

INDIANA BAT SURVEY GUIDANCE FOR KENTUCKY



(Photo courtesy of J. MacGregor)

May 10, 2011

Developed by:

U.S. Fish and Wildlife Service
Kentucky Field Office
J.C. Watts Federal Building
330 West Broadway, Room 265
Frankfort, KY 40601

And

Kentucky Department for Fish & Wildlife Resources
#1 Sportsman's Lane
Frankfort, KY 40601

TABLE OF CONTENTS

BACKGROUND INFORMATION	1
WHITE-NOSE SYNDROME	3
DETERMINING PRESENCE OF POTENTIAL SUMMER AND WINTER HABITAT FOR INDIANA BATS IN KENTUCKY	5
Information to Determine if Potential Summer Habitat is Present	5
Information to Determine if Potential Winter Habitat is Present	5
SUMMER HABITAT SURVEY PROTOCOLS	6
Mist Netting	6
Acoustical Sampling	8
Radio Telemetry	11
FALL AND SPRING SURVEY PROTOCOLS FOR IDENTIFYING POTENTIAL INDIANA BAT HIBERNACULA	13
LITERATURE CITED	17
Appendix 1. Survey Report Format	1
Appendix 2. Phase I Habitat Assessment Sample Data Sheet	2
Appendix 3. Sample Data Sheets for Indiana Bat Surveys	3
Appendix 4. Sample Data Sheet for Fall or Spring Surveys of Potential Hibernacula	5
Appendix 5. Acoustical Monitoring Site Flowchart	6
Appendix 6. Step-by-step instructions on the analysis of Anabat calls	7
Commonly Used Anabat Commands	8
Appendix 7: Other Relevant Information to Indiana Bat Consultations	10
Appendix 8: KDFWR Project Proposal Form	11
Appendix 9: Bat Handling/Disinfection Protocol for Researchers	12
Caves, mine portals and hibernacula	12
Notification of Signs of WNS	12
Appendix 10: Recommended Weatherproofing System	14

BACKGROUND INFORMATION

The Indiana bat (*Myotis sodalis*) was listed as an endangered species by the U.S. Fish and Wildlife Service (Service) on March 11, 1967 (32 FR 4001) and is currently protected under the Endangered Species Act of 1973, as amended (Public Law 93-205). Critical Habitat was designated for the species on September 24, 1976 (41 FR 41914) and included 11 caves and 3 mines in six states. In Kentucky, these critical habitat designations include Bat Cave (Carter County) and Coach Cave (Edmonson County). For additional information concerning the ecology and life history of the Indiana bat, please refer to the Indiana Bat Recovery Plan (Service 1983), the numerous scientific articles, books, and other publications relating to the species, and the recently released Draft Revised Indiana Bat Recovery Plan (Service 2007).

In recent years, the Kentucky Ecological Services Field Office (KFO) of the U.S. Fish and Wildlife Service (USFWS) has reviewed hundreds of Indiana Bat summer and winter survey results for development projects and other activities in the Commonwealth. In reviewing these survey results, it became evident that the survey guidance could be improved in three ways, which are to: (1) improve the accuracy of the survey data and results; (2) use improved survey methodologies and technologies; and (3) provide survey protocols for potential hibernacula. These are described in greater detail below.

Improving Data and Results

During the review of survey data and results, some results were determined to be insufficient, invalid, or of poor quality due to a variety of factors. These factors include, but are not limited to, (a) poor mist net placement; (b) a lack of suitable mist netting locations on the project area (i.e., Indiana bats can be more difficult to capture in heavily forested areas where high quality mist netting sites do not exist.); (c) conducting surveys during unsuitable weather conditions; (d) not tending mist nets at frequent intervals; (e) overlooking potential hibernacula; (f) uncontrollable changes in site habitat conditions; (g) use of inadequately trained or experienced personnel to gather or develop survey results; and (h) lack of sufficient personnel and other resources to investigate potential hibernacula with many entrances (i.e., mine or quarry portals and their associated underground chambers). As a result, it became apparent that consistent, statewide guidance on how, where, and when to conduct Indiana bat summer and winter habitat surveys was needed and that the guidance should be sufficiently detailed to control these types of data collection or survey errors.

Using Improved Methods and Technology

It is also well documented that Indiana bats, even when we know they are present, are difficult to capture using currently accepted mist netting survey techniques. In response to this limitation, studies have been conducted to investigate the efficiency of mist netting to determine Indiana bat presence (Robbins et al. 2008) and several other studies have been conducted to directly compare mist netting and acoustical monitoring (Kunz and Brock 1975, Kuenzi and Morrison 1998, Murray et al. 1999, O'Farrell and Gannon 1999, Flaquer et al. 2007, Robbins et al. 2008). Robbins et al. (2008) found that acoustical detectors were more effective than mist nets in identifying Indiana bats using the study area. This study also suggested that mist netting effort would have to be modified from the basic mist netting survey guidelines to achieve at least a similar probability of capture. Murray et al., (1999) deployed mist nets and acoustical monitoring equipment at the same locations on the same nights and found that acoustical monitoring consistently detected bat species that mist netting missed, including Indiana bats. The authors of that study stated that "the combination of both survey methods provides the most effective means of determining bat species composition in an area".

Based on this data, we have high confidence that the combination of mist netting and acoustical sampling will provide improved survey accuracy throughout Kentucky.

The current technology used to isolate and analyze Indiana bat calls detected through acoustical monitoring, while promising, is currently being tested on a large (i.e., state-wide) scale. Therefore, acoustical sampling alone will not be relied upon to confirm Indiana bat presence or absence within a project area. Instead, mist netting and acoustical sampling will be used together. The detection of echolocation calls that are consistent in structure to calls of Indiana bats recorded through acoustical monitoring will be used, similar to a Habitat Suitability Model, as an indicator that additional mist netting is necessary. Once this technology has been more extensively tested, we may also rely on acoustical sampling alone to determine presence or absence.

