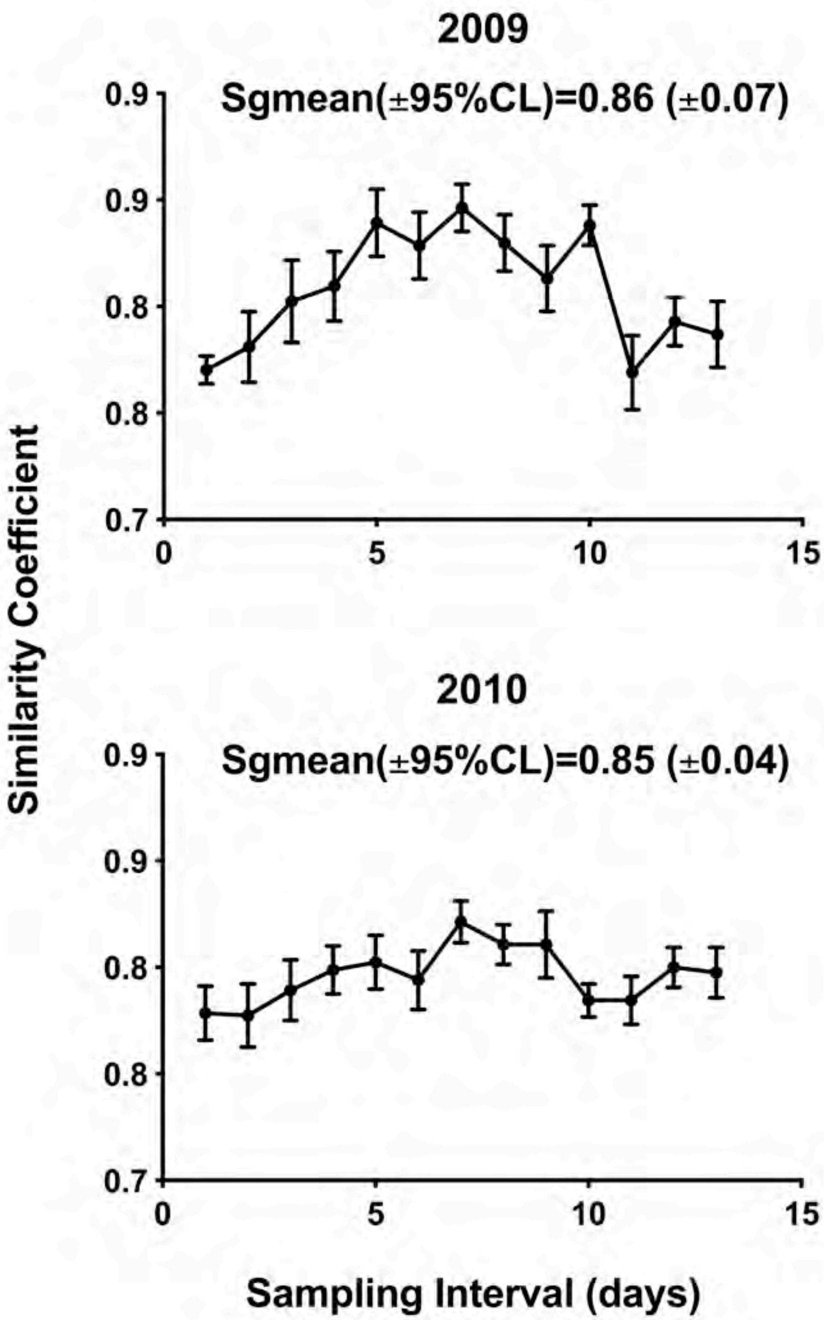

Figure 3.3: Similarity coefficients of mean pairwise similarities for the sampling intervals by summer study period of insect faunal succession on white-tailed deer carcasses in southwest Virginia during the summers of 2009 and 2010.

