FINAL PERFORMANCE REPORT

FEDERAL AID GRANT NO. E-65-R-2

EXAMINATION OF AMERICAN BURYING BEETLE REPRODUCTIVE HABITAT

OKLAHOMA DEPARTMENT OF WILDLIFE CONSERVATION

October 1, 2005 through October 31, 2010

FINAL PERFORMANCE REPORT

STATE: Oklahoma **GRANT NUMBER:** E-65-R-2

GRANT PROGRAM: Endangered Species Act Section 6

GRANT TITLE: Examination of American Burying Beetle Reproductive Habitat

GRANT PERIOD: 1 October 2005 - 31 October 2010

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A: ABSTRACT:

There have been many hypotheses proposed to explain the decline of the endangered American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae) including habitat alteration and limited availability of appropriate sized carcasses for reproduction. Here we examine the reproductive habitat preferences and effect of carcass availability on the burial and subsequent reproductive success (production of larvae) of beetles in Cherokee Wildlife Management Area (CWMA), Cherokee County, Oklahoma. Beetles were trapped using standard baited pitfall traps. Breeding pairs were placed on a rat carcass of known mass (grams) and covered with a five gallon bucket to reduce scavenging. This was repeated until a set of nine breeding pairs were established within each of 15 study sites distributed among the three most abundant habitat types (five post oak-blackjack oak-hickory, six post oak-winged elm, and four American elm-chestnut oak-hackberry sites). Carcasses were checked repeatedly to determine time of burial. Two Tidbit temperature probes were buried at 15 cm deep at two burials at each of the 15 study sites to record soil temperature. All burials were exhumed at least 12 days post

burial. Any measurable larvae (>2cm) were weighed. Burial chambers were measured (length, width, depth) along with soil parameters (compaction, soil texture, and moisture). Sixty-five percent of carcasses were buried. Burial chambers averaged 11.5 cm long, 7.9 cm wide and 7.6 cm deep. Burial was least likely in the American elm-chestnut oak-hackberry habitat (P=0.0001) and in soils with high compaction (P=0.0008) and clay content (P=0.001) Larvae were found at 61 burials. Larvae weighed an average of 1.46 g (range 0.1-2.7g). Brood size ranged from 4-35 (mean 20). The size of rat carcass was not correlated with the number of larvae (P=0.50), mean mass of larvae (P=0.20) or total mass of the brood (P=0.54). Soil temperature varied with habitat type (P=0.0006) but did not significantly influence larval mass (P=0.22) or brood size (P=0.36). Reproductive success was eleven fold greater in this study than in previous ones where scavenging removed 54% of available carcasses and decomposers (dipterans) utilized 26%. Carcass size did not impact brood size or larval mass so size of carcasses may not be as much of a limiting factor as competition for carcasses and subsequent reproductive success.

B: OBJECTIVE:

Evaluate the use of habitat by reproducing American Burying Beetles by examining their selection of suitable animal carcasses across a range of habitat variables.

C: INTRODUCTION:

The endangered American burying beetle, *Nicrophorus americanus* (Coleoptera: Silphidae) is unique among non-social insects in providing parental care of young. Male beetles search for a suitable carrion item using sensitive chemoreceptors on the antenna. When a male beetle locates a carcass, he attracts females with pheromones. Competition with other *N.*

americanus and congeners occurs until one pair of beetles occupies the carcass, although evidence exists for intraspecific (Scott and Williams 1993) and interspecific communal brooding (Smith and Clifford 2006).

Reproduction occurs between late April and mid-August depending on latitude (USFWS 1991). The carcass is typically buried during the first night, cleaned of fur or feathers, and coated with anal and oral secretions to retard decomposition (USFWS 1991). Eggs are laid in an escape tunnel near the carrion item. At least one parent, usually the female (Smiseth and Moore 2004), stays with the carcass throughout growth and development of the larvae which ranges from 21-60 days (Wilson and Fudge 1984; Wilson et al. 1984; Smith and Clifford 2006). During this time, parents provide food for larvae and defend them from predators and competitors (Eggert and Müller 1997; Eggert et al. 1998; Scott 1998). *Nicrophorus* larvae are able to self-feed upon hatching but are provided with predigested carrion by the parents (Featherston et al. 1990; Rauter and Moore 1999; Smiseth and Moore 2002; Smiseth et al. 2003). The larva of at least one *Nicrophorus* species (*N. vespilloides*), begs for food from the parents (Rauter and Moore 1999; Smiseth and Moore 2007). The begging behavior ceases after 72 hours of age (Smiseth et al. 2003). Hatching is asynchronous resulting in large size variation within broods as larvae increase body mass rapidly within 24 hours after hatching (Smiseth et al. 2003).

Work on *N. americanus* has been carried out since it was listed as endangered in 1989 (USFWS 1991), but there still remains much speculation as to the proximate cause(s) of the 90% decrease in this species range (Lomolino and Creighton 1996). Among the numerous hypotheses proposed to explain the decline of the American burying beetle are: habitat alteration, competition with vertebrates, competition with congeners, lack of appropriately sized carcasses for feeding and reproduction, use of pesticides and rodenticides, light pollution, and pathogens (Sikes and Raithel 2002 and citations within). Considered a habitat generalist (Creighton et al. 1993; Lomolino and Creighton 1996), habitat alteration may not be as important in the decline of the beetle as the indirect loss of appropriate sized carcasses such as the extinct passenger pigeon (*Ecopistes migratorius*) for feeding and reproduction (Sikes and Raithel 2002).

Carrion is a high-quality resource that is limited and competition among Nicrophorines for carcasses of the appropriate size is presumably high. Based on the number of beetles caught per trap night during annual surveys of Camp Gruber (personal observation), it is probable that *N. americanus* compete with congenerics for carcasses. Intraspecific and interspecific competition would further limit the availability of carcasses for reproduction. In burying beetles, both parents care for larvae. Male defense of the brood and carcass is effective in preventing losses to conspecifics but is not effective against larger invertebrate species (Koulianos and Schwarz 2000) and vertebrate scavengers. In 2008, 54% of carcasses were scavenged in CWMA and an additional 26% were used by flies and decomposed on site leaving 20% of carcasses available for beetles (Smith and Clifford 2008).

While *N. americanus* can be drawn into baited pitfall traps in a variety of habitats, little is known about its reproductive habitat preferences (Lomolino et al. 1995). In a study by Lomolino and Creighton (1996) beetles were placed on carrion, covered with a bucket, and forced to bury at sites located in either grasslands or forested habitats. They found that breeding success was significantly higher in forested habitats. While they observed higher breeding success in forested habitats, the study did not address environmental factors that may be associated with reproduction.

Nicrophorus spp. appear to exhibit soil preferences for reproduction. In a study in Nebraska by Bishop et al. (2002) *Nicrophorus* were more abundant in undeveloped riparian areas with alluvial soils. Reproductive studies at the adjacent Camp Gruber Military Training site in Muskogee County, Oklahoma indicate that *N. americanus* show site preferences for reproduction. Burial of carcasses was higher at sites with lower soil compaction and clay content in studies at Camp Gruber (Smith and Clifford 2006; Smith and Clifford 2007) but was not significant at CWMA in 2008 (Smith and Clifford).

It is unlikely that a single hypothesis will explain the decline of *N. americanus*. The decline is probably due to many factors that are connected. So the most productive avenue of research would be to study several factors simultaneously. Here we examine the effect of carcass availability and habitat variables on the reproductive dynamics of the endangered American burying beetle.

Beetles were trapped and potential breeding pairs were placed on carcasses and covered with buckets to encourage breeding and to reduce competition with vertebrate scavengers and invertebrate decomposers. Breeding pairs were placed on carcasses at three different habitat types within Cherokee Wildlife Management Area, Cherokee County, Oklahoma. Burial sites were later checked to address the following questions: 1) What percentage of carcasses are buried by Nicrophorines when competition is reduced? 2) Do Nicrophorines exhibit habitat preferences for burial? 3) Do soil parameters influence burial? 4) What are the burial characteristics (depth of burial, width burial chamber, and length of chamber)? 5) Once buried, what percentage of burials results in reproductive success as determined by the presence of larva? 6) If larvae are present, how large are broods? 7) Does larval mass or brood size vary with the mass of the carcass?

