THE AMERICAN BURYING BEETLE (NICROPHORUS AMERICANUS) POPULATION AT CAMP GRUBER, OK BREEDING AND OVERWINTERING SURVIVAL

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Abstract: The American burying beetle (ABB), *Nicrophorus americanus* Olivier (Coleoptera: Silphidae), once occurred across two thirds of North America but now occupies <10% of its historic range. Reasons for the decline have not been determined but are generally associated with changing land use and vertebrate communities, although competition with other carrion beetles has also been suggested. The ABB overwinters as an adult underground and emerge in the spring looking for a mate for reproduction. Overwintering has been suggested as one of the main causes of death in ABB populations. The objective of this study was to characterize the ABB population and silphid community at Camp Gruber Training Base near Braggs, OK and investigate habitats where it will survive during overwintering. Bi-weekly sampling was conducted from 2016 to 2020 following the U.S. Fish and Wildlife protocol. A minimum of 15 baited above ground traps were used during each period with a minimum of two days of sampling in three different habitat categories. Two male/female pairs were collected per sampling trip for reproduction studies in 2019 and 2020. A five-day sample period with mark and release sampling was used to estimate the yearly ABB population using 20 trap locations. The estimated population on the 133 km^2 Camp Gruber ranged from 1,136 to 4,657 ABB with activity occurring from April until October. An overwintering survival study was conducted by placing 30 lab-reared ABB in a lakeside, forest and open area habitat during the winter and a laboratory study was conducted to investigate the months of reproduction. The beetles in the overwintering experiment were placed in October of 2019 at the Cherokee Nation Sallisaw Creek State Park and checked of survival in March of 2020. Most ABB survived the overwintering period in all habitats. Despite a longer activity period than ABB in northern regions, only one peak of newly emerged ABB was observed in July, suggesting a single brood each year. In the laboratory ABB pairs were placed into containers, with moist peat moss and provided a large rat (175 grams) as a food/reproduction source. In the laboratory ABB only bred in late July. The results of these studies show ABB use all habitats at Camp Gruber and strongly suggest that ABB reproduction is limited to one period each year. Observed declines in population and individual size suggest potential impacts of climate change on the Oklahoma population.

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CHAPTER I

LITERATURE REVIEW OF THE AMERICAN BURYING BEETLE, *NICROPHORUS AMERICANUS*

INTRODUCTION

Background

The carrion beetles (Coleoptera: Silphidae) are a relatively small group of beetles, with approximately 200 species described (Sikes, 2008). The family Silphidae belongs to the superfamily Staphylinoidea and is divided into two subfamilies, the Nicrophorinae and the Silphinae (Dekeirsschieter et al., 2011). The beetles belonging to the subfamily Nicrophorinae are all in the genus *Nicrophorus* and referred as the burying beetles, because of the behavior in which they bury small vertebrates carcasses to use for reproduction (Ratcliffe, 1996).

The Silphidae are distributed worldwide, however, they are most commonly found in the temperate regions (Dekeirsschieter et al., 2011; Ratcliffe, 1996). In tropical areas, Silphidae are usually absent or rare to find, most likely because they are out-competed by flies, ants, and vertebrates (Dekeirsschieter et al., 2011).

Carrion beetles help recycle nutrients by using a small vertebrate carcasses for food and reproduction (Conley, 2014; Walker & Hoback, 2007). In temperate forests, carrion beetles are the main decomposers of these carcasses along with flies (Gibbs & Stanton, 2001). Carrion beetles also help to control populations, of flies, by burying the carcass and limiting the access of these insects (Raithel, 1991).

The family Silphidae can be recognized by a combination of characteristics, including clubbed antennae, shortened elytra, and generally dark color. In North America, it is possible to find 8 genera of the 13 described and 30 species of the total of 208 found worldwide (Ratcliffe, 1996; Sikes, 2008). Substantial research on the genus *Nicrophorus* has been conducted because of their ecological importance as scavengers of carrion, their bi-parental care and the existence of the threatened species, *Nicrophorus americanus*, the American burying beetle (Lingafelter, 1995).

The American Burying Beetle (ABB), is the largest *Nicrophorus* species in North America with adults that can reach 35 mm in length (Raithel, 1991; Holloway & Schnell, 1997). They are easily identified by their unique red-orange pronotum marking (U.S. Fish and Wildlife Service, 2014).

The ABB historically occurred in 35 states in the eastern temperate zone of the United States, but the rapid decline to approximately 10% of its historic range beginning in early 1900 caused it to be listed as an endangered species by the United States Fish and Wildlife Service in 1989. In 2020 it was considered more secure and down-listed to threatened (Raithel, 1991; U.S. Fish and Wildlife Service, 2014; Gray & Parham, 2020). The precise reason for decline is unknown but several hypotheses have been proposed, including loss of their ideal carrion, use of pesticides, increase of artificial lighting, habitat loss, vegetation changes, pathogens, vertebrate competition for food sources, and congener competition (Sikes & Raithel, 2002). Remaining ABB populations are threatened by urban development, usage of land for agricultural purposes and climate change (U.S. Fish and Wildlife Service, 2019). Carcass availability during the overwinter period may also affect the survival of ABB that breed during summer (Schnell et al., 2008).

To assess ABB population and range, surveys are conducted in areas where populations are known to exist and also in areas where the ABB could potentially be found based on vegetation, soil characteristics, and absence of human disturbance (Raithel, 1991). The ABB is currently found in Kansas, Nebraska, South Dakota, Rhode Island, Arkansas, and Oklahoma. Reintroduction has been attempted in Missouri, Massachusetts, and Ohio with limited success (U.S. Fish and Wildlife Service, 2019).

The potential effects of climate change on many animal species has been modeled and have produced variable results (Pearson et al., 2002; Ortega-Huerta & Peterson, 2004; Shepard et al., 2007). Taxonomy and life history suggest that most *Nicrophorus* do not tolerate warmer temperatures, contributing to the higher diversity and abundance of the genus *Nicrophorus* in temperate climates. Possible reasons for this lack of success in warmer temperatures can be related to the increased competition between the ABB and other insect scavengers such as ants and flies. In warmer climates, the carcass decomposition rate also increases, which could possibly affect its usage by the ABB as a source for food and reproduction. The U.S. Fish and Wildlife Service predicts that, if temperatures keep rising with the current rates, the remaining ABB populations will be negatively impacted leading to a possible loss of southern populations of ABB (U.S. Fish and Wildlife Service, 2019).

Life Cycle

Species of the genus *Nicrophorus* are social and have strong biparental care of their brood, in which both parents participate in the feeding and protection of their offspring (Creighton & Schnell, 1998; Lomolino et al., 1995). Their biparental care is so strong that even when one of the beetles is injured the other will increase its effort to compensate and maintain the offspring (Creighton et al., 2014). All the *Nicrophorus* have a one year life cycle but species will develop during the spring, summer or fall (Smith, 2002).

Like other *Nicrophorus,* the ABB depends on vertebrate carcasses to bury and use as a food source for its offspring. ABBs use larger carcasses (80-250 g preferred) than other *Nicrophorus* for reproduction (Backlund et al., 2008; Schnell et al., 2008; Scott, 2002). Using the chemoreceptors in their antennae, ABB find carcasses for food and reproduction (Backlund et al., 2008; Ratcliffe, 1996; Schnell et al., 2008). Recent research suggests that in the southern population, ABB may have two broods per year due to the longer active season compared to northern areas with longer winters (U.S. Fish and Wildlife Service, 2019). However, no research has been published that documents the months of reproduction of ABB in the southern regions of its range.

When a *Nicrophorus* male finds a carcass that is suitable for reproduction, it will stand on top of it and emit a sex pheromone to attract a female (Dekeirsschieter et al., 2011). A fierce competition for the carcass will occur among other beetles of the same species and other species, until only the dominant pair remains (Beeler et al., 1999; Trumbo, 1991). If a male arrives at the carcass before a female, it may bury the carcass superficially and use pheromones to call for a female. When another male is at the carcass, but no female, males will fight, but when there are a female and two males, the males will compete over the carcass and also for the opportunity to mate (Beeler et al., 1999).

When in possession of carrion, larger males will increase their calling for females while smaller ones will decrease calling effort because smaller beetles usually lose the contest over the carrion. When males compete for a carcass the less dominant male will transfer greater quantities of sperm per mating, to compensate for their disadvantage when competing with a dominant male for mating (Pettinger et al., 2011). Even though rare, communal breeding may happen when multiple males and females of different *Nicrophorus* species find a larger carcass (Scott, 1997). When the carcass found can support more offspring than what a single female can produce, a male will attempt to attract other females by pheromones emission (Eggert & Sakaluk, 1995).

After securing a suitable carcass the *Nicrophorus* pair will examine the soil conditions (Ratcliffe, 1996). When the carcass is too large for burial, burying beetles can still use it as a food source. When the carcass is adequate for burial, the mating pair will test the surrounding soil and if found to be too hard for them to bury in, the pair working together can move the carcass about 1 meter per hour, for as long as 3 hours, until they find a suitable area for burial (Ratcliffe, 1996).

Using their heads, the pair of beetles will move the soil from beneath the carcass, causing it to settle into the ground and be buried (Ratcliffe, 1996). The breeding chamber can be located up to 60 centimeters under the ground (Scott, 1998). After burying the carcass, the beetles will remove the fur or feathers and mold it into a ball. Then they will deposit anal and oral secretions on the surface of the brood ball, which will delay the decomposition of the carcass (Ratcliffe, 1996; Scott, 1998).

After the carcass has been placed in the chamber, the female will lay eggs in a separate chamber where, after a couple of days, the larvae will hatch. The parents make a small opening on top of the carcass and treat it with oral fluids, where the larvae will be able to feed (Scott, 1998). The adult beetles feed the larvae regurgitated carcass until the larvae begin feeding themselves (Butler et al., 2012).

The normal brood size for ABB averages 15 larvae, but it is possible to be more than 25 if the conditions are appropriate (U.S. Fish and Wildlife Service, 2014). In cases where the brood is larger than will be supported by the available carcass, adults will commit infanticide so the larvae that remain have enough for their development (Scott, 1998). The female will stay with the larvae throughout the entire development of the brood, while the male will leave the chamber a few days before full development (Dekeirsschieter et al., 2011). After reaching their maximum size, the larvae will disperse from the carcass and pupate in the soil for 48-60 days (Trumbo, 1991; Backlund et al., 2008).

American Burying Beetle Habitat Association

Historically, the ABB ranged throughout most of the eastern and central U.S. and some parts of Canada. Remnant populations remain in about 10% of its historic range in the states of Arkansas, Kansas, Oklahoma, Nebraska, South Dakota, and Rhode Island, and reintroduction has been attempted in Massachusetts, Ohio and Missouri (Lomolino et al., 1995; Raithel, 1991; U.S. Fish and Wildlife Service, 2019). As a result of this drastic decline in distribution, the U.S. Fish and Wildlife Service designated the ABB as a federally endangered species in 1989 (Raithel, 1991).

Based on the historic distributions and surveys made of the remaining populations, ABB is characterized as a habitat generalist when food resources are available (Holloway & Schnell, 1997; Leasure & Hoback, 2017). Since the ABB is the largest burying beetle in North America, it requires larger carcasses than other burying beetles, and to find an appropriate carcass covers large areas (Leasure & Hoback, 2017; Lomolino et al., 1995).

Over the last century in North America, large areas of native forests were removed leading to one hypothesis for ABB decline. Based on historic distribution, most of the ABB's range would have been forested (Anderson, 1982). Fragmented forests have reduced richness of carrion beetle species compared to intact forests (Gibbs & Stanton, 2001; Kozol et al., 1988). Forest fragmentation may lead to the extirpation of species through reduction of total habitat area and disconnected fragments, which interfere with species dispersal (Wilcove et al., 1986). In studies of forest fragmentation, forests that contain dirt roads have more carrion beetle species richness than those that contain paved and heavily used roads (Dunn & Danoff-Burg, 2007).

Breeding success of different species of burying beetles, has been related to the amount of forest available for foraging (Lomolino & Creighton, 1996; Wolf & Gibbs, 2004). The declining numbers of *Nicrophorus* in fragmented forests, has been shown to be more related to the environment lost than to the urbanization around the forests fragments (Wolf & Gibbs, 2004). The reduction or absence of burying beetles in some habitats, may be related to space required for them to successfully find appropriate carcasses (Trumbo & Bloch, 2000). Several studies have also related the type of soil (percentages of clay, silt and loam) with the diversity of burying beetles and soil factors have been suggested to be an important factor when considering conservation areas (Lomolino et al., 1995; Looney et al., 2004).

In response to the classification of ABB as an endangered species, the U.S. Fish and Wildlife Service created a recovery plan in 1991. The short term objective was to reduce the extinction threat to the ABB and the long term objective was to downlist the ABB from endangered to threatened (Raithel, 1991). Even with the finding of new populations of the American burying beetle, the U.S. Fish and Wildlife Service still classified it as an endangered species until 2020 (U.S. Fish and Wildlife Service, 2008). Recently the USFWS down-listed the ABB to threatened because of it wider distribution and larger populations reducing the threat of extinction (U.S. Fish and Wildlife Service, 2020).

