OVERWINTERING BIOLOGY AND TESTS OF TRAP AND RELOCATE AS A CONSERVATION MEASURE FOR BURYING BEETLES

NDOR Research Project Number M330
Nebraska Research Work Program

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ABSTRACT

Burying beetles are carrion beetles and utilize dead animal carcasses for feeding and reproductive efforts. They assist with decomposition, prevent the spread of disease, and reduce the number of pest species. The largest species of carrion beetle, the American burying beetle, is a federally endangered insect and its distribution has been reduced by 90%. The conservation of this species is important in maintaining a healthy ecosystem. Overwintering biology and trap and relocation were studied to determine how this beetle survives freezing temperatures and to find whether trap and relocation could be a suitable conservation management measure.

Trap and relocation is a technique often used to relocate organisms from an area where human and animal habitats overlap. In this study, we test the efficacy of a trap-relocate technique with a surrogate species of burying beetle, *Nicrophorus marginatus*, to determine the implications of this technique on the conservation management of the federally endangered American burying beetle, *Nicrophorus americanus.* Baited pitfall traps were used for capture, and the comparison of percent recaptures at different trap sites (control, source, and destination) was used to determine the effects of relocating

beetles. The results showed that percent recaptures were lower for the beetles relocated to the new location; however, there was no statistical difference between trap sites. Trap and relocation, in circumstances where there is substantial threat to the American burying beetle's habitat, should be considered for conserving this species.

Insects are poikilotherms and have evolved strategies to survive freezing temperatures through changes in behavior and physiology. Overwintering insects either utilize a freeze tolerant or freeze avoidant strategy. Freeze avoidant insects cannot withstand their cellular fluids freezing solid, while freeze tolerant insects can survive this. Burying beetles from their northern range in Nebraska were put in a simulated, natural environment to determine whether they are freeze avoidant or freeze tolerant. The results showed that there was a strong relationship between beetle depth and temperature. Beetles from the northern range buried at or below the frost line to survive freezing temperatures. This could have further implications in the conservation of the American burying beetle if there are differences between the overwintering behavior of northern and southern range beetles.

DISCLAIMER

"This report was funded in part through grant[s] from the Federal Highway Administration [and Federal Transit Administration], U.S. Department of Transportation. The views and opinions of the authors [or agency] expressed herein do not necessarily state or reflect those of the U. S. Department of Transportation."

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CHAPTER 1. NORTHERN OVERWINTERING STRATEGY AND SURVIVAL OF BURYING BEETLES (*NICROPHORUS ORBICOLLIS)*

1.1 BACKGROUND

Insects are poikilotherms and must adapt to avoid tissue damage in sub-zero temperatures (Block et al. 1990). Invertebrates that overwinter in northern climates have both physical and behavioral changes to survive freezing temperatures (Denlinger and Lee 2010). Cold temperatures may inhibit normal development in invertebrates, and the line between normal development and halted development is not entirely temperature based, such as freezing point but based on supercooling points and physiological capabilities (Salt 1961). Overwintering also involves metabolic depression to overcome metabolic functions that decline with decreasing temperatures during winter months (Mansingh 1971, Leather 1995). There are three basic ways which insects employ to survive cold temperatures: freeze avoidance, freeze tolerance, and migration (Sinclair 2003). However, freeze tolerant insects can be further divided according to supercooling point and lower lethal temperature (Sinclair 1999). Factors affecting survival and mortality are dependent upon the cold hardiness of the insect, temperature, periods of exposure experienced by the insect, presence of chill injury, and specific developmental stage (Bale 1987, Lee 1989, Bale 1993).

Freeze avoidant insects must avoid having their extracellular and intracellular fluids freeze in order to survive, while freeze tolerant insects can allow this ice formation within their cellular fluids (Somme 1999, Denlinger & Lee 2010). Freeze avoidant insects survive through migration, burying vertically in the soil or withstanding freezing temperatures by using supercooling methods, such as ice-nucleating proteins (Sinclair

2003). Water content and desiccation can influence the supercooling point of an insect (Cannon et al. 1985, Lee 1989). This is because supercooling capabilities are inversely related to fluid volume, and insects have relatively high water content for their small size (Angell 1982). Insect strategies are usually correlated with climate, with insects from the Northern hemisphere often exhibiting freeze tolerance, and those from the Southern hemisphere exhibiting freeze avoidance (Sinclair 2003).

Insects found in temperate climates often spend a majority of their lifecycle in the overwintering phase (Leather 1995). During the overwintering period, predation and starvation risks are greatly reduced, since these insects are not active during winter months (Mansingh 1971, Leather 1995). However, the insects' immobility can be a disadvantage if the conditions become unfavorable, such as during drought or too much precipitation (Tauber et al. 1986, Leather 1995).

For many overwintering insects the soil substrate is an important aspect of survivability (Leather 1995). Soil offers warmth and more stable temperatures than the surface of the soil. Factors that can influence this buffer include depth, composition, moisture content, and snow covering (Leather 1995, Bennett et al. 2003). While insects often bury far enough into the soil to obtain warmth and stable temperatures, it is likely that they do not bury to unnecessary depths because such activity would waste needed energy reserves (Leather 1995).

This study examines burying beetles, *Nicrophorus orbicollis,* in Nebraska. Burying beetles are an important part of an ecosystem because they act as decomposers utilizing dead animals for food and reproduction and they aid in the prevention of disease (Gibbs and Stanton 2001, USFWS 2008). Understanding life history traits are important in the conservation of the federally endangered American burying beetle, *Nicrophorus americanus*. The reasons for its decline have not been fully elucidated (Lomolino et al. 1995, Sikes and Raithel 2002, USFWS 2008).

Schnell et al. (2008) found that American burying beetles from Arkansas have an overall survival rate of 59.6% in a simulated field experiment testing overwintering survival. They also tested survival rates between beetles in provisioned (77.1%) and non-provisioned buckets (44.6%) and found that beetles that buried in the soil buried at shallow depths (averaged 6 cm) (Schnell et al. 2008). The study was completed in Arkansas, part of the ABB's southern range, and temperatures never reached below freezing throughout the duration of the experiment.

Our study experimented with beetles from Nebraska, part of the ABB's northern range, and temperatures reach below freezing with a frost line present throughout most of winter. The objective was to determine the overwintering strategy of northern range American burying beetles by using a surrogate species, *N. orbicollis.* In addition to implications for reintroduction, learning how burying beetles overwinter in climates where freezing occurs also impacts construction activities in these areas. Temperature, water content, and beetle burial depth (in relation to the frost line) is the focus to determine overwintering patterns of these beetles, although many factors, including habitat, respiration, and other cold adaptations, may influence this behavior (Danks 1978, Danks 1991, Kukal 1991, Block 1994b, Danks et al. 1994).

1.2 METHODS

1.2.1 Experimental Pipes

Polyvinyl chloride (PVC) pipes were used to house beetles in the field for the duration of the experiment. Two halves of PVC pipes were put together and wrapped with duct tape. Tubes of varying sizes were used – long, medium, and short. These tubes all had a diameter of 10.16 cm (4 inches), and the length of the long tubes was 1.22 m (4 feet). The medium tubes had a length of 1.07 m (3.5 feet), while the short tubes were 0.61

m (2 feet) long. Small slits were made around all sides of the tube to allow water exchange with the surrounding soil. A square piece of screen, 20.32 cm (8 inches), was placed on the top and bottom of the tube, and a PVC cap was placed on both the top and bottom to prevent beetles from escaping.

Holes on each side of the tube were drilled through the top and bottom caps. A rope was threaded through the top holes to provide a hoist for tube extraction. Once the tubes were placed in the ground, a sand/dirt mixture from Broadfoot Sand and Gravel of Kearney was placed inside, filling the tube to the top. Two *Nicrophorus orbicollis* beetles, one male and one female, were placed at the top of each tube, except in 2013 when most tubes only had one beetle. A lid was then placed on the PVC cap for the entirety of the experiment. This prevented beetles from escaping and prevented precipitation and debris from accumulating inside the tube and injuring the beetles.

In 2013 some of the tubes were modified to test whether low soil moisture had an impact on the survivability of beetles. Tubes were modified by wrapping them in 47.32 L (50 quart) plastic trash bags so that water could not infiltrate the tube. Rain shields were added above the tubes to prevent precipitation from entering the tops of the tubes. The rain shields consisted of small Tupperware containers placed over the cap with a stone weight.

1.2.2 Tube Arrangement

Testing occurred at Fort Kearny State Park, Kearney County, Nebraska. An area was cleared of vegetation and mowed. A Bobcat with an auger attachment was used to dig 1.52 meter-deep holes. Tubes were placed about half a meter apart and placed into rows. Tubes were buried to the bottom of the top cap, so that the cap was above ground and the rest of the tube was underground. A wooden stake was placed next to each tube to identify it. A total of 60 tubes were installed in 2011, 140 tubes total were installed in

2012, and 135 tubes total were installed in 2013.

1.2.3 Weather

Weather conditions were recorded using a HOBO weather station placed next to the tubes. This station recorded the ground temperature and humidity, and a PVC pipe buried in the ground was used to measure depth to the water table. A long, measured wooden stick was placed inside the PVC pipe until the stick hit the bottom. The stick was removed and the line where the water reached was where the water table occurred. The thermocouples from the HOBO weather station were placed on the surface, 10 cm, 20 cm, 30 cm, and 60 cm beneath the soil around the HOBO weather station. The temperature was recorded once per hour of each day.

1.2.4 Data Collection

The tubes were set in the ground during late September, and the beetles were added in October each year. Beetles were collected using baited pitfall traps in Garfield and Buffalo counties of Nebraska. Beetle status, whether they were alive or dead, and beetle activity, whether they were active or buried in the soil, was checked each once a week between October and April. The depth to water table, surface activity of beetles, and dead beetles at the surface were noted. If beetle mortality had occurred before November, *N. orbicollis* were replaced if available with beetles, *N. marginatus*, housed in the laboratory.

1.2.5 Removal of Tubes

Tubes were randomly chosen for removal. Beetles were added in October each year, and removed the following winter/spring months. Refer to Table 1 for the removal dates.

An engine hoist was used to extract the tubes by attaching rope at the top of the tubes and slowly lifting the tubes from the ground. Once removed, the tube was laid on its side, the duct tape surrounding the tubes was cut, and the upper half of the tube removed. The measurement from the top of the tube to the top of the soil was taken, and dirt was carefully sifted by hand until a beetle was found. Beetles found dead on the top of the soil were not counted, while beetles found dead at any depth were recorded. Beetle depth was measured from the top of the soil to where the first body part was found. All dirt was completely sifted until none remained in each tube.

1.3 RESULTS

1.3.1 Totals for 2011-2014

Three trials were completed for this experiment, showing an overall similar trend of beetles burying deeper in the soil during the colder months, such as January and February, and shallower by later in the spring, such as May (Figure 2). The data from year to year started with the peak of the bell curve at 10-20 cm and 20-30 cm in 2011-2012, then did not peak as much with a wider spread of data across beetle burial depths in 2012-2013, and finally ended with a more defined bell curve with the peak at 40-50 cm in 2013-2014 (Figure 3, 4, 5). This trend and movement of the bell curve towards deeper burial depths each year seemed to be associated with differing temperature each year, with the frost line staying present for a longer duration each year with the longest frost line presence occurring in 2013-2014.

While the temperature and frost line was different each year a trial was conducted, it seemed as though the trend for beetle burial depth related to month was similar regardless of this (Table 3). The average depth by month peaked in February, with January and March slightly lower, and May the lowest bar on the graph (Figure 2). Tubes were only exhumed January-May (Table 1), so it is unknown if the bell curve would follow the trend for the months of October, November, and December. Also, the results of the average depth of beetle burial by month supported the idea that burial depth and month follow a bell-curve distribution (Figure 2). Every year the average beetle burial depths for the first pull and third pull was shallower than the second pull.

Measuring burial depth of beetles that survived the winter showed that deepest, shallowest, and average depths increased each year and trial (Table 2). Burial depth measurements of beetles that did not survive the winter showed that deepest, shallowest, and average depths decreased each year and trial. For every year and trial beetle survival was much higher for beetles that buried compared to those that did not (Table 4).

1.3.2 2011-2012

In the fall and winter of 2011-2012, sixty-five beetles buried in the soil, with 53 alive and 12 dead. Four were found dead on the surface. The survival rate for beetles once buried was 81.54%, and 44.17% for beetles overall (Table 4). The deepest burial depths were observed in January and March, and very shallow burial depths, with some beetles already on the surface, were observed in May (Figure 3). The deepest depth a beetle buried and survived was between 70 to 80 cm, and the shallowest depth a beetle buried and survived was 2.5 cm (Table 2). One dead beetle was found at 25 cm and the shallowest buried beetle was found at 1 cm.

Twenty-eight beetles were removed from tubes during the first pull of tubes on January 4, 2012. Twenty-seven beetles were alive, while 1 beetle was dead. Thirty-one beetles were removed from tubes during the second pull of tubes on March 3, 2012.

Twenty beetles were alive, while 11 beetles were dead. Six beetles were removed from tubes during May 2012. All six were found alive. Beetles stayed close to the surface, with most beetles (30) found from 10-30 cm below the surface (Figure 3). Fourteen beetles buried deeper than 30 cm and 5 beetles buried shallower than 10 cm.

While there were no data for air and 10 cm temperatures for the months of October, November, and December, the 30 cm temperature was shown to stay above 0°C for the majority of the winter (Figure 6). Air temperature and 10 cm temperature was more variable and reached below 0°C more often than 30 cm below the surface. The frost line seemed to be around 20-30 cm and was present through the months of January, February, and March. The frost line depth for October-December was unknown.

1.3.3 2012-2013

In the fall and winter of 2012-2013, fifty-two beetles buried in the soil. Forty-eight survived, 4 died, while 188 died on the surface. The survival rate of buried beetles was 92.31% and 17.14% for beetles overall (Table 4). The deepest burial depth was 82 cm, and the shallowest burial depth with the beetle still alive was 8 cm (Table 2).

Thirty beetles were found total on the first date of removal. Seventeen out of thirty beetles were found alive, while thirteen were found dead either on top of the soil or buried at a shallow depth (<7.5 cm). The range of data then is 74 cm, and the average being 19.6. Thirteen beetles were found total on the second date of removal (March 15, 2013). Seven out of the thirteen beetles were found alive, while six were found dead at a shallow depth (<7.5 cm). Twenty-nine beetles were found total on the third and final date of removal (April 26, 2013). Twenty-five beetles were found buried alive, while four beetles were found dead at shallow depths (<5.0 cm).