4 Development of *Phormia regina* Meigen (Diptera: Calliphoridae) on Pork and Venision *in vitro*

4.1 ABSTRACT

Laboratory rearing of larval *Phormia regina,* Meigen, on pork and venison was conducted in southwest Virginia. Environmental rearing conditions were 30° C, 75% RH and 14:10 hour light dark cycle. Maggot samples were collected from rearing containers every eight hours until more than 90% of the maggots reached the 3rd instar wandering stage. Significant differences in lengths of 3rd instar and combined overall maggot lengths were found for maggots reared on the different meat sources. Mean adult weights and wing lengths of venison-reared flies were significantly greater than those reared on pork at α=0.05. Venison appears to be more nutritious than pork for larvae of *P. regina*, causing 3rd instar maggots to grow larger in size. This difference could introduce error into PMI estimations from third instar maggots if they were constructed using data from pork reared maggot studies.

Keywords Postmortem Interval, *Phormia regina*, Venison, Pork

4.2 INTRODUCTION

The black blow fly, *Phormia regina* (Meigen), is widely distributed throughout the United States, except for the southernmost portion of Florida. *P. regina* is active in the cooler weather of spring and fall but is inactive in the southern United States during the summer months (Byrd and Castner 2001). Because of its long season of activity, *P. regina* is often the most prevalent species to colonize a carcass in an outdoor setting. The black blow fly rapidly locates and colonizes a carcass and as such, it is often among the first insects to arrive. The age of the oldest blow fly maggots developing on a carcass generally approximates the minimum time since colonization, assuming that the maggots arose from eggs deposited shortly after death. An estimation of the postmortem interval

(PMI) can be extrapolated by "aging" the oldest maggots collected. *P. regina* is thus of significant forensic importance and extensive research has been devoted to its life cycle and development. The black blow fly is known to colonize carcasses of white-tailed deer and larger wildlife species in North America (Anderson 1999, Watson 2003, 2005) and therefore may prove useful for PMI estimation in poaching cases. A number of developmental studies have been conducted on this species, most of which concentrate on testing the effect of temperature and diet on development, and in turn, the validity of the PMI estimate (Table 1).

The difference in crude fat content of the meat of wild white-tailed deer, *Odocoileus virginianus,* Rafinesque, compared with that of domestic pig, *Sus scrofa,* L., could have a significant effect on the growth and development of *P. regina* larvae, and could lead to differences in PMI estimation. Previous development studies have not compared a specific diet of venison with commonly used diets such as pork. The purpose of this study was to rear the larvae of wild-caught *P. regina* on a diet of venison and pork under the same conditions. We hypothesized that there would be differences in the development rates of *P. regina* depending on diet.

4.3 MATERIALS AND METHODS

Adult *P. regina* were collected off of caged fetal pig carcasses located at Moore Farm in Blacksburg, Virginia (37.24° N, 80.43° W), where previous forensic entomology studies have been conducted (Tabor et al. 2005a, Tabor et al. 2005b, Monthei 2009). Adults were collected over a 20-day period, from August 4 - August 23, 2010, and maintained in a mesh-sided cage (Plexiglas® and nylon) with a food source (50/50 mix of sucrose and instant nonfat dry milk fortified with vitamins A and D). Fresh water was provided in a separate container with a sponge wick to prevent the flies from drowning. The identification of flies was confirmed using stereomicroscopy following chilling in a 4° C cold storage room. This process yielded between 150 and 200 adult *P. regina*.

Initial attempts to induce oviposition by placing warmed ground pork in a plastic container with a mesh lid in the fly cage were not successful. However, the addition of liquid material from thawed ground pork to the mesh lid did induce oviposition. Eggs were collected after a two-hour period and weighed into batches of 20 mg, or approximately 200 eggs (Monthei 2009). The meat samples were placed in 473 ml plastic cups and consisted of either previously frozen, ground venison (harvested in 2009), or store bought, ground, center-cut, pork loin. The eggs were then placed on 100 g portions of room temperature ground pork or venison.