Developing Protocols for Potential Hibernacula

Specific guidance related to surveys of potential winter habitat (i.e., caves, quarries, and/or abandoned mines) was needed, because no standardized guidance was available. Development of these protocols is important in Kentucky, because large areas of the state contain karst areas or abandoned mines from either coal or limestone mining that could be used by Indiana bats for hibernation. Some of these areas, such as the Mammoth Cave region of south-central Kentucky, the Cumberland Plateau (including the Daniel Boone National Forest), and Pine Mountain in the eastern Kentucky coalfield, are known to contain Indiana bat hibernacula. However, other areas of Kentucky that are subject to development and other activities that could adversely affect Indiana bat hibernacula have not been adequately surveyed and could, thus, benefit from the development of survey guidance for potential hibernacula.

As a result of these factors, the KFO and Kentucky Department of Fish and Wildlife Resources (KDFWR) developed this survey guidance to assist Kentucky's permitted bat biologists in (a) consistently implementing surveys to document presence or absence of Indiana bats on proposed project areas and (b) using data gathered from these surveys to develop biological assessments or evaluations (BAEs) that are based on the best scientific and commercial data available for a proposed project area. In developing this guidance, the KFO consulted with a number of recognized Indiana bat experts including, but not limited to, two Indiana Bat Recovery Team members, one of the acknowledged experts on the use of acoustical monitoring equipment and surveying methods, and other biologists with considerable experience relating to Indiana bat survey methods and research. The KFO also coordinated with other State and Federal agencies (e.g., KDFWR and United States Forest Service) and a number of the USFWS' Indiana bat and/or Endangered Species Act (ESA) experts in Regions 3, 4, and 5 in formulating these survey protocols and this guidance.

This Indiana bat survey guidance provides the standard survey protocols necessary to determine the presence or absence of Indiana bats on a given site in Kentucky. This information is typically necessary for consultations under section 7(a)(2) of the ESA. Use of this information may also be useful to the KFO and project proponents in identifying actions that may avoid and/or minimize impacts to the Indiana bat resulting from a proposed project or activity or that may assist with the species' recovery. We have also included other relevant information to consider during Indiana bat consultations in Appendix 7. If implemented as described in the sections below, the KFO will generally accept the results of these surveys to determine presence or absence for the purposes of Section 7 consultation. Survey results that are derived using this guidance will be valid for two survey seasons (e.g., a survey completed in 2011 is accepted for the 2011 and 2012 survey seasons or through May 14, 2013) from the date the survey is completed. Project Proposal Forms (or study plans) and an 8.5" x 11" topographical map of action area must be submitted to KDFWR at least 15

days prior to project initiation (see Appendix 8). The submittal of study plans to the KFO prior to completing surveys is no longer necessary as long as surveys are implemented as described in the sections below; however, study plans to the KFO should be submitted if you are proposing any modifications to this guidance. Please attach a copy of the KDFWR Project Proposal Form approval letter when submitting your BAEs to the KFO for Indiana bat consultations.

This guidance closely follows but expands upon the Indiana bat survey guidance that many states have used to determine Indiana bat presence or absence and the proposed general guidance provided in the Draft Revised Indiana Bat Recovery Plan (USFWS 2007). The additional detail provided in this guidance is intended to minimize the number of situations where insufficient, invalid, or poor quality survey data is collected; the addition of the acoustical sampling component is intended to improve the accuracy of the survey data that is collected by utilizing new survey technologies that are highly accurate; and the identification of fall survey protocols for potential hibernacula is designed to address an unmet need for guidance in that situation. This guidance also supports several of the recovery tasks identified in the Draft Revised Indiana Bat Recovery Plan (USFWS 2007), including tasks 2.7 (Refine and develop standardized monitoring protocols), 2.7.1 (Refine mist netting protocols), 2.7.2.6 (Use of bat detection systems to survey for Indiana bats), and 3.1.2.3 (Revise the winter bat survey protocol to include newly developed survey techniques).

Guidance Modifications

This guidance is subject to change as more information on Indiana bat ecology, distribution, and habitat use is acquired. It is also likely that the survey protocols will be modified as more information is gained on their effectiveness and applicability in different situations. For example, summer surveys at potential hibernacula are no longer necessary, because the available data on Indiana bat use of these sites in Kentucky shows that the loss of these sites as summer roosts is likely a discountable adverse effect to Indiana bats. Also, the use of mist netting to determine Indiana bat presence or absence at some sites may be replaced by acoustical sampling if the technology associated with acoustical sampling improves to the point that such sampling provides better survey results on Indiana bat presence or absence and habitat use.

Since this document can be modified as new information becomes available, the KFO welcomes any comments or suggestions for improvement to the guidance. Any comments or suggestions should be sent to Mike Armstrong of the KFO at 502/695-0468, ext. 101.

WHITE-NOSE SYNDROME

Due to the rapid spread of white-nose syndrome (WNS) from the epicenter in New York (first discovered in February 2006) to West Virginia and Virginia in 2009, Tennessee, Vermont, Missouri, and Oklahoma in 2010, [and Kentucky, Indiana, Ohio, and North Carolina thus far in 2011](#), the Service's Field Offices in several states, in coordination with their state wildlife agency partners, have decided to delay the start of the 2011 summer bat survey season until June 1, 2011. The decision to delay direct capture bat surveys was solely based on the current research related to the transmission potential of WNS. Current information indicates that the *Geomyces destructans* fungus actively grows on bats within caves as late as May 6th thus it is possible that transmission of WNS could occur during spring migration and possibly during the remainder of the summer. Infected bats captured during these first few weeks of the survey season could contaminate survey gear which

may have the potential to transfer WNS to other uninfected bats captured in the same or subsequent nights.