Beetle activity observably decreased as the experiment progressed. Beetles were active on the surface in December and even in January. Forty-four beetles buried at or deeper than the 10-20 cm frost line, and only 3 were found closer to the surface at 0-10 cm (Figure 4). Buried beetles died in many tubes when burial did not exceed 10 cm.

Temperature was very variable for air temperatures (0 cm) (Figure 7). Temperature became less variable as depth increased with 0 cm depth being most variable and 30 cm being the least variable. Air temperature and 10 cm thermocouples showed that temperatures reached below freezing multiple times throughout the duration of the overwintering experiment. The frost line seemed to stay between 10-20 cm during the months of December, January, and February.

1.3.4 2013-2014

In the fall and winter of 2013-2014, there were seventy-six beetles that buried in the soil. Seventy-one of the beetles that buried survived, 5 died, and 36 died on the surface before burying. The survival rate for beetles buried beetles was 93.42% and 49.31% for beetles overall (Table 4). The deepest burial depths were seen in January and March, and some more shallow burial depths were seen throughout all months. There was not a clear month where burial depth was shallower. The deepest depth a beetle buried and survived was 105 cm, and the shallowest depth a beetle buried and survived was 5 cm. The deepest depth of a beetle that died was 8 cm, and the shallowest was 2 cm.

Twenty-seven beetles were removed from tubes during the first pull of tubes on January 9, 2014. All 27 beetles removed had buried and were alive. Twenty-five beetles were removed from tubes during the second pull of tubes on February 7, 2014. Twenty-one beetles were alive, while 4 beetles were dead. Twenty-four beetles were removed from tubes during the third and final pull of tubes on March 7, 2014. Twenty-three beetles were alive, while only 1 beetle was dead. Sixty-six beetles were found at or deeper than the 20-30 cm mark, and only 5 beetles were found shallower than this mark (Figure 5).

Similar to previous years' data, the thermocouples showed considerable variation closer to the surface. The air temperature and 20 cm reached below 0°C for a period of around 2 months (December-February) (Figure 8). The 30 cm thermocouple showed temperatures staying near the 0°C mark, and the frost line was estimated at around 20-30 cm below the surface. The frost line was present during the months of November, December, January, February, and March. This was the longest the frost line was present compared to data from 2011-2013.

1.3.5 Water Fraction per Volume (WFV) Measurements

When soil water fraction per volume (WFV) was measured and compared between the moisture-controlled tubes and regular tubes, the average WFV measurement was equal for both (0.04 WFV). The average beetle depth for moisture-controlled tubes (49.33 cm) was similar to regular tubes (50.32 cm) and was not statistically different ($p=0.69$). WFV was measured in the soil of tubes with beetles alive and dead, but there was no significant differences found between them when statistically compared with a Mann-Whitney U-test. Alive beetle tube soil was measured 0.05 WFV and dead beetle tube soil was measured at 0.04 WFV ($p=0.66$).

1.3.6 Beetle Depth and Temperature

A regression compared the relationship between average beetle depth and average temperature (Figure 9). The temperatures at the surface and depths of 20 cm, 30 cm, and 60 cm were compared to the average depths beetles buried at these temperatures. A strong relationship was found at each of these depths (R^2=0.90, 0.91, 0.94, 0.81, respectively). The colder temperatures became, the deeper beetles would bury, and the warmer temperatures became, the shallower beetles would bury. This indicates beetles base their burial depth on temperature and avoided freezing temperatures (Figure 9, Table 3).

1.4 DISCUSSION

Beetles had a high survival rate once they buried into the soil but would die if they did not bury. Beetles would at least double their survival rates if they buried (2011-2012: 44.17% and 81.54%; 2012-2013: 17.14% and 96%; 2013-2014: 49.31% and 93.42%). However, the beetles that died at the surface did not seem to attempt burial and stayed active on the surface until they died of cold injury, desiccation, or starvation. This could have occurred because of a variety of reasons, including the difference between senescent and teneral beetles used or beetles could possibly search for a specific, habitable microclimate.

Precipitation may have also been a factor for the large amount of beetles that died in 2012-2013. Precipitation was higher in the months of October and November in 2011 and 2013, and it is these months that beetles were placed in the tubes and buried before freezing temperatures occurred. In October and November of 2012, precipitation was very low, and thus, dehydration could have accounted for large amount of beetles dying on the surface during the 2012-2013 trial.

Temperature may also be an important factor in the beetles' decision to bury and begin the overwintering process. Temperatures were higher during the 2012-2013 trial so beetles may not have been ready for burying to start the overwintering process. Beetles were then "stranded" because it was too warm for overwintering but could not escape tubes to find food, and thus, died from starvation or desiccation. Burying beetles have high water-loss rates because of transpiration through exposed, shortened elytra, and oral and anal excretions secreted during disturbances, so it is likely that these beetles died from desiccation (Bedick et al. 2006).

These results supported the findings from Schnell et al. (2008), where temperatures stayed above freezing, and beetles needed food provisions to survive their overwintering period. In Nebraska, where temperatures usually stay below freezing in the winter, the beetles that overwinter slow their metabolic processes to survive. They are able to stay the duration of the winter in soil below the frost line and do not need food provisions that beetles in above freezing temperatures utilize for survival. The warmer temperatures in 2012-2013 resulted in beetles that usually slow their metabolic rate and survive cold temperatures, but instead died at the surface of the soil because they were likely unable to find these much needed food provisions. While Schnell et al. (2008) found that beetles overwintering above freezing temperatures needed food provisions, this study found that food provisions were unnecessary for survival, considering the high survival rates of beetles without food provisions.

The following trial from 2013-2014, soil water content was controlled for by adding dry soil and keeping moisture and precipitation out of the tubes to see if beetles would die from desiccation. Bedick et al. (2006) found that in low-humidity conditions with little access to water, *N. marginatus* was unable to survive. The WFV of the moisture-controlled tubes was similar to that of the regular tubes, so it could not be directly determined if desiccation was the reason for the high mortality in 2012-2013. More beetles did bury in the moisture-controlled trial, compared to the trial from 2012-2013 where beetles stayed active on the surface until December and January. When tubes were pulled, WFV was taken in tubes where beetles were alive and dead, but the soil moisture content did not seem to affect beetle survival.

Some beetles found in soil with 0.00 WFV were able to successfully overwinter and survived these drought-like conditions. A hard, cement-like mixture formed at the top of some of the tubes (0-20 cm) in the 2013-2014 trial, and beetles were still found in these seemingly inhospitable conditions, even when soil moisture was higher towards the bottom of the tube. It may be possible that beetles are selective when deciding where to bury and overwinter and may be able to withstand less favorable conditions once buried.

Costanzo et al. (1997) studied the influence of soil moisture on the overwintering of the Colorado potato beetle and found that moisture, texture, water potential, and other physiological properties of the soil have an effect on cold hardiness and survival. Soil properties were not focused on in this study, with the exception of soil moisture, but studying more physiological properties of the soil and beetle microhabitat could elucidate why beetles died on the surface or buried. Insect cold-hardiness and mortality is affected by temperature, soil moisture, substrate, and relative humidity, so these are important factors to consider in overwintering success of terrestrial invertebrates (Leather 1984, Costanzo et al. 1997, Bennet et al. 2003, Ellsbury and Lee 2004).

While beetles that buried in the soil had a greater chance at survival, the depth that they buried was mostly chosen based on the depth of the frost line and presence of colder temperatures. Some beetles buried deeply at the very bottom of the tubes and might have been able to avoid having to move with the fluctuation of warmer and colder temperatures. Other beetles may move throughout their overwintering period, considering some of the beetles were found at more temperature variable, shallower depths during colder months with the frost line present. There were likely local differences, but there were no beetles found in ice. Mean beetle depth data was strongly correlated with mean temperature data, indicating that burying beetles are freeze avoidant.

The movement of beetles with temperature is evident as seen with the regression between mean beetle depth and mean temperature data. The average temperatures for each month are very similar, and as shown by beetle burial depth, there are a similar number of beetles found at each burial increment, especially the shallower and deeper ones. The 10-20 cm, 90-100 cm, and 100< cm increments all had the same number of beetles each month that tubes were pulled. This could have been because temperatures were similar each month below 20 cm during the 2013-2014 trial where this was seen.

Beetles had high mortality when burying at shallow depths, usually 0-10 cm in colder months. It is unknown why these beetles died or why they did not bury deeper to avoid freezing temperatures, but many of the *N. marginatus* died at shallow depths or on the surface. These beetles were added to tubes as replacements to tubes where *N.*

orbicollis had died, so because of this later addition they may not have acclimated properly to the cooler temperatures.

Burying beetles are freeze-avoidant, as evident with this experiment, and beetles were able to survive by burying very deep in the soil or moving with the fluctuation of temperature. However, more research should be done on the movement of burying beetles during their overwintering period. There also does seem to be a trend with the beetle burial depth exhibiting a bell curve distribution with shallower depths for early winter months, deepest depths for the middle of winter months, and again, shallower depths for later winter months. This pattern of beetle burial depth could be explained by the presence and absence of the frost line.

Knowing how these beetles react to the influence of temperature is important for learning more about overwintering biology (Zachariassen 1985). Overwintering biology could be important to the conservation management of the American Burying Beetle, because it could have implications for reintroductions, as well as construction in beetle habitat. Reintroductions in the ABBs' northern range utilized beetles reared in the lab from a southern range population for their reintroduction efforts. The different overwintering behaviors of southern range (Schnell et al. 2008) and northern range beetles may contribute to the lack of success in places of reintroduction, such as Ohio.

Also, construction in beetle habitat during winter months may affect the compaction of the soil or physically injure or kill beetles that overwinter close to the surface of the soil. However, in the northern range of ABB, beetles were found to bury beneath the frost line, so construction in beetle habitat would pose little threat to those overwintering beetles (Hoback unpublished).

Southern and northern populations of burying beetles exhibit different overwintering behaviors and strategies to elicit survival in colder temperatures (Schnell et al. 2008). These differences in overwintering behavior may have implications on reintroduction efforts and also for construction efforts in beetle habitat. Knowledge of the frost line's presence (or absence), where the frost line occurs, and temperature fluctuations may help to avoid harm to these beetles during winter months. Further research should also be done on determining the lower lethal temperatures of northern and southern range burying beetles, as there may be differences in these characteristics with latitude (Addo-Bediako et al. 2000).

1.5 ACKNOWLEDGEMENTS

This work was funded through the Nebraska Department of Roads and the University of Nebraska Research Services Council. We thank Broadfoot Sand and Gravel of Kearney for their donation of our sand/dirt mixture used every year. We also thank John Henderson, Gary Phillips, Elisabeth Jorde, Scout Wilson, Josh Weise, and Erik Prenosil for aid in this experiment.

TABLES

Table 1. Dates of tube removal by year. Tubes were removed in thirds in the winter/spring months.

Removal of Tubes	Dates of Tube Removal by Year		
	2011-2012	2012-2013	2013-2014
1st Tube Removal	January 4, 2012	February 7, 2013	January 9, 2014
2nd Tube Removal	March 3, 2012	March 15, 2013	February 7, 2014
3rd Tube Removal	May, 2012	April 15, 2013	March 7, 2014

Table 2. Descriptive statistics of the beetles once they had buried in the soil. Alive and dead beetles' shallowest, deepest, and average depths are shown by year.

Beetle Status and Depth		Year/Trial		
		2011-2012	2012-2013	2013-2014
Alive (#)		53	48	71
	Shallowest (cm)	0	1.50	5
	Deepest (cm)	70-80	82	105
	Average (cm)	25.92	33.65	50.78
Dead (#)		12	2	5
	Shallowest (cm)	1	5.50	2
	Deepest (cm)	25	7.50	8
	Average (cm)	8.25	6.50	3.40

Table 3. Average burial depth and average monthly temperature at each depth. Bolded numbers show between what depths the average beetle buried and the corresponding temperature at that depth. The “-“ indicate where the HOBO unit could not retrieve data or where thermocouples were not installed yet during the 2011-2012 trial.

Year and Month	Average Beetle Burial Depth (cm)	Average Temperature (Celsius)				
		0 cm	10 cm	20 cm	30 cm	60 cm
2011-2012						
January	28.03	-8.82	-	**-1.69**	**0.50**	-
March	29.34	4.62	-	**7.77**	**9.55**	-
May	3.04	**19.30**	**-**	16.83	18.48	-
2012-2013						
February	34.1	-0.85	1.65	4.28	**7.15**	**8.97**
March	41.9	2.45	4.91	6.34	**7.95**	**9.04**
April	31.0	5.81	8.08	9.15	**9.70**	**10.05**
2013-2014						
January	49.94	-3.26	-	-0.51	**1.65**	**3.89**
February	56.31	-4.71	-	-0.34	**1.76**	**3.87**
March	46.71	-6.16	-	-0.29	**2.04**	**4.01**

Table 4. Beetle survival rates for all 3 trials was calculated by looking at how many beetles buried and if these beetles were alive or dead.

