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Effects of carrion decomposition on arthropod community structure and habitat seeking behavior

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Submitted in Partial Completion of the
Requirements for Departmental Honors in Biology

Bridgewater State University

May 9, 2017

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1. Introduction

Carrion Ecology

Carrion is a term used to describe decaying animal flesh. In an ecological context, this is sometimes compared to detritus, which comprises mostly plant matter (Swift et al. 1979). However, carrion serves as resource patches for many different communities to coexist with each other (Benbow et al. 2016). To understand the nature of decomposition and its effects on organisms living within an ecosystem, a community ecological perspective can be used to observe the complex interactions and dynamics that occur during decomposition.

An important concept for understanding the complex relationships that occur during decomposition is that of the ecological niche, which can be defined as the resource preference of a species or the location in which it resides (Elton 1927, Peterson et al. 1999). Niche dynamics can be observed in carrion insects as successional patterns in arthropods throughout decomposition. As carrion decays, the characteristics of the habitat change, favoring different species. Therefore, successional patterns can be viewed as decomposition proceeds. These patterns are typically driven by behavior impacted by competition and coevolution over millions of years; however, there are many immediate environmental factors that dictate successional patterns as well, including but not limited to humidity, temperature, rainfall, geographic location, and anthropogenic factors (Elton 1927, Schoenly 1992, Leibold 1995).

Another way succession can be impacted is through the local extinction or immigration of species at the decomposition site, often resulting from competition between species. Species that are good colonizers will be present at high abundance and over time be outcompeted by stronger competitors, which drive the good colonizing species to local extinction (Levins and Culver 1971). Interspecific competition occurs between two species that have a similar niche and

share at least one limiting resource. This process can shape entire ecological communities (Tilman 1982). When one species completely outcompetes another, it will lead to competitive exclusion: the weaker competitor will become extinct in the community where the competition took place (Hardin 1960). Carrion insects can avoid this through niche partitioning. This is a process in which multiple species share niches but coexist, defying the competitive exclusion principle by each exploiting only a subset of the possible habitats or resources (Rockwood 2015).

Variation of carrion insects within the same geographic location can be a direct reflection of the landscape and the arthropod community that resides there prior to the introduction of carrion (Benbow et al. 2016). Landscape ecology observes the ways that organisms are distributed within a given habitat (Forman 1995). Community composition reflects characteristics of a group of individuals that are from different species, such as species diversity (Hooper et al. 2005). When carrion is introduced into a landscape, community structure is considered stable because decomposition is so localized that it does not have any significant impact on the overall community composition (Forman 1995). Rather, carrion decomposition presents small patches of nutrient islands of organic compounds in the immediate vicinity (Yang 2006).

Decomposition

Several major environmental factors affect the rate at which carrion decomposes. Warm ambient temperature encourages microbial growth, the rapid accumulation of volatile organic compounds, and, ultimately, the attraction of large numbers of invertebrate and vertebrate scavengers (Carter et al. 2007). Moisture is another key factor in the rate of decomposition, as arthropods and bacteria need moist environments to successfully reproduce (Bass 1997). In

contrast, cooler temperatures retard microbial growth and reduce the abundance and activity of arthropods (Mann et al. 1990). Environmental factors can also influence insect behavior, indirectly changing the way carrion will decompose. Sunlight, for example, affects the location where fly larvae feed. Carrion exposed to direct sunlight will have larvae burrowing under the skin, whereas shaded carrion will have larvae visible on top of the skin. By altering the behavior of the most abundant carrion insect, decomposition rates can be altered (Bass 1997).

Not only do direct environmental factors alter decomposition, but the physical environment in which the carrion is found can alter the rate at which it decomposes. The number of volatile organic compounds (VOCs) emitted from the microbial breakdown of the carrion are altered in different habitat types. For example, more VOCs are detected in agricultural sites than urban; this will attract different species and abundances of arthropods because many species are attracted to specific VOCs (Dekeirsschieter et al. 2009).

Decomposition can be categorized into major events that are defined by changes in biological and chemical processes, which interact with each other throughout decomposition. These events are described as five stages of decomposition: Fresh, Bloat, Active, Advanced, and Dry decay (Payne 1965). Each stage has a several names used by different authors; however, for the purpose of this thesis, these will be the names used. The following descriptions of the stages assume optimum temperature, which is approximately 20-25° C (Payne 1965).

The first stage of decomposition is the Fresh stage. This typically occurs within the first 72 hours of decomposition (Payne 1965) and exhibits the most dramatic changes in the body at the cellular level (Forbes and Carter 2016). The start of the stage is marked by the complete halt of circulation of lymph and blood, as well as the cessation of breathing (Clark et al. 1997). Due

to the lack of circulation and breathing, cellular metabolism ceases, causing the body to become anoxic (Clark et al. 1997, Gill-King 1997). Thereafter, muscles and sphincters relax, allowing bodily fluids to leave the body, especially if the body is moved postmortem (after death) (Clark et al. 1997). The relaxing of muscles leads to a sunken look in the face around the eyes. After only one day, the cornea dries, creating a white cloudy color over the eyes (Janaway 1996). The blood initially clots in the vessels of the organism, but as the body continues to decompose, pH drops, leading to the re-liquefaction of the blood. The blood then begins to pool at the lowest part of the body in a process called Livor Mortis; however, this phenomenon typically is seen only in animals with little hair, such as domesticated pigs and humans, as in other animals, fur will prevent its detection (Clark et al. 1997). During life, vertebrates rely on adenosine triphosphate (ATP) to allow for muscle contraction; after death, the production of ATP ceases, but it continues to be consumed by muscles, so they tighten, creating a stiffening of the muscles called Rigor Mortis, which starts at about 2 hours after death and continues for 12-18 hours (Dix and Gram 2000).

The second stage of decomposition is referred to as the Bloat stage, due to the bloated look that the organism gains. This is particularly obvious in the gut of the organism, as if it were pumped up with air. The Bloat stage typically takes place between 4 and 10 days after death (Payne 1965). As the oxygen and pH begin to drop within the body, the cellular structure of the soft tissues is disrupted, releasing enzymes that subsequently begin to break down soft tissues and lipids (Janaway 1996, Clark et al. 1997, Carter et al. 2007). Additional enzymes are added with the introduction of microbes and insects that visit the decomposing organism (Carter et al. 2007). As the tissues liquefy under the epidermis, gaseous byproducts build up within the body,

creating the bloated appearance (Weigelt 1989). This environment is ideal for bacteria, which will become abundant, increasing the rate of decomposition of softer tissues (Clark et al. 1997).

As pressure within the tissues builds, the skin will begin to tear and rupture, causing liquefied tissues and gases to escape the body (Weigelt 1989). This is the first indication of Active decay. The liquefied tissues and fats are released into the soil immediately surrounding the body (Dekeirsschieter 2009). During this time, a Cadaver Decomposition Island (CDI) will appear. CDI is a byproduct of liquefied chemical components, such as short-chain alcohols, polysulfide compounds, volatile fatty acids, and aromatics. These are produced by decaying proteins, tissues, and cells. They seep into the surrounding soil and create a black ring around the carcass (Carter et al. 2007). This stage of decomposition typically lasts 10-20 days (Payne 1965).

As the soft tissues decay and are consumed by vertebrate scavengers, arthropods, and bacteria, the skeletal remains and other hard tissues can become visible. The skin dries out and becomes leather-like (Dix and Graham 2000). This marks the next stage of decomposition, Advanced decay. This stage is slower than those prior, as insect and microbial activities slow and the majority of vertebrates interested are rodents and lagomorphs coming to gnaw on the bones (Anderson 2010). The bones will soon be all that is left, with a small amount of hide and ligaments left to hold the shape of the now highly decomposed body. The body can stay this way for 50 days after death or for years, depending on environmental conditions (Payne 1965). Plants and weathering will begin to fracture bones and leave markings on them (Janaway 1996). At this point, the body has reached the end of decomposition.

Arthropods

Arthropods (mostly insects, Class Hexapoda) that have direct impacts on decomposition and are commonly present at carrion will be referred to as carrion-associated arthropods, or simply associated arthropods. Arthropods that are adventitious in the presence of carrion or that are not known to be drawn to or colonize decomposing carrion will be considered non-carrion-associated, or simply non-associated. Arthropods located around carrion are typically categorized into one of four groups, three of which are associated arthropods: necrophages, parasitoids and predators, omnivores, and adventitious (non-associated) species (Rivers and Dahlem 2014a).

Investigations of carrion ecology typically involve successional patterns of invertebrates, specifically insects that colonize (Payne 1965, Pechal et al. 2014). Environmental conditions have effects on community abundance and taxon richness, as arthropods rely on warm, humid conditions (Benbow et al. 2013). Arthropod succession comes in waves, where a previous arthropod species that was at the carrion creates an environment that is suitable for the species that colonize thereafter (Rivers and Dahlem 2014a).

Necrophagous insects are those that feed directly on carrion in their adult or larval stages. These include many commonly known Diptera (true flies) and Coleoptera (beetles). Two notable Dipterans that are commonly referred to as “filth flies” include blowflies (calliphorids), such as *Lucilia spp.*, and flesh flies, which are in the genus *Sarcophaga*. Larval masses of these two genera are strong indicators of the length of time an organism has been dead (Rivers and Dahlem 2014a). Scuttle flies (*Phoridae*) are also common necrophagous dipterans that arrive after calliphorid flies have retreated to the soil to pupate (Smith 1986). Scuttle flies are often a problem in museums, as they are able to access burial crypts to feed and become adults, leaving preserved vertebrate specimens at risk, even in sealed cases (Katz 1987). Other notable flies

include cheese skippers (*Piophilidae*) and Soldier Flies (*Stratiomyidae*), which also lay eggs in decomposing plant and animal matter (Hall and Gerhardt 2009). Necrophagous coleopterans such as carpet beetles (*Dermestidae*) are often late colonizers, consuming the leathery flesh and cartilage that has been left by the flies. Carpet beetles are very small and are often used in taxidermy to clean bones, as they can quickly consume carrion (Rivers and Dahlem 2014a).

Parasitoids and predators directly impact the abundance of necrophagous insects, thereby having an indirect effect on decomposition rates. These insects are defined as necrophilous: attracted to vertebrate decomposition, but not feeding on the carrion (Rivers and Dahlem 2014a). They include beetles from the family *Staphilinidae* (Rove beetles), which can be found in any type of decomposing matter; however, there are few species that frequent decomposition sites. These, such as the hairy rove beetle (*Creophilus maxillosus*), are predatory in their adult life but act as parasites in their larval stages (Rivers and Dahlem 2014a). Parasitoid wasps are another type of insect that affect dipteran abundance at carrion. They lay eggs in the larvae of other insects, such as caterpillars and fly larvae, for their offspring to feed on until they develop into adults. This process inevitably kills the host, unlike the effects of other parasites, which typically do the least damage possible to the host (Rivers and Dahlem 2014b, Godfray 1994). *Nasonia vitripennis* is a well known parasitoid wasp found in the United States that primarily parasitizes fly larvae, typically of the genera *Sarcophaga* and *Lucilia* (Saunders 1966). *Nasonia vitripennis* is an ectoparasite (resides on the outer cuticle or “skin”) to these fly larvae and infects them during their pupation (Godfray 1994).

Omnivorous species are those that consume both carrion and other arthropod species that reside near carrion. Carrion beetles are the most abundant of this group. These beetles are in the family *Silphidae* and include the endangered American burying beetle (*Nicrophorus*

americanus). Like others of its genus, *N. americanus* specializes on vertebrate carrion as food and shelter for larvae. Males and females make a crypt of a small vertebrate, such as a rodent, in which to raise offspring, exhibiting a biparental lifestyle (Ratcliffe 1996). *Necrophila americana*, the American carrion beetle, does not show parental care; however, it is present on larger carrion, often in high abundance (Ratcliffe 1996). Another notable order in decomposition is Hymenoptera, which includes bees, wasps, and ants. Ants have been seen as forensically important, as forensic entomologists can establish the time carrion has been in a location by observing when winged adults are produced, which indicates the length of time since the founding of the colony. Ant colonies are established rapidly near carrion, due to the rich resource base it provides (Goff & Win 1997). Yellow jackets (*Vespidae*) have also been recorded as forensically important, as they utilize the corpse and fly larvae as food sources (Moretti et al. 2011).