The containers were divided into two groups of six, with three containers of pork and three containers of venison in each group. All containers were placed in an environmental growth chamber (model 1-35LL, Percival Scientific, Inc., Boone, Iowa) without lids and observed every 30 minutes until the first eggs hatched. The growth chamber was maintained at 30° C (± 1°) and 75% RH, with a 14:10 hour light dark cycle. Beginning with the first sampling event, the two groups of six were labeled as cohort A, the first cohort to be sampled, and cohort B, the group to be sampled eight hours afterward. Each cohort was sampled every sixteen hours so that eight maggots were removed from a single container every 24 hours. Sampling began eight hours after egg eclosion and continued in eight-hour intervals until over 90% of the larvae in the container had reached the post-feeding stage. Each sample consisted of four randomly selected maggots that were immediately fixed in a KAA solution (Catts and Haskell 1990).

Maggot length (mm) was plotted against time (hours) and spiracular slits were used to identify the instar. A Kolmogorov-Smirnov test (http://www.physics.csbsju.edu/stats/KS-test.html) was used to compare the lengths of larvae reared on venison and pork. The test program compares the two curves (pork and venison data) to each other and measures the distance between them to determine if there is a significant difference at $\alpha=0.05$ (Conover 1999). A significant difference in the two curves would indicate a difference in the growth and development of maggots on the two diets. Kolmogorov-Smirnov tests were also run to compare the treatments among cohorts to test for in-chamber effects. Significance in this test would indicate that the cohort position in the chamber affected the growth rate.

Past studies have found positive correlations between the size of adults and diet, as well as between the size and reproductive success of adult *P. regina* (Bennettova and Fraenkel 1981, Stoffolano et al. 2000). As such, adults were collected and placed in plastic sealable bags in a -20° C freezer. After freezing the adult colony overnight, 10 females and 10 males from each container were randomly sampled for measurement of adult weights. The weights were analyzed using a two-way ANOVA (JMP 8.0.1, SAS Institute). Additionally, five females and five males were randomly selected from each of the rearing containers for wing measurement. A total of 120 flies were examined (60 for each diet and 30 of each sex within each diet). The right wing of each adult was removed and placed on a microscope slide for measurement with an ocular micrometer on a microscope fitted with a digital camera. Measurements were made of the distance from the humeral cross-vein, at the intersection of the sub-costal, to the end of the radial 4+5 vein (Stoffolano et al. 2000); (Fig. 1). Wing length measurements were fitted to a normal distribution. A *t*-test was conducted to determine if significant differences existed between wing lengths of venison-reared and pork-reared adults (JMP 8.0.1, SAS Institute).

Crude fat and pH of the ground meat used for rearing were analyzed in triplicate samples for each meat type by the Virginia Tech meat science laboratory using a semicontinuous solvent extraction method (the Soxhlet method). The Soxhlet method is the industry standard for the extraction of meat fat and utilizes anhydrous ether as the solvent (Min and Ellefson 2010, Nielsen 2010). During sample preparation the meat science lab determined that an analysis of protein content was not necessary because of the apparent similarity between the two meats (Virginia Tech Meat Science and Muscle Biology Laboratory, personal communication).

4.4 RESULTS

Maggot lengths were tabulated by diet and instar with corresponding means and standard deviations (Table 2). Second order polynomial trend lines were fitted to each set of maggot length data (Figures 2 and 3).

Separate Kolmogorov-Smirnov tests were conducted between the pork and venison data for first, second, and third instars. Comparisons of first and second instar larval lengths between diets were not significantly different. Tests of the third instar and all instar data combined yielded significance at $\alpha=0.05$ ($D=0.3238$, $P< 0.001$ and $D=0.2133$, $P= 0.003$, respectively).

The adult weight data were normally distributed for both the venison and pork diets. The adult weights of 30 male and 30 female flies reared on venison ranged from 30.6 mg to 49.1 mg with a mean of 38.9 mg (±SD 3.0). The weights for 30 adult male and 30 adult female flies reared on pork ranged from 28.4 mg to 45.6 mg with a mean of 37.1 mg (±SD 3.5). The analysis of the adult weight data was conducted using a Fit Least Squares, factorial to degree, model to test the interaction levels between sex and diet (JMP 8.0.1, SAS Institute). A significant difference in adult weight was found between adults that were reared on venison versus pork with $P < 0.0001$, indicating that the venison diet yielded significantly heavier adults. There were no significant interactions between sex and adult weight.