Recent studies have compared ABB in northern (South Dakota and Nebraska) and southern (Oklahoma and Arkansas), suggest that ABB do not have specific habitat requirements but are likely to avoid areas disturbed by humans when foraging (Leasure & Hoback, 2017). In the norther ABBs were positively correlated to depressional wetlands, topographic wetness and areas of high precipitation, in the southern population ABBs were associated with sandy soils, native forests and grasslands and hay fields (Leasure & Hoback, 2017).

Different studies have shown that the ABB is a generalist when looking for carcasses. However, it has been suggested that when looking for a place for breeding and burial of a carcass ABB and other *Nicrophorus* are likely more habitat specific (Anderson, 1982; Lomolino et al., 1995). Previously, ABB breeding usage at Camp Gruber was reported (Creighton et al., 1993). The authors suggested that ABB had a similar habitat preference when reproducing as *Nicrophorus tomentosus* and that ABB was a habitat generalist when seeking food sources (Creighton, Vaughn, & Chapman, 1993; Lomolino et al., 1995).

Dense forests can reduce the ability of ABB to detect carrion because of the smaller wind movement, suggesting that open areas such as prairies or savannas, might facilitate carrion detection when foraging (Walker & Hoback, 2007). When seeking food sources, the ABB does not appear to have a preference for type of carrion or environment (Lomolino & Creighton, 1996). In a study carried out in Camp Gruber, OK Schnell et al. (2014) suggested that ABB are attracted to areas with high abundance of mammals and birds, because they are potential breeding resources. In the same studies the authors hypothesize that the same environmental conditions are good for the three animal groups, making the overall numbers higher than other areas (Schnell et al., 2014).

Oklahoma American Burying Beetle

In 2010, a predictive map of ABB occurrence in the eastern area of Oklahoma was created to improve directed conservation actions. However, the map was found to be inaccurate due to its low predictive success, potentially from data deficiencies or incorrect model specifications (Crawford & Hoagland, 2010).

Surveys conducted in eastern and central Oklahoma, suggested that the ABB, preferred forested areas instead of open areas when seeking carcasses for breeding because of compacted soil and lack of organic material in open grassland areas (Lomolino & Creighton, 1996). In a study at Camp Gruber, the presence of ABB was strongly related to soil texture, being more abundant in parts of the military training facility where the percentage of sand in the soil was higher compared to areas with silt or clay (Lomolino et al., 1995). The same study showed that in Oklahoma and Arkansas, the soil depth in the areas with occurrence of ABB was not related to the abundance of ABB as suggested by Anderson (1982).

Studies of forest harvest effects on ABB populations were conducted in southeastern Oklahoma, and suggest ABB are impacted by clear cutting (Anderson, 1982; Creighton et al., 2009). When surveying for ABB before and after deforestation and in nearby areas with minimal disturbance, ABB was not found in the deforested area but remained in the less disturbed area (Creighton et al., 2009). Two possible explanations were given, that beetles close to emergence were killed during the harvesting process and beetles did not have the time to recolonize the region or alternatively the adult ABB could actively avoid the deforested area (Creighton et al., 2009). Previous studies in Oklahoma examined ABB habitat associations for periods when air temperatures were above 60 F at midnight and thus, had short sampling periods between May and September. Studies outside the normal breeding season that examine the same areas over multiple seasons are needed to better determine if ABB is a habitat generalist or specialist.

Overwintering

Arthropods are poikilotherms, and temperate arthropods survive freezing conditions using one of two overwintering strategies (Block, 1990). When the temperature of the habitat starts declining, the insect metabolism will decrease until reaching chill coma and eventually death will occur by freezing (Block, 1990; Bale & Hayward, 2010). A few can be tolerant to freezing within their bodies, while most avoid freezing (Bale & Hayward, 2010). Insects that survive the formation of ice in their bodies are classified as freeze-tolerant while those that die immediately after being frozen are classified as freeze tolerant (Bale & Hayward, 2010; Sinclair et al., 2003). Previous research in Nebraska revealed that *NIcrophorus* stay close to the frostline during the winter suggesting that they are freeze avoidant (Conley 2014). A study of ABB overwintering in Arkansas found that access to a carcass improved winter survival (Schnell et al. 2008).

Another factor that may increase the survival rate of *Nicrophorus* beetles during overwintering, is the size of the insect entering the overwintering stage (Smith, 2002).

Nicrophorus species that overwinter as adults can select the overwintering habitat and bury into the ground prior to the onset of freezing temperatures. If the beetles that overwinter as adults prefer a certain type of environment, and the overwintering phase has a high mortality rate, being able to conserve the required habitats for overwinter survival is crucial for maintaining the ABB population.

With climate change increasing temperatures insect overwintering may be affected (Bale & Hayward, 2010). The beetles on the genus *Nicrophorus*, will overwinter in different stages according to the species (Schnell et al., 2008). Even with a high mortality rate during overwintering, there is not enough data to conclude that the population reduction may be related to it (Bedick et al., 1999; Mckenna-Foster et al., 2016). Provision with a food source during overwintering, increased ABB survival rates in comparison with those without a food source (Schnell et al., 2008), suggesting that beetles remained active during part of the winter and were able to feed.

OBJECTIVES

The objectives of this project were (1) to investigate the seasonal activity and population characteristics of ABB at Camp Gruber, (2) document the breeding months of the southern population of ABB and investigate overwintering survival of ABB.

CHAPTER II

NICROPHORUS AMERICANUS OLIVIER (COLEOPTERA: SILPHIDAE) POPULATION CHARACTERISTICS IN CAMP GRUBER, OKLAHOMA

ABSTRACT

The endangered American burying beetle (ABB), *Nicrophorus americanus* Olivier (Coleoptera: Silphidae), once occurred across two thirds of the United States but now occupies <10% of its historic range. Reasons for the decline have not been determined but are generally associated with changing land use and vertebrate communities, although competition with other carrion beetles has also been suggested. To better understand the population in Camp Gruber, OK I conducted extensive field surveys to characterize the ABB population and carrion beetle community inside the military base. Bi-weekly sampling was conducted from 2016 to 2020. A minimum of 15 baited aboveground traps were used during each period with a minimum of two days of sampling. Three habitats, grassland, savannah and forest were sampled. Once per year an intensive five-day sample period with mark and release sampling was conducted to estimate the yearly ABB population at 20 trap locations. The estimated population on the 133 km^2 Camp Gruber ranged between 1,136 and 4,657 ABB with activity occurring

from April until October. ABB occurred in all sampled habitats, suggesting that ABB might be a habitat generalist. Despite a longer activity period than ABB in northern regions, only one peak of newly emerged ABB was observed in July, suggesting a single brood each year.

INTRODUCTION

Carrion beetles (Coleoptera: Silphidae) are a relatively small group of Coleoptera, containing approximately 200 species (Sikes, 2008). The family Silphidae is divided into two subfamilies, the Nicrophorinae and the Silphinae (Dekeirsschieter et al., 2011). The beetles in the subfamily Nicrophorinae are referred as the "burying beetles", because of the behavior of members of the genus *Nicrophorus*, which bury small vertebrates into the ground (Ratcliffe, 1996). Because of their ecological importance as carrion scavengers, bi-parental care and the endangered species *Nicrophorus americanus* Olivier (Coleoptera: Silhpidae), substantial research on the burying beetles has been conducted (Lingafelter, 1995).

The ABB is the largest North American species of Silphidae and uses larger carcasses (80-250 g preferred) than other *Nicrophorus* for reproduction (Scott, 2002; Backlund et al., 2008; Schnell et al., 2008). Even though the ABB uses larger carcasses, other *Nicrophorus* species also search for carcasses for food and reproduction, and can compete for the resources leading to reduction in reproduction (Howard & Hall, 2019; Keller et al., 2019). All *Nicrophorus* species have a one-year life span with communities showing niche portioning with differences in daily activity patterns and seasonal differences in development (Smith, 2002).

The ABB is nocturnal and will usually only breed once per year. Creighton and Schnell (1998) found that the ABBs located in Oklahoma and Arkansas fly an average of 1.23 km per night or a maximum of 10 km in 6 nights in search for carrion (Creighton & Schnell, 1998). In Nebraska, the ABB has been found to travel further reaching a maximum of 29.19 km in a single night (J. Jurzenski et al., 2011). Chemoreceptors in their antennae allow the ABB to locate an animal carcass up to four km away within one hour of the animal's death (Ratcliffe, 1996).

In the early 1990's a population of ABB was discovered in northeastern Oklahoma, in the Muskogee Management Area/Camp Gruber area (Creighton, Vaughn, & Chapman, 1993). Past surveys conducted in eastern and central Oklahoma, suggested that ABB, prefer forested areas to breed in deep soil associated with organic matter in forests (Lomolino & Creighton, 1996); however, previous surveys have only been conducted during the peak active season and have been limited to a few weeks each year. No studies have characterized the characteristics of ABBs located inside Camp Gruber across the full active season to examine seasonal habitat use nor has any study compared population characteristics for Spring when overwintered adults emerge to fall when the new generation of ABB become active.

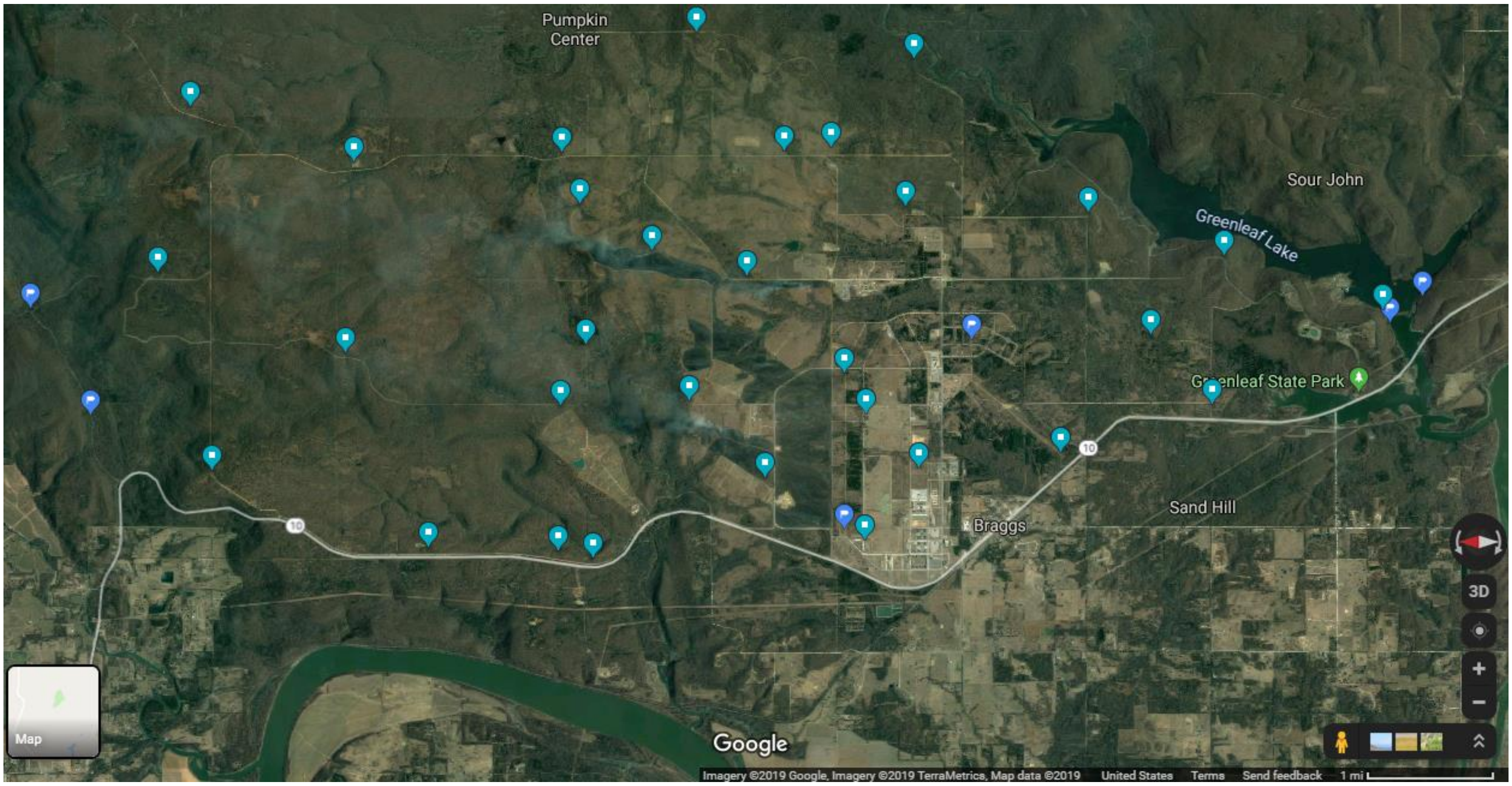
To better understand the characteristics of the ABB population in Camp Gruber, I estimated the ABB population at Camp Gruber from 2016 to 2020, examined ABB association with different habitats inside Camp Gruber, analyzed sex ratios, age ratios, and ABB size as measured by pronotum width and.

