RESPONSE OF SILPHIDAE TO BURNED CARCASSES AND USE FOR BREEDING BY BURYING BEETLES AND FLIES

By

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RESPONSE OF SILPHIDAE TO BURNED CARCASSES

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BY BURYING BEETLES AND FLIES

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Abstract: When an animal dies, the resources in its body are used by a range of organisms including bacteria, fungi, invertebrate and vertebrate necrophores. Among these organisms, burying beetles in the Genus *Nicrophorus* are specialized to detect the carcass soon after death, bury it if it is an appropriate size, and use biparental care to rear offspring in an underground brood chamber. Many factors can cause the death of an animal, including wildfires and prescribed burns, but the effects of burned bodies on response and utilization of insect necrophores, including burying beetles has been poorly studied. Self-sustaining populations of the threatened *Nicrophorus americanus* occur at Camp Gruber, a military training facility and wildlife management area, near Braggs, OK. Because of training activities and planned prescribed burns to manage vegetation, studies were conducted to understand how *Nicrophorus* species respond to fire and whether burned carrion would be used as a food and or reproductive source. Three separate five-day sampling studies were conducted from May to July. Hanging pitfall traps were baited with either unburned, slightly burned, moderately burned, or severely burned rat carcasses. All carrion beetles were identified and counted. In the laboratory, reproduction on burned mice was tested along with survival of beetles buried in soil that had a fire burn above it. *Nicrophorus*, including American burying beetles, used burned carcasses for food and reproduction was similar on slightly burned carcasses as unburned controls. Additionally, in a lab experiment house flies had higher reproductive succcess on moderate and severely burned carcasses, although differences were not statistically significant. Because burying beetles survived beneath the surface, and were capable of utilizing burned carcasses, prescribed fire is unlikely to harm American burying beetles, and potentially can benefit them. Flies can also use burned carcasses for reproduction and populations of flies and beetles should be monitored after wild fires and prescribed burns.

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LITERATURE REVIEW OF THE AMERICAN BURYING BEETLE, *NICROPHORUS AMERICANUS*, AND THE COMMON HOUSE FLY FAMILY, MUSCIDAE, CARRION BEETLES, FLIES, AND BURNED CARCASSES

Background: Carcass Succession

When a vertebrate animal dies, it undergoes five distinct stages of decomposition: fresh, bloated, active decay, advanced decay, and skeletonization/dry remains (Wolff et al., 2001). The length of each of these stages can vary, depending on many factors, including the environment in which the carcass is present (Haddadi et al., 2019). Upon death, different species of insects will visit and make use of a carcass. This is a phenomenon known as insect succession. Often, these visitations occur in a predictable order, and can even be used as a tool to estimate a corpse's time and place of death (Matuszewski, 2021).

The order of which insects visit a carcass depends heavily on the insect's feeding preferences, and for some species, reproductive requirements. Additionally, carrion use by insects is influenced by cues from the carcass itself through its microbial flora

(Tomberlin et al., 2011). The pattern of insect succession can be influenced by the state of the carcass itself, since insect succession differs depending on if a carcass is burned or not (Mashaly, 2015). Flies are often the first to arrive at a body, with extensive research showing that the family Calliphoridae is often the first, and arrives rapidly upon death (Mohr and Tomberlin, 2015). Although Calliphoridae colonize most carcasses, Muscidae have proven to be very capable of colonization and are deemed forensically important (Wolff et al., 2001; Grzywacz et al., 2017). House flies are found in a variety of places and can consume a wide range of things, from fruit to flesh (Scott et al., 2014).

Shortly after arrival of flies, beetles will visit the corpse. Among these beetle are carrion feeders belonging to the family Silphidae. There are two subfamilies within Silphidae: Silphinae and Nicrophorinae (Dekeirsscheiter et al., 2011). The subfamily Silphinae consists of the non-burying carrion beetles, including *Oiceoptoma noveboracense*, *Oiceoptoma inaquale*, *Necrodes surinamensis* and *Necrophila americana*. Unlike Nicrophorinae, Silphinae contains some flightless species that reproduce by laying eggs in the soil (Ikeda et al., 2008). However, the Silphinae species that are capable of flight tend to oviposit near carcasses (Ikeda et al., 2008).

Burying beetles belong to the subfamily Nicrophorinae, and the genus *Nicrophorus*, which feed on a range of small vertebrate carcasses, including mammals and birds (Bedick et al. 1999, 2004; Lomolino and Creighton, 1996). In addition to using the carcass as a food source, *Nicrophorus* will also potentially use the carcass to raise their offspring (Scott, 1998). After obtaining a suitable carcass for reproduction, burying beetles will bury the carcass underground (Ratcliffe, 1996).

Burying Beetle Background

Burying beetles are an essential part of nature's clean-up crew, and are found in temperate zones worldwide. As a whole, the Genus *Nicriophorus* is temperate and has been suggested to be outcompeted in tropical regions by other insects, including flies and ants (Sikes and Capinera, 2008). Burying beetles reproduce annually and typically reproduce in the summer months, with adults overwintering underground in the winter months. Both day active and night active species are present in the *Nicrophorus* genus. (Keller et al., 2019; Ratcliffe, 1996).

American burying beetles (ABB) are nocturnal habitat generalists that best tolerate forests and grasslands. The ABB, like other *Nicrophorus*, have prominent orange and black features that aid in their identification (Creighton and Schnell, 1998; Lindstedt et al., 2017). Along with being the largest *Nicrophorus* beetle species in North American, these beetles have an orange area on the pronotum, which is unique from their *Nicrophorus* counterparts (USFWS). This orange coloration is also found at the tips of their clubbed antennae, and as markings along their elytra, which contrasts with the black body (USFWS). Because of their large size, being approximately 8 cm long (1.5 inches), ABB utilize larger carrion, up to 300g, than their smaller *Nicrophorus* counterparts (Lomolino et al., 1995; Lomolino and Creighton, 1996).

Although specific carcass size is critical for reproduction, ABB will make use of both smaller and larger carcasses for feeding (Bedick et al. 1999, 2004). When carrion is unavailable, burying beetles are known to be insectivorous, and even cannibalistic (Bartlett, 1987).

Reproduction

Typically, upon finding a carcass, male ABBs will compete with each other and other attracted carrion beetles to "win" the carcass. Often, the successful beetle is larger in size than its competition. The successful beetle will then bury the carcass superficially to lay claim on it and secrete pheromones to attract a potential mate (Beeler et al., 1999). Surprisingly, even if a male does not have a carcass for his potential mate, he may still secrete pheromones to try to attract a mate (Beeler et al., 1999).

If the soil is unsuitable for burying, *Nicrophorus* will move the carcass to an area with suitable soil (Scott, 1998). Then, preparation of the carcass for reproduction begins. This is done by removing all the potential hair or feathers from the carcass, and then burying the carcass into the ground. To bury the carcass, *Nicrophorus* uses their heads to move soil, and this burying is done in order to reduce competition from other insect species including flies and ants that are attracted to vertebrate remains (Ratcliffe, 1996). After burying the carcass, the beetles then form the carcass into a ball-like shape and cover the entire carcass in secretions (Milne and Milne, 1976). Usually, these secretions are antimicrobial, and act as a preservation tool to ensure that the beetles and their soon-to-come offspring will have a food source.

***Nicrophorus* Host Shift**

Like other *Nicrophorus* species*, N. pustulatus* often uses small carcasses for reproduction. However, this species has undergone a host shift and can utilize living snake eggs for reproduction, making it a parasitoid of vertebrates (Smith et al., 2007). Currently, this is the only known Silphidae species with this capability. In laboratory studies, when presented with both a mouse carcass and a snake egg for reproduction, *N. pustulatus* would utilize both simultaneously for the same brood (Smith et al., 2007). Additionally, although most other burying beetles have antimicrobial activity in their oral secretions, *N. pustulatus* does not (Ayala-Ortiz et al., 2021).

Parental Care

Unlike most insect species, beetles in the *Nicrophorus* genus exhibit biparental care, with both parents are involved in preparation and maintenance of the carcass for their future young (Eggert and Muller, 1997). The females lay their eggs near the brood ball, and the hatched larvae migrate to the brood ball where they undergo three larval instar stages. During early stages, the adults feed their offspring by regurgitating carrion (Benowitz and Moore, 2016). Afterwards, the larvae pupate in nearby soil, and then emerge as teneral adults. The parental beetles also reemerge and persist in the environment, although the ability to raise a second brood in the wild has not been documented.

Normally, with burying beetle broods, the male parent will leave the brood chamber before the larvae finish developing, while the female will continue to tend to the larvae, eventually leaving as well (Ward et al. 2009). Females leave the brood chamber roughly one week after mating with the male (De Gasperin et al., 2015). Burying beetle parents are also known to reduce their brood size themselves if not enough resources are available. This reduction of offspring is done to ensure the success and size of the larvae that they can raise (Bartlett, 1987).

The extent of which both parents are involved in brood rearing depends on the species, with *Nicrophorus orbicollis* having some of the most involved parents in the *Nicrophorus* genus. Laboratory studies showed that the presence of both parents were integral in the overall fitness and number of offspring raised (Benowitz and Moore, 2016).

In contrast for some *Nicrophorus* species, including *N. vespilloides*, the presence of both parents is not necessary, and broods do not appear to be affected. In this small species, parental abandonment is a relatively common phenomenon (Ward et al., 2009). Often a single parent leaves early and it is thought to be capable of reproducing again at some point in the future (Ward et al. 2009). Usually, the male abandons the brood first, however, in laboratory studies when the female was removed, the males produced similar brood success rates as their single female counterparts (Ward et al., 2009).

Habitat

Nicrophorus americanus is classified as a habitat generalist, and is found in a variety of areas, but tend to occur away from human disturbance. Historically, the ABB was found across 35 states in the eastern half of the United States, along with parts of Canada. Now, the threatened American burying beetle only occupies approximately 10% of its historic range. Although many hypotheses for its decline have been advanced, including the use of pesticides, habitat loss, climate change, electric lighting, and loss of passenger pigeons (*Ectopistes migratorius*) and other appropriately sized vertebrate carcasses (Sikes and Capinera, 2008), currently, no consensus has emerged. Naturally occurring populations of ABB are found in Oklahoma, Kansas, Arkansas, Nebraska, South Dakota, and on an island off the shores of Rhode Island (USFWS).

