A TEST OF THE HABITAT ASSOCIATION AND SEASONAL ACTIVITY OF THE AMERICAN BURYING BEETLE (*NICROPHORUS AMERICANUS*) AT CAMP GRUBER, OK AND THE BENEFITS OF NICROPHORUS TO SOIL FERTILITY

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Abstract:

Within the past decade, the American burying beetle, *Nicrophorus americanus* (ABB) have disappeared from approximately 90% of its former range. Efforts have been made to protect existing populations in the wild; however, ABB is considered a habitat generalist, which makes prioritizing conservation areas difficult. I sampled three habitat types: savannah, grassland, and forested areas. To compare across seasons, ABB were sampled from July 16, 2016 to November 12, 2017. I trapped a total of 1,756 unique individual ABB along with 10,448 individuals of 9 other Silphidae species. During the surveying period I found no significant difference in the habitat association of ABB. Overall trapping, forests had 6.0 ± 0.8, grasslands had 8.1 ± 1.2, and savannah had 8.2 ± 1.3 ABB per trap night. This suggests that across their active season, ABB are associated with all habitats at Camp Gruber. My data support the conclusion that ABB is a habitat generalist and is moving among the patches of different habitat types at Camp Gruber.

In order to determine influence of Eastern redcedar and brood success on soil fertility, I conducted a laboratory experiment with *Nicrophorus marginatus* and various soil mixtures. Test containers consisted of: soil/rat/beetles (14) and soil/cedar/rat/beetles (14). Control containers consisted of the following combinations: soil only (3), soil/cedar (3), soil/rat (4) and soil/cedar/rat (4). Burial success and adult survival were recorded. Live weight and dried weight were taken for each larva produce from successful brood balls and the soil mixtures were analyzed. Cedar needles did not have a significant difference to the soil nutrients in each treatment type. Experimental containers with larva had significantly higher average soluble salt content when compared to all other treatments ($df = 3$; $p < 0.001$). There was significantly higher nitrate level in the containers with beetles and rats, especially those with larval broods ($df = 3$; $p < 0.05$). Experimental containers with successful broods had significantly higher levels of phosphorus compared to soil-only treatments ($df = 3$; $p < 0.05$) and potassium was significantly higher in treatments containing beetles ($df = 3$; $p < 0.05$). It appears that *Nicrophorus* species may improve soil fertility while rearing broods.

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CHAPTER I

LITERATURE REVIEW OF THE SILPHIDAE, INCLUDING THE AMERICAN BURYING BEELTE, *NICROPHORUS AMERICANUS*

INTRODUCTION

Background

Silphidae and subfamilies are common in temperate regions of the globe (Ratcliffe, 1996). They are rare and almost entirely absent in tropical regions likely because of competition by other insects including flies and ants, along with some mammals and birds (Ratcliffe, 1996; Lognay et al., 2011). Heat may also play a role with increased rates of carcass decomposition (Scott, 1998). Nicrophorinae is less widely distributed than Silphinae, which appear to be more tolerant of warmer climates and use maggots for larval nutrition (Lognay et al., 2011).

First described by Olivier in 1790, *Nicrophorus americanus* Olivier, the American burying beetle, is a member of Order Coleoptera: Family Silphidae (USFWS, 1991). Carrion beetles participate in the important ecological role of scavenging and utilizing decaying organic matter by utilizing nutrients found in small vertebrates and birds (Lomolino, Creighton, Schnell, & Certain, 1995; Walker & Hoback, 2007). In doing so, carrion beetles reduce the spread of microorganisms and the number of fly species (Hoback & Conley, 2014). American burying beetles (ABB) are the largest North American members of the Family Silphidae, ranging from 30 to 35 millimeters in length as adults (Holloway & Schnell, 1997). They can be readily identified by their characteristic shiny, black body, red-orange pronotum and frons, and four-scalloped, red-orange marking on their elytra (Ratcliffe & Jameson, 1992; USFWS, 2014).

Family Silphidae is comprised of 13 genera and 208 described species that are found worldwide. North America has eight of these genera and 30 species (Ratcliffe, 1996). The family is divided into two subfamilies: the Silphinae and the Nicrophorinae, which contains one genus, *Nicrophorus*. The genus *Nicrophorus* has about 85 species spread across Europe, Asia, North and South America; however, most species occur in Europe and Asia. There are 15 species in North America (Ratcliffe, 1996). The genus *Nicrophorus* are known as burying beetles and have been extensively studied for three reasons. First, the ecological service they provide as scavengers; second, their behavior of biparental care; and thirdly, the designation as a federally endangered species of the American burying beetle (ABB), *Nicrophorus americanus* (Lingfelter, 1995).

Because of the decline of American burying beetle, the U.S. Fish and Wildlife Service (1991) developed a recovery plan focusing on monitoring and protecting remaining populations in the wild. Surveys have been conducted to better understand the suitable habitat for ABB. Species distribution models have been employed to better understand ABB distribution, environmental requirements, and design land conservation, detect new population, and calculate future habitats in regards to climate change (Ortega-Huetra & Peterson, 2004; Pearson et al., 2006; Camarero et al., 2005; Anderson, 2003;

Costa et al, 2008; Pearson et al., 2006; Berry et al., 2002). Crawford and Hoagland (2010) utilized this method to create a map of eastern Oklahoma to predict ABB habitat and range. Through efforts like these, predictive habitat maps may provide insight for conservation by determining suitable habitats for the ABB. However, they stress that additional research is still needed to determine ABB critical habitat. The researchers noted that since the beetle is currently considered a habitat generalist, habitat requirements are difficult to model. Research is on-going in order to better understand ABB habitat requirements (Leasure & Hoback, 2017).

Life History

The ABB relies on vertebrate carcasses for feeding, breeding and raising their offspring. Ideally, carcasses between 80-100 g and up to 200 g are used for breeding and rearing offspring, while other vertebrate carcasses are utilized for feeding (Trumbo, 1991; Kozol, Scott & Traniello, 1987). Living approximately one year, burying beetles are nocturnal and have a remarkable sense of smell for detecting carrion (Milne & Milne, 1976; Ratcliffe, 1996). Nicrophorinae find carrion with chemoreceptors on their antennal club (Kalinová et al., 2009). Burying beetles can locate a carcass within a day of death from several kilometers away (Jurzenki et al., 2011).

In 1933, Pukowski hypothesized that a male beetle first finds the carrion, then, standing on its head, emits a pheromone that attracts a female. Competition for viable carcasses can be extreme, with a single mated pair eventually claiming it (Trumbo, 1991). Recent studies show that male burying beetle behavior differs based on carcass size and resources held by the individual (Beeler, Rauter, & Moore, 1999). Without carrion, smaller males released more pheromones than larger males. When carrion was available, smaller males decreased calling, while larger males increased their frequency. Researchers concluded that these changes in behavior are based on the cost and benefits of attracting mates and competitors, which vary for males of different sizes (Beeler, Rauter, & Moore, 1999). The largest of each sex typically claims the carrion and buries the carcass (Pukowski, 1933; Suzuki, 2000; Pettinger et al., 2011). Communal breeding may take place for some *Nicrophorus* if the remains are large enough; however, this occurrence is rare (Scott, 1997). If a carcass can support a greater number of offspring than a single female burying beetle can produce, a male may attempt to attract a second mate to increase fecundity (Eggert & Sakaluk, 1995).

Once arriving at the carcass, the mating pair will evaluate its size by attempting to move it (Ratcliffe, 1996). If it is determined too large to bury, the carcass can still be used as a food source for the pair. If the carcass is determined suitable for burial, the pair may relocate the remains up to several meters if the soil is too hard or unsuitable for burial (Ratcliffe, 1996).

Burying beetles are social and both parents participate in caring for their young (Lomolino et al., 1995). Both sexes work to conceal and defend vertebrate carcasses underground to later be used by their offspring (Scott, 1998). Recent models suggest that bi-parental care is so strong that when one partner reduces its levels of care, the other compensates for the loss by increasing activity (Creighton, Smith, Komendat & Belk, 2014). Both parents have the capacity to take on all parental care in the absence of the other (Creighton, Smith, Komendat & Belk, 2014). In all burying beetle species, egg laying and larval development take place in the spring, summer, or fall (Smith, 2002).

To make the carcass ready for their larvae, the parents remove the hair or feathers, roll the animal into a ball, bury the remains, and then apply anal and oral secretions to prevent decay (Ratfcliffe, 1996; Scott, 1998). Using their heads, the beetles force soil upward, displacing it on the sides of the carcass, causing the carcass to sink and bury (Ratcliffe, 1996). The remains may be buried up to 60 centimeters underground (Scott, 1998). Burial typically takes five to eight hours to complete. Burying and preparing the carcass stimulates ovarian development in the female burying beetle (Scott, 1997; Scott & Panaitof, 2004). Once burial is completed, the female then lays her eggs in a separate chamber near the buried carcass and the larvae hatch within 5 to 7 days (Butler, Jurzenski & Hoback, 2012; Creighton, Smith, Komendat & Belk, 2014). The larvae are born altricial, or require parental care and are typically fed by one or both parents (Scott, 1998). Just before the brood hatches, the parents create a small opening in the carrion that they treat with regurgitated oral fluid (Scott, 1998). The larvae can feed directly from this source, or from their parents (Scott, 1998).

Johnston et al. (2012) analyzed the salivary secretions of *Nicrophorus* to determine their role and mechanisms for defense from bacteria and fungi. Janzen (1977) hypothesized that competition between animal consumers and microbes for carrion could result in selection for strategies that allow one to repel the other. *Nicrophorus* beetles and their interactions with carrion and bacteria make them an excellent system to study competition between insects and microbes (Johnston et al., 2012).

Previous studies have reported that *N. vespilloides* larvae pay a fitness cost due to the presence of bacterial competition (Rozen, et al. 2008). The spread of microbial pathogens, along with reducing the quality of the food source by secreting toxic compounds, can be harmful to larvae feeding on a carcass (Johnston et al., 2012). Johnston et al. (2012) analyzed the impact of anal secretions on bacteria by measuring change in colony-forming units in a culture of cells incubated with dilute *Nicrophorus* secretions. They found that lytic activity varied with breeding cycle. Anal secretions taken before a female received a carcass had no lytic activity, while females that had been exposed to carcasses were found to have anal secretions with significant lytic activity. The closer the eggs were to hatching, the stronger the female's lytic activity became. The researchers found that bacterial cell walls lysed when exposed to anal secretions. This suggests that the major component of the *Nicrophorus* secretion is an insect lysozyme. To test the lysozyme impact on larvae survival, larvae were reared on liver coated in anal secretions at varying concentrations, while others were given a control. They found that twice the number of larvae reared on secretion-coated food sources survived when compared to those reared without the secretion (Johnston et al., 2012).

In a similar study, Hall et al. (2011) tested the hypothesis that *Nicrophorus* anal and oral secretions are made up of antimicrobial peptides (AMP). They found that the antimicrobial components reduced bacteria and yeast number that naturally occur in soils used by the beetle. Testing a variety of naturally occurring microorganisms likely to compete with carrion beetles by degrading food resources and how the beetle secretions inhibited bacterial growth, their findings suggest that selection pressure drove the production of antimicrobial secretions. The researchers found that the oral and anal secretions of the beetle inhibited the growth of microorganisms.

A typical brood of ABB is composed of 12 to 15 larvae, but some broods have been recorded to be as large as 35 offspring (Amaral, Kozol, & French, 1997). In the case that a brood is too large, parents may reduce the number of larvae through infanticide. By managing brood size, parents can control the amount of resources available (Scott, 1998). When a carcass is taken over by another pair, or by a stronger member of either of the sexes, infanticide often takes place (Trumbo, 1991). Trumbo (1991) suggests this activity prevents a waste of time, energy, and resources on young unrelated to the victorious individual and allows for a replacement brood. The male leaves the larvae sooner than the female. After abandonment, the larvae separate into the soil to pupate (Scott, 1998; Trumbo, 1991). After approximately two weeks, the larvae pupate and 45-65 days later emerge as teneral adults (Ratcliffe, 1996).

Habitat

Over the past 100 years, ABB populations have declined and disappeared from roughly 90% of their historic range. The majority of this decline is from the eastern half of the United States. As a result, the U.S. Fish and Wildlife Service added the species to the endangered species list in 1989 (USFWS, 1991; Lomolino & Creighton, 1996). Remaining populations can be found on Block Island, Rhode Island, southern Kansas, eastern Oklahoma, central Nebraska, western Arkansas, and southern South Dakota (Kozol, Scott, & Traniello, 1988; Sikes & Raithel, 2002). Sike and Raitthel (2002) proposed eight potential causes for the species' decline: habitat fragmentation, resource competition with vertebrates, competition with congeners, lack of appropriately sized carrion for breeding and feeding, rodenticides and pesticides, light pollution, and pathogens. Schnell et al. (2007) suggest that carcass availability during times of overwintering has significant effects on species survival. The American burying beetle is currently found mostly in areas undisturbed by human activity (USFWS, 1991).

Historically, ABB was found in 35 U.S. states across mostly of eastern North America (Creighton & Schnell, 1998). Because of its wide distribution, the ABB is considered a habitat generalist (Holloway & Schnell, 1997) that utilizes a variety of habitats like forests, prairies and woodlands. Some studies suggest ABB prefer forests with moderate undergrowth and deep, loose soil, while others show association with grasslands (Creighton & Schnell, 1998; Jurzenki et al., 2014). As the largest of the burying beetles, mated pairs require larger carcasses for breeding. Larger carcasses are also more difficult and time consuming to bury. Because of their larger size and need for larger carrion, which is less prevalent, ABB requires a larger home range (Lomolino & Creighton, 1995).

Anderson (1982) hypothesized that declines in ABB distribution are because of a decrease in preferred sized carcasses in areas with deep, loose soil as a result of deforestation and agricultural conversion. Reduction of habitat areas and lack of connection among fragments are the two leading causes of extinction as a result of habitat fragmentation (Wilcove, McLellan, & Dobson, 1986). In a study done in Maryland, Wolf and Gibbs (2004) examined how forest fragmentation and urbanization affects beetle community diversity and ecological function. They found that forest fragmentation greatly reduced burying beetle diversity and abundance. Forest size was the primary factor that explained variations in the beetle community. Researchers concluded that carrion beetle diversity declined along an urban-rural gradient as a result of forest fragmentation and loss. Declines in beetle abundance and diversity correlated with declines in the ecological services provided by the silphids (Wolf & Gibbs, 2004).

Considerable research has been conducted to determine which vegetative habitat best supports ABB. Schnell & Holloway (1996) hypothesized that ABB occur in areas of high abundance of small to medium size mammals, where soil is appropriate for burial. They concluded that ABB occur in many habitats, where these criteria are met. When carrion is available it is likely that the most important characteristic of a viable habitat is soil texture and composition (Scott, 1998). Soil compaction and soil moisture are all directly correlated to the speed of carcass burial and removal from competition (Scott, 1998). Sikes and Raithel (2002) hypothesized that declines in ABB population size are a result of competition for carrion with vertebrate scavengers. Jurzenki et al. (2011) found that opossums fed directly on ABB and suggested that direct predation of ABB may prove to be a limiting factor in some areas of Nebraska.