MATERIAL AND METHODS

Study Site

The Oklahoma Army National Guard's (OKARNG) Camp Gruber Training Center is located 22.5 kilometers southeast of Muskogee, Oklahoma. The training center has an area of 133 square kilometers and serves as a military training site during weekends and summer field exercises (Oklahoma National Guard, n.d.). The training center hosts Army National guard units, Law Enforcement and Joint Services for a variety of training activities, including live fire exercies. Camp Gruber is also used as a wildlife management area, that is open to public hunting for part of the year. The OKARNG is composed by a mixture of grassland, savannah and forests with streams and some ponds.

Figure 1. Trap locations for *Nicrophorus americanus* sampling at Camp Gruber, OK. 15 locations were used for bi-weekly sampling and 20 locations were used for 5-day population estimates. Sites were selected with guidance from the U.S. Army National Guard based on training activities.



Site Sampling

Sampling for ABB was conducted according to the modified (2014) U.S. Fish and Wildlife Services (1991) protocol using above-ground carrion pitfall traps. Sampling occurred twice a month during the ABB active season (April to October) using 15 traps for 2 nights that were chosen with the aid of OKARNG personnel to ensure that the traps would be accessible and undisturbed. Thus, trap locations varied among dates and years with a total of 36 sites used at least once between 2016 and2020. Because of the presence of scavengers and bedrock at Camp Gruber, the pitfall traps were attached to trees with bungees cords (Leasure et al., 2012). The traps were composed of a five-gallon (18.92 Liter) plastic bucket. A quarter of the bucket (approximately 10 centimeters) was filled with Sphagnum Peat Moss to allow the beetles attracted to the trap to bury themselves to avoid high temperatures, competitors, and desiccation.

The bucket used for the trap had 3mm holes in the sides every 5 centimeters vertically and every 7.62 centimeters diagonally to prevent the bucket from flooding in case of a rain event and also to disperse the carrion scent more easily. In order to prevent beetles from escaping and debris and rainfall from entering the trap, a piece of plywood (70 centimeters by 40 centimeters) was placed on top of the trap and secured by j-hooks. This plywood board also provides a landing area for the beetles so they can enter the trap. The entrance to the trap was an 18-centimeter diameter hole located in the center of the board with a funnel that leads to the interior of the trap and prevents beetles that enter the trap from escaping. Above the entrance a 23-centimeter diameter Frisbee disk was attached by four nails housed in plastic tubing. The Frisbee protects to the trap entrance from debris and rainfall.

The traps were baited with previously frozen 275-375-gram laboratory rats (*Rattus norvegicus*) purchased from RodentPro.com. The rats were thawed and left to age inside a bucket for approximately 5 days at environmental temperatures prior to use as baits. The beetles could access the bait while in the trap.

All captured beetles from the family Silphidae were identified to species. All ABB were measured for pronotal width, sexed, and aged as teneral or mature/senescent. During the 5-day sampling population monitoring, ABB were marked using a cauterizer to obscure one of the markings on the elytra. After all the procedures were made, the ABBs were released individually in holes and then covered with loose soil.

During the month of July of 2016-2019, a 5-day mark-recapture survey was conducted using 20 traps with at least 1.6 km of distance between each trap. In 2020, population estimates were conducted in August. The population size was estimated using the Schnabel index for multiple sampling events and a 95% confidence interval was generated, with each trap was considered a repetition (Schumacher & Eschmeyer, 1943).

To test for differences in size among years pronotal widths for males, females, and for teneral and mature ABB were checked for normality and then compared by year. A Kruskal-Wallis one-way analysis of variance to compare the medians of the pronotal sizes for teneral ABB and to test for differences between ABB collected in the Fall and Spring. For the male and female mature ABB, the normality test passed and a One-Way Analysis of Variance was used followed by a Tukey test.

Seasonal Surveys

To determine the age and sex ratio of the ABB at Camp Gruber, 15 locations were sampled bi-weekly from April to October. The years analyzed were collected between July, 2016 and September 2020. Two-day trapping periods occurred approximately every other weekend, setting the traps on Friday afternoon and checking the traps on Saturday and Sunday. After checking the traps, they were removed from the sites. The location of the trap's sites varied according to military training activity and hunting season. To ensure trap independence they were placed a minimum of 1 mile (1.6 km) apart from each other.

Habitat Usage

To determine if ABB used certain habitats inside Camp Gruber more frequently, 18 sites that were used for at least 3 years of sampling and had at least 5 sampling nights per year were examined. I calculated the percentage of sampling nights that had at least one ABB inside the trap to determine if there is a difference among traps set in savannah, forest and grassland inside Camp Gruber.

For the habitat comparison, the months of April and October were excluded due to the low numbers of ABB collected, because of weather conditions. The traps were classified by the frequency (%) of sample nights with at least one ABB captured as not present (0-24%)(class 1), rare (25-49%)(class 2), probable (50-74%)(class 3), very probable (75-90%)(class 4) and always present (91-100%)(class 5).

Occurrence of ABB and other Silphidae was also examined using GIS to create heat maps. I analyzed the number of ABB captured at each site, spatially, using the Getis-Ord Gi* statistic in ArcMap version 10.8 (ESRI, 2020). This approach looks at each feature within the context of neighboring features. If a feature value is high, and the values for all its neighboring features are also high, and the conclusion is that of the area is a hotspot.

First, I used the Getis-Ord General G high/low clustering tool (ESRI, 2020) to determine the optimal threshold distance within 100 meters. Second, I conceptualized and calculated spatial relationships relating sample sites within the study area using a fixed distance band. The threshold distance used for this analysis was 2,700 meters. Third, I implemented Euclidian distance to give an output of a z-score and p-value for each site within the sampling area. Sites with high z-scores (greater than 1.645) and small p-values indicated spatial clustering and thus, an ABB hotspot. Sites with low z-scores (less than -1.645) and high p-values indicated a spatial clustering of absence of ABB.

I then implemented the Kriging interpolation tool in ArcMap (ESRI, 2020) using the z values calculated in the Getis-Ord Gi* analysis to generate the output surface. Kriging is a geostatistical procedure that generates an estimated surface from a scattered set of points with z-values.

RESULTS

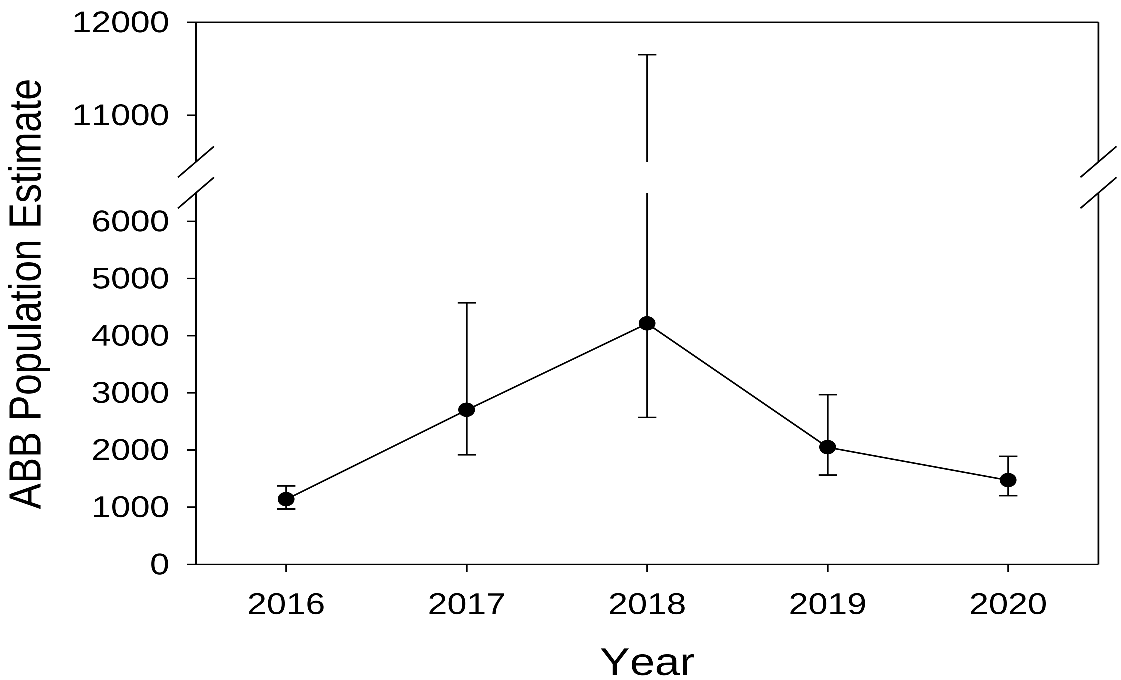
During surveys, 10 species of Silphidae were captured. The rarest *Nicrophorus* species were *N. carolinus*, *N marginatus*, and *N. tomentosus* which are all diurnal species.

Between 2016 and 2020, a total of 6,161 ABB were captured at least once, with 346 recaptured at least once. The estimated population for ABB ranged between 1,136 and 4,212 individuals and showed an increase up until 2018, followed by a decrease (Table 1). Across years, the average population was 1,232 *Nicrophorus americanus*. Populations ffor 2017, 2018 and 2019 were not significantly different from each other while the year of 2020 was significantly smaller than 2018 (Figure 2).

Table 1. Number of total silphids caught at Camp Gruber, Braggs, OK and populations estimates for *Nicrophorus americanus* with the 5-day sampling period recaptures.

	2016	**2017**	**2018**	**2019**	**2020**
Trap Nights per year	15	30	27	27	25
Nicrophorus americanus	607	1,263	1,738	1,316	1,237
N. amerianus recaptures (5-day)	18	27	54	26	32
Population estimate	**1,136**	**2,701**	**4,212**	**2,047**	**1,469**
N. carolinus	2	2	9	1	1
N. marginatus	11	39	14	6	1
N. orbicollis	1,191	1,532	1,372	1,549	1,422
N. pustulatus	373	875	1,580	1,446	1,430
N. tomentosus	48	249	300	324	149
Necrodes surinamensis	872	4,802	4,282	15,633	5,608
Necrophila americana	75	363	336	1,066	1,147
Oiceoptoma novaboracense	0	3	7	18	6
O. inaequale	0	10	198	99	742
Total Captures per year	3,179	9,138	9,836	21,458	11,609

Figure 2. Populations estimates and 95% confidence intervals using the Schnabel method for *Nicrophorus americanus*



In 2019, the ABB population estimate decreased inside Camp Gruber while the number of other carrion beetles increased. The increase was greatest for *Necrodes surinamensis*, which increased from 4,282 in 2018 to 15,633 in 2019. Between 2016 and 2020, the number of *N. pustulatus* also increased substantially.

The ratio between ABB males and females showed females to be generally more common than males throughout the year (Table 2). In 2019 the only time female numbers were slightly less than male members was in the beginning and end of the active season, while in 2020 this only happened in May. For the other years, the ratio between males and females was consistent with females being captured more often than than males throughout the entire year.