Carrion Feeding Flies

The Dipteran family Calliphoridae is well-studied in the forensic sciences. Commonly known as blow flies, calliphorids are attracted to the odor of decomposing carcasses and are among the first to arrive at a fresh carcass (Anderson, 2019; Mohr and Tomberlin, 2015). Because of their near immediate arrival after death, blow fly larvae are used to estimate the post mortem interval (PMI) of a carcass, and this method is one of the few reliable techniques used to estimate a time of death for bodies that are undiscovered for more than 24 hours (Heaton et al., 2014; Mohr and Tomberlin, 2015).

Many factors influence the use of bodies by flies, including access (burial and wrapping in materials reduce flies on the body), temperature, moisture, and enclosing bodies in buildings or vehicles (Mohr and Tomberlin, 2015). Another factor that influences blow fly use of a body is previous exposure to fire.

Research has shown that exposure to fire may be favorable for some insects (Vanin et al., 2013); however, calliphorid oviposition was delayed by one day on carcasses subjected to extensive fire exposure. The amount of burning of a body is rated on the Crow-Glassman Scale and at level 3 and above, calliphorid use is reduced (Mahat et al., 2016). Carcasses subjected to less fire exposure did not have delayed oviposition, and calliphorid development was similar to carcasses untouched by fire (Mahat et al., 2016).

Muscidae Biology

Muscidae are Dipterans that are found worldwide, existing on every inhabited continent (Keiding, 1986). The family Muscidae encompasses many common species including house flies, stable flies, face flies, horn flies, etc. These flies serve vital roles as decomposers and are known to also be forensically important (Scott et al., 2014). However, Muscidae are much more difficult to identify to species level, leading to limited use to calculate PMI (Al-Shareef, 2016; Grzywacz et al., 2017). House flies are difficult to identify even as adults, so much of the identification stays either at the family or genus level (Grzywacz et al., 2017).

Like Calliphoridae, Muscidae are holometabolous. Female house flies lay 4-6 clutches of eggs with each clutch consisting of up to 150 eggs (Iqbal et al., 2014). Larva hatch from an egg and then develop through three separate instars (Grzywacz et al., 2017). After the third instar, Muscidae larvae “wander” away from where they originally hatched. Shortly after wandering, they begin pupation, with their pupa being dark and relatively large. The house fly’s lifecycle progresses faster if conditions are warmer (Kieding 1986).

Because female flies lack ovipositors or other ways to penetrate flesh, eggs are deposited at natural openings. This behavior causes large amounts of larvae to be concentrated in one area (Iqbal et al., 2014). Large concentrations of maggots in an area lead to a maggot mass, which can produce heat, thus accelerating decomposition. Although the maggot mass phenomena has been only well-documented in Calliphoridae species, it is likely Muscidae maggots are capable of this phenomenon as well (Heaton et al., 2014).

Muscidae are generally attracted to feces and are important in both abuse cases and forensic investigations when feces in present. In addition, Muscidae have been noted with a number of forensic cases involving burning of victims (Al-Shareef, 2016), especially when burning has been more severe. Often with severe burns, the abdomen ruptures, exposing feces which may be attractive to house flies.

Muscidae as Vectors of Disease

Species belonging to the family Muscidae are found wherever humans are present and are a prominent pest (Iqbal et al., 2014; Scott et al., 2014; Sukantason et al., 2006). Muscidae tend to feed on rotting matter where pathogens are frequently found (Iqbal et al., 2014; Scott et al., 2014; Sukantason et al., 2006). Because of their range of foods, along with presence near humans, Muscidae have become well known for their abilities to transmit pathogens mechanically on mouthparts, wings, legs, or the adhesive device located between the tarsi (Scott et al., 2014; Sukontason et al., 2006). Animals can carry a myriad of disease causing agents (van Doorn, 2013). Upon death, house flies are among the first insects to arrive to a carcass, and are thus exposed to these disease causing agents early on (Grzywacz et al., 2017).

Bacteria such as *E. coli*, *Enterococci*, *Shigella*, and *Salmonella* species can be carried by flies, leading to the spread of over one hundred human diseases (Macovei et al., 2008; Iqbal et al., 2014). While these pathogens don't stay with the fly forever, it was found that *Yersinia pseudotuberculosis* can stay in a house fly's digestive track for up to 36 hours (Zurek et al., 2001). The staying power of this human and animal pathogen suggests that other disease causing agents can stay with a fly for just as long or longer.

Rationale

Prescribed burning is a type of controlled burning done to a particular chosen area under specific conditions to achieve desired results (AFAC, 2012). Prescribed burning is not the same as a wildfire, since wildfires are not planned or managed in the same way that prescribed burns are (Morgan et al., 2020). When prescribed burning is implemented, it is a valuable tool that can help reduce the severity of wildfires while managing undesirable vegetation and increasing availability of nutrients (Ryan et al., 2013). However, when a site is picked to burn, if it's on federal land, an ecological survey of the area is required to assess if any protected species are present and is mandated by The National Environment Protection Act (Pilloid et al., 2003).

There is a self-sustaining population of ABB located near Braggs, Oklahoma, on Camp Gruber, a military training facility. Camp Gruber serves as a military training area as well as a wildlife management area (Schnell et al., 2014). Although there is a newly approved prescribed fire plan that requires assessment of ABB populations, historically, the area has had frequent fires that were started through training exercises and though the spread of wildfires. Questions included whether the carrion-feeding ABB would respond to burned carcasses as a food source, if carcasses could be used as a reproductive source, and whether surface fires would affect buried beetles.

Additionally, there is little information on the effects of burning on fly groups other than Calliphoridae that can be used in forensic cases. Muscidae are often attracted to feces and moist areas and could be competitors if attracted to burned carcasses.

OBJECTIVES

The objectives of this project were (1) to understand how burying beetles would respond to wildfires when buried in the soil and if they would use burned carcasses as a food and breeding resource, (2) to investigate development of house flies on burned carcasses.

CHAPTER II

THE EFFECTS OF FIRE ON *NICROPHORUS AMERICANUS*

ABSTRACT

The threatened American burying beetle (ABB), *Nicrophorus americanus*, has a self-sustaining population within Camp Gruber a National Guard training facility. Camp Gruber is near Braggs, OK that acts as both a military training base and wildlife management area. Because of its purpose, accidental and incidental fires often occur at Camp Gruber. To better understand how ABB respond to animals burned by wildfire, tests were conducted to determine if *Nicrophorus* species would utilize burned carrion for both food and reproductive purposes. In addition, survival of buried beetles was measured when exposed to a fire. Three treatments containing rats that were slightly, moderately, or heavily burned were set at Camp Gruber for three five-day sampling cycles. ABB, along with other *Nicrophorus* species, were captured using every level of burned carrion, with ABB showing preference towards severely burned rats in May. *Nicrophorus* use of burned carcasses was tested in 28 breeding chambers, with 7 mice for each burn level. *Nicrophorus orbicollis* were able to use the moderately burned carcasses,

as well as the severely burned carcasses, produced much fewer offspring. Reproductive success and number of offspring between the control mice and slightly burned mice were comparable. To test *Nicrophorus* survival while burned, *Nicrophorus carolinus* were placed in jars filled with moistened peat moss and allowed to settle for approximately 24 hours. The jars were then placed under a fire table, where a straw-fueled fire burned directly above the jars. Out of 24 beetles tested, only one died. These data show that prescribed and accidental fires are unlikely to harm inactive beetles directly and may benefit *Nicrophorus* by providing vertebrate carrion for food and reproduction.

INTRODUCTION

Burying beetles are carrion feeding beetles within the family Silphidae, which contains two subfamilies, Silphinae and Nicrophorinae (Dekeirsschieter et al., 2011). Currently, there are approximately 200 species within Silphidae (Sikes and Capinera, 2008). The Nicrophorinae contains one genus *Nicrophorus*, which are burying beetles. Small vertebrate carcasses (<300 grams) serve as both a food and reproductive source for *Nicrophorus* species, and because of this, *Nicrophorus* are more likely to be found in areas with larger numbers of vertebrates (Lomolino and Creighton, 1996; Schnell et al., 2014). Carcass size is crucial for reproduction, with *Nicrophorus* species using different sizes of mammals and birds. *Nicrophorus* will feed on carcasses of all sizes (Bedick et al. 1999, 2004; Lomolino and Creighton, 1996).

Nicrophorus are called burying beetles because of their reproductive method of burying a small vertebrate carcass underground. Before burying, *Nicrophorus* will test the soil underneath the carcass to determine if burial is possible. If not, the beetles will move the carcass to an area with more suitable soil. After finding suitable soil, the beetles will then strip the carcass of any potential feathers or hair and form the carcass into a ball as they bury it (Scott, 1990).

This reproductive method limits competition from disease-spreading insect counterparts, including flies, and limits scavengers from having access to the carcass (Ratcliffe, 1996). In addition to limited carcass access from outside competition, it also limits other burying beetles from accessing the carcass and taking it over, killing existing offspring to replace with their own (Scott, 1994). However, competition with flies can sometimes lead to cooperative communal breeding chambers (Scott, 1994).

Nicrophorus species most often exhibit biparental care and are generally known to be extremely attentive parents (Eggert and Muller, 1997). Both the male and female participate in parental care, and will feed their offspring by regurgitating parts of the buried carcass (Benowitz and Moore, 2016). Typically, males leave the brood chamber first, while the female parent remains to finish rearing their offspring (Ward et al. 2009). While parental involvement varies among *Nicrophorus* species, *Nicorphorus orbicollis* is noted to have of the most involved parents (Benowitz and Moore, 2016).

The most recognizable member of the Silphidae family is the American burying beetle, *Nicrophorus americanus*, which was down listed from federally endangered to threatened in 2020 (U.S. Fish and Wildlife Service, 2021). Occupying only 10% of its historic range, the American burying beetle (ABB) is the largest burying beetle in North America, measuring 30-35mm in length (Schnell et al., 2014; Sikes and Raithel, 2002). To reach a large size, the ABB uses larger carrion, up to 300g, than its congeners

(Lomolino and Creighton, 1996). One of the few places American burying beetles occupy 10% of their historic range with large population in Nebraska and Oklahoma. In Oklahoma, Camp Gruber, which is a military training facility located near Braggs, Oklahoma consistently supports a large population.

Because of Camp Gruber's different functions, accidental and incidental fires commonly occur within base. Despite frequent fires at gun ranges, and occasional wildfires, prescribed burning is restricted because fires have unknown effects on ABB. This information is becoming increasingly important elsewhere as the effects of climate change worsen and wildfires likely become more commonplace (Amiro et al., 2001; Wotton et al., 2009).