Because of the decline of ABB, the U.S. Fish and Wildlife Service (1991) developed a conservation and recovery plan. The ABB recovery plan was created after the species was listed as endangered and used the best information available at that time. In 1991, there were two known locations for ABB populations: four counties in eastern Oklahoma and an island located off the coast of Rhode Island (USFWS, 2008). The focus of the recovery plan, “due to the species’ profound decline and uncertainty regarding the reasons for decline” focused on two main objectives: 1) “reduce the immediacy of the threat of extinction” and 2) “improve its status so it can be reclassified from endangered to threatened” (USFWS, 2008). In a five-year review of the recovery plan, it was determined that the recovery criteria did not demonstrate the best and most up-to-date information on ABB and its habitat. The plan failed to improve the species status or reduce the threat of extinction. Reintroduction efforts failed and survey efforts in ABB historic range were unsuccessful in locating additional wild populations (USFWS, 2008). With additional research, the known range of ABB was greatly expanded to include South Dakota, Nebraska, Kansas, Arkansas and Texas (Bedick et al., 2014; Jurzenski et al., 2011). Attempts to re-establish ABB failed in Ohio but have had limited success in New Hampshire and Missouri. In 2015, the American Stewards of Liberty, the Independent Petroleum Association of America, the Texas Public Policy Foundation, and Dr. Steven W. Carothers petitioned for the delisting of the species due to lack of information on the existence and magnitude of threats to the ABB and the efficiency of current recovery plans (USFWS, 2016). Upon reviewing the petition, the U.S. Fish and Wildlife Service concluded there was enough scientific to warrant delisting; however, further review is now underway to determine all potential threats to the species and if conservation efforts have helped reduce those threats (USFWS, 2016). As a result, the U.S. Fish and Wildlife Service is requesting any information on the species' endangerment and other possible threats for status review. In the interim, conservation is still underway and research on going. ABB is still a federally endangered species.

Research in Oklahoma

In Oklahoma, monitoring wild populations and habitat mitigation in the form of conservation banks is the primary action. In other states, monitoring of wild and captive populations is implemented, along with protection of existing habitats, breeding programs for future reintroduction purposes, and in some areas, reintroduction of the ABB

(USFWS, 2016). Surveys have been conducted to better understand the suitable habitat for the ABB. Species distribution models have been developed to better understand ABB distribution, and environmental requirements, and to design land conservation models, detect new populations, and calculate future distribution in regards to climate change (Leasure & Hoback, 2017). Crawford and Hoagland (2010) utilized this method to create a map of eastern Oklahoma for potential ABB habitat and range. Through efforts like these, predictive habitat maps may provide insight for conservation by determining suitable habitats for the American burying beetle. However, they stress that additional research is still needed to determine ABB critical habitat in Oklahoma. The researchers noted that since the beetle is currently considered a generalist, the species habitat requirements are difficult to model. More research is needed in order to better understand ABB habitat requirements.

Originally, Anderson (1982) hypothesized that ABB relied on habitats with deep, loose soil where it would be easiest to bury the large carcasses they rely on for feeding and breeding. He attributed the decline of the population to the deforestation of eastern deciduous forests in North America, which were rich in loose, deep soil.

In the summer of 1991, a population of ABB was discovered on the Cherokee Wildlife Management Area, adjacent to Camp Gruber, in northeastern Oklahoma (Creighton, Vaughn, & Chapman, 1993). Thirteen sites were surveyed between July 10, 1991 and October 4, 1991. Study plots were located in three habitat types: grassland, bottomland forest areas, and oak-hickory forests. ABB showed a significant difference in numbers caught among the different habitat sites. With most found in oak-hickory forests with a relatively low number found in bottomland forests. Higher numbers were also captured in grasslands when compared to bottomland forest, where foraging could be hampered by denser undergrowth (Creighton, Vaughn, & Chapman, 1993).

Further studies took place and in 1992, when Lomolino et al. (1995) tested the hypothesis that ABB is a habitat specialist when searching for food. Traps were set in grassland and forested sites on Camp Gruber, Oklahoma. Niche breadths were calculated for *N. orbicollis*, ABB, and *N. marginatus*, the three most common species found (Lomolino et al., 1995). ABB demonstrated the broadest niche and was the only species recorded from all habitat categories. Lomolino et al. (1995) also discovered that trapping success of ABB was not significantly correlated with soil depth. As a result, Anderson's (1982) suggestion that ABB are contained to habitats with deep soil was not supported (Lomolino et al., 1995). However, the authors found correlations with soil types. ABB increased with higher percentages of sand and declined with higher percentages of clay and silt (Lomolino et al., 1995). The authors concluded that ABB was a generalist in its search for food, but was likely to be more selective of habitats in regard to burial and breeding.

In June 1994, Lomolino and Creighton (1996) studied the abundance of beetles breeding in grasslands and upland forests at Camp Gruber and the Cherokee Wildlife Management Area in Oklahoma. They found that habitat selectivity was high for all species of burying beetles. Sites having moderate to well-developed forests with moderate to deep soils, and moderate shrub cover, had the most abundant populations (Lomolino & Creighton, 1996). They continued their study in the Tiak District of the Ouachita National Forest in southeastern Oklahoma where they found *N. orbicollis, N. tomentosus* and ABB to display distributions that coincided with habitat conditions. ABB displayed a noticeable avoidance of clear-cut or deforested habitats, and a higher affinity for mature forested sites (Lomolino & Creighton, 1996). The authors concluded that the breeding success of the ABB was lower in grassland habitats when compared to forested habitats. The results were attributed to the difficulty in burying carrion in grassland areas where soil was compact and lacked ground litter.

The hypothesis that deforestation is correlated with declining beetle populations was never directly tested (Creighton, et al., 2007). In 1995, the U.S. Forest Service removed trees located in an area of the Ouachita National Forest, along with a site previously surveyed for ABB, where it was abundant. Providing an opportunity to test the deforestation hypothesis directly. In the area where deforestation occurred, ABB disappeared, but it remained abundant in the sites nearby where disturbance was minimal (Creighton et al., 2007). Researchers provided two possible explanations for the disappearance of ABB. The first potential explanation was the beetles were killed during the soil disturbance as a result of the tree removal and new individuals didn't have time to repopulate. The second potential explanation was the adult beetles chose to avoid the new clear-cut area and relocated to less disturbed habitats (Creighton et al., 2007).

To further study ABB and forest habitats, a breeding study in various forest habitat types was conducted at the Cherokee Wildlife Management Area (Smith, 2009). Investigators found that the likelihood of burial varied with forested habitat types, but soil proved important (Smith, 2009). Burial occurrence was lowest in American elm-chestnut oak-hackberry forests and two times higher in the other two habitat sites composed of post-oak blackjack oak-hickory and post oak-winged elm. Regardless of habitat, burial was most likely in soils with minimal compaction measured at a depth of 7 centimeters.

This depth corresponds with the calculated average depth of burial chambers (Smith, 2009).

Research in Nebraska

In Nebraska, the American burying beetle has been located in three areas: the Loess Canyons in south-central Nebraska, the Sandhills in the north-central Nebraska (Jurzenki et al., 2011) and in the northern prairie ecoregion extending into South Dakota (Jenkins et al., 2018) In 1969, six specimens of ABB were collected in the Wess Canyons (Ratcliffe & Jameson, 1992). The Sandhills are the largest grass-stabilized sand dunes in the world and are used primarily as grazing land. According to Ratcliffe (1996), contrary to the early hypothesis that ABB was associated to eastern deciduous woodlands, greater populations of beetles have been found in grassland prairie, scrubland, and forest edges.

Bishop et al. (2002), studied the effects of soil texture and land use in Kearny County, Nebraska. Five soil types: alluvial, loam, sand, loess, and mixed sand and loam, were tested for carrion beetle abundance, along with three land use types: river, range, and agricultural (Bishop et al., 2002). The results supported the hypothesis that soil composition is a key component in determining burying beetle habitat. Nine of the eleven species tested, showed a preference for a specific soil type. Five species, O. *inequale, Necrophila. americana, O. novaboracense, N. orbicollis,* and *N. pustulatus* were all found almost entirely in undeveloped riparian areas with alluvial soil.

Varying grass species were also tested to determine effects on *Nicrophorus* burial of carrion by *N. marginatus* (McPherron, 2011). Bare soil, smooth brome grass, needle-and-thread grass, and little bluestem grass were tested to determine effects on carcass burial. Burying beetles were forced to relocate carcasses from the smooth brome grass habitat more frequently than other grass habitats. However, though burying beetles buried fewer carcasses and moved carcasses most often away from smooth brome grass, no significant effects on grass species on burial behavior were found (McPherron, 2011).

In 1994, a large population of ABB was found in south-central Nebraska near the town of Gothenburg (Peyton, 2003). As a result, between 1995 and 1996, field surveys took place to determine the range and density of this discovered population (Bedick et al., 1999). Five counties (Dawson, Gosper, Phelps, Lincoln, and Frontier), centered on Gothenburg were tested. The areas of the study encompassed the Loess Hills, made up of a highly erodible, loess/sand mixture, featuring few trees, sparsely human population, and used primary for cattle grazing. Their goal was to determine if the Gothenburg population exceeded 500 individuals. Meeting the 1991 U.S. Fish and Wildlife Service's conditions to become the third, self-sustaining breeding population in the Midwest Region (Bedick et al., 1999). The Gothenburg population was found to be one of the largest remaining populations of ABB (Peyton, 2003).

Bedick's research provided further evidence that fragmentation is associated with ABB decline. In altered and disturbed habitats around Gothenburg, few ABB individuals were found (Bedick et al, 1999). It was noted that surveys of traps located alongside cornfields occasionally produced ABB. Researchers determined that while agricultural practices are likely to not be the sole source of decline, agriculture may influence carrion availability. Habitat fragmentation likely results in a decrease in carrion and an influx of vertebrate scavengers (Bedick et al., 1999; Jurzenski & Hoback, 2011).

Life Span

The life history of ABB is similar to other burying beetles species (Scott & Traniello, 1987; Kozol, Scott, & Traniello, 1988; Wilson & Fudge, 1984). ABB are active in the summer months and burrow underground for the winter (USFWS, 2014). Teneral, or freshly emerged beetles, usually eclose late in the fall, over-winter as adults, and make up the breeding population the following summer (Kozol, 1990). Studies suggest that over-wintering is a significant result of beetle mortality (Bedick et al., 1999).

In Nebraska, ABB are univoltine and it appears unlikely that adults live past a single year (Bedick et al., 1999). In contrast to the beetle's lifecycle in Nebraska, Lomolino and Creighton (1996) found that some beetles will produce a second set of offspring in Oklahoma. In the laboratory-based study, five of eight ABB pairs continued produced a second brood in the same year; thus, the Oklahoma ABB populations may be able to have two generations in a year.

In Oklahoma, ABB are active from mid-May to the end of October (USFWS, 2014). The reproductive process takes approximately 50-60 days (Kozol, 1990). In Nebraska, most ABB appear in early June and by the start of July, are underground with a carcass and their offspring. By early August, the new tenerals and mature senescents have been found in Nebraska survey traps (Bedick et al., 1999; Jurzenki et al., 2011).

Populations for ABB fluctuate because of mortality, availability of carrion and effects of weather (USFWS, 2014). The lifecycle of an ABB is one year, so population rates are highly dependent on the reproductive success of the year prior. As a result, populations may be cyclic in response to factors including carrion availability, weather, and disease (USFWS, 2008).

To survive cold temperatures experienced in temperate climates, insects that do not migrate utilize one of two strategies: freeze avoidance, where insects die upon freezing, or freeze tolerance, where they can survive the formation of internal ice (Bale, 2002; Sinclair, Addo-Bediako, & Chown, 2003). Like many insects of the temperate regions, burying beetles are freeze-avoidant (Schnell et al., 2008). To survive winter months, behavioral and physiological changes take place, including locating a site at which to overwinter, increasing fat content, and reducing body water content (Bale, 2002). In addition, to live through colder months, burying beetle must burrow beneath the frost line to avoid freezing (Hoback & Conley, 2014). Frost line depth also varies from season to season and by location.

Schnell et al. (2008) attributed overwintering survival to food availability in the form of a carcass. They found that overall, 59.6% of 104 ABB tested survived winter. However, 77.1% of the beetles that were provided a rat upon over-wintering survived, while only 44.6% of beetles not given a rat, survived. They found no significant evidence for survival and habitat type, or survival and body size or gender; yet, the age of the beetle proved to be a crucial predictor of survival. Older beetles had a high rate of survival, but only if the not provisioned with a rat (Schnell et al., 2008). However, in this study winter temperatures remained above freezing. Potentially, ABB remain active across longer time periods in Oklahoma.

Additionally, soil enrichment within the vicinity of the carcass leads to changes to the local plant community (Parmenter & MacMahon, 2009). Collins (1970) study found that herbaceous plants found within 20 centimeters of a decaying rat carcass in a temperate oak forest grew denser and taller for the following 18 months, suggesting a combination of soil-enhancing effects from carcass leaching and soil disturbance by insects associated with carrion.

OBJECTIVE

The objectives of this project are to investigate the seasonal activity and use of habitat by ABB at Camp Gruber. By conducting field surveys of the ABB, sampling soil moisture and percent relative humidity levels, throughout the season, this research will provide a better understanding of how the species utilizes a patchy environment. The results will aid conservation of this endangered species and assist in recovery plans.

Habitat Association

1. Conduct field surveys of ABB at savannah, forest, and grassland sites to determine if ABB utilize a specific habitat across the season.
2. Measure the temperature and relative humidity of savannah, forest, and grassland habitats to determine correlations between habitat characteristics and ABB association.