	2011-2012	2012-2013	2013-2014
Total Beetles Used (#)	120	280	144
Beetles Buried (#)	65	52	76
Beetles Alive (#)	53	48	71
Beetles Dead (#)	12	4	5
Buried Beetle Survival Rate (%)	81.54	92.31	93.42
Total Beetle Survival Rate (%)	44.17	17.14	49.31

FIGURES

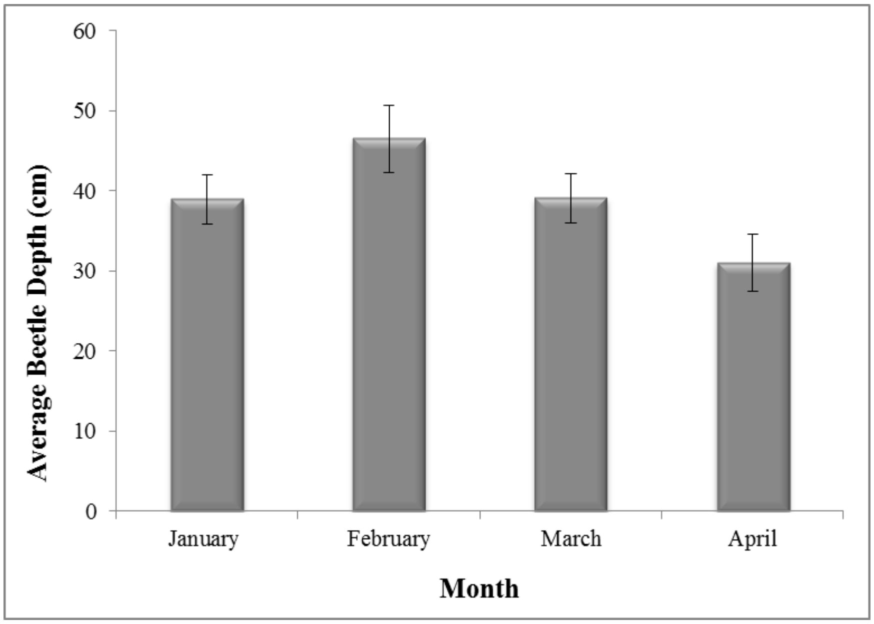
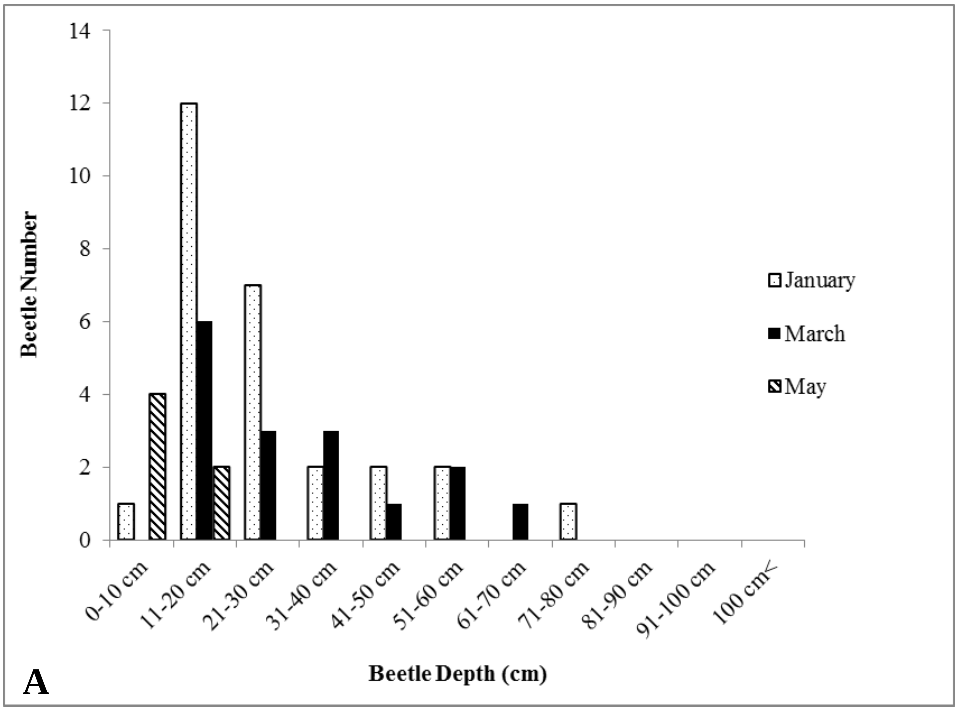


Figure 2. Average beetle burial depth and standard error by month of pull. Averages were compiled from every trial (2012-2014).



14
12
10
8
6
4
2
0
Beetle Number
January
March
May
0-10 cm
11-20 cm
21-30 cm
31-40 cm
41-50 cm
51-60 cm
61-70 cm
71-80 cm
81-90 cm
91-100 cm
100 cm<
Beetle Depth (cm)
A

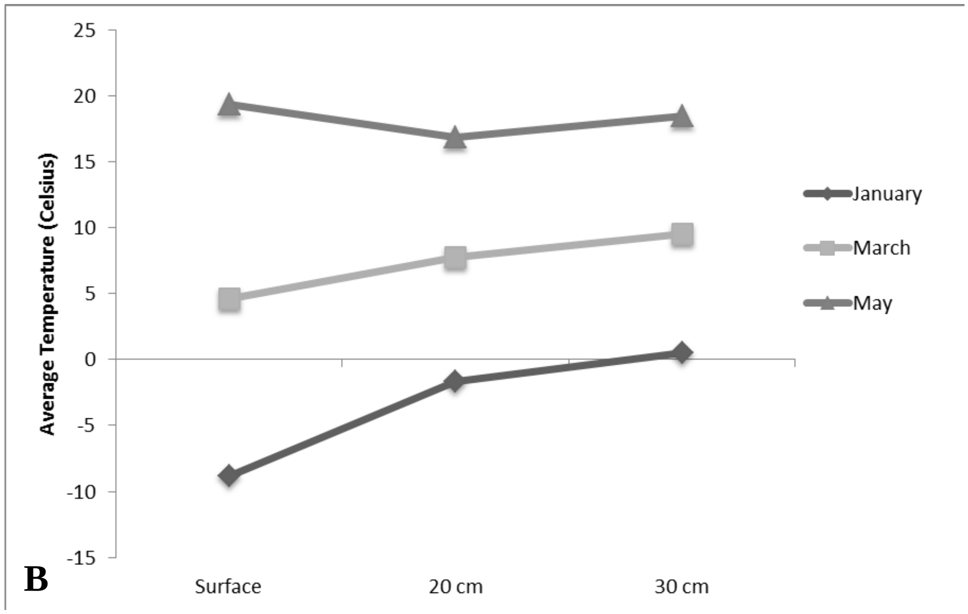
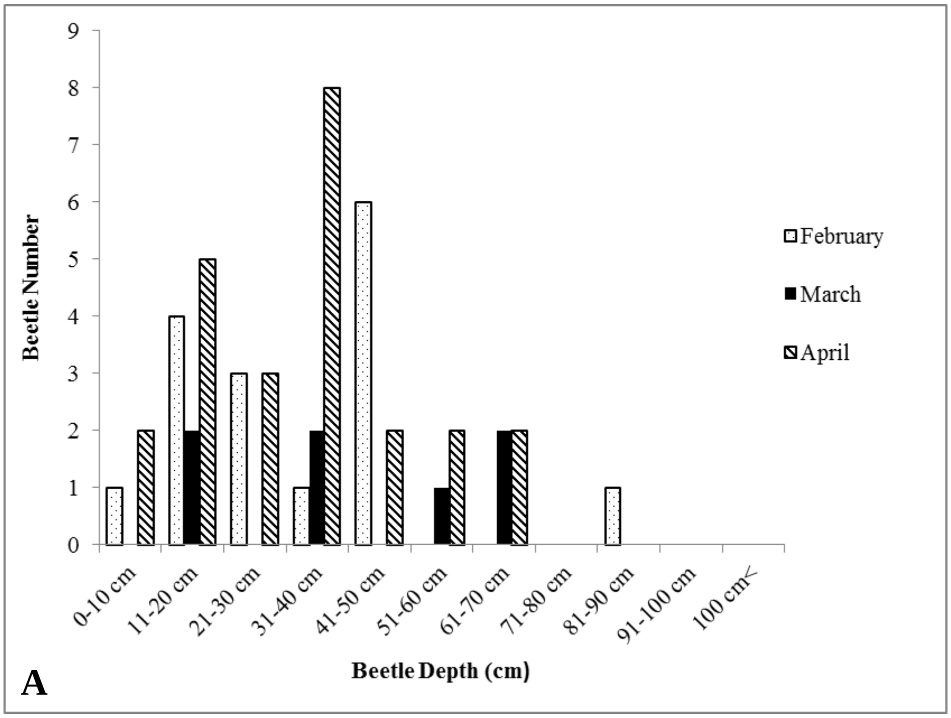


Figure 3. 2011-2012 beetle burial depths (A) and average temperature by month (B). Samples were pulled in January, March, and May of 2012.


9
8
7
6
5
4
3
2
1
0
Beetle Number
February
March
April
0-10 cm
11-20 cm
21-30 cm
31-40 cm
41-50 cm
51-60 cm
61-70 cm
71-80 cm
81-90 cm
91-100 cm
100 cm<
Beetle Depth (cm)
A

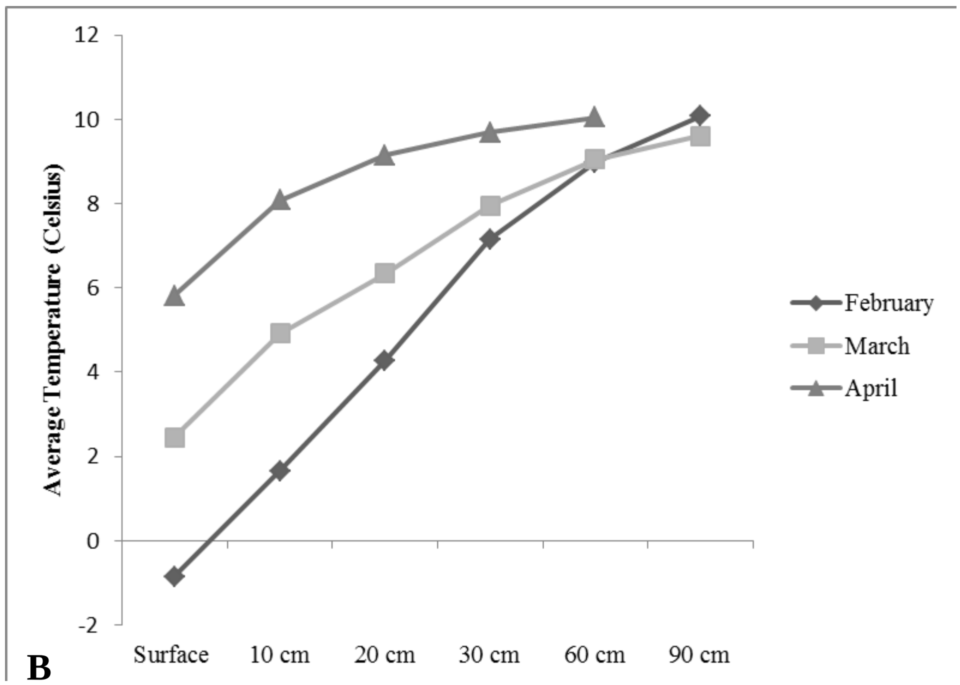
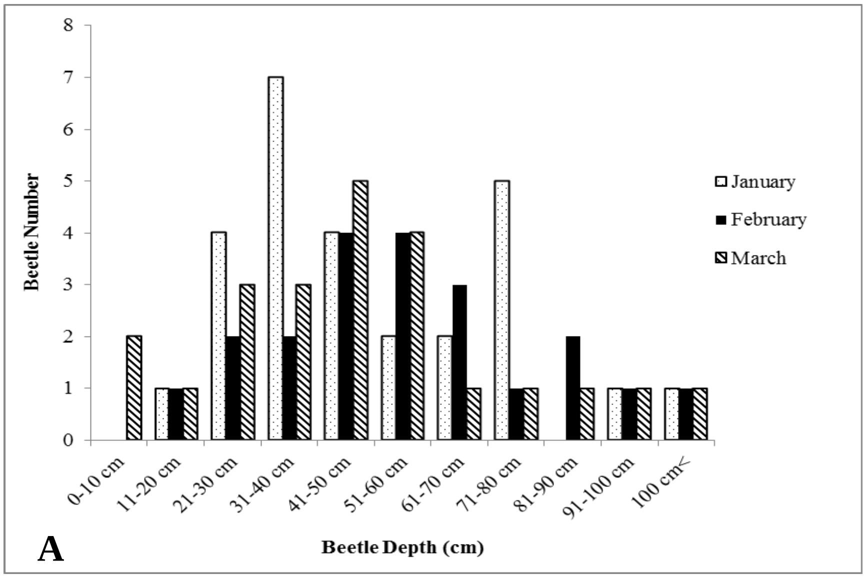


Figure 4. 2012-2013 beetle burial depths (A) and average temperature by month (B). Samples were pulled in February, March, and April of 2013.



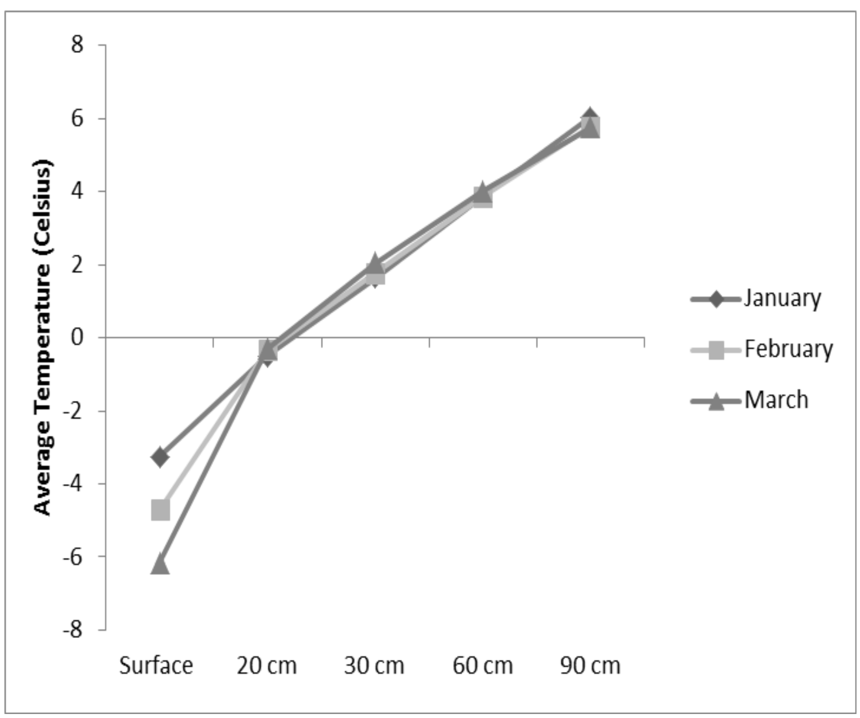


Figure 5. 2013-2014 beetle burial depths (A) and average temperature by month (B). Samples were pulled in January, February, and March of 2014.

