United States Department of the Interior
FISH AND WILDLIFE SERVICE
Division of Ecological Services
9014 East 21st Street
Tulsa, Oklahoma 74129-1428
918/581-7458 / (FAX) 918/581-7467

American Burying Beetle *Nicrophorus Americanus* Survey Guidance for Oklahoma

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Introduction

The goal of this document is to provide guidance in designing and conducting presence/absence surveys for the ABB as a means of complying with section 7 and 9 of the Endangered Species Act (ESA). Section 9 of the Endangered Species Act (ESA) prohibits all persons from the taking of federally listed species. Take includes harming, harassing, or killing. Section 7 of the ESA requires federal agencies to consult with the Service if a project they authorize, fund, or carry out may adversely affect a federally listed species. Baited pitfall traps are the most effective method known for surveying for ABBs (Creighton *et al.*, 1993; Service, 1991; Bedick *et al*,. 2004). The below baited pitfall survey methodology has proven to be successful in safely capturing the ABB. Following this guidance should help to ensure the validity of survey results. Implementing other survey methods, not recommend by the Service, may not result in confidence of survey results. Due to the ABBs life history, false negative survey results can occur. Further, data gathered using this guidance will allow for comparison of results between surveys. Surveys for ABBs that are conducted for research purposes are beyond the scope of this guidance and should be coordinated with the Service.

Time for Surveys

As a means of determining the presence or absence of ABBs, the Service recommends surveys be conducted during the ABBs primary active season, which is between May 20 and September 20 in Oklahoma. Surveys should begin no earlier than May 20 and end no later than September 17 in Oklahoma (dates may vary among states and will need to be verified for each state). Additional nights of survey are needed for each night during a survey effort where the night-time ambient temperature falls below 60 degrees Fahrenheit [ºF] and when rain events deposit ½ inch or more during 7 pm to 7 am.

The ABB's nocturnal activity and trapping success decreases or is absent when temperatures drop below 60 (Bedick *et al*. 1999, Kozol 1991). Further, ABB nocturnal activity is shown to be delayed when temperatures are greater than 75ºF (Bedick *et al*. 1999).

The determination of the ABB's activity period in Oklahoma is based on the past eleven years of weather data in eastern Oklahoma (Oklahoma Mesonet) where nighttime ambient temperatures are consistently above 60ºF. Mesonet data from the Tahlequah and Broken Bow stations were analyzed. May 20 is typically the day commencing the onset of a five day period were nighttime ambient temperatures remain 60ºF or higher, and September 20 is the first day of a five day period with temperatures below 60ºF. There is the potential that the temperature will drop below 60ºF for a night or two, but typically not for five consecutive nights or more.

Timing

Although the capture rates of ABBs are known to be higher during certain dates during their prime active period, May 20 and September 20, the Service is not recommending restricting survey implementation to specific dates during this prime active period.

Both Bedick *et al.* (2004) and the U.S. Fish and Wildlife Service (Service) (1991) reported an increase in capture rates during certain times during the ABBs active season and during the ABBs nightly activity. Capture rates for ABBs are highest from mid-June to mid-July and again in mid-August. Bedick *et al.* (2004) reported that a peak in capture success also occurred in mid-August. In Nebraska, Bedick et al (1999) reported two peak activity periods. One in late June and early July when ABB are most actively searching for carrion, and in late August and early September when teneral ABBs

emerge.

Surveying during the above two peak timeframes would result in the greatest potential for trapping success and the Service would prefer that surveys be conducted during these periods. However, we understand that limiting surveys to these time periods is too restrictive and therefore conducting surveys during these time periods is not a requirement. Further, this survey protocol is only a method to determine presence/absence and not an abundance or population survey.

Timeframe a Survey is Valid
Survey results are only valid for one calendar year from the last date of the survey effort. This determination is based on the fluctuating nature of ABB populations. Data indicates that ABBs likely have moved and numbers have fluctuated within a given area from year to year.

Much of the long-term information concerning the life history of the ABB pertinent to Oklahoma has come from work done at Fort Chaffee, Arkansas; McAlester Army Ammunition Plant (McAlester), Oklahoma; and Camp Gruber, Oklahoma. While the land use at Fort Chaffee, McAlester, and Camp Gruber is different among each entity, each entity maintains a relatively consistent land use pattern of its own through time. However, Hiott and Schnell (2003) reported the number of ABBs captured and the location of high density ABB concentrations typically varies annually or biennially at each site. This trend indicates ABBs are cyclic, where there are high numbers and abundance for one or two years, followed by a decline in numbers the following year or two, and repetition of the cycle over time. In addition, each year they reported that the high concentration areas of ABBs appeared to shift annually throughout the sites. Further, the ABB is an annual species (living for only one year) and the following year's numbers are dependent upon the reproductive success of the previous year.