Table 2. Sex ratio (M/F) of *Nicrophorus americanus* from 2016-2020 during active season in Camp Gruber, Braggs, OK

	2016			**2017**			**2018**		
	Male	**Female**	**Ratio**	**Male**	**Female**	**Ratio**	**Male**	**Female**	**Ratio**
April	-	-	-	8	8	1:1	0	0	0
May	-	-	-	16	33	1:2.1	98	147	1:1.5
June	-	-	-	30	55	1:1.8	43	47	1:1.1
July	97	145	1:1.5	160	288	1:1.8	472	536	1:1.1
August	90	144	1:1.6	125	200	1:1.6	172	183	1:1.1
September	25	41	1:1.6	64	143	1:2.2	15	25	1:1.7
October	25	39	1:1.6	48	85	1:1.8	0	0	0
Total	**237**	**369**	**1:1.6**	**451**	**812**	**1:1.8**	**800**	**938**	**1:1.2**

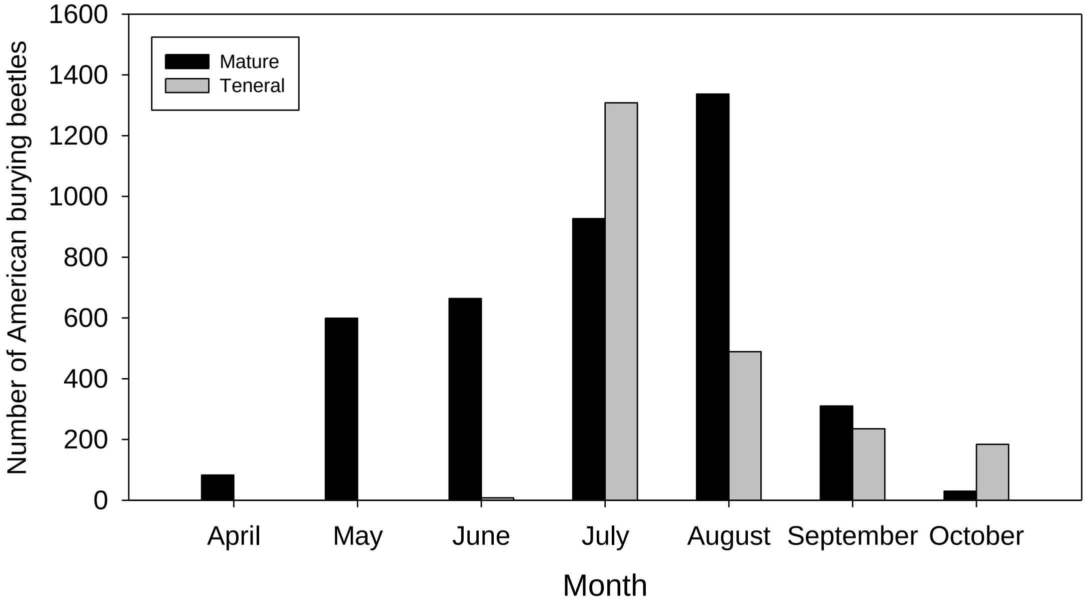
	2019			**2020**		
	Male	**Female**	**Ratio**	**Male**	**Female**	**Ratio**
April	40	27	1:0.7	-	-	-
May	6	0	1:0	157	142	1:0.9
June	148	163	1:1.1	85	88	1:1
July	156	246	1:1.6	55	79	1:1.4
August	148	184	1:1.2	263	317	1:1.2
September	76	105	1:1.4	24	27	1:1.1
October	9	8	1:0.9	-	-	-
Total	**583**	**733**	**1:1.3**	**584**	**653**	**1:1.1**

The age ratio of ABBs throughout the season was similar across the five years (Table 2). Up until July only mature beetles were trapped, with the exception of 8 tenerals in the month of June in 2020. After a peak of teneral beetles in July, the number of tenerals decreased, with the exception of October of 2017, where the amount of tenerals had a slight increase in the last month of the year. Compared to 2016-2018, the number of tenerals in July was smaller than the number of mature ABB in 2018 and 2020. When looking at the total proportion between mature and teneral beetles throughout the years, there is only one peak in numbers of tenerals in July followed by a decrease in both mature and teneral beetles (Figure 3).

Table 3. Mature (M) and Teneral (T) ratio for *Nicrophorus americanus* in Camp Gruber, Braggs, OK.

	2016			**2017**			**2018**			**2019**			**2020**		
	M	**T**	**Ratio**	**M**	**T**	**Ratio**	**M**	**T**	**Ratio**	**M**	**T**	**Ratio**	**M**	**T**	**Ratio**
April	-	-	-	16	0	1:0	0	0	1:0	67	0	1:0	-	-	-
May	-	-	-	49	0	1:0	245	0	1:0	6	0	1:0	299	0	1:0
June	-	-	-	85	0	1:0	103	0	1:0	311	0	1:0	165	8	1:0.1
July	61	182	1:3	106	342	1:3.2	387	621	1:1.6	309	93	1:0.3	64	70	1:1.1
August	87	147	1:1.7	148	177	1:1.2	319	36	1:0.1	248	84	1:0.3	535	45	1:0.1
September	14	52	1:3.7	95	112	1:1.2	31	9	1:0.3	150	31	1:0.2	20	31	1:1.5
October	9	55	1:6.1	10	123	1:12.3	0	0	0	11	6	1:0.5	-	-	-

Figure 3. Total captures of mature (M) and teneral (T) of *Nicrophorus americanys* from years 2016-2020 in Camp Gruber, Braggs, OK



Across the years of the study, ABB showed a trend in declining size with mean pronotal widths decreasing from about 10 mm to 9.4 mm for males and 9.3 to 8.98 for females. The pronotal width (mm) of *N. americanus* senescent males in 2016 (10.015 ± 0.102) had no statistical difference from the ones in 2017 but was larger than the senescent males for the years 2018, 2019 and 2020. The senescent females had no statistical difference among years. The teneral males had the same statistical difference of senescent males, where males of 2016 and 2017 did not differentiate between them but were larger than the following years. Teneral females did not differentiate in size when comparing 2016 and 2018, the years of 2017 and 2019 were no different from either 2016 or 2020 (Table 4) (Figure 4). There is no apparent relationship between the size of the beetles in the last months of the season and the size of the beetles in the first months of the next season.

Table 4. Mean (± 1 S.E.) pronotum width (mm) by age of *Nicrophorus americanus* at Camp Gruber, OK from 2016-2020

	2016						2017						2018			
	Teneral			Senescent			Teneral			Senescent			Teneral			Senescent
Male	9.90	±	0.07	10.01	±	0.10	9.75	±	0.06	9.99	±	0.06	9.52	±	0.06	9.63
Female	9.31	±	0.07	9.43	±	0.11	9.11	±	0.07	9.39	±	0.07	9.37	±	0.07	9.19

	2019						2020					
	Teneral			Senescent			Teneral			Senescent		
Male	9.35	±	0.10	9.49	±	0.04	9.30	±	0.14	9.53	±	0.04
Female	9.05	±	0.10	9.22	±	0.04	8.98	±	0.13	9.30	±	0.05

Figure 4. Mean (± 1 S.E.) pronotum width (mm) by age of *Nicrophorus americanus* at Camp Gruber, OK from 2016-2020. Mature individuals were produced the previous year while tenerals are the result from current year breeding

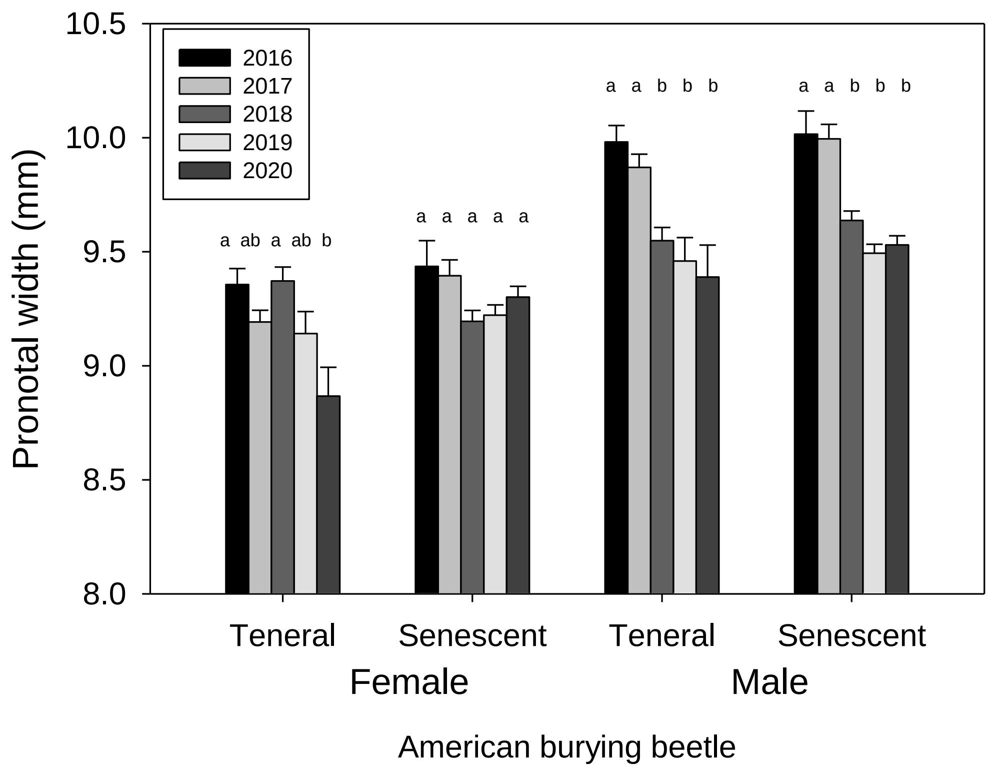


ABB were commonly captured in all habitat types. Of the 18 traps that met criteria to test for frequency of ABB capture, no traps were classified as class 1 (no ABB) or class 5 (always with ABB present), and 50% of the traps were classified as class 3.When heat maps were generated, ABB were found in different areas across years inside Camp Gruber. In 2018 for example, it was possible to see a higher density of positive traps on the east part of the base and a lower number in the cantonment area (Figure 5), but other years differed (Appendix).

Figure 5. Heat map for *Nicrophorus americanus* in 2018. White dots indicate traps/ Blue areas had less occurrence and red areas had more occurrence.

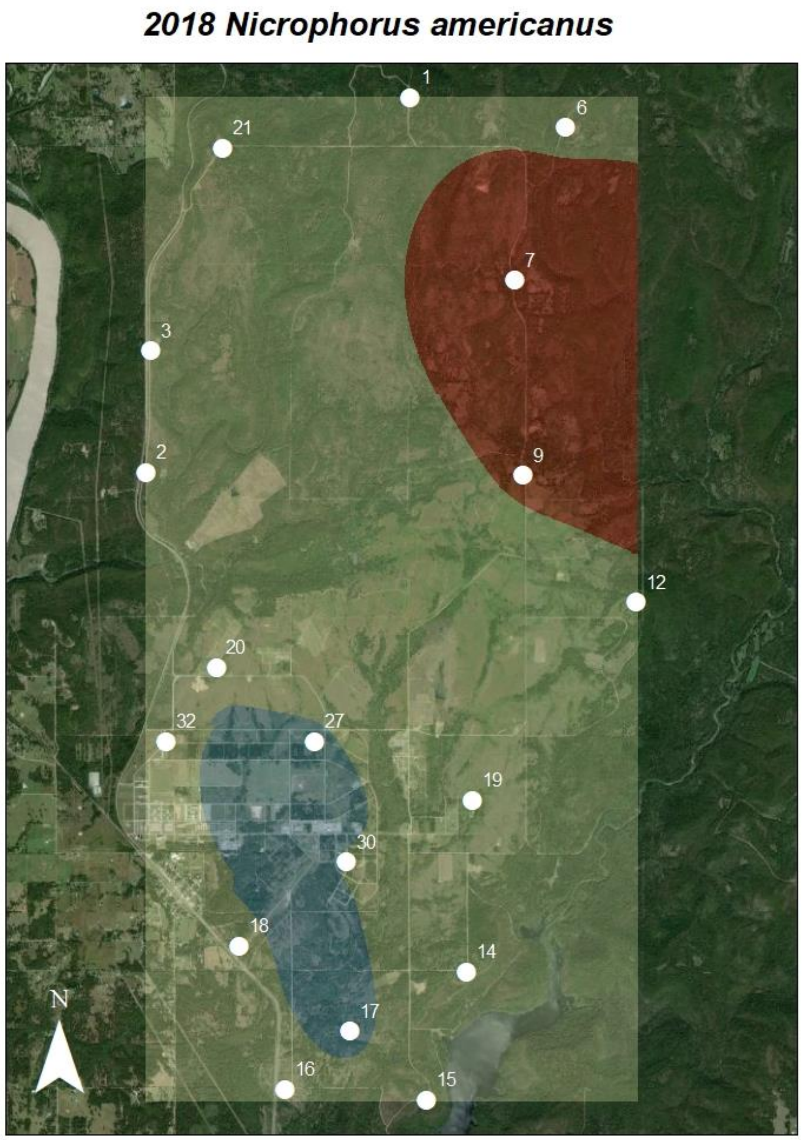


Table 5. Distribution of *Nicrophorus americanus* per habitat in Camp Gruber, Braggs, OK using number of nights that had at least one ABB.

Trap #	Habitat	Nights with ABB (%)	Class
1	Savannah	75%	4
2	Savannah	90%	4
3	Forest	75%	4
6	Forest	71%	3
7	Savannah	69%	3
9	Grassland	75%	4
12	Savannah	73%	3
13	Forest	28%	2
14	Forest	70%	3
15	Forest	74%	3
16	Savannah	70%	3
17	Forest	76%	4
18	Savannah	50%	3
19	Grassland	72%	3
20	Grassland	79%	4
21	Savannah	76%	4
27	Savannah	47%	2
30	Grassland	50%	3

DISCUSSION

The number of ABB in Camp Gruber increased from 2016 to 2018, reaching a maximum population estimate of 4,657. After 2018, the estimated population size decreased. In the same time period, the pronotal width of the beetles collected decreased consistently, which could be related to limitations of food sources. The ABB inside Camp Gruber exhibited generalist behavior, and was frequently found in all the different habitats inside the base.

In 2019, Oklahoma had record breaking rainfall. From January to May of 2019, Muskogee received more rain than the total rainfall for 2018 (Mesonet). ABB occurrence has been positively related to rainfall in Nebraska, but the impacts to ABB from high amounts of rain in a short period of time are not recorded (J. D. Jurzenski et al., 2014). The unusual rainfall started in May of 2019, and the active season of ABB in Oklahoma starts in April. This time frame made it possible for ABB to emerge from overwintering and attempt reproduction. The U.S. Fish and Wildlife Service (USFWS) suggests that ABB could have two broods per year because of the longer active season in southern populations (U.S.F.W.S., 2019). If the first brood is produced during the first months after overwintering, the soil saturation could have drown ABB broods and adults.