D: METHODS:

Site description

Cherokee Wildlife Management Area (CWMA) is a 31,360 acre area located in Cherokee County, Oklahoma. It was originally part of the Camp Gruber Military Training Site, but was deeded to the state of Oklahoma by the U.S. government in 1949. It is divided into a 16,000 acre public hunting area and a 15,360 acre area that is used for game management with annual controlled hunts.

CWMA is within the Ozark Highlands – Dissected Springfield Plateau (Figure 1 showing EPA ecoregions). Oak-hickory forests dominate the majority of this area which lies on limestone geology. Five additional habitats occur at CWMA including post oak-winged elm, post oak-blackjack oak-hickory, American elm-chestnut oak-hackberry, post oak-blackjack oak-little bluestem, and prairie threeawn-western ragweed herbaceous.

Experimental Design

Three distinct habitat assemblages were examined at CWMA to determine what habitat variables impacted *Nicrophorus americanus* reproductive success. Reproductive success was defined as the presence of measurable larvae 14-18 days after burial of a carcass.

Site Selection

Land Condition Trend Analysis (LCTA) sites were selected to represent each of the three dominant habitat types within CWMA (post oak-blackjack oak-hickory, post oak-winged elm, and American elm-chestnut oak-hackberry). LCTA sites were selected based on replication of habitats and accessibility of sites. When readily accessible LCTA sites were not available, habitats were located along roadways. All sites were a minimum of 50 meters away from roadways and ½ mile from other study sites. We were able to locate five post oak-blackjack oak-hickory sites, six post oak-winged elm sites, and four American elm-chestnut oak-hackberry sites (Figure 2) that met the criteria. Sites classified as American elm-chestnut oak-hackberry forests often only had a single representative of the tree species. This was true for all LCTA sites designated as this habitat type as well, so each of the sites used in this study were considered suitable representatives.

Trapping of adult *N. americanus*

Adult *N. americanus* were captured between 22 May and 2 June 2009 using standard baited pitfall traps. Eight pitfall traps were placed along transects at 20-meter intervals. The traps consisted of two nested 24 oz Solo© cups placed into the soil with a two cm lip extending above the ground surface. Soil was built up below the cup lip to form a berm. Two nested 24 oz cups were used so the inner trap (which would contain any beetles) could be removed easily without damaging the trap structure. Moistened soil was placed in the bottom of the interior cup to an approximate depth of 2.5 cm (as per US Fish and Wildlife Service Survey Guidance recommendations 2005) to prevent desiccation of specimens.

Traps were baited with approximately 1.5 to 2 oz of chicken gizzards that were allowed to decompose for a minimum of two days prior to use. Gizzards and associated liquid were placed into a 3 oz bait cup that was suspended over the trap cups with bailing wire inserted through the upper edge of the bait cup. One-gallon, plastic nursery pots with side drainage holes and solid bottoms were inverted and placed over traps to protect them from precipitation and heat. Three holes were cut equidistantly along the lip of each pot to allow insects to enter the trap. All traps were set and baited before 6:00 p.m. (1800 hours) and checked starting shortly after dawn the next day.

All *Nicrophorus* species captured were marked by cutting a small triangular notch into the posterior margin of the left elytra edge. Individual *N. americanus* were additionally marked with a uniquely colored and enumerated bee tag (Figure 3). Tags were secured to the right elytra using gel superglue. Gel superglue was far superior to the adhesive provided with the bee tags because its higher viscosity allowed for greater control of glue application, faster drying time, and better adherence. The age and sex of each *N. americanus* was recorded.

Establishing breeding pairs

A randomly selected male and female *N. americanus* were placed on a rat carcass of known mass (grams) and covered with a five gallon plastic bucket to reduce scavenging. Holes were drilled in the sides of buckets to provide airflow (Figure 4). This process was repeated as male and female *N. americanus* were available until a set of nine reproductive units were established within each of the 15 study sites. Buckets were located at least 30 m from each other in a grid pattern within the site. Unused *N. americanus* were released at the site.

Carcasses were checked repeatedly to determine the time of burial. Burial was designated as the point where the carcass was no longer visible. All burials were exhumed within 14-18 days after the carcass was made available and at least 12 days after the carcass was buried. Larvae attain their maximum size between 12 and 14 days post burial and generally leave the carcass after 15 days (Louis Perrotti, Roger Williams Park Zoo, personal communication). Any large larvae (> 2cm) observed were weighed using a Pocket-Pro PP401 digital scale (Figure 5). The burial chamber was measured (length, width, depth). Larvae were returned to the chamber unharmed and the chamber was restored.

Measurement of habitat variables

To elucidate soil preferences at CWMA, two replicate soil samples were collected from each site. Topsoil samples were collected for analysis of soil texture (silt, sand, and clay) and were processed by Oklahoma State University Soil Lab. Soil compaction was measured in the field at each bucket location (153 total) at 7cm, 15cm, and 23 cm depth. Soil moisture was determined by collecting two topsoil samples at random from each of the 15 study sites.

Samples were kept in Ziplock© bags on ice until returned to the laboratory where they were stored in a freezer until processed. Subsamples were weighed to determine the original moisture content. Subsamples were then dried until they attained a constant mass. The difference in mass was determined to be due to moisture.

The developmental rate of arthropods can be impacted by temperature. To determine if soil temperature varied at sites, two Tidbit temperature probes were buried 15 cm deep into soil at two of the burial sites at each of the 15 study sites (30 total). Tidbits recorded soil temperature every two hours throughout the study.

Data were analyzed using JMP statistical software. Relationships between soil and chamber variables were determined with a multivariate Principle Components Analysis (PCA). Those that explained more than 20% of the variation were retained for further analysis. The effect of variables on the burial of carcasses by *N. americanus* and subsequent reproduction was analyzed with analysis of variance (ANOVA) and the nonparametric Wilcoxon/Kruskal-Wallis test (W/K-W) with $\alpha=0.05$.

E: RESULTS:

A total of 445 *N. americanus* individuals were captured from the 15 sites studied with a trapping rate of 1.1 *N. americanus* per trap night. The majority of *N. americanus* were male (55%; G test P=0.03; $\chi^2 = 4.28$) and a significantly greater number were young (94%; G test P<0.0001; $\chi^2=458.29$). During this period 49 (10%) *N. americanus* were recaptured and none were found dead in traps.

Nine reproductive units were placed at each of the 15 sites (N=135). Carcasses ranged in mass from 79.8 g to 265 g with a mean of 182 g. Sixty-five percent of the carcasses were buried within two days of when they were made available. Those that were not buried were presumed to be abandoned by one or both potential *N. americanus* parents. Burial chambers were on average 11.5 cm long, 7.9 cm wide and 7.6 cm deep.

The likelihood of burial varied with habitat type (ANOVA P=0.0001; F_{ratio}=21.01). Burial was least likely in the American elm-chestnut oak-hackberry forests. Burial was twice as frequent in the other two habitat types (Figure 6).

Soil compaction (7, 15, and 23 cm depth) was compared using Principle Component Analysis (PCA). Soil compaction at all depths explained 43% or more of the variation (Eigenvalue=3.53). Burial was more likely to occur in soils with low compaction (ANOVA P=0.0008; F_{ratio}=7.19) measured at 7 cm depth, which corresponded with the average depth of burial chambers.

Soil temperature varied with habitat type (ANOVA P=0.0006; F_{ratio} =0.77). The American elm-chestnut oak-hackberry forests were generally cooler than other habitat types (Figure 7). The temperature probe at site OS elm was planted late. It was a statistical and methodological outlier and was not included in analysis.

Soil texture impacted burial of carcasses. Burial was least likely in clay loam soils (ANOVA P=0.0001; F_{ratio}=12.78). The frequency of burial did not vary significantly between the other identified soil textures (Figure 8). The clay content of soils varied with habitats (ANOVA P=0.0010; F_{ratio}=7.23). The American elm-chestnut oak-hackberry forests had soils with the highest clay content and were previously identified has having the least number of burials (Figure 9).