Length measurements of adult wings were normally distributed (after omission of a single outlier). For adults reared on pork wing length ranged from 5.2 mm to 5.7 mm with a mean of 5.4 mm (±SD 0.2). For adults reared on venison, length measurement varied from 5.3 mm to 5.8 mm with a mean of 5.1 mm (±SD 0.1). There was a significant difference between the two groups ($t = 3.44$, $DF = 114$ with a p-value < 0.05), indicating that the wing lengths of adults reared on venison are significantly larger than those of adults reared on pork.

4.5 DISCUSSION

To the knowledge of the authors, no other studies have been conducted on the rearing suitability of venison as a diet for the black blow fly and no studies have compared venison with pork diets. In addressing the suitability of venison as a rearing medium, adults were measured by weight and size to see if the effects found in larvae carried over to the eclosed adults. Past studies have shown that adult size correlates to both larval diet and the reproductive success of the adult (Bennettova and Fraenkel 1981, Stoffolano et al. 2000). Bennettova and Fraenkel (1981) found that the size of *Sarcophaga bullata*, Parker, and *Phormia regina* related to the availability of food during the larval stages and the number of ovarioles developed as an adult. The larvae that had access to food for the longest amount of time yielded larger flies and more ovarioles. Observations of reproductive fitness were also made by Stoffolano et al. (2000), who determined that pairing small males and large females of wild *P. regina* resulted in a reduction of successful insemination. Analysis of adult weights in this study showed that venison-reared adults were significantly larger than pork-reared adults.

Adult wing lengths were also found to be significantly larger in venison-reared adults. Stoffolano et al. (2000) found that head width correlated to wing length in *P. regina* and could be used as an accurate measure of size relative to reproductive success. The significant differences in adult weights and wing lengths in this study indicate that venison may be a better rearing medium than pork. Likewise, the reproductive success of venison-reared adults may be greater than that of pork-reared adults. *P. regina* larvae may assimilate protein more efficiently from venison because the percentage of crude fat in the venison samples is less than half of that found in the pork samples, 2.9 ± 0.36% lipid in venison and 8.6 ± 0.16% in pork. The percentage of usable protein is higher in the venison samples and should have a lower handling cost than the higher fat containing pork. The pH values of the meats were 5.38 in the venison and 5.76 in the pork. The pH values of the pork were affected by the additives commonly found in store-bought pork but is not thought to have any significant affect on the growth of maggots (Virginia Tech Meat Science and Muscle Biology Laboratory, personal communication).

The analysis of maggot length provides a comparison of diet effects on the development of *P. regina*. The significant differences found in the third instar maggot lengths and the combined overall curves of lengths over time suggest that venison is a more suitable diet than pork. The use of rearing data and maggot length to determine age may be affected by the variation in development on different diets (Clark et al. 2006). Clark et al. (2006) found that *Lucilia sericata* (Meigen) larvae reared on pork were significantly larger than those reared on beef. The common practice of choosing pork or beef as a rearing medium (Catts and Haskell 1990) may not account for this potential variation and, as a consequence, PMI calculations based on pork diet data may overestimate the ages of venison-reared larvae (Fig. 3).

The difference in the growth of maggots reared on venison compared with those reared on pork indicates the possibility of errors in PMI estimation when maggot length is used to determine age. If standard forensic rearing practices were used in a deer poaching investigation, where the growth curves used to estimate age were based on pork, the potential error could be as much as 12-24 hours, based on data from this study. Anderson (1999) used *P. regina*, among other species, to construct a PMI in the unlawful slaying of black bear cubs, *Ursus horribilis* (L.). The timeline was based on the time until egg eclosion and subsequently led to the conviction of the poachers (Anderson 1999). Had the carcasses of the bear cubs been found later and third instar maggots used in the estimate, error may have been introduced into the PMI based on previous development times and common practices.

Watson and Carlton (2003, 2005) conducted faunal succession studies that included Louisiana black bear *Ursus americanus luteolus* (Griffith) and white-tailed deer and could provide baseline data for long-term PMI determination for both host species. However, there have not been any subsequent larval blow fly rearing studies that would provide the data necessary for short-term PMI estimates on either species. Additional studies examining different temperature regimes and other rearing mediums would prove useful for the application of forensic entomology in wildlife and veterinary forensics. Further investigations into the effects of diet could also help to quantify possible error or provide reference data more appropriate for wildlife forensics.