Soil Moisture and Percent Relative Humidity of Habitat Types

1. Measure the soil moisture, percent relative humidity, and permeability of savannah, forest, and grassland habitats to determine if correlations can be found to ABB occurrence

Effects of Burying Beetles on Soil Nutrients During Brood Rearing

1. Analyze the effects *N. marginatus* brood rearing has on the fertility of the surrounding soil

2. Research possible effects Eastern redcedar (*Juniperus virginiana*) has on *N. marginatus* survival and brood success

CHAPTER II

HABITAT USE BY THE AMERICAN BURYING BEETLE *(NICROPHORUS AMERICANUS)* ACROSS SEASONS AT CAMP GRUBER TRAINING FACILITY, BRAGGS, OK

ABSTRACT

Within the past century, the American burying beetle, *Nicrophorus americanus* (ABB) disappeared from roughly 90% of its historic range of 35 central and eastern U.S. states. As a result, the U.S. Fish and Wildlife Service listed the species as federally endangered in 1989. Although there are many proposed causes for the decline in ABB populations, the most widely accepted is that the population declined as a result and alterations in habitat and changes in carrion availability. At this time, ABB is hypothesized to be a habitat generalist. I conducted field surveys at Camp Gruber Training Center located in northeastern Oklahoma to determine if ABB utilize the same habitat throughout the season. I sampled three habitat types: savannah, grassland, and forested areas. To compare across seasons, ABB were sampled from July 16, 2016 to November 12, 2017. I trapped a total of 1,756 unique individual ABB along with 10,448 individuals of 9 other Silphidae species. Few diurnal beetles were captured while nocturnal beetles were more abundant. During the sampling period, I found no significant difference for the numbers of ABB found in each habitat type. Over all trapping, forests had 6.0 ± 0.8, grasslands had 8.1 ± 1.2, and savannah had 8.2 ± 1.3 ABB per trap night. This suggests that while ABB may show a preference for a certain habitat during a

specific time of the year, across their active season, ABB are associated with all habitats at Camp Gruber. Measures of temperature and relative percent humidity were similar among the tested habitat types as was soil moisture.

These data support the conclusion that ABB is a habitat generalist and is moving among the patches of different habitat types at Camp Gruber.

INTRODUCTION

Most burying beetles (Silphidae: *Nicrophorus*) breed on the carcasses of small vertebrates ranging from 80 to 100 grams (Trumbo, 1991). In North America, the nocturnal ABB is the largest member of the *Nicrophorus* genus (Creighton & Schnell, 1998) with adults weighing up to two grams. To attain these sizes, ABB need larger carcasses weighing between 100 and 300 grams (Kozol et al., 1988), which are likely to be more difficult to bury when compared to smaller carcasses (Lomolino & Creighton, 1996).

Carcasses are a rare and unpredictable resource, especially those of larger size, and thus, organisms using larger prey may also utilize a greater diversity of habitats. Carcasses are often a limited resource and both intraspecific and interspecific competition among adult beetles can be intense (Scott, 1998). The wide distribution of other *Nicrophorus* species across North America has led to niche partitioning likely in response to interspecific competition (Lomolino et al., 1995). ABB is believed to require a larger home range than other species (Lomolino & Creighton, 1996) and ABB have been documented to disperse more than 1 mile per night across their range (Creighton & Schnell 1998, Bedeck et al. 1999, Jurzenski et al. 2014).

Anderson (1982) hypothesized that declines in ABB population range are due to a decrease in adequately sized carcasses in areas with deep, loose soil as a result of deforestation and habitat conversion. Reduction of habitat areas and lack of connection among fragments are the two leading causes of extinction as a result of habitat fragmentation (Wilcove, McLellan, & Dobson, 1986). Because of its former wide distribution encompassing 35 U.S. states, the ABB is considered a habitat generalist (Holloway & Schnell, 1997). Unfortunately, this makes ABB habitat requirements difficult to model, and there is no designated critical habitat for ABB (USFWS, 2014).

Remaining western ranges with high populations of ABB are associated with a mixture of various woodland or forest types or prairie (Walker & Hoback, 2007; Leasure and Hoback 2017). Creighton et al. (1993) hypothesized that American burying beetle prefer well-developed forests with understory shrubs and moderate to deep soils. He further suggested that before their decline, ABB were most often in oak-hickory forest. In contrast, Walker and Hoback (2007) found more ABB in open grassland habitats rather than cedar-dominant habitats. They concluded that the closed canopy cover of dense stands of redcedar made getting to carrion difficult for the beetle, along with reducing wind flow, which hindered carrion detection. Unfortunately, the lack of understanding of the distribution of American burying beetle makes it difficult to successfully prioritize areas of conservation. Though ABBs utilize a variety of habitat types there may be other underlying factors within an ecoregion that influence ABB occurrence (Jurzenki et al., 2014; Leasure and Hoback 2017).

This study had three objectives: 1) test habitat associations of populations of ABB in Oklahoma; 2) estimate population sizes; 3) document seasonal activity and identify environmental factors that are associated with activity. Specifically, I tested the hypothesis that ABB is a habitat generalist and occurs equally in grassland, forest, and savanna conditions at Camp Gruber in north-central Oklahoma. I also tested the hypothesis that ABB has a defined seasonal pattern in Oklahoma and that its numbers are influenced by habitat environmental conditions including temperature and moisture.

Materials and Methods

Sampling

Sampling for ABB was conducted in accordance with U.S. Fish and Wildlife Services (1991) protocol as modified by the USFWS 2014 protocol. To avoid interference by predators and because of the presence of bedrock in areas of Camp Gruber Training Center, pitfall traps were elevated off the ground through attachment to trees or t-posts by bungee cords (Leasure et al., 2012). Five gallon (18.92 liter) plastic buckets were used for the above ground traps. Roughly 5-8 centimeters of damp peat moss was placed in the bottom of bucket to allow for captured beetles to burrow, avoiding high temperatures, competitors, and low moisture levels. To prevent buckets from flooding in the event of rain and to disperse carrion scent, holes were drilled vertically every 5 centimeters and diagonally every 7.62 centimeters, into the sides of the bucket, to allow for drainage. To prevent beetle escape and additional debris and rainfall from entering the trap, a rectangular piece of plywood (70 centimeters by 40 centimeters) was secured to the top of the trap by j-hooks. The board also acts as a landing area for burying beetles to enter the trap. To allow for entrance into the bucket an 18 centimeters diameter hole was located in the center of the plywood lid with a fitted funnel that leads into the bucket. Beetles that enter are unable to escape from the trap. Attached above this opening is a 23 centimeters diameter Frisbee disk supported by four nails housed in plastic tubing. The Frisbee works to shield the entrance to the trap from debris and rainfall, as well as serve as a notice of the trap's purpose to citizens who encounter the trap. Each disk has a brief statement that explains the purpose of the trap.

All traps were baited with previously froze 275-375 gram laboratory rats (*Rattus norvegicus)* obtained from RodentPro.com. The rats were thawed and left to age for approximately 4 days at environmental temperatures. An open bait design was used, allowing beetles to access the bait when in the trap.

All captured silphid beetles were identified to species. All captured ABBs were marked using a cauterizer (Jenkins et al., 2015) to follow recaptures and all captured ABBs were released in individual holes that were then covered with loose vegetative material approximately 50 meters from the trapping site once data were recorded. For ABB, age (teneral or senescent) and sex were recorded.

Study Site

The Oklahoma Army National Guard's (OKARNG) Camp Gruber Training Center is located 22.5 km southeast of Muskogee, Oklahoma. The site encompasses more than 33,027 acres or 140 square km (Figure 1) (Oklahoma National Guard, n.d.). Camp Gruber Training Center serves as a training base for weekend training and summer field exercises. The Oklahoma National Guard, Department of Homeland Security, and federal and state law enforcement agencies utilize the training site for its wide variety of training activities, including firing ranges for weapons like pistols, machine guns, mortars, and demolitions (Army National Guard, 2016). There are also Military Operations in Urban Terrain facilities, land navigation courses, and urban and ambush assault courses. The property is also used as a wildlife management area and houses an elk, *Cervus canadensis*, population along with deer and other wildlife.

Figure 1. Camp Gruber Training Center military installation map (Oklahoma National Guard). Trap locations indicated by stars.

Table 1. Trap Location GPS Coordinates and habitat designation at Camp Gruber, OK

Trap Site	Latitude	Longitude	Habitat Type
1	35°45'39.0"N	95°09'44.9"W	Savannah
2	35°43'46.9"N	95°12'06.4"W	Savannah
3	35°42'52.9"N	95°12'09.0"W	Forest
4	35°42'41.5"N	95°10'22.3"W	Savannah
5	35°44'21.1"N	95°10'26.7"W	Forest
6	35°45'25.7"N	95°08'20.3"W	Forest
7	35°44'18.1"N	95°08'48.3"W	Savannah
8	35°44'18.1"N	95°08'48.3"W	Grassland
9	35°42'51.5"N	95°08'43.6"W	Grassland
10	35°42'13.9"N	95°09'34.1"W	Grassland
11	35°41'18.6"N	95°08'42.5"W	Grassland
12	35°41'55.2"N	95°07'41.8"W	Savannah
13	35°40'24.1"N	95°07'54.7"W	Forest
14	35°39'11.4"N	95°09'14.3"W	Forest
15	35°38'14.5"N	95°09'36.4"W	Forest
16	35°38'19.1"N	95°10'53.4"W	Savannah
17	35°38'44.9"N	95°10'17.7"W	Forest
18	35°39'22.8"N	95°11'18.4"W	Savannah
19	35°40'27.5"N	95°09'11.0"W	Grassland
20	35°41'26.4"N	95°11'30.7"W	Grassland
21	35°45'16.7"N	95°11'27.0"W	Savannah
22	35°40'58.8"N	95°08'40.8"W	Grassland
23	35°40'45.0"N	95°12'03.4"W	Grassland
24	35°42'38.3"N	95°12'12.4"W	Forest
25	35°42'51.7"N	95°10'54.1"W	Forest
26	35°41'58.1"N	95°10'51.1"W	Savannah
27	35°40'53.6"N	95°10'37.4"W	Savannah
28	35°41'34.1"N	95°09'47.3"W	Forest
29	35°42'44.1"N	95°09'09.8"W	Grassland
30	35°40'00.1"N	95°10'19.8"W	Grassland
31	35°40'22.314"N	95°11'26.322"W	Cantonment
32	35°40'53.544"N	95°11'57.7608"W	Cantonment
33	35°40'44.4396"N	95°10' 57.9396"W	Cantonment

Population Estimates

One 5-day survey was conducted each July utilizing traps to sample the dominant habitat types (savannah, forest, grassland) at Camp Gruber Training Center. Twenty traps were set with a minimum of 6 traps per habitat type. Sampling dates were July 26, 2016 to July 30, 2016 and July 12, 2017 to July 16, 2017. Population size was estimated using the Schumacher and Eschmeyer (1943) method based on multiple samples with marking occurring each day. For the five-day period, the population was assumed to be closed.

Seasonal Surveys

To determine if ABB utilize the same habitat throughout the year, 12 locations were sampled approximately bi-weekly August to October 2016 and April to November 2017 in savannah, grassland, and forested areas with a minimum of three traps per habitat type. Two-day trapping periods took place every other weekend with traps set on Friday afternoon and checked Saturday and Sunday before 10 a.m. Traps were then removed after sampling. Locations varied depending on military activities and hunting season, but all traps were spaced at least one mile (2.2 km) away from each other to increase independence of samples (Bedick et al., 1999). ABB can fly up to 6 kilometers per night (Jurzenski et al. 2014).

Designation of habitat types was determined using Google Earth to assess vegetation type and with the aid of the Oklahoma Military Department personnel. Grassland sites were characterized as having a dominant vegetation of grasses and few or no trees within 50 meters. Savannah sites were characterized as grasslands accompanied by scattered trees and shrubs and forested sites as those composed of completely enclosed canopy and presence of dense understory.

Captures per trap night among habitat types were compared using Analysis of Variance. Sex ratios were compared using Chi-squared goodness-of-fit tests.

Habitat Climate Conditions

Local climatological data were obtained from the nearest weather station at Muskogee Airport found on www.weatherunderground.com. To measure the temperature and relative humidity in different habitat types HOBOware U23 Pro v2 Temperature/Relative Humidity Data Loggers were set at least one trap in each habitat type (forest, grassland, and savannah) and began recording the temperature and relative humidity of the site at 5:00 P.M. on the day the traps were set, until 11:00 A.M. on the day the traps were removed. Readings were gathered every 30 minutes and the average temperature and percent relative humidity was then found for each habitat type.

Soil Conditions

Soil temperature, moisture, and permeability were gathered at all survey site locations while traps were being checked. Soil temperature and moisture data were gathered using an Extech Humidity Content Meter. The probe was inserted into the soil adjacent to the pitfall trap. Soil permeability was determined using a Turf-Tech Infiltrometer. When using the infiltrometer, the double ring cutting blades are inserted into the ground at an approximate two-inch depth. The time it takes for 2.5 cm of water to drain into the soil was recorded at each trap site every time the trap site was used.

Results

Between July 26, 2016 and November 12, 2017, 1,870 ABB were captured. Of those 1,870 ABB, 114 were recaptured throughout the survey season (Table 2). 1,235 of the 1,756 ABB captured were teneral, while 635 were senescent. Across all samples, the population was strongly female-biased with 1,124 females and 632 males captured and was significantly different than the expected 50:50 ratio (Chi-square Goodness of Fit, df= 1, $p < 0.01$).

In addition to ABB, 9 other species of Silphidae were captured (Table 2). Of these, two other night-active species of *Nicrophorus* were captured frequently, *N. orbicollis* (2,723 individuals) and *N. pustulatus* (1,248 individuals). Three additional day-active *Nicrophorus*, *N. tomentosus* (297 individuals), *N. carolinus* (4 individuals), and *N. marginatus* (50 individuals) were rarely encountered.

Among the subfamily Silphinae that use dead animals and maggots for feeding as adults and larvae, the most commonly encountered species was the night-active *Necroides surinamensis* (5,674) followed by day-active *Necrophila americana* (438 individuals) and two *Ocioptoma* species (Table 2).

Table 2. Carrion beetle captures in baited pitfall traps in Camp Gruber Training Center, OK, 2016-2017

Active time		2016-07	2016-08	2016-09	2016-10	2017-04	2017-05	2017-06	2017-07	2017-08	2017-09	2017-10	2017-11	Totals
Night	*N. americanus*	(243)* 225	(234) 228	(66) 65	(64) 61	16	(49) 48	(84) 83	(449) 416	(326) 311	(207) 188	(132) 115	0	(1,870) 1,756
Day	*N. carolinus*	1	0	0	1	0	1	1	0	0	0	0	0	4
Day	*N. marginatus*	1	2	3	5	4	2	12	2	3	4	12	0	50
Night	*N. orbicollis*	101	614	300	176	62	36	163	488	217	401	135	0	2,723
Night	*N. pustulatus*	86	205	62	20	72	32	89	449	127	62	44	0	1,248
Day	*N. tomentosus*	0	3	20	25	0	80	89	4	0	31	44	0	297
Night	*Ne. surinamensis*	437	320	73	13	16	39	526	1,042	963	2,158	31	0	5,674
Day	*Ne. americana*	60	8	5	2	2	8	70	127	104	40	9	0	438
Day	*O. novaboracense*	0	0	0	0	2	1	0	0	0	0	0	0	3
Day	*O. inaequale*	0	0	0	0	6	4	0	0	0	0	0	0	10

*For *N. americanus* during each trapping period, the number in parentheses includes all recaptures, second number is total first-time captures.

Population estimates

During population sampling in 2016, a total of 243 ABB were captured with 18 recaptures (Table 3). In July 2017, 449 ABB were captured with 28 recaptures. Using Schumacher and Eschmeyer's (1943) population-size estimator 1,313 ABB were present in the 10,000 acres sampled with an estimated range between 969 and 1,373 in 2016. In 2017, 2,603 ABB were estimated to be present in the 10,000 acres sampled with a range between 1,883 and 4,388. Some caution should be used in interpreting these results because recapture rates for ABB were less than 10% in both sample periods.