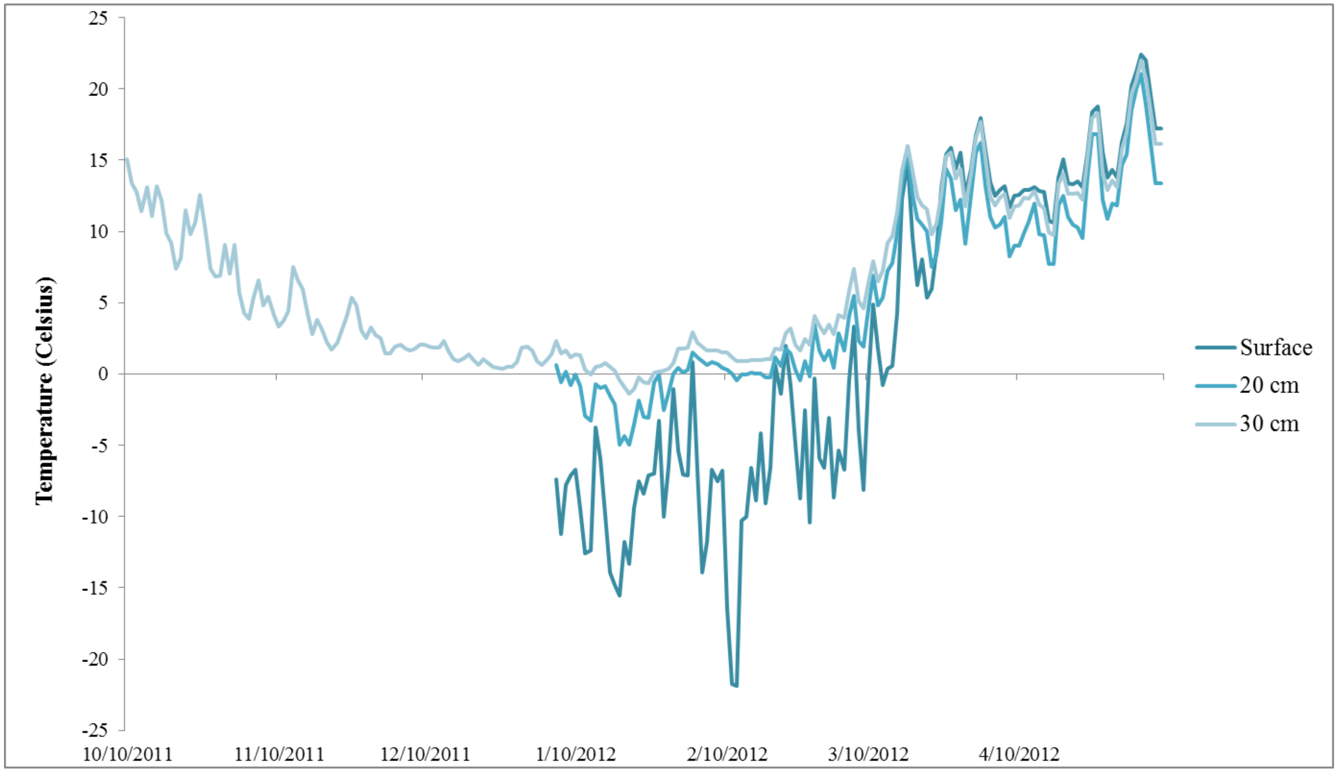


Figure 6. Temperatures taken from the HOBO unit and thermocouples corresponding to different incremental depths (surface, 20 cm, 30 cm) from 2011-2012.

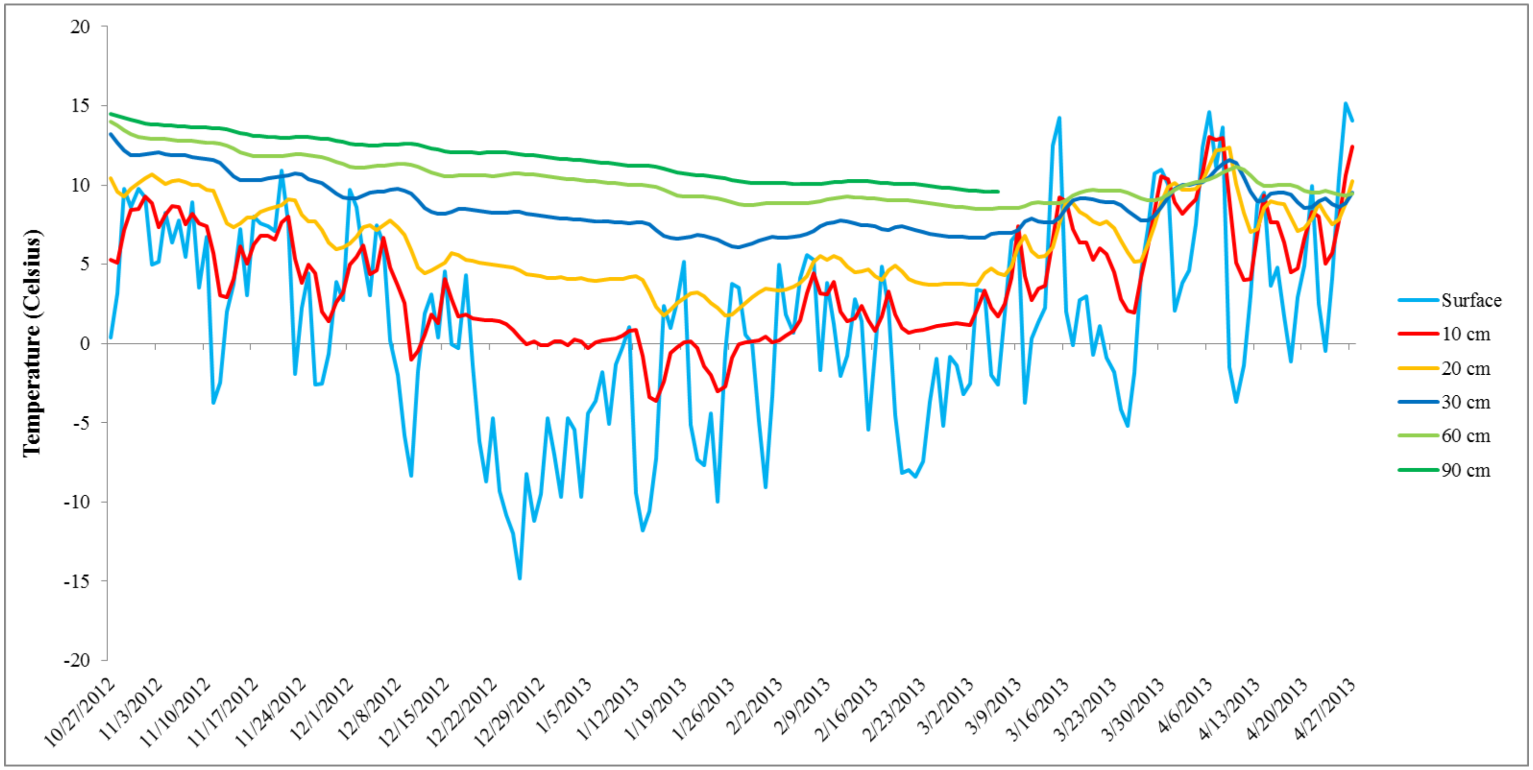


Figure 7. Temperatures taken from the HOBO unit and thermocouples corresponding to different incremental depths (surface, 10 cm, 20 cm, 30 cm, 60 cm, 90 cm) from 2012-2013.

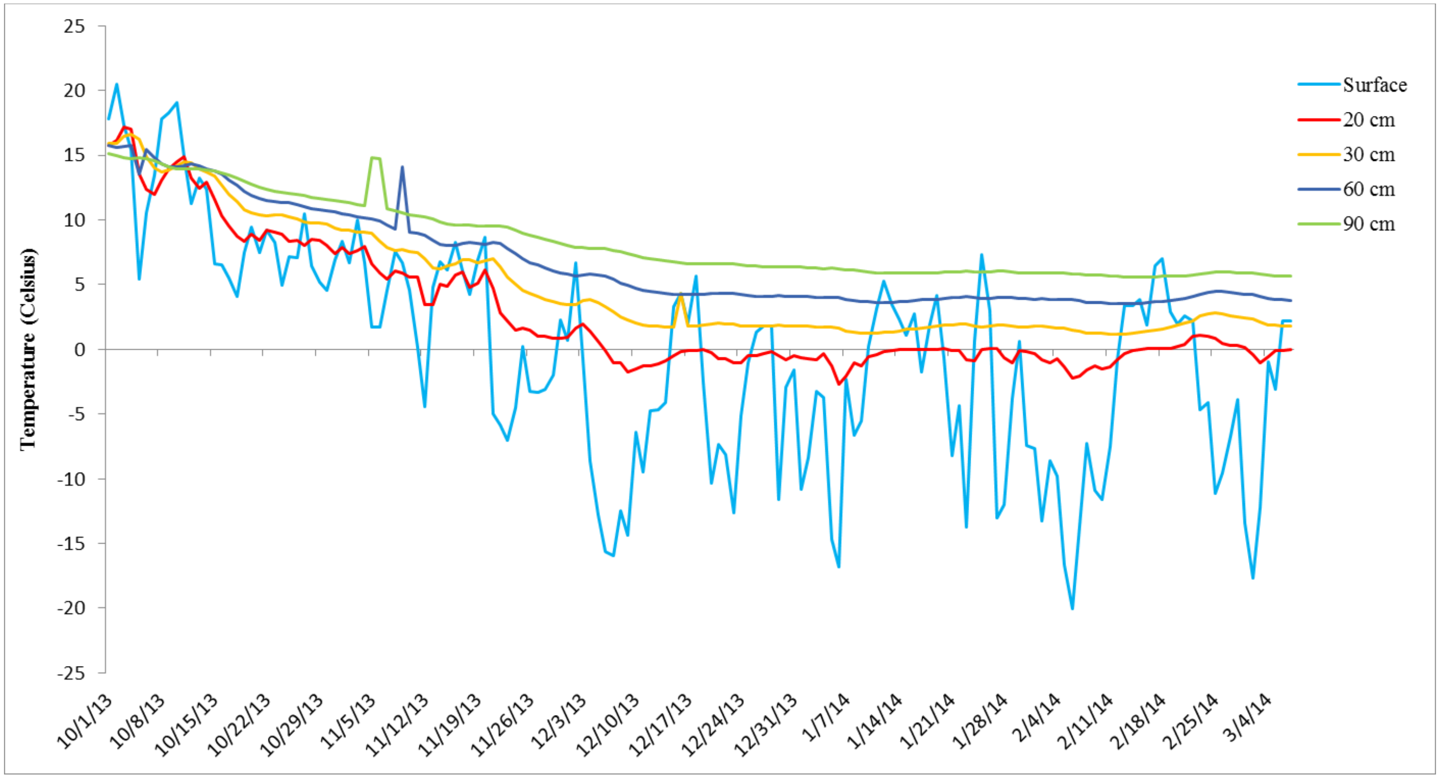


Figure 8. Temperatures taken from the HOBO unit and thermocouples corresponding to different incremental depths from 2013-2014.

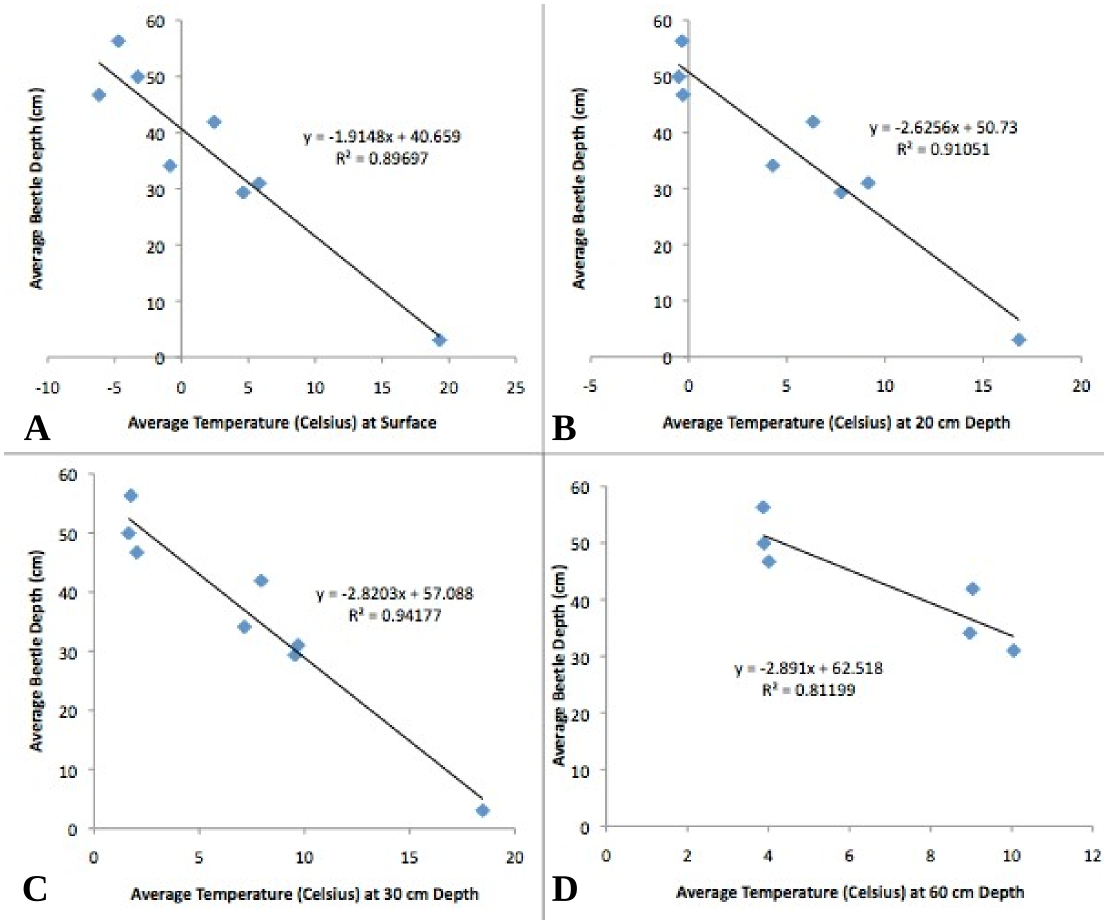


Figure 9. Regression analysis from all 3 trials (2011-2014) of average beetle temperature and average temperature at different depths at the surface (A), 20 cm (B), 30 cm (C), and 60 cm (D).