Researchers should follow the “Bat Handling/Disinfection Protocol for [Researchers](#)” (Appendix 9) to minimize the potential for transmission of WNS after June 1st. In addition, please regularly visit the Service’s WNS Website (<http://www.fws.gov/whitenosesyndrome/>) to ensure implementation of the most current [decontamination](#) protocols.

Given the temporary, voluntary moratorium on entering caves (see USFWS Cave Advisory from March 26, 2009), presence/absence surveys (see “Fall and Spring Survey Protocols for Identifying Potential Indiana Bat Hibernacula” and Appendix 9) of caves/abandoned mines have been temporarily modified. Until further notice, all caves/mines identified as potential bat habitat by a Phase 1 Habitat Assessment should be coordinated through the KFO and KDFWR as a further precaution related to the transmission of WNS.

As a reminder, the white fungus is only one of the signs of WNS, and finding bats with fungus on them during the summer (once they are active and grooming) is not expected. However, other abnormal characteristics may be indicative of WNS. Abnormal characteristics observed in summer may include: extremely underweight bats; flaky, dehydrated or wrinkled wing/tail membranes; wing lesions; discolored spots /scarring of flight and tail membranes; multiple small to medium sized holes in wing membranes; torn or necrotic areas at the trailing edge of wing and tail membranes. If you capture a bat that exhibits signs of WNS or abnormal characteristics, inform the Kentucky Department of Fish and Wildlife Resources or U.S. Fish and Wildlife Service – Kentucky Field Office personnel via the email addresses listed below, and:

- Record a wing score for each bat using the Reichard WNS Wing Damage Index, found on the Northeastern USFWS page:

http://www.fws.gov/northeast/white_nose2.html#research

- Photograph all suspicious bats
- Report suspicious bats (and send photos) scoring a 2 or higher using the Wing Damage Index within 24 hr to: brooke.slack@ky.gov or mike_armstrong@fws.gov

DETERMINING PRESENCE OF POTENTIAL SUMMER AND WINTER HABITAT FOR INDIANA BATS IN KENTUCKY

The information below is provided to assist applicants, consultants, and/or project proponents (hereinafter termed the “applicant”) in establishing whether summer and/or winter surveys for Indiana bats should be conducted. Surveys results, and the results of any subsequent radio telemetry work (if applicable), will be used to complete a project-specific analysis to determine if proposed activities will adversely affect Indiana bats and/or their habitat. The applicant is responsible for developing and providing sufficient information as to whether potential summer and/or winter Indiana bat habitat exists within a proposed project area. In order to accomplish this, the applicant must have a detailed knowledge of the project area that is sufficient to adequately and accurately describe the potential Indiana bat habitat conditions that may or may not exist on-site. This knowledge can be derived from any number of sources including, but not limited to, on-site visits, review of aerial photography and other maps, previous mining records (if applicable), forest inventories, previous species survey reports, and the work of the applicant’s consultants or other designees. At a minimum, however, the applicant must determine if potentially suitable Indiana bat summer roosting habitat and/or potentially suitable Indiana bat winter hibernation habitat is present. The following sets of information, which are not all-inclusive, can be useful in determining if either of these two types of Indiana bat habitat is present:

Information to Determine if Potential Summer Habitat is Present

- a) Acreage of forests or other lands with roost tree and/or snags $\geq 5''$ dbh that have exfoliating bark, solar exposure, and that are present on project area;
- b) Distance to available water in miles from project area (e.g., ponds, streams, rivers, lakes);
- c) Maps or photographs of the project area (e.g., forested area and water sources); and
- d) Summary of the acreage of potential summer habitat as identified in a-c above (e.g., forested vs. non-forested areas) that adequately and accurately describes the habitat relative to the proposed project (i.e., Is habitat present and will it be adversely affected or otherwise impacted?)

Information to Determine if Potential Winter Habitat is Present

- a) Review of karst occurrence maps (e.g., Kentucky Geological Survey)
- b) Mining history of the area (e.g., Do underground mines or quarries exist within or adjacent to the project area?);
- c) Summary of interviews with landowners and/or mineral rights owners regarding presence/absence of potential caves, rockshelters, and/or abandoned mine portals, when available;
- d) Geologic core sample data from exploration, if applicable;
- e) Copy of topographic, mining, and environmental resources information maps; and
- f) Results of field inspections of areas containing potential hibernacula as identified in items a-d above.

SUMMER HABITAT SURVEY PROTOCOLS

If summer maternity colony and roosting habitat surveys are to be conducted, they must be conducted between May 15 and August 15 and prior to any tree clearing on the project area. However, minor tree clearing/trimming is permissible to improve conditions for acoustical monitoring (e.g., creating small forest openings) and improve suitable mist net locations (e.g., pinch points along flight corridors). Such minor clearing is only permissible if (a) suitable openings do not already exist and (b) such clearing will not require the removal of potential Indiana bat summer roost trees. In no case, shall such openings be more than 50 meters in diameter or created less than 2 weeks prior to conducting the survey (Personal Communication, John MacGregor, KDFWR, 2006). Larger openings and recently-created openings are expected to reduce capture and data collection efficiencies. Summer maternity and roosting habitat surveys will consist of a mist netting component, an acoustical sampling component, and a radio telemetry component (if Indiana bats are captured) as described in the sections below.

Mist Netting

The following protocols apply to all mist net surveys:

1. A minimum of two nights of mist netting is required. The second night of netting is not required if a female or juvenile Indiana bat is captured on the first night of sampling.
2. For every 1 km² (247 acres) of potential summer habitat, 2 net sites for 2 nights are required.
3. For linear projects (e.g., electrical transmission lines, natural gas pipelines, highways, haul roads), 1 net site for 2 nights per 1 km length of potential Indiana bat summer habitat associated with the project area is required.
4. A qualified biologist¹ must identify each mist net site and must establish those net sites in the areas that are most suitable for capturing Indiana bats (e.g., travel corridors with canopy closure, abandoned mine portals, water sources, etc...). Mist net sites should be located on the project area or as close as possible to the project area. At a minimum, the qualified biologist should identify the required number of mist netting locations for the project and a sufficient number of suitable alternate mist netting sites that could be used if the original sites become unavailable for any reason.
5. A net site consists of two net locations that are not closer than 30 meters apart. If nets are placed closer than 30 meters apart, the qualified biologist must provide sufficient justification to the USFWS in the survey report as to why the modification was appropriate and did not inhibit the determination of Indiana bat presence or absence.