Seasonal surveys

ABB were captured on the first sample day of April 1st, 2017 and as late as October 30th, 2017 (Figure 2). Throughout the season, the rate of ABB captured generally increased during the summer, with a drop in the third week of June. The population continued to increase in fall months with low numbers at the end of September and a large increase on the last sampling date in October (Figure 2).

ABB have a life cycle where adults from the previous year overwinter and then emerge to find carcasses. Successful pairs bury a small carcass and spend approximately 6 weeks rearing offspring prior to the original pair emerging as senescents along with their offspring which are classified as tenerals. The percentage of teneral and senescent ABB was measured by sampling date and revealed early season by adult beetles followed by an increasing number of tenerals (Figure 3). In 2017, peaks of tenerals are observed in July, September, and October (Figure 3).

All sampled habitats were used by ABB across the season. Overall more ABB were captured in grassland habitats than in savannah or forest; however, the differences were not significant (Figure 4).

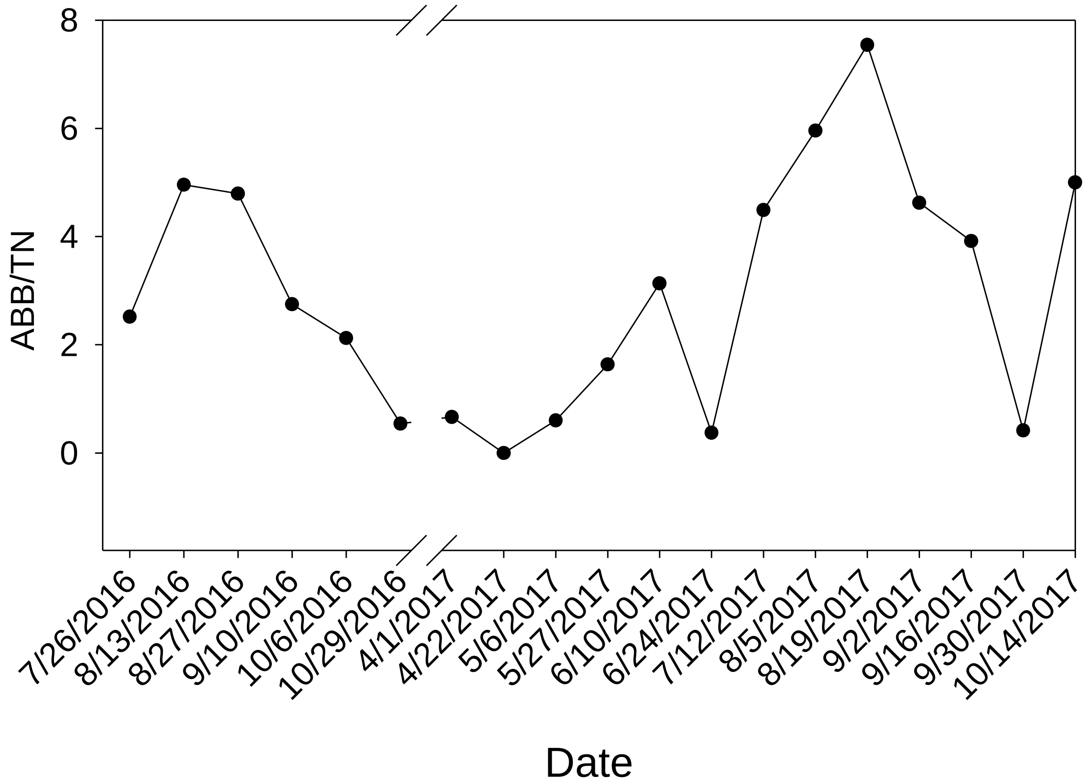

Figure 2. Seasonal activity of ABB per trapping night at Camp Gruber, OK 2016-2017

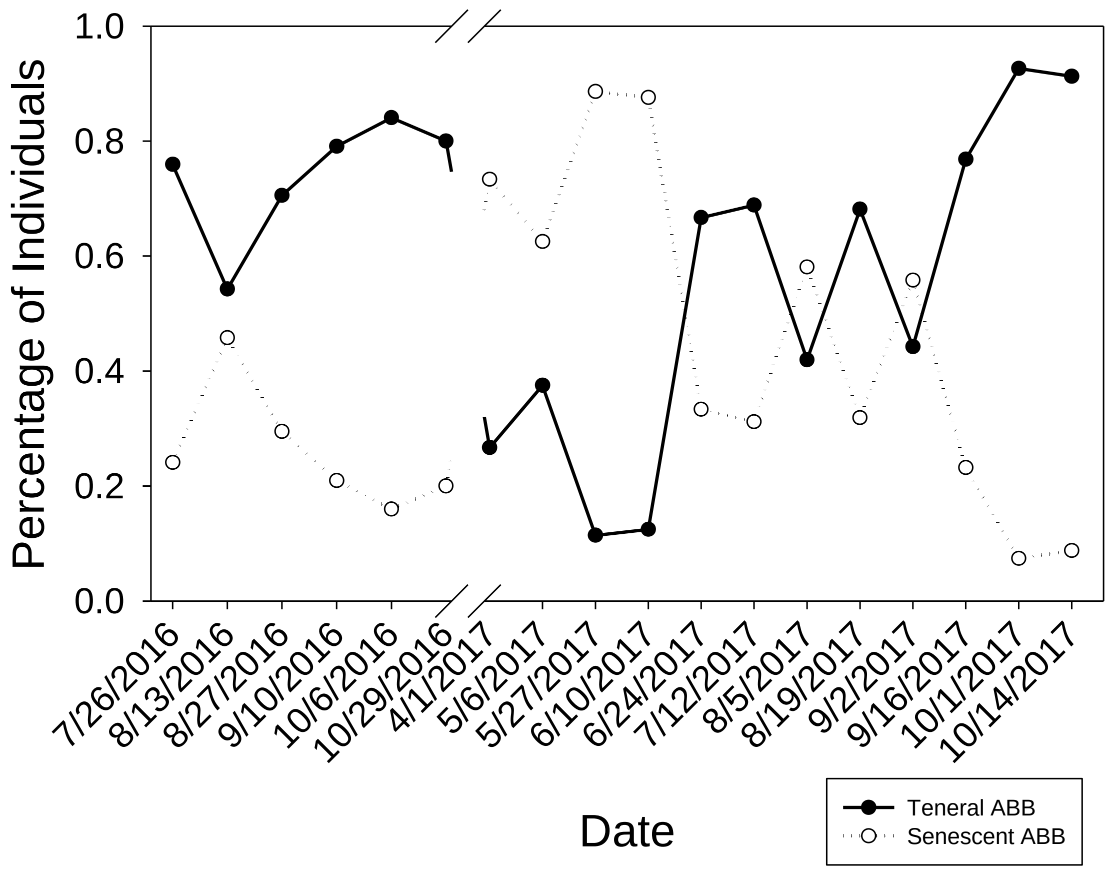

Figure 3. Percent of teneral and senescent ABB per trapping night at Camp Gruber, OK 2016-2017

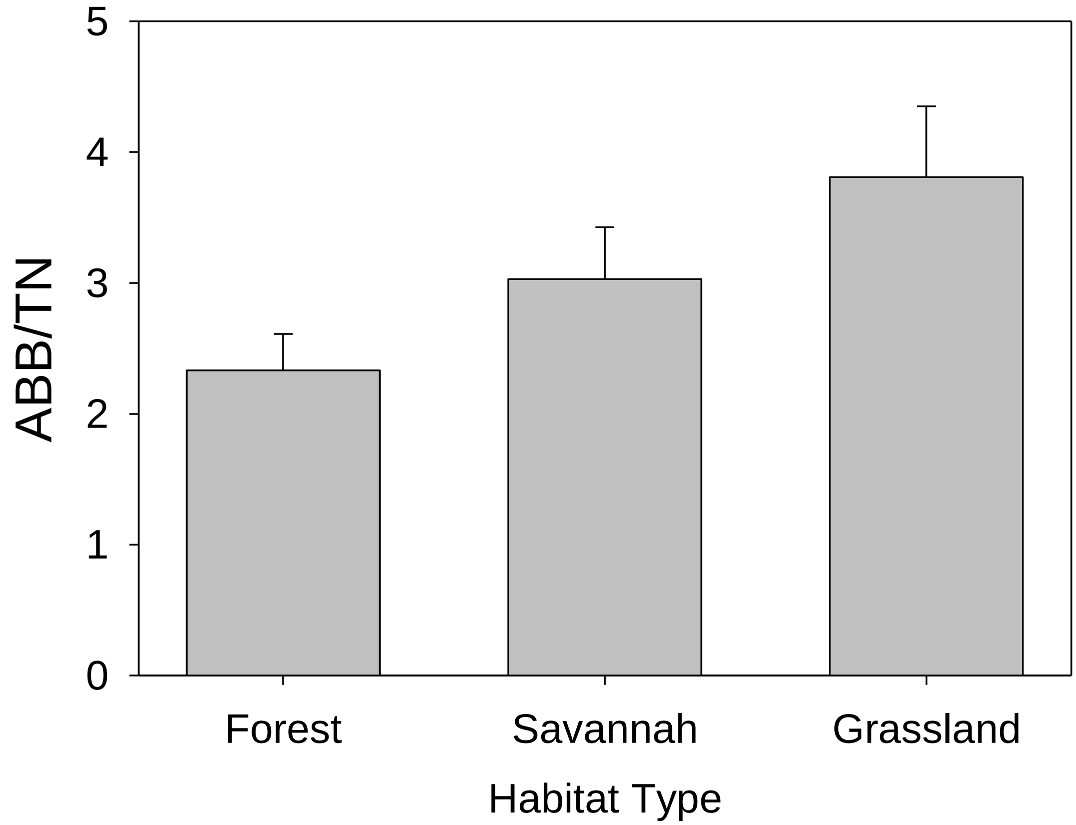

Figure 4. Number of ABB per trapping night at each habitat type across the season at Camp Gruber, OK 2016-2017.

Seasons did not substantially alter habitat use by ABB and there were no clear trends of a habitat preference based on season (Figure 5). Habitat association during the presumed breeding period (May-June) was uniform and variability among traps was greater than differences among traps in different habitats.

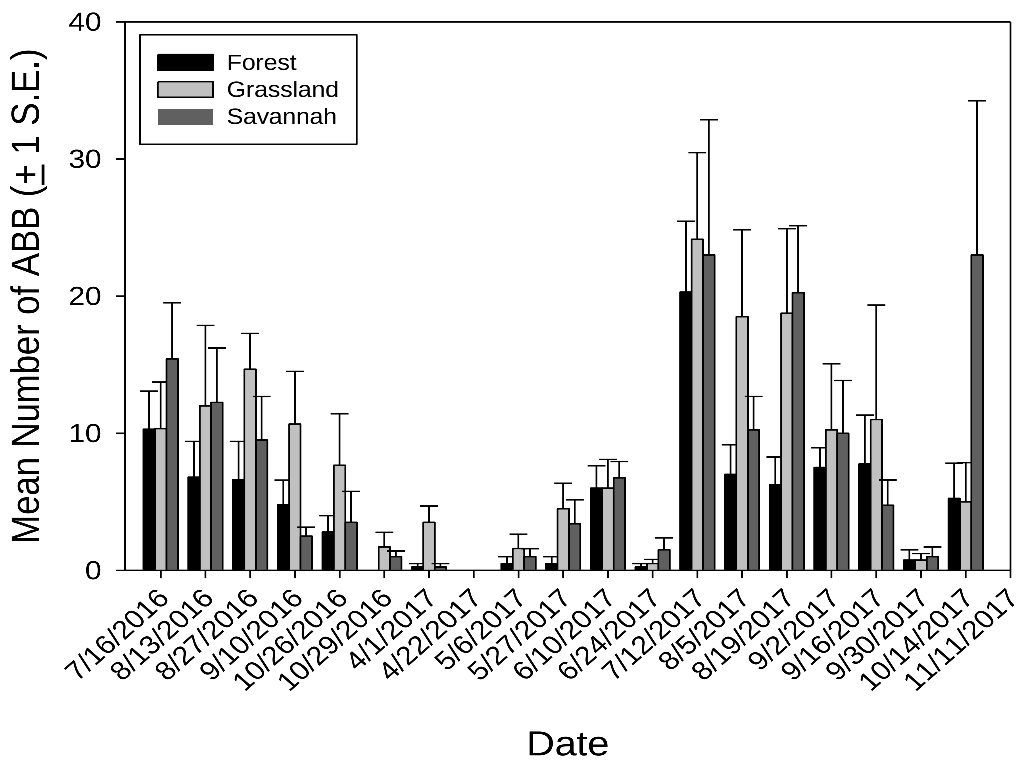

Figure 5. Habitat association of ABB across the season at Camp Gruber, OK 2016- 2017

Habitat Climate Conditions

The minimum and maximum recorded temperature for each habitat type was observed from May 2017 to October 2017. While fluctuations occurred, the air temperature of each habitat type was similar during each sampling period (Figure 6). I compared the maximum air temperature at each habitat type to the number of ABB captured (Figure 7). While the number of ABB varied with by sampling period, the numbers of ABB captured were not strongly associated with habitat temperature. Similarly, I compared the minimum air temperature at each habitat type to the number of ABB captured (Figure 8). The minimum air temperature by date was similar among habitat types and did not correlated with the number of ABB captured.