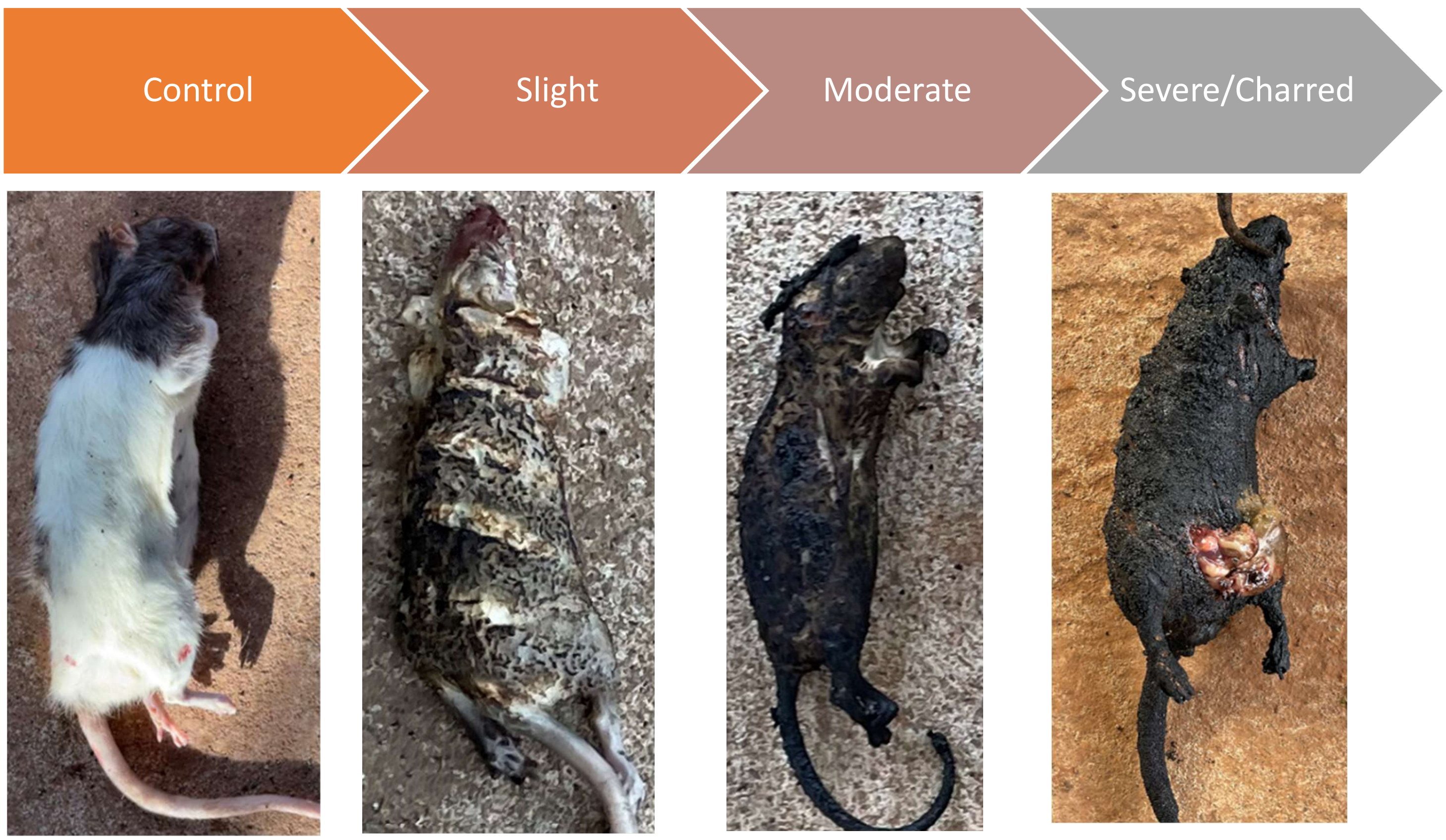
In this study, I analyzed how *Nicrophorus* species respond to various fire-driven events: burned carcasses as food, burned carcasses as a sourced of reproduction, and the effects of fire burning above buried beetles.

MATERIALS AND METHODS

Burn Scale

The Crow Glassman scale was not used in this experiment due to its lack of reliability beyond level 1, being singed carcasses (Keyes, 2019). Using a portable gas grill, previously frozen rodents were burned to desired levels with firestarter wood used as the fuel source. The control rodents were thawed but not exposed to fire. Larger carcsses, such as rats, were exposed to fire longer than smaller carcasses, as smaller carcasses would move to the next burn stage at a faster pace. Slightly burned rats were slightly singed and subjected to fire for up to 90 seconds. Moderately burned rats were characterized by burned fur, but still remained intact without open wounds, and fleshy. Moderately burned were subjected to fire for approximately 5-6 minutes. Severely burned rats were entirely burnt, with fire exposure greater than 6 minutes. Few distinguishable features were present, but severely burned rats also tended to have abdominal rupture from the fire, exposing the insides.

Figure 1. A visualization of the burn scale, as shown with rat carcasses.



Nicrophorus Attraction to Burned Carcasses

Experiments were conducted at Camp Gruber, with sites being spread across base. Camp Gruber is approximately 26,000 hectares and serves as a wildlife management area as well as military training grounds (Schnell et al., 2014). Above ground pitfall traps were used for sampling (Leasure et al., 2012). Pitfall traps were strapped tightly to trees using bungee cords, and consisted of five gallon buckets filled with approximately 10 centimeters of moistened Sphagum peat moss. To prevent the traps from flooding if rainfall were to occur, each bucket had 3mm holes in the sides. To prevent beetles from escaping, the buckets were covered with plywood (measuring 70cm by 40cm). For each trap, the bucket and the plywood cover was secured with j-hooks. Each piece of plywood had a hole in the center with an attached funnel. Frisbees were secured above the plywood, using screws with gaps to allow beetles to easily enter the trap.

To test if Silphidae species, specifically those in *Nicrophorus*, would make use of burned carrion as a food source, previously frozen extra-large rats were thawed overnight and then burned to desired levels using a grill. Control rats, which were untouched by fire, were compared with rats that were: slightly burned, moderately burned, and charred.

After burn treatments, all rats were placed in airtight buckets and held for 3-5 days, depending on environmental temperatures so that carcasses were at bloat stage. The hanging traps were then baited with rats. For each burn level, two traps were set. To allow for comparison between burned and unburned traps, traps containing burned rats were placed approximately 0.5 kilometers from traps containing control rats. Sampling consisted of three five-day periods, occurring once a month from May to July, with baiting occurring on the first day and rebaiting on the third day. Traps were checked daily, and all Silphidae captured in traps were identified to species and counted.

Nicrophorus **Reproduction on Burned Carcasses**

Nicrophorus use small vertebrate carcasses for reproduction. The effect of burning was tested using 28 breeding chambers. Mouse carcasses were prepared as above and pairs of *Nicrophorus orbicollis* were placed with carcasses.

Breeding chambers consisted of Tupperware capable of holding 1.2L. The Tupperware was filled most of the way with moistened top soil, so that only about an inch of space was available at the top of the chamber. The mice were thawed overnight, and then burned on a grill to varying degrees. As with the food preference experiment, there were four levels tested: control, slight, moderate, and charred. Seven breeding chambers were created for each level.

A pair of *N. orbicollis* were placed in each chamber with individuals collected from Camp Gruber used randomly after sexing. Successful broods were removed carefully from the chamber during the pupal stage, weighed, and placed individually in 2oz cups containing moistened peat moss until emergence.

Nicrophorus **Response to Surface Fire While Buried**

To test how *Nicrophorus* respond if a fire burned directly above, a fire table was constructed. The fire table was built with wood and a shelf was created by stretching metal chicken wire tightly across the table. A metal sheet was set on top of the fire table, leaving several inches of room between it and the metal shelf.

Pint sized mason jars were used for testing. Jars either contained nothing, 3.81cm of plaster, or 7.62cm of plaster to constrain beetle depth of burial. The jars were then filled most of the way with moistened peat moss, leaving approximately 1.27cm of room at the top of the jar for air flow. With each jar measuring at 15.5cm tall, the depth of the peat moss in each jar level was approximately 14.23cm, 10.42cm, or 6.61cm. The depth of the peat moss is the furthest that the beetles were able to bury away from the fire. Small holes were drilled into the mason jar lids to allow for air flow.

Nicrophorus carolinus, a diurnal species, were individually placed into a mason jar and allowed approximately 24 hours to settle. Jars were then placed on the wire shelf of the fire table, and lids were replaced with mesh to allow beetles to be exposed to the temperatures caused by the fire, but to prevent escape. Twelve centimeters of straw was placed on top of the metal sheet and was ignited. Six *Nicrophorus carolinus* beetles were subjected to fire tests at a time, two in each condition. This was repeated four times. To measure the temperature of the fire, the Milwaukee M12 Laser Temperature Gun was used. Data recording ended when active flames became embers.

RESULTS

Attraction to Burned Carcasses

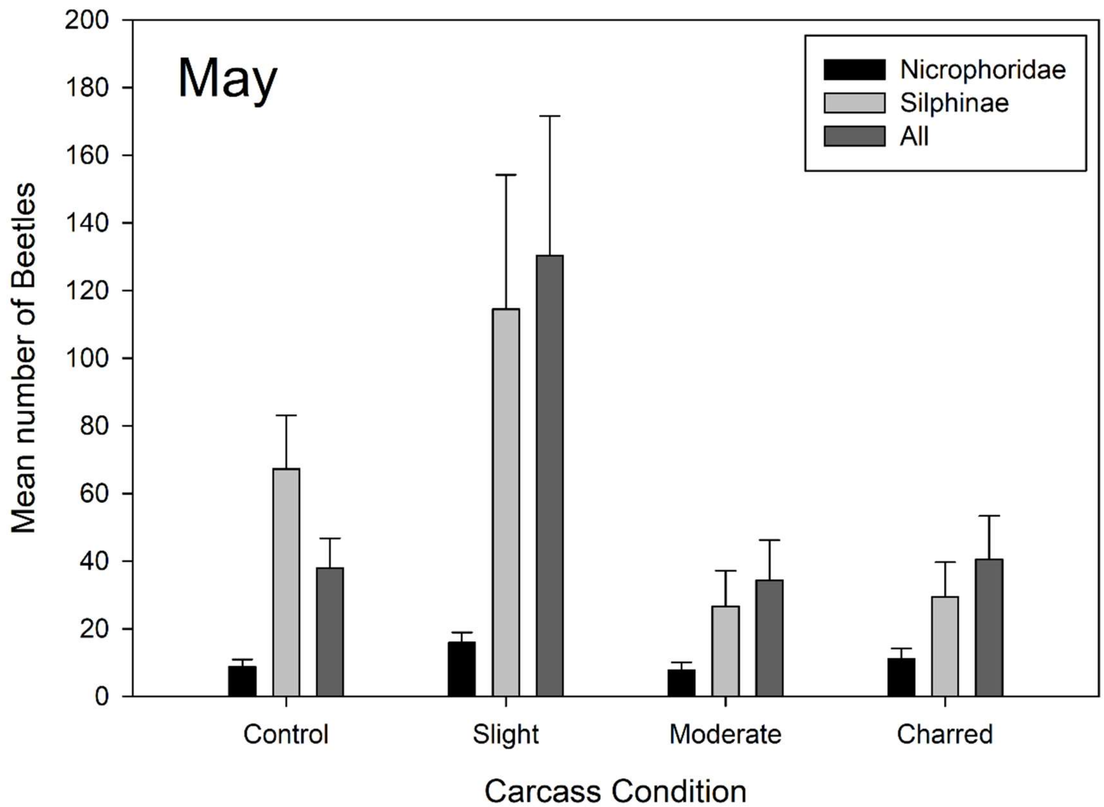
In total, 4,093 carrion beetles were captured in traps containing burned carrion (Table 1). Six *Nicrophorus* species were captured with the following totals: 274 ABB, 1 *N. carolinus*, 4 *N. marginatus*, 196 *N. orbicollis*, 653 *N. pustulatus*, and 101 *N. tomentosus*. Four Silphinae species were captured in traps containing burned carrion with the following totals: 2,140 *N. surinamensis*, 21 *O. noveboracense,* 373 *O. inaequale*, and 307 *N. Americana*. The most abundant species captured was *N. surinamensis.*