¹ A qualified biologist is an individual that holds a USFWS Recovery Permit (Federal Fish and Wildlife Permit) for federally listed bats in Kentucky (starting in 2009) and has been authorized by the KDFWR to survey for bats.

6. A net site may be relocated to a suitable alternate location if no bats are captured or if bat activity is very low during the first night of netting at the original net site. The qualified biologist must provide sufficient justification to the USFWS in the survey report for relocating a net site.
7. The qualified biologist should use the following document as additional guidance on the identification, establishment, and placement of mist net sites:

Kiser, J.D. and J.R. MacGregor. 2004. Indiana bat (*Myotis sodalis*) mist net surveys for coal mining activities. Pages 45-61. *In: The Proceedings of The Indiana Bat and Coal Mining: a Technical Interactive Forum* (K.C. Vories and A. Harrington, eds.). Office of Surface Mining, U.S. Department of the Interior, Alton, Illinois.

If low quality mist netting sites are used, the survey results will not necessarily be accepted by the USFWS.

8. Mist nets should be made of the finest, lowest visibility mesh commercially available and should be from 6 to 9 meters long and capable of being extended at least 7 meters high. Currently, this is 2-ply, 50-denier nylon (denoted 50/2). The mesh should be approximately 1.5-inch in size. No other specific mist netting hardware is required.
9. The mist netting sampling period should begin at sunset and continue for at least 5 hours. During this time, mist nets should be monitored for captured bats on 10-minute intervals to minimize the number of bats that escape the nets. If bat activity or captures increase during the survey or if 6 or more bats were captured during the last hour of monitoring, the survey effort must continue until activity declines or fewer than 6 bats are captured per hour.
10. Severe weather adversely affects the activity levels of bats. If any of the following weather conditions exist during the mist net survey, the time and duration of such conditions must be noted on the data sheets and within the survey report (Appendices 1 & 3), and the survey effort must be repeated for that day: (a) temperatures below 10°C (50°F); (b) winds sufficiently strong and variable to move mist nets more than 50 percent of the time; and (c) precipitation, including rain and/or fog, that does not stop within 30 minutes or continues intermittently during the survey period. The qualified biologist can request a variance from the USFWS to repeat survey efforts if the biologist believes these adverse weather conditions did not inhibit their ability to determine the presence/absence of Indiana bats within the project area. This request must be made in writing, and the USFWS must concur in writing prior to submittal of the survey report for the survey results to be valid.
11. At least one member of the survey crew at each mist-net site must hold, and have in his or her possession, a valid endangered species collection permit issued by USFWS and KDFWR that allows the qualified biologist to collect bats, including federally listed species.

12. If one or more Indiana bats or other federally listed species are captured, immediate notification to the USFWS, Kentucky Field Office (502-695-0468) and KDFWR (502-564-7109) is required. Please reference Appendix 1 for data collection requirements.
13. If a bat from the genus *Myotis* is captured during mist netting and that bat cannot be readily identified to the species level, species should be verified through fecal DNA analysis. To accomplish this, one or more fecal pellets (i.e., guano) must be collected from the bat. To collect fecal pellets, the bat should be temporarily placed in a holding bag or other container that does not interfere with the bat's ability to breathe for 15 minutes or until one or more fecal pellets can be collected. Holding bats is stressful (e.g., some pregnant females may abort) and lactating females are prevented from returning to the roost to nurse their young; therefore, pregnant and/or lactating female bats of the genus *Myotis* should not be held for longer than 30 minutes. The pellet (or pellets) should then be placed in a 1.5 ml vial with silica gel desiccant, with pellets from individual bats stored in separate vials and out of direct light. Samples should then be shipped to Dr. Jan Zinck, Department of Biology, Portland State University, 630 SW Mill St., Portland, Oregon, 97201 for fecal DNA analysis to assign or confirm the species of bat collected. The current cost for DNA sequencing is approximately \$50 per individual pellet of guano. Contact Dr. Zinck (e-mail: zinckj@pdx.edu) prior to shipping samples. To our knowledge, this is the only lab that currently provides this service; however, the use of other labs can occur if those labs are first approved by the USFWS in writing. Any additional information (or additional sources) on this technique will be made available on the Indiana bat webpage on the Service's Region 3 website (www.fws.gov/midwest).

Acoustical Sampling

Acoustical sampling equipment is used in conjunction with mist netting to provide presence/absence survey results that have a greater **efficiency** of documenting Indiana bat use of a project area. Sole reliance on mist netting survey results to determine the presence or absence of Indiana bats is problematic due to the inherent difficulty in capturing Indiana bats even if they are present. **An automated program is being developed that allows for analysis of large amounts of echolocation data. As this program is currently in development,** acoustical sampling alone will not be relied upon to confirm Indiana bat presence or absence within a project area. Instead, mist netting and acoustical sampling will be used together, and the detection of bat calls **that are consistent in structure to the echolocation calls of** Indiana bats will be used as an indicator that additional mist netting is necessary to (a) determine Indiana bat presence or absence and (b) focus mist netting efforts at locations where Indiana bats likely forage and/or travel (Appendix 5). Currently, **methods developed by Britzke et al. (in press) provide the only methodology** capable of discerning among species of **eastern** bats to an acceptable confidence level. If other acoustical sampling models are also shown to discern species at an acceptable confidence level, they may also be used if first approved by the USFWS in writing.