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CHAPTER 2: THE EFFECTS OF TRAP AND RELOCATION OF BURYING BEETLES (*NICROPHORUS MARGINATUS, NICROPHORUS CAROLINUS*) ON SURVIVAL AND RECAPTURE RATE

2.1 BACKGROUND

Mark and recapture has been used for population estimates, recruitment, and survival estimation in biological and ecological studies (Pradel 1996). These studies focus on marking a large number of organisms in their natural habitat, releasing them back into their habitat, and comparing the number of marked organisms that are recaptured to the number unmarked individuals captured during subsequent sampling (Pradel 1996, Miller et al. 2005). This experimental method enables researchers to use an equation, such as the Lincoln-Peterson Index, to estimate the population size (Hawes 2008). Estimations of population size are very important in monitoring threatened or endangered species, as well as species that are potentially at risk.

In order to obtain accurate estimates with a mark-recapture study, a relatively high fraction of the population, usually 10% or more, must be initially marked (Krebs and Boonstra 1984). In addition, the life history of the organism being studied must be considered, because assumptions to the population estimate are made including that the population is closed (no immigration and emigration) and that the marks do not influence mortality or behavior (Krebs and Boonstra 1984, Pradel 1996). Trapping likelihood should also be known because it highly affects the results of a mark-recapture study, and different methods for capture should be thoroughly considered (Krebs and Boonstra 1984). Further, techniques for capturing and marking should not inhibit the organisms' survival or behavior (Hagler and Jackson 2001). The marks should be environmentally safe, cost-effective, and easy for researchers to use (Hagler and Jackson 2001). Marks should also be retained for the study period and be readable because the loss of marks increases the population estimates based on proportion recaptured (Butler et al. 2012).

Many different insect populations have been estimated using mark-recapture techniques, and these efforts have been useful in documenting demography and behavior of various insects (Hagler and Jackson 2001, Jurzenski et al. 2011). Insects that have been studied using mark-recapture studies include the American burying beetle (ABB), gypsy moth (*Lymantria dispar*), stag beetle (*Lucanus cervus*), and various dung beetles (*Aphodius*) (Weseloh 1985, Creighton and Schnell 1998, Roslin 2000, Hawes 2008).

The marking techniques for each insect species are different and specific to the organism being studied. Insects can be marked by clipping or cutting small areas of their exoskeleton. For example, stag beetles have been marked with a hot pin leaving small holes in the elytra (Hawes 2008). Dung beetles were marked by cutting a small portion of their elytra (Roslin 2000). Gypsy moths were marked by removing the prolegs of the larvae (Weseloh 1985), and ABBs have been marked by cutting part of the elytra, using a cauterizer to burn a small hole in the elytra, painting elytra, and issuing individual bee tags (Creighton and Schnell 1998, Butler et al. 2012).

Burying beetle population size and individual movement have been monitored by marking and recapturing beetles (Bedick et al. 1999, Jurzenski et al. 2011), and through this method ABB have been found to move more than 7.24 km in one night, which influenced how far apart traps were placed in this study (Jurzenski et al. 2011). Another study by Creighton and Schnell (1998), found that the average movement per night was around 1.23 km with beetles moving as much as 10.00 km over six nights. There were no differences in recapture rates between males and females or teneral (younger) and senescent (older) beetles (Creighton and Schnell 1998).

For conservation purposes, a trap and relocate technique may be used in situations where there will be significant habitat disturbance because of construction. This habitat loss is usually caused by human encroachment and human development. Mark-relocate may help individuals thrive in new, suitable habitats. However, the process of, collecting, marking, and releasing these organisms in a new habitat may cause harm.

In this study, trap-mark-relocate methods were used on *Nicrophorus marginatus*, a species closely related to the ABB to study the efficacy of this method as a conservation technique when beetle habitat will be disturbed by contruction. *N. marginatus* is a habitat generalist and is one of the most common species of burying beetle in Nebraska. It is easily caught using pit-fall traps baited with rotting rats. In this study, beetles were marked in two ways – cauterization and paint. Elytral cauterization is a permanent marking method and causes very low mortality when applied properly (Butler et al.2012). Acrylic paint applied to the elytra of the beetles lasts <10 days, (Butler et al. 2012), and in this study beetles were marked with paint for only up to 5 days. Both ways were shown to be safe, effective, and allow the beetles to continue to behave naturally without any known detrimental effects (Butler et al. 2012).

2.2 MATERIALS AND METHODS

Sites for study were chosen in the south-central and northern Sandhills regions of Nebraska. Sites were chosen based on rangeland conditions with little human disturbance and were placed in roadside ditches. Baited pit-fall traps were used to collect *N. marginatus.* The traps consisted of an 18.9-liter (5 gallon), plastic bucket dug into the ground with only 4-5 cm of the bucket above ground level (NGPC, USFWS 2008). A dead rat (>200 g) that had been allowed to decay for 3-5 days was then placed inside the bucket to attract the beetles. An appropriate amount of moist soil, about 5-8 cm, depending on how many beetles were usually caught in the trap, was placed inside the bucket to provide the beetles with substrate to limit aggressive interactions and prevent desiccation (Bedick et al. 1999).

Two wooden sticks were placed across the top of the buckets, and wooden plywood boards were placed on top of the sticks. These created a gap between the buckets and a wooden board, allowing beetles to enter the traps, but not allowing them to climb or fly out of the traps. The boards also prevented rainfall, debris, and small animals from entering the traps (NGPC, USFWS 2008). Traps were checked every day, for a consecutive 10 days, to complete one trial.

Both north (sandhills) and south (loess canyons) populations of ABB were represented in this study (Figure 10). Traps were located in Garfield, Sherman, Holt, Dawson, and Lincoln counties in Nebraska, USA (Figure 11). Lincoln and Dawson county trap locations were designated “south sites”, and Sherman, Holt, and Garfield county traps were designated “north sites.” Specific trap locations were chosen through previous collection success or finding potentially suitable beetle habitat, such as tall grasses and/or sandy loam (Jurzenski et al. 2011). The south sites were placed in the loess canyons area, both north and south of the Platte River.

A global positioning system was used to record coordinates and photographs of each trap were taken at each trap location. Seven trials, lasting 10 days each, were completed in the months of May, June, July, and August 2012. Six trials, also lasting ten days, were completed in the months of June, July, and August 2013. For one trial, Brady 2013, an additional 10 trap nights were completed, giving this trial a 20-day time period.

The seven sites in 2012 were located near the towns of Burwell, Chambers, Gothenburg, Farnam, Brady, and Lexington (2 trials were conducted in Chambers). The six sites in 2013 were located near Brady, Gothenburg, Burwell, Maxwell, and Loup City (2 trials were conducted in Burwell). Three different locations were chosen at each site, and each location was approximately 8.05 km (5 miles) apart (Figure 12). One location was designated the control, another was designated the source, and the last was designated the destination. At each location there were 4 traps set at 1.61 km (1 mile) apart. Thus, there were 12 traps total at each location (Figure 12).

The control location was used to determine beetle recapture rates; no beetles were moved from these locations, but were marked and immediately released at the trap site. The source location was where the beetles were taken from, and the destination location was where the source location beetles were taken to and released. For the first five trap nights, the captured beetles were cauterized or clipped on their left or right elytra depending on their location, in order to tell locations apart. The source and destination location beetles were oppositely marked, and the controls were marked on the left or right at random. The control beetles were cauterized and released at the site of capture, while the source and destination beetles were collected and kept in the laboratory with moist soil until the fifth trap night.

Beetles caught from the source and destinations sites were housed in the laboratory in buckets separated by location (source and destination). The beetles were fed hamburger meat *ad libitum* before being released to the destination location. After the fifth trap night, the beetles were divided into 4 equal groups, and released at each of the 4 destination trap locations. After their stay in the laboratory, source location beetles were relocated and destination beetles were released where they were caught. Both source and destination beetles were released at the destination locations. Beetles were released approximately 15 meters away from the baited pitfall traps at these destination locations. During the next five trap nights, captured beetles were marked with different colors of car scratch repair paints (Rust-Oleum Scratch and Chip Repair Marker), based on captive location.

The control site had both clipped and painted beetle recaptures, because beetles were not relocated to other locations. The source site had only painted beetle recaptures because all beetles collected at the source site within the first five trap nights were relocated to the destination site. Therefore, captures at the source site represent individuals that were not captured in the first five days of sampling. These individuals were painted and could be recaptured. The destination site included clipped beetles from the source site (the moved beetles) and the destination site (from there or "resident" beetles). For days 6-10, all captured beetles received paint and could be recaptured additional times.

Data were separated by north and south locations to compare whether trap and relocation as a conservation technique is similarly effective in both small and large populations of burying beetles. The loess canyons area (trial done in Dawson and Lincoln counties) is thought to have a smaller population of ABBs than the sandhills ABB population (trials done in Garfield, Sherman, and Holt counties). Years and locations (control, source, destination – residents, and destination – moved) were also compared to determine differences between 2012 and 2013. The statistical package InStat was used statistical testing with significance judged at $p \leq 0.05$. Statistics for trap nights 1-5 and 6- 10 were separately considered because trap nights 1-5 are before relocation and 6-10 is after relocation.

2.3 RESULTS

Total Beetle Numbers and Recapture Rates (2012 and 2013)

Recapture rates were not statistically different between trap sites for beetles caught trap nights 1-5 or 6-10 (Figure 13, 14). There were high standard errors because of the variability within the data. While there was no statistical difference, there was a lower recapture rate of destination-moved beetles compared to destination-residents and control trap sites for trap nights 1-5 (cauterized) (Figure 14). Trap nights 6-10 (painted) showed no statistical difference because recapture rates all remained around 4% with the exception of the north control site, which was higher (Figure 13). There were high standard errors and variability within the data, as there was with recapture rates from trap nights 1-5.

In 2012, there were 21,217 *N. marginatus* captured, marked, and released (Table 5). In 2013, substantially fewer (7,768) beetles were marked and released (Table 6). The number of beetles recaptured in 2012 was 1,136, and there were 343 beetles recaptured in 2013. The recapture rates were 5.35% in 2012, 4.42% in 2013, and 5.10% for both years combined.

In 2012 and 2013, there were 8,013 *N. carolinus* captured, marked, and released (Table 7). There were 5,124 beetles marked (cauterized) trap nights 1-5 at all locations and 2,889 beetles marked (painted) trap nights 6-10 at all locations. The overall recapture rate for cauterized and painted beetles was 11.13%, which was substantially higher than the overall recapture rates for *N. marginatus* (5.10%). The range of recapture rates for beetles was 2.21-25.00%. In the south, recapture rates ranged from 5.41-20.12% with an average of 11.52%, and in the north 2.21-25.00% with an average of 10.74%. There was only one location included from 2012 with recapture rates ranging from 9.09-15.05% with an average of 12.34%. In 2013, the recapture rates ranged from 2.21-25.00% with an average of 10.72%.

The recapture rates of *N. marginatus* from both years and all locations ranged from 0.00-37.50% with high variation but similar average recapture rates (Table 5, 6). In the south, recapture rates ranged from 0.00-10.73 with an average of 4.50%, and in the north recapture rates ranged from 0.00-37.50% with an average 7.06%. In 2012 recapture rates ranged from 0.00-28.57% with an average of 5.64%, and in 2013 recapture rates ranged from 0.00-37.50% with an average of 5.73%.

The numbers of *N. marginatus* caught and marked from both years and all locations ranged from 14-1911 with high variation across sites and locations (Table 5, 6). In the south, beetles captured and marked ranged from 49-1911 with an average of about 478 beetles, and in the north beetles captured and marked ranged from 14-782 with an average of about 247 beetles. In 2012, beetles captured and marked ranged from 14-1911 with an average of about 505 beetles, and in 2013, beetles captured and marked ranged from 32-510 with an average of about 216 beetles.

The numbers of *N. carolinus* caught and marked from both years and all locations ranged from 28-1249 with high variation across sites and locations (Table 7). In the south, beetles captured and marked ranged from 28-1249 with an average of about 357 beetles, and in the north beetles captured and marked ranged from 31-900 with an average of about 311 beetles. In 2012, beetles captured and marked ranged from 28-1249 beetles with an average of about 450 beetles. In 2013, beetles captured and marked ranged from 31-900 beetles with an average of about 295 beetles.

There were differences in the number of *N. marginatus* caught between north and south locations, and in 2012 north recapture rates were significantly higher than in the south (Table 9). There was a difference in beetle recapture rates in 2012, but there was no difference between recapture rates in 2013. More beetles were caught and marked in the north in 2013, and more beetles were caught and marked in the south in 2012. Recapture rates of a location were independent of how many or few beetles were captured and marked.

Recapture Rate Statistics: 2012

The control recapture rates were compared for *N. marginatus* caught trap nights 1-5 and 6-10 using a Mann-Whitney U-test (Table 8). The p-value is 0.1649 with a Mann-Whitney U-test statistic of 13.000. In 2012, north recapture rates were significantly higher than the south recapture rates (p=0.04).

The data were not normally distributed, so Kruskal-Wallis ANOVA was used to compare the recapture rates of the control, source, and destination groups for beetles marked on trap nights 6-10. A Kruskal-Wallis ANOVA was also used to compare the control, destination-resident, and destination-moved for beetles marked on trap nights 1-5. There were no significant differences in recapture rates between treatments for beetles caught trap nights 1-5 (p=0.17) or 6-10 (p=0.36).

Recapture Rate Statistics: 2013

The control recapture rates were compared for trap nights 1-5 and 6-10 using an unpaired t-test (Table 8). The p-value is 0.04 with a t-statistic of 2.348 and was significantly different. There was a higher recapture rate for trap nights 1-5 (8.76%) than 6-10 (4.38%). North and south recapture rates were compared using a Mann-Whitney U- test and were not found to differ significantly (p=0.89) (Table 9).

A Kruskal-Wallis test was used to compare the recapture rates between the different treatments for beetles marked on trap nights 1-5 and 6-10, as the data did not pass the normality test. There were no significant differences in recapture rates among treatments for beetles caught trap nights 1-5 (p=0.12) or 6-10 (p=0.24).

Beetle Number Statistics: 2012

The number of beetles captured and marked at the controls for trap nights 1-5 and 6-10 were compared using a Mann-Whitney U-test, as the data did not pass the normality test (Table 8). The p-value is 0.4428 with a Mann-Whitney U-test statistic of 18.000. North and south beetles caught and marked were compared using a Mann-Whitney U-test as well. The p-value is 0.0003 with a Mann-Whitney U-test statistic of 73.000 (Table 9). This comparison was considered statistically significant ($p=0.0003<0.05$). More beetles were captured and marked in the south than in the north locations.