Non-associated arthropods include detritus feeders that consume only decomposing plant matter, as well as generalist predators. In the simplest terms, any non-associated arthropod is one that is not mentioned previously under associated arthropods. Examples of non-associated arthropods include spiders (Chelicerata: Araneae), isopods (Crustacea: Isopoda), and field crickets (Hexapoda: Orthoptera).

Mediators that influence arthropod behavior

Chemicals are located throughout the environment and can be useful tools for many animals to sense potential mates, food, and even individuals of different species. Insects rely on chemical stimuli to communicate with others of the same species (intraspecific) and other species within the community (interspecific) (Gullan and Cranston 2010). For example, predatory insects can pick up on intraspecific communication between necrophagous species to

locate the site of decomposition and feed on the individuals inhabiting it (Giao and Godoy 2007). Auditory, visual, and chemical stimuli are all used by insects at carrion. In addition, the carrion itself will emit volatile organic compounds (VOCs) that are helpful with chemical communication (Rivers and Dahlem 2014b). These VOCs can be used by some species to detect a decomposing vertebrate from miles away in order to locate food or an oviposition site (Tomberlin et al. 2011).

The chemicals that are used for communication between individuals are referred to as semiochemicals and modify the behavior of the recipient (Rivers and Dahlem 2014b). The individuals receiving these chemical signals pick them up using smell (olfaction). The chemical receptors that take in the information are referred to as sensilla and relay it to the sensory neuron (Chapman 1998). Olfactory chemoreceptors are referred to as multiparous sensilla (Gullun and Cranston 2010). Intraspecific communication relies on pheromones, whereas interspecific communication relies on allelochemicals, which are both produced by exocrine glands (Rivers and Dahlem 2014b).

Pheromones are separated into two categories: primers and releasers. Primers are required for the receiver to experience behavioral change in the future, ranging from days to months. Events such as development and reproduction are thought to be influenced by primers. Releasers, on the other hand, trigger an immediate behavioral response and can control things such as sexual attraction, mate recognition, and alarm (Chapman 1998).

Allelochemicals are much more diverse than pheromones. There are four types of allelochemicals, categorized by the effect they have on the receiver: allomone, synomone, kairomone, and apneumone (Leblanc and Logan 2010). Allomonones are chemicals that cause

harm to an individual of another species and are typically used for defense (Gullan and Cranston 2010). Synomones are chemicals that generate a response in the receiver that leads to a positive outcome for both species. This is commonly seen in situations where plants are under attack by an insect and synomones attract parasitoid wasps that will attack the insect and benefit the plant and the wasp (Nordlund and Lewis 1976, Wood 1982). A kairomone is a chemical that benefits the recipient but has no benefit to the emitter and, in fact, can potentially harm the emitter if it attracts a predator or a parasitoid (Grasswitz and Jones 2002). This may happen when dipteran eggs or larvae emit kairomones and attract rove beetles, for example. Apneumones are extremely important to carrion ecology, as these are chemical signals released from a non-living object (Vass et al. 2002).

Apneumones can vary depending on the environment in which carrion is lying. Carrion located in a forest will have fewer VOCs present than carrion at an agricultural site but more than carrion located in an urban location. This is because the local microclimate generates “background noise” and can mask cadaver VOCs (Dekeirsschieter et al. 2009). This can drastically change the behavior and community assembly of arthropods at the site of decomposition, depending on the environment in which carrion is placed.

Practical uses for Carrion Ecology

An understanding of carrion ecology can be exploited to answer many practical ecological and legal questions. The different subfields of applied carrion ecology include conservation and forensic science. This section will describe how subfields can use carrion ecology.

Conservation biologists use information in the environment to attempt to preserve a population from going extinct. Carrion scavengers, such as carrion beetles, occupy high trophic positions, which causes them to be more susceptible to alteration of the environment by humans (Hocking et al. 2006). The resource availability of carrion also influences the mass of carrion beetles, allowing beetles with a larger carrion resource to have larger wing loads (Barton et al. 2013). *Nicrophorus* is a good model species for carrion insect conservation because it is at a higher trophic level relative to other carrion-associated species and is attracted to small carrion (such as a rodent or bird). *Nicrophorus* carrion choice can be strongly affected by human activity and construction, which may limit the number of small carrion *Nicrophorus* can access (due to its inability to fly long distances quickly) that are located on appropriate soil for crypt production. Anthropogenic factors preventing *Nicrophorus* from accessing carrion will also prevent them from finding mates, as this is often where they will meet to reproduce (Hocking et al. 2006, Hocking and O'Regan 2016).

Conservation biologists may also need to use carrion insects to determine the time in which an endangered animal has been poached. Animals that are accidentally shot are often left at the site to avoid a citation or potential loss of a hunting license. On the contrary, deliberate poachers will harvest the animal for the desired parts, with remains left behind. Carrion insects can be used to determine how long the animal had been dead, indicating the time when it was poached. This evidence, along with witness accounts, can lead to the finding of poachers and those who do not report accidental shootings, leading to the further protection of endangered animals (Anderson 1999).

Forensic Entomology is a field of study in which specialists study insect behavior and use this information to answer legal questions. The subject of questions can range from animal or

child abuse and/or neglect to homicide. For example, in 2001, a study was conducted by a forensic entomologist to answer two questions about a potential homicide of a child: (1) was the child neglected prior to death, and (2) did the estimated time of death match the time the child was known to be in the mother's care. The entomologist collected fly larvae from both the face and genital regions of the child. There were larvae of multiple species, but the larvae from species that are attracted to fecal matter were in later stages of their life cycles than the larvae that are attracted to decomposition. This indicated that the child was neglected long before death, and the estimated time of death showed that the child was in the mother's care at death (Benecke 2001).

The purpose of this thesis was to determine how chemical mediators and nutrients that are released into the environment during carrion decomposition affect the community ecology and behavior of arthropods. Studies have been done to examine associated carrion insects to a great extent; however, not much has been done to observe non-associated arthropods and secondary carrion insects in regards to how carrion impacts behavior and ecology. To have a better understanding of this, two studies were conducted, addressing the following research questions:

- 1) How does the introduction of vertebrate carrion impact soil nutrient content and abundance and biodiversity of arthropods not normally associated with decomposition?
- 2) Do different carrion species trigger different olfactory responses in the carrion insect *Nasonia vitripennis*?

2. Effects of carrion decomposition on litter arthropod assemblages and soil nutrient content

Introduction

All animals contribute to the carrion cycle, whether in life by consuming carrion or preying on necrophagous insects, or at death, unless they have fallen prey to another individual and been consumed (Barton 2016). The dispersal of nutrients is strongly affected by arthropods and scavengers that consume carrion and the predators that feed on these animals (Payne et al. 1968, Putman 1978, Devault et al. 2003). In temperate forests, leaf litter covers most of the ground, and plants, fungi, and microbes in the soil rely on nutrients from decomposing organisms, primarily leaf matter. Carrion is nutrient-dense, whereas leaf litter has poor quality of macronutrients, including nitrogen (the most limiting nutrient for most terrestrial plants; Elser et al. 2007). For example, carrion inputs up to 5 times the concentration of nitrogen into the soil that leaf litter does (Swift et al. 1979, Moore et al. 2004). Generally, concentrations of nutrients such as nitrogen and phosphorous increase in the soil as decomposition progresses (Petersen and Luxton 1982, Dent et al. 2004, Carter et al. 2010). Consequently, some plants gain a competitive advantage when large game, such as the white-tailed deer (*Odocoileus virginianus*), decomposes nearby (Bump et al. 2009).

Leaf litter provides shelter to an extensive community of arthropods, bacteria, and fungi that live within it. Arthropods, such as millipedes, and earthworms break down large bits of leaf litter into small enough pieces for fungi and bacteria to utilize and decompose into chemical compounds (Johnson and Catley 2002). Trees in temperate woodland can produce 1,500-5,000 kg of leaf litter, wood debris, and insect excrement per year (Yahner 1995). Without the aid of the leaf litter community, this debris would pile up, and nutrients would never be recycled, completely changing what current woodlands would look like today.

Saprophagous arthropods (those that feed on decaying organic material) that live in the leaf litter can act as adventitious species if carrion is placed on top of the leaf litter. Adventitious species will use the carrion as habitat for protection prior to the onset of Advanced decomposition and in late stages of decomposition, when skin is leather-like. There is no successional pattern to these arthropods; however, they can be used to determine whether carrion has been moved or disturbed (Rivers and Dalhem 2014b).

Arthropod abundance at carrion most likely follows a normal distribution over time (Benbow et al. 2016). There is little arthropod activity during the Fresh stage of decomposition, a peak at the Active stage, and a decline until the Dry stage (Payne 1965). Community processes and patterns have been extensively studied for necrophagous insects (Benbow et al. 2016). However, little has been studied on arthropod assemblages that reside in the area in which carrion is introduced (referred to hereafter as the local community).

In Kings Park, Western Australia, Bornemissza (1957) observed the succession of arthropods on carrion and the influence of the carrion on surrounding soil fauna. Guinea pigs were placed in individual cones, laid on a wire grate. A vial of 96% ethanol was placed below each cone to collect soil fauna; samples were collected daily for the first 21 days, then every other day from days 21-30 and every three days from days 30-40. To study the impacts on soil fauna, guinea pigs were also placed on soil, and soil characteristics were measured beneath the carrion and at 10, 20, and 40 cm from the carcass. Arthropods were extracted from soil samples by using Berlese funnels (Bornemissza 1957).

Contrary to most other research (e.g., Payne 1965), Bornemissza (1957) concluded that soil fauna had little impact on the rate of decomposition. However, the impact of decomposition

on arthropods could not be measured, as most, if not all, individuals (excluding Diptera and parasitoid Hymenoptera) that visited the carrion were collected by the funnels placed below the carrion (Bornemissza 1957). This would effectively prevent arthropods from accessing the carrion and affecting the rate of decomposition. It remains unknown whether the absence of carrion-associated species alters dispersal rates of carrion nutrients (Barton et al. 2013).

Australia and New Zealand are located in the Australasian biogeographic region, whereas the United States is located in the Nearctic biogeographic region (Rivers and Dahlem 2014a). Biogeographic regions differ in species composition (Huggett 2011). Therefore, because the study in question (Bornemissza 1957) occurred in Australia, it would involve different arthropod communities than those seen in the United States, at least at the species level and potentially at higher taxonomic levels. This justifies repeating such studies in other biogeographic regions, as the accuracy of data used in legal cases depends on using research from the appropriate biogeographic region.

Smaller carrion may attract different species of carrion-associated insects than larger species of carrion. Specifically, species of the genus *Nicrophorus* (sexton or burying beetles) prefer small vertebrates, such as rodents and birds. These beetles will bury small carrion to create crypts of carrion to rear offspring (Pukowski 1933, Milne and Milne 1976). However, more arthropods visit larger carrion, and smaller animals decompose more slowly due to low arthropod abundance (Hewadikaram and Goff 1991). Because it attracts different assemblages of arthropods with different species richness, small carrion cannot be used to predict the dynamics of arthropod assemblages on larger carrion. Smaller carrion will also have a smaller effect on the environment surrounding it (e.g., soil nutrient concentrations) than would larger carrion.

The purpose of this study is to better understand the effects of carrion on local arthropod assemblages and to address the concerns outlined above. By observing insect succession near larger carrion (i.e., pig heads) and in a temperate North American forest, this research will test the degree to which the results of Bornemissza (1957) are applicable in a broader context.

Methods

Field Setup:

Five pig heads (*Sus scrofa*) were placed at least 7 m apart near The Great Hills Walking Trails, a temperate forest site at Bridgewater State University in Bridgewater, Massachusetts (41.984726°, -70.957484°), on 25-V-2015. Heads were covered by cages that were constructed of wood (L × W × H: 76.2 cm × 60.96 cm × 60.96 cm), covered in 2.54-cm hexagonal chicken wire, and staked to the ground on two sides to prevent vertebrate scavengers from accessing the carrion. Pig heads were not thawed prior to the experiment, to ensure they all were in the same initial condition.