The percentage of soil moisture did not vary with habitat type (ANOVA P=0.20; F_{ratio} =1.66) but it did vary among the study sites (ANOVA P=0.0001; F_{ratio} =69.16) (Figure 10). Soil moisture was not associated with the mean depth of burial chambers (ANOVA P=0.76; F_{ratio} =0.09). Soil moisture varied significantly with the clay content (ANOVA P=0.0025; F_{ratio} =10.98) but this observation was driven by a two high measurements at the OS elm site. If this site is treated as an outlier and eliminated from the analysis, then no significant relationship is observed (ANOVA P=0.09; F_{ratio} =3.05).

Larvae were found at 61 burials. Larvae weighed an average of 1.46 g (mode=1.5; range = 0.1-2.7; CI 1.4-1.5; N=691). Brood size ranged from 4-35 (mean=20; mode=23; CI=18-22; N=61). An additional 41 larvae were observed dead in chambers. While we can not be certain about the cause of death, many of these were found in chambers with standing water, presumably from heavy rains in the area (Figure 11).

The size of the rat carcass was not correlated with the number of larvae (ANOVA P=0.50; F_{ratio}=0.45) nor did it influence the mean mass of larvae (ANOVA P=0.20; F_{ratio}=1.66) or total biomass of the brood (Bivariate fit ANOVA P=0.54; F_{ratio}=0.38). While not significant (ANOVA P=0.055; F_{ratio}=3.87), larval size tended to increase with increased brood size (Figure 12). The amount of time that lapsed before excavation of larvae did not significantly impact larval size (ANOVA P=0.67; F_{ratio}=0.17) but was associated with brood size (ANOVA P=0.006; F_{ratio}=4.01) (Figure 13).

The size of the carcass influenced the burial chamber characteristics. Large carcasses were not buried at a greater depth than small ones (ANOVA P=0.37; F_{ratio}=0.21) but chamber width was increased presumably to accommodate larger carcasses (ANOVA P=0.0024; F_{ratio} =4.01).

Although temperature is known to impact arthropod growth rates, soil temperature did not significantly influence larval mass (P=0.22; F_{ratio}=0.066) or brood size (P=0.36; F_{ratio}=0.036) in this study. The range of temperatures observed in this study (18.73-21.29 °C) may not vary enough to impact growth differentially.

E: DISCUSSION:

A large number of *N. americanus* were captured for use in the reproductive study at Cherokee Wildlife Management Area (CWMA). A total of 445 *N. americanus* individuals were captured between 22 May and 2 June, 2009 with a trapping rate of 1.1 *N. americanus* per trap night. Ten percent of the individuals were recaptured. The adjacent Camp Gruber Army Training Center was the site of an additional survey. Only 415 *N. americanus* were captured there between 23 June and 2 July with a 19% recapture rate and a trapping rate of 0.33 *N.*

americanus per trap night. The Camp Gruber population exhibited a 20% decline in beetles per trap night since the 2008 survey. The difference in the trapping success between CWMA and Camp Gruber is likely due to biased trapping efforts. Since the purpose of trapping at CWMA wasn't to survey the population but to collect mating pairs for the reproductive study, traps were located in areas which were thought to result in the greatest success. Transects were relocated when trapping success dwindled. At Camp Gruber, transects were placed at Land Condition Trend Analysis sites as dictated by the long term study design. Transects were examined for three consecutive days as required by the US Fish and Wildlife Service protocol. Twice as many beetles were recaptured at Camp Gruber than at CWMA. This is likely because most *N. americanus* captured at CWMA were placed directly into the reproductive study and were confined to a carcass (presumably reproducing), while those captured at Camp Gruber were immediately released.

As adult *N. americanus* were captured, they were placed on carcasses of known size (mass in grams) and covered with a bucket to encourage reproduction and decrease scavenging. Carcasses were typically buried within two days of when they were made available. Sixty-five percent of carcasses were buried. In a similar study conducted in 2008 20% of carcasses were buried at CWMA (Smith and Clifford 2008). The large increase in burial rate in the current study was probably due to decreased competition with scavengers and dipterans (flies). As much as 54% of rat carcasses used in the 2008 study were scavenged and 26% were used by dipterans. One cannot discount possible increased burial success by "forcing burial" with the male and female placed on the carcass. Smith and Clifford (2008) allowed *N. americanus* to find carcasses on their own and no protection was provided to them. Carcasses that were not buried in the 2009 field season were presumed to be abandoned by one or both potential *N. americanus* parents. No scavenging was confirmed.

Burial chambers were on average 11.5 cm long, 7.9 cm wide, and 7.6 cm deep. This was similar in size to chambers observed during previous studies (Smith and Clifford 2006, 2007, and 2008).

The likelihood of burial varied with habitat type. Burial was least likely in the American elm-chestnut oak-hackberry forest association and twice as likely in the other two habitat types (post oak-blackjack oak-hickory sites and post oak-winged elm). During the 2008 study at CWMA, Smith and Clifford found that the burial of carcasses did not significantly vary with habitat type (ANOVA F=2.237; P=0.081) although burials were more numerous in the post oak-winged elm and post oak-blackjack oak-hickory habitat association. A replicate study at Camp Gruber found that burial numbers were greater in the oak hickory association (Smith and Clifford 2008). While it may appear that *N. americanus* prefers oak-hickory habitats for reproduction in 2008 and 2009 this is not clear cut. The most productive habitat for beetles based on emergence of *N. americanus* in 2007 at Camp Gruber was the post oak-winged elm association with five newly eclosed individuals. In the study by Smith and Clifford (2006) at the same sites, the most productive habitat for beetles and the only site in which *N. americanus* emerged, was the big bluestem-little bluestem herbaceous assemblage found at one site (LCTA 10).

Regardless of habitat preferences, burial was more likely to occur in soils with low clay content and low compaction measured at 7cm depth which corresponded with the average depth of burial chambers. This trend has been observed in previous studies at CWMA and Camp Gruber since 2006, although it is not always statistically significant. Soils with lower compaction are easier to dig through and therefore bury carcasses.

Soil temperature varied with habitat type. Soil temperature varied from a mean of 20.45°C in the post oak-blackjack oak-hickory association to 19.34°C in the American elm-chestnut oak-hackberry forests. Nisimura et. al (2002) found that *N. quadripunctatus* in Japan suppressed reproductive behavior at 25°C. During this "summer diapause" no larvae hatched in 85% of broods. Temperatures of 20°C to 22.5°C resulted in normal reproductive output. If *N. americanus* respond similarly to *N. quadripunctatus*, then it appears soil temperatures observed at CWMA are not a limiting factor for reproduction.

Soil moisture was not associated with habitat type nor clay content but did vary among study sites. Forty-one larvae were found dead in standing water in chambers at sites 11, 12, 16, 13, 18 and 19. One would expect that soil moisture would be high at these sites; however that was not the case. The chambers with standing water were well within the range of soil moisture values associated with healthy larva. Samples for soil moisture were taken at a different time than the chambers were excavated. Soil moisture could be so variable through time that the saturation of some chambers was not evident at the time of soil sampling.

Reproductive success was much higher in this study than in previous ones. Larvae were found at 69% of burials and 49% of the total number of carcasses provided. This was an eleven fold increase over the reproductive success observed at CWMA in 2008 of 4% of all carcasses available (Smith and Clifford 2008). This dramatic increase in reproductive success is probably due to the use of buckets which decreased loss of carcasses to scavengers and dipterans. This result implies that competition is a limiting factor for reproductive success in *N. americanus*. However it does not explain their endangered status. Congeners would presumably face the same competitive pressures, but they are not endangered.

The sole energy source for larval growth is the carcass selected by the parents. Larger *Nicrophorus* larvae pupate into larger and presumably more successful adults (Trumbo 1990). One would expect that *N. americanus* would produce larger broods when larger carcasses were available, or that the larvae would be bigger. The size of the rat carcass (mean 182 g; range 79.8-265 g) did not determine the number of larvae, nor did it influence the mean mass of larvae in this study or total biomass of the brood. While not significant, larval size tended to increase with increased brood size. In a study of the effects of elevation on reproductive success of captive beetles in the western Rocky Mountains, Smith et al. (2000) observed that *Nicrophorus investigator* reproductive strategies varied somewhat with elevation. At high elevations (approximately 3,200 m) the number of larva did not increase with increased carcass mass but the average larval mass increased. At low elevations (approximately 2,800 m), brood size increased with carcass mass and larval mass remained about the same.