A Kruskal-Wallis test was used to compare the number of beetles captured and marked between the different treatments for beetles marked on trap nights 1-5 and 6-10, as the data did not pass the normality test. There were no significant differences between beetles captured and marked on trap nights 1-5 or 6-10. The p-value for comparing different treatments of beetles marked on trap nights 1-5 is 0.9743 with a Kruskal-Wallis statistic of 0.052. The p-value for comparing different treatments of beetles captured and marked on trap nights 6-10 is 0.4294 with a Kruskal-Wallis statistic of 0.3859.

Beetle Number Statistics: 2013

The number of beetles captured and marked at the controls for trap nights 1-5 and 6-10 were compared using an unpaired t-test, as the data did pass the normality test. The p-value is 0.6318 with the t-statistic of 0.4943 (Table 8). North and south beetles caught and marked were compared using a Mann-Whitney U-test, as the data did not pass the normality test (Table 6). The p-value is 0.0354 with a Mann-Whitney U-test statistic of 95.000. This comparison was considered statistically significant. More beetles were caught and marked in the north than in the south.

A Kruskal-Wallis test was used to compare the number of beetles captured and marked between the different treatments for beetles marked on trap nights 1-5 and 6-10, as the data did not pass the normality test. There were no significant differences between beetles captured and marked on trap nights 1-5 or 6-10. The p-value for comparing different treatments of beetles captured and marked for trap nights 1-5 is 0.3971 with a Kruskal-Wallis statistic of 1.847. The p-value for comparing different treatments of beetles captured and marked on trap nights 6-10 is 0.4293 with a Kruskal-Wallis statistic of 0.4327.

Trial Extension: Recapture Rates After 10-Day Trial

In 2013, one trial near Brady, Nebraska was extended for an additional 10 days to see if recapture rates would differ from the previous 10 days of trapping (Table 10). A total of 2,225 marked *N. marginatus* were released in the area during the first 10 trap nights. There were 80 beetles recaptured during the trial, eliciting a 3.60% recapture rate. During the additional sampling of trap nights 11-20, there were 3,013 *N. marginatus* captured. Fourteen beetles were recaptured during trap nights 11-20, eliciting a 0.01% recapture rate. This additional ten days of sampling did show a change in recapture rates with a decrease from 3.60% to 0.01%. There were 788 more beetles caught total from trap nights 11-20 (3,013 beetles) compared to trap nights 1-10 (2,225 beetles).

Control, Source, Destination Traps

Control, source, and destination trap beetle (*N. marginatus, N. carolinus*) numbers were compared between trap nights 1-5 and 6-10 (Table 11, 12). The largest difference seen between early (1-5) and late (6-10) recaptures at the source was 29 beetles at the Maxwell 2013 location. The number of beetles captured at the source before and after relocation was compared to see if beetle numbers declined, since beetles were taken from the source locations.

At source trap sites there were 8 sites out of 13 where *N. marginatus* numbers decreased, and 5 out of 13 sites where beetle numbers increased (Table 11). The largest decrease at a source location was 720 beetles at the Lexington site in 2012. The smallest decrease was 41 beetles at the Burwell 1 site in 2013. The largest increase at a source location was 134 beetles at the Gothenburg location in 2013, and the smallest increase was 10 beetles. Also, a decrease in beetle numbers at the source location seemed more prevalent at north sites (5 out of 6) than south sites (3 out of 7). *N. carolinus* numbers decreased at 3 out of the 4 source trap locations (Table 12). The largest decrease was 274 beetles at the Burwell 1 site in 2013, and the smallest decrease was 27 beetles at the Brady site in 2012. The only increase in beetle numbers was 34 beetles at the Maxwell site in 2013.

At the destination trap sites there were 7 out of 13 sites where *N. marginatus* numbers decreased, and 6 out of 13 sites where beetle numbers increased (Table 11). The largest decrease at a destination location was 691 beetles at the Brady site in 2012. The smallest decrease was 49 beetles at the Maxwell location in 2013. The largest increase at a destination location was 236 beetles at the Burwell site in 2012, and the smallest increase was 8 beetles at the Gothenburg site in 2012. *N. carolinus* numbers decreased at 3 out of 4 destination trap locations (Table 12). The largest decrease was 538 beetles at the Burwell 2 site in 2013, and the smallest decrease was 73 beetles at the Maxwell site in 2013. The only increase in beetle numbers was 10 beetles at the Burwell 1 site in 2013.

At the control trap sites there were 8 out of 13 sites where *N. marginatus* numbers decreased, and 5 out of 13 sites where beetle numbers increased (Table 11). The largest decrease at a control location was 593 beetles at the Chambers 2 site in 2012, and the smallest decrease was 6 beetles at the Gothenburg site in 2013. The largest increase at a control location was 230 beetles at the Gothenburg site in 2012, and the smallest increase at a control location 34 beetles at the Chambers 1 site in 2012.

N. carolinus numbers decreased at all locations at the control sites (Table 12). The largest decrease was 598 beetles at the Brady 2012 site, and the smallest decrease was 41 beetles at the Burwell 1 site in 2013.

Effect of Trap and Relocate in Same Trapping Locations

There were 3 sites that had the same placement of traps and treatments in years 2012 and 2013. These sites were Burwell, Brady, and Gothenburg. The most notable result of being able to trap in the same locations both years was the pattern of decreasing beetle numbers at all the control locations. Also, while there was a sometimes severe decrease in beetle numbers, as much as a difference of 1329 beetles, the recapture rates did not seem to be affected by high or low beetle numbers. In contrast to the other sites, Burwell did show a decrease at the control, source, and destination locations, as well as a decrease in the recapture rates at these locations.

N. marginatus* and *N. carolinus

Both beetle species, *N. marginatus* and *N. carolinus*, showed no differences between trap sites for both trap nights 1-5 (cauterized) and 6-10 (painted). However, *N. carolinus* showed higher recapture rates for trap nights 1-5 than *N. marginatus* (Table 13). At the control site *N. carolinus* percent recaptured (13.17%) was higher than *N. marginatus* (6.64%). There were slightly more destination-residents of *N. carolinus* recaptured (6.74%) than *N. marginatus* (4.47%). There were a lot more destination- moved of *N. carolinus* recaptured (12.38%) compared to *N. marginatus* (3.80%).

Recapture rates were the highest for *N. carolinus* destination-moved beetles, whereas recapture rates were the highest for *N. marginatus* at the control sites. Overall, recapture rates for *N. carolinus* (11.13%) were higher on average than *N. marginatus* (5.10%).

2.4 DISCUSSION

The results of this study show that there is no difference between beetle recaptures at different locations, including the control, where beetles were removed (source), and where beetles were added (destination). This supports the hypothesis that moving beetles does not negatively affect the beetle population from where beetles were taken or added. There was a reduction in beetle numbers from 2012 to 2013 but this is most likely due to the previous year's drought conditions (Bedick et al. 2007), considering the similar pattern of fewer beetles caught at the control sites in 2013 compared to 2012.

We did not observe consistent declines in captures at the site from which beetles were taken. This suggests that *N. marginatus* and *N. carolinus* are filling in areas where numbers are reduced by dispersion. Recapture rates were similar across sites, indicating that burying beetles distribute randomly. In the case of ABB, trap and relocate is conducted until no ABB are caught for three consecutive days, which is generally achieved in Nebraska. It is likely that areas of high occupancy will not eliminate ABB from the area but will substantially reduce the number that may be harmed through construction activities.

There seems to be no detrimental effect in moving beetles from one habitat to another making trap and relocate a possibly effective conservation measure. Further, in Creighton and Schnell (1998), it was found that 71% of the ABBs captured and relocated were found in a different habitat than the one they were first caught in. This re-enforces that these beetles are habitat generalists and can survive in different habitats (Creighton and Schnell 1998). There was a general trend that there were less beetles at the source after removal and relocation, but the exceptions where beetle numbers increased may come from the mobility of these beetles to find carrion sources. This also may be the reason that at some locations there were no recaptures (Gothenburg – 2012, Chambers Trial 2 – 2012). It is possible that these beetles leave the habitat they were released into and seek habitats that are more favorable (moisture, carrion, etc.).

Control trap sites showed a higher recapture rate than destination-resident and destination-relocated trap sites for both species of *N. marginatus* and *N. carolinus.* Resident and relocated beetles were collected, brought back to the laboratory, and fed ground beef while they were in our care, while control beetles were released immediately on site. This difference in treatment between the control and destination beetles could have accounted for the differences in percent recaptures among the groups.

No significant differences were found between the destination-residents and destination-moved groups of beetles caught and marked on trap nights 1-5 despite a difference in the numbers of beetles caught at each site. No significant differences were found between the source and destination groups of beetles marked on trap nights 6-10. Recapture rates were also similar between treatments for both beetles marked on trap nights 1-5 and 6-10 (<≈10%). Recapture rates also stayed similar after the 11-20 day extension, although they did decrease after the 1-10 day trial.

Based on these data, marking beetles with paint, on trap nights 6-10, and a cauterizer on trap nights 1-5 does not interfere with their natural movement or life history (Butler et al. 2012). Painted beetles were considered "residents" because they were caught and returned to traps in the same location. There were no detrimental effects observed between recapture rates attributed to the respective marking technique (paint versus cauterized). If there was a negative impact, we would have seen a significant difference between the treatments (source and destination) and the control trap recapture rates, and this was nonexistent regardless of mark, location, site, and year.

This could have further implications for conservation management of the ABB, such as being able to use this method in areas of large or small populations or even varying habitat conditions (ie. drought). Although this study found that beetle populations in the north and south were dynamic, with more beetles found in the south in 2012 and more beetles found in the north in 2013, we were able to see the effects of trap and relocate on these changing populations. The use of this congener species in varying habitats, years, and environmental conditions for testing the efficacy of trap and relocate is important to ABB conservation management implications.

The ABB has been listed as federally endangered since 1989 due to a significant reduction in its numbers and range of occurrence (USFWS 1991). Within the last century, the ABB has disappeared from over 90% of its original range (Lomolino et al. 1995). It is a habitat generalist and is a highly vagile insect, traveling over large areas in search of appropriately sized carrion (Lomolino and Creighton 1996, Bell et al. 2013). ABB show a general preference for clay-based soil, mixed grass prairie, and wet meadow habitat in Nebraska (Panella 2012). While these beetles can be found in many areas of large, unfragmented areas, they do not seem to inhabit agricultural areas and may avoid areas with large numbers of eastern red cedars (Jurzenski 2012, Walker and Hoback 2007).

Conservation efforts are advocated at both state and federal levels, with the goal to prevent extinction, and improve population numbers. Wild populations and introduced populations of ABBs are extensively monitored using sampling by baited pitfall traps (Amaral et al. 1997, Bedick et al. 2004). Management techniques have also been studied to improve ABB numbers, including the removal of competing beetle species, inverted buckets to protect breeding pairs from scavengers, and bait-away stations to attract beetles away from areas of potential threat (USFWS 1991, Hoback and Jurzenski 2011). However, bait-away station efforts were tested and found to be unsuccessful, and they also attracted undesired predators to the beetles, such as opossums and leopard frogs (Hoback and Jurzenski 2011, Jurzenski and Hoback 2011). Mark-relocate is another possible avoidance technique, but it has not been thoroughly studied for use with burying beetles.

The ABB has considerably large and established populations in both the loess canyons and the sandhills of Nebraska, and *N. marginatus* also has high population numbers in these areas. The similar habitat between these beetle species offers insight into how ABB would similarly react to relocation and a new habitat. The surrogate species, *N. marginatus* and *N. carolinus*, were used to test the effectiveness of this conservation measure. The mortality rate of relocating *N. marginatus* was conservatively 0.67%, with resident beetle recaptures (4.47%) being higher than relocated beetle recaptures (3.80%). *N. carolinus* had more relocated beetles recaptured (12.38%) than the resident beetles recaptured (6.47%) at destination sites. These differences in recapture rates between species shows that different species of burying beetle may react differently to relocation and trapping in general.

There was a difference between beetle recapture rates at trap sites between *N. marginatus* and *N. carolinus* when a Mann-Whitney U-test was used to test for significant differences (p=0.003). More cauterized *N. carolinus* were caught at every trap site (control, resident, relocated). However, there still was a similar pattern across trap sites, years, and beetle species. Beetle numbers captured at trap sites after relocation were most notably reduced compared to beetle numbers before relocation. We predicted that there would be more beetles at the destination sites because of the added, relocated beetles, less at the source sites because of the beetles removed, and a mixture of increases and decreases at the control sites.

The prediction was not correct, and there were decreases in beetle numbers caught after the relocation (trap nights 6-10) at the great majority of locations. Both species, *N. marginatus* and *N. carolinus*, both years, 2012 and 2013, and all trap sites, control, source, and destination, showed this similarity. Trap avoidance, weather, or any of the previous factors discussed, rather than an indication that trap and relocation is detrimental to beetle populations, could cause this decrease in numbers after relocation. If there were a negative effect from relocation causing these lower numbers after relocation, there would be differences between control, source, and destination trap sites. However, beetle numbers decrease at a similar numbers of trap sites, regardless of the treatment.

While there were these low recapture rates, and many possible reasons for the variation, there is no evidence suggesting any detrimental effects of relocation. However, the recapture rates were observably low. These low percent recapture rates for the traps may be caused by many reasons, including the high vagility of this insect and the ephemeral resource they need. While these recapture rates are low, they are similar to other mark-recapture studies involving marking and releasing ABB (Bedick et al. 1999, Peyton 2003, Bedick et al. 2004). For example, a mark-recapture study tested ABB from the loess canyon region, and found that recapture rates ranged from only 6.2-7.8% (Peyton 2003).

Two assumptions are used when determining a population size and these are that population size is to remain static and organisms are available to be recaptured during the sampling period (Peyton 2003). These criteria may not be met with burying beetles, because they are highly mobile and are dependent upon carrion availability for survival and reproductive purposes. Beetles may not remain in the same habitat or sampling area because of this. Also, when beetles find a suitable carrion source for reproduction, they stay with the offspring and exhibit biparental care, so it is likely that the timing of sampling periods affected the recapture rates of our study (Peyton 2003).

Sampling was done through the months of May-September, which includes emergence and reproductive times for burying beetles. In emergence months more beetles were likely caught, while in reproductive months fewer beetles were likely caught. This not only had the ability to affect collection numbers but also recapture numbers. Another way in which beetle capture and recapture numbers could have varied is because of weather conditions. Beetle movement is dependent upon hotter temperatures (>15°C) and fair weather conditions, because finding carrion involves the beetles' use of chemoreceptors located on their antennae (Carde and Willis 2008, Scott 1998). Storms, rain, and colder and/or windy conditions may make it difficult for beetles to navigate to the carrion source. Different weather conditions may have affected how many beetles were captured, marked, recaptured, and could have influenced these numbers before and after the beetle relocations.