Insect Collection/Identification:

Insect samples were collected each week, from 25-V-2015 to 31-VII-2015, and once per month from 19-VII-2015 to the first freeze (18-VIII-2017), by removing leaf litter approximately 0.3m in diameter down to the soil surface. Three samples were collected from each pig head: one immediately next to the head (i.e., 0.0 m from the head), one 1.5 m from the head, and another 3.0 m away, in random directions. Distances were measured from the center of the skull between the eyes outward. Due to equipment limitations, insects were collected from no more than two pigs on any particular day, although all five heads were sampled each week. Sampling was done at mid-day (approximately 1100 h to 1300 h), when diurnal insect activity

should be greatest, as this is when most carrion-associated arthropod activity occurs (Payne 1965). Once sampling was done, cages were replaced over the pig heads.

Tullgren funnels (Macfadyen 1953) were used to extract arthropods from the leaf litter samples (Fig. 2.1). Leaf litter was placed in the Tullgren funnels on the day of collection and remained in each Tullgren funnel under a lamp for at least 24 hours. During this time, the lamp dried the leaf litter, forcing arthropods to retreat to the bottom, where they fell into Whirl-Pak bags that were filled with 70% ethanol to euthanize and preserve arthropods. Afterwards, arthropods were sorted and identified to species. If it was impossible to identify a specimen to species, the individual was identified to the lowest taxonomic level possible and classified as a morphospecies. All specimens were placed into vials labeled by species, date collected, and location of sample, and were preserved in 70% ethanol.

Soil Samples:

At the beginning of each stage of decomposition (Fresh, Bloat, Active, Advanced, and Dry decay), three soil samples were collected. On each occasion, a single pig head was randomly selected, and soil cores (approximately 30 cm length) were collected from three locations: within 30 cm of the head, 1.5 m from the head, and 3.0 m from the head, in one random direction. No head was sampled more than once. Samples were sent to the Louisiana State University Agricultural Center for analysis of nutrient composition of the soil. Analyses included pH (McLean 1982), percent organic matter (Nelson and Sommer 1982), nitrogen content, and carbon content (Matejovic 1997).

Statistical Methods:

Repeated-measures analysis of variance (ANOVA) was conducted using IBM SPSS v28 software to test whether stage of decomposition, distance from carrion, or an interaction of the two affected species richness, diversity (calculated using the exponential form of the Shannon Diversity Index), or abundance of total arthropods, as well as arthropods separated into carrion-associated and non-carrion-associated groups. Species abundance was log-transformed to meet the assumption of normality. Spearman's rank correlation was used to test whether soil pH, percent organic matter, nitrogen, or carbon increased monotonically over time. For these analyses, stage of decomposition was treated as a surrogate for time; Spearman's rank correlation was used rather than Pearson's product-moment correlation coefficient because it accounts for potential nonlinearities introduced by the different lengths of the stages of decomposition.



Fig. 2.1: Tullgren funnels in use at Bridgewater State University.

Results

Abundance

Abundances of carrion-associated, non-carrion associated, and total arthropods changed significantly over time, with advanced decomposition seeing the highest abundance (Figs. 2.2, 2.3, 2.4; Table 2.1). Abundance was significantly greater at the carrion site (0 m) than at other distances for associated and total arthropods (Figs. 2.1, 2.2; Table 2.1).

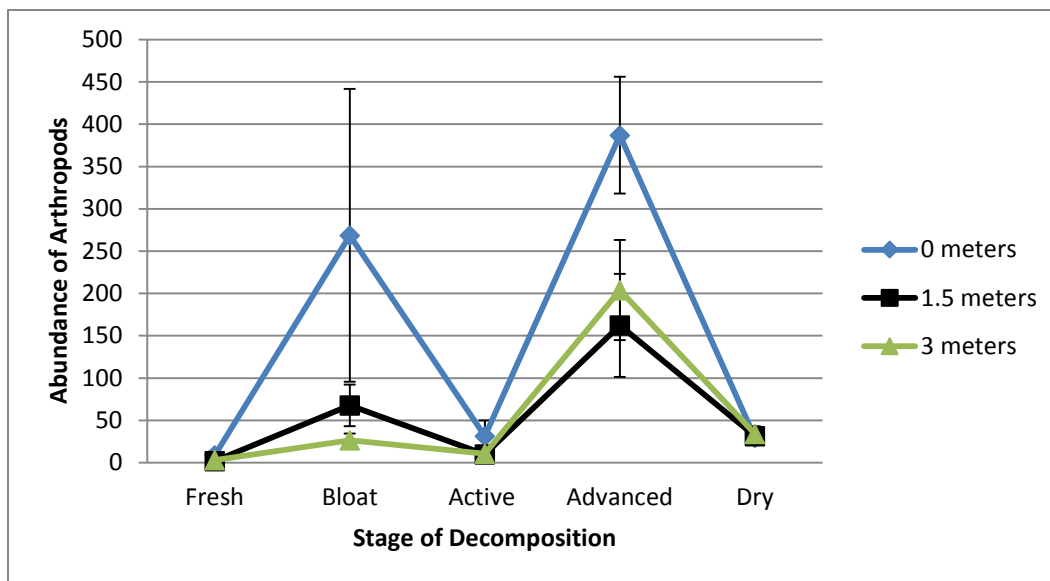


Fig. 2.2: Mean abundance of arthropods (\pm standard error) from leaf litter samples collected at each distance from pig heads and each stage of decomposition.

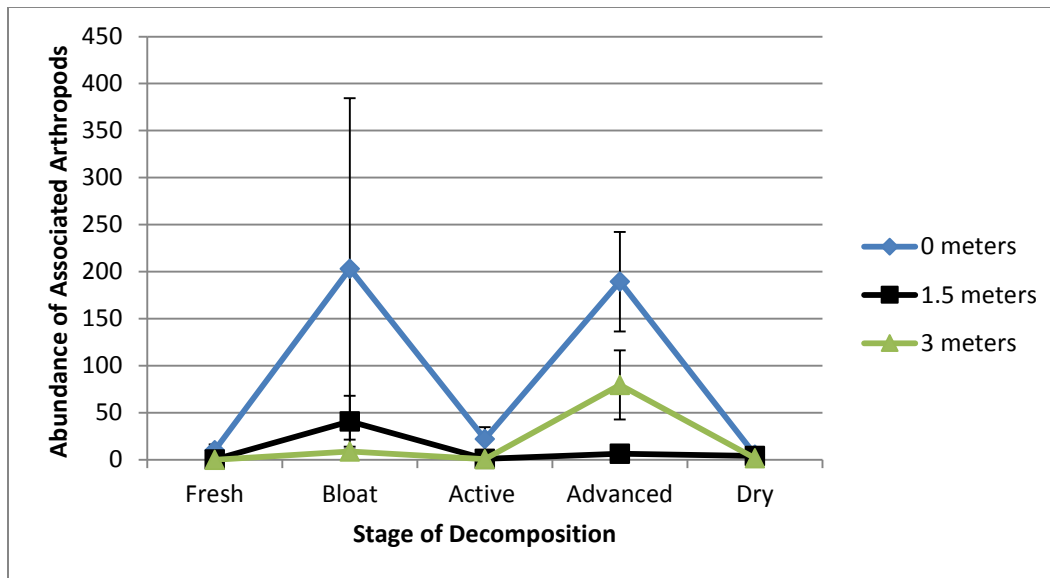


Fig. 2.3: Mean abundance (\pm standard error) of arthropods associated with carrion decomposition at each distance from pig heads and each stage of decomposition.

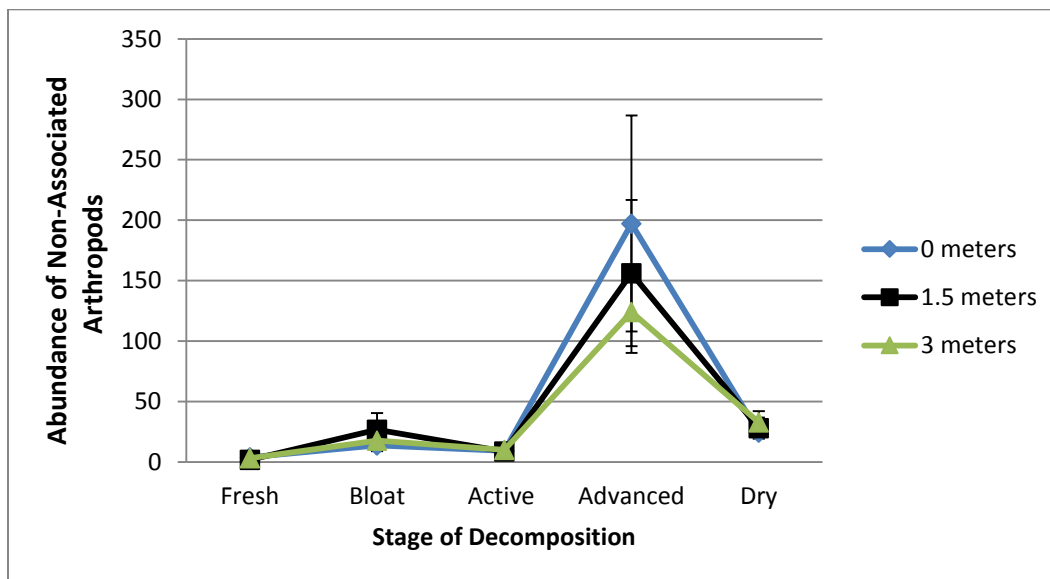


Fig. 2.4: Mean abundance (\pm standard error) of arthropods not associated with carrion decomposition at each distance from pig heads and each stage of decomposition.

Species Richness

Species richness of total arthropods and non-carrion-associated arthropods changed significantly over time, with advanced decomposition seeing the greatest species richness (Fig.

2.5, 2.7; Table 2.1). Species richness was greatest at the carrion site (0 m) and 3 m for total arthropods (Fig. 2.5), and 3 m from carrion for non-carrion associated arthropods (Fig. 2.7).

Stage and distance from carrion had an effect on species richness for carrion-associated arthropods, such that species richness was greatest during intermediate stages of decomposition at the carrion site (0 m) (Fig. 2.6; Table 2.1).

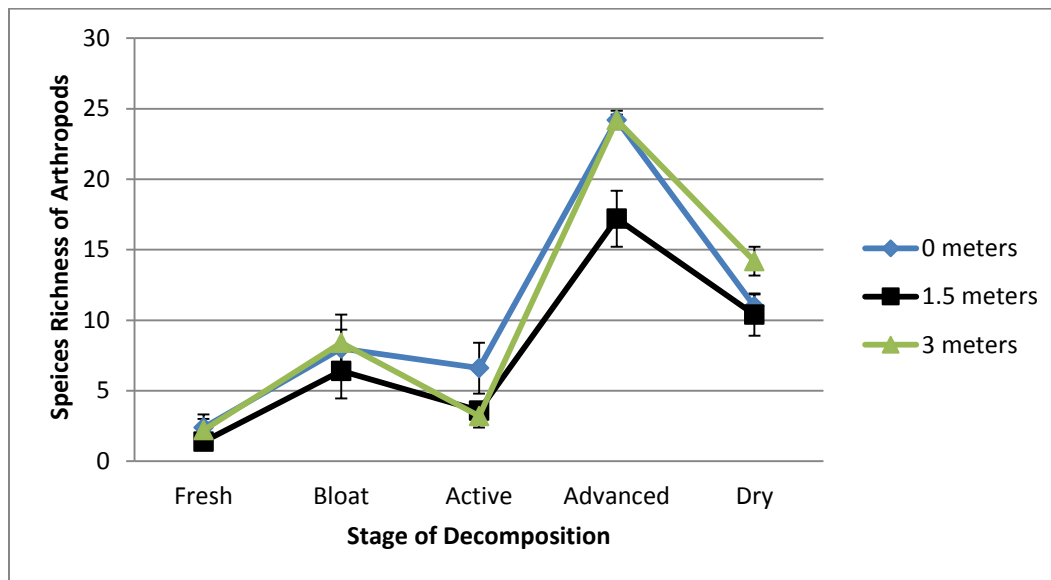


Fig. 2.5: Mean species richness of arthropods (\pm standard error) at each distance from pig heads and each stage of decomposition.