False negatives are possible outcomes of with ABB surveys. The Service (1991) reported that during late July ABBs were easy to attract to carrion bait but were difficult to capture in pitfall traps. Standard transects on Camp Gruber that resulted in ABB captures in one year showed no capture of ABBs in another year. Other surveys conducted in a given area of Oklahoma have resulted in ABB captures during one survey but surveys conducted in the same given area and the same active season have resulted in negative ABB captures.

Survey effort radius
A survey is valid for a 5 mile radius. Considering ABBs mobility, small size, recorded movement distances, and distance from which they can detect carrion, a presence/absence survey was effective only over 5 miles and that this erred on the side of the species.

An ABB may move as little as 0.15 mile per night to a maximum of 6.2 miles in 6 nights (1.03 miles per night), with a mean movement distance of 0.52 mile per night (Schnell and Hiott 1995, 2003). Bedick *et al.* (2004) reported average nightly movements of 0.62 mile with the large proportion (85%) of recaptures moving distances of 0.31 mile per night. Creighton *et al.* (1992) reported that individual ABBs moved over 4 miles in only a few days. Creighton and Schnell (1998) reported an average nightly movement of 0.76 mile, and maximum distances of four miles in five nights (0.8 mile per night) and 6.2 miles in 6 nights (1.03 miles per night), a minimum distance of 0.15 mile in one night, and a mean nightly movement of 1.67 miles.

While this data could be interpreted to imply that an ABB could move 95 miles [0.62 (mean nightly movement) X 154 days (May 20 – September 20)] during the active season, the Service does not believe this is an accurate interpretation. Mark and recapture data at Camp Gruber and Fort Chaffee did not find any ABBs that moved between these installations, a distance of about 54 miles (Schnell and Hiott, 1997-2003). Even if ABBs moved such long distances, the Service assumes it is unlikely ABBs move in such a consistently linear direction.

The group of ABBs at Camp Gruber has persisted for at least 11 years. While the numbers and high density areas of ABBs have changed annually or biennially, indicating ABBs are typically a cyclic species (Schnell and Hiott, 2003), they appear to be self-sustaining. The Service assumed it was not likely that all, the majority, or even a single ABB on these installations moved from 54 to 95 miles away from Camp Gruber. Further, for a species to survive it cannot exert more energy than it consumes and would only expend the minimal amount of energy needed to secure necessary resources.

Minimum Survey Effort

To effectively determine presence or absence of ABBs in a given area, surveys need to be conducted for a minimum of 3 consecutive nights [or 24 trapnights, 8 traps (or 1 transect) x 3 nights = 24 trapnights] not hampered by rain or temperature as described below. The effective radius of a transect is 0.5 miles. So to effectively survey a particular area, transects should be spaced at 0.5 mile increments across the project area until the entire project area is within the effective trapping area of at least one transect. Each transect deployed should be open for 3 consecutive nights. Keep in mind that a transect does not have to be linear.

For projects with less than a 0.5 mile radius from the center to any point along the perimeter, a minimum of 1 complete transect (8 traps spaced 66 feet apart) is required. One transect with 8 traps for 3 nights results in 24 trapnights (the number of traps times the number of survey nights). Again, the placement of the transect does not need to be linear

Based on ABB movement data described above, the effective trapping area of a transect is a 0.5 mile radius. Creighton *et al.* (1993) and the Service (1991) recommend surveying for a minimum of 3 consecutive nights to adequately determine the presence or absence of ABBs in an area. Bedick *et al.* (2004) recommends a range of 3 to 5 nights, preferably 5 nights. However, after 3 consecutive nights of trapping, surveyor observations report trapping success can decline. If ABBs have not been captured within the 3 consecutive nights of surveying then ABBs are unlikely, although not impossible, to be captured (Personal communication with Ana Hiott, 2003) with additional trapnights.

Minimum project acreage

According to our 2005 analysis of ABB density estimates, projects which disturb less than 1.2 acres would have, on average, no more than a one percent chance of impacting an individual ABB. In addition, the Service evaluates numerous other factors including the project: type, construction/installation duration, permanency, location, land use, implementation methods, habitat, and time/season.