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Table 4.1: Past rearing studies of *Phormia regina* (Meigen) including diet and temperature.

Author (Year)	**Diet**	**Temperature (°C)**
(Anderson 2000)	Beef Liver	16.1, 23
(Byrd and Allen 2001)	Center Cut Pork	10, 15, 25, 30, 35, 40
	Center Cut Pork	10-15, 15-25, 25-35, 35-45
(Cyr 1993)	Beef Liver	26.6
(Greenberg 1991)	Ground Beef	22, 29
(Kamal 1958)	Beef Liver	26.7
(Monthei 2009)	Ground Pork (A portion treated with ETOH)	23
(Nabity et al. 2006)	Beef Liver, Ground Beef	12, 14, 15, 20, 26, 32
	Beef Liver, Ground Beef	12, 14, 15, 20, 26, 32
(Nabity 2007)	Beef Liver	20, 25
(Stoffolano et al. 2000)	Pork Liver	27
(Tabor et al. 2005b)	Ground Pork (A portion treated with ETOH)	Ambient

Table 4.2: Lengths (in mm) of *Phormia regina* maggots reared on either pork or venison diet at 30° C, 75% RH and a 14:10 hour light dark cycle. For each instar of each treatment n=24, for 3rd instar pork reared maggots n=91 and for 3rd instar venison reared maggots n=92.

	1st Instar		2nd Instar		3rd Instar	
Diet	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Pork	3.4 ± 0.7	2.0 – 5.0	6.6 ± 1.1	5.0 – 9.0	12.6 ± 1.4	8.0 – 15
Venison	3.3 ± 0.7	2.0 – 4.8	6.4 ± 0.7	5.3 – 8.0	13.3 ± 1.9	8.0 – 18