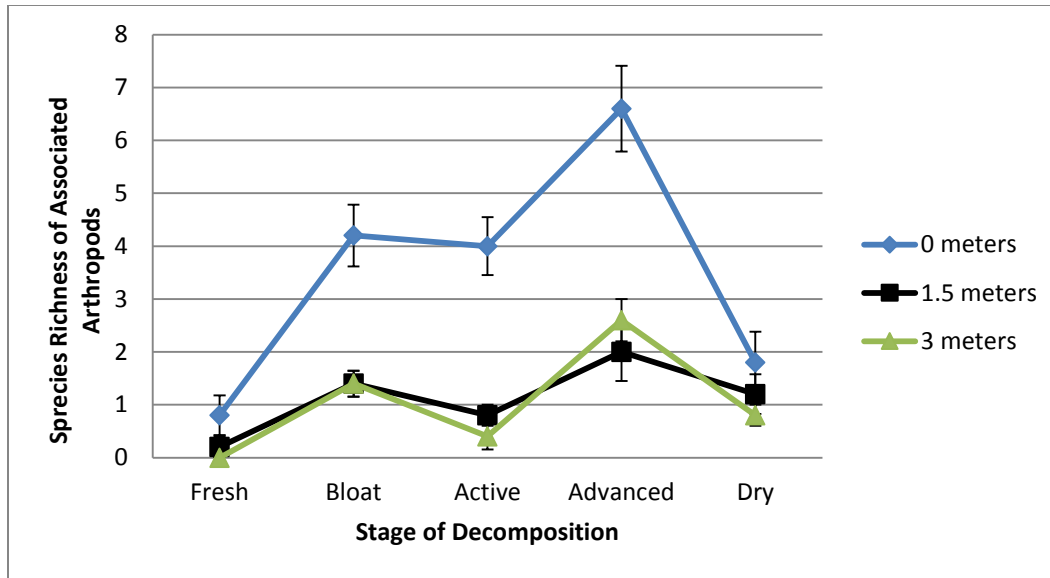


Fig. 2.6: Mean species richness of arthropods associated with carrion decomposition (\pm standard error) at each distance from pig heads and each stage of decomposition.

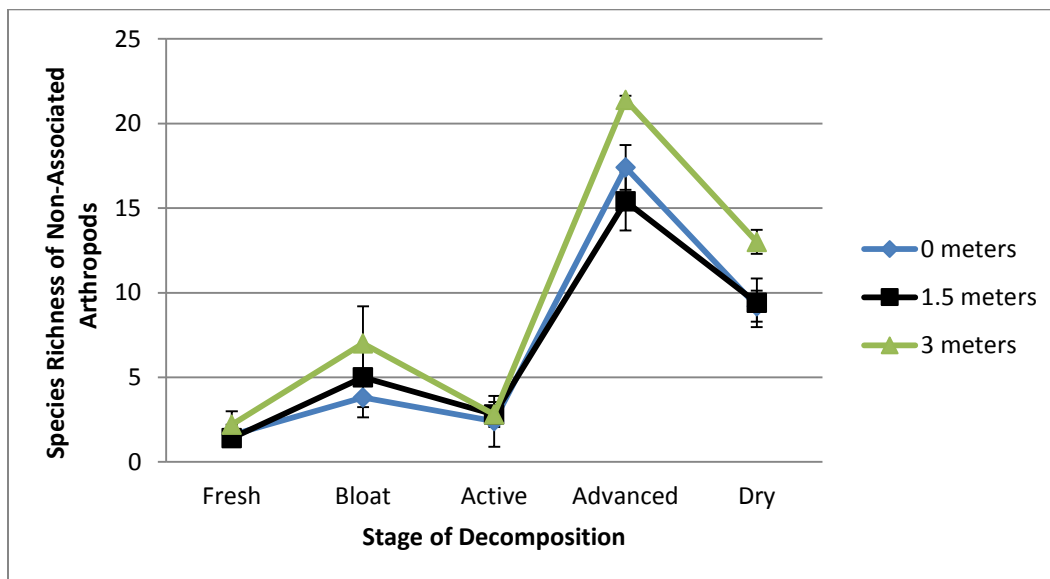


Fig. 2.7: Mean species richness of arthropods not associated with carrion decomposition (\pm standard error) at each distance from pig heads and each stage of decomposition.

Diversity

Diversity of total arthropods increased significantly over time (Fig. 2.8; Table 2.1). Diversity was greatest at 3m from the carrion site for total arthropods (Fig. 2.8; Table 2.1). In contrast, diversity of carrion-associated arthropods was greatest at the carrion site (0 m) (Fig. 2.9; Table 2.1). Non-carrion-associated arthropod diversity was significantly affected by stage of decomposition and increased during late stages of decomposition (Fig. 2.10; Table 2.1).

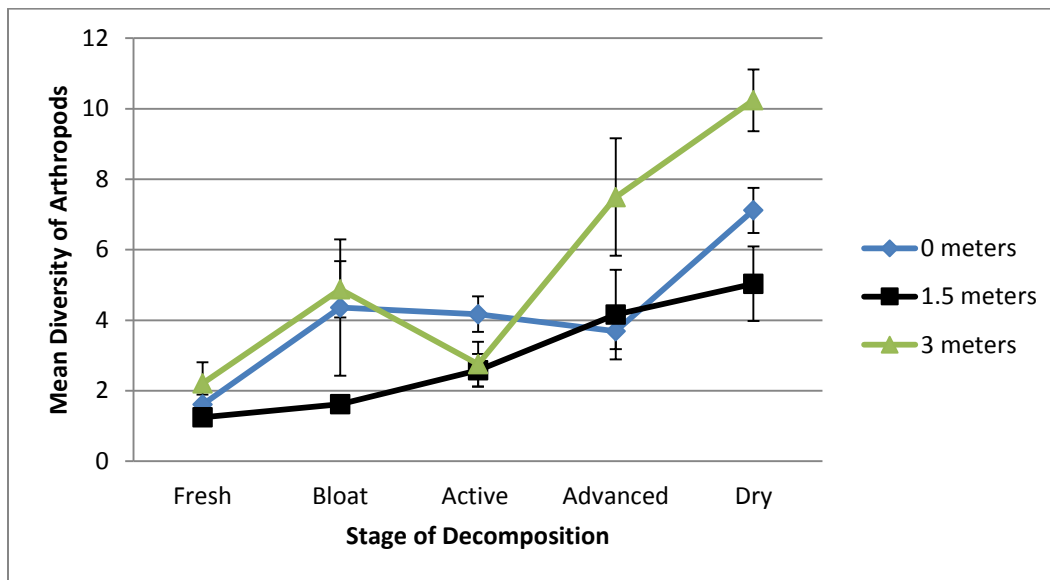


Fig. 2.8: Mean diversity of arthropod (\pm standard error) at each distance from pig heads and each stage of decomposition. Diversity was estimated using the exponential form of the Shannon Diversity Index.

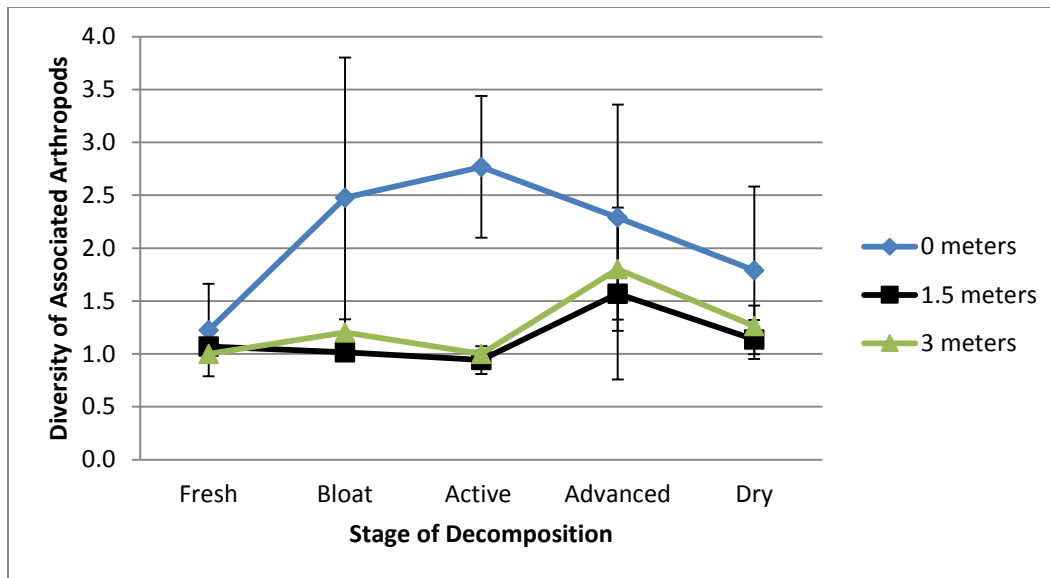


Fig. 2.9: Mean diversity of arthropods associated with carrion decomposition (\pm standard error) at each distance from pig heads and each stage of decomposition. Diversity was estimated using the exponential form of the Shannon Diversity Index.

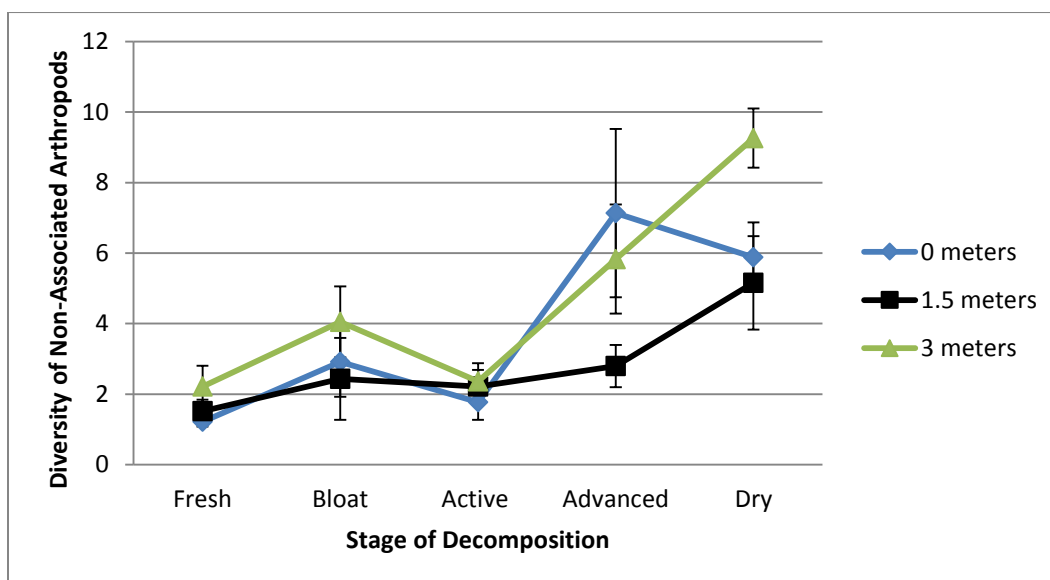


Fig. 2.10: Mean diversity of arthropods not associated with carrion decomposition (\pm standard error) at each distance from pig heads and each stage of decomposition. Diversity was estimated using the exponential form of the Shannon Diversity Index.

Table 2.1: Results of repeated measures ANOVAs testing for differences in arthropod abundance, species richness, and Shannon diversity among five stages of carrion decomposition, three distances from carrion, and their interaction. Bold type indicates statistical significance.