N. americanus exhibit asynchronous hatching (Smiseth et al. 2006) that results in marked size differences within a brood because larvae increase seven-fold in mass during the first 24 hours after hatching (Smiseth et al. 2003). Therefore, the amount of time that lapsed between egg laying and excavation of the larvae is likely to affect the mean larva mass. In our study, the time until excavation did not significantly impact larval size but was associated with brood size. Presumably more larvae could have hatched and attained measurable size at sites where excavation was delayed.

The mass of the carcass influenced the burial chamber characteristics. Large carcasses were not buried at a greater depth than small ones but chamber width was increased presumably to accommodate larger ones.

F: CONCLUSIONS:

Based on these observations, the reproductive success of *N. americanus* increases significantly when the competition for carcasses with vertebrate scavengers and invertebrate decomposers is reduced. Carcass size did not impact brood size or larval mass, therefore size of carcasses may not be as great of a limiting factor as competition for carcasses and subsequent reproductive success. Burial was least likely in the American elm-chestnut oak-hackberry forest association and twice as likely in the other two habitat types (post oak-blackjack oak-hickory sites and post oak-winged elm). Burial was more likely to occur in soils with low compaction and clay content.

Acknowledgments

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G. SIGNIFICANT DEVIATIONS: none

H. COST: $30,042.98

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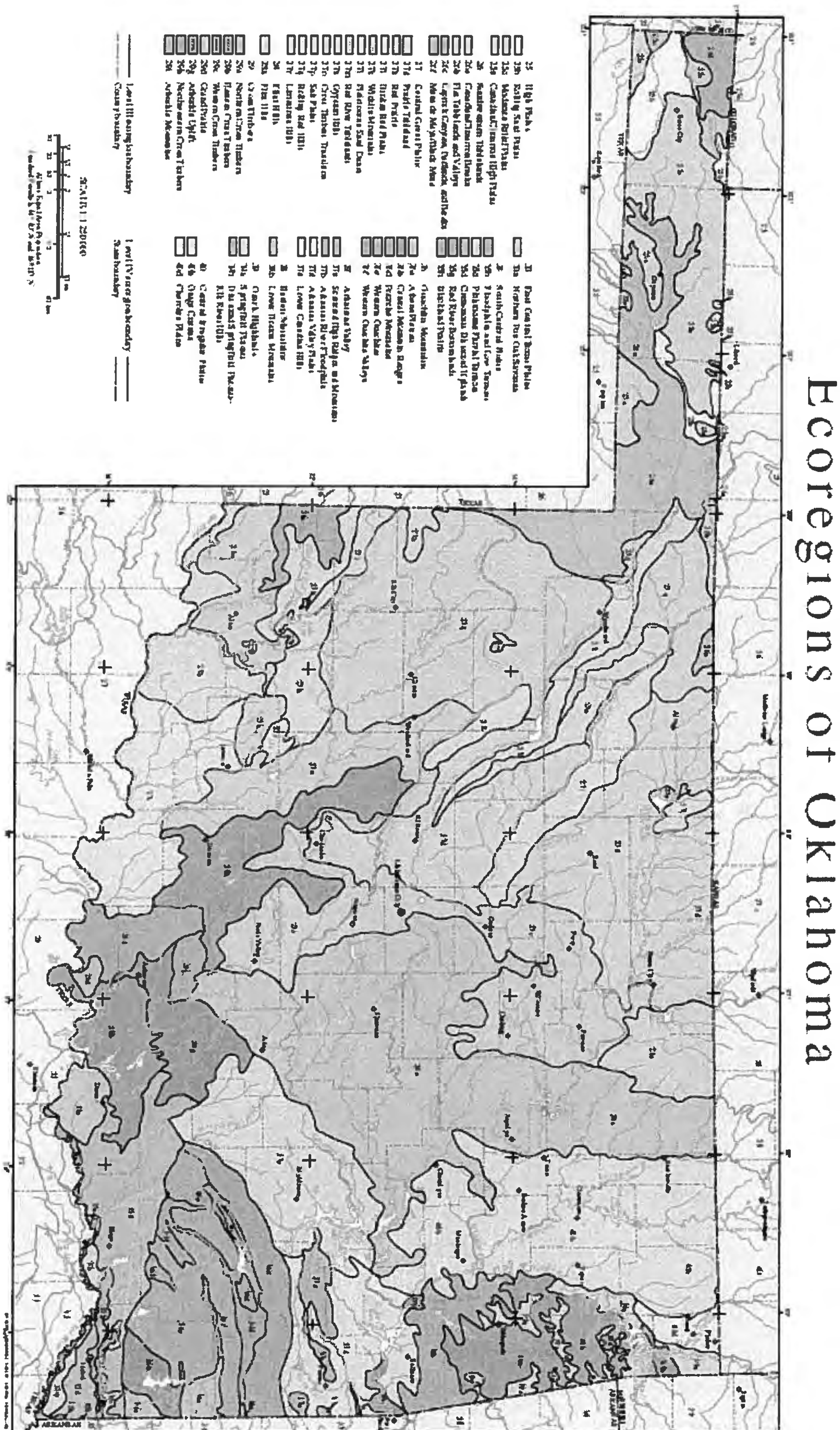

Figure 1.