Using recently collected survey data, the Service's Oklahoma Ecological Services Field Office derived densities of ABBs in their known range within Oklahoma. We used all known survey records to determine the total number of ABBs captured and total transects deployed per survey. In using this information, we assumed all captures were obtained using the methodology described by Creighton, *et al*. (1993) unless stated otherwise. Data that were not collected using the Creighton *et al*. (1993) methodology were excluded from the analysis.

The Service then estimated the area each transect would effectively trap. Creighton, *et al*. (1993) determined, based on known movements of ABBs, that transects do not need to be spaced any closer than 0.5 miles. Past and ongoing research demonstrates this trapping recommendation is still appropriate. Schnell and Hiott (1997-2003) annually determined the average nightly movements of the ABB, using marked individuals over a nine-year period at Camp Gruber to be 0.62 miles. The smallest average nightly movement for any given active season over that same period was 0.52 miles. Consequently, we believe each transect effectively traps beetles from an area of 0.5 mile. As such, the effective trapping area (ETA) for one transect is about 153.5 acres. Using the ETA and number of ABBs collected, we estimated average ABB densities to be 0.0084 ABBs/acre for their known range in Oklahoma. The Service then used a standard z test to determine the probability of encountering an individual ABB in a given area. We determined that disturbance of less than 1.2 acres would have, on average, no more than a one percent chance of impacting an individual ABB.

Weather Requirements

An additional night of surveying is required when the temperature falls below 60ºF between 7:00 PM and 7:00 AM and/or when rainfall greater than ½ inch occurs between 7:00 PM and 7:00 AM. All weather data for surveys should be collected using the Oklahoma Mesonet website, www.mesonet.org. Directions for using the Oklahoma Mesonet website are provided below. All additional nights of surveys conducted due to weather need to be specified in the "*ABB Survey Data Collection Form*" and the "*ABB Survey Summary Report*".

Bedick et al. (1999) reported the highest number of ABB captures 3 to 4 hours after sunset. However, captures occurred from 1 to 11 hours after sunset during this study. In Oklahoma, sunset times range from 7:18 PM to 8:44 PM between May 20 and September 20. Based on Bedick *et al*.'s (1999) findings, the peak timeframe for ABB captures in Oklahoma would be from 10:18 PM to 12:18 PM and the nightly timeframe of ABB activity would be from 10:18 PM to 7:24 AM.

This is just one study and the study site was in Nebraska, so to err on the side of the species, the Service has determined that the nighttime active period for ABBs to be between 7:00 PM to 7:00 AM in Oklahoma. The Service also considered the limiting factors of this type of survey effort, specifically we considered the fact that this type of survey provides only the presence or absence data of the ABB in a given area and that the minimum duration of the survey effort is 3 nights. To err on the side of the species, all feasible variables that could result in a false negative survey need to be eliminated if possible. Temperature and rainfall are variables that can be easily monitored and adjusted for.

Oklahoma Mesonet Instructions:

1. At www.mesonet.org, click on "Past Data and Files" under "Mesonet Data" (Left side of the screen)
2. Click on the Mesonet station closest to the survey site. Then under the "Station Monthly Summary" heading select the month and year of the survey. Then click "Get summary."

Temperature:

- In "Summary Report" of this Mesonet page, find the date of the survey. Daily Mesonet data is measured from midnight to midnight, so if traps are set on June 3rd, temperature data from both the 3rd and the 4th will be needed to address the entire trapping night, which is between 7:00 PM to 7:00 AM, and because the nightly low temperature most often occur past midnight.

Rainfall:

- In "Summary Report" of this Mesonet page, find the date of the survey. Once again, if the traps are set on June 3rd, Mesonet data from the both the 3rd and 4th need to be reviewed to address the entire trapping night, which is between 7:00 PM to 7:00 AM. Rainfall over ½ inch during a trap night requires further analysis and reporting. The time of the rainfall needs to be determined. This Mesonet page only reports the entire rainfall that occurred in a 24 hour period (midnight to midnight). To determine when during a 24 hour period rainfall events occurred and how much rain fell, proceed to the paragraph below for instructions to navigate through Mesonet to the proper page.

- Click on "Past Data and Files". Then under the heading "Data Files" on the bottom right of screen click on "Mesonet MTS Files". Then click on the relevant date. Then select the Mesonet station nearest to the survey area. Rain totals are given in 5 minute increments on this Mesonet page. Remember that daily Mesonet data is provided for a 24 hour period, beginning at 12:00 AM and ending at 12:00 AM. So the date the traps were set and the following date need to be reviewed to determine the rainfall for the entire trapnight.