Table 1. Mean (± 1 S.E.) number of carrion beetles by carcass condition collected during May, June and July, 2021 at Camp Gruber, Oklahoma.

Month	Condition	ABB	*N. carolinus*	*N. marginatus*	*N. orbicollis*	*N. pustulatus*	*N. tomentosus*	*N. surinamensis*	*O. noveboracense*	*O. inaequale*	*N. americana*
May	Slight	5.0±1.87	0.0±0.00	0.0±0.00	1.3±0.700	8.0±1.99	0.0±0.00	77.5±28.46	0.3±0.21	13.3±4.87	11.9±5.27
	Moderate	3.7±1.52	0.0±0.00	0.0±0.00	0.8±0.42	1.7±1.11	0.0±0.00	2.4±1.02	0.3±0.21	13.1±5.28	5.5±4.47
	Charred	8.2±2.74	0.0±0.00	0.0±0.00	0.4±0.40	2.5±1.29	0.0±0.00	13.6±5.46	0.1±0.10	12.4±5.30	3.3±2.02
June	Slight	0.8±0.39	0.0±0.00	0.0±0.00	0.3±0.15	4.5±1.57	2.2±0.89	2.3±0.78	0.0±0.00	0.1±0.10	0.6±0.267
	Moderate	1.4±0.79	0.0±0.00	0.0±0.00	1.5±0.37	19.4±5.77	3.2±8.9	8.6±5.82	0.2±0.13	0.5±0.17	3.0±1.94
	Charred	0.4±0.27	0.0±0.00	0.0±0.00	2.7±1.07	2.4±1.10	4.3±1.2	0.0±0.00	1.1±0.99	0.2±0.13	0.2±0.13
July	Slight	4.0±1.56	0.0±0.00	0.3±0.3	1.3±0.75	9.8±3.15	0.2±0.13	96.3±26.78	0.0±0.00	0.0±0.00	2.2±0.84
	Moderate	2.7±0.75	0.0±0.00	0.1±0.10	2.5±1.44	8.0±2.68	0.2±0.20	17.3±5.11	0.0±0.00	0.0±0.00	2.0±0.67
	Charred	2.7±1.12	0.1±0.10	0.0±0.00	9.1±5.11	9.7±2.62	0.0±0.00	4.2±1.55	0.1±0.10	0.0±0.00	2.4±0.85

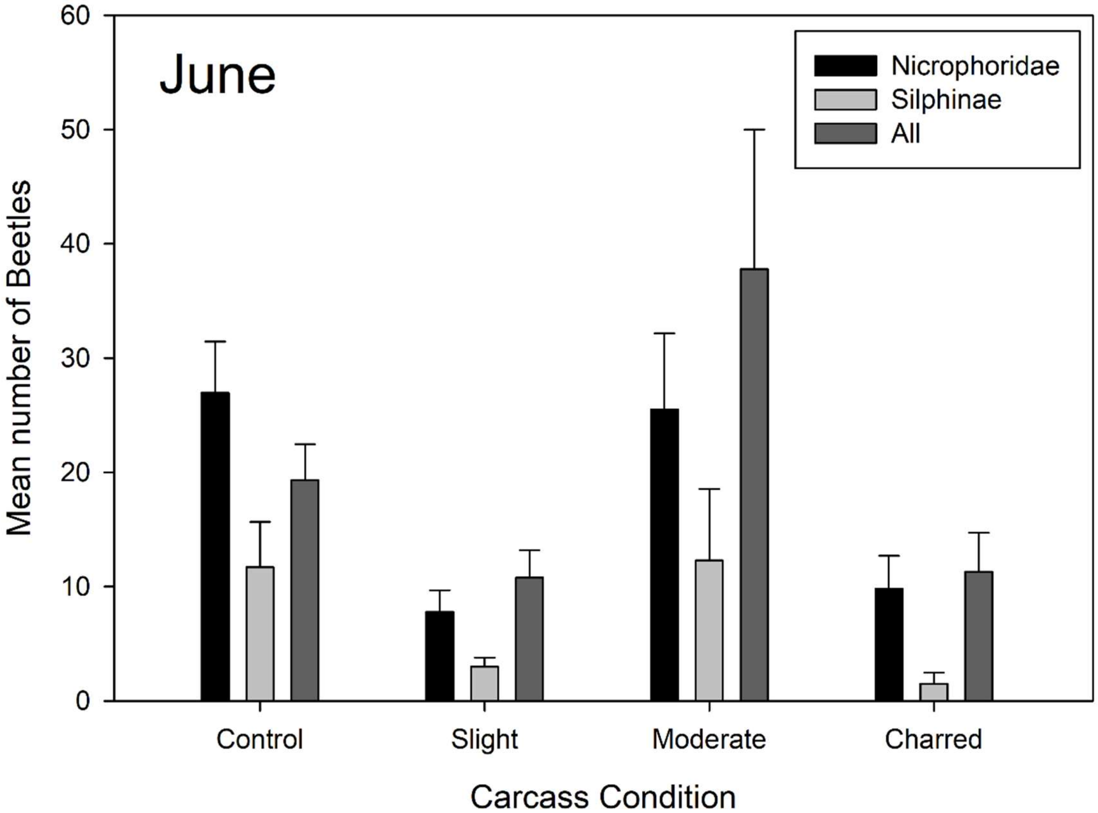
In May, most captured species were attracted to slightly burned carcasses. Moderately burned carcasses and charred carcasses attracted similar amounts of Silphidae (Figure 2). Although about twice as many beetles were collected from slightly burned rats, differences were not significant (Kruskal-Wallis ANOVA, P > 0.05).

Figure 2: Mean number (±S.E.) of all Silphidae caught each trap type in May.



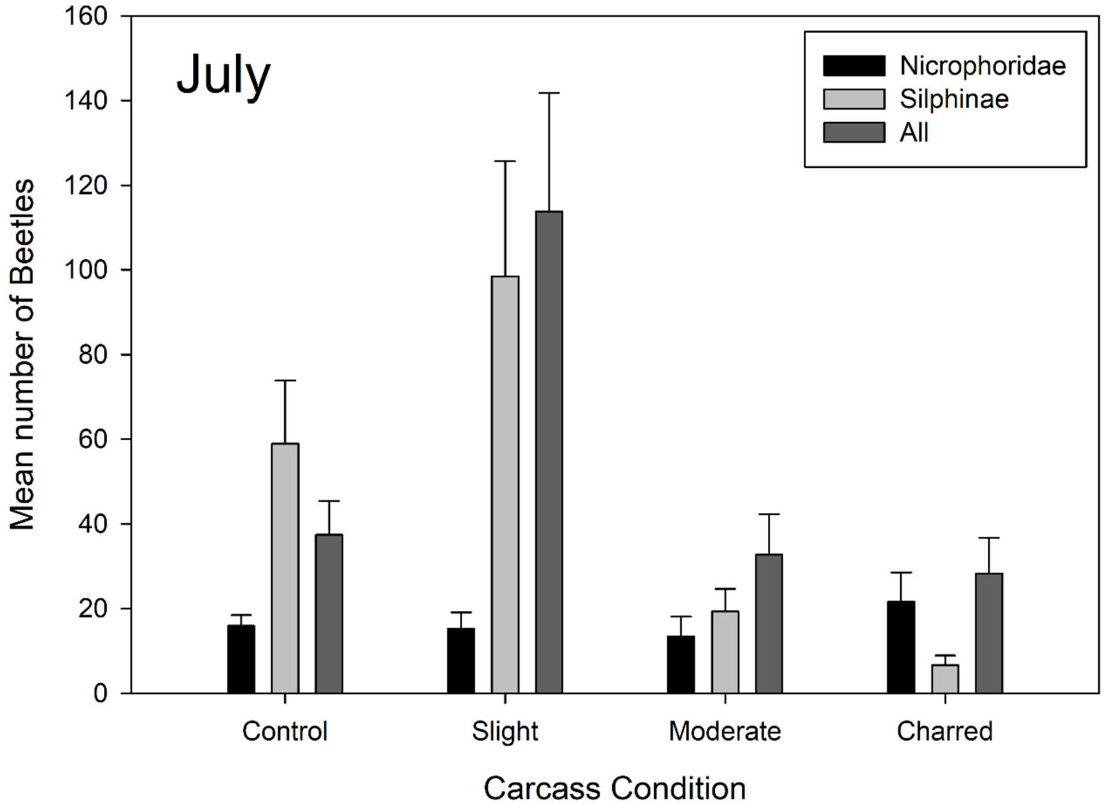
In June, moderately burned carcasses appeared to be most attractive to Silphidae, but there was no significant preference. Fewer Silphidae were collected in June (Figure 3). Reduction in captures could be a result of beetles breeding during this period.

Figure 3. Mean number (±S.E.) of all Silphidae caught in each trap type in June.