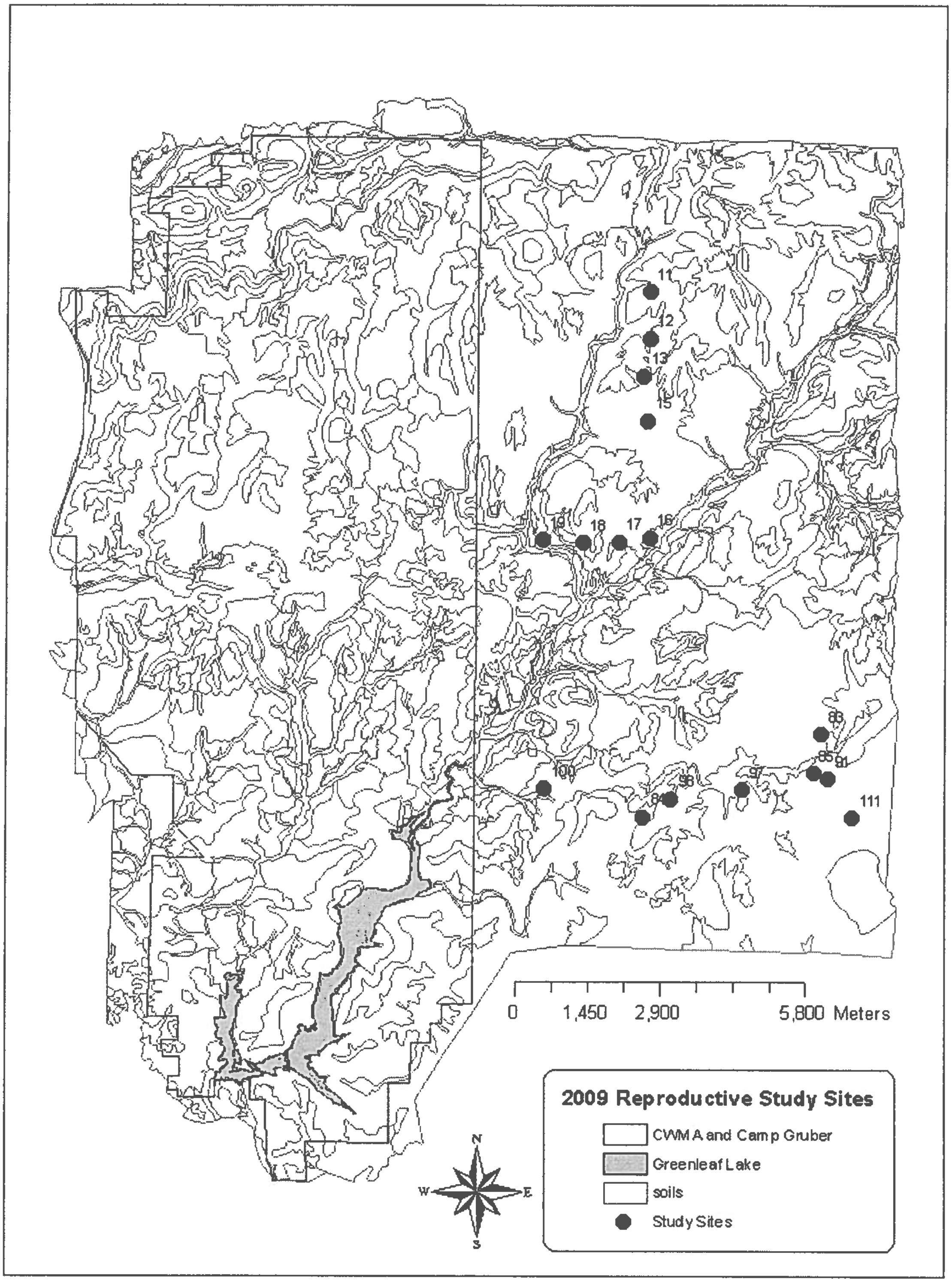

Figure 2. This map shows the locations of the study sites at Cherokee Wildlife Management Area, Cherokee County, Oklahoma and its proximity to Camp Gruber Army Training Site. Camp Gruber is the area west of the demarcation line. Note that lines are not topographic lines but are associated with soil types.

Figure 3. Adult *N. americanus* were marked with unique identifying bee tags and their elytra were clipped.

Figure 4. A randomly selected male and female *N. americanus* were placed on a rat carcass of known mass (grams) and covered with a five gallon plastic bucket to reduce scavenging. Holes were drilled in the sides of buckets to provide airflow. Photo by Dr. Craig Clifford.

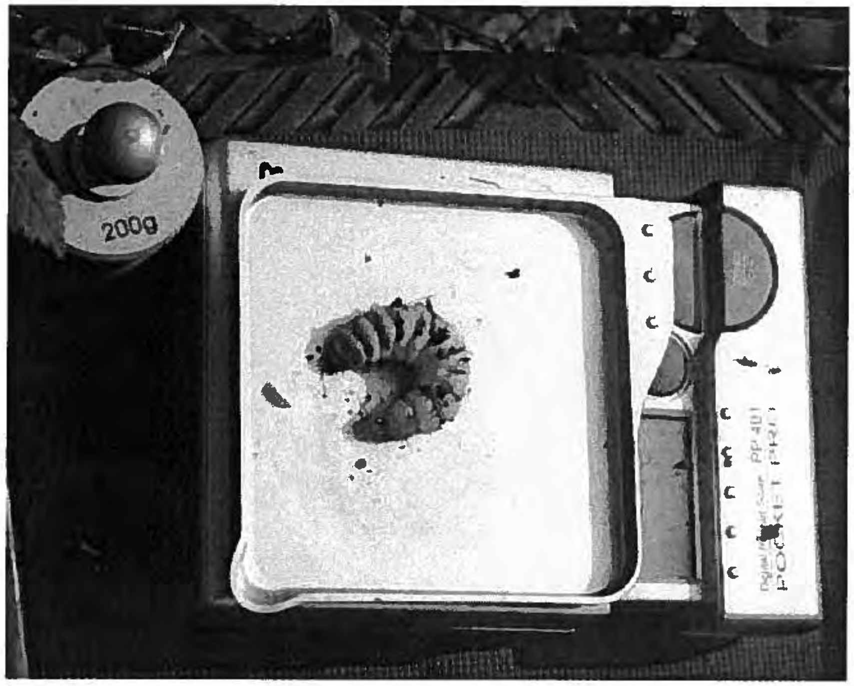

Figure 5. Larvae were weighed using the Pocket-Pro PP401 digital scale. Photo by Dr. Craig Clifford.

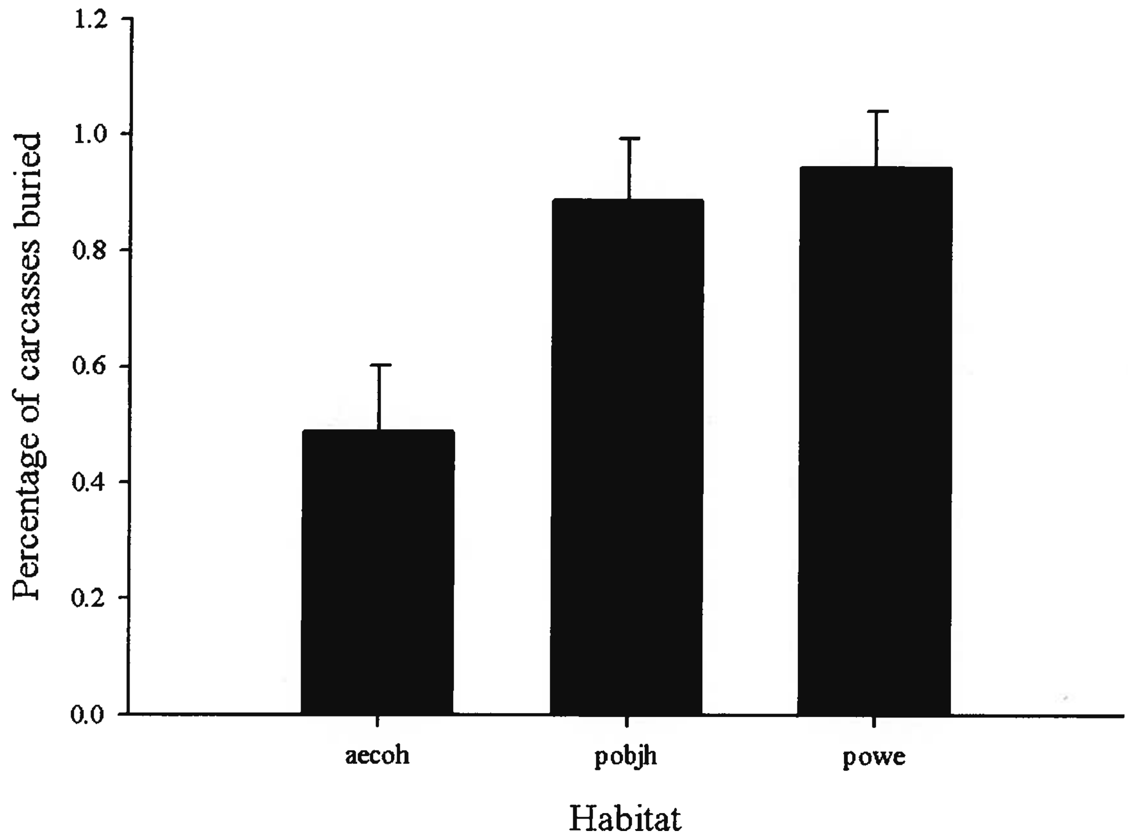

Figure 6. Burial of carcasses was much lower in the American elm-chestnut oak-hickory forest association (aecoh) than in the post oak-blackjack oak-hickory (pobjh) or post oak-winged elm association (powe). Error bars represent ± 2 standard errors of the mean.