Transect spacing

Transects should be spaced a minimum of 0.5 mile apart on small to medium sized projects and a maximum of 1 mile apart on large projects to achieve reliable survey results. The Service defines large projects as those that are over 20 square miles or over 20 linear miles.

Bedick *et al.* (2004) recommended that 0.31 mile be used as a minimum transect spacing for traps with large bait. Creighton *et al.* (1992) concluded that transects less than 0.5 mile apart were not required. In large sample areas, Creighton *et al.* (1992) stipulated that transects can be located as much as 1 mile apart. The Service's rationale considered these recommendations and the ABB movement data discussed previously in determining an effective transect spacing.

Transect Placement

Transects should be placed in a configuration that best represents the different habitat types present in the survey area and on the highest spots in the survey area. Transects are not limited to linear arrangements, they can be aligned to suit the shape of the project area for which you are surveying. For example, if a project is kidney bean shaped, transects can be curved to fit a kidney bean shape. However, transect spacing of 0.5 mile needs to be followed.

American burying beetles are feeding habitat generalists (Creighton *et al*, 1993). Consequently, it is recommended that transects be placed in all the different habitat types present in a survey area. Bedick *et al.* (2004) reported ABBs were

significantly more attracted to traps placed on ridges than those placed in valleys. They believed that increased ABB captures on ridge tops may be a result of increased odor movement, thus increasing the possibility of detection by ABBs.

Ants

Traps should not be placed within 23 feet (7 meters) of ant hills. If ants are discovered in a trap it should be relocated at least 23 feet away. Ants can swarm and kill an ABB that is trapped in a pitfall trap (Creighton *et al,*. 1993 and Bedick *et al,*. 2004).

Transect

A transect is defined as 8 pitfall traps, spaced 66 feet (20 meters) apart (Figure 1), for a total length of 460 feet (140 meters). This definition is based on the transect protocol described in Creighton *et al.* (1993) and Bedick *et al.* (2004) which all utilize or recommend the same type of transect. Transects are not limited to linear arrangements, they can be aligned to suit the shape of the project area for which you are surveying. For example, if a project is kidney bean shaped, transects can be curved to fit a kidney bean shape. However, the above described quantity of traps per transect and the spatial arrangement (traps 66 feet apart) of traps must be followed.

Traps

The Service recommends using baited pitfall traps for ABB presence/absence surveys. Each pitfall trap consists of a trap cup, bait cup, wire, and cover. The traps are designed to utilize carrion to attract ABBs, keep captured ABBs alive, minimize bait and ABB contact, and prevent ABBs from escaping until traps are checked. The ABB flies toward the bait odor, lands near the trap, and crawls under the cover and into the trap cup. Once in the trap, the slick sides of the cup prevent the ABB from crawling out, and the cover and suspended bait cup keep the ABB from flying out of the trap cup. Figures 2 and 3 display the baited pitfall trap setup. If the utilization of other trap design and equipment is proposed this must be coordinated with and approved by the Service.

Traps in the form of 24 oz (0.7 L) plastic cups (similar to Solo cups) have proven effective. The Service recommends that the trap cup consist of two 24-oz Solo cups. Cups must have smooth sides, free of any texture or ridges to prevent ABBs from climbing out. American burying beetles can climb a surface with textured/ridged sides but cannot climb a smooth, slick surface. The 2 cups are stacked together and placed in an appropriate sized hole in the ground. Stacking the cups one inside the other facilitates removal of trapped insects. The top cup can easily be pulled out and replaced while the second cup remains in the ground to maintain the integrity of the hole. The lip of the trap cups should be 0.5 to 0.75 inch above ground level. This prevents water runoff from filling the cup. The cup should not exceed 0.75 inches from the ground because this could prohibit ABBs from being able to crawl over the lip and into the trap. A 1 to 2 inch squared piece of wetted sponge soil should be placed in the bottom of the trap cups. This has been shown to decrease mortality by desiccation of ABBs and providing a floatation device should the cup be inundated with water.