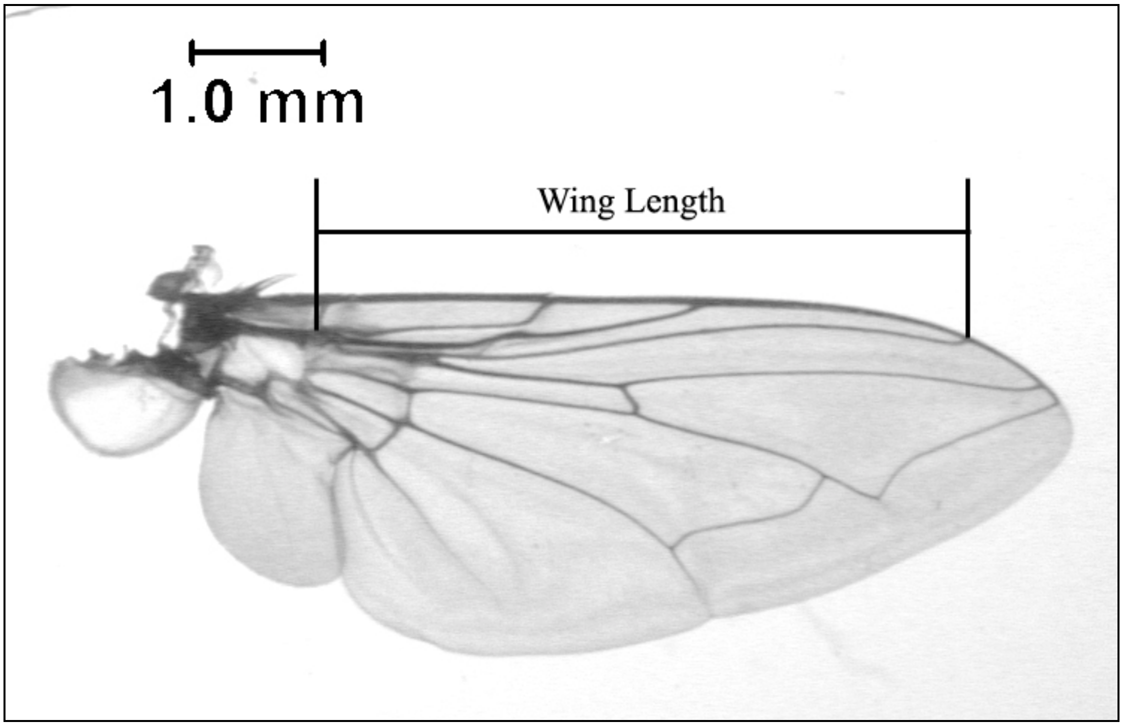

Figure 4.1: Wing of *Phormia regina*, showing measurement points from humeral cross-vein, at the intersection of the sub-costal vein, to the end of the radial 4+5 vein.

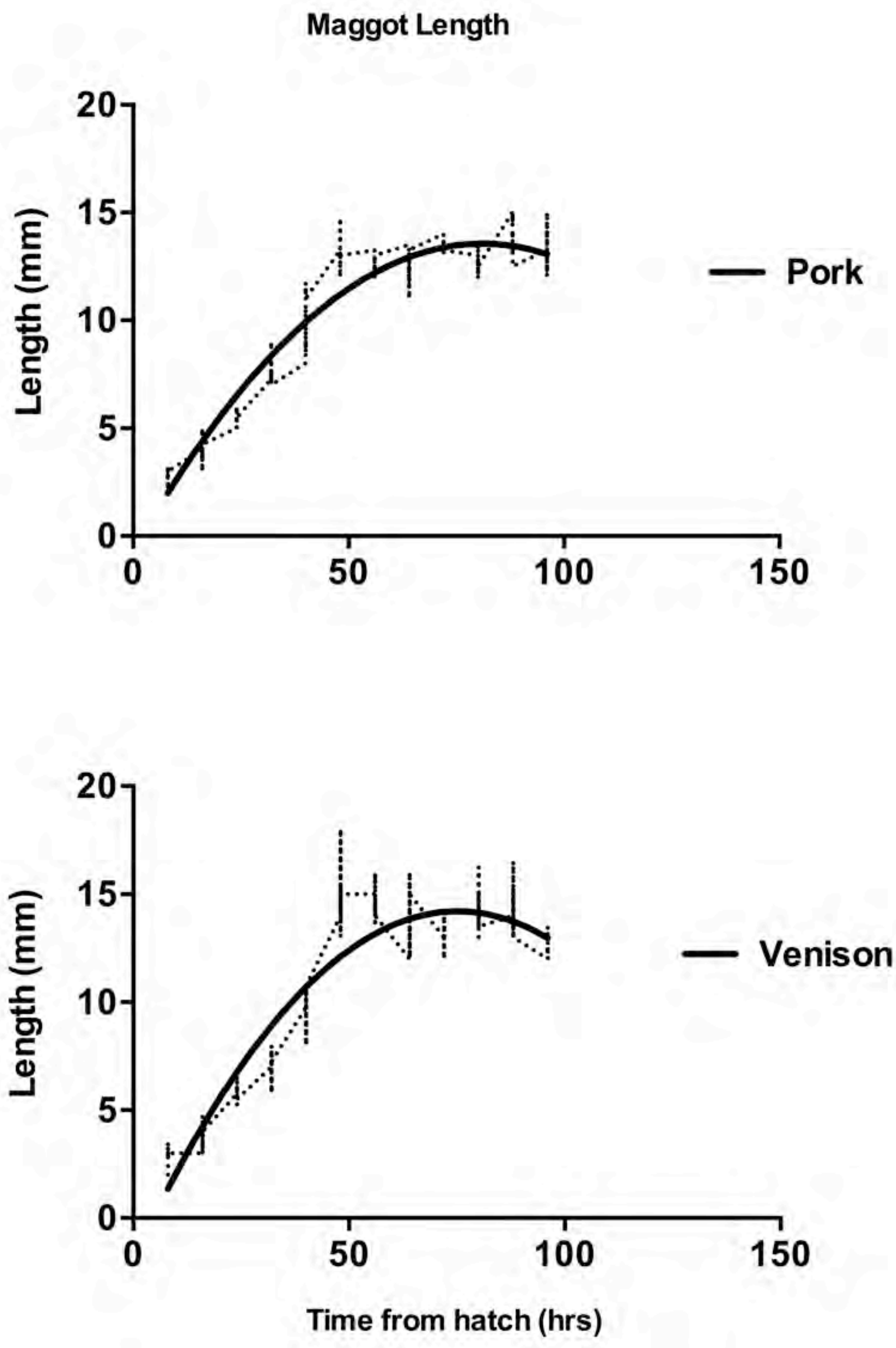

Figure 4.2: Fitted curves and measured values of maggot lengths against time from maggots reared on either pork or venison diet at 30° C 14:10 hour light dark cycle 75%RH.