Repeated Measures ANOVA		Abundance		Richness		Diversity	
Focal taxa	Factor	F	P	F	P	F	P
All Arthropods	Stage of Decomposition	39.939	<.001	107.710	<.001	17.603	<.001
	Distance	5.457	.021	10.261	.003	8.616	.005
	Stage x Distance	.682	.642	2.025	.108	2.106	.078
	N		5		5		5
Carrion-associated	Stage of Decomposition	23.392	<.001	25.517	<.001	2.692	.074
	Distance	15.309	<.001	69.961	<.001	26.606	<.001
	Stage x Distance	2.251	.059	4.734	.005	1.584	.196
	N		5		5		5
Non-carrion-associated	Stage of Decomposition	30.579	<.001	89.500	<.001	15.837	<.001
	Distance	.185	.834	9.072	.004	2.401	.133
	Stage x Distance	.244	.945	1.115	.373	1.734	.175
	N		5		5		5

Soil

Of the four soil characteristics tested, only percent organic matter displayed a positive trend over time (Figs. 2.11-2.14; Table 2.2). This was true for soil samples 3 m from carrion (Table 2.2) and for all soil samples, regardless of distance (Fig. 2.11; Table 2.2).

Table 2.2: Spearman's rank correlation of soil composition at different distances from carrion over time. Bold type indicates statistical significance. *Significant with an alpha of 0.05. **Significant with an alpha of 0.01.

Spearman's Rank Correlation		Carbon (%)	Nitrogen (%)	Organic Matter (%)	pH
0m	Correlation Coefficient	.800	.800	.700	.600
	Sig. (2-tailed)	.104	.104	.188	.285
	N	5	5	5	5
1.5m	Correlation Coefficient	.600	.700	.600	-.400
	Sig. (2-tailed)	.285	.188	.285	.505
	N	5	5	5	5
3m	Correlation Coefficient	.000	.000	.900*	-.700
	Sig. (2-tailed)	1.000	1.000	.037	.188
	N	5	5	5	5
All Distances	Correlation Coefficient	.497	.480	.775**	-.115
	Sig. (2-tailed)	.060	.070	.001	.684
	N	5	5	5	5

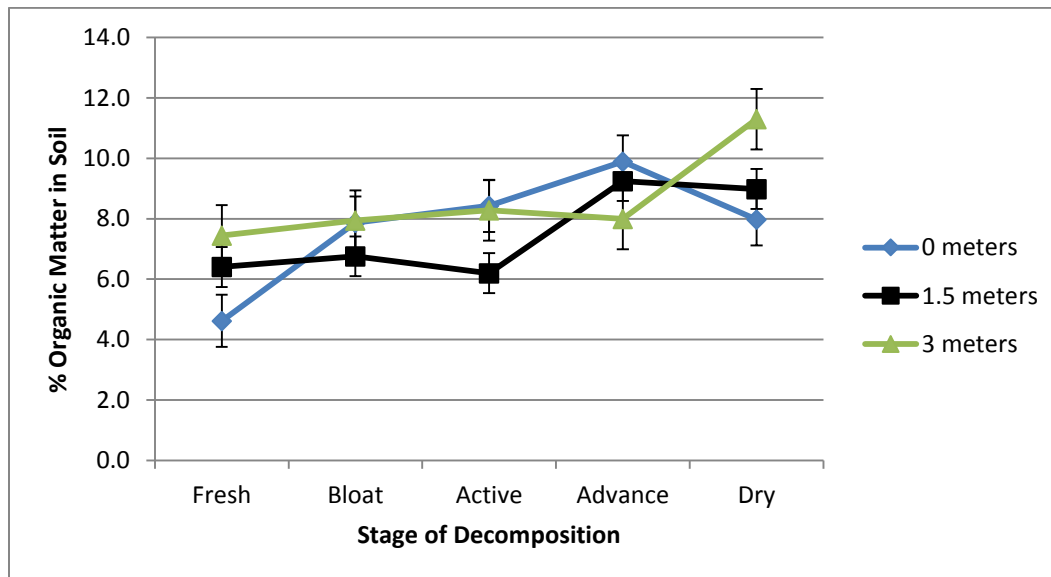


Fig. 2.11: Percent organic matter (mean \pm standard error) in soil at each distance from pig heads and stage of decomposition. Organic matter (%) generally increases over time (Spearman's rank correlation: $r_s = 0.78$, $P = 0.001$).

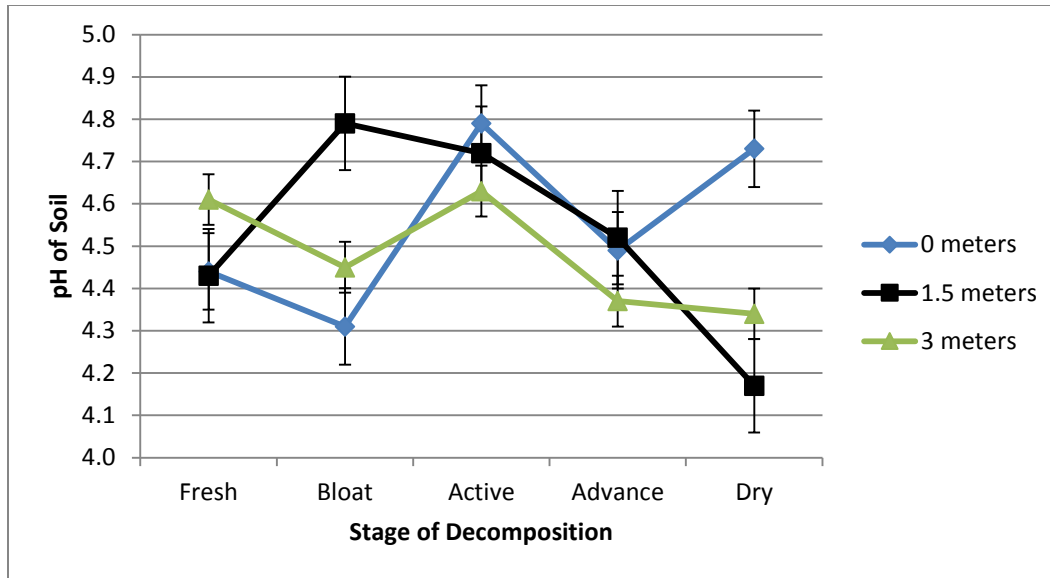


Fig. 2.12: pH of soil (\pm standard error) at each distance from pig heads and stage of decomposition. pH generally does not change over time (Spearman's rank correlation: $r_s = -0.12$, $P = 0.684$).

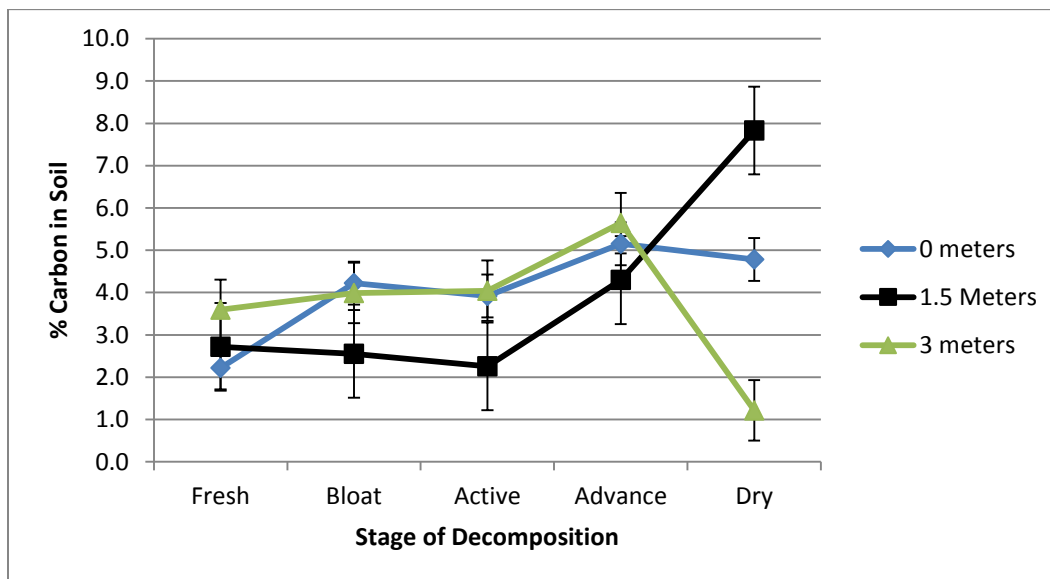


Fig. 2.13: Percent carbon in soil (\pm standard error) at each distance from pig heads and stage of decomposition. Carbon generally does not change over time (Spearman's rank correlation: $r_s = 0.50$, $P = 0.060$).

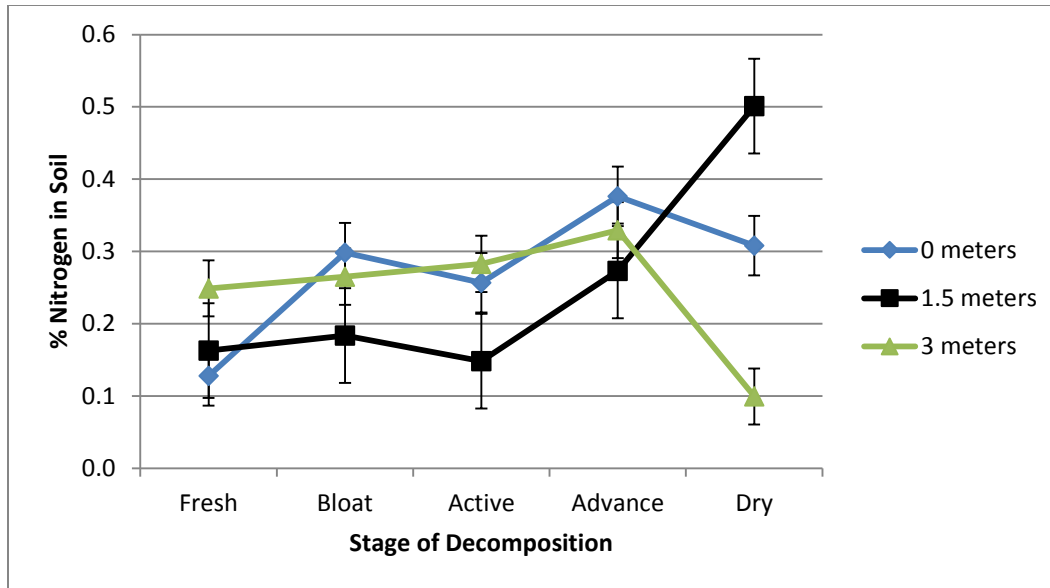


Fig. 2.14: Percent nitrogen in soil (\pm standard error) at each distance from pig heads and stage of decomposition. Nitrogen generally does not change over time (Spearman's rank correlation: $r_s = 0.48$, $P = 0.070$).

Discussion

When an animal dies, its body will be broken down by microbes, arthropods and vertebrate scavengers. Chemical compounds and nutrients from the carrion will be cycled into the environment and made available to other organisms (Petersen and Luxton 1982, Dent et al. 2004, Carter et al. 2010).

Carrion insects have been extensively studied to understand successional patterns and species interactions for use in forensic cases, such as poaching and homicide (Anderson 1999, Anderson 2010). Carrion insect activity increases in the beginning stages of decomposition, with the peak of activity when the most nutrients are readily available (Benbow et al. 2016). As carrion insects are attracted to the carrion, it is no surprise that abundance, species richness, and diversity in the current study were greatest at the carrion site (Fig. 2.3, 2.6, 2.9, Table. 2.1).

Carrion-associated arthropods are often used to determine how long an animal has been deceased

(Benbow et al. 2016); this is because of their predictable successional patterns related to the stage of decomposition (Payne 1965). This was represented in the results, as abundance and species richness changed over time (Table 2.1), with abundance being greatest at the Bloat and Advanced stages of decomposition (Fig. 2.3) and richness being greatest at Advanced decomposition (Fig. 2.6). Diversity, however was not affected by stage of decomposition (Table 2.1). Due to the competition for resources at the carrion site, the species associated with early decomposition are often replaced by those associated with late decomposition, so diversity does not change even though the dominant species are different (Peschke et al. 1987).

Carrion placed in different habitats will be exposed to different arthropod species that were present prior to exposure. Not many studies have been conducted on the impact carrion can have on these non-carrion-associated arthropods or whether they can give insight to the time the carrion has been exposed. Terrestrial isopods and field crickets are often found using carrion as shelter like they would a wet log, and successional patterns cannot be derived from these arthropods (Rivers and Dalhem 2014b). However, it is still important to investigate these arthropods and effects carrion has on them, as location and weather conditions can have an impact on the arthropods present and the overall behavior of the adventitious community living in leaf litter (Wolff et al. 2001).