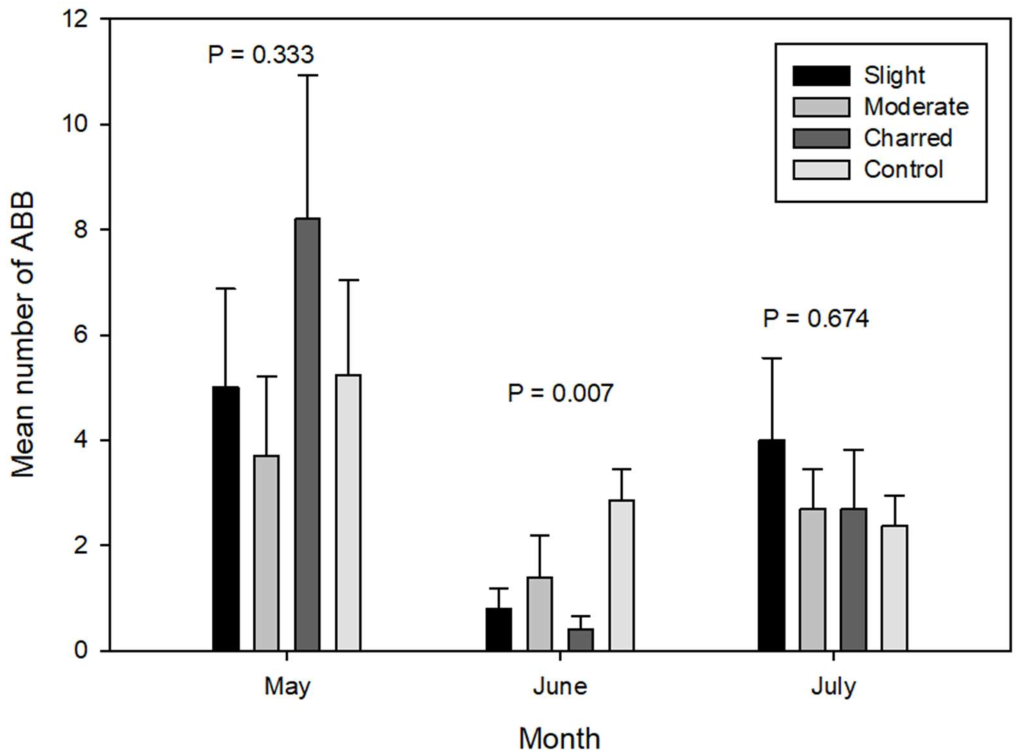
In July, slightly burned carcasses appeared to be preferred by most species, but there was not a significant preference. The amount of captured Silphidae increased compared to Silphidae captured in June (Figure 4). The amount of Silphidae captured in July, along with potential carcass preference, was similar to May.

Figure 4. Mean number (±S.E.) of all Silphidae caught in each trap type in July.



In May and July, American burying beetles, responded equally to carcasses in all conditions. In June, significantly more (Kruskal-Wallis ANOVA, P = 0.007). ABB responded to control carcasses compared to all carcasses. All numbers in June were lower, likely because adults were underground and reproducing.

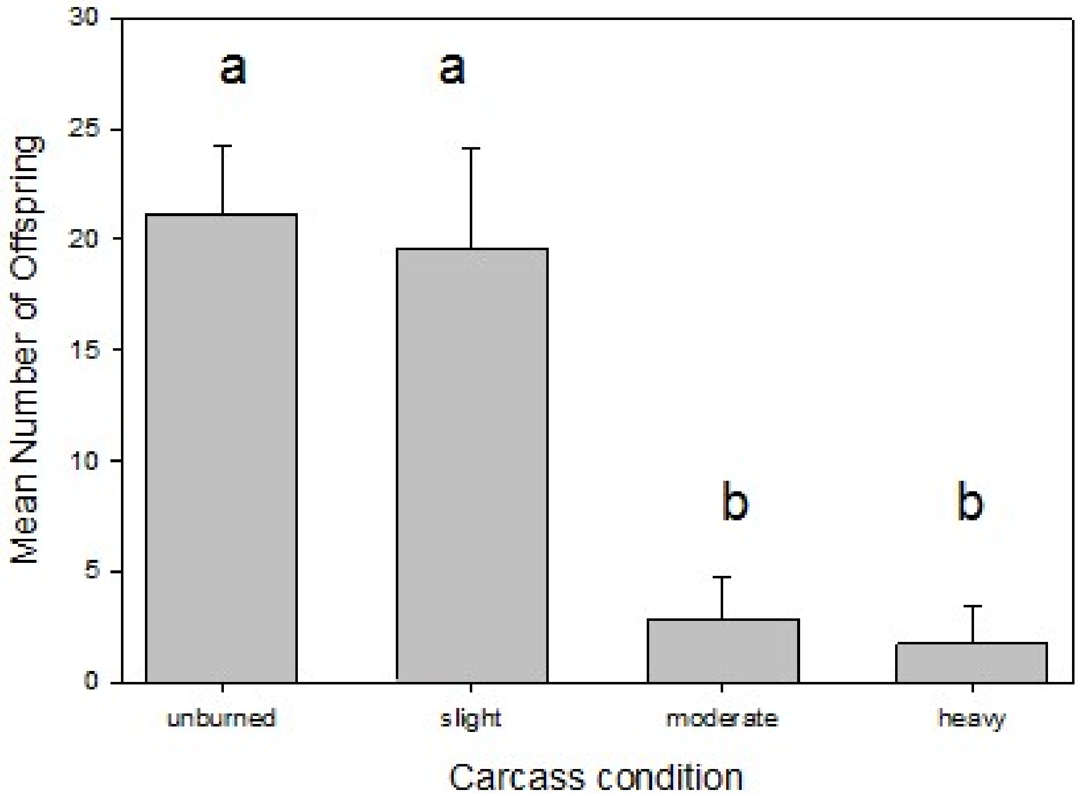
Figure 5. Mean capture (±S.E.) of *Nicrophorus americanus* per trap type over each five-day period.



Reproductive Capability

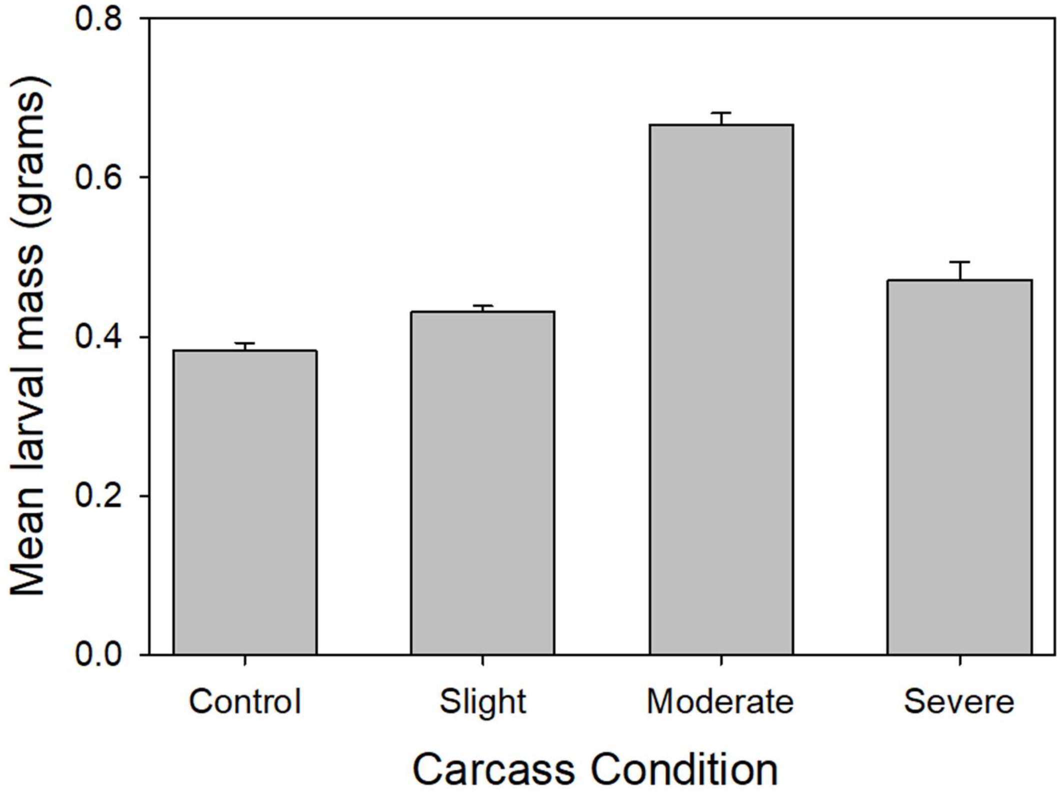
Nicrophorus orbicollis produced similar numbers of offspring on control and slightly burned carcasses (Figure 6). Offspring numbers declined for moderately burned carcasses and charred carcasses. However, *Nicrophorus orbicollis* were still capable of reproducing on these carcasses.

Figure 6. Mean number (±S.E.) of *Nicrophorus orbicollis* offspring per burn level of carcass. Results with the same letters show no significant difference (ANOVA, F = 12.142; P < 0.001).



Moderately burned carcasses had the largest offspring than its counterparts, with a value of P<0.001. This is likely due to selective filicide.

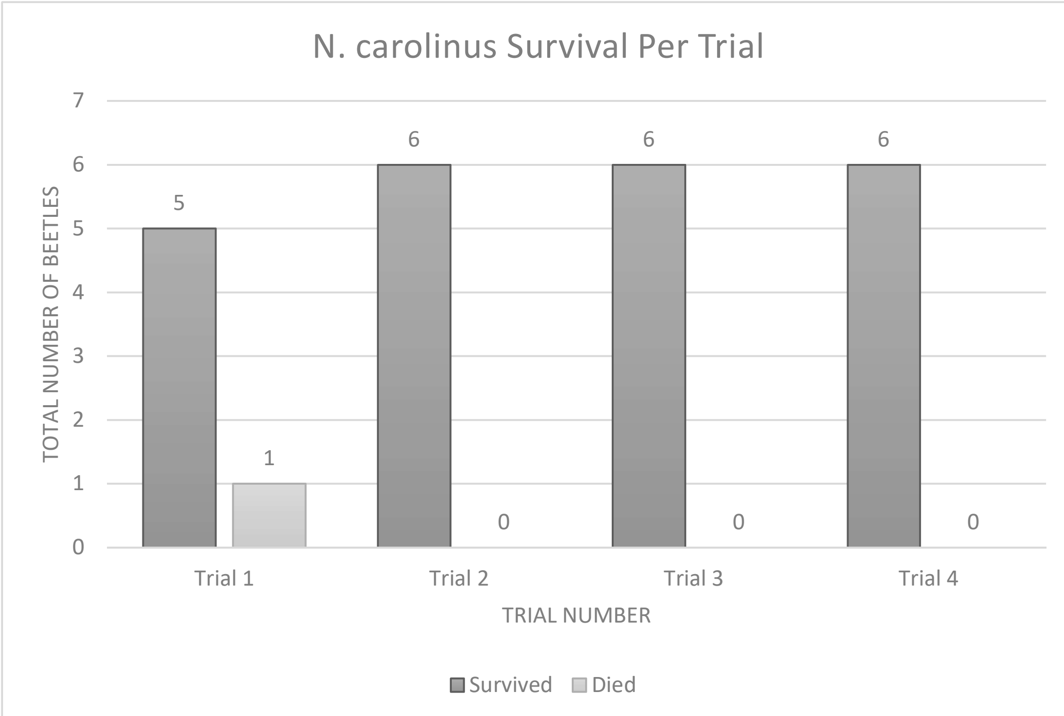
Figure 7. Mean weight (±S.E.) of *Nicrophorus orbicollis* offspring per burn level of carcass.