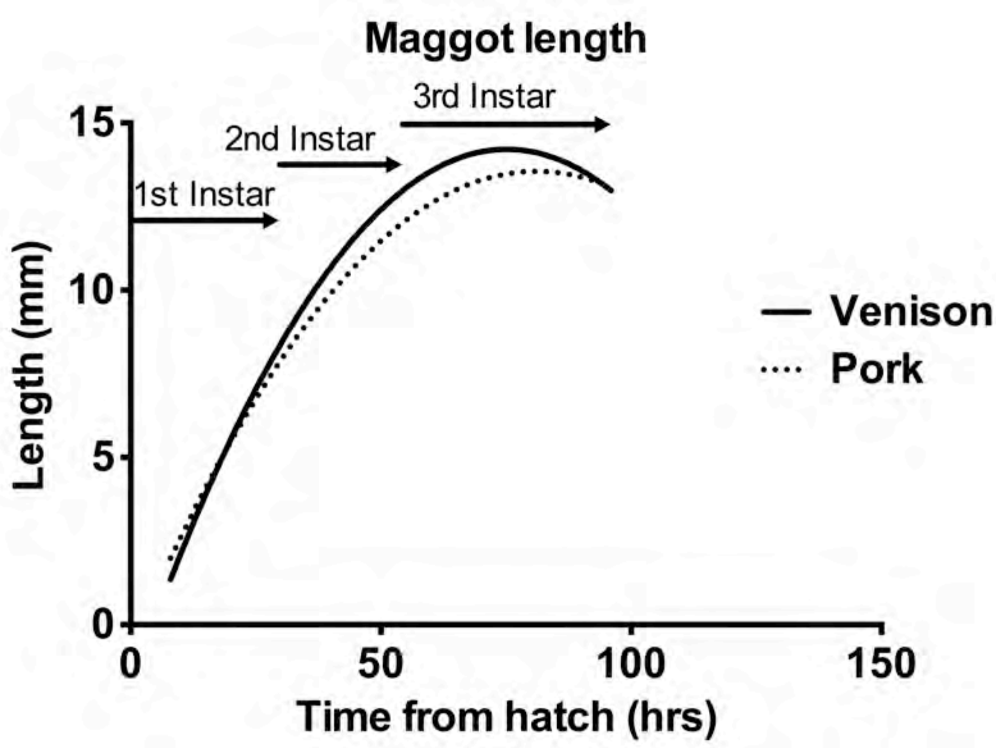

Figure 4.3: Combined fitted curves of maggot lengths and observed instar against time of maggots reared on either pork or venison diet at 30° C 14:10 hour light dark cycle 75%RH.

5 Summary

The ecology of death and decomposition involves many organisms, some of which have evolved to become specialized at utilizing carcasses. These specialists and their specific predators make up a guild of organisms referred to as necrophages. Humans utilize the insects of this guild as evidence in cases of wrongful death, neglect, and the abuse of humans and wildlife; this use represents a part of forensic entomology (Keh 1985, Anderson 1999).

Necrophagous insects arrive and utilize a carcass in a predictable pattern. This pattern has been studied so extensively that investigators can collect insects from a given point in the insect faunal succession and estimate how long the host has been dead (Payne 1965, Byrd and Castner 2001, Tabor et al. 2004). This approach provides one method for determining the minimum postmortem interval, PMI_{MIN}. The initial colonizing insects are mainly flies and the biology of these colonizers is well documented (Byrd and Castner 2001). Investigators can collect members of these early colonizing species and determine the ages of those insects based on the biology of the species and the environmental conditions around the time of collection (Catts and Haskell 1990, Benecke 1998, 2001). Determining the age of the collected insect provides a minimum time since the carcass was colonized and serves in the estimate of a short-term PMI_{MIN}. The overall objective of this study was to explore the role of necrophagous insects on the decomposition of white-tailed deer carcasses in southwest Virginia, determine how these findings compare with pig studies conducted in the same area, and evaluate the application of forensic entomology techniques to wildlife forensics and poaching.