The following protocols shall apply to all acoustical sampling efforts:

1. The number of acoustical sampling sites required for a project will be equal to the number of mist net sites required for the project. For example, a project area containing 240 acres of suitable habitat would require the deployment of 2 detectors for two nights for a total of 4 detector nights.
2. Detectors must be placed separately (i.e., greater than 100 meters) from net sites. [Placement of detectors should be made independently from mist netting locations and should be deployed in areas that maximize detection. Effort should be made to minimize overlap in sampling between the 2 methods.](#) Detectors allow sampling of habitats that cannot be effectively sampled with mist nets (e.g., forest edges, large streams, large ponds, etc.) Deployments of detectors in closed-canopy locations that typically are good for mist netting are not appropriate for use of the data analysis. If closer spacing was determined to be necessary or beneficial (e.g., multiple habitats immediately adjacent to each other), sufficient justification must be provided by the qualified biologist within the survey report submitted to USFWS.
3. In most cases, detector sites should not be located closer than 200 meters. If closer spacing was determined to be necessary or beneficial, the qualified biologist must provide sufficient justification to the USFWS in the survey report.
4. A qualified biologist must identify each detector placement site and must establish those sites in the areas that are most suitable for detecting Indiana bat calls.
5. Recording of high quality bat calls is critical to their proper analysis. For these reasons, weatherproofing should only be used for acoustic sampling when absolutely necessary. Detectors must be properly placed at suitable monitoring sites, because such placement is critical to the successful isolation of bat calls for later analysis. If detectors are placed in unsuitable locations (e.g., heavily cluttered sites), effective data analysis may be impossible, and the results of the sampling effort may be invalid. The following locations, and others not listed below, are likely to be suitable sites for detectors: (a) forest canopy openings that are no more than 50 meters wide; (b) water sources that are too large to sample effectively with mist nets; (c) wooded fence lines that are adjacent to large openings or connect two larger blocks of suitable habitat; (d) large blocks of “high-graded” or recently logged forest where potential roost trees remain due to their undesirable condition as commercial trees; (e) road and/or stream corridors with open tree canopies; and (f) small grassy openings that are no more than 50 meters wide ([Britzke et al. 2010](#)).
6. Acoustical sampling period should begin [before](#) sunset and continue throughout the entire night on the first night of sampling, when possible. If theft of equipment is a concern, the acoustical sampling period on the first night must occur, at a minimum, for the duration of the mist net survey. On the second night of mist net surveys, the acoustical sampling must occur, at a minimum, for the duration of the mist net survey.

7. If weatherproofing of detectors is desired, then the sampling period must continue throughout the night for both nights of sampling. The only currently accepted weatherproofing methodology is found in Appendix 10.
8. Severe weather adversely affects the activity levels of bats. If any of the following weather conditions exist during the acoustical sampling, the time and duration of such conditions must be noted, and the acoustical sampling effort must be repeated for that day: (a) temperatures below 10°C (50°F); (b) winds sufficiently strong and variable to move mist nets more than 50 percent of the time; and (c) precipitation, including rain and/or fog, that does not stop within 30 minutes or continues intermittently during the survey period.
9. Detectors should be used to prioritize prospective mist netting areas and to evaluate suitable habitat within the permit area that is not easily or effectively sampled with mist netting equipment. This type of additional habitat evaluation should be done in advance of the mist netting and acoustical monitoring surveys that will be done; however, this additional, advance work is suggested (i.e., not required) because it would likely further improve survey results.
10. At the conclusion of each acoustical sampling period, collected calls must be downloaded and processed through 2 filters provided by the USFWS and/or KDFWR. The first filter is designed to remove as much of the ‘background’ noise (e.g., insects, leaves, wind) from the call files as possible. [In particularly noisy environments, the files should be visually checked to ensure only files containing bat echolocation calls remain.](#) The second filter is designed to [extract parameters describing the frequency, time, and slope components of each pulse.](#) These files will then be read using the automated program for analysis.
11. If the analysis of collected calls results in the [identification of calls of endangered species with high levels of certainty \(\$P < 0.05\$ \)](#), then an additional mist netting site (i.e., 2 nets for 2 nights) must be conducted. This additional survey work should follow the mist netting guidance. The additional mist netting site should be located as close to the acoustical sampling site as possible with the goal of capturing the detected bats with the additional mist netting effort. The acoustical sampling site must also be re-sampled during the additional mist netting effort near the site, but during the additional mist net effort the [further identification of endangered species](#) will not require any further mist netting effort near that site. Additional mist netting resulting from [analysis of bat echolocation calls recorded](#) on August 14 and/or 15 should be completed by August 17. If weather conditions prevent the completion of the additional mist netting effort by August 17, contact the USFWS for guidance on how to proceed. As a reminder, the purpose of the acoustical sampling is to provide improved data that shows that Indiana bats either potentially use or do not use a site. If this data shows that Indiana bats are potentially using a site, the additional mist netting then either confirms the presence or absence of Indiana bats on the site.

12. The complete project directory, which includes all “.DAT” files, all noise-filtered bat call files, [the parameter files](#), [as well as the output file](#) must then be saved and submitted to the USFWS and/or KDFWR (if requested). Results of the analysis of filtered call files will be provided to the qualified biologist for educational purposes upon review and confirmation of those calls by the USFWS. Only the complete project directory for surveys resulting in the [identification of files](#) should be submitted within 30 days of completion of the project survey effort. These files should be zipped, burned to a compact disc, and mailed to the following address – USFWS, Kentucky Field Office, 330 West Broadway, Room 265, Frankfort, KY 40601 – using the following directory and file structures. As shown in the example below, the Project Name should be the KFO project number that is assigned to the project and provided to the applicant in the initial correspondence sent to the applicant for the project.

Sample Directory Structure

11BXXXX (USFWS project number that will be provided)

Site 1

19May11

20May11

Site 2

19May11

20May11

Appendix 6 contains a template of instructions on how to analyze bat calls using Anabat technology and ANALOOK software.