The only carrion beetle species that appeared to increase after 2018 were *Necrodes surinamensis* Fabricius (Coleoptera: Silphidae) and *Necrophila americana* Linnaeus (Coleoptera: Silphidae). These two species feed on maggots in decomposing carcasses, *N. surinamensis* is active all year long and *N. americana* has an active season starts in late May (Ratcliffe, 1996). The increase in population of these species possibly related to more food resources from larger vertebrates that drown. Although all Silphidae use carcasses for feeding, members of the Silphinae are not considered to be direct competitors with *Nicrophorus.*

Other than the record amount of rain that occurred in 2019, temperatures during winter (ex. December 1st– February 28th for the active season of the next year) have generally increased. For example, in 2018, which had a population estimate of 4,657 ABB, the lowest temperature at Camp Gruber in the winter period reached -16.25 °C and 58 days had minimum temperatures below 0 °C. For 2017, which had the second largest population estimate, the lowest temperature during the winter was -21.59 but there were 38 days with temperatures below 0 °C. In 2016, 2019 and 2020, the years with the lowest population estimates, the lowest temperature in the winter was between -6.19 °C and -7.92 °C and there were an average of 45 days with temperatures below 0°. The rise in temperature has been suggested as one of the risks for the current populations of ABB, especially in the southern region, which could relate to lower winter survival as the temperature during winter rises (U.S. Fish and Wildlife Service, 2019).

Temperate female insects often need some environmental cues, such as temperature, for their neuroendocrine system to release their reproductive diapause (Pener, 1992). In monarch butterflies, *Danaus plexipus*,, if the temperature fluctuates too much, both males and females will have reproductive complications, including male sterility (Goehring & Oberhauser, 2002). It has been Other *Nicrophorus* species will avoid the hottest hours of the day to avoid over-heating and dehydrating, showing that hot temperatures may affect the behavior of the beetles in this genus (Bedick et al., 2006). More research is needed to understand the effects of increasing temperatures on *Nicrophorus*, including ABB.

In 2020, the period of the 5-day sampling to estimate the population of ABB inside Camp Gruber had to be moved to August because of active military training in the base. Thus compared to the previous years, the decline in population could also be a result of later sampling when fewer senescent beetles are still alive. The decline in ratio between senescent and teneral beetles in 2020 during July is because there were less trap nights when compared with the past years, but this change in dates also contributed to more beetles in August in 2020 than in any other year.

Interspecific competition may also contribute to declines in ABB. Previously, *Nicrophorus orbicollis* has been shown to have niche overlap with ABB, suggesting competition for carrion for reproduction and food (Howard & Hall, 2019). During this five-years study, the ABB population estimate had a large increase in 2018, followed by a decrease in 2019 and 2020. Along with the shift in estimated population sizefor ABB, *N. orbicollis* increased along with, *N. pustulatus*. These results suggest increased competition

The higher number of females when compared to males throughout all the years, could be related to ABB reproductive behavior. When a male finds a suitable carcass it will stay at the carcass and emit pheromones to attract females (Beeler et al., 1999). This behavior might explain why there is a higher number of females at the traps, if males release their attraction pheromones.

The higher number of females, along with the decrease in population, could also be related to ABB infection by the bacteria *Wolbachia*. This bacteria is maternally inherited and can cause feminization in insects, leading to skewed sex ratios (Kern et al., 2015). ABB have not been tested for infection by *Wolbachia* in Camp Gruber, and it is recommended that the population at Camp Gruber be investigated for *Wolbachia* in order to draw any conclusions.

The smaller number of males could also be reflected by males finding another carcass, other than the one in the trap, and guarding it. At a carcass, males attracts females, but also other males that are looking for a suitable carcass (Beeler et al., 1999). The lack of female sex bias in the early and late season, might indicate that ABB are only searching carcasses as food resources and not as breeding resources. If outside of the reproductive period, males would likely not advertise for females.

Previous studies have shown that bigger beetles have better chances of surviving the overwintering period (Smith, 2002). However, when looking at the sizes of beetles in August and September and comparing them with the beetles captured in the months of April and May of the following year, no apparent relationship is observed. Although no relationship in sizes between before and after overwintering periods were observed, the overall sizes of the beetles declined since 2016. The decrease in size of beetles can be an indicative of lack of food sources, since the more the larvae eat the larger they will grow. This could also indicate that smaller beetles are having more reproductive success, as will be discussed in chapter 3. In *Nicrophorus,* bigger parents usually have bigger offspring while smaller parents have smaller offspring.

The ABB only lives for one year. During this year it will reproduce in the summer months and overwinter as an adult (U.S. Fish and Wildlife Service, 2014). During the first half of the active season, April through June, all the beetles collected were classified as mature, which indicates they are old enough to reproduce but still have not reproduced (Table 4). Teneral beetles differ in color and are distinguished by having uniform coloration on the pronotum and elytra. The numbers of both, senescent and teneral beetles decreased as the active season ends, which could indicate that the senescent ABB are dying from old age and the tenerals are starting the overwintering process. In all five years, the peak of tenerals occured in July, strongly suggesting that the ABB reproduces in the late spring and beginning of summer, so that tenerals emerge in July. The peak of senescent beetles, which emerge after brood success, in July also supports this hypothesis.

When examining habitat associations, half of the traps were considered in class 3, which indicates that it is probable that ABB will be caught. The frequency of ABB in the traps per habitat supports the hypothesis that in Camp Gruber, ABB is a habitat generalist when food is available (Holloway & Schnell, 1997; Leasure & Hoback, 2017). These data contradict the previous conclusions that ABB prefer forests in the Camp Gruber region (Creighton et al., 1993). Based on attraction to baited traps, the ABB is a habitat generalist and ABB will occur in different habitats, including developed areas within cantonment at Camp Gruber while searching for carrion (Lomolino et al., 1995; J. D. Jurzenski et al., 2014).

The heat maps suggest that areas of the highest density change among years. In 2018 a cold spot was found in the cantonment area, while the central area of the base had the greatest number of ABB. Because of live fire exercises, sampling at Camp Gruber cannot be uniform and the results of heat mapping must be viewed with caution.

CHAPTER III

REPRODUCTIVE CHARACTERISTICS AND OVERWINTERING OF THE AMERICAN BURYING BEETLE *(NICROPHORUS AMERICANUS)* IN OKLAHOMA, USA

ABSTRACT

The endangered American burying beetle (ABB), *Nicrophorus americanus* Olivier (Coleoptera: Silphidae), depends on small vertebrate carcasses that it buries and uses as a food source for its offspring. The species occurs in two separate populations in the Midwest, a southern population centered in Oklahoma and a northern population centered in Nebraska. Although it is an annual species, seasonal activity occurs from April to October in the southern population and June to August in the northern population leading to the possibility of double brooding in the south. The ABB overwinters as adults underground and emerge in the Spring to reproduce. Overwintering has been suggested as one of the main causes of death in ABB. The objectives of this study were to determine overwintering survival and document the months of ABB reproduction in Oklahoma. A field study to determine overwintering survival was conducted by setting 30 laboratory raised ABB. A laboratory study was conducted to investigate the months of reproduction, by collecting two male/female pairs twice per month from April to

October in Muskogee County Oklahoma. The beetles in the overwintering experiment were placed in October of 2019 at the Cherokee Nation Sallisaw Creek State Park and checked of survival in March of 2020. In the laboratory ABB pairs were placed into containersand provided a large rat (175 grams) as a food and reproduction resource. More than 50% of ABB successfully overwintered. The first successful brood happened with ABB collected in July of 2019, with the mean ($\pm$ 1 S.E.) brood size of 15.71 ± 2.9 in 2019. ABB collected in 2020 generated only 2 successful broods. Based on these results, it appears that ABB survives in different environments during winter and generally reproduces only once and later than was previously believed in the southern population.

INTRODUCTION

The subfamily Nicrophorinae which is composed of members of the genus *Nicrophorus,* are commonly referred to as 'burying beetles', because of their behavior of burying small vertebrate carcasses (Ratcliffe, 1996). The genus *Nicrophorus* has been intensively studied for several reasons, including its bi-parental care and because of the federally endangered species, the American burying beetle (ABB), *Nicrophorus americanus* Olivier (Lingafelter, 1995). This species disappeared from over 90% of its historical range, which caused it to be protected in 1989 (Lomolino & Creighton, 1996).

The ABB is the largest North American species of Silphidae and buries carcasses to be used as a food source for their offspring. Usually this species uses larger carcasses (80-250 g) than other *Nicrophorus* species when reproducing (Backlund et al., 2008; Schnell et al., 2008; Scott, 2002). The ABB is a nocturnal species and is considered univoltine in northern populations (Rhode Island, South Dakota, and Nebraska), but recently the southern population of ABB has been suggested to produce two broods in one year (U.S. Fish and Wildlife Service, 2019). In Oklahoma teneral beetles were observed emerging in August and September (Ferrari, 2014), but no recent data has investigated this activity in Camp Gruber, Oklahoma where a large self-sustaining population exists.

In this experiment, the reproduction of ABB collected from Camp Gruber, near Braggs OK was tested to better understand the reproduction patterns of Oklahoma ABB.

The beetles in the genus *Nicrophorus* generally overwinter as adults, and are thought to avoid freezing (Schnell et al., 2008). Carcass availability during the overwinter period is an important factor in reducing mortality, and access to a food source during overwintering, has been shown to increase ABB survival rates compared to those without a food source (Schnell et al., 2008). In addition to food, the size and age of ABB influenced survival perhaps as a result of beetles that emerged earlier having more fat reserves. Overwintering beetles without a food source survive until activity becomes possible using stored energy and because metabolism is driven by environmental temperatures, warmer temperatures or highly fluctuating temperatures could cause individuals to use up fat reserves too quickly.

The difference in temperatures among habitats has been linked with chance of survival of other insects (Bale & Hayward, 2010; Sorvari et al., 2011). It is possible that in environments with less vegetation covering, such as prairies, and with the rise in winter temperature from climate change, that the mortality of overwintering insects may increase (Sorvari et al., 2011). Overwintering by ABB in Oklahoma has not been previously tested.

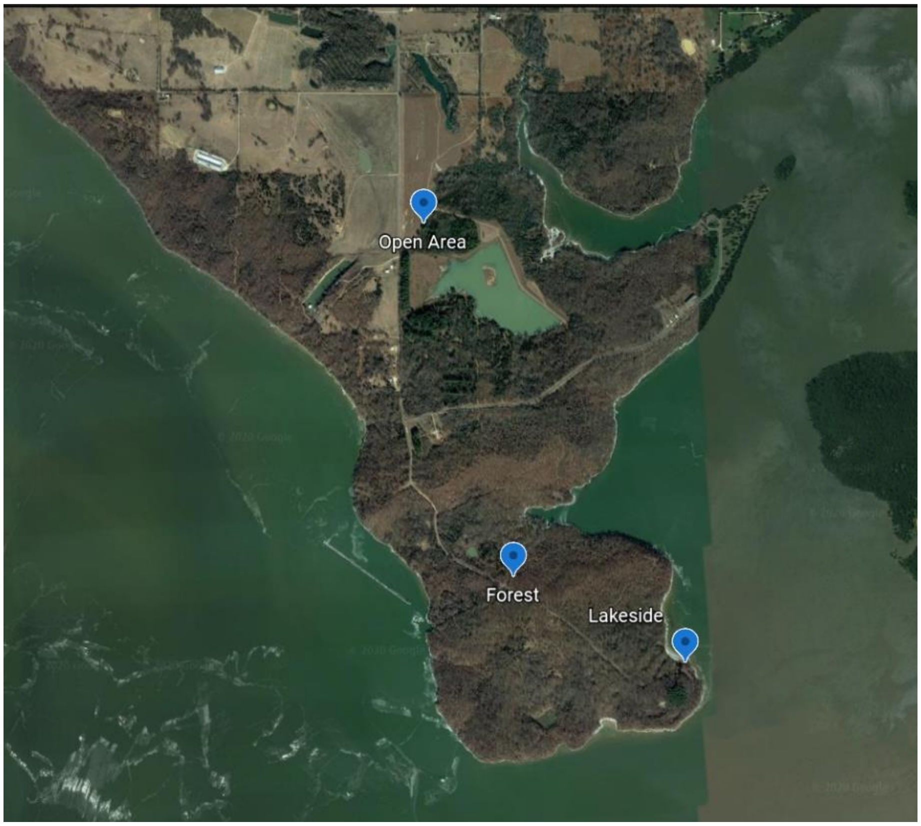
This study investigated survival rates of Oklahoma ABB in a lakeside, forest and open environment and characterized the months of ABB reproduction in Oklahoma.