It was predicted that adventitious arthropod communities would benefit from the carrion not just by utilizing it as protection, but from the nutrients provided. Carrion produces five times the amount of soil nitrogen as does leaf litter (Swift et al. 1979, Moore et al. 2004). This was not the case, as nitrogen did not significantly increase over time; however, organic matter, which contains nitrogen, did see a significant increase over time (Table 2.2). This increase in soil nutrients should reduce competition among arthropods and support greater abundance and

diversity. This hypothesis was supported, as stage of decomposition affected non-carrion-associated abundance, species richness, and diversity (Table 2.1). Abundance and diversity were homogeneous at all distances from the carrion, suggesting nutrients were dispersed relatively far from the carrion site, and increased primarily at Advanced decomposition, while diversity continued to increase into Dry decay (Figure 2.4, 2.10). Nutrients being dispersed far from the carrion site was not expected, as they should be more concentrated at the source (0m). Species richness was greatest at 3m from the carrion site and peaked at the Advanced stage of decomposition (Fig. 2.7). Organic matter (%) generally increased over time (Table 2.2); however, when analyses focused on particular distances from the carrion, organic matter increased over time only at 3m from the carrion (Fig. 2.11 Table 2.2). This is unusual, as the significant increase over time should be seen at the carrion site, which is the source of nutrients. It is unclear why increased soil organic matter would generate an increase in species richness but not clearly affect abundance or diversity. Probably other factors (e.g., attraction of predators, changes in environmental conditions) that were not a part of this study contributed to the overall successional pattern.

Total arthropod abundance was driven primarily by carrion-associated arthropods, suggesting that carrion will introduce a large number of individuals that would not typically be found in the area (Table 2.1, Fig 2.2). Changes in species richness and diversity were driven by both carrion-associated and non-associated arthropods, suggesting that carrion-associated arthropods do not impact the overall species richness of the community significantly. However, diversity of carrion-associated species was greatest at the carrion site (0 m) and did not vary significantly over time (species richness was consistent over time), while non-carrion-associated

species diversity increased during late decomposition, most likely due to an increase in species richness, rather than evenness (Table 2.1, Fig. 2.5, 2.8).

Soil nutrient content such as pH, nitrogen, and carbon, did not see a significant increase or decrease over time (Table 2.2, Fig. 2.12-2.14). This may result from small sample size due to a limited budget. Soil samples were taken only at the start of each stage of decomposition; if soil samples were taken more frequently, carbon and nitrogen may have produced a significant increase over time as organic matter did.

In conclusion, carrion has a strong impact on non-carrion-associated community composition. Arthropods are utilizing the readily available nutrients from the carrion to allow them to thrive. By better understanding the impact carrion has on non-carrion-associated arthropods, carrion ecology develops a holistic understanding of decomposition, rather than focusing solely on arthropods that are attracted to the carrion. Viewing decomposition as a process that exists in a vacuum excludes potentially useful information predicting behavior. For this reason, when studying decomposition, everything that is potentially affected by carrion should be investigated.

3. Behavioral Response of *Nasonia vitripennis* (Hymenoptera) When Exposed To Different Species of Carrion

Introduction

Hymenopteran parasitoids often do not find suitable hosts at the sites where they emerge as adults and often mate; instead, females interact with their environment to locate hosts in which to oviposit (Roitberg et al. 2001). This process occurs in four steps: the female must first locate the host's habitat, then find the host, accept the host, and as larvae (and sometimes adult females), regulate the host's growth and development (Brodeur and Boivin 2004, Vinson and Iwantsch 1980, Voss et al. 2009, Roitberg et al. 2001). Females will often locate the host's habitat using visual, vibrational, and tactile cues; however, the most important mechanism involves semiochemicals (Meiners et al. 2003, Rains et al. 2004; Vinson 1976, Fellowes et al. 2005), which are detectible at long ranges (Voss et al. 2009). This four-step process allows females to maximize their fitness by searching for the most suitable host, and is genetic by nature, as it is passed down to subsequent generations (Carton et al. 1986, Roitberg et al. 2001).

Nasonia vitripennis is a parasitoid wasp. Parasitoid wasps lay eggs in the larvae of other insects, such as caterpillars and fly larvae, for their offspring to feed on until they develop into adults, killing their hosts, unlike other parasites that do the least damage possible to the host (Rivers and Dalhem 2014a; Godfray 1994). Primarily parasitizing fly larvae, *N. vitripennis* typically uses hosts from the families Sarcophagidae, Calliphoridae, and Muscidae (Saunders 1966, Whiting 1967). Classified as an ectoparasite (residing on the outer cuticle or "skin" of the fly larvae), *N. vitripennis* infects hosts during their pupation (Godfray 1994).

Frederickx et al. (2014) examined the mechanism of habitat location in *N. vitripennis*. In this study, females were placed in a Y-tube olfactometer and given a choice between several

options: pork meat alone, pork meat that had flies being reared on it, and pork meat that had reared flies and had the flies removed prior to the test. Female *N. vitripennis* were attracted to the meat only on day four, and only to the meat that did not come in contact with fly larvae. On day eight of decomposition, the meat elicited a repulsive behavior (Frederickx et al. 2014).

Furthermore, electroantennography (EAG) revealed that there were three chemical compounds that stimulated the olfactory nerve of *N. vitripennis* (butanoic acid, butan-1-ol, and dimethyl disulfide). Dimethyl disulfide, however, was the only compound to elicit an attractive response.

Cablk et al. (2012) investigated potential risks in the training of cadaver dogs that used pig remains to train for locating human remains at crime scenes, based on the belief that dogs have the potential to differentiate human remains from those of other animals. Because dogs have a strong olfactory sense, Cablk et al. (2012) used solid-phase microextraction, gas chromatography/mass spectrometry to compare three meat types (beef, chicken, and pork), then compare them to existing data on humans. The chemical profile of volatiles produced from human remains was more similar to chicken than pig, which is the most commonly used non-human surrogate in carrion studies (Cablk 2012).

Like canines, *N. vitripennis* has an acute olfactory sense. Because *N. vitripennis* depends on this sense for reproductive fitness, it is likely that differences in chemical cues between carrion species can be detected. The objective of this study is to determine if *N. vitripennis* is able to differentiate between different species of carrion, and, if so, which carrion species is preferred. If there is a preference, or difference in behavior of *N. vitripennis* between species of carrion, using other species of carrion in addition to pigs for research to determine insect behavior and succession should be considered.

Methods

Live cultures:

Nasonia vitripennis were kept in one culture vial as pupae and kept at 20°C. Drops of 3% sucrose solution were given daily to feed adults as they emerged. When adults emerged, they were moved to individual vials. Thereafter, they were maintained on 3% sucrose solution every other day. To prevent any semblance of a reward system, feeding occurred only on days when wasps were not exposed to carrion. Cultures were kept in a fume hood to prevent exposure to volatiles from decomposing meat and other sources.

Carrion cultures:

Beef, pork, and chicken were purchased from a local grocery store. Meats were organic, antibiotic free, and grass-fed or cage-free. All meats were kept in separate containers in individual resalable bags to prevent contamination of bacterial cultures and to keep odor down. To prevent the advancement of decomposition before the experiment, all meats were stored frozen and thawed immediately before being placed in the incubator. All meats were incubated at 25°C for a total of 8 days.

Set-up:

On days 2, 4, 6, and 8, behavior of *N. vitripennis* was monitored by using a standard Y-tube olfactometer. Individuals were placed in vials that measured 4.25 cm in length and connected to a Y-tube connector so the entrance to the Y-tube connector and the opening of the vial were flush. On each branch of the Y-tube connector, 1.27-cm silicon tubing was connected, using Parafilm to create a seal. For each trial of the study, a vial containing meat was attached to

one randomly selected branch of the Y-tube. On the other branch, a new and clean empty vial was attached as a control. The main branch of the constructed Y-tube was 4.25 cm in length, with each branch measuring 10 cm from the Y-tube to the vial (Fig. 3.1).

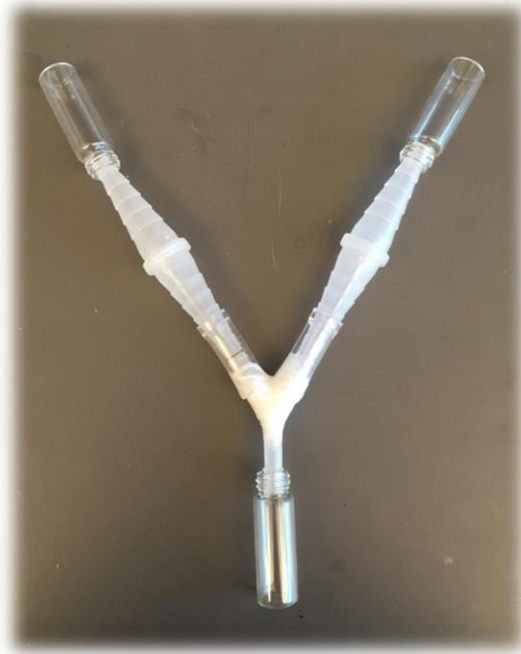


Fig 3.1: Y-Tube olfactometer constructed of vials, silicone tubing, and tube connectors. Parafilm was used to seal any creases.

Tests:

Three tests (one test for each meat type) were conducted on days 2, 4, 6, and 8. For each test, 32 trials were conducted. For each trial, one female *N. vitripennis* was placed in the main vial and connected to the Y-tube connector. The wasp was allowed 10 min. to make a decision; if it did not enter the Y-tube in that time, it was considered not to have made a choice. If an individual did make a choice between the control or the carrion, it was allowed only to enter the

linear tube connector, never reaching the vial. All tests were conducted in a fume hood to prevent external air from impacting results.

At the end of each trial, the apparatus was deconstructed and cleaned. All tubing was soaked in 70% ethanol to kill any bacteria and eliminate any volatiles that might be present. After being soaked, materials were rinsed in water and dried prior to use in another trial. In most cases, an individual *N. vitripennis* was used only once per test. The exception was toward the end of the study, when 6 individuals died before use, and previously used individuals were needed to complete the study. The few individuals that were reused were randomly selected.

Statistical Analyses:

Chi-square tests of independence (i.e., contingency tables) were used to detect whether a particular species of carrion was more attractive or repulsive than the other carrion species on the same day. The analysis was performed in three ways. The first was the standard approach for similar studies: individuals that did not enter the Y-tube olfactometer in 10 min. were excluded. The second approach was an attempt to determine whether including individuals who were non-choosers (those who did not enter the Y-tube olfactometer in 10 min.) could provide additional information on *N. vitripennis* behavior. For this analysis, non-choosers were considered to be repulsed by the carrion. The third analysis considered only whether individuals made a choice (entered the olfactometer, regardless of their choice thereafter) or not (completely repulsed).

Results

Chi-Square tests of independence (Table 3.1) indicated that there were days that elicited nonrandom behavior of *N. vitripennis*. The particular day depended on how data were analyzed. If individuals that did not enter the olfactometer were excluded from analyses or were treated as

repulsed, nonrandom behavior was observed on day 4. When the analysis compared the proportion of individuals that entered the Y-tube olfactometer (regardless of which choice they ultimately made) to the proportion of individuals that did not, there was nonrandom behavior on day 2 (Table 3.1).

Table 3.1: Chi-Square tests of independence to evaluate behavioral choices of *N. vitripennis*. Bold type indicates statistical significance.

	Day 2			Day 4			Day 6			Day 8		
	N	χ^2	P (2-tailed)	N	χ^2	P (2-tailed)	N	χ^2	P (2-tailed)	N	χ^2	P (2-tailed)
No Choice Excluded	44	0.18	0.913	50	8.85	0.012	48	4.52	0.104	54	1.92	0.384
Attracted vs. Repulsed	96	1.86	0.395	96	9.56	0.008	96	2.40	0.301	96	1.91	0.385
Choice vs. No Choice	96	9.15	0.010	96	2.59	0.274	96	1.75	0.417	96	0.25	0.881

No Choice Excluded

Nasonia vitripennis behavior when individuals who did not enter the olfactometer were excluded from analyses was significantly nonrandom on Day 4 (Table 3.1). On Day 4, individuals were attracted to pork significantly more than to any other meat type. However, when in the presence of chicken, *N. vitripennis* approached significance for repulsive behavior on Day 4 (Fig. 3.2, Table 3.2).