Another factor possibly influencing beetle recapture rates was the effect of trapping, holding, and releasing the beetles. During a burying beetle's life history they are only in a group setting while fighting and securing a carrion source. If they lose, they leave the area (Müller et al. 1990, Otronen 1988). However, in this study beetles were forced to stay at the carrion site because they were enclosed in the baited pitfall trap, were housed together in large quantities, and were released together in a large group. Because beetles are not naturally inclined to be in these large groups, beetles once released could have traveled even further from the area than they would have naturally, if not for the unnatural group living conditions. This could explain the lower recapture rates at all trap sites and both species after relocation and trapping for 5 days.

Moving beetles seems to have no to little effect on beetle population, and because these beetles are habitat generalists and are known to move throughout habitats in search of carrion, trap and relocate may allow these beetles to thrive in a different habitat with little harm to individual beetles. Adding hundreds to thousands of beetles to an area could arguably create more competition for the beetles already at the area and the beetles moved into the area. However, burying beetles are univoltine and a large increase in beetle population mirrors the competition during a successful reproductive year when natural selection ordinarily occurs.

Trap and relocation as a conservation method for the ABB may be successful in cases where there is imminent threat to their current habitat. When deciding when to use the trap and relocate method, a risk assessment should be considered for removing/relocating beetles and leaving the beetles at the site of endangerment. If risks outweigh the benefits of moving the beetles, then the option to leave the beetles where they are may be more beneficial, depending on why the beetles' habitat is being threatened.

2.5 ACKNOWLEDGEMENTS

This work was funded through the Nebraska Department of Roads and the University of Nebraska Research Services Council. We thank Mike Fritz and the Nebraska Game and Parks Commission for use of a vehicle. We also thank Stephanie Butler, Elisabeth Jorde, Scout Wilson, Kelly Willemssens, Carlos Henrique Braz Giorgenon, Kaue Shera, Guillerme Guarnieri, JaeHong Lim, SooMin Son, Kari Page, Sean Farrier, Jess Lammers, and Mike Cavallaro.

2.6 TABLES

Table 5. For 2012, beetles marked trap nights 1-5 (cauterized) (A) and 6-10 (painted) (B) and their correlating recapture rates at each site, which show the percentage of beetles returning to the site they were released. Destination-R indicates resident beetles from the destination, and Destination-M indicates moved beetles from the source that were relocated to the destination. A Kruskal-Wallis ANOVA was used to determine if there were significant differences between the data.

A

	Beetles Marked Trap Nights 1-5 (#)			Recapture Rates		
	Control	Destination-R	Destination-M	Control	Destination-R	Destination-M
North						
Chambers 1	14	21	267	21.43%	28.57%	0.00%
Chambers 2	782	396	31	6.78%	7.83%	9.68%
Burwell	422	43	474	7.58%	9.30%	2.11%
South						
Gothenburg	451	74	157	5.76%	2.70%	0.00%
Farnam	451	1170	616	5.10%	1.28%	0.81%
Brady	1566	1359	490	10.34%	3.46%	2.45%
Lexington	306	975	1911	4.58%	9.95%	10.73%
	Kruskal-Wallis Statistic: 0.052		P-value: 0.9743	Kruskal-Wallis Statistic: 3.5720		P-value: 0.1676

B

	Beetles Marked Trap Nights 6-10 (#)			Recapture Rates		
	Control	Source	Destination	Control	Source	Destination
North						
Chambers 1	48	35	60	2.08%	5.71%	5.00%
Chambers 2	189	47	119	12.70%	4.26%	2.52%
Burwell	337	378	279	7.42%	5.29%	2.15%
South						
Gothenburg	681	207	82	0.29%	0.97%	1.22%
Farnam	369	626	1059	6.50%	1.12%	1.51%
Brady	1488	199	668	2.69%	2.01%	4.79%
Lexington	258	1191	921	3.49%	10.58%	4.02%
	Kruskal-Wallis Statistic: 0.3859		P-value: 0.4294	Kruskal-Wallis Statistic: 0.7421		P-value: 0.3576

Table 6. For 2013, beetles marked trap nights 1-5 (cauterized) (A) and 6-10 (painted) (B) and their correlating recapture rates at each site, which show the percentage of beetles returning to the site they were released. Destination-R indicates resident beetles from the destination, and Destination-M indicates moved beetles from the source that were relocated to the destination. A Kruskal-Wallis ANOVA was used to determine if there were significant differences between the data.

A

	Beetles Marked Trap Nights 1-5 (#)			Recapture Rates of Beetles Trap Nights 1-5		
	Control	Destination-R	Destination-M	Control	Destination-R	Destination-M
North						
Burwell 1	75	292	105	14.67%	6.85%	9.52%
Burwell 2	180	264	182	8.33%	1.52%	2.75%
Loup City	109	109	278	6.42%	6.42%	5.04%
South						
Brady	237	359	474	5.06%	4.18%	1.05%
Gothenburg	69	32	510	11.59%	37.50%	5.69%
Maxwell	246	318	54	6.50%	2.52%	1.85%
	Kruskal-Wallis Statistic: 1.847		P-value: 0.3971	Kruskal-Wallis Statistic: 4.224		P-value: 0.1210

B

	Beetles Marked Trap Nights 6-10 (#)			Recapture Rates of Beetles Trap Nights 6-10		
	Control	Source	Destination	Control	Source	Destination
North						
Burwell 1	49	64	224	2.04%	4.69%	1.79%
Burwell 2	97	67	82	2.06%	1.49%	0.00%
Loup City	208	227	190	7.69%	1.76%	9.47%
South						
Brady	392	383	380	3.57%	6.01%	2.89%
Gothenburg	63	644	108	7.94%	2.17%	7.41%
Maxwell	302	126	269	2.98%	1.59%	3.35%
	Kruskal-Wallis Statistic: 0.4327		P-value: 0.4293	Kruskal-Wallis Statistic: 1.719		P-value: 0.2269

Table 7. *N. carolinus* marked trap nights 1-5 (cauterized) (A) and 6-10 (painted) (B) and their recapture rates by location for 2012 and 2013. Destination-R indicates resident beetles from the destination, and Destination-M indicates moved beetles from the source that were relocated to the destination.

A

	Beetles Marked Trap Nights 1-5 (#)			Recapture Rates		
	Control	Destination-R	Destination-M	Control	Destination-R	Destination-M
2012						
Brady	1249	468	55	15.05%	10.04%	9.09%
2013						
Maxwell	582	74	237	9.11%	5.41%	6.75%
Burwell 1	72	623	64	13.89%	7.70%	25.00%
Burwell 2	301	499	900	14.62%	3.81%	8.67%
	Kruskal-Wallis Statistic: 1.423		P-value: 0.2866	Kruskal-Wallis Statistic: 4.654		P-value: 0.0485

B

	Beetles Marked Trap Nights 6-10 (#)			Recapture Rates		
	Control	Source	Destination	Control	Source	Destination
2012						
Brady	651	28	248	11.06%	14.29%	14.52%
2013						
Maxwell	418	108	164	16.27%	6.48%	20.12%
Burwell 1	31	349	74	12.90%	9.17%	6.76%
Burwell 2	190	266	362	12.12%	12.03%	2.21%
	Kruskal-Wallis Statistic: 0.8077		P-value: 0.3859	Kruskal-Wallis Statistic: 0.9615		P-value: 0.3785

Table 8. Beetle numbers caught and marked and the recapture rates of these beetles compared at the control traps in 2012 (A) and 2013 (B). Beetles marked and recaptured on trap nights 1-5 and 6-10 were compared using a Mann-Whitney U-test.

	Beetles Marked		Recapture Rates	
	Control (1-5)	Control (6-10)	Control (1-5)	Control (6-10)
Average	570.29	481.43	8.80%	5.02%
Range	14 - 1566	48 - 1488	4.58 - 21.43%	0.29 - 12.70%
A	Mann-Whitney U-test Statistic: 18.000	P-value: 0.4428	Mann-Whitney U-test Statistic: 13.000	P-value: 0.1649

	Beetles Marked		Recapture Rates	
	Control (1-5)	Control (6-10)	Control (1-5)	Control (6-10)
Average	152.67	185.17	8.76%	4.38%
Range	75 - 246	49 - 392	5.06 - 14.67%	2.04 - 7.69%
B	Mann-Whitney U-test Statistic: 18.000	P-value: >0.9999	Mann-Whitney U-test Statistic: 6.000	P-value: 0.0649

Table 9. Beetle numbers caught and marked and the recapture rates of these beetles compared at the north and south traps in 2012 (A) and 2013 (B). Beetles marked and recaptured were compared using a Mann-Whitney U-test.

A

	Beetles Marked		Recapture Rates	
	North	South	North	South
Average	219	719.79	7.97%	3.91%
Range	14 - 782	74 - 1911	0.00 - 28.57%	0.00 - 10.73%
	Mann-Whitney U-test Statistic: 73.000 P-value: 0.0003		Mann-Whitney U-test Statistic: 134.50 P-value: 0.0396	

B

	Beetles Marked		Recapture Rates	
	North	South	North	South
Average	275.89	155.67	6.33%	5.14%
Range	32 - 644	49 - 292	1.05 - 37.50%	0.00 - 14.67%
	Mann-Whitney U-test Statistic: 95.000 P-value: 0.0354		Mann-Whitney U-test Statistic: 157.00 P-value: 0.8868	

Table 10. *N. marginatus* were sampled 10 days following the Brady 2013, 10-day trial and recaptures were noted. No beetles were further marked during days 11-20 of sampling. Beetle numbers for the first 10-day trial and consequent 10-day trial (trap nights 11-20), as well as recaptures for the last 10 days are shown.

	Clipped	Painted
Beetles Marked	1070	1155
Beetles Recaptured (1-10)	32	48
Recapture Rate	2.99%	4.16%
Beetles Recaptured (11-20)	11	3
Recapture Rate	1.03%	0.02%

Table 11. *N. marginatus* caught early (trap nights 1-5) and beetles caught late (trap nights 6-10) at the control (A), source (B), and destination (C). The highlighted areas show where beetle numbers decreased from trap nights 1-5 (early) to 6-10 (late).

Year	Location	Control	
		Beetles (1-5)	Beetles (6-10)
2012	Chambers 1	14	48
	Chambers 2	782	189
	Burwell	422	337
	Gothenburg	451	681
	Farnam	451	369
	Brady	1566	1488
2013	Lexington	306	258
	Brady	237	392
	Gothenburg	69	63
	Maxwell	246	302
	Burwell 1	75	49
	Burwell 2	180	97
	Loup City	109	208
		Source	
		Beetles (1-5)	Beetles (6-10)
2012	Chambers 1	267	35
	Chambers 2	31	47
	Burwell	474	378
	Gothenburg	157	207
	Farnam	616	626
	Brady	490	199
2013	Lexington	1911	1191
	Brady	474	383
	Gothenburg	510	644
	Maxwell	54	126
	Burwell 1	105	64
	Burwell 2	182	67
	Loup City	278	227
		Destination	
		Beetles (1-5)	Beetles (6-10)
2012	Chambers 1	21	60
	Chambers 2	396	119
	Burwell	43	279
	Gothenburg	74	82
	Farnam	1170	1059
	Brady	1359	668
2013	Lexington	975	921
	Brady	359	380
	Gothenburg	32	108
	Maxwell	318	269
	Burwell 1	292	224
	Burwell 2	264	82
	Loup City	109	190

Table 12. *N. carolinus* caught early (trap nights 1-5) and beetles caught late (trap nights 6-10) at the control (A), source (B), and destination (C). The highlighted areas show where beetle numbers decreased from trap nights 1-5 (early) to 6-10 (late).

A

Year	Location	Control	
		Beetles (1-5)	Beetles (6-10)
2012	Brady	1249	651
2013	Maxwell	582	418
	Burwell 1	72	31
	Burwell 2	301	190

B

Year	Location	Source	
		Beetles (1-5)	Beetles (6-10)
2012	Brady	55	28
2013	Maxwell	74	108
	Burwell 1	623	349
	Burwell 2	499	266

C

Year	Location	Destination	
		Beetles (1-5)	Beetles (6-10)
2012	Brady	468	248
2013	Maxwell	237	164
	Burwell 1	64	74
	Burwell 2	900	362

Table 13. A comparison of recapture rates between trap sites and species, *N. marginatus* and *N. carolinus*, was statistically compared using a Mann-Whitney U-test. A total comparison by species of all trap sites was also compared.