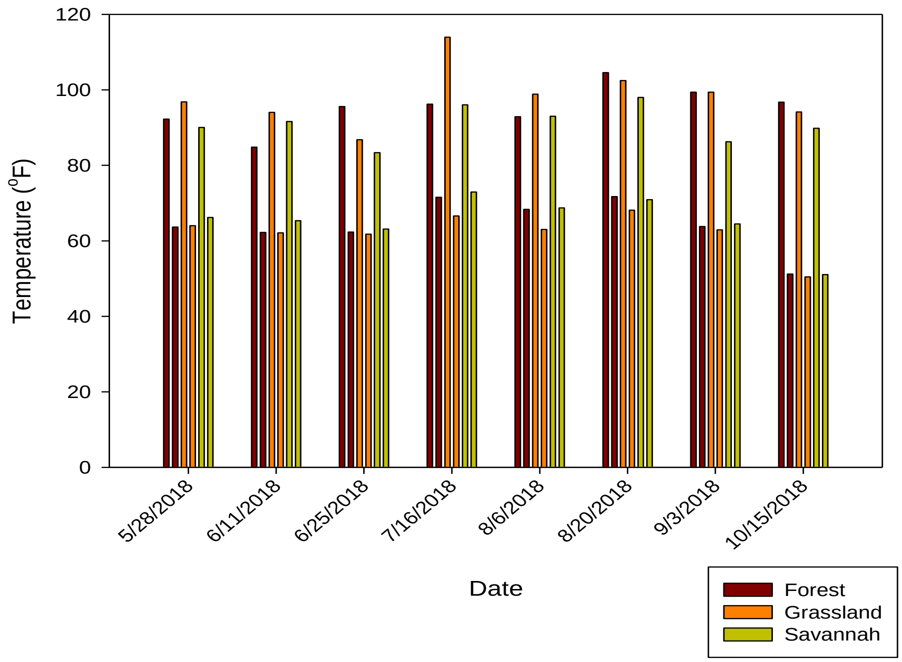

Figure 6. Maximum and minimum air temperature at each habitat type at Camp Gruber, OK 2017

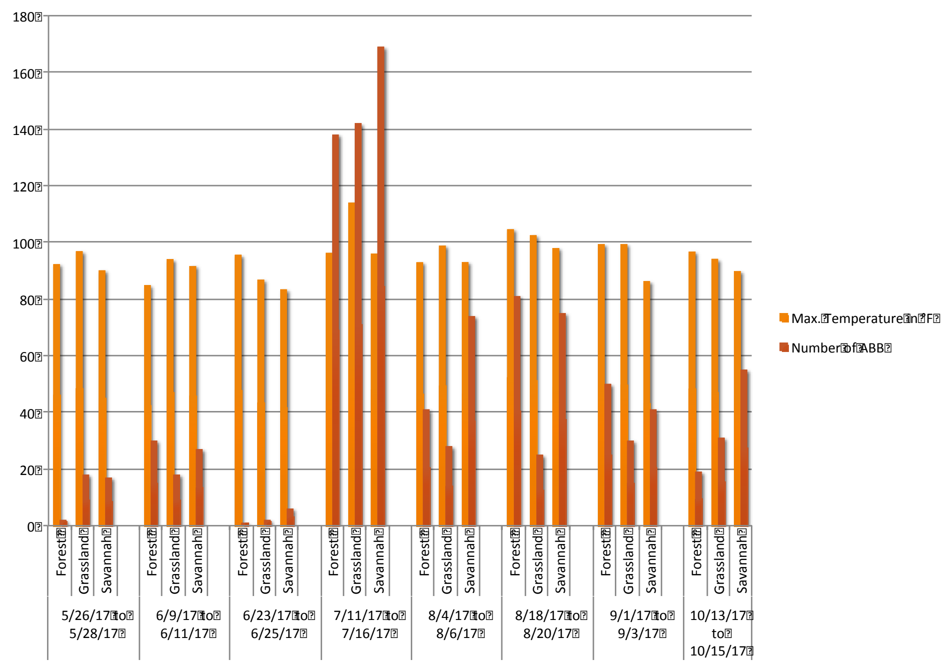

Figure 7. Maximum air temperature and the number of ABB at each habitat type at Camp Gruber, OK 2017

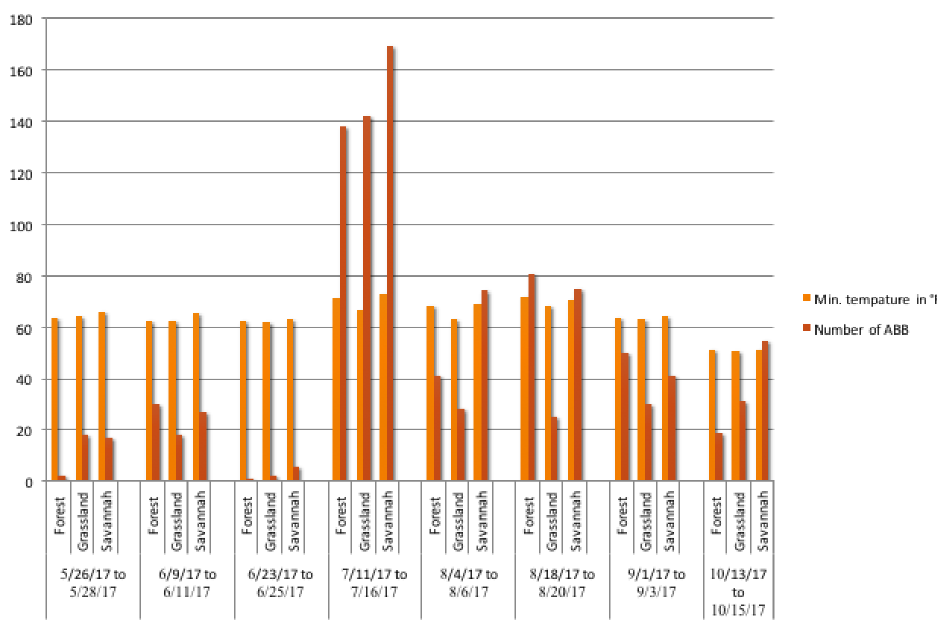

Figure 8. Minimum air temperature and the number of ABB at each habitat type at Camp Gruber, OK 2017

Soil Conditions

The average infiltration rate of 2.5 cm (one inch) of water at forest sites was 85 $\pm$ 29 seconds. Similarly, savannah sites had an average infiltration rate of one inch of water in 64 $\pm$ 33 seconds. Grasslands had the fastest average infiltration rate of one inch of water in 54 $\pm$ 19 seconds. The differences in soil infiltration was significant between forest and grassland sites Kruskal-Wallis ANOVA (df = 2, p= 0.011).

The soil moisture was similar among habitats throughout the season, and significant differences by habitat type were not observed (one-way ANOVA, df = 2; p = 0.76) (Figure 9). The soil temperature at 4 cm depth for each habitat type was also similar during each sampling period. When tested using one-way ANOVA, the soil temperature was not significantly different across the habitat types throughout the sampling period (df= 2; p = 0.33) (Figure 10).

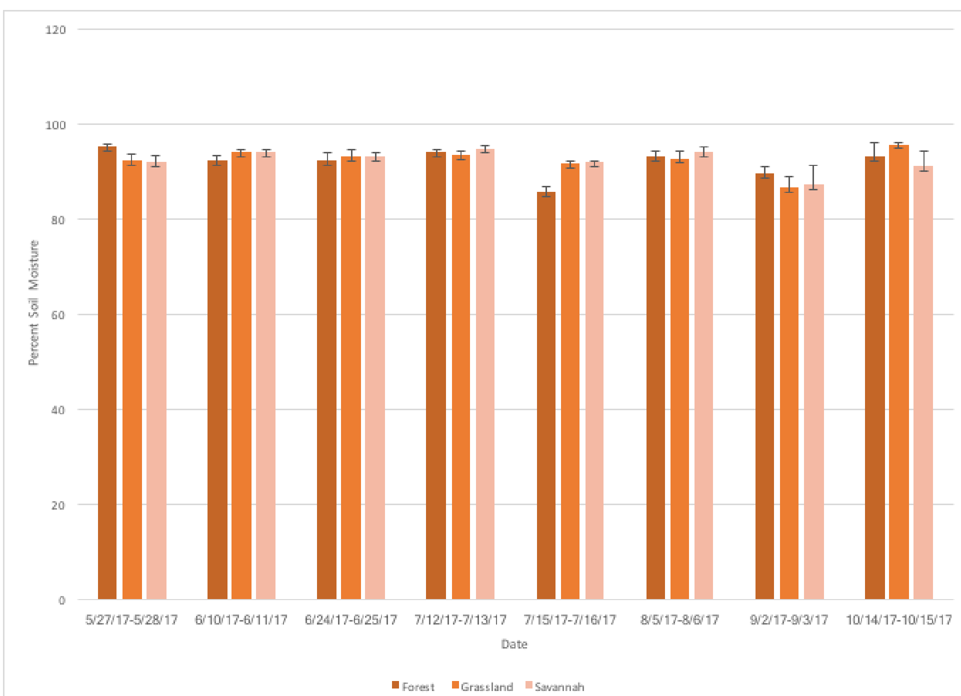

Figure 9. Percent soil moisture at each habitat type at Camp Gruber, OK

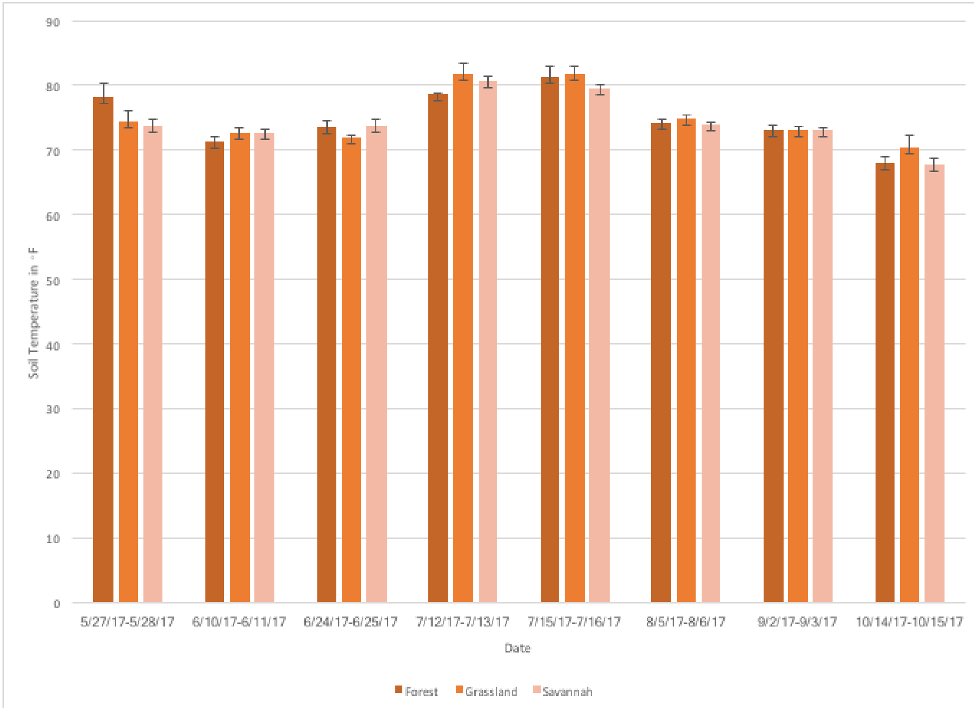

Figure 10. Soil temperature at each habitat type at Camp Gruber, OK

Discussion

Over the sampling years, a total of 1,870 ABB were captured, sex ratios were strongly female based, and teneral ABB were collected throughout the sampling season. Previous studies in Oklahoma have suggested the possibility of double brooding by adult ABB (Lomolino & Creighton 1996). Our data do not allow evaluation of this hypothesis because ABB that are active as adults may breed when a carcass is available and thus produce brood throughout the season. The extended active season observed at Camp Gruber in 2017 (Figure 2 and 3) may have allowed individuals to produce two broods since ABB require approximately 6 weeks for rearing brood (Kozol, 1988). However, the data collected are not sufficient to determine if individuals double-brooded or if later tenerals were a result of adults that did not get carcasses in the early season found suitable carcasses later in the year.

In Oklahoma, the U.S. Fish and Wildlife Service (2014) reports ABB to be active from mid-May to mid- September. During our survey period, we found ABB during our earliest sample on April 1st and as late as October 30th. ABB activity was strongly impacted by the maximum or minimum air temperature, perhaps because the mean air temperature at each habitat type was similar for each sampling date. On the last sample date, when nighttime air temperatures were below 60 ^{o}F, I captured large numbers of ABB (Figure 8), suggesting that the USFWS protocols restricting sampling when air temperatures are below 60 ^{o}F should be re-examined.

During the five-day intense sampling period, mark-recapture results suggested an estimated population size of 1,313 ABB in 2016. In 2017, the population estimate doubled to 2,603 ABB. Caution in interpreting the population estimates should be employed because recapture rates were <10% each year. Low recapture rates despite permanent marks suggests ABB are moving out of trap ranges and likely dispersing to neighboring areas including the Cherokee Wildlife Management Area. In fact, across the entire study, recapture rates remained low, supporting this conclusion.

This study shows that ABB breed and emerge throughout the season (Figure 2). From April to June 2017, senescent ABB emerge from overwintering. As the summer progresses, ABB numbers diminish in June as adult's brood underground. From July to August 2017, a peak in numbers is observed as senescent's emerge from brooding and teneral's eclose. The increase in ABB numbers in October 2017 may indicate a second breeding cycle (Figure 3). The activity of ABB at different life stages differs from previous studies of ABB population dynamics in Nebraska (Bedick et al. 1999). A greater percentage of senescent ABB were captured early in the season. Mid-season (late May, early June) captures demonstrated peaks in both teneral and senescent ABB numbers indicating a transition period where both life stages are present. The senescent's percentage then decreased in late June and early July as mature ABB go belowground to brood. Within six weeks, senescent numbers increase as they complete their breeding cycle. As fall approaches, senescent percentages decline as they die and teneral numbers increase as they eclose and search for carrion. The peak in teneral numbers in October 2017 may indicate a second breeding cycle, or increased activity because of above-normal temperatures.

When the average number of ABB per trap night was compared between the three habitat types across the sampling season (Figure 4), grasslands had the highest capture rates. Though not significant, this suggests that grasslands are utilized more by ABB than forests. Several factors may influence this result, including carrion sources in grasslands or the ability of ABB to detect carcasses (Holloway & Schell, 1997). Tree covers and dense underbrush may inhibit the detection and access of carrion by ABB in forested sites. However, Lomolino and Creighton (1996) have demonstrated that ABB are more successful burying of carrion occurs in forests, a prerequisite to breeding. Follow up studies of breeding of beetles in the three habitat types is warranted.

I calculated ABB/ Trap night across the season to compare with published values for studies conducted at Camp Gruber. Across 2016 and 2017, 3.0 ABB were captured per trap night. In contrast, in 1993, Creighton, Vaughn and Chapman captured 102 ABB with 0.35 beetles per trap night at Camp Gruber. In 1995, Lomolino et al. (1995) reported 215 ABB with 0.10 beetles per trap night. In 1996, Lomolino and Creighton caught 207

ABB with 0.05 beetles per trap night, and in 1998, Creighton and Schell reported 221 ABB with 0.10 beetles per trap night (Table 3).

Table 3. Total published capture rates of *N. americanus* at Camp Gruber, OK in comparison to current study

Reference	Trap Nights	*N. americanus* Male	Female	Total	BTN
Creighton and Schnell 1998	300	110	111	(228) 221*	0.74
Lomolino et al. 1995	260	94	121	215	0.10
Lomolino and Creighton 1996	537			207	0.05
Creighton, Vaughn and Chapman 1993	38			102	0.35
Current study	578	632	1,124	(1,870) 1,756	3.00

*For *N. americanus* during each trapping period, the number in parentheses includes all recaptures, second number is total first-time captures.
*BTN represents beetles for trap night

Higher numbers of ABB captured in the current study could show population increases for the species. Alternatively, increased numbers could reflect changes in trapping protocols. Prior to 2014, Oklahoma trapping protocol used transects of 8 cups baited with small amounts of rotten chicken or gizzards. The use of whole rats in a five-gallon bucket likely increases the trap attractiveness and may explain the observed higher numbers, although Butler et al. (2012) showed that conversion of captures per 8 cup transects to one trap night was equivalent to one bucket trap and are the numbers compared above.