Temperature Endurance

Among trials, one death occurred in a jar containing 7.62cm of plaster. Each jar used was 15.5cm tall. Survival was 95.83% regardless of depth that beetles could bury. For all trials, temperatures measured at the surface exceeded 550 degrees Celsius.

Figure 8: Survival rates of *Nicrophorus carolinus* per trial.



DISCUSSION

In total, 9,927 Silphid beetles were captured during surveys. Traps containing burned bait captured 4,093 Silphids, while traps containing control bait captured 5,834 Silphids. Out of the 4,093 beetles captured in burn traps, 274 of those Silphids were ABB. A total of 323 ABB were captured in control traps. ABB were attracted to all levels of conditions of carrion, suggesting their ability to detect and use it as a food source (Fig. 5). ABB in May and July showed no significant preference towards any type of carcass, with their respective P-values being P=0.333 and P=0.674. However, ABB had a significant preference towards control carcasses in June, with the P-value being 0.007.

The preference ABB show towards control carcasses in June could be influenced by goals of reproduction. ABB are an annual species that are known to have one reproductive season, which normally occurs in June. (Mckenna-Foster et al., 2016). The lack of significant preference in May and July is likely because ABB are responding to all rotting carcasses for feeding.

This lack of preference could be due to the impacts that fire had on these heavily burned carcasses. When subjected to extensive fire exposure, carcasses would undergo an abdominal rupture. This rupture would expose much of the intestines, making it desirable due to ease of access. Characteristics present in charred carcasses are related to more advanced decomposition compared to unburned carcasses (Gruenthal et al., 2011; Keough et al., 2014). Notably, heavily burned carcasses were often completely skeletonized by beetles while its slightly burned counterparts were not. This skeletonization could be due to the advanced decomposition, making it easier for Silphidae to eat the flesh off of the bone.

All captured Silphidae numbers decreased substantially in June (Fig. 3). The overall reduction in captured beetles is likely due to reproduction, as many of the captured beetles belong to the *Nicrophorus* genus. Out of the burned carcasses, Silphidae captured in June showed a preference towards the moderately burned carcass, rather than slightly burned or charred. This was especially true for Nicrophorinae. In May and July, Silphidae that visited the burned carcasses appeared to favor slightly burned carcasses, though there was no statistical significance (Fig. 2 and 4).

When only comparing Silphidae captured in traps containing burned bait, *N. pustulatus* significantly preferred slightly burned carcasses over both moderately and severely burned carcasses in the month of May, with P=0.024. This preference shifted in June, with *N. pustulatus* then preferring moderately burned carcasses instead of slightly burned or severely burned, with P= 0.001. In July, *N. pustulatus* did not show a significant preference towards any burned carcass level. The preference towards slightly burned carcasses in May could be due to an early reproduction cycle, and with slightly burned carcasses being the most similar to the control carcasses, slightly burned carcasses were more sought after. Since carcass seeking for reproductive purposes ended, the preference towards moderately burned carcasses in June could just be a food preference.

When only comparing traps containing burned bait, *N. orbicollis* significantly preferred severely burned carcasses in June, with a P value of P=0.017. Similar to *N. pustulatus*, this preference could be due to an earlier reproduction cycle, with reproduction occurring in May instead of June. Thus, this preference of heavily burned carcasses could be due to a preference in food rather than carcass seeking for reproductive purposes.

Necrodes surinamensis is a carrion feeder, but is primarily predatory towards fly larva (Ratcliffe, 1972). When only comparing *N. surinamensis* caught in traps containing burned bait, *N. surinamensis* had a significant preference towards slightly burned carcasses in every month sampled. For May, June, and July, the respective P values were P=0.007, P=0.001, and P<0.001. These significant preferences are likely due to the slightly burned carcasses being most similar to control carcasses. Maggots were found on every type of carcass sampled, but no *N. surinamensis* were caught in traps containing severely burned carcasses in the month of June. The lack of *N. surinamensis* on severely burned carcasses may be due to there being possible peaks of activity during May and July (Lingafelter, 1995).

Because of its close relation to the American burying beetle, we used the round-necked burying beetles, *Nicrophorus orbicollis* to test suitability of burned carcasses on burying beetle. Typically, breeding chambers are prepared using moistened sphagnum peat moss, but this method led to several failed chambers that became overridden with mold. This is not a problem that typically occurs to such an extent. Since carcasses influence soil pH, it's possible that the presence of burned carcasses caused the pH of the peat moss to change, making it more suitable for mold (Freeman et al., 2020). As an attempt to remedy the problem, moistened top soil was used instead of peat moss, and breeding was successful. It's also possible that the burned rats picked up fungi during the burning and transportation process, but we are unsure.

Using SigmaPlot for analysis*, N. orbicollis* exhibited similar numbers of offspring with both the control mouse and the slightly burned mouse (Fig. 6). *N. orbicollis* reared an average of 21.1 offspring on control carcasses, 19.6 offspring on slightly burned carcasses, 2.9 offspring on moderately burned carcasses, and 1.7 offspring on severely burned carcasses. *Nicrophorus* species are known to be very attentive parents, and will feed their offspring with regurgitated carrion, taken from the carcass they buried (Benowitz and Moore, 2016; Eggert and Muller, 1997).

If the parental beetles gauge that there is not enough food to feed all of their offspring, they will kill and consume some offspring during the first instar to ensure survival of the remaining offspring (Bartlett, 1987). This is likely what happened with the moderately burned carcasses and the heavily burned carcasses, as these carcasses likely did not offer the necessary amount of resources needed to raise a large brood. *N. orbicollis* was successful at raising a brood on both the moderately burned and severely burned carcasses, but had a much lower success rate, as well as lower number of offspring. In relation to this, there was a single first instar larva that successfully hatched on a heavily burned carcass, only to be eaten by its parents by the next day, likely due to lack of food availability. However, despite the amount of offspring being lower, moderately burned carcasses produced significantly larger offspring than its counterparts, with a P value of $P<0.001$ (Fig. 7). It is possible that the parental beetles become more K-selected when using carcasses exposed to fire, leading to larger, more successful offspring.

Most *Nicrophorus carolinus*, a day active species, survived when buried directly below temperatures reaching over 550 degrees Celsius (Fig. 8). It's possible that the death that occurred was due to a lack of moisture in the mason jar the beetle was placed in, resulting in the beetle drying out and dying before the trial took place. However, despite one death, these results suggest that if a wildfire were to take place during a beetle's inactive period, when beetles are buried underground, the beetles have a very high chance of survival if a fire burns directly above them.

While individual beetles have a high chance of survival, it is still unknown if offspring present on a buried brood ball would be able to survive burning right above it. Since *Nicrophorus* reproduction occurs in June with tenerals emerging in July, it's likely that fires occurring in the months of May, August, and September would cause the least possible harm to brood balls. More research needs to be conducted in this area in the future.

If a small mammal were to get caught in a fire, it is unlikely that the resulting carcass would be severely burned. More than likely, the carcass would have some singed fur, and would have succumbed to smoke inhalation (Fitzgerald et al., 2006). This description best matches that of the slightly burned carcasses used in the both the food capability and reproductive success experiments, leading to the conclusion that *Nicrophorus* species would be able to utilize carcasses caught in a fire as both a food and reproductive source without leading to a drastic decline in population.

Additionally, because *Nicrophorus* species can use burned carcasses, this can limit other insects from making use of these carcasses. Depending on which insect might make use of the carcass if *Nicrophorus* cannot, this carcass utilization can limit the spread of disease and unwanted pathogens. Such is the case with Dipterans, which serve as mechanical vectors of disease (Carn, 1996).

CHAPTER III

A TEST OF HOUSE FLY, *MUSCA DOMESTICA* REPRODUCTION ON BURNT CARCASSES

ABSTRACT

After death, an animal undergoes decomposition aided by different insects that utilize the corpse for food and reproductive purposes. House flies, *Musca domestica*, are among the first to arrive to corpse, especially when feces are present, and are forensically important species. While numerous studies have reported house flies associated with corpses, relatively few have examined the effect of burning on reproduction and larval survival. Previously frozen large rats were slightly burned, moderately burned, and severely burned and compared with unburned control carcasses. Carcasses were placed in cages along with house fly pupa that selected carcasses of their choice. Flies reproduced on the moderately burned and severely burned carcasses, with few new adults emerging from control carcasses. Unlike studies of Calliphoridae, it appears that burning enhances Muscidae larval success.

INTRODUCTION

When an animal dies, it undergoes five distinct stages of decomposition: fresh, bloat, active decay, advanced decay, and skeletonization (Wolff et al., 2001). Within each of these stages, insects visit the corpse in what is usually a predictable order, known as insect succession (Archer, 2004). Among the first to arrive to a fresh corpse are Dipteran families Calliphoridae and Muscidae (Marchenko, 2001; Rodriguez and Bass, 1983).

Since they are among the first to arrive, Calliphoridae are especially important forensically since their larvae can be used as a tool to pinpoint the time of death of a corpse, known as the post-mortem interval (PMI) (Heaton et al., 2014; (Mohr and Tomberlin, 2015). Members of the family Calliphoridae have long been a focus of study in the forensic entomology realm due to their abundance on corpses and their near immediate presence upon death. However, Muscidae appear soon after an animal dies as well, but are understudied, often due to the similarities between species causing identification to be more difficult (Grzywacz et al., 2014).