Trap Site		Beetle Species Statistics	
Control		*N. marginatus*	*N. carolinus*
	Average	6.64%	13.17%
	Range	5.06 - 10.34%	9.11 - 15.05%
	Mann-Whitney U Statistic	1.000	
	U'	23.000	
	P-value	0.0190	
Destination-R			
	Average	4.47%	6.74%
	Range	1.28 - 9.95%	3.81 - 10.04%
	Mann-Whitney U Statistic	6.000	
	U'	18.000	
	P-value	0.2571	
Destination-M			
	Average	3.80%	12.38%
	Range	0.81 - 10.73%	6.75 - 25.00%
	Mann-Whitney U Statistic	3.000	
	U'	21.000	
	P-value	0.0667	
Total			
	Average	5.10%	11.13%
	Range	0.81 - 10.73%	3.81 - 25.00%
	Mann-Whitney U Statistic	36.000	
	U'	180.00	
	P-value	0.0025	

FIGURES

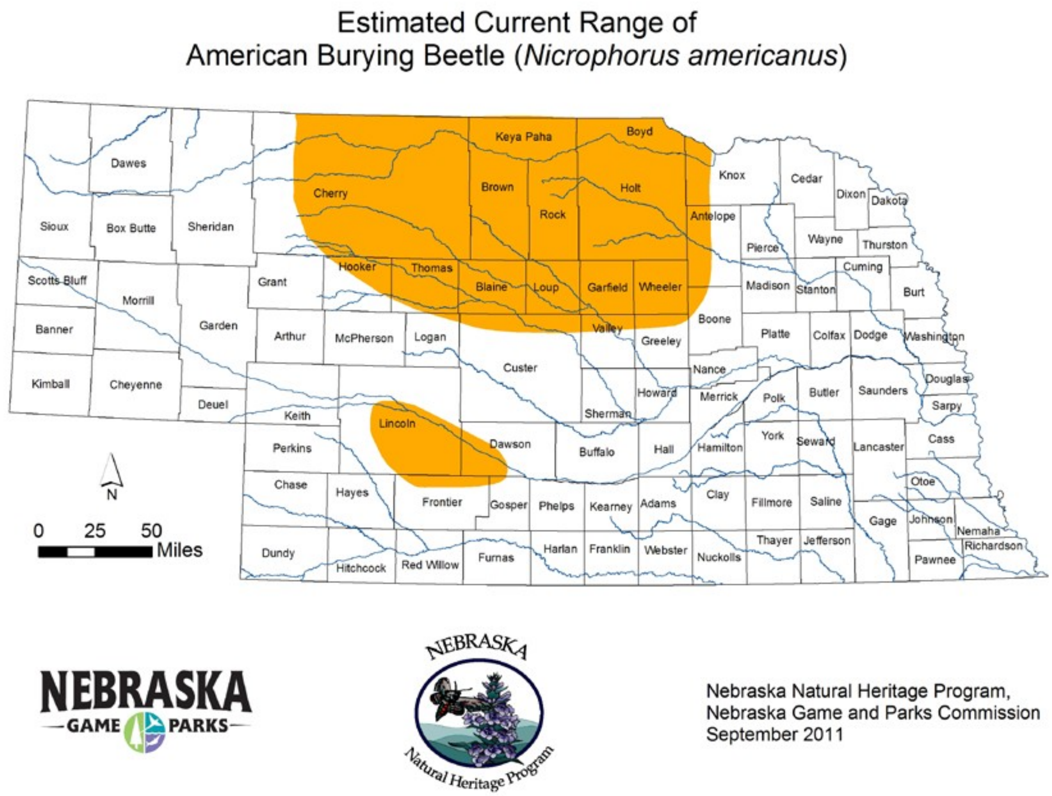

Figure 10. Two populations of ABB are distributed in Nebraska. The north population is present in the larger, sandhill region of the state. The south population is present in the smaller, loess canyon region of the state.

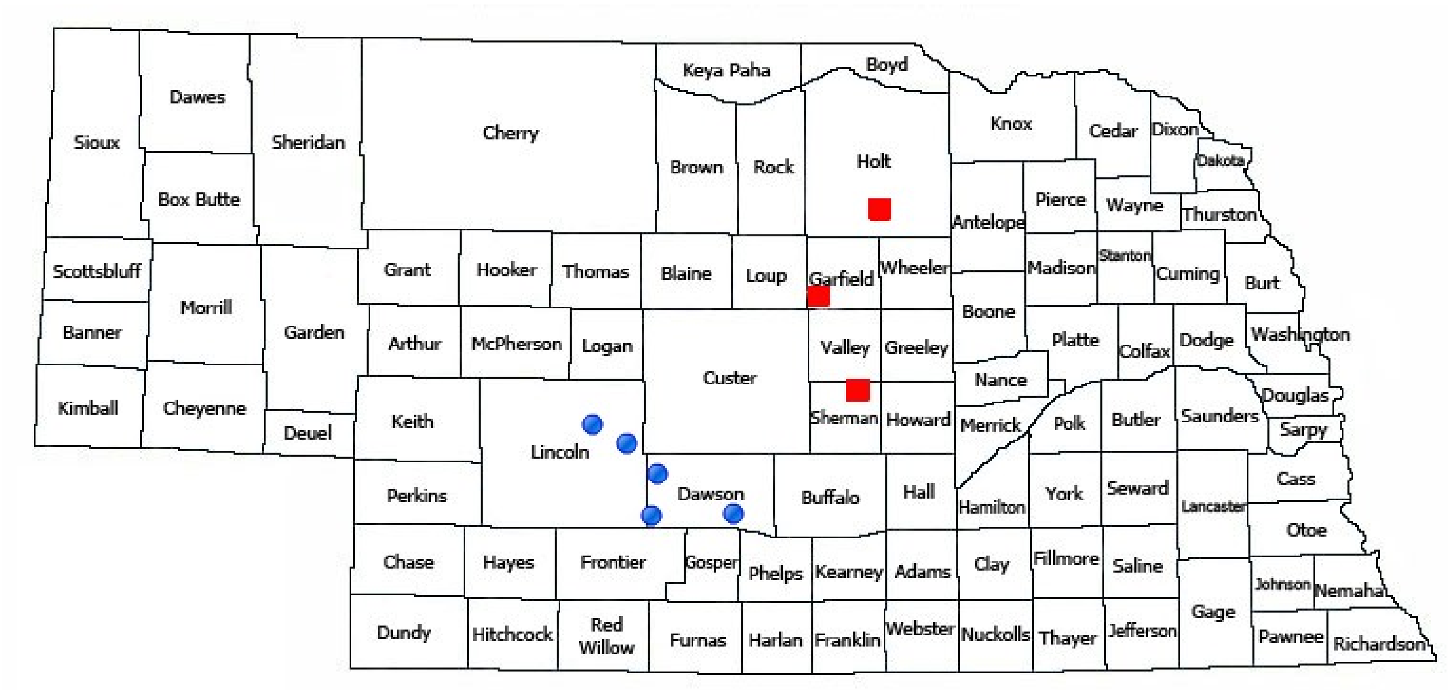

Figure 11. *N. marginatus* and *N. carolinus* trapping locations in the north, sandhills region are marked by a square, and trapping locations in the south, loess canyons region are marked by a circle.

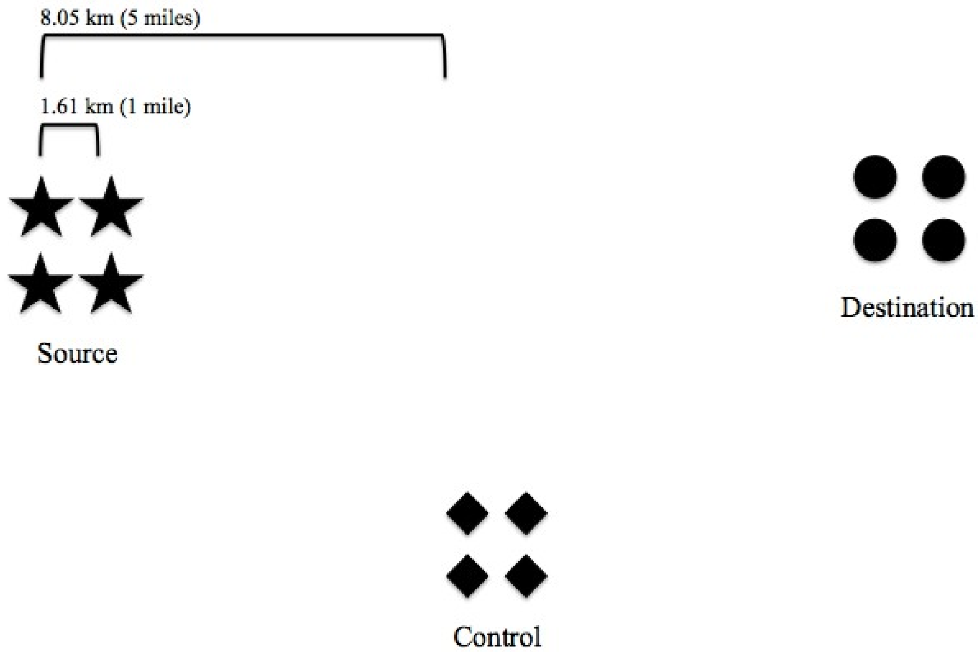

Figure 12. An example of the control, source, and destination locations showing the distance between each trap location and treatments. There are 4 traps at each location and 12 traps total at each site.

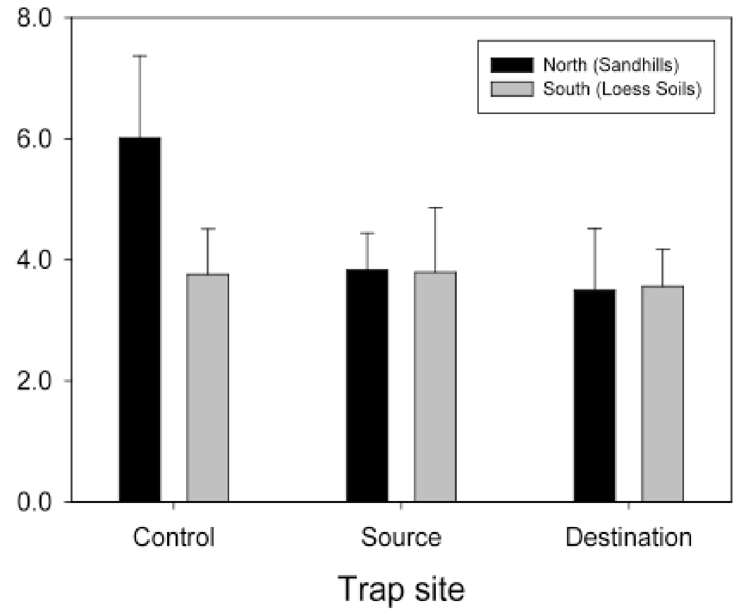

Figure 13. A comparison of the recapture rates with standard error at the control, source, and destination sites (trap nights 6-10). North and south locations are shown for each trap site.

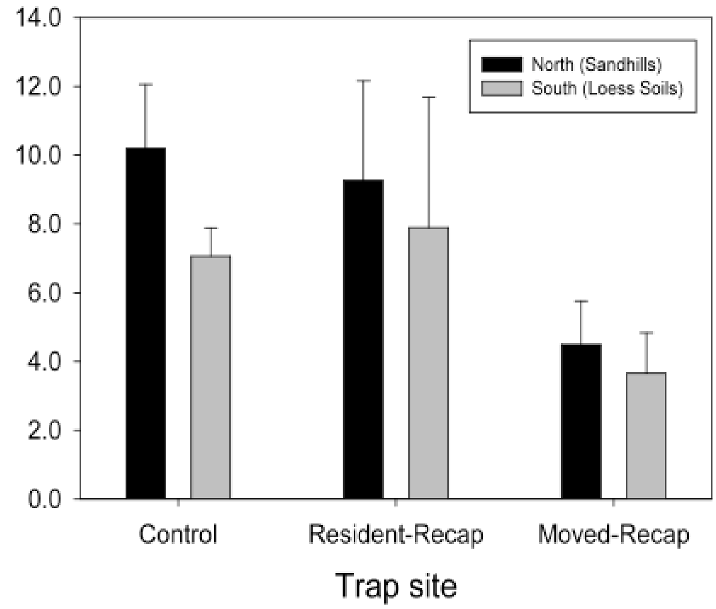

Figure 14. A comparison of the recapture rates with standard error at the control, destination-residents, and destination-moved sites (trap nights 1-5). North and south locations are shown for each trap site.

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Chapter 3.0 FUTURE WORK AND IMPLICATIONS

The objectives of this project were to use surrogate species, *Nicrophorus marginatus, Nicrophorus carolinus,* and *Nicrophorus orbicollis,* to determine the American burying beetles' overwintering biology and to investigate the efficacy of trap and relocation as a conservation measure. Overwintering behavior is an integral life history trait to study because it may provide more information on conservation methods vital to protecting the federally endangered ABB from both construction efforts and aiding in determining optimal reintroduction efforts. Trap and relocation may be an important conservation measure to protect burying beetles from construction disturbances that threaten major ABB habitat.

Human encroachment is limiting and fragmenting known areas of ABB populations such as prairies, grasslands, and woodlands. If human disturbance must occur in known ABB habitat, it would be beneficial to know how to administer this process in a way that is least likely to negatively impact the beetles. Construction work in areas of current beetle habitat may have a detrimental effect, and knowing how close to the surface of the soil that beetles overwinter is important in the conservation of this species. Northern range burying beetles bury deeper than the frost line for overwinter survival, so construction and preconstruction activities such as tree clearing and grubbing and clearing, which may otherwise result in crushing the ABB in the summer, can be safely accomplished in the winter. The species is below the frost line during this time and the ground is frozen above the frost line.

Determining overwintering biology of the ABB is not only important for avoiding harm to ABB during construction but also for their reintroduction efforts. The ABB that were reintroduced to establish sustainable populations were often reared in the lab before being released. These beetles were collected and reared in Arkansas and released in Ohio. Ohio reported a high success rate of ABB reproducing on carcasses, the occurrence of larval development, and the emergence of new, teneral beetles. However, they did not find these teneral beetles the following year.

Burying beetles from the southern range, like Arkansas and Oklahoma, do not bury deep in the soil and need food provisions to survive the winter. Burying beetles from the northern range, like Nebraska and Ohio, bury deep beneath the frost line to avoid the freezing temperatures, slow their metabolic rate, and thus, do not need food provisions to survive the winter. Because burying beetles from the northern and southern ranges have such behaviorally and metabolically different strategies to overwinter, the difference between using beetles from the northern or southern range could be the difference between success and failure of a sustainable ABB population. Reintroduction sites in northern ranges should release beetles originally from the northern range, and reintroduction sites in southern ranges should release beetles originally from the southern range.

Future work should include testing northern overwintering strategy of ABB from Nebraska to see how similar our findings were to the surrogate species', *N. orbicollis.* It would also be beneficial to determine the low temperature tolerance of both *N. orbicollis* and ABB to act as a sort of control for our simulated, natural overwintering environment.

Finding out more about the overwintering biology of these beetles would be helpful for conservation, and would also validate that our experiment created a suitable environment for overwintering beetles.

We also wanted to better understand the effects of trap and relocation on burying beetles species, *Nicrophorus marginatus* and *Nicrophorus carolinus*. These surrogate species were used in the experiment to determine survival and recapture rates, and the results were implicated for the conservation management of the American burying beetle. There were no differences between recapture rates at trap sites for *N. marginatus* and *N. carolinus*. The mortality rate calculated for *N. marginatus* was 0.67% and for *N. carolinus* there was a higher percent recapture for relocated beetles than resident beetles.

While surrogate species were used for this experiment and there were different mortality and recapture rates by species, there were no differences between recapture rates and low mortality rates within these species. The ABB should similarly have these results with trap and relocation, considering these species are comparable in genetics, life history, behavior, and habitat. Our data suggests that relocation may benefit beetles in circumstances where mortality from relocation is less than mortality from construction in beetle habitat. Beetle mortality should be the main consideration in determining whether to trap and relocate beetles. Other considerations include habitat, handling, month of release, and weather.