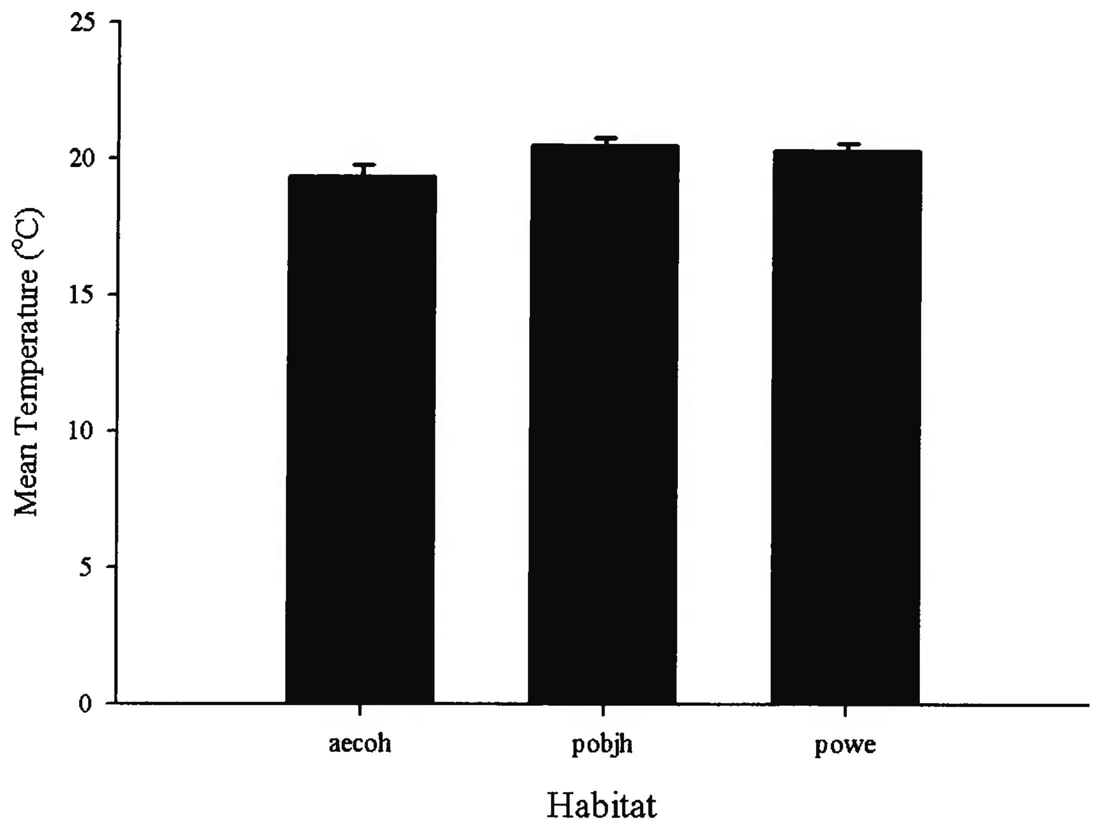

Figure 7. Temperature varied significantly with habitat. The habitat with the coolest temperatures was the American elm-chestnut oak-hickory forest association (aecoh). No significant difference in temperature was noted between the post oak-blackjack oak-hickory (pobjh) and post oak-winged elm association (powe). Error bars represent ± 2 standard errors of the mean.

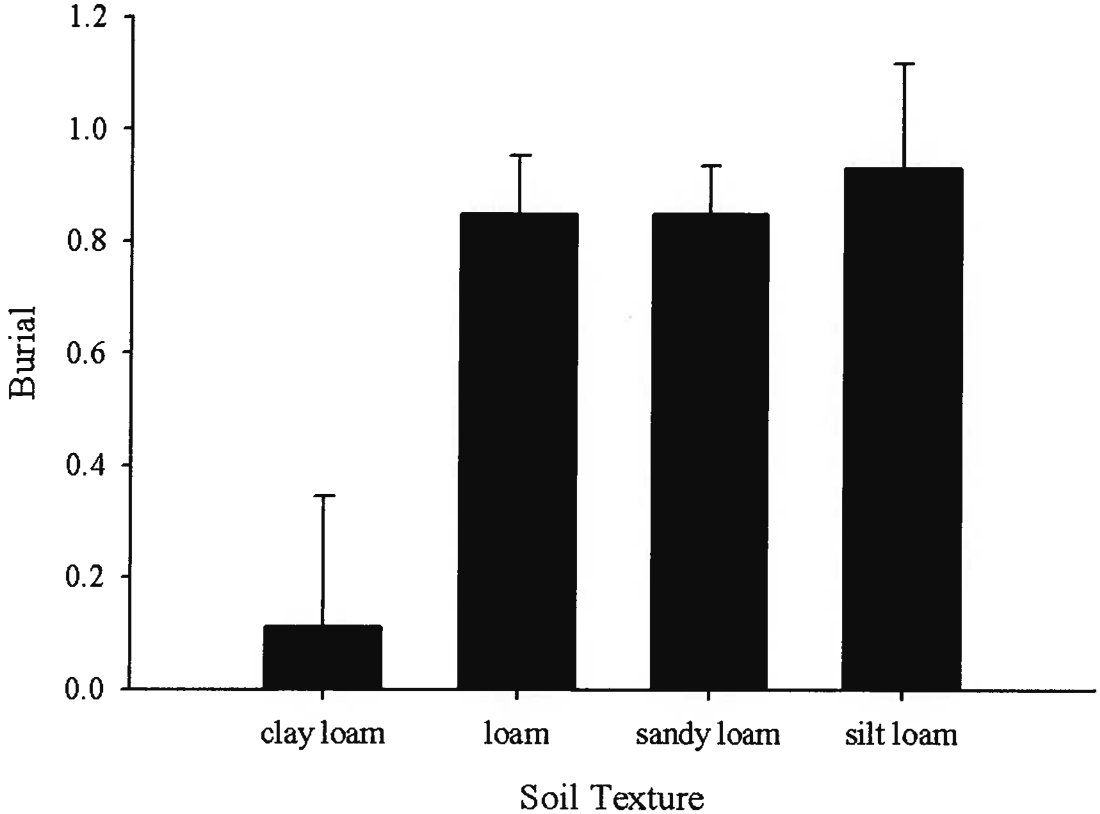

Figure 8. Burial of carcasses by *N. americanus* varied with soil texture. Burial was least likely in clay loam soils. The likelihood of burial did not vary significantly between loam, sandy loam, and silt loam. Error bars represent ± 2 standard errors of the mean.

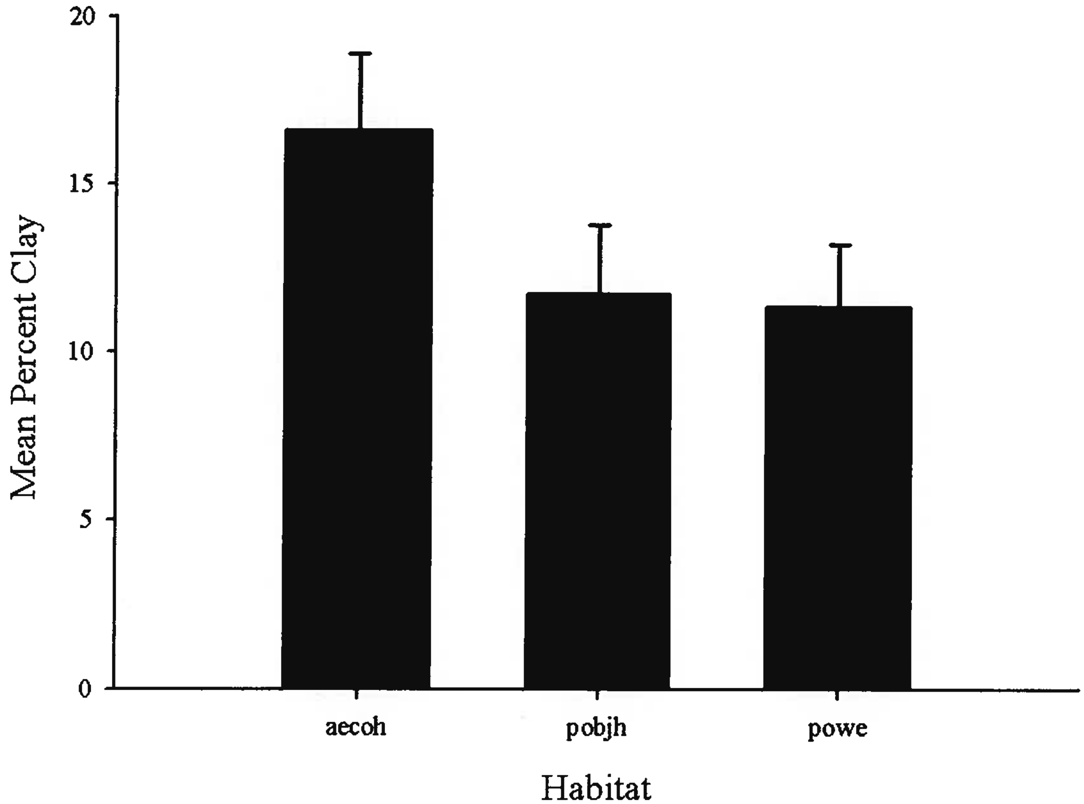

Figure 9. The clay content (percent) varied with habitat. The American elm-chestnut oak-hickory forest association (aecoh) had the greatest amount of clay. No significant difference in clay content was noted between the post oak-blackjack oak-hickory (pobjh) and post oak-winged elm association (powe). Error bars represent ± 2 standard errors of the mean.