The bait cup consists of a plastic cup about 5 – 6 ounces (20 milliliters) in size. Examples of bait cup types that have proven effective are cups similar to those used in restaurants for carry out of salad dressing or styro-foam coffee cups with the top portion cut off leaving only the bottom 1 inch of the cup. The type of bait cup used is not as important as the trap cup; however the size of the bait cup is important. The bait cup should be large enough to hold the proper amount of bait (described below) but small enough to be suspended over the trap cups and still allow ABBs to crawl into the trap cup. Bait suspended over the pitfall trap via skewer or similar device is not recommended. The bait dries out and the odor emitted is greatly weakened.

Wire is used to secure and suspend the bait cup over the trap cup. To accomplish this, the wire needs to be hand malleable but sturdy enough to support a full bait cup. The wire is inserted into one side of the bait cup near the top and pushed all the way through the cup and out the other side of the cup; about 3 inches of wire should extend on either side of the exterior of the bait cup. Bend wire down and push into the ground to suspend and secure the bait cup over the trap cup. The reason for separate bait and trap cups is to protect the ABB from coming in contact with the bait. As the bait liquefies or becomes gummy ABBs could be harmed if they come into contact with the bait.

The cover should be hard, not transparent or opaque, weighted or secured to the ground, at least 6 inches in depth, at least 12 inches in diameter at its widest point, and raised off the ground about 1 to 2 inches. A hard, plastic dome structure has proven effective at protecting the trap (e.g. inverting nursery plant containers with holes on the side, or small gray paint

buckets). A hard structure, secured to the ground is needed to prevent scavenger and rainfall from accessing the trap cup, and to provide shade to captured insects to avoid desiccation. Scavengers absconding with bait is common and potentially results in harm to ABBs. In addition, bait loss during trapping reduces the effectiveness of the trapping effort and therefore ABB capture success. The cover structure should be raised off the ground about 1 inch or holes should be cut along the lip of the container flush to the ground to allow ABBs to crawl into the trap. At least 40% of the lip should be open to allow ABBs access to trap. In lieu of a plastic dome structure, other similar structures can be used. For example, a piece of wood raised on legs can be used. However, all covers must be secured to the ground. Shingles or other such flimsy materials are not to be used.

In areas where scavengers are a significant problem wire mesh can be installed between the pitfall trap and the cover (Appe). The piece of wire mesh should be a minimum of 6 inches squared and mesh size should be at least 1 inch to allow ABBs access to trap but prevent larger animals from stealing the bait. The wire mesh should be secured to the ground with stakes, and a hard cover will still need to be used.

Exposure to full sunlight and temperatures over 77ºF for even a few hours can result in ABB mortality (Service, 1991). According to Bedick *et al.* (2004) wetted soil in the bottom of the trap helped maintain high humidity. This improved the longevity of ABBs and therefore decreased the mortality of ABBs from desiccation. They found this was feasible to use with stacked cup traps. However, saturated soil in the bottom of a trap cup can also pose a threat to ABBs. The Service (2008) found that saturated soil can result in mortality of ABBs through clogging of spiracles or drowning. Other permittees have used a 1 to 2 inch squared piece of wetted sponge in the bottom of traps. The Service recommends utilization of wetted sponges. Soil can still be placed in the bottom of the trap cup; however, this soil should not be wetted.

Bedick *et al.* (2004) found the primary cause of ABB mortality was drowning due to traps flooding with water. However, on multiple occasions, floating and apparently dead beetles were removed from traps, and they subsequently recovered after 10 to 20 minutes. Consequently, ABBs that appear dead or are lethargic should be held for at least 20 minutes to determine actual condition. Monitored ABBs should be placed in a roomy, open container, with air circulation, out of direct sunlight.

Bedick *et al.* (2004) evaluated six types of pitfall traps for capturing ABBs ranging from stacked cups, to PVC pipe, to buckets. They determined buckets with a dome cover had the greatest trapping success because it allowed for the largest piece of bait. However, they realized that this was not the most practical or economically feasible method to implement. They rated stacked cups as the second best trapping method. Creighton *et al.* (1993) also determined that stacked cups as baited pitfall traps proved effective at capturing ABBs and recommended this method.

Creighton *et al*. (1993) recommended using traps that are smooth, free from any bump or ridge to prevent ABBs from climbing out of the trap. Metal cans should be avoided because as soon as any rust appears, ABBs may be able climb out of the trap (Service, 1991). Creighton *et al.* (2004) also recommended the lip of the cup be flush with the ground. However, Bedick et al. (2004) and Hiott (2002 personal communication) recommend the lip of the pitfall cup trap be a 0.5 to 1 inch above the ground surface to prevent water runoff from filling up the trap. Bedick *et al.* (2004) took additional measures by building a berm around the raised portion of the cup.