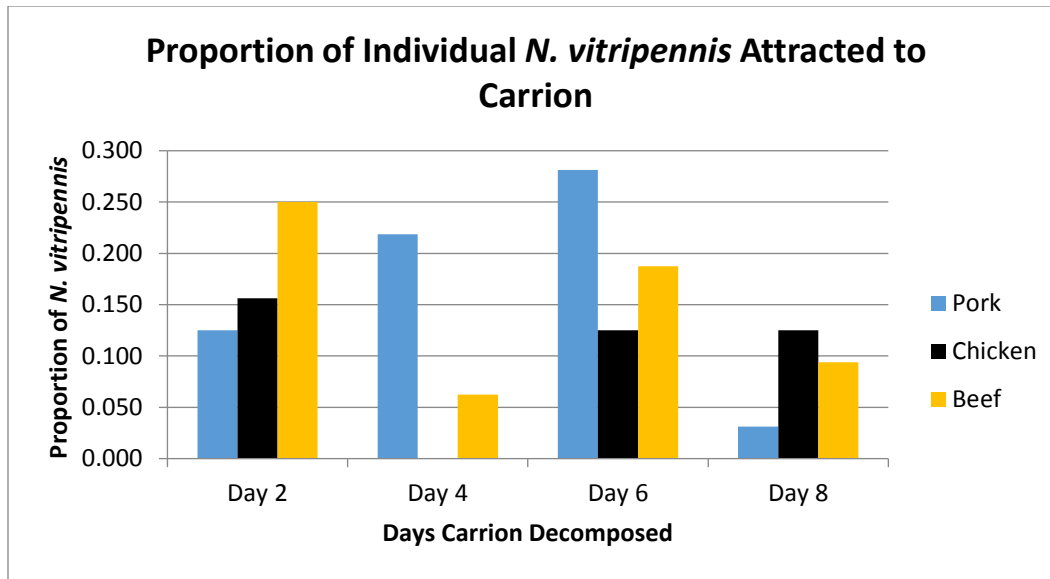


Fig. 3.2: Proportion of individual *N. vitripennis* attracted to carrion species over time. For this analysis, individuals that did not make a choice within 10 min. were excluded.

Table 3.2: Raw counts and standardized residuals of Chi-Square tests of independence for *N. vitripennis* repulsed by (1) and attracted to (2) carrion species. Individuals that did not enter olfactometer excluded.

No Choice Excluded		Day 2			Day 4			Day 6			Day 8		
		1	2	Total	1	2	Total	1	2	Total	1	2	Total
Beef	Count	13	8	21	17	2	19	12	7	19	14	3	17
	Standardized Residual	0.0	0.0		0.4	-0.8		0.3	-0.3		-0.1	0.3	
Chicken	Count	9	5	14	13	0	13	11	4	15	15	4	19
	Standardized Residual	0.1	-0.2		0.7	-	1.5	0.8	-0.9		-0.3	0.7	
Pork	Count	5	4	9	11	7	18	5	9	14	17	1	18
	Standardized Residual	-0.2	0.3		-1.0	2.1		-	1.3		0.4	-1.0	
Total Count		27	17	44	41	9	50	28	20	48	46	8	54

Attracted vs. Repulsed

Nasonia vitripennis behavior when attracted or repulsed by carrion species over time, considering individuals who did not enter the olfactometer to be repulsed by carrion, was significantly nonrandom on Day 4 (Table 3.1). On Day 4, individuals were repulsed by pork

more so than by any other meat type (Fig. 3.3c, Table 3.2). However, when in the presence of chicken, *N. vitripennis* approached significance for repulsive behavior on Day 4 (Fig. 3.3b, Table 3.3).

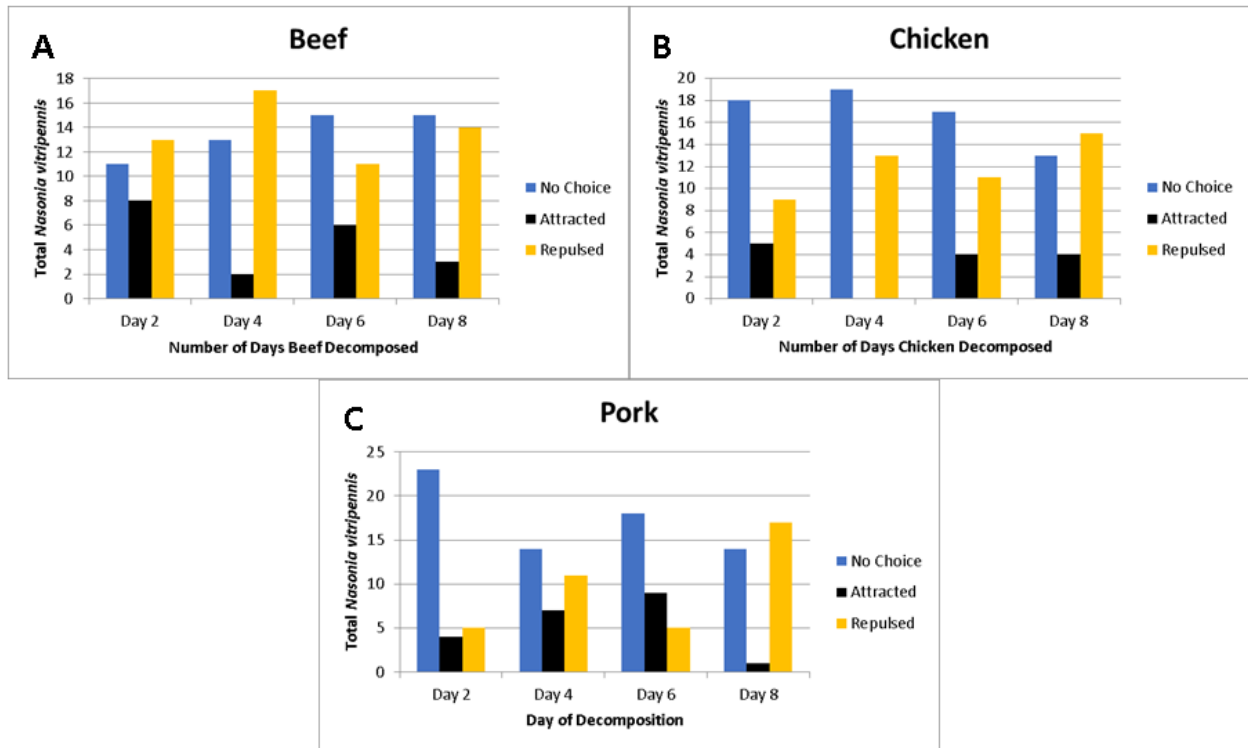


Fig. 3.3: Number of *N. vitripennis* repulsed by or attracted to (A) beef, (B) chicken, and (C) pork at 2, 4, 6, or 8 days of decomposition.

Table 3.3: Chi-Square Contingency Table for *Nasonia vitripennis* repulsed by (1) and attracted to (2) carrion species. All individuals including those that did not enter olfactometer included and considered to be expressing repulsive behavior (1).

Attracted Vs. Repulsed		Day 2			Day 4			Day 6			Day 8		
		1	2	Total	1	2	Total	1	2	Total	1	2	Total
Beef	Count	8	24	32	2	30	32	7	25	32	3	29	32
	Standardized Residual	1.0	- .5		- .6	0.2		0.1	- .1		0.2	-1.0	
Chicken	Count	5	27	32	0	32	32	4	28	32	4	28	32
	Standardized Residual	- .3	0.1		-1.7	0.6		- 1.0	0.5		0.8	- .2	
Pork	Count	4	28	32	7	25	32	9	23	32	1	31	32
	Standardized Residual	- .7	0.3		2.3	- .7		0.9	- .5		-1.0	0.3	
Total Count		17	79	96	9	87	96	20	76	96	8	88	96

Choice vs. No Choice

When the analysis compared the proportion of individuals that entered the Y-tube olfactometer (regardless of which choice they ultimately made) to the proportion of individuals that did not, there was significant nonrandom behavior on Day 2. Although the overall test was significant, standardized residuals revealed no definitive significant behavior for *N. vitripennis*. Beef and pork, however, elicited behavior that approached significance (Table 3.4). Individuals appeared more likely to enter the olfactometer to make a choice for beef and less likely to enter the olfactometer for pork (Fig. 3.4, Table 3.4).

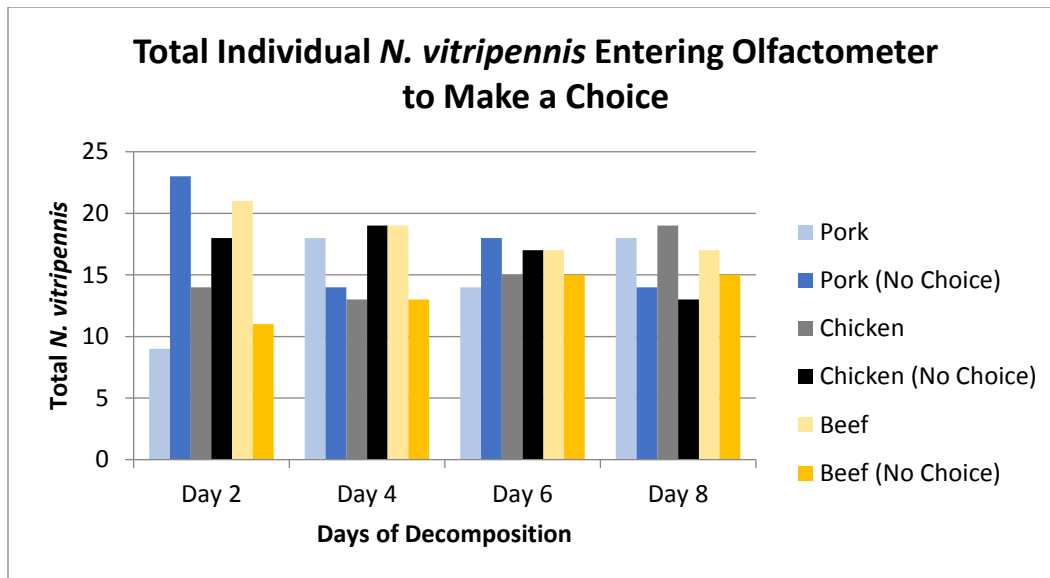


Fig. 2.4: Number of *N. vitripennis* entering or failing to enter the olfactometer to make a choice in the presence of different carrion species over time.

Table 3.4: Chi-Square tests of independence for *N. vitripennis* that entered the olfactometer to make a choice (1) or did not enter the olfactometer (2), for three carrion species at various days of decomposition.

Choice Vs. No Choice		Day 2			Day 4			Day 6			Day 8		
		1	2	Total	1	2	Total	1	2	Total	1	2	Total
Beef	Count	21	11	32	19	13	32	19	13	32	17	15	32
	Standardized Residual	1.7	-1.5		0.6	-0.6		0.8	-0.8		-0.2	.3	
Chicken	Count	14	18	32	13	19	32	15	17	32	19	13	32
	Standardized Residual	-0.2	0.2		-0.9	0.9		-0.3	0.3		.2	-0.3	
Pork	Count	9	23	32	18	14	32	14	18	32	18	14	32
	Standardized Residual	-1.5	1.4		0.3	-0.3		-0.5	0.5		0.0	.0	
Total Count		44	52	96	50	46	96	48	48	96	54	42	96

Discussion

Different species of carrion, as they decompose, produce different suites of chemical compounds, which can potentially affect the ability of animals sensitive to these cues to locate the carrion site and the degree of attraction they exhibit toward the carrion (Cablak et al. 2012).