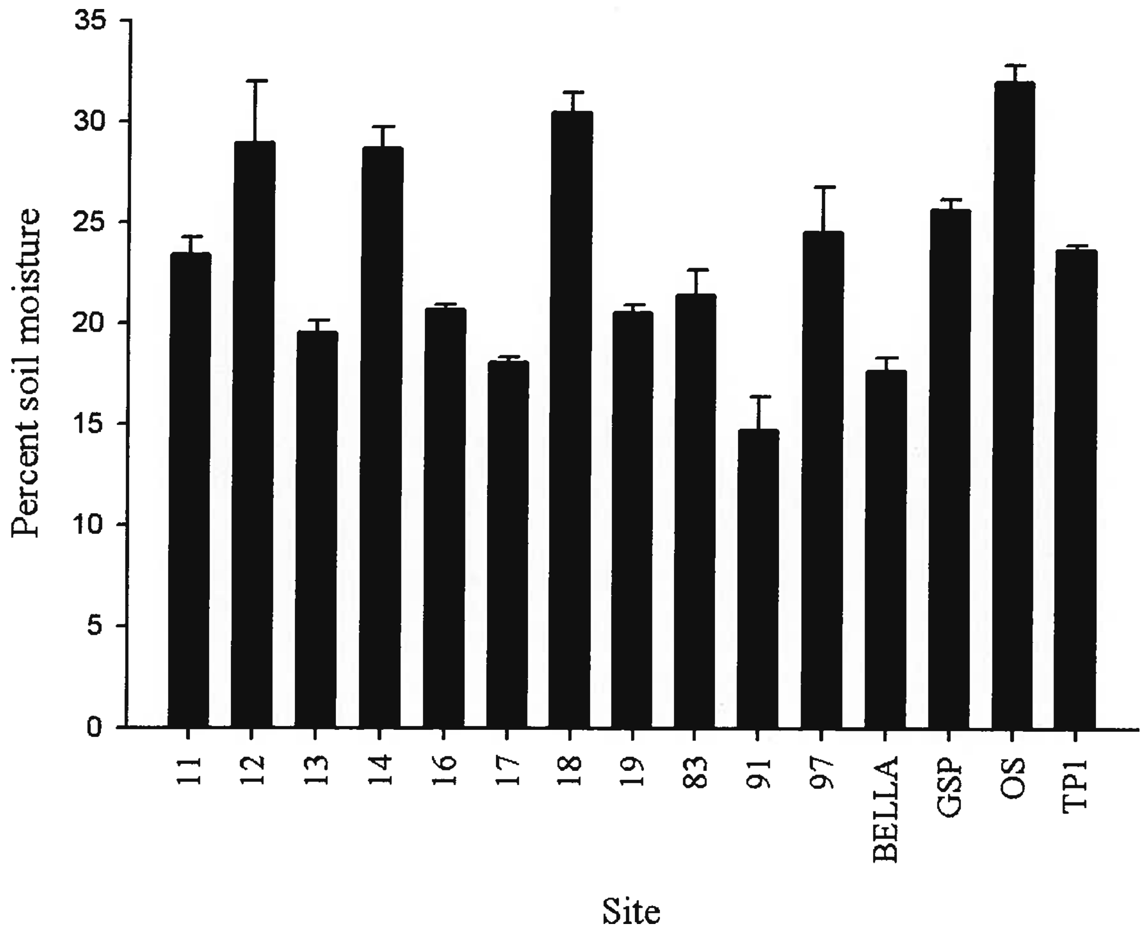

Figure 10. Soil moisture significantly varied among study sites. The greatest percentage of soil moisture was found at OS elm. The driest site was 91. Error bars represent ± 2 standard errors of the mean. Dead larvae were found at sites 11, 12, 13, 16, 18 and 19. The cause of larval death could not be attributed to soil moisture.

Figure 11. Forty-one larvae were found dead in standing water in chambers at sites 11, 12, 16, 13, 18 and 19. The adult in this picture was an *N. orbicollis*. We can not be sure at this time if the larva were *orbicollis* or *americanus*. However, *N. americanus* parents were placed with the carcass and later observed with the burial. Photo by Dr. Craig Clifford.

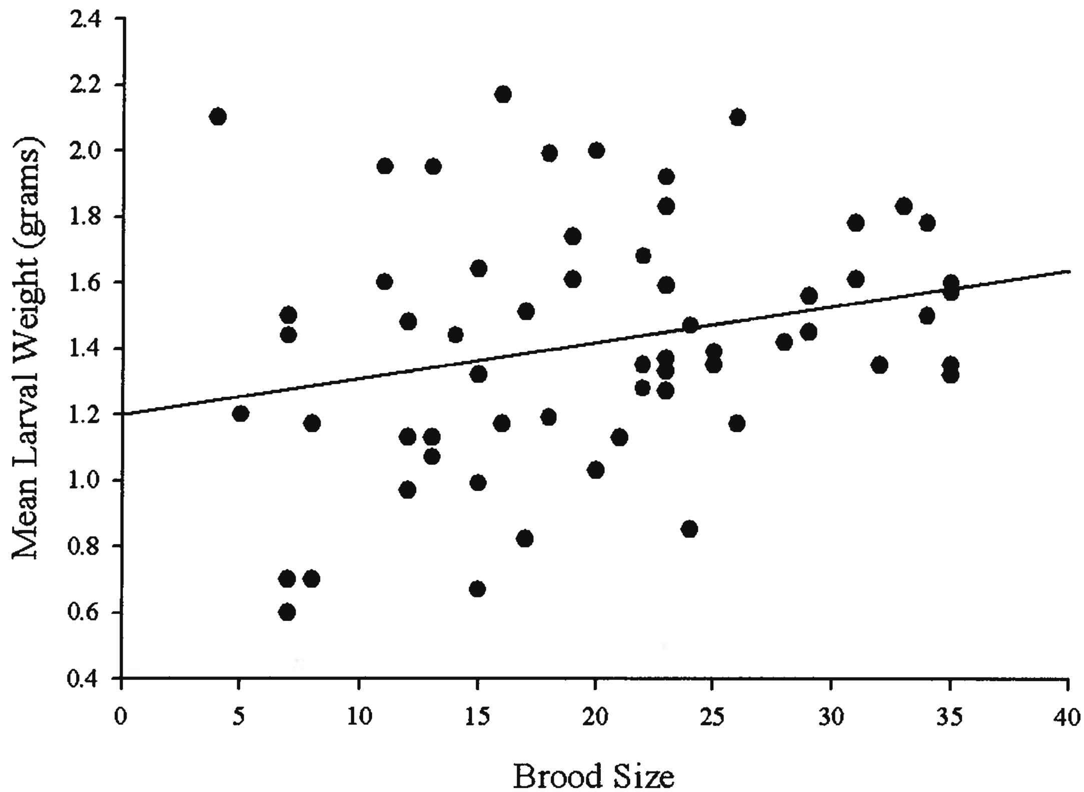

Figure 12. While not significant, there appears to be a linear trend between the size of larvae (mass) and the number of larvae in a brood. Larger brood sizes appear to contain larger larvae. This was not correlated with the size of the rat carcass used.