Radio Telemetry

If one or more Indiana bats are captured during survey efforts, the following radio telemetry protocols will apply. Radio telemetry will provide vital data regarding roosting habitat and could provide information on home range and foraging behavior for use during the ESA consultation process. In particular, this information would provide valuable insight into the selection of appropriate avoidance and minimization techniques and assist the applicant and/or the federal action agency in satisfying their requirements under the ESA.

The following protocols apply to all radio telemetry efforts:

1. A qualified biologist that is experienced in handling Indiana bats and attaching radio transmitters shall attach radio transmitters to all female, juvenile, and adult male (≥ 6.0 grams) Indiana bats captured at each site. [The placement of transmitters on adult males is not necessary when the individual is captured within known swarming zones of hibernaculum.](#) Please see: http://www.fws.gov/frankfort/indiana_bat_procedures.html for the most recent hibernaculum swarming zone shapefile.
2. The radio transmitter and adhesive shall not weigh more than 10 percent of a bat’s total body weight. However, in all cases, the lightest transmitters capable of accomplishing

the required task should be used, especially with pregnant females and newly volant juveniles. Please reference Appendix 1 for data collection requirements.

3. Radio telemetry equipment (e.g., receivers, antennas, and transmitters) must all utilize the 172 MHz frequency range. This frequency is the most commonly used in Kentucky and will generate consistency and allow for increased opportunities for cooperation among biologists, researchers, and agencies.
4. The qualified biologist or technician must track all radio-tagged bats to their diurnal roosts for at least 5 consecutive days and must conduct a minimum of two evening emergence counts at each identified roost tree during that period. If radio telemetry shows roost trees exist in areas that are off of the project area, the adjacent landowner(s) must be contacted and the landowner(s) must grant access to those areas prior to conducting these activities. If access is denied, roost tree locations should be determined using triangulation. Persons conducting radio telemetry work should never trespass during radio telemetry work. Please reference Appendix 1 for data collection requirements.
5. Daily radio telemetry searches for roost trees must be conducted during daylight hours and must be conducted until the bat(s) is located or for at least 4 hours each day.
6. Qualified biologists are encouraged to continue radio tracking efforts, on a voluntarily basis, for the life of each transmitter. This will generate better data related to Indiana bat roosting behavior on the project site and will further assist applicants and the Service in completing the ESA section 7 consultation.

FALL AND SPRING SURVEY PROTOCOLS FOR IDENTIFYING POTENTIAL INDIANA BAT HIBERNACULA

Entry of abandoned mine portals, quarries, or caves can be extremely dangerous because of the potential for ceiling collapse and presence of toxic gases. Safety or health problems may occur as a result of entering abandoned mines. The USFWS does not authorize or require anyone to enter any potential hibernaculum that is or could be unsafe while implementing these survey protocols. In some cases, entry is prohibited by the Kentucky Department of Mines and Minerals (KRS 352.100) and Mine Safety and Health Administration (30 CFR 75.202(b)). These guidelines do not require any applicant or applicant employee, consultant, lessee, or other such designee to enter into any cave, quarry, or mine portal.

Indiana bats have been documented using caves, quarries, and abandoned mine portals (and their associated underground workings) as winter hibernation habitat. A fall or spring survey of such potential hibernacula is necessary to determine if such sites are utilized as hibernacula by Indiana bats. Caves that have large enough openings to allow the safe entrance of surveyors should be directly surveyed for the presence of federally listed bat species, including the Indiana bat. Only properly trained and qualified individuals with the appropriate equipment should attempt these surveys. If the qualified biologist does not have the necessary experience to complete cave survey work, then this portion of the project should be subcontracted to another individual or group that does. If the cave is impossible to enter or it is believed that significant portions of the cave system are inaccessible, then it should be treated like an abandoned mine portal or quarry and the following guidance should be used to determine presence or probable absence of federally listed bat species, including the Indiana bat.

The following protocols shall apply to all such surveys:

1. Prior to initiating fall or spring surveys of potential Indiana bat hibernacula, the KDFWR and/or KFO must be contacted to determine if any identified cave or abandoned underground mine portal or quarry (and their associated underground workings) have been previously documented as habitat for federally listed bat species. Any proposed surveys of previously documented hibernacula must be coordinated directly with the KFO and KDFWR to ensure that adverse affects to listed species do not occur as a result of the survey.
2. A qualified biologist² must conduct a Phase 1 Habitat Assessment (Appendix 2) on all potential hibernacula that could be affected by the proposed project. The following information should also be considered when completing a Phase 1 Habitat Assessment:
 - a. The openings should be at least one (1) foot in diameter or larger.
 - b. The passage should continue for at least 100 feet (Note: This may not be verifiable by surveyor due to safety concerns.).
 - c. Some airflow, either in or out of entrance, should exist (Note: Air flow is not always detectable and changes by day and/or season.).

² A qualified biologist is an individual that holds a USFWS Recovery Permit (Federal Fish and Wildlife Permit) for federally listed bats in Kentucky and has been authorized by the KDFWR to survey for bats.

- d. Entrances that are flooded or prone to flooding (i.e., debris on ceiling), collapsed, or otherwise inaccessible to bats should be excluded from consideration.
- e. Openings that have occurred recently (i.e., within the past 12 months) due to creation or subsidence can be excluded from consideration if the applicant provides a written description and photographs of the opening in the survey report or biological evaluation/assessment.
- f. Bats will use vertical shafts. Vertical passages should be at least two (2) feet in diameter with some airflow.
- g. Foliage and other vegetation in front of openings do not stop use by bats. The animals can navigate through foliage.
- h. Bats can access mines via old buildings such as a fan house.