The first objective of this study was to observe the insect faunal succession on white-tailed deer carcasses in southwest Virginia during the summers of 2009 and 2010. Each summer two deer were obtained immediately after being killed and were placed in predator exclusion cages on research farms outside of Blacksburg, Virginia. The carcasses were then observed and sampled for larval and adult insects for the first 13 days after death. Adults were identified to species where possible; larvae were reared to adult and then identified. Twenty-eight taxa were present in the summer of 2009 and 26 taxa were present in the summer of 2010. The dominant early colonizing species for both years was *Phormia regina* (Calliphoridae). Predaceous species were dominated by *Euspilotus assimilis* (Histeridae) in 2009 and *Necrophila americana* (Silphidae) in 2010. The patterns of succession were analyzed using a permutation analysis to compare the overall similarity based on similarity coefficients derived from a simple matching coefficient equation. The overall succession patterns from 2009 and 2010 were similar. These findings follow successional patterns observed in past pig studies conducted in the region. The similarity between deer carcasses and pig carcasses was 52% in the occurrence of necrophagous insect species, whereas the two pig studies were 63% similar to each other despite large differences in carcass size. Our data support the adoption of forensic entomology techniques to wildlife forensics with proper utilization of appropriate (species-specific) baseline data.

The second objective of this study was to determine if the growth of the forensically important blow fly, *Phormia regina*, could be affected by a diet of either pork or venison. Pigs serve as human analogues in forensic entomology and pork is commonly used as a rearing medium (Payne 1965, Catts and Haskell 1990). Ground venison was obtained from deer harvested on the same property where the summer succession studies were conducted. Ground pork was obtained from the local grocery store. Wild-caught *Phormia regina* eggs were reared concurrently in one growth chamber until 90% pupation on either diet, pork or venison. The chamber maintained the rearing containers at 30° C (± 1°) and 75% RH, with a 14:10 hour light dark cycle. After eclosion, maggots were collected at 8 hour intervals, fixed in KAA solution, and measured for length (Catts and Haskell 1990). The lengths of venison-reared maggots were compared to pork-reared maggots using a Kolmogorv-Smirnov test and significant differences (α= 0.05) were found in overall lengths and in the lengths of third instar maggots. Adult wing lengths were measured after eclosion. Wing lengths were compared using a *t*-test with $\alpha = 0.05$. Venison-reared maggots were significantly larger, as were the wing lengths of adults reared on venison. The difference in the length of maggots could contribute error to the PMI_{MIN} estimation if pork were used as a rearing medium in a wildlife poaching investigation or if development times of venison-reared maggots were calculated from pork-reared maggot data.

Forensic entomology has previously been applied to wildlife forensics (Anderson 1999) and research in wildlife has validated the need for host and region specific succession studies for accurate adoption of forensic entomology techniques (Watson 2003, 2005). The studies conducted here contribute to the baseline data necessary to further utilize forensic entomology in wildlife poaching and forensics. Regional studies provide specific reference material that enable more accurate estimations of PMI_{MIN} to be made in investigations nearby. Carcass species-specific insect succession studies provide data on the patterns and species of insect succession that can later be utilized as baseline data in wildlife poaching investigations. Without the appropriate baseline data, investigators would be limited in their ability to determine PMI_{MIN}. The findings of these studies provide additional reference material to insect faunal succession and the rearing biology of *Phormia regina.* There are additional variables surrounding the ecology of death and wildlife forensics that have not been explored here, for example, the effects of scavenging, limb removal, disembowelment, injury level, burial, or carcass exposure to the elements. Continued research in wildlife forensics could improve our understanding and strengthen our investigative potential in this field.

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