Creighton *et al*. (1993) and Bedick *et al.* (2004) recommend suspending bait cups over trap cups to lure ABBs, and prevent contact between ABBs and the bait. While carrion beetles are seemingly well-adapted to moving around in carrion, Bedick *et al.* (2004) found that under some conditions spiracle blocking could occur. Bait placed in a small container with a water-tight bottom can decay into a near liquid state. Bait in this liquid form could adhere to ABBs, harden, and then clog spiracles. They did not think this was a common occurrence but could potentially occur.

Bedick *et al.* (2004) recommends using weighted, hard plastic rain-shields larger than the opening of the trap cup to protect the trap from rain. The cover over the pitfall trap should be secured to the ground to effectively protect the trap from rainfall and predators (Creighton *et al.*, 1993). Bedick *et al*. (2004) also recommended using wire mesh secured to the ground between the trap and the cover (Figure 4) to prevent scavenge of bait. Mesh was secured to the ground with three to four bent wire stakes, 4 inches (10 cm) long, driven into the ground. Covers were raised between 1-2 inches above the ground to allow ABBs access to trap.

Bait

Any type of carrion is suitable for use as bait for ABB pitfall traps. However, un-skinned chicken is preferred by multiple surveyors because it is inexpensive and remains moist longer than other baits due to the fat content of the skin. Others have successfully used liver, gizzard, or road kill. The only requirements are that the bait must be the appropriate size in correlation with trap size and must produce a pungent odor that ABBs are able to detect. The appropriate size of bait for stacked cup traps is between 0.5 to 0.7 ounces (15-20 grams). The bait must be rotten and emit a pungent odor to be effective. Adding a small amount of water to the bait cup is effective at prolonging the moisture content and odor. During trapping efforts any bait that has dried out or no longer emits a pungent odor must be replaced with new bait.

Fresh bait is not an attractant to ABBs (Creighton *et al.,* 1993). To prepare bait for use, cut into cubes about 0.5-0.7 ounces (15-20 grams) in size. Place the cubes into a sealed container or bag. Do not fill the container or bag completely full, as the bait rots gas pressure is increased and the extra room is need for this expansion. Place the container or bag in the sun for a minimum of one day (Creighton *et al.*, 1993). If the day is relatively cool (less than 85ºF [29ºC]), the bait should stay in the sun longer.

Once bait is prepared, the packaged (container or bag) bait should then be further enclosed in a larger sealed container, such as an ice chest or bucket with lid. The bait should be used within the next few days. Use of the larger sealed container prevents odor from escaping into unwanted areas and is ideal for transporting bait. The Service recommends that the bait or any containers holding bait not be placed inside a vehicle. You will never get the smell out of your vehicle! Instead, place the containers in the bed of a pick-up or secure to outside of vehicle. In addition, discarded or old bait should not be left at or near the current trapping area. This could lure ABBs away from the baited pitfall traps.

Bedick *et al.* (2004) reported no significant difference between capture rates of ABBs using various types of bait (Appendix 1). However, they did find a significant difference between the sizes of bait used and the number of ABBs captured. A large piece of bait positively correlated with an increase in the number of ABBs captured. Bedick *et al.* (2004) recommended that bait of 7 ounces (200 grams) be used. This amount of bait is feasible if a bucket trap is used but not if a cup is used. Creighton *et al.* (1993) and Service (1991) recommend that 0.5-0.7 ounces (15-20 grams) of bait be used.

Setting and Checking Traps

Each trap must be checked by 10:00 A.M. each morning. Checking traps entails: collecting all trapped ABBs, recording and releasing other *Nicrophorus* species; replacing any missing or dry bait, re-moistening sponge in trap cup if needed, replacing floatation device if needed, and replacing/resituating any disturbed parts of the trap.

Any injured or lethargic ABBs should be released immediately. ABBs that appear to be dead should be collected and monitored for at least 20 minutes, as described below under ***Processing ABBs***, to accurately determine their condition. Any dead ABBs should be handled as described below under ***Accidental Death of ABBs***.