This assessment includes all entrances or openings that will be directly or indirectly impacted by the proposed project. This would include those caves, quarries, or portals that are within the project site or that are connected to any underground mine or quarry workings that will be directly or indirectly impacted by the proposed project and that are shown on the underground mine or quarry workings maps maintained by the Department for Natural Resources, Division of Mine Permits. This assessment can be completed at anytime of year. The results of the assessment for all entrances must be submitted with the Indiana Bat Survey Report.

- 3. For linear projects (e.g., transmission lines, natural gas pipelines, highways, haul roads), a field survey, where access can be obtained, of all land within 1 km of the edge of the project footprint and documentation (i.e., literature search) of all known caves and abandoned mine portals within 5 km of the outside edge of the project footprint should be conducted.
- 4. Fall portal/cave surveys must be conducted between September 1 and October 31 and prior to any tree clearing by the project applicant. A minimum of two nights of sampling is required at each suitable entrance as determined by the Phase 1 Habitat Assessment. Each night of sampling must be separated by at least two weeks of the survey window. This sampling is in addition to any summer habitat mist netting that is required. The data sheet contained in Appendix 4 is used to document this work.
- 5. Spring portal/cave surveys must be conducted between April 1 and April 21 and prior to any tree clearing by the project applicant. Conducting surveys during the spring emergence is typically more complex than conducting fall surveys due to a greater number of uncontrollable factors (e.g., weather related factors). Thus, a minimum of three nights of sampling per week for three weeks (i.e., 9 nights of sampling) is required at each suitable entrance as determined by the Phase 1 Habitat Assessment. Due to the need to monitor weather conditions closely, each proposed spring portal/cave survey must be coordinated with the KFO prior to sampling to ensure that adequate survey results are achieved. This sampling is in addition to any summer habitat mist netting that is required. The data sheet contained in Appendix 4 is used to document this work.

6. The sampling period should begin at sunset and continue for at least 5 hours each night. During this time, harp traps and/ or mist nets should be monitored for captured bats on 10-minute intervals to minimize the number of bats that escape the nets.
7. If bat activity or captures increase during the survey or if 6 or more bats were captured during the last hour of monitoring, the survey effort must continue until activity declines or fewer than 6 bats are captured per hour. If bat activity declines during the first 2.5 hours, the survey must be postponed. The USFWS can accept partial night surveys but only if a minimum of 2.5 hours of surveys, beginning at sunset, have been accomplished and all other requirements in this Portal/Cave Survey guidance are met. However, a total of 10 hours of sampling must take place for a portal/cave survey to be approved.
8. Severe weather adversely affects the activity levels of bats. If any of the following weather conditions exist during the fall or spring cave/portal survey, the time and duration of such conditions must be noted on the data sheets and in the survey report (Appendices 1 & 4), and the survey effort must be repeated for that day: (a) winds sufficiently strong and variable to move equipment more than 50 percent of the time; and (b) precipitation, including rain and/or fog, that does not stop within 30 minutes or continues intermittently during the survey period.
9. Harp traps are the preferred method for sampling entrances as they are less stressful on captured bats. Mist nets should also be deployed along corridors immediately adjacent to the entrance to increase survey effectiveness. Mist nets can also be used at the entrance when the portal or cave configurations are not suitable to harp trapping. Mist nets should be made of the finest, lowest visibility mesh commercially available. Currently, this is 2-ply, 50-denier nylon (denoted 50/2). The mesh should be approximately 1.5-inch in size. No other specific mist netting hardware is required.
10. When harp trapping, entrances must be entirely enclosed by the survey gear. If mist nets are used, entrances should not be entirely enclosed by the survey gear.
11. In cases where one team of surveyors cannot feasibly sample all entrances in one night, a modified method could also be used. This method can only be used in situations where the entrances are known to be interconnected. In this modified method, half of the interconnected entrances are surveyed on the first night, because the other half of the entrances are completely blocked using plastic or other impervious material. On the second night, survey efforts are reversed. Disconnected entrances do not have to be surveyed simultaneously.
12. If Indiana bats (or other federally listed species) are captured during fall or spring portal/cave surveys, the data discussed in Appendix 1 must be collected and immediate notification to USFWS and KDFWR is required. Radio telemetry of captured female and juvenile Indiana bats is optional. Although telemetry work may require a significant amount of effort (e.g., transmittered bats may be underground), applicants could demonstrate that the portal/cave is not an Indiana bat hibernaculum in some

circumstances, which would reduce the amount of additional survey effort (See #14 below) and the avoidance and/or minimization requirements for the portal(s)/cave(s).

13. If a bat from the genus *Myotis* is captured during survey efforts that cannot be readily identified to the species level, species can be verified through fecal DNA analysis. Collect one or more fecal pellets (i.e., guano) from the bat in question by placing it temporarily in a holding bag (15 minutes is usually sufficient, no more than 30 minutes is recommended). Holding bats is stressful (e.g., some pregnant females may abort) and lactating females are prevented from returning to the roost to nurse their young; therefore, pregnant and/or lactating female bats of the genus *Myotis* should not be held for longer than 30 minutes. The pellet (or pellets) collected should be placed in a 1.5 ml vial with silica gel desiccant; pellets from each individual bat should be stored in separate vials. Samples should be stored out of direct light. Samples should be shipped to Dr. Jan Zinck, Department of Biology, Portland State University, 630 SW Mill St., Portland, Oregon, 97201 for subsequent fecal DNA analysis to assign or confirm the specimens' identification to the species level. The current cost for sequencing is approximately \$50 per individual pellet of guano. Contact Dr. Zinck (e-mail: zinckj@pdx.edu) prior to shipping samples. To our knowledge, this is the only lab that currently provides this service. Any additional information (or additional sources) on this technique will be made available on the Indiana bat webpage on the Service's Region 3 website (www.fws.gov/midwest).
14. At least one member of each survey crew must hold, and have in his or her possession, a valid endangered species collection permit issued by USFWS and KDFWR that allows the qualified biologist to collect bats, including federally listed species.
15. The capture of an Indiana bat during a fall or spring portal survey requires that the applicant complete three additional nights of sampling per week for three consecutive weeks in order to determine the significance of the portal(s) and/or cave(s) and their associated underground workings to the Indiana bat. If the portal/cave survey season (i.e., September 1 to October 31 for fall sampling and April 1 to April 21 for spring sampling) ends prior to the completion of the required sampling, any additional sampling must be completed the following fall or spring.