MATERIAL AND METHODS

Field Study Site

The Cherokee Nation Sallisaw Creek State Park is located 11.7 km southwest of Sallisaw, Oklahoma. This park is open for the public and has several horseback trails. areas for archery and is managed for hunting and fishing opportunities. The park has different ecological areas and three were chosen to examine overwinter survival: a lakeside (35°22'47.3"N 94°52'09.3"W) that had small trees and some bushes next to a large body of water, a mature forest area (35°23'01.3"N 94°52'43.5"W) that had large trees covering the entire area and a deep layer of organic material on the ground, and an open area (35°23'58.4"N 94°53'01.3"W) at the edge of the forest which had no trees or bushes and was covered in cold season short grass (Figure 4).

Figure 6. Cherokee Nation Sallisaw Creek State Park map.10 enclosures were placed in each habitat with 5 male and 5 female *N. americanys* that were laboratory reared



Overwintering Tubes

To house the beetles in the field during the experiment, high-density polyethylene tubes with 4 mm perforations (Pentair Aquatic Eco-systems) were used. Ten tubes were placed per site with a spacing of approximately 1 meter from each other. All tubes were 55.88 cm long and 10.16 cm diameter. Closing both ends of the tubes were two metal screens, the first layer was a soft metal mesh that covered the entire opening and was held with a tightened hose clamp. The second metal layer was a strong chicken wire screen that was glued with epoxy to the softer metal mesh covering and placed at the top which remained above ground and prevented beetles from escaping or scavengers from gaining access.

The perforations allowed the soil in the tube to experience environmental conditions, including rainfall, subsurface moisture, and temperature fluctuations. To standardize the soil for all habitats, organic soil was mixed with topsoil purchased from a hardware store. Holes were made using an auger and each tube was closed with the screen and the hose clamp and put in the hole. After the tubes were in the ground, they were filled with organic soil, leaving approximately 5 centimeters of space above the surface.

A medium size rat (approximately 180 grams) that had been previously frozen was placed in the tube to provide a food source during the overwintering period. Single ABBs were added to each tube so that five males and five females were placed in each habitat, all the beetles were measured before placing them in the overwintering tubes. The ABB used in the experiments were lab reared from beetles collected in July from Camp Gruber. When extracting the tubes from the soil, the depth of burial of the beetles was visually assessed

Weather Data

The surface temperature in each habitat were recorded using a HOBO weather station tied to a cinder block placed in the middle of each site. The air temperature was recorded every 8 hours daily. Rainfall data were obtained through the Mesonet website (www.mesonet.org) from the Sallisaw weather station. Temperature was also obtained from the same station to compare for site-specific differences.

Data Collection

The overwintering tubes were placed on October 12th, 2019. Tubes were extracted on March 17th, 2020 and ABB were checked for survival. All tubes were extracted from the ground manually and then dumped in approximately 5 cm sections onto a wire screen until the ABB was discovered. Survival and approximate depth of burial were recorded. Surviving beetles were returned to the laboratory and placed in breeding chambers.

ABB Laboratory Reproduction

The field collection for ABB followed the U.S. Fish and Wildlife Service protocol and used modified above-ground traps baited with rotten rats. During each sample period, two male and two female ABB were collected and taken into the laboratory. Once in the laboratory a male-female pair was placed into a container (13.97 x 29.21 x 34.29 cm) with moist Sphagnum Peat Moss. A thawed large rat (275-375 grams) was placed on top of the soil. One pair was placed in a controlled environmental room (23°C, 70% RH, and 14 Light: 10 Dark) and the other in a darkened laboratory room at 23°C. The chambers were checked for reproduction, without disturbing the inside of the chamber, twice per week and distilled water was used to keep the environment moist.

When the adult beetles surfaced, they were removed and placed in individual containers with moist paper towel and provided mealworms as food source. When the teneral beetles surfaced, they were also placed in individual containers with water and food. All teneral ABB were counted and measured.

RESULTS

A total of 17 of 29 ABB survived the field overwintering trial. In both the open and lakeside areas, 50% of the ABB survived the winter, 3 males and 2 females in the open area and 3 females and 2 males in the lakeside. In the forest seven out of nine beetles, 5 males and 2 females, survived and one ABB was not found, although the tube had no apparent openings.

The average depth of burial of the survivors in the open area was 3.05 ± 2.03 cm while the dead ones were found at an average depth of 7.11 ± 4.98 cm. In the lakeside area, survivors were found at an average depth of 21.34 ± 5.71 cm while the dead ones were found in 1.02 ± 1.02 cm. In the forest area the average depth of the survivors was 15.60 ± 7.57 cm and the dead ones were found at 1.27 ± 1.27 cm. the high variation in burial depth partially reflects that ABB were active with several individuals attempting to escape the tubes during field collections in March. The average survivor pronotal width was 10.52 ± 0.11 mm while the average pronotal width was 10.06 ± 0.14 mm for dead beetles. The surviving beetles were significantly larger than the ones that died (Students t-test, $P = 0.014$).

The temperatures among the sites and the Sallisaw meteorological station were different. The temperatures recorded for the open area site (Figure 5) showed a maximum temperature of 39.67 °C in October and a minimum of -6.78 °C in November, with 43 out of 155 days (27.74%) having the minimum temperature below freezing (0°C). In the lakeside area the maximum recorded temperature was 32.81 °C in February and the minimum was -5.67 °C in November (Figure 6). The minimum temperature was below freezing (0°C) for 26 out of 155 days (16.77%). In the forest area the HOBO weather station stopped working in February 2020 (Figure 7). The maximum temperature registered was 25°C in October and the minimum was -6.82°C in November, 24 days out of 111 (21.62%) had minimum temperatures below freezing (0°C).

Figure 7. Temperature at the open area of the Cherokee Nation Sallisaw Creek State Park where *Nicrophorus americanus* were tested for overwinter

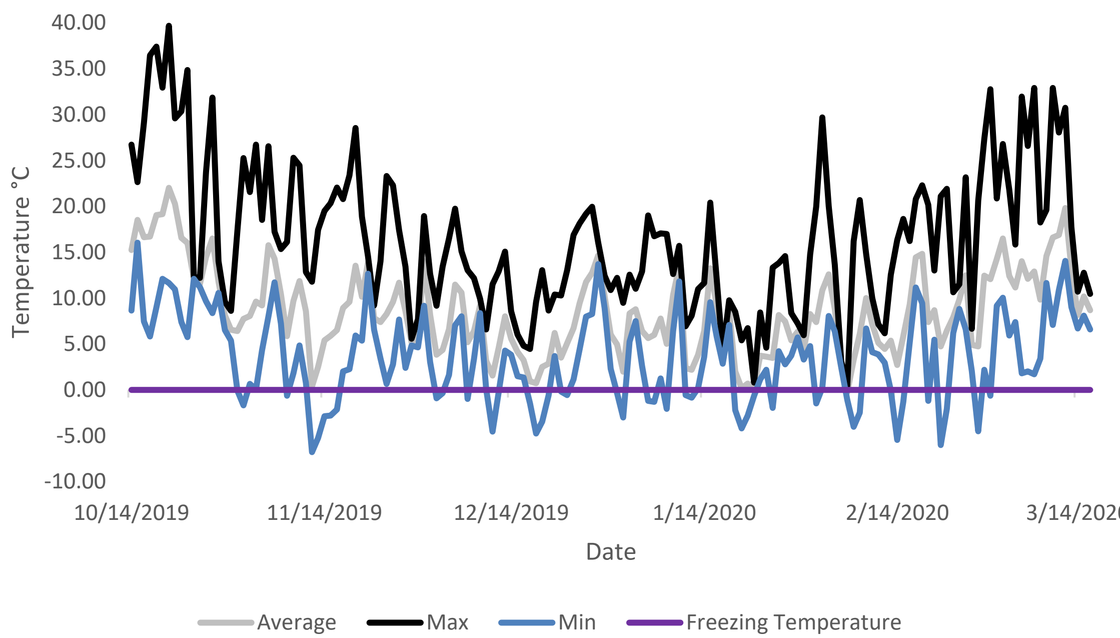


Figure 8. Temperatures at the lakeside area of the Cherokee Nation Sallisaw Creek State Park where *Nicrophorus americanus* were tested for overwinter

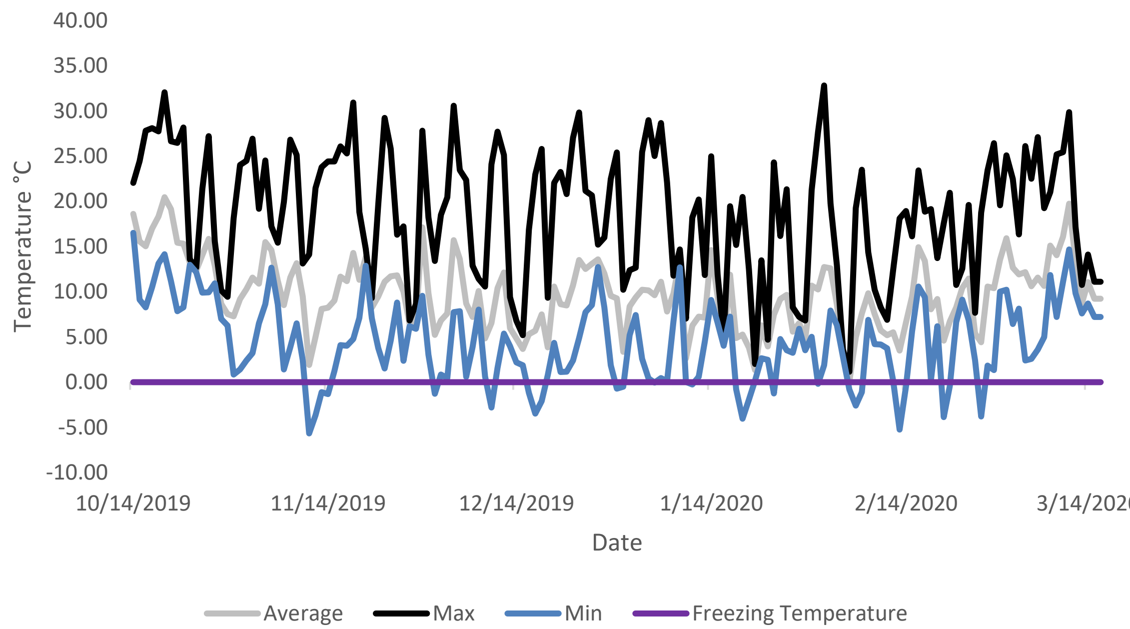
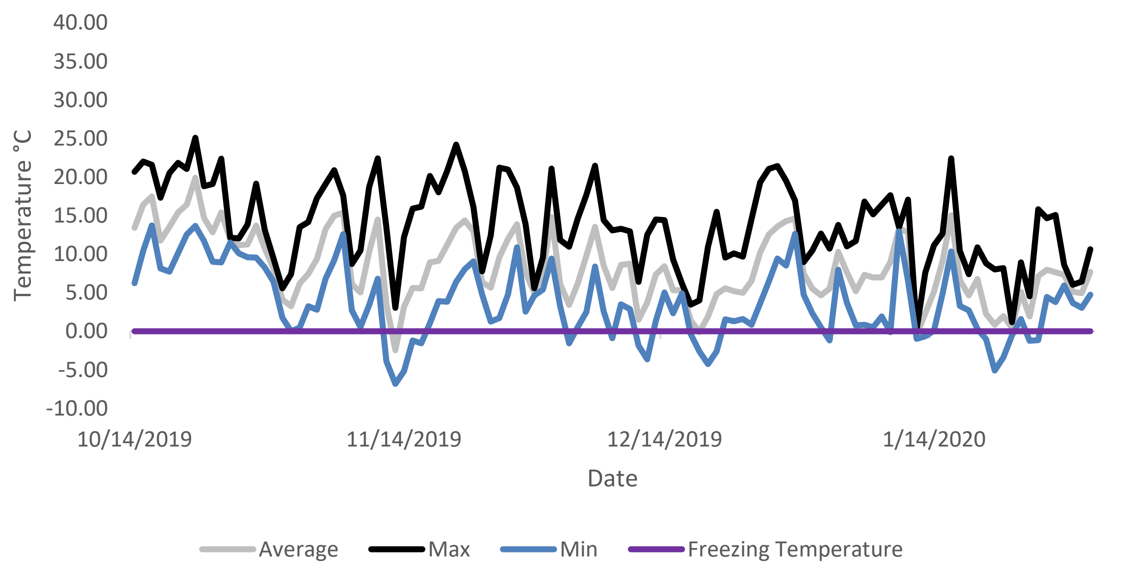
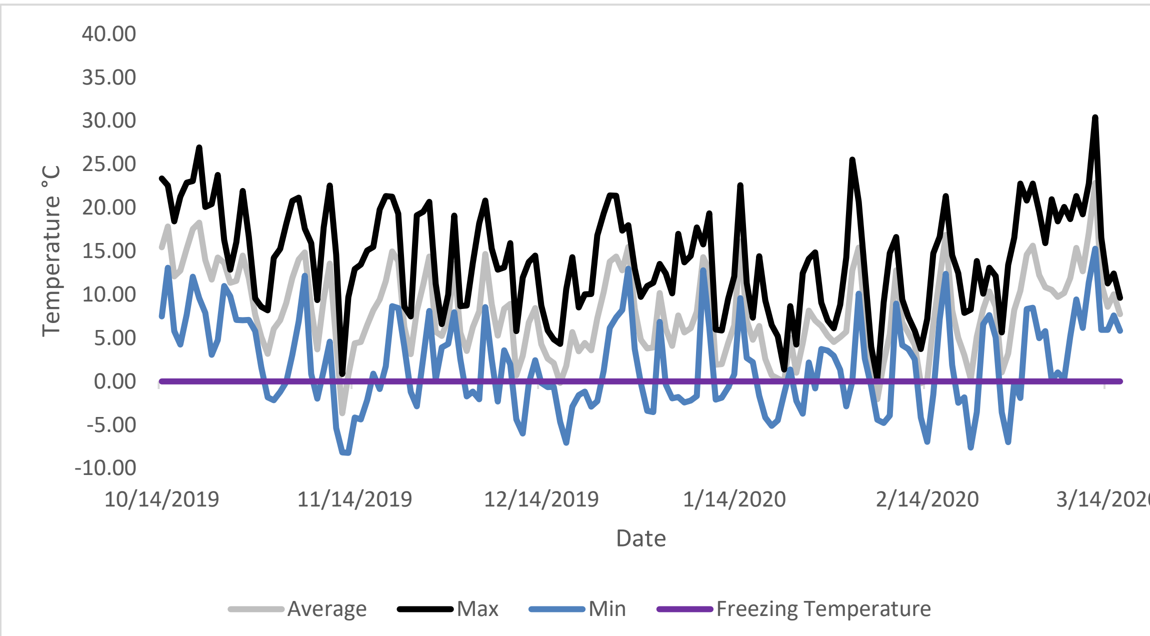