Parasitoid wasps use chemical cues originating from carrion to locate hosts in a process referred to as host-habitat location (Frederickx et al. 2014, Brodeur and Boivin 2004, Vinson and Iwantsch 1980, Voss et al. 2009). Butonic acid, Butan-1-ol, and Dimethyl disulfide (DMDS) are stimulating to *N. vitripennis*, but only DMDS clearly attracts the wasps (Frederickx et al. 2014). DMDS is attractive to several other carrion insects, including carrion beetles and carrion flies (Podskalska et al. 2009). The particular compounds present differ between different species of carrion. For example, in a study comparing chicken, pork, and beef, 3-methyl-butonic acid was found in fresh and decomposed chicken and pork, whereas DMDS was found in all carrion at all stages of decomposition (Cablak et al. 2012, Dekeirsschieter et al. 2009). Due to the differences in chemicals among carrion species, it can be hypothesized that *N. vitripennis* and other carrion insects, such as carrion flies and beetles that are attracted to similar chemicals, such as DMDS, will all behave differently in the presence of carrion species with varying chemical compounds.

It is hypothesized that butonic acid is a cofactor for dimethyl disulfide, creating a stronger affinity in the binding to the neuro-receptors of *N. vitripennis*. Butan-1-ol is the decomposed form of butonic acid, present after the fresh stage of decomposition (Dekeirsschieter et al. 2009), missing a carbonyl group and one oxygen. It may bind to the neuro-receptor, blocking DMDS from binding, thereby preventing an attractive behavior from occurring. This would be advantageous for *N. vitripennis*, as by the end of the fresh stage of decomposition, carrion flies would already have laid their eggs. Especially in hot, humid environments, in which larvae develop rapidly through instars (Wall et al. 1992), this could lead to a missed opportunity to seek out suitable hosts. However, there are no current studies to support this hypothesis. If it is true, different carrion species are likely to elicit different behaviors in *N. vitripennis*.

In Frederickx et al. (2014), *N. vitripennis* was observed to be significantly attracted to pork on the fourth day of decomposition. This was also observed during the current study when individuals that had not entered the olfactometer were excluded from the data. *Nasonia vitripennis* was attracted to pork significantly more so than to other carrion species on the fourth day of decomposition (Tables 3.1 and 3.2, Fig. 3.2).

There is an inherent flaw in the design and analysis of choice experiments using Y-tube olfactometers. In a typical Y-tube olfactometer study, the experimental subject is placed in the main arm of the olfactometer (the “stem” of the Y), and a potential attractant is placed in each of the other arms. For example, in the current study, the attractants were meat of various types and stages of decomposition compared to a control (no stimulus). To express a repulsed behavior, the individual would first have to approach the Y junction (i.e., move *toward* the meat), then be repulsed and make the decision to enter the control. However, many individuals remained toward the back of the main arm, not attempting to approach the Y junction. The typical protocol is to exclude these individuals from analysis (e.g., Frederickx et al. 2014, Kogel et al. 1999). In contrast, individuals that did approach the Y junction would take a moment before making the choice to go to the control or the carrion. The behavior to not approach the junction at all seems more like a repulsive behavior than to approach the junction, seemingly curious about the carrion to see if it was suitable, then deem it not so and select the control. This has important implications for the interpretation of results.

To test whether there was an impact on the results if the non-choosers were considered repulsed, they were categorized with the repulsed individuals against those who were attracted to the carrion. During this analysis, a significant behavior was again expressed on day 4; however, this analysis suggested that pork was more repulsive than the other carrion types (Table 3.1 and

3.3, Figure 3.3) , as opposed to more attractive, as it was when individuals that did not enter the olfactometer were excluded from analyses. The protocol for the Y-tube olfactometer should be reconsidered for this reason. Results can be completely altered when data are excluded, potentially leading to false conclusions about insect behavior.

Different carrion types affected whether *N. vitripennis* chose to enter the olfactometer. Individuals were more likely than expected by chance to enter the olfactometer on day 2 in the presence of beef and less likely than expected by chance to enter the olfactometer in the presence of pork on the same day (Table 3.1 and 3.4, Figure 3.4).

Other productive future studies would be to determine whether different bacterial colonies are driving *N. vitripennis* behavior. Different bacterial colonies will be dominant on different carrion species and in different habitats, which may provide additional chemical cues to attract or repel *N. vitripennis*. *Cochliomyia hominivorax*, the new world screwworm, is a host species of *N. vitripennis*. Studies suggest that contamination with gram-negative bacteria is a prerequisite for *C. hominivorax* attraction and oviposition (DeVaney et al. 1973, Eddy et al. 1975).

In conclusion, *N. vitripennis* exhibits different responses to different carrion and on different days of decomposition. Because *N. vitripennis* uses host-habitat location tactics such as chemoreception that is sensitive to the same compounds as its host (Godfray 1994), studies should be conducted on carrion flies to determine if female carrion flies express similar behaviors. Dekeirsschieter et al. (2013) used a Y-olfactometer to determine if different chemical compounds were attractive to blow flies and determined of all compounds tested, DMDS was the strongest attractant, similar to the Frederickx et. al (2014) study. However, there are no published

studies on how different species of carrion elicit different behaviors. Future studies should be conducted to see how different carrion species affect other important carrion species and if community structure or insect succession are impacted because of it.

4. Conclusion

The purpose of this thesis was to determine how chemical mediators and nutrients that are released into the environment during carrion decomposition affect the community ecology and behavior of arthropods. Studies have been done to examine carrion-associated insects to a great extent; however, little has been done to observe behavior and ecology of non-associated arthropods.

In a constantly adapting field that is applied to legal cases such as homicide and poaching, these studies can aid in solving problems that have not been addressed previously. This includes how different species of carrion can affect insect behavior and succession. Insects that are attracted to one species may not exhibit the same behavior for all species of carrion. This is important in homicide and poaching cases, as the absence of a species in a recreated scenario, that is found at a crime scene, may alter the rate of decomposition, and therefore estimates of time of death, by several hours. For these reasons, researchers place carrion in a variety of environmental scenarios (e.g., inside houses, under porches). Behavior of a parasitoid wasp, *N. vitripennis*, suggests that the species of carrion utilized in these studies may be as important as the environmental context. Therefore, studies on other species of carrion besides pigs will be necessary.

There is also an issue that studies revolving around decomposition are not widespread geographically. There are very specific locations that decomposition research is done, such as Australia, Netherlands, and the United States (Texas, Alaska, and Tennessee), to name a few. Legal authorities rely on this research to provide justice. If research has not been conducted in the area in which a crime has been committed, this creates a potential for information to be

missed and someone to get away with a crime or, worse, be charged with one they did not commit.

There is much to learn about carrion ecology, and this research is used for important, practical applications. However, it is important to remember that overall community studies are just as important in understanding entomology in a forensic context. This study has contributed to the field by developing a better understanding of the effects carrion decomposition has on the non-associated arthropod community. Non-carrion-associated arthropods benefit from the introduction of organic material that is made available during later stages of decomposition. Future studies should look at a variety of different locations, such as open fields or urban sites, to determine if the same results apply. It was also found that insect behavior will change in the presence of different carrion species, calling into question whether utilizing different carrion species to compare community composition for comparison is useful in a legal setting. Future studies should investigate whether this same difference in behavior is seen with carrion insects that are used to determine the postmortem interval, because of the potentially important legal ramifications of these differences in behavior.

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Appendix:

Table A1: All species found in leaf litter and stage of decomposition they were present. Individuals were identified to the lowest taxonomic group identifiable, if species name could not be determined, morpho-species was created.

Species	Associated (A)					
	Non-Associated (N)	Fresh	Bloat	Active	Advanced	Dry
Acari	N		x	x	x	x
Achatinoidea	N			x	x	
<i>Aethina</i>	N		x		x	
Agelenidae	N				x	x
Agelenidae 1	N					x
Amaurabiidae	N		x		x	x
Amaurabiidae 1	N				x	
<i>Anthocoris</i>	N		x	x	x	x
Araneidae 1	N		x		x	
Araneidae 2	N				x	x
<i>Blaniulus guttulatus</i>	N	x	x	x	x	x
<i>Blatta orientalis</i>	N				x	x
<i>Blattaria</i> 1	N				x	
<i>Blattaria</i> 2	N					x
Braconidae 1	N				x	
Braconidae 10	N				x	
Braconidae 2	N				x	
Braconidae 4	N				x	
Braconidae 5	N				x	x
Braconidae 6	N				x	
Braconidae 7	N				x	
Braconidae 8	N				x	
Braconidae 9	N				x	
<i>Calopteron terminale</i>	N		x	x	x	x
<i>Camponotus pennsylvanicus</i>	N	x			x	x
<i>Catacala antinympha</i>	N	x				
<i>Cheiracanthium</i>	N	x			x	
<i>Cheliosia caerulescens</i>	A			x	x	
Chironomidae	N				x	x
Cicadellidae	N			x		
Cicadellidae 1	N				x	
Cicadellidae 2	N				x	
Cicadellidae 3	N				x	
Coleoptera 2	N			x		

Species	Associated (A)					
	Non-Associated (N)	Fresh	Bloat	Active	Advanced	Dry
Coleoptera L2	N				x	x
Coleoptera L5	N		x			
Collembola	N	x	x	x	x	x
<i>Corpis minutus</i>	N	x				
<i>Creophilus maxillosus</i>	A		x	x	x	x
Culcidae	N				x	
Culicidae 1	N		x			
Culicidae 2	N		x			
Curculionoidea	N			x	x	x
Cydnidae	N				x	
Diptera	A		x	x	x	
Elateridae	N	x	x	x	x	x
<i>Erynis baptisiae</i>	N	x				
Formicidae	N	x				
Geophilomorpha	N		x		x	x
Gnaphosidae	N				x	
Gryllinae	N				x	
<i>Haylomorpha halys</i>	N				x	
<i>Hister furtivus</i>	A		x		x	
<i>Ixodes scapularis</i>	N	x	x			
<i>Lephtothorax affinis</i>	A		x	x	x	x
Lepidopetra 3	N				x	
Lepidoptera 1	N		x			
Lepidoptera 2	N				x	
Lepidoptera 4	N				x	x
Lepidoptera 5	N					x
Lepidoptera 6	N					x
<i>Leptoglossus occidentalis</i>	N			x		
Limacodidae	N				x	x
Lyctidae	N				x	
<i>Malacosoma americanum</i>	N				x	
Megadrilacea	N		x	x	x	
<i>Megaselia scalaris</i>	N			x	x	
<i>Melolontha melolontha</i>	N	x				
Miridae	N			x		
<i>Necrobia rufipes</i>	A		x			
<i>Necrophila americana</i>	A	x		x	x	
Nitidulidae	A		x	x	x	x
<i>Oiceoptoma novaboracense</i>	A		x	x	x	
Oniscidea	N			x	x	x
<i>Operophtera bruceata</i>	N	x			x	

Species	Associated (A)					
	Non-Associated (N)	Fresh	Bloat	Active	Advanced	Dry
Pisauridae	N				x	x
Pisauridae 1	N				x	x
<i>Plodia interpunctella</i>	N	x	x	x	x	x
<i>Podisus maculiventris</i>	N	x			x	x
Pseudoscorpiones	N		x		x	x
Psocoptera	N				x	x
Pseudoscorpiones	N	x	x	x	x	x
<i>Pterosichus melanrius</i>	N		x			
<i>Ptomaphagus</i>	A		x			
Reduviidae	N				x	
Rhopalocera 1	N		x			
Rhopalocera 2	N					x
Salticidae	N				x	x
Salticidae 1	N		x		x	
Scarabaeidae	N				x	
Scarabaeidae 1	N		x			
Segestriidae 1	N			x		
Sicariidae 1	N				x	
Sicariidae 2	N					x
Staphylinidae	N		x	x	x	x
<i>Stelidota octomaculata</i>	A				x	
<i>Stenolophus comma</i>	N		x	x	x	x
Tetragnathidae 1	N					x
Tetragnathidae 2	N		x		x	x
Theridiidae	N		x		x	x
Thomisidae	N		x	x	x	x
Thysanoptera	N			x	x	x
<i>Xestia smithii</i>	N	x	x	x	x	x
<i>Xysticus elegans</i>	N		x			
Zoropsidae 1	N			x	x	x
Zoropsidae 2	N					x
Zoropsidae 3	N					x