LITERATURE CITED

- Britzke, E. R., B. A. Slack, M. P. Armstrong, S. C. Loeb. 2010. Effects of Orientation and Weatherproofing on the Detection of Bat Calls. *Journal of Fish and Wildlife Management* 1(2) 136-141.
- Flaquer, C., I. Torre, and A. Arrizabalaga. 2007. Comparison of sampling methods for inventory of bat communities. *Journal of Mammalogy* 88:526-533.
- Kuenzi, A. J., and M. L. Morrison. 1998. Detection of bats by mist-nets and ultrasonic detectors. *Wildlife Society Bulletin* 26:307-311.
- Kunz, T. H., and C. E. Brock. 1975. A comparison of mist nets and ultrasonic detectors for monitoring flight activity of bats. *Journal of Mammalogy* 56:907-911.
- Murray, K. L., E. R. Britzke, B. M. Hadley, and L. W. Robbins. 1999. Surveying bat communities: a comparison between mist nets and the Anabat II bat detector system. *Acta Chiropterologica* 1:105-112.
- O'Farrell, M. J., and W. L. Gannon. 1999. A comparison of acoustic versus capture techniques for the inventory of bats. *Journal of Mammalogy* 80:24-30.
- Robbins, L. W., K. L. Murray, and P. M. McKenzie. 2008. Evaluating the Effectiveness of the Standard Mist-netting Protocol for the Endangered Indiana Bat (*Myotis sodalis*). *Northeastern Naturalist* 15(2): 275-282.

Appendix 1. Survey Report Format

- 1) Introduction
 - a) Why and for whom the survey is being conducted.
 - b) Objectives of the survey.
- 2) Description of Study Area
 - a) General physiographic description.
 - b) Detailed description based on site-specific reconnaissance and existing data (e.g. county, USGS quadrangle, latitude/longitude location, elevation, watershed, stream(s), land use history, proposed action, acreage, major vegetative cover types, etc.).
- 3) Materials and Methods
 - a) Describe net and acoustical monitoring locations, net set-up (include net height), survey dates, duration of survey, weather conditions.
 - b) Include copies of data sheets (as an Appendix C).
 - c) Map identifying mist net and acoustical monitoring locations.
 - d) Identify the personnel that were present during surveys and that made any bat species identifications.
- 4) Results
 - a) Table with information on all bats captured during the survey including: capture site, date of capture, time of capture, sex, reproductive condition (if female), age, weight, direction of flight (if a cave or portal survey). A sample data sheet shown in Appendix B.
 - b) Table with information on acoustical monitoring and resulting data including but not limited to: acoustical monitoring equipment settings, deployment data (i.e., deployment site, habitat, date, time started, time stopped, number of files, orientation), and type of call filters used.
 - c) Include sufficient photographs of the net and acoustical monitoring locations, **as well as all Indiana bat(s) and little brown bat(s) (*Myotis lucifugus*)**, if captured, so that the placement of equipment and identification of species can be verified. **Photographs of bats should include all distinguishing characteristics that resulted in the identification of the bat to species level.**
 - d) Include a map showing location of roost tree(s) identified during follow-up radio telemetry of transmitted Indiana bat(s) as well as photo(s) and lat/long coordinates of tree(s).
 - e) Include roost tree(s) data (e.g., tree species, condition, percent canopy cover, number of Indiana bats counted during emergence counts, etc...).
- 5) Discussion of Results
 - a) Description of climate conditions that occurred during the survey and any effects they might have had on the survey.
 - b) Description of bat habitat (i.e., presence of water, tree density of canopy, understory and travel corridors) at each site and on the overall permit area.
 - c) General description of bat findings relative to site conditions, habitat adjacent to proposed permit area and regional expectations.
 - d) Conclusion to be drawn from findings regarding impacts to the Indiana bat.
 - e) If Indiana bats are to be impacted during the proposed project action(s), include protective and enhancement measures to be taken during and after project construction.

Appendix 2. Phase I Habitat Assessment Sample Data Sheet

Location _____
Observers _____
Latitude _____ **Longitude** _____
Date _____ **Time** _____ **Temp (outside)** _____

	Portal #1	Portal #2	Portal #3	Portal #4
Opening (cave, quarry, shaft, or adit)				
Opening Size: Height x Width (or Diameter)				
Internal Dimensions: Height x Width				
Slope (up or down from entrance)				
Entrance Stable?				
Direction of Airflow (In or out?)				
Amount of Airflow (e.g., none, slight, heavy)				
Air warmer or cooler than outside temp.				
Evidence of collapse?				
Ceiling Condition				
Amount of water in opening				
Evidence of past flooding?				
Observed length of portal				
Distance to nearest water source				
% Canopy Cover at portal entrance				
Foraging Signs? (e.g., moth wings)				

Are any portals suspected or known to be connected? Which ones?

Any observable side passages?

Additional comments:

Entry of abandoned mine portals, quarries, or caves can be extremely dangerous because of the potential for ceiling collapse and presence of toxic gases. Safety or health problems may occur as a result of entering abandoned mines. The USFWS does not authorize or require anyone to enter any potential hibernaculum that is or could be unsafe while implementing surveys. In some cases, entry is prohibited by the Kentucky Department of Mines and Minerals (KRS 352.100) and Mine Safety and Health Administration (30 CFR 75.202(b)). These guidelines do not require any applicant or applicant employee, consultant, lessee, or other such designee to enter into any cave, quarry, or mine portal