Figure 9. Temperatures at the forest area of the Cherokee Nation Sallisaw Creek State Park where *Nicrophorus americanus* were tested for overwinter



The temperature data obtained from the Mesonet station in Sallisaw (Figure 8) showed that the maximum temperature was 30.41°C in March and the minimum temperature was -8.22°C in November. Out of the 155 days registered for the experiment, 68 (43.87%) had the minimum below freezing temperatures (0°C). The rainfall data showed an average of 3.85mm of rain across all days, with the maximum rainfall in one day of 75.43 mm and the cumulative amount 549.91 mm during the 155 days.

Figure 10. Temperatures at the Sallisaw Mesonet Weather Station for October 2019 through March 2020

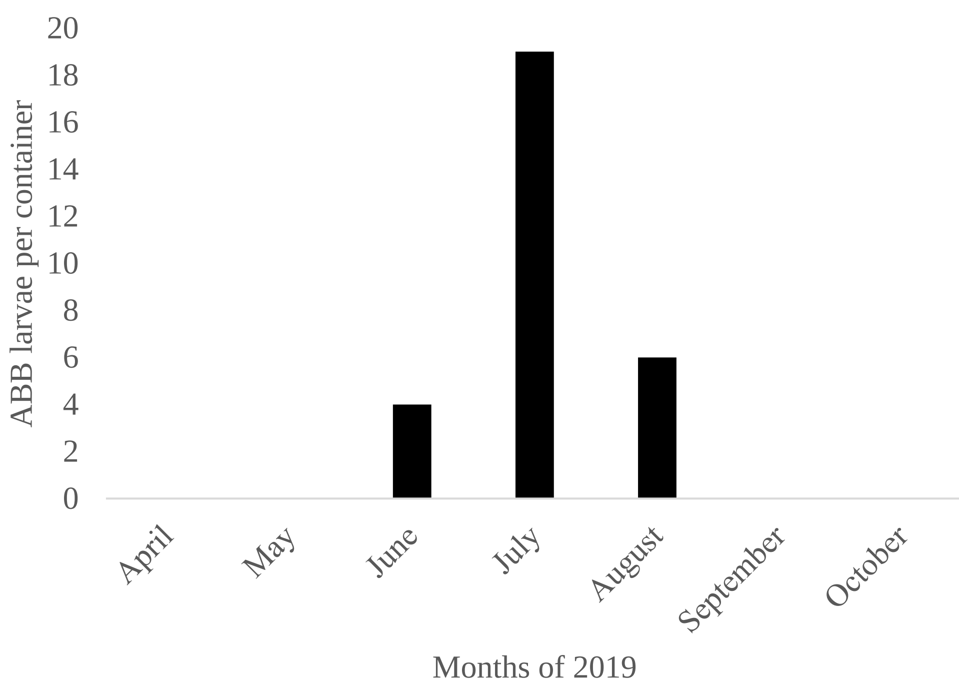


Despite breeding attempts of 4 pairs of ABB each two week period, emergence of teneral ABB only occurred in July (Figure 11). The successful chambers were established between June 27 and August 13. Only 14 chambers were successful out of 62 chambers that were set for reproduction from April to October of 2019. From the 14 successful chambers 215 beetles emerged and 9 larvae failed to emerge from pupae. A mean of 15.7 ± 2.9 offspring per brood were successful. Out of the 14 successful chambers, 8 were in the darkened laboratory room, generating 150 beetles, while 6 broods were from the controlled environment, generating 65 beetles.

The males and females used for reproduction were chosen randomly in the field and then matched with a similar sized partner in the laboratory. In the successful breeding chambers, the males had an average pronotal width of 10.57 ± 0.29 mm and females 10.34 ± 0.24 mm. The average pronotal width of the offspring was 9.85 ± 0.07 mm.

Out of the 215 teneral beetles that emerged in the laboratory, 30 were released at the Sallisaw Park, and thirty were used to test for overwintering. The remaining ABB were tested for ability to reproduce but all died without reproduction. Every beetle was tested for reproduction with different mates at least twice with new rats and containers. Most pairs would bury the rats but produce no offspring.

Figure 11. Number of *Nicrophorus americanus* larvae per breeding pair in laboratory conditions per month of the year of 2019



DISCUSSION

Laboratory breeding attempts resulted in successful broods for only one period in July despite attempts using beetles collected in April, May, June and Septemeber. This result contradicts the hypothesis that ABB in southern populations produce two broods per year, in this study was found that ABB collected from Camp Gruber reproduces only once per year in the month of July. Beetles that emerge as tenerals in July or August appear to search the environment for food and then seek an area to overwinter beginning in September or October.

The reproductive cycle of ABB is believed to start in May and end in September, when the nighttime temperatures are adequate for activity (U.S. Fish and Wildlife Service, 2014). Because of warmer temperatures in the southern regions where ABB is found, the U.S. Fish and Wildlife Service suggests that a second brood in these regions is possible (U.S. Fish and Wildlife Service, 2019). With my two-year data in rearing ABB in laboratory conditions, it appears that a second brood in one year is very unlikely.

In laboratory condition the ABB only reproduced in the month of July in 2019 while the ones that reproduced in 2020 did so in mid-August. The beetles brought from the overwintering experiment, that were previously reared in the laboratory in 2019 and placed in the field as tenerals, died before June of 2020 suggesting that some factor was missing for these beetles to reproduce. All beetles used in breeding chambers were given resources at least twice with the same conditions but different mates, but only reproduced once before senescing and dying.

The low reproduction in 2020 compared to 2019 has unknown causes because laboratory conditions were identical between years and beetles were collected from the field over the same period. Potentially, increases in temperatures during overwintering affected reproduction or constant laboratory conditions prevented maturation of eggs. Temperatures can be different in microhabitats, as observed inside the Cherokee Nation Sallisaw Creek State Park. All beetles brought from the field worked as pairs to bury the rat, but no reproduction was observed. This could indicate that even though the beetles were sexually mature, they might not have had the necessary environmental cues to produce oocytes (Pener, 1992). It has also been shown in insects that temperature can be relevant to the deposition of diapausing eggs, which could explain why they would bury the rat but no reproductive success would occur (Mousseau & Dingle, 1991).

Little is known about how climate change will affect insect overwintering (Bale & Hayward, 2010). Even with a high mortality rate during overwintering, there is not enough data to conclude that population reduction will occur (Bedick et al., 1999; Mckenna-Foster et al., 2016). In 2008 a study made in northwestern Arkansas, had 34.7% of the days with the minimum temperatures below 0 °C, which is about 7% more than the highest percentage observed at Cherokee Nation Sallisaw Creek State Park. In the Arkansas study, survival difference between grasslands and woodlands were tested and showed that 59.6% of the ABB survived the winter, without significant differences between the habitats, and that their survival was improved by the presence of a carcass (Schnell et al., 2008).

This experiment tested the survival of the ABB in three different habitats: forest, open area and lakeside. The same soil was used in all three areas and the same food source so that the temperature effects and vegetation could be tested. In this experiment 17 out of 29 (58.62%) ABB survived across all habitats. The highest survival rate was observed in the forest area (77.77%) while both the open area and the lakeside area had 50% survival. More tests are necessary to determine ABB survival under different conditions in Oklahoma.

The survival rate of *Nicrophorus* beetles during overwinter, has also been shown to relate to the size of the insect entering the overwintering stage (Smith, 2002). Smith (2002) tested *Nicrophorus* from Colorado, and found that the survival of bigger beetles was usually is related to the amount of stored energy they accumulated before overwintering. However, in the Smith (2002) study there were no days with temperatures reaching below freezing.

The average size of the survivors in the current experiment was approximately 0.5 mm larger than the ones that did not survive. All the rats offered as food sources appeared to have been consumed, but since tubes were not checked between setting and retrieving, it is not known what influenced decomposition. It is notable that the provided rats were not buried more than a couple of mm in the overwintering tubes, making it possible for other insects such as flies to reach the carcass through the tube openings. If another insect consumed the rat provided, then the beetles would have to rely on their energy stores and this could be the reason that larger ABB survived better than smaller ABB.

Adult ABB are sexually mature, around one month after emergence, have better chances of survival without a food source than younger ones (Bedick et al., 1999; Schnell et al., 2008). The higher survival rates of mature beetles is suggested to be due to the fat deposits an older beetle have by the time it achieves sexual maturity, granting it more energy to use during the winter (Schnell et al., 2008; Sorvari et al., 2011). The lack of survival of newly emerged adults could also be due to environmental factors such as temperature fluctuation, causing stress to the beetle leading it to use more energy to keep itself alive (Schnell et al., 2008).

An experiment in Nebraska tested overwintering of *Nicrophorus marginatus* and showed that there was a strong relationship between temperature and depth of burial. In this experiment, the beetles stayed under or at the frostline, i (Conley, 2014). The average observed depth for buried beetles was 19.6 cm while the average burial depth in Oklahoma was approximately 3.13 cm. The reason I believe the depth was so much shallower, is because of when the tubes were checked in March of 2020, several beetles were already active and trying to escape, lowering the depth of burial. For a more precise depth of burial analysis in Oklahoma, I suggest measuring the burial of the beetles periodically during winter.

Nicrophorus species that overwinter as adults have the advantage of being able to select suitable areas in which to overwinter, and likely chose the best environment for survival (Schnell et al., 2008). If the beetles that overwinter as adults prefer a certain type of environment, maintaining the preferred environmental condition for overwinter is crucial for maintenance of the population.

Testing different food availability levels, such as smaller or larger carcasses, with different size beetles would indicate the usage of a food source and a more precise relationship between beetle size and food needs for winter. It would be interesting as well to measure precipitation in order to investigate the effects of flooding during the overwinter period (Cavallaro et al. 2017).

CHAPTER IV

CONCLUSION

During 2016 to 2020 the American burying beetle (ABB) and other carrion beetles were sampled in Camp Gruber, near Braggs OK using a modified U.S.F.W.S. protocol. Camp Gruber was chosen because of the presence of a known ABB population and because of requirements associated with Army training activities that may affect this endangered species. Trap locations were chosen to include forests, savannah and grasslands, along with areas of disturbance in cantonment and in areas scheduled for tree clearing. Currently the ABB population inside Camp Gruber, appears to be declining after an increase in 2018. In 2018 the population estimate was 4,657 while it was 1,469 in 2020. The reasons for the decline have not yet been discovered but may be related to weather conditions or carrion availability inside Camp Gruber.

The frequency of traps with ABB in different habitats were similar, contradicting previous conclusions that ABB inside Camp Gruber prefer forests (Creighton et al., 1993). Heat maps for occurrence of ABB showed less frequent occurrence in the cantonment area. ABB are unlikely to complete their life cycle in these areas because of frequent human disturbance. To limit ABB from being attracted to the cantonment area, excessive lighting, especially with lights generating UV wavelengths such as mercury vapor lights should be avoided, and trash dumpsters should be cleaned regularly.

The ABB population inside Camp Gruber appears to be female-biased. Skewed sex ratios may be related to the male calling behavior for females or infection by the bacteria *Wolbacchia* that can cause feminization in some insects. Follow up research to examine attraction to carcasses with males present should be conducted along with genetic analysis to determine presence of *Wolbachia*.