A significantly greater number of female ABB were captured compared to males (Chi-square Goodness of Fit, $p < 0.01$). In previous surveys at Camp Gruber, the sex-ratio was not skewed (Lomolino et al. 1995; Chi-square Goodness of Fit, $p = 0.07$).

Similarly, Creighton and Schell (1998) didn't report a significant difference between male and female ABB captures (Chi-square Goodness of Fit, $p = 0.95$). A potential explanation for the skewed female sex ratio for captured ABB is a *Wolbachia* infection. *Wolbachia* is a bacteria found in roughly 66% of arthropods (Roy, Girondot, & Harry, 2015). The bacteria ensure vertical transmission by transforming populations into predominately females. A female-biased sex ratio is an indication of potential infection (Roy, Girondot, & Harry, 2015). Another possible reason for the skewed sex ratio is that female ABB may eclose more quickly than their male counterparts (Hoback unpublished data), though this wouldn't explain the shift in sex ratios from previous studies to now.

In addition to ABB, other *Nicrophorus* species were captured in greater numbers compared to previous studies at Camp Gruber, though day active burying beetles were far less prevalent than night active species during this surveying period (Table 4). Low numbers of *N. marginatus* and *N. carolinus* may be the result of the prevalence of forest at Camp Gruber as both of these species occur in greater frequencies in grassland sites with *N. carolinus* preferring very sandy soils (Bishop et al. 2002). The higher numbers of both *N. orbicollis* and *N. pustulatus* than previously observed (Table 4) is potentially a concern as both are night active and likely compete directly with *N. americanus* for intermediate sized carcasses.

Table 4. Total published capture rates of other *Nicrophorus* species at Camp Gruber, OK in comparison to current study

Reference	Trap Nights	*N. marginatus*	*N. orbicollis*	*N. pustulatus*	*N. tomentosis*	*N. carolinus*
Lomolino et al. 1995	260	18	2,481	17	68	15
Lomolino and Creighton 1996	537	19	1,674	20	411	18
Current study	578	50	2,723	1,248	297	4

Across the season, ABB did not show habitat preference for one type of habitat tested. This result varies from previous habitat association surveys at Camp Gruber. Creighton, Vaughn, and Chapman (1993) examined the number of ABB found in grassland, bottomland forests, and oak-hickory forest at Camp Gruber from July 10, 1991 to October 4, 1991. They found that more ABB were found in oak-hickory forests than grasslands ($p < 0.001$) or bottomland forests ($p < 0.001$). However, when Lomolino et al. (1995) compared the niche breathe of ABB at Camp Gruber, open grassland, bottomland forests, and mixed deciduous forests were surveyed. ABB demonstrated a 93% occurrence at all three habitat types. They concluded ABB to be a habitat generalist at Camp Gruber.

Soil infiltration, which measures the ability of water to enter soil was significantly faster in grasslands than forest habitats. This is in contrast to early hypotheses that ABB uses forest sites because of the ease of carcass burial in loose deep soils. However, caution should be exercised because ABB are drawn to baited pitfall traps from an area of approximately 2 kilometers in diameter (Bedick et al., 1999). Similarly, the measures of local conditions were not significantly different for traps placed in different habitats. Soils had similar temperatures at 4 cm depth and held similar moistures. Jurzenski et al. (2014) looked at potential variables for habitat suitability for ABB in Nebraska. The researchers found that loamy sand soil, wetland land cover and precipitation were positively associated with ABB. Negative influences to ABB included: loam soil, developed land cover and maximum temperature. In modeling the northern and southern ABB populations, Leasure and Hoback (2017) found a positive correlation with soil moisture in Nebraska but no relationship with moisture in Oklahoma. These authors interpret the findings based on more frequent precipitation events in Oklahoma compared to Nebraska. The lack of observed differences between the air temperature and soil conditions (temperature and percent relative humidity) of each habitat type, likely explains why ABB are not showing an affinity for a specific habitat type at Camp Gruber. During both years of this study, precipitation was normal or above normal and follow up studies should be conducted during periods of drought.

The objective of this study were to investigate the seasonal activity and use of habitat by ABB. By conducting field surveys of the ABB in different areas of Camp Gruber Training Center, OK, this research provides a better understanding of how the species utilizes a patchy environment including grassland, savannah, and forested areas.

It would be beneficial to investigate ABB food resources on the training center and how habitat disturbances affect food availability of ABB, along with how competition between ABB and *N. orbicollus* or *N. pustulatus* impacts resource partitioning. Greater sampling of habitat air temperature and soil temperature and percent relative humidity are required to better understand how habitats vary. In addition, determining how and where ABB overwinter at Camp Gruber is important. Soil analysis at the different habitats, along with trapping sites located near water may help in this regard as well. The different training activities and the number of people present at Camp Gruber on a weekend may have an impact on the number of ABB captured during weekend sampling. It would be advantageous to compare the capture rates of ABB to the influx of people and activity at Camp Gruber to better understand its effect.

CHAPTER III

BURYING BEETLE (SILPHIDAE: *NICROPHORUS*) BREEDING BEHAVIOR IMPROVES SOIL FERTILITY

ABSTRACT

Burying beetles utilize small vertebrate carcasses for breeding by burying the carcass and preparing it with secretions. The effects of carcass and brood rearing on local soil chemistry is unknown. In order to determine influence of buried mammal carcasses and brood success on soil fertility, I conducted a laboratory experiment with *Nicrophorus marginatus* and various soil mixtures. Test containers consisted of: soil/rat/beetles (14) and soil/cedar/rat/beetles (14). Control containers consisted of the following combinations: soil only (3), soil/cedar (3), soil/rat (4) and soil/cedar/rat (4). Burial success and adult survival were recorded. Live weight and dried weight were taken for each larva produce from successful brood balls and the soil mixtures were analyzed. Adult survival and brood averages where not significantly different ($p > 0.05$) when comparing across soil mixtures using a Students' t-test. However, larval dry weights within the beetle/soil/rat containers were 0.22 ± 0.01 grams ($n = 219$), and significantly less (Student t-test, $p = 0.039$) at 0.20 ± 0.01 grams ($n = 169$) than for cedar needles treatment. In additional, cedar needles had no significant effect on the soil nutrients of each treatment. Rats buried in the soil changed the soil composition by increasing the pH,

soluble salts, nitrate, phosphorus, and potassium content. These amounts increased with beetles and larvae. Experimental containers with successful broods had significantly higher average soluble salt content when compared to all other treatments (df = 3; $p < 0.001$). There was significantly lower nitrate level in the soil-only treatment when compared to containers with beetles and rats, especially those with larval broods (df = 3; $p < 0.05$). Experimental containers with successful broods had significantly higher levels of phosphorus compared to soil-only treatments (df = 3; $p < 0.05$) and potassium was significantly higher in treatments containing beetles (df = 3; $p < 0.05$) when compared to control treatments. The results of this study support the theory that *Nicrophorus* increase soil fertility, especially when breeding.

INTRODUCTION

When a vertebrate dies, it provides a valuable resource, resulting in intense intra- and interspecific competition by a wide variety of necrophores. Small carcasses are often consumed by vertebrate scavengers and larger carcasses may be dismantled by these species. Other organisms that feed and/or breed on carrion include ants, flies, beetles, soil-dwelling fungi and bacteria (Bornemissza,1956; Scott et al., 1987; Ohkawara et al., 1998) and even plants. Carcasses that remain at the site of death create distinguishable changes to vegetation as a result of changes in soil fertility (Bornesmissza, 1957; Towne, 2000; Parmenter and MacMahon 2009). This effect varies based on the size of the carcass, the time of year that the animal died, and its placement in the environment.

As small vertebrate carcasses undergo decomposition, the underlying soil absorbs the nutrients released (Bornesmisszza, 1957; Putnam, 1978). The soil enrichment within the vicinity of the carcass leads to changes to the local plant community (Parmenter & MacMahon, 2009). Vertebrate carrion creates a nutrient-rich resource. Potassium and nitrogen are the first nutrients to be lost from the body through seepage of bodily fluids into the soil and through feeding on the soft tissues by insects (Parmenter & MacMahon, 2009). Later in the decomposition process, phosphorus, found in bone and soft tissues, is lost, followed by calcium and magnesium. Plants require primary macronutrients including nitrogen, phosphorus, and potassium, along with secondary macronutrients such as magnesium, sulfur, and calcium (Mitra, 2017).

Collins (1970) found that herbaceous plants found within 20 centimeters of a decaying rat carcass in a temperate oak forest grew denser and taller for the following 18 months, suggesting a combination of soil-enhancing effects from carcass leaching and soil disturbance by insects associated with carrion. Decomposing animal carcasses often result in localized areas of increased soil fertility, leading to influences of surrounding plant and invertebrate populations (Parmenter & MacMahon, 2009).

The effects of carcass access to abiotic and biotic factors influences the effects it has on soil nutrients. For example, Parmenter & MacMahon (2009) found total nitrogen increased in the soils below the carcass in the first two years of exposure, representing approximately 16% of the total nitrogen available in the live animal. Rodent carrion that was buried in a "burrow" environment in arid and semiarid ecosystems had better microclimate conditions for microbial processes and arthropod activities when compared to surface environments and retained more of the original nutrients of the body (Parmenter & MacMahon, 2009).

Carrion that is not removed by vertebrate scavengers undergoes decomposition aided by microbes and invertebrates (Towne, 2000). The decomposition of a vertebrate carcass typically follows a progression of necrophores that arrive at the carcass at various stages of decay (Reed, 1958; Payne, 1965; Johnson, 1975; McKinnerny, 1978). Carrion beetles (Silphidae) are among the first to arrive at a carcass using olfactory organs located on their antennae to locate the carcasses, often within one hour of death and from as far away as six kilometers (Jurzenki et al., 2011).

Among the Silphidae, the subfamily Silphinae arrive during the early to mid-stages of carcass decomposition and lay eggs on, or near, the carcass, where their young will hatch and feed on the carcass and maggots (Ratcliffe, 1996). The burying beetles, subfamily Nicrophorinae, also arrive during early to mid-stages of decay but have a different breeding strategy. They display a unique behavior of burying appropriately sized carcasses for reproductive attempts (Milne & Milne, 1976).

Upon discovery of a suitable carcass, males broadcast pheromones to attract potential mates (Bartlett, 1987; Eggert & Muller, 1989; Eggert, 1992). Males and females of different *Nicrophorus* species often compete with each for the carcass, which is typically won by the largest male/female pair (Scott, 1998). The pair bury the carcass by removing soil from underneath it until the carcass sinks into the ground (Milne & Milne, 1976). Within the burial chamber, the feathers or fur are removed and the carcass is coated with anal and oral secretions to slow decay. The oral secretions of *N. marginatus* are composed of digestive phospholipase PLA_2 (Rana et al. 1997; Dirrigl & Perrotti, 2014). Carcass burial and preparation suppresses fungal growth (Suzuki, 2001), prevents fly infestation (Suzuki, 2000), and reduces detection by other Silphidae (Shubeck, 1985; Trumbo, 1994). In addition, burying beetle secretions from most species have anti-microbial properties (Hoback et al., 2004), which limit growth of bacteria.

In addition to limiting bacteria, burial of carcasses also reduces carrion-breeding flies. There are 47 species of carrion-breeding flies that are associated with the transmission of foodborne pathogens, including *E. coli, Salmonella,* and *Shigella* (Olsen, 1998). Scott (1994) found that carcasses used by *Nicrophorus* produced lower numbers of fly larvae. Thus, declines in silphid abundance and diversity are likely correlated with declines in the ecological services provided by the beetles, including fly suppression and reduction in transmission of potential pathogens (Gibbs & Wolfs, 2004).

After the *Nicrophorus* pair coats the carcass with secretions, the female lays eggs nearby (USFWS, 1991). One or both parents will remain with the brood until larval development is complete (Wilson & Fudge, 1984; Wilson et al., 1984), a behavior which is unique to this family of insects (Scott, 1989). Parental care increases offspring survival and growth (Eggert et al., 1998) and the presence of both parents reduces the chances of conspecific or congeneric take-over of the carcass (Scott, 1989; Scott, 1990; Trumbo, 1991; Trumbo 1994).

The objective of the study was to investigate the effects of *Nicrophorus* brooding behavior on soil fertility along with the impacts of Eastern redcedar needles on brood ball production, juvenile survival, and soil chemistry.

MATERIALS AND METHODS

A laboratory experiment was conducted to test the influence of rat carcasses and brood success on soil fertility. Soil was collected from a sandpit in Buffalo County, Nebraska. Soil textural class was determined to be sandy soil (90.6% sand, 3.8% silt, 5.6% clay) using the hydrometer method (Thien and Graveel, 2003). Approximately 4 kilograms of dry soil was added to 4.25-liter plastic containers. 200 milliliters of distilled water was added to each container and mixed thoroughly. Resulting soil moisture was determined to be 3-5% using the gravimetric method. Fresh eastern redcedar (*Juniperous virginiana*) needles (100-110 grams) collected from beneath cedar trees were placed two centimeters thick on the surface of the soil in the designated containers.

Test containers (28) consisted of: soil/rat/beetles (14) and soil/cedar/rat/beetles (14). Control containers consisted of: soil only (3), soil/cedar needles (3), soil/rat (4) and soil/cedar needles/rat (4). Frozen 300-350-gram laboratory rats (*Rattus norvegicus* from RodentPro.com) were allowed to thaw for three days and were placed into the appropriate containers.

Nicrophorus marginatus beetles were collected in Kearny County, Nebraska using open-baited pitfall traps modified from Bedick et al (1999). Beetles were sexed and one male/female pair was placed on each of the rat carcasses in experimental conditions. Beetles were checked after two days to determine the progress of carcass burial. If no signs of burial activity were observed, a new male/female pair was used to replace the initial pair. After burial action was observed the carcasses were left at room temperature (24 ^{o}C) for 18 days. In the control containers with rats, rats were buried by hand four centimeters under the surface of the soil or soil/cedar needle combination on the second day and left for 18 days.

After 18 days, burial success, adult survival, and brood success were recorded. Carcasses from controls and unsuccessful brood balls were exhumed and discarded. Cedar needles were also collected and discarded from all containers. Successful brood balls were carefully excavated from the soil and larvae were removed and counted.

Once carcasses and larvae were removed from each treatment container, soils were thoroughly mixed to assume homogeneity and 500 grams of soil was placed in soil sample bags for further chemical analysis. Samples were sent to Ward Laboratories, Inc. in Kearney, Nebraska for analysis, which included pH, soluble salts, and nutrient level.

Live and dried mass were taken for each larva produced from successful brood balls. Dried weights were determined by gravimetrically drying larvae to a constant weight in a drying oven for 48 hours at 70^{o} C.