The Service recommends using a 5-gallon bucket or similar container to carry the needed equipment used during trap checking. The items you will need to carry include: a container to hold ABBs, prepared bait, water, a trowel for digging, tongs and the data sheet. Use a hard, plastic container with a lid and air holes to hold ABBs collected along a transect. Carry a small container of prepared bait (and tongs to handle bait) to replace missing or dried bait. Carry a 20 oz plastic bottle of water to re-moisten sponge in the bottom of the cup trap, and the bait. Replace or re-situate any disturbed traps (this is where you may need the trowel to re-dig the hole for the trap). Record the species and number of any other *Nicrophorus* captured and then immediately release. The other *Nicrophorus* species should be released several feet away from a transect to avoid being crushed by foot traffic. Once all the traps along a transect have been checked, proceed in processing all the captured ABBs either at the end of the transect or at the vehicle. If processing occurs at the vehicle, the vehicle must be within 500 feet of the transect. This is to minimize handling time and for ABBs to be released in the area they were captured.

All traps must be in place and baited by 5:00 P.M. each night. Traps should be cleared of ABBs by 10:00 A.M. every day. Exposure to full sunlight and temperatures over 77ºF (25ºC) for even a few hours can result in ABB mortality (Service 1991). Traps can be baited at the same time they are checked each morning provided the bait does not dry out.

Since ABBs are nocturnal, there is not a risk of baited traps capturing ABBs during the day.

Disturbed bait or traps

Additional nights of trapping will need to be implemented if 4 or more traps and/or bait are disturbed, and no ABBs have been captured during a 3 night survey period. Predators or scavengers can cause bait loss and/or trap disturbance during ABB surveys. This can affect the results of the trapping effort. Disturbed traps or traps missing bait, reduces or eliminates the effectiveness of attracting and/or containing ABBs and therefore ABB capture success. Any negative results from these traps are not reliable. The Service calculated that a trap disturbance percentage of 20 was acceptable and still allowed for surveys that provided valuable data results.

The specific number of additional nights of trapping that will be needed is dependent on the number of traps and/or bait disturbed. The table below outlines the amount of additional trapping needed. Transects in which ABB have been captured, regardless of whether or not any traps have been disturbed or are missing bait, do not require additional nights of trapping since ABB have been confirmed detecting and finding baited pitfall traps along a transect.

# of traps &/or bait disturbed	# of additional nights to survey
0-4	0 additional trapping needed
4-8	1 transect for 1 night
9-16	2 transect for 1 night
17-24	3 transect for 1 night

All trap disturbances must be recorded on the "*ABB Survey Data Form*". There are specific sections on this form pertaining to bait disturbance and any additional nights of survey required. In addition, the amount of disturbed bait and/or traps needs to be summarized in the "*ABB Summary Report*".

Processing ABBs

Processing includes sexing, aging, taking measurements, marking (if authorized) and data recording.

Captured ABBs should only be held for a maximum of 30 minutes, preferably much less than this. ABBs held for longer than 10 minutes should be placed in a hard, plastic container with a damp sponge and then the container placed in an iced cooler. ABBs are sensitive to prolonged heat exposure.

An efficient method to process ABBs is to collect all the ABBs from all 8 traps along a transect and then process all the ABBs at one time. Processing of ABBs can then be completed at the end of the transect or at the vehicle. Multiple permittees find processing ABBs at their vehicle facilitates the task. This eliminates the need to carry all of the equipment during trap checking, quicker processing of ABBs, availability of a cooler if needed, and other advantages.

Clipping of the elytra is only applicable for mark and recapture surveys and is not appropriate without specific authorization from the Service. The survey protocol described here is to determine the presence or absence of ABBs only. Clipping of elytra causes hemoglobin to be secreted by ABBs. Although, there is currently no conclusive evidence that this is a direct or indirect adverse impact to the ABB, it is not recommended. It is the Service's responsibility to err on the side of the species. Further based on past survey reports, recaptures are highly unlikely during such a short survey effort. If a research project is being conducted and identification of individuals is needed then this research project must be approved by the Service.

Measuring the pronotum is voluntary; however the Service would appreciate the collection of this data. Measuring of the pronotum should be done with digital calipers.

Sexing

The sex of ABBs can be distinguished based on the orange-red marking located between the frons and mandibles on the head. These markings are rectangular on males and triangular on females (Figure 5).

Aging

Adults that have pupated during the current active season are known as newly eclosed. Newly eclosed ABBs (young) can be distinguished from ABBs produced the previous year (old) by their softer bodies and more shiny appearance (Creighton *et al.*, 1994). Also, the orange-red pronotum appears to be lighter and more orange in color in newly eclosed adults. Older adults often are missing body parts, especially legs or antennae. In addition, the mandibles of older adults appear to be a bit more worn at the tip.