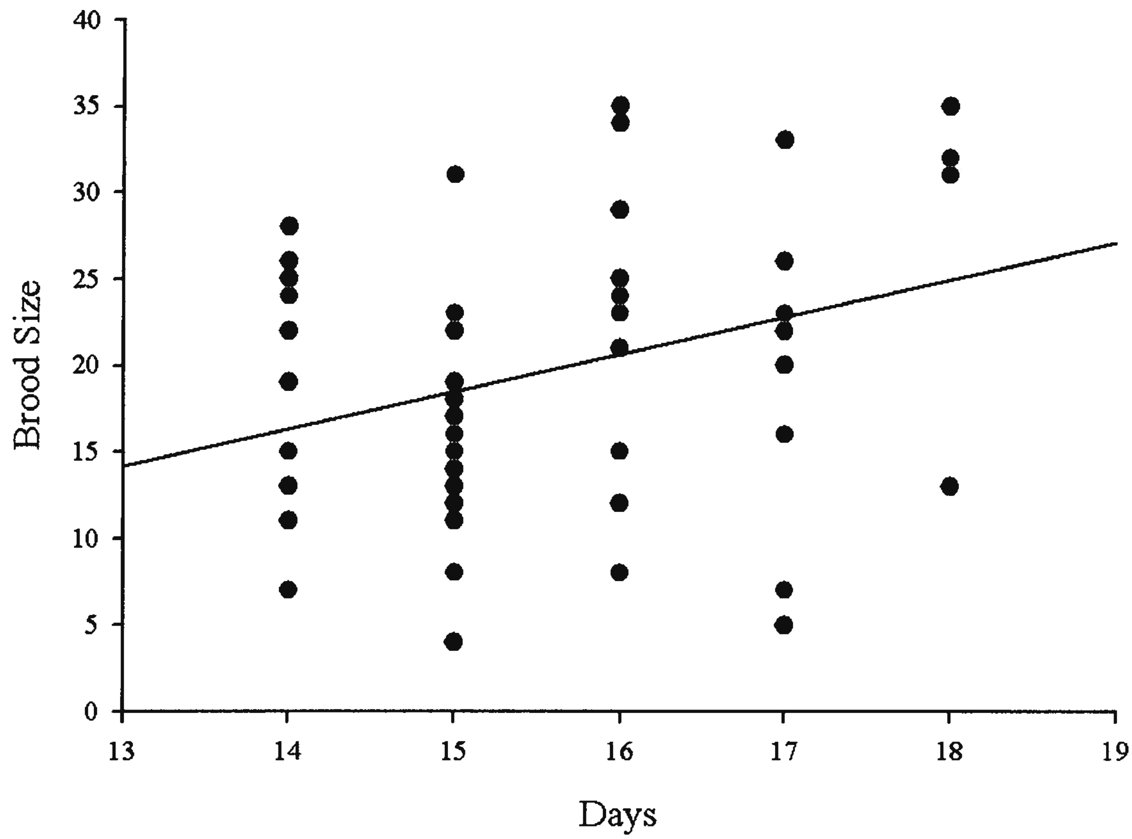

Figure 13. Brood size appears to increase linearly with increased time from the date carcasses were made available to the time of excavation.

Citations

Bishop. A. A., W. W. Hoback, M. Albrecht, K. M. Skinner. 2002. A comparison of an ecological model and GIS spatial analysis to describe niche partitioning amongst carrion beetles in Nebraska. *Transactions in GIS* 6(4); 457-470.

Creighton, J. C., C. C. Vaughn, and B. R. Chapman, 1993. Habitat preference of the endangered American burying beetle (*Nicrophorus americanus*) in Oklahoma. *Southwestern Naturalist* 38: 275-306.

Eggert, A. K. and J. K. Müller. 1997. Biparental care and social evolution in burying beetles: lessons from the larder. In The Evolution of Social Behavior in Insects and Arachnids (ed. By J. E. Choe and B. J. Crespi) pp216-236. Cambridge: Cambridge University Press.

Eggert, A. K., M. Reinking, and J. K. Müller. 1998. Parental care improves offspring survival and growth in burying beetles. *Animal Behaviour* 55:97-107.

Featherston, I. A., M. P. Scott, and J. F. A. Traniello. 1990. Parental care in burying beetles: the organization of male and female brood-care behavior. *Ethology* 85: 177-190.

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

Lomolino, M. V., J. C. Creighton, G. D. Schnell, and D. L. Certain. 1995. Ecology and conservation of the endangered American burying beetle (*Nicrophorus americanus*). *Conservation Biology* 9:605-614.

Koulianos S. and H. H. Schwarz. 2000. Probability of intra- and interspecific encounters, and the duration of parental care in *Nicrophorus investigator* (Coleoptera: Silphidae). *Ecology and Population Biology* 93(4): 836-840.

Nisimura, T, M. Kon, H. Numata. 2002. Bimodal life cycle of the burying beetle *Nicrophorus quadripunctatus* in relation to its summer reproductive diapause. *Ecological Entomology* 27: 220-227.

Rauter, C. M. and A. J. Moore. 1999. Do honest signaling models of offspring solicitation apply to insects? *Proceedings of the Royal Society of London, Series B* 226: 1691-1696.

Scott, M. P. 1998. The ecology and behavior of burying beetles. *Annual Review of Entomology* 43: 595-618.

Scott, M. P. and S. M. Williams. 1993. Comparative reproductive success of communally breeding burying beetles as assessed by PCR with randomly amplified polymorphic DNA. *Proceedings of the National Academy of Science* 90: 2242-2245.

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

Smiseth, P. T., C. T. Darwell, and A. J. Moore. 2003. Partial begging: an empirical model for the early evolution of offspring signaling. *Proceedings of the Royal Society of London, Series B* 270: 1773-1777.

Smiseth, P. T. and A. J. Moore. 2004. Behavioral dynamics between caring males and females in a beetle with facultative biparental care. *Behavioral Ecology* 15: 621-628.

Smiseth, P. T. and A. J. Moore. 2007. Signaling of hunger by senior and junior larvae in asynchronous broods of a burying beetle. *Animal Behaviour* 74: 699-705.

Smiseth, P. T., R .S. J. Ward, and A. J. Moore. 2006 Asynchronous hatching in *Nicrophorus vespilloides,* an insect in which parents provide food for their offspring. *Functional Ecology* 20: 151-156.

Smith, A. D. F. and C. W. Clifford. 2006. Examination of the reproductive dynamics of *Nicrophorus americanus* at Camp Gruber Army Training Site, Oklahoma. Final Report to the Department of the Army ARNG Training Site, Camp Gruber, Oklahoma Army National Guard.

Smith, A. D. F. and C. W. Clifford. 2007. Examination of the reproductive dynamics of *Nicrophorus americanus* at Camp Gruber Army Training Site, Oklahoma. Final Report to the Department of the Army ARNG Training Site, Camp Gruber, Oklahoma Army National Guard.

Smith, A. D. F. and C. W. Clifford. 2008. Examination of the reproductive dynamics of *Nicrophorus americanus* at Camp Gruber Army Training Site, Oklahoma. Final Report to the Department of the Army ARNG Training Site, Camp Gruber, Oklahoma Army National Guard.

Smith, A. D. F. and C. W. Clifford. 2008. Examination of the reproductive dynamics of *Nicrophorus americanus* at Cherokee Wildlife Management Area, Cherokee County, Oklahoma. Final Report to the Oklahoma Department of Wildlife Conservation.

Smith, R. J., A. Hines, S. Richmond, M. Merrick, A. Drew, and R. Fargo. 2000. Altitudinal variation in body size and population density of *Nicrophorus investigator* (Coleoptera: Silphidae). *Environmental Entomology* 29 (2): 290-298.

Trumbo, S. T. 1990. Reproductive success, phenology, and biogeography of burying beetles (Silphidae, Nicrophorus). *The American Midland Naturalist* 124:1-11.

U.S Fish and Wildlife Service. 1991. American Burying Beetle (*Nicrophorus americanus*) Recovery Plan. Newton Corner, Massachusetts. 80 pp.

Wilson, D. S. and J. Fudge. 1984. Burying beetles: intraspecific interactions and reproductive success in the field. *Ecological Entomology* 9: 195-203.

Wilson, D. S., W. G. Knollenberg, and J. Fudge. 1984. Species packing and temperature dependent competition among burying beetles (Silphidae, *Nicrophorus*). *Ecological Entomology* 9: 205-216.