RESULTS

Burying Beetle Reproduction

All but two carcasses from the soil/rat/beetles and one carcass from the soil/cedar needles/rat/beetles container showed signs of burial activity after two days. New mating pairs were added to each carcass, and all showed signs of burial activity within two days. After 18 days 53.6% (15/28) of mating adults were alive in the beetles/soil/rat containers while 67.9% (19/28) remained alive in the beetle/soil/cedar needles/rat containers. In the containers with just soil, 35.7% (5/14) carcasses had successful broods with 219 total larvae averaging 43.8 larvae per brood. In comparison, 28.6% (4/14) in the containers with cedar needles had broods totaling 169 larvae with an average of 42.3 larvae per brood (Table 5). Mean live weights for larvae were similar 0.545 grams (n=219) for soil and 0.553 grams (n=169) for soil with cedar needles. None of these differences were significant (Students t-test, P > 0.05). However, larval dry weights within the beetle/soil/rat containers were 0.22 ± 0.01 grams (n = 219), and significantly less (Student t-test, P = 0.039) at 0.20 ± 0.01 grams (n = 169) for cedar needles treatment.

Table 5. Brood success and size in the presence and absence of Eastern red cedar needles.

	Burial Success			Percentage of successful broods	Average larvae number per brood	Total larvae
	Buried	Partial burial	No burial			
beetle/soil/rat	11	3	2	35.70%	43.8 ± 10.8	219
beetle/soil/rat/cedar	10	3	3	28.60%	42.3 ± 9.4	169

Soil Analysis

There was no statistical significant difference between soil nutrients in all treatments containing cedar needles versus those without cedar needles (ANOVA, p > 0.05). Thus, I combined experimental containers with and without cedar needles and tested for significance among the treatment means. All data were first tested for normality and a One-Way ANOVA was used for normally distributed data followed by a Tukey test when significance was detected. For data that did not fit a normal distribution, a Kruskal-

Wallis One Way ANOVA followed by the Dunn's Method Multiple Comparison test was used. Comparisons were made among the controls (soil-only and soil/rat), carcasses with beetles but no larvae, and carcasses with beetles and larvae. Rats buried in the soil changed the soil conditions by increasing the pH, soluble salts, nitrate, phosphorus, and potassium content. These amounts increased with beetles and larvae (Figures 11-15).

The addition of cedar needles did not affect soil pH (Figure 11). When rats were added with or without beetles, soil pH was lowered and found to be similar among all conditions (Figure 9).

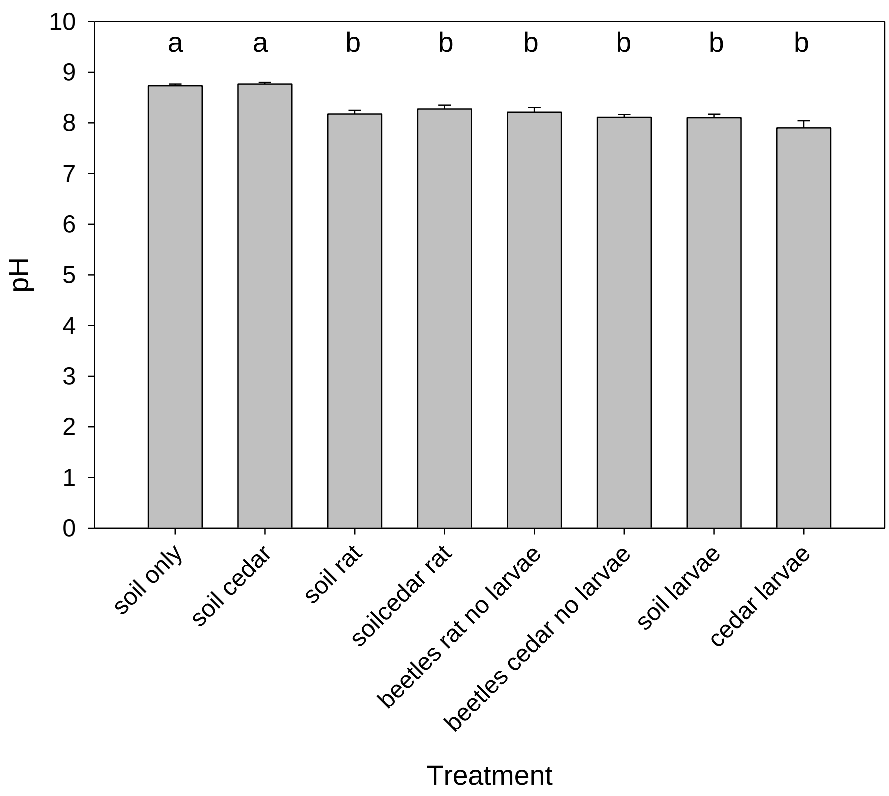

Figure 11. Mean (± 1 S.E.) soil pH across experimental conditions. Different letters indicate significance (ANOVA, P < 0.05).

The amount of soluble salts was compared (mean ± S.E.) across treatments (Figure 12). Soil-only containers had significantly lower soluble salt content, while the experimental containers containing rats and adult beetles were similar to containers with only rats. Containers with larvae had the highest amounts of soluble salts among treatments (df = 3; $p < 0.001$).

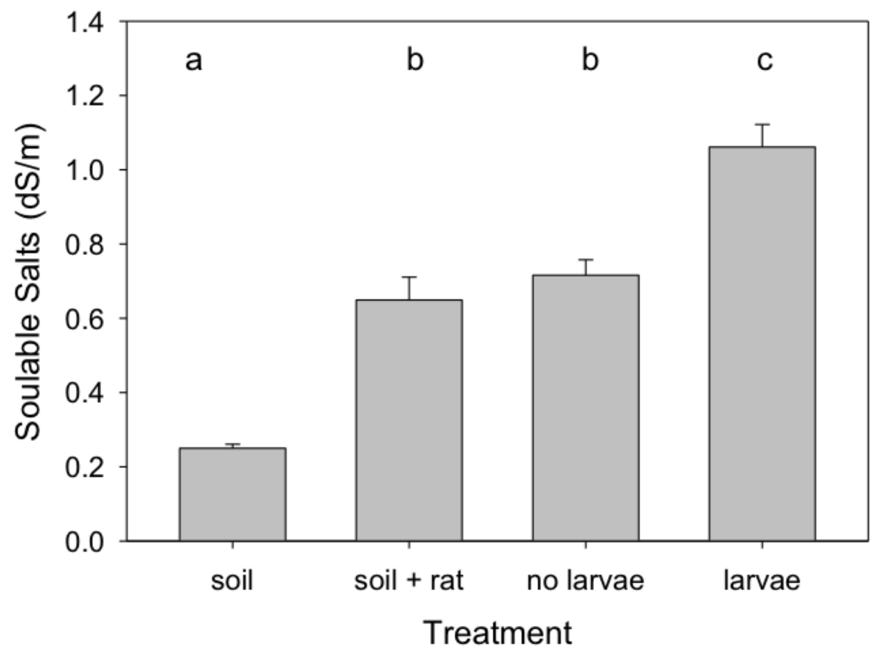

Figure 12. Mean (± 1 S.E.) soluble salts (dS/m) for each treatment. Different letters above bars represent significant differences (ANOVA, P < 0.001).

Breeding by beetles significantly affected the amount of nitrate, phosphorus, and potassium levels in the soil (Figure 13, 14,15; Table 6). There was significantly lower nitrate level in the soil-only treatment when compared to containers with beetles and rats, especially those with larval broods (df = 3; $p < 0.05$) (Figure 13). The presence of adult beetle feeding on the carcass increased the amount of nitrate in the soil to similar levels as the rats supporting brood (Figure 13).

Experimental containers with successful broods also had significantly higher levels of phosphorus and potassium compared to soil-only treatments (df = 3; $p < 0.05$) (Figure 14 and Figure 15). The addition of a rat significantly increased the level of phosphorus. Similarly, for potassium, the presence of beetles significantly increased the amount of potassium in the soil (Figure 15). Furthermore, the addition of rats to soil, increased most micronutrients (Table 6).

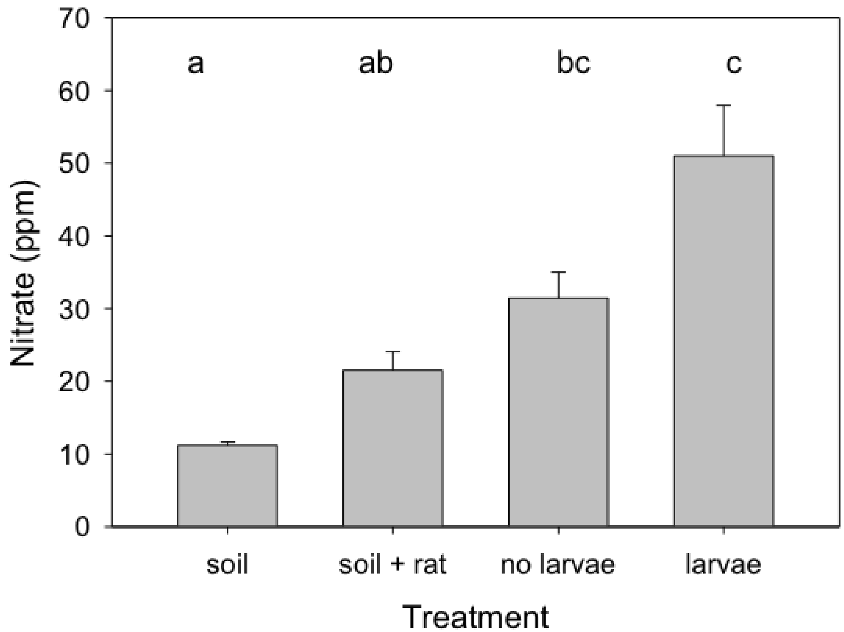

Figure 13. Mean (+ 1 S.E.) nitrate (ppm) in soil. Different letters above bars indicate significance (Kruskal Wallis ANOVA, P >0.05).

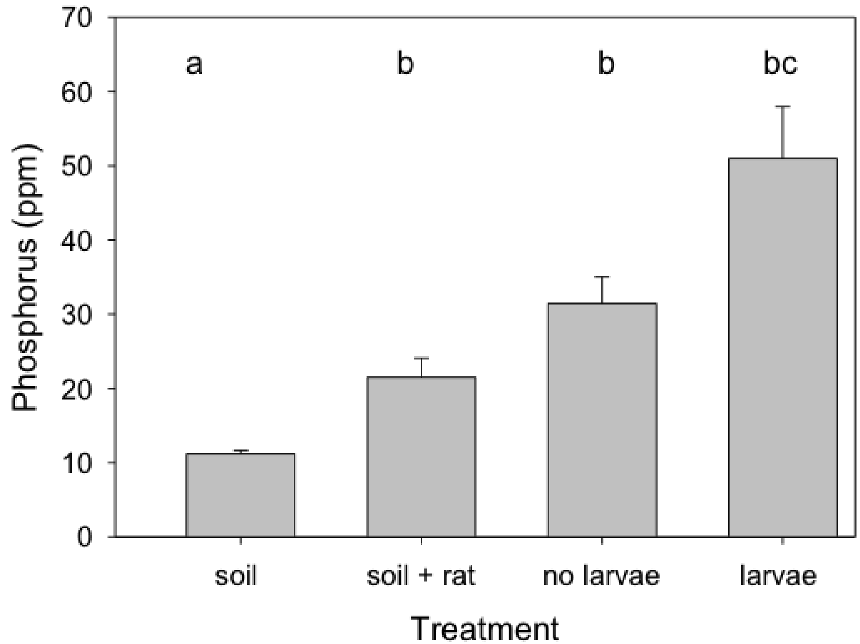

Figure 14. Mean (+ 1 S.E.) phosphorus (ppm). Different letters indicate significance (Kruskal-Wallis ANOVA, P > 0.05).

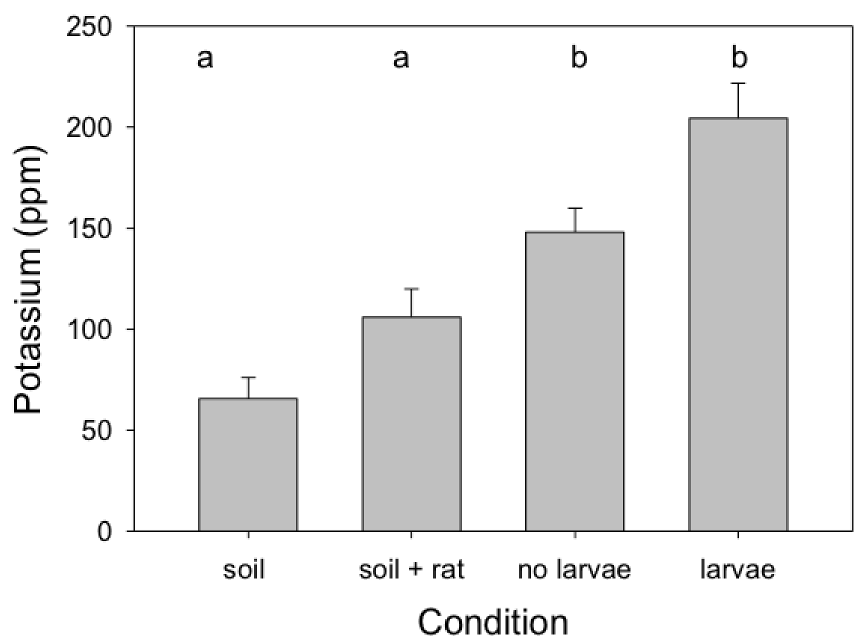

Figure 15. Mean (+ 1 S.E.) potassium (ppm) under experimental conditions. Bars with different letters significantly different (Kruskal Wallis ANOVA, P < 0.05).

Table 6. The effects of experimental treatment on micronutrients (ppm).

Sample Type	Sulfate ppm	Zinc ppm	Iron ppm	Manganese ppm	Copper ppm	Calcium ppm	Magnesium ppm
Soil only	7.33	0.41	7.37	3.97	0.533	617	41.667
Soil/Rat	30.75	0.508	7.38	9.35	0.6	525	32.25
Soil/Cedar	10	0.417	8.4	7.67	0.57	739	40
Soil/Cedar/Rat	24	0.435	7.08	11.4	0.57	919	48.5
Beetle/Soil/Rat	36.5	0.729	4.81	12.4	0.71	878	58.625
Beetle/Soil/Rat/ Cedar	43.75	0.73	4.38	16.3	0.694	884	61.25
Beetle/Soil/Rat/ Larva	41.5	0.83	6.7	14.9	0.86	1,154	82.5
Beetle/Soil/Rat/ Cedar/Larva	45.67	0.767	4.57	16	0.727	1,004	73.667

DISCUSSION

As expected, the addition of vertebrate carrion to soil increased soil nutrient availability. The action of burying beetles burying and preparing the carcass significantly increased the availability of nitrate, phosphorus, and potassium, which are all essential plant nutrients (Figures 12-15). In all cases, successful production of brood had the greatest results on soil nutrients. The presence of cedar needles did not affect nutrient assimilation in the soil, nor did it affect brood size or wet mass of larvae (Table 5).