ABB Release

ABBs should be released along transects where they were captured or within about 500 feet of the transect. Further, ABBs should be released a minimum of about 100 feet away from vehicle or foot traffic and outside of the pathway of vehicle and foot traffic to avoid trampling.

Identification and Other Nicrophorus Species

There are 6 other *Nicrophorus* species in Oklahoma that resemble the ABB. The ABB can usually be distinguished from other *Nicrophorus* species by the large orange-red spot on its pronotum (body segment between the head and abdomen) and on the frons (Figure 6). No other mature *Nicrophorus* species has an orange-red marking covering the pronotum and frons. However, newly eclosed (within 2 weeks after emerging from the ground) *N. orbicollis* can have a burnt orange marking on the pronotum. However, red-orange frons present on the ABB should allow for distinction from new *N. orbicollis*. The *N. orbicollis* has black frons. A description of the other *Nicrophorus* species in Oklahoma and an identification key is located in Appendices 2 and 3, respectively. In addition, photos of other *Nicrophorus* species in Oklahoma are available on our website for comparison.

Accidental Death of ABBs

The handling of all endangered species is strictly regulated by the Service. All accidental mortalities of ABBs must be accounted for and an "*ABB Accidental Death Form"* must be completed for each individual specimen and submitted within 14 calendar days to the Oklahoma Ecological Services Field Office. An "*ABB Accidental Death Summary Report"* must be completed in electronic and hardcopy formats and submitted to the Oklahoma Ecological Services Field Office by October 15 of each year and to the Regional Permit Coordinator along with your annual report.

Dead ABBs should be placed in cotton within a sealable, rigid container to prevent jostling of the ABB causing limb and antennae damage. Each specimen must have a unique alphanumeric name assigned. This alphanumeric name should be the first letter of the first 2 words of the permittee company or individual (e.g. Acme Company, first dead ABB = AC001). A label with the date found dead, permittee, legal description (down to quarter section at least), and specimen alphanumeric name should be placed inside each container to ensure future identification. Only place one ABB specimen per container to avoid mixing up specimens. Place the container on ice until the ABB can be prepared. Dead ABBs are to be submitted to the Service or a Service approved facility with their corresponding "*ABB Accidental Death Form"*.

Reporting

The Service has prepared a standard "*ABB Survey Data Collection Form*" (Appendix 4). Use of this form ensures that all of the needed data is recorded by all permittees. This form is to be completed for each transect, each night during a survey effort. Copies of all forms are to be submitted to the Oklahoma Ecological Services field office (see address in heading).

In addition, an "*ABB Survey Summary Report*" (Appendix 5 and 6) is to be completed for each survey effort. This is to be submitted electronically in excel file format to ABBcontact@fws.gov. Additionally, a hardcopy form is to be mailed to the Oklahoma Ecological Services Field Office along with the corresponding "*ABB Survey Data Collection Forms*". A description of the required fields to complete in the "*ABB Survey Summary Report*" is provided in Appendix 7.

All latitude and longitude data should be reported in decimal degrees and the coordinate system/projection should be in NAD 83. If a survey is conducted in compliance to the Endangered Species Act or the National Environmental Protection Act, project names and numbers need to correctly correspond. Only complete and accurate reporting forms will be accepted. Incomplete and/or inaccurate forms will be returned and the surveys will be considered invalid until the forms are corrected and/or properly completed, and submitted. When sending corrected forms, indicate that it is a correction,

what specifically has been corrected, and the project name.

Protocols and Forms

All forms can be downloaded from the Oklahoma Ecological Services Field Office's website <http://www.fws.gov/southwest/es/oklahoma/beetle1.htm>.

References

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This guidance was developed from the above references, U.S. Fish and Wildlife Service's July 14, 2005, "ABB Survey Guidance" and U.S. Fish and Wildlife Service Working Group on May 6, 2004, and other meetings between Service personnel and permittees in March and April 2009. The Oklahoma Ecological Services Field Office, in coordination with other Field Offices, update this survey protocol as necessary due to new findings. This guidance strives to streamline and update American burying beetle survey recommendations among the Arkansas, Oklahoma, Kansas, Nebraska, South Dakota, and Arlington, Texas Field Offices. However, due to the current habitat, land-use, development, other environmental considerations, and etc. there is variation among the different states. However, each state protocol may be different in some manners due to the land use and actions that occur in the different states. Each state Service office should be contacted for their most current protocols.