While extensive studies have been conducted on insect succession and bodies that died under different circumstances, little research has been conducted on how insects involved in insect succession react to burned carcasses, even though bodies have been found that have been burned (Anderson, 2019; McIntosh et al., 2017; Chin et al., 2008). However, it has been found that insect succession does change if a carcass has been exposed to fire (Mashaly, 2016). Generally, burnt carcasses tend to attract more beetles than flies during decomposition, with the exception of the fresh stage (Mashaly, 2016). There is debate regarding whether exposure to fire accelerates decomposition (Keough et al., 2015; Mashaly, 2016). Studies on Calliphoridae have suggested that they have the same reproductive capability on slightly burned carcasses that they do on control carcasses, though their reproduction was delayed on the carcasses that underwent more advanced burning (Mahat et al., 2016). However, studies on Muscidae and burned carcasses are few in number.

Members of the family Muscidae, containing the insects commonly known as house flies, are deemed forensically important for medico-legal purposes (Grzywacz et al., 2016). However, house flies are also medically important due to serving as mechanical vectors of disease, and are capable of spreading unwanted pathogens through touch (Carn, 1996; Iqbal et al., 2014). Since Muscidae are found worldwide, and feed on anything wet or decaying, giving special preference towards animal dung, unwanted pathogens are easily spread from decaying matter to humans and animals (Iqbal et al., 2014)

The objective of this study was to determine the reproductive capabilities of house flies on varying levels of burned carcasses: control, slightly burned, moderately burned, and severely burned.

MATERIALS AND METHODS

In total, 16 previously frozen extra-large rats were used. The rats were thawed overnight, and then burned on a grill to varying degrees. There were four levels tested: control, slight, moderate, and severe. Four rats were untouched by fire, serving as controls. Four rats were burned to the slight level, four were burned to a moderate level, and the four remaining rats were severely burned.

Each rat carcass was individually placed in a small open top plastic container that contained a thin layer of sand on the bottom, serving as substrate. All containers used were transparent in nature, but varied in color, being clear, gray, or blue. Due to the differences in color, each level of rat was placed in at least one of each color.

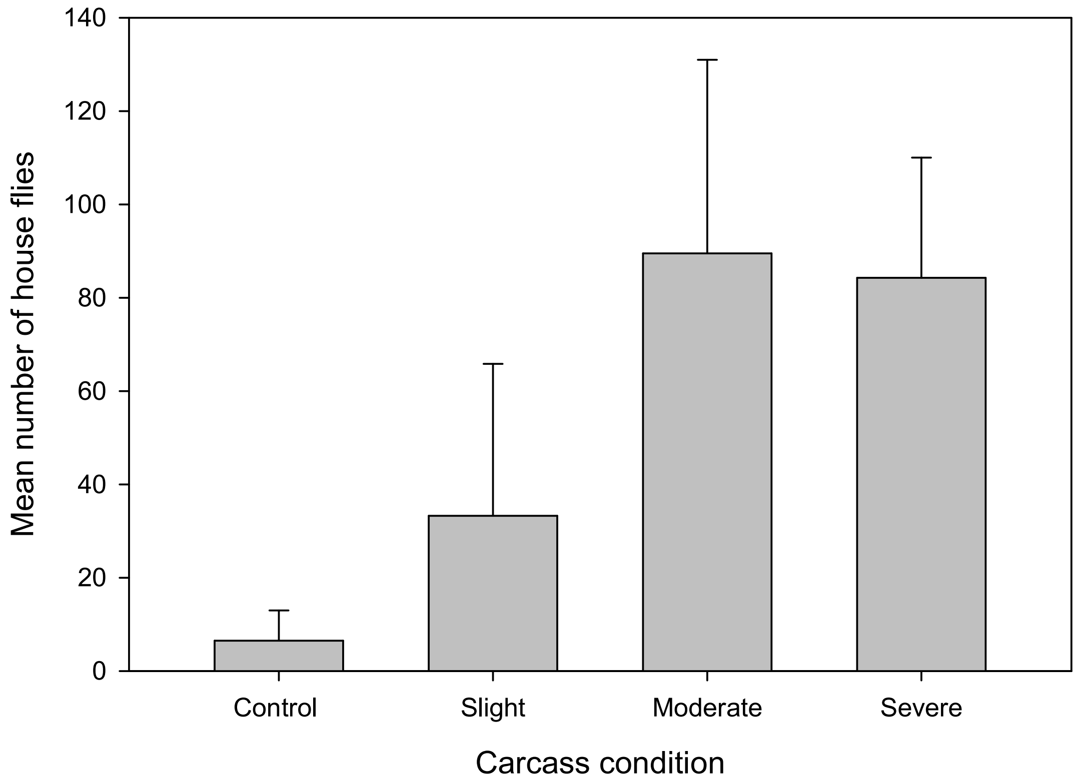
Each color group stayed together. Containers used in Cage 1 were white, containers used in Cage 2 were gray containers, and containers used in both Cages 3 and 4 were blue. The open top containers with rats were then placed in cages, using a total of four cages. For each of the four cages used, every level of burning was present. This was done to test the preference that house flies might have.

Within the four cages containing each level, 200 pupae were placed, along with a dish of water. All cages were placed inside of a room with ideal conditions, being 26.7 degrees Celsius and 80% humidity, to allow for as many pupae to emerge as possible. Additionally, a light system was set up to simulate day/night conditions.

The pupae were the allowed to hatch into adults, which were counted. After visible eggs were on the carcasses, the carcasses were removed from the cages, covered with a lid, and placed in pillowcases. Pillowcases were then secured with rubber bands. The fly eggs were allowed to hatch and continue to adulthood, where the flies then died, and were counted.

RESULTS

Figure 9. The mean (± 1 S.E.) number of adult houseflies recovered from each carcass level from all cages.



The amount of recovered offspring is extremely low, and no significant preference between carcasses is shown.

DISCUSSION

The emergence of original pupae was similar in each cage, and matched expected levels of emergence. House flies showed no significant preference between carcasses, but had the most reproductive success on carcasses moderately and severely burned. Cage 3 is the only instance where flies chose to reproduce on a control carcass, and did so successfully. All of the fly cages were placed in the same room together due to that room possessing ideal conditions for house fly development. It is unlikely that different container colors used would lead to different results. Though not all of the pillowcases were the same, the color of pillowcases seemed to have little to no effect on the amount of adult offspring extracted from each of the carcasses.

Midway through the study, it was discovered that members of the family Phoridae had somehow infiltrated the experiment. This may have occurred by the Phorid flies directly infiltrating by squeezing through the small holes of the cages, or by laying eggs through the cage (Zuha et al., 2014). This more than likely occurred before each container was individually secured in a pillowcase, as Phoridae pupae were found alongside Muscidae pupae, but not adult Phoridae. It is possible that this infestation impacted results. Members of the Phoridae family also appeared to favored carcasses that were subjected to fire, especially those severely burned.

House flies are capable of reproducing on burned carcasses, but more studies need to be conducted to better understand if there is a significant preference between carcass levels. Assuming that the 200 pupae placed within each cage had a relatively equal sex distribution, and a female housefly is capable of laying 4-6 clutches of up to 150 eggs, the amount of emerged offspring collected from all carcasses is extremely low (Iqbal et al., 2014). This could be due to interference from Phorids.

Because they are capable of reproducing on burned carcasses, it is likely that they spread unwanted pathogens found on these carcasses to other surfaces. Previous studies indicate that Calliphoridae had similar reproductive abilities on slightly burned carcasses as they normally would with controls, while delayed reproduction occurs on carcasses that are more heavily burned (Mahat et al., 2015). In our trials, Muscidae had higher numbers of offspring on moderately and severely burned carcasses, indicating a potential preference, but more research needs to be done to confirm this.

REFERENCES

Al-Shareef, L. A. H. (2016). *Houseflies* (Diptera: Muscidae) associated with rabbit carcasses in Jeddah city*, Kingdom of Saudi Arabia*. 9.

Amiro, B. D., Stocks, B. J., Alexander, M. E., Flannigan, M. D., & Wotton, B. M. (2001). Fire, climate change, carbon and fuel management in the Canadian boreal forest. *International Journal of Wildland Fire*, *10*(4), 405–413.

Anderson, G. S. (2019). Factors that Influence Insect Succession on Carrion. In *Forensic Entomology* (3rd ed.). CRC Press.

Archer, M. S. (2004). Annual variation in arrival and departure times of carrion insects at carcasses: Implications for succession studies in forensic entomology. *Australian Journal of Zoology*, *51*(6), 569–576.

Australasian Fire and Emergency Service Authorization Council (AFAC). (2012). *Bushfire-terminology.pdf*.

Ayala-Ortiz, C. O., Farriester, J. W., Pratt, C. J., Goldkamp, A. K., Matts, J., Hoback, W. W., Gustafson, J. E., & Hagen, D. E. (2021). Effect of food source availability in the salivary gland transcriptome of the unique burying beetle *Nicrophorus pustulatus* (Coleoptera: Silphidae). *PloS One*, *16*(9), e0255660.

Bartlett, J. (1987). Filial cannibalism in burying beetles. *Behavioral Ecology and Sociobiology*, *21*(3), 179–183.

Beeler, A. E., Rauter, C. M., & Moore, A. J. (1999). Pheromonally mediated mate attraction by males of the burying beetle *Nicrophorus orbicollis*: Alternative calling tactics conditional on both intrinsic and extrinsic factors. *Behavioral Ecology*, *10*(5), 578–584.

Benowitz, K. M., & Moore, A. J. (2016). Biparental care is predominant and beneficial to parents in the burying beetle *Nicrophorus orbicollis* (Coleoptera: Silphidae). *Biological Journal of the Linnean Society. Linnean Society of London*, *119*(4), 1082–1088.

Carn, V. M. (1996). The role of dipterous insects in the mechanical transmission of animal viruses. *The British Veterinary Journal*, *152*(4), 377–393.

Chin, H. C., Abdullah Marwi, M., Firdaus Mohd. Salleh, A., Jeffery, J., Kurahashi, H., & Omar, B. (2008). Study of insect succession and rate of decomposition on a partially burned pig carcass in an oil palm plantation in Malaysia. *Tropical Biomedicine*, *25*(3), 202–208.

Creighton, J. C., & Schnell, G. D. (1998). Short-term movement patterns of the endangered American burying beetle *Nicrophorus americanus*. *Biological Conservation*, *86*(3), 281–287.

De Gasperin, O., Duarte, A., & Kilner, R. M. (2015). Interspecific interactions explain variation in the duration of paternal care in the burying beetle. *Animal Behaviour*, *109*, 199–207.

Dekeirsschieter, J., Verheggen, F., Lognay, G., & Haubruge, E. (2011). Large carrion beetles (Coleoptera, Silphidae) in Western Europe: A review. *Biotechnol. Agron. Soc. Environ.*, 13.