Walker and Hoback (2007) captured more ABB in grasslands than in cedar-dominated habitats. They hypothesized that the dense stands of cedar inhibited detection and the ability of ABB to get to carrion. Cedar-dominated habitats also demonstrated lower air temperature, soil temperature, and less wind movement (Walker & Hoback, 2007). In this study, burying beetles were provisioned with a rat and were able to bury through a thick (~2 cm) layer of needles without apparent effect. After 18 days, 53.6% (15/28) of mating adults were alive within the beetle/soil/rat containers while 67.9%

(19/28) remained alive in the beetle/soil/cedar/rat containers. Thus, Eastern redcedar had no apparent effect on the survival of adult beetles, burial of carrion, or brood success.

Pierce and Reisch (2010) found that Eastern redcedar had no impact on soil pH but retained higher soil moisture when compared to plots of native prairie grass. This is likely why adult beetle survival and brood success and averages were not impacted by the presence of cedar needles because *Nicrophorus marginatus* is sensitive to water loss (Bedick et al. 2004). In this study, cedar needles were not found to significantly alter soil pH under control conditions. In previous studies, a body can influence soil pH with the soil underneath a carcass often becoming more acidic as a result of NH_4^+ accumulation in the soil (Benninger et al., 2008). In this study, pH decreased in all conditions with the addition of a rat (Figure 11) but the presences of beetles or larvae feeding on the carcass did not result in pH differences compared to rat only.

It is important to note that Benninger et al. (2008) found that soil pH fluctuated significantly through time. Between days 14 to 23 of burial, they found that the soil had a significantly higher pH, which was followed by a decrease to significantly lower pH on day 30. For this experiment, soil samples were taken after day 18, then sent for laboratory analysis. Time may have been a factor that influenced observed pH.

Soil mixtures with rats and beetles present, especially those with larval broods, had higher soil nutrients. Limited nutrients required for healthy plant life like nitrogen, phosphorus, and potassium all demonstrated increased levels in the presence of burying beetles. Typically, potassium and sodium are the first nutrients to leave the body, followed by nitrogen and sulfur, then magnesium and phosphorus and finally calcium (Parmenter & MacMahon, 2009). Although this study did not follow soil nutrients through time, the feeding and excretion by adults and larvae increased soil nutrient availability compared to only buried rats.

The control containers with soil only, and soil with rats had significantly lower soluble salt content, while the experimental containers with larva had significantly higher soluble salts (Figure 12). This result was expected because soluble salts (sodium and potassium) typically occur in the bodily fluids of the carcass (Parmenter & MacMahon, 2009). Containers without larva had lower averages than those with successful broods likely because of feeding and larval excretion. High soluble salt concentrations in the soil can reduce plant health; however, the highest observed amount in this study was 1.1 mS/d, which is considered a low salinity and unlikely to cause plant damage (Cavins et al., 2000).

Containers provisioned with a rat and beetles had significantly higher nitrate level averages than those without (Figure 13). This result was somewhat unexpected. A decomposing carcass is nitrogen rich. The nitrogen concentration of a rat has been reported to be approximately 32 g kg^{-1} (Widdowson, 1950). Benninger et al. (2008) reported a significant increase in total N concentrations in the soil beneath a carcass. Thus, I anticipated that all treatments provisioned with a rat would have similar nitrate content. One potential explanation for the significant differences in nitrate levels in the rat and beetle containers is that the soft tissue- high in nitrogen, was disturbed by the beetles and thus, better distributed into the soil (Parmenter & MacMahon, 2009). Nitrogen may have also been transferred into the soil via insect feces.

Experimental containers with successful broods had significantly higher levels of phosphorus and potassium when compared to soil-only containers (Figure 14 and 15).

Phosphorus is a compound found in low concentrations in soft tissue and in high concentrations in bones. Because it is contained in bones, it is lost at a slower rate than nitrogen, while potassium is released earlier as a soluble salt (Parmenter & MacMahon, 2009). The higher levels of phosphorus and potassium observed in association with brood are likely a result of carcass destruction by the adult and larval beetles. Dirrigl and Perrotti (2014) analyzed the taphonomy of quail skeletons (*Coturnix japonica*) after being used by ABB. They found that the beetle produced observable bone damage and modifications as a result of both successful and unsuccessful breeding attempts. The scratching, flaking, and pitting of the rat bones likely sped the release of phosphorus into the surrounding soil. The presence of larva may have contributed to greater bone modification.

Carcass decomposition has been found to contribute less than 1% of the nutrient budget in ecosystems, but can be significant in increasing local soil health (Macdonald et al., 2014). As a result, islands of soil fertility are created, promoting biodiversity across landscapes. In a tropical savannah ecosystem in Australia, Carter et al. (2008) reported a 400% increase in the microbial biomass under a rat carcass. Although small, carcasses used by burying beetles can have a substantial effect on nutrients. By burying carrion, *Nicrophorus* species improve soil fertility and plant health. In many pasture ecosystems, suppression of wildfires has caused increases in Eastern redcedar (Walker &Hoback, 2007). Although burying beetles appear to use redcedar habitats less, cedar does not appear to directly impact brood behavior.

CHAPTER IV

SIGNIFICANCE OF THE AMERICAN BURYING BEETLE, *NICROPHORUS AMERICANUS* TO RANGELAND HEALTH AND LIVESTOCK PRODUCTION

INTRODUCTION

Healthy ecological communities encompass a diverse number of species with important aesthetic, functional, and economic value (Levines & HilleRisLambers, 2009). Unfortunately, loss of biodiversity can alter the ecological processes necessary to functional ecosystems (Cardinale et al., 2003). In Oklahoma, livestock production makes up a large portion of the economy. In 2017, pasturelands per acre were estimated to be worth $1,470 per acre, representing a 2.5% increase across the Southern Plains (USDA, 2017). Nitrogen, potassium, and phosphorus are the primary macronutrients necessary for a healthy pastureland, along with micronutrients including calcium, sulfur, and magnesium (Mitra, 2017). Most grasses found in grazing lands have high nitrogen requirements (Bidwell & Woods, n.d.). Unfortunately, grazing of pasturelands often causes degradation and loss of nutirents leading to reduction in livestock production (Bidwell & Woods, n.d.). Furthermore, a variety of flies can have significant economic impacts on cattle herds through nuisance biting and transmission of various diseases (Boxler, 2016).

The American burying beetle (*Nicrophorus americanus*) or ABB (Figure 16) along with other Silphidae, benefits pasturelands and livestock production. ABB and other burying beetles participate in the nutrient recycling of organic matter, which increases soil fertility and removes breeding resources of flies. Once prevalent in 35 U.S. states, ABB was listed as federally endangered by the U.S. Fish and Wildlife Service in 1989 having disappeared from over 90% of its historic range (USFWS, 1991). One of the largest remaining populations of the species exists in the eastern half of Oklahoma. This area of the state is also used extensively for livestock production.

Figure 16. An American burying beetle (*Nicrophorus americanus*) found at Camp Gruber, Braggs, OK

SOIL HEALTH

Soil health is the ability of the soil to sustain animal and plant productivity, maintain or enhance air and water quality, and support animal and plant health (Doran & Ziess, 2000). Unfortunately, almost 40% of the earth's agricultural land is degraded because of human activity. Erosion, atmospheric pollution, over-grazing, tree clearing, extensive soil cultivation, and other changes have resulted in the loss of productive agricultural land and is one of the world's greatest ecological concerns (Doran & Ziess, 2000). Soils and their biota provide necessary ecosystem functions that benefit humans. The ecosystem processes supported by healthy soil include decomposition of plants and animals, storing and controlled release of water, recycling nutrients, detoxification of contaminates, and suppressing pathogens that affect plant health (Contanza et al., 1997).

Annually, 2-3% of cattle herds are lost to old age, injury, or other natural causes and while management polices dictate carcass removal, in more remote areas, carrion is left to rot (Towne, 2000). Carcasses that are not consumed quickly by vertebrate scavengers will undergo invertebrate decomposition and microbial decay. While ABB cannot bury cattle, they can provide the benefit of recycling small vertebrate carcasses that occur much more frequently in the environment (Trumbo, 1991). ABB increase soil fertility and health by burying small vertebrates including birds and rodents and using the carcasses to rear their offspring. ABB is the largest North America member measuring between 30 to 35 millimeters (Holloway & Schnell, 1997) and as a result, ABB buries larger sized carrion like young turkey, quail and rabbit (between 80-300 g). The beetles are active at night and can detect a carcass from up to six kilometers away within one hour of death (Jurzenski et al., 2014).

Upon detection of a carcass, the beetles will determine the size and, if appropriate, will bury the carcass, or move it up to a meter to a more suitable burial site (Kozol et al., 1998). The fur or feathers are removed, the carcass is rolling into a “brood ball”, and the covered in anti-microbial secretions (Hall et al., 2011). Burial is rapid, taking only a couple of hours and complete by the following morning. The female will lay eggs near the carcass so the larvae can feed on the carrion once they hatch (Scott, 1998).

Not surprisingly, rodents buried underground in arid and semiarid environments decompose more slowly, allowing a more complete decomposition of the carcass (Parmenter & MacMahon, 2009). Belowground, carcasses provide nutrients including nitrogen, sulfur, phosphorus, and potassium more completely to the soil than when decomposition occurs aboveground.

The soils around the carcass will gain nitrogen as the body fluids and body particulates leach from the body. Both successful and unsuccessful reproductive attempts by ABB result in modifications to the bones of a carcass, including: flaking, cracking, and pitting, all of which help in the distribution of the nutrients like calcium and phosphorus contained in the carcass (Dirrigl & Perrotti, 2014). Plants benefit from nutrients and studies have shown that those plants located within 20 cm of a small vertebrate carcass (rat) in a temperate oak forest grow more densely and taller for the 18 months following decomposition (Collins, 1970).

Because of large densities of small mammal bird species, most with short lifespans, the function of carcass decomposition in recycling important, limited nutrients like nitrogen and phosphorus, likely has considerable influence on ecosystems though the creation of patches of fertility (Parmenter & MacMahon, 2009). By burying these small vertebrate carcasses, ABB and other burying beetles prove beneficial in enhancing soil fertility; and ultimately, pasture health.

REDUCTION OF FLY SPECIES

It’s difficult to estimate the cost fly species have on the livestock industry in Oklahoma and beyond, but it is substantial. The most economically important fly species include horn fly (*Haematobia irritans*), face fly (*Musca autumnalis*) and stable fly (*Stomomxys calcitrans*) (Boxler, 2016; Klauch et al., 2014). It's estimated that horn flies result in more than $1 billion dollars in cattle losses per year in the United States. Horn flies are blood feeders and can cause cattle irritation, decreasing grazing efficiency, weight reduction, and limited milk production (Boxler, 2016). Another blood feeder, the stable fly, congregates around the cattle's legs, feeding and causing painful bites. Face flies feed on animal secretions and dung. While they don't feed directly on the animal, they cluster around the eyes, nose, and mouth of the animal, creating annoyance and in some cases, pink-eye (Boxler, 2016). Additionally, house flies (*Musca domestica*) can spread disease by contaminating the surfaces on which they land (Klauch et al., 2014; Olsen, 1998). *Muscidae* will visit decomposing carrion and most will breed in both carrion and feces.

ABB activity occurs in the late spring through late summer months, when fly are also seeking breeding opportunities (Kozol, 1990). Flies are one of the primary competitors of burying beetles for carcasses (Scott, 1994). Ultimately, if fly eggs are not destroyed by ABB, the carcass may be lost as the maggots consume the carcass. Scott (1994) found that carcasses used by burying beetles had lower numbers of flies and fly larvae. The adult beetles, beetle larvae, and the phoretic mites found on the beetle's body all work to destroy fly eggs; thus, reducing fly numbers (Wilson & Knollenberg, 1987; Scott, 1994). In addition, Wilson (1983) suggested that not only do burying beetles reduce fly competition by destroying fly eggs, but also limit fly numbers by burying carcasses deeply so fly oviposition cannot occur. Because ABB is active at night, carcasses are removed before flies become active in the day. By rolling the carcass into a ball and coating the body in anti-microbial secretions, the beetle may also be creating an anaerobic environment that limits fly development (Wilson & Knollenberg, 1987). By competing with flies, ABB decrease the potential for the spread of pathogens by flies to humans and livestock.

ADDITIONAL BENEFITS OF ABB

Burying beetles are well-studied for a number of reasons. First, the Genus *Nicrophorus* demonstrates the characteristic of bi-parental care, where both the male and female beetle care for their young, which occurs in less than 200 species of insects. Numerous studies have been conducted on burying beetles to gain insights on selection pressures and adaptations for this unique behavior. Second, the endangerment of the largest of the Silphidae, the ABB, has prompted many studies aimed at understanding its ecology and current distribution in order to understand how its range could be reduced from 35 U.S. states to only six. Because of its size and formerly wide distribution, it likely played an important role in intact ecosystems.

The Oklahoma agricultural and livestock industry has an estimated $40 billion economic impact; however, the degradation of pasturelands threatens this economy (Reese, Hundl & Coon, 2015). The hay and fodder made on Oklahoma pasturelands plays a large role production of 4,000,000 head of cattle that makes Oklahoma the third largest cow producing state (Love, n.d.). Aside from cattle, the hay produced in the state accounts for roughly $500 million to hay producers annually. To maintain pasture health, fertilization is almost always recommended; however, variation in soil, climate, pests, and prices makes this a complicated decision (Love et al., 2006). In the presence of burying beetles, fertilization may not be necessary. Nitrogen, phosphorus, and potassium are limiting resources necessary to health plant life. By burying carcasses often found in pasturelands, burying beetles may distribute these limited resources more completely into the soil, improving pastureland health.

While the monetary value of ABB and other burying beetles cannot be readily estimated, the annual value of the ecological services insects provide is estimated to be at least $57 billion (Losey, 2006). Furthermore, fertilizing pasturelands is costly, averaging $700 per ton for nitrate (Love et al., 2006). Unfortunately, as ABB numbers decline, so do the benefits it provides. The beetles provide multiple important ecological services. By utilizing carrion, burying beetles create areas of enhanced soil fertility by better distributing nutrients to the soil. Additionally, by burying carrion and consuming fly eggs, the beetles work to reduce fly species that can prove harmful to humans and livestock. Burying beetles also provide unique research opportunities and sources for novel compounds that may become antibiotics for humans or livestock. Finally, a large brightly colored beetle provides aesthetic value and educational opportunities to engage members of the public in conservation issues. The presence of burying beetles, especially ABB, benefits Oklahoma ranchers, researchers, and the public and efforts should continue that are aimed at understanding its endangerment allowing the species to recover.

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