The size of adult beetles, including ABB, is related to food resources while the individual is a larva. The average pronotum size of the ABB inside Camp Gruber have declined since 2016, suggesting that individuals are getting smaller. The decline in average size could be related to the absence of appropriate food sources, either as a direct result of a change in vertebrates or indirectly as a result of change in vertebrate condition. In addition, as temperatures rise the decay process of carcasses also increases potentially limiting food for developing larvae. Smaller ABB are more susceptible to vertebrate predators, losses in interspecific competition and higher overwinter mortality and research to determine the factors leading to decreased size should be prioritized. Surveys of vertebrate prey are recommended along with the possibility of supplementing the population by providing carcasses during the breeding period for ABB.

Past survey data suggest that a second generation of ABB is possible in the southern United States because of an extended active season for adults. However, laboratory breeding experiments in this study indicate a single brood that emerges in August and September. Field data also show that a single peak of teneral ABB occur in July. These results suggest that the southern ABB population will have the same reproduction cycle of the northern population, reproducing only once per year (and once in their lifetime).

Overwintering has been suggested as a limiting factor to ABB populations. Limited tests found relatively high levels of survival among three habitats (lakeside, open, and forest). All beetles were provided a food source, and all were collected in March. Surviving beetles were found to be larger than those that died. The temperatures among habitats were different and it is possible that stable temperatures throughout the winter benefit the survival of ABB. Additional studies of habitat and access to food during overwintering should be conducted, especially with the predicted effects for global climate change.

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APPENDICES

APPENDIX A

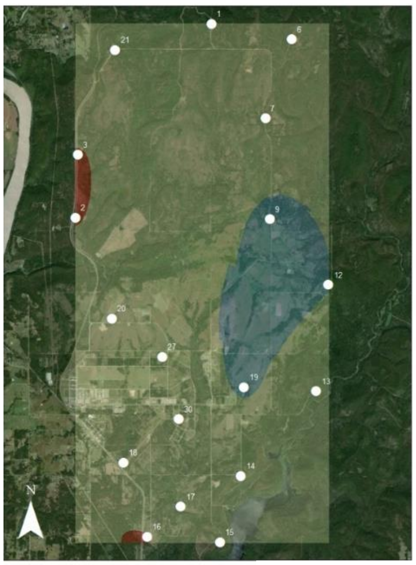
Trap locations for *Nicrophorus americanus* sampling with habitat designation at Camp Gruber, OK

Habitat Designation	Latitude	Longitude	Habitat Designation	Latitude	Longitude
Cantonment	35°40'22.314"N	95°11'26.322"W	Grassland	35°42'13.9"N	95°09'34.1"W
Cantonment	35°40'53.544"N	95°11'57.7608"W	Grassland	35°41'18.6"N	95°08'42.5"W
Cantonment	35°40'44.4396"N	95°10'57.9396"W	Grassland	35°40'27.5"N	95°09'11.0"W
Firebreak	35°46'59.6"N	95°10'41.5"W	Grassland	35°41'26.4"N	95°11'30.7"W
Firebreak	35°46'33.6"N	95°10'03.9"W	Grassland	35°40'58.8"N	95°08'40.8"W
Firebreak	35°46'08.3"N	95°10'58.8"W	Grassland	35°40'45.0"N	95°12'03.4"W
Forest	35°42'52.0"N	95°12'09.0"W	Grassland	35°42'44.1"N	95°09'09.8"W
Forest	35°44'21.1"N	95°10'26.7"W	Grassland	35°40'00.1"N	95°10'19.8"W
Forest	35°45'25.7"N	95°08'20.3"W	Savannah	35°45'30.0"N	95°09'44.9"W
Forest	35°40'24.1"N	95°07'54.7"W	Savannah	35°43'46.9"N	95°12'06.4"W
Forest	35°39'11.4"N	95°09'14.3"W	Savannah	35°42'41.5"N	95°10'22.3"W
Forest	35°38'14.5"N	95°09'36.4"W	Savannah	35°44'18.1"N	95°08'48.3"W
Forest	35°38'44.9"N	95°10'17.7"W	Savannah	35°41'55.2"N	95°07'41.8"W
Forest	35°42'38.3"N	95°12'12.4"W	Savannah	35°38'19.1"N	95°10'53.4"W
Forest	35°42'51.7"N	95°10'54.1"W	Savannah	35°39'22.8"N	95°11'18.4"W
Forest	35°41'34.1"N	95°09'47.3"W	Savannah	35°45'16.7"N	95°11'27.0"W
Grassland	35°44'18.1"N	95°08'48.3"W	Savannah	35°41'58.1"N	95°10'51.1"W
Grassland	35°42'51.5"N	95°08'43.6"W	Savannah	35°40'53.6"N	95°10'37.4"W

APPENDIX B

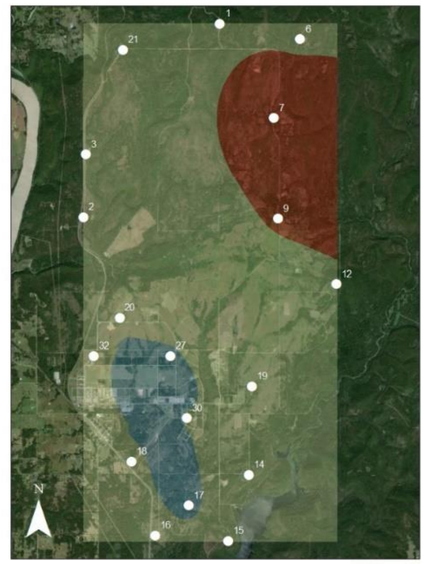
Heat maps for common silphid species encountered at Camp Gruber. Blue areas have less occurrence and red areas have more occurrence.

2016 *Nicrophorus americanus*



2017 *Nicrophorus americanus*

2018 *Nicrophorus americanus*



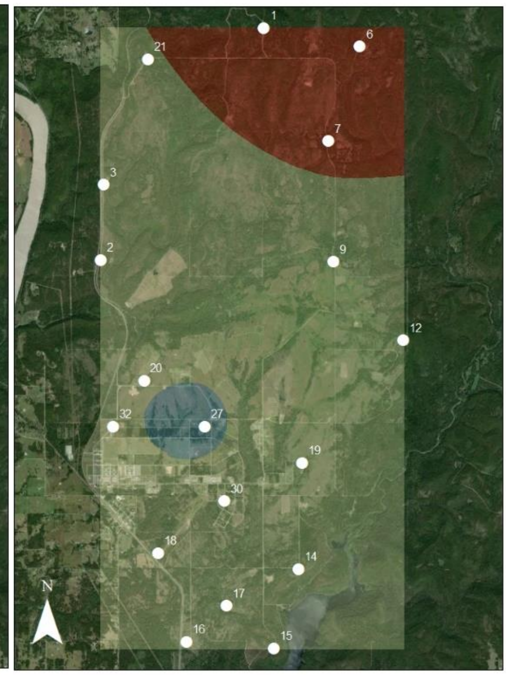
2019 *Nicrophorus americanus*

2020 *Nicrophorus americanus*

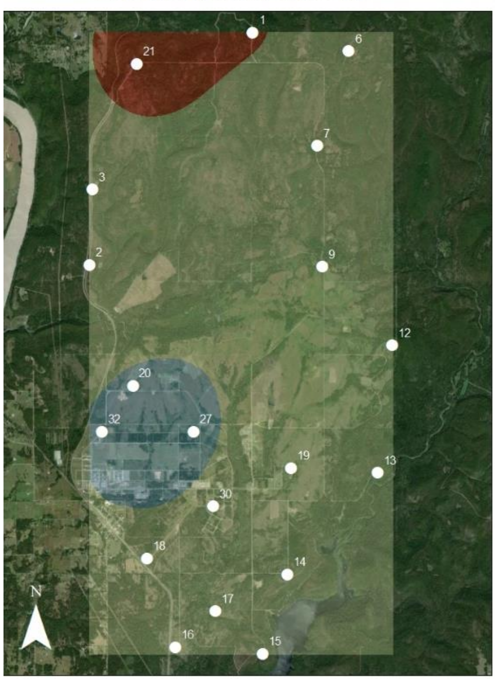
2016 Nicrophorus orbicollus

2017 Nicrophorus orbicollus

2018 Nicrophorus orbicollus

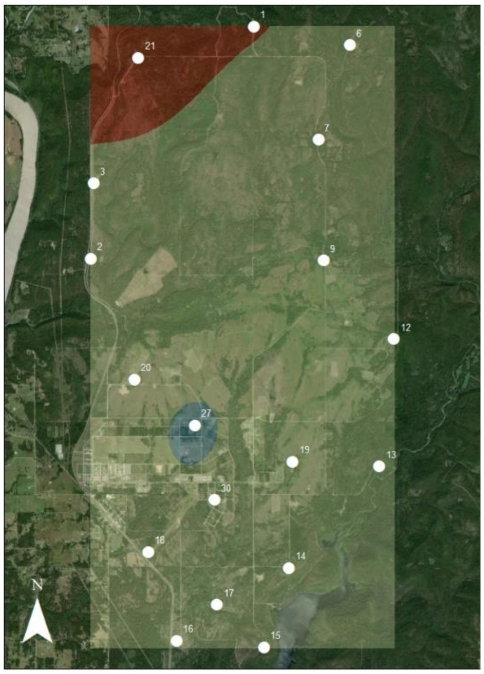


2019 Nicrophorus orbicollis



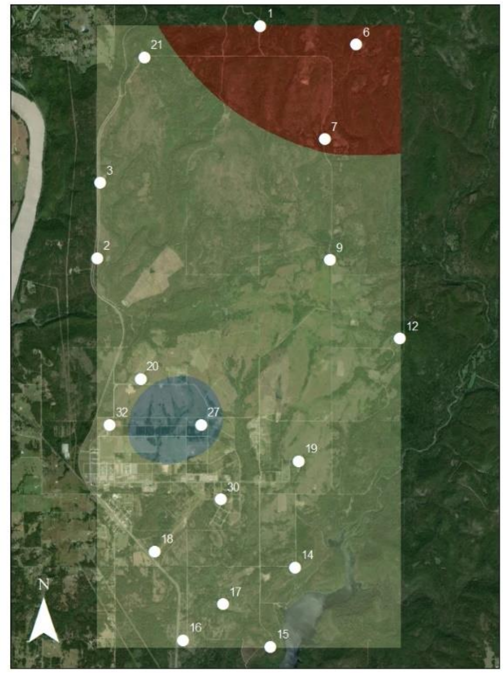
2020 Nicrophorus orbicollis

2016 Nicrophorus pustlatus



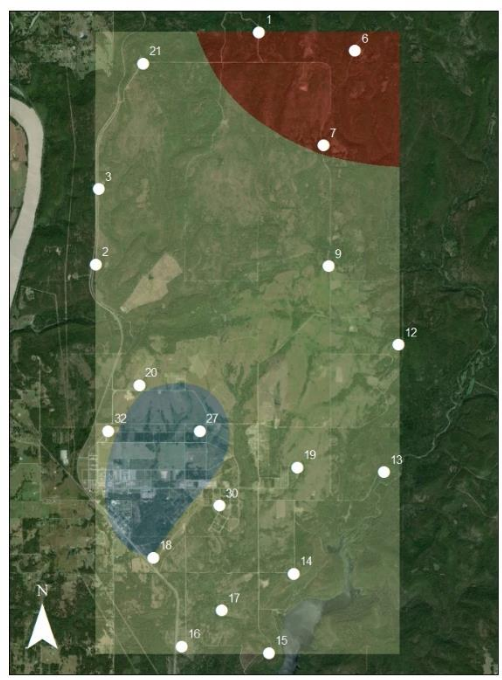
2017 Nicrophorus pustulatus

2018 Nicrophorus pustulatus



2019 Nicrophorus pustulatus

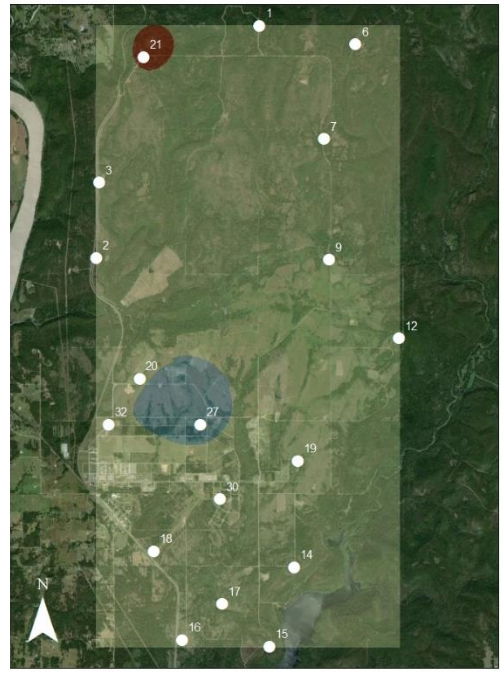
2020 Nicrophorus pustulatus



2016 Necophilia americana

2017 Necophilia americana

2018 Necrophilia americana



2019 Necrophilia americana

2020 Necrophilia americana

2016 Necrodes surinamensis

2017 Necrodes surinamensis

2018 Necrodes surinamensis

2019 Necrodes surinamensis

2020 Necrodes surinamensis

VITA

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Presentations in regional and national meetings for the Entomological Society of America