Eggert, A. K., & Muller, J. K. (1997). Biparental Care and Social Evolution in Burying Beetles: Lessons from the Larder. Cambridge University Press.

Fitzgerald, K. T., & Flood, A. A. (2006). Smoke inhalation. *Clinical Techniques in Small Animal Practice*, *21*(4), 205–214.

Freeman, L., Hoback, W. W., Payton, M., & Peterson, B. C. (2020). Burying Beetle (Coleoptera: Silphidae: *Nicrophorus Fabricius*) Brooding Improves Soil Fertility. *The Coleopterists Bulletin*, *74*(2), 427–433.

Gruenthal, A., Moffatt, C., & Simmons, T. (2012). Differential decomposition patterns in charred versus un-charred remains. *Journal of Forensic Sciences*, *57*(1), 12–18.

Grzywacz, A., Hall, M. J. R., Pape, T., & Szpila, K. (2017). Muscidae (Diptera) of forensic importance—An identification key to third instar larvae of the western Palaearctic region and a catalogue of the muscid carrion community. *International Journal of Legal Medicine*, *131*(3), 855–866.

Haddadi, R., Alajmi, R., & Abdel-Gaber, R. (2019). A comparative study of insect succession on rabbit carrion in three different microhabitats. *Journal of Medical Entomology*, *56*(3), 671–680.

Heaton, V., Moffatt, C., & Simmons, T. (2014). Quantifying the temperature of maggot masses and its relationship to decomposition. *Journal of Forensic Sciences*, *59*(3), 676–682.

Ikeda, H., Kagaya, T., Kubota, K., & Abe, T. (2008). Evolutionary relationships among food habit, loss of flight, and reproductive traits: Life-history evolution in the Silphinae (coleoptera: Silphidae). *Evolution*, *62*(8), 2065–2079.

Iqbal, W., Malik, M. F., Sarwar, M. K., Iram, N., & Rashda, A. (2014). Role of housefly (*Musca domestica*, Diptera; Muscidae) as a disease vector; a review. *Journal of Entomology and Zoology Studies*, 5.

Keiding, J. (1986). Vector Control Series: The housefly training and information guide. *World Health Organization Vector Biology and Control Division*.

Keller, M. L., Howard, D. R., & Hall, C. L. (2019). Spatiotemporal niche partitioning in a specious silphid community (Coleoptera: Silphidae *Nicrophorus*) | SpringerLink. *The Science of Nature*, *106*(56).

Keough, N., L'Abbé, E. N., Steyn, M., & Pretorius, S. (2015). Assessment of skeletal changes after post-mortem exposure to fire as an indicator of decomposition stage. *Forensic Science International*, *246*, 17–24.

Keyes, C. A. (2019). How reliable is the charred body scale? An interobserver reliability study on scoring burned remains. *Burns*, *45*(7), 1673–1679.

Leasure, D., Rupe, D., Phillips, E., Opine, D., & Huxel, G. (2012). Efficient new above-ground bucket traps produce comparable data to that of standard transects for the endangered American burying beetle, *Nicrophorus americanus* Olivier (Coleoptera: Silphidae). *The Coleopterists Bulletin*, *66*, 209–218.

Lindstedt, C., Boncoraglio, G., Cotter, S., Gilbert, J., & Kilner, R. M. (2017). Aposematism in the burying beetle? Dual function of anal fluid in parental care and chemical defense. *Behavioral Ecology*, *28*(6), 1414–1422.

Lingafelter, S. W. (1995). Diversity, habitat preferences, and seasonality of Kansas carrion beetles (Coleoptera: Silphidae). *Journal of the Kansas Entomological Society*, *68*(2), 214–223.

Lomolino, M. V., & Creighton, J. C. (1996). Habitat selection, breeding success and conservation of the endangered American burying beetle *Nicrophorus americanus*. *Biological Conservation*, *77*(2), 235–241.

Mahat, N. A., Zainol-Abidin, N. L., Nordin, N. H., Abdul-Wahab, R., & Jayaprakash, P. T. (2016). Patterns of oviposition and development of *Chrysomya megacephala* (Fabricius) (Diptera: Calliphoridae) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) on burned rabbit carcasses. *Forensic Science International*, *260*, 9–13.

Marchenko, M. I. (2001). Medicolegal relevance of cadaver entomofauna for the determination of the time of death. *Forensic Science International*, *120*(1), 89–109

Mashaly, A. M. (2016). Entomofaunal succession patterns on burnt and unburnt rabbit carrion. *Journal of medical entomology*, *53*(2), 296-303.

Matuszewski, S. (2021). Post-mortem interval estimation based on insect evidence: Current challenges. *Insects*, *12*(4), 314.

McIntosh, C. S., Dadour, I. R., & Voss, S. C. (2017). A comparison of carcass decomposition and associated insect succession onto burnt and unburnt pig carcasses. *International Journal of Legal Medicine*, *131*(3), 835–845.

Mckenna-Foster, A., Perrotti, L., Blyth, J., LoPresti, E., & Kennedy, R. S. (2016). Measuring success of a reintroduced population of the American burying beetle (*Nicrophorus americanus* Olivier) to Nantucket Island, MA. *Journal of Insect Conservation*, *20*(5), 895–904.

Milne, L. J., & Milne, M. (1976). The social behavior of burying beetles. *Scientific American, 235*(2), 84–89.

Mohr, R. M., & Tomberlin, J. K. (2015). Development and validation of a new technique for estimating a minimum postmortem interval using adult blow fly (Diptera: Calliphoridae) carcass attendance. *International Journal of Legal Medicine*, *129*(4), 851–859.

Morgan, G. W., Tolhurst, K. G., Poynter, M. W., Cooper, N., McGuffog, T., Ryan, R., Wouters, M. A., Stephens, N., Black, P., Sheehan, D., Leeson, P., Whight, S., & Davey, S. M. (2020). Prescribed burning in south-eastern Australia: History and future directions. *Australian Forestry*, *83*(1), 4–28.

Pilliod, D., Bury, R., Hyde, E., Pearl, C., & Corn, P. (2003). Fire and amphibians in North America. *Forest Ecology and Management*, *178*, 163–181.

Ratcliffe, B. (1996). The carrion beetles (Coleoptera: Silphidae) of Nebraska. *Bulletin of the University of Nebraska State Museum*, *13*, 1–100.

Ratcliffe, B. C. (1972). The natural history of *Necrodes Surinamensis* (Fabr.) (Coleoptera: Silphidae). *Transactions of the American Entomological Society (1890-)*, *98*(4), 359–410.

Rodriguez, W. C., & Bass, W. M. (1983). Insect activity and its relationship to decay rates of human cadavers in east Tennessee. *Journal of Forensic Sciences*, *28*(2), 11524J

Ryan, K. C., Knapp, E. E., & Varner, J. M. (2013). Prescribed fire in North American forests and woodlands: History, current practice, and challenges. *Frontiers in Ecology and the Environment*, *11*(s1), e15–e24.

Schnell, G., Goetzinger, J., Hiott, A., Estevez, B., & Brennan, S. (2014). Effects of available food resources on numbers of the endangered American burying beetle (*Nicrophorus americanus*). *The Southwestern Naturalist*, *59*, 173–180.

Scott, J. G., Warren, W. C., Beukeboom, L. W., Bopp, D., Clark, A. G., Giers, S. D., Hediger, M., Jones, A. K., Kasai, S., Leichter, C. A., Li, M., Meisel, R. P., Minx, P., Murphy, T. D., Nelson, D. R., Reid, W. R., Rinkevich, F. D., Robertson, H. M., Sackton, T. B., … Liu, N. (2014). Genome of the house fly, *Musca domestica L*., a global vector of diseases with adaptations to a septic environment. *Genome Biology*, *15*(10), 466.

Scott, M. P. (1994). Competition with flies promotes communal breeding in the burying beetle, *Nicrophorus tomentosus*. *Behavioral Ecology and Sociobiology*, *34*(5), 367–373.

Scott, M. P. (1998). The ecology and behavior of burying beetles. *Annual Review of Entomology*, *43*(1), 595–618.

Scott, M. (1990). Brood guarding and the evolution of male parental care in burying beetles. *Behavioral Ecology and Sociobiology*, *26*(1).

Sikes, D. S., & Capinera, J. L. (2008). Carrion beetles (Coleoptera: Silphidae). *Encyclopedia of entomology*, *1*, 749-758

Sikes, D. S., & Raithel, C. J. (2002). A review of hypotheses of decline of the endangered American burying beetle (Silphidae: *Nicrophorus americanus* Olivier). *Journal of Insect Conservation*, *6*(2), 103–113.

Smith, G., Trumbo, S. T., Sikes, D. S., Scott, M. P., & Smith, R. L. (2007). Host shift by the burying beetle, *Nicrophorus pustulatus*, a parasitoid of snake eggs. *Journal of Evolutionary Biology*, *20*(6), 2389–2399.

van Doorn, H. R. (2014). Emerging infectious diseases. *Medicine*, *42*(1), 60–63.

Vanin, S., Zanotti, E., Gibelli, D., Taborelli, A., Andreola, S., & Cattaneo, C. (2013). Decomposition and entomological colonization of charred bodies – a pilot study. *Croatian Medical Journal*, *54*(4), 387–393.

Ward, R. J. S., Cotter, S. C., & Kilner, R. M. (2009). Current brood size and residual reproductive value predict offspring desertion in the burying beetle *Nicrophorus vespilloides*. *Behavioral Ecology*, *20*(6), 1274–1281.

Wolff, M., Uribe, A., Ortiz, A., & Duque, P. (2001). A preliminary study of forensic entomology in Medellín, Colombia. *Forensic Science International*, *120*(1), 53–59.

Wotton, B. M., Nock, C. A., Flannigan, M. D., Wotton, B. M., Nock, C. A., & Flannigan, M. D. (2010). Forest fire occurrence and climate change in Canada. *International Journal of Wildland Fire*, *19*(3), 253–271.

USFWS: American Burying Beetle Fact Sheet. (2021).

Zuha, R. M., See, H. W., Disney, R. H. L., & Omar, B. (2014). First record of genus *Puliciphora Dahl* (Diptera: Phoridae) associated with rabbit carcasses placed in concealed environments in Malaysia. *Forensic Science International*, *245*, e36–e37.

Zurek, L., Denning, S. S., Schal, C., & Watson, D. W. (2001). Vector competence of *Musca domestica* (Diptera: Muscidae) for *Yersinia pseudotuberculosis*. *Journal of Medical Entomology*, *38*(2), 333–335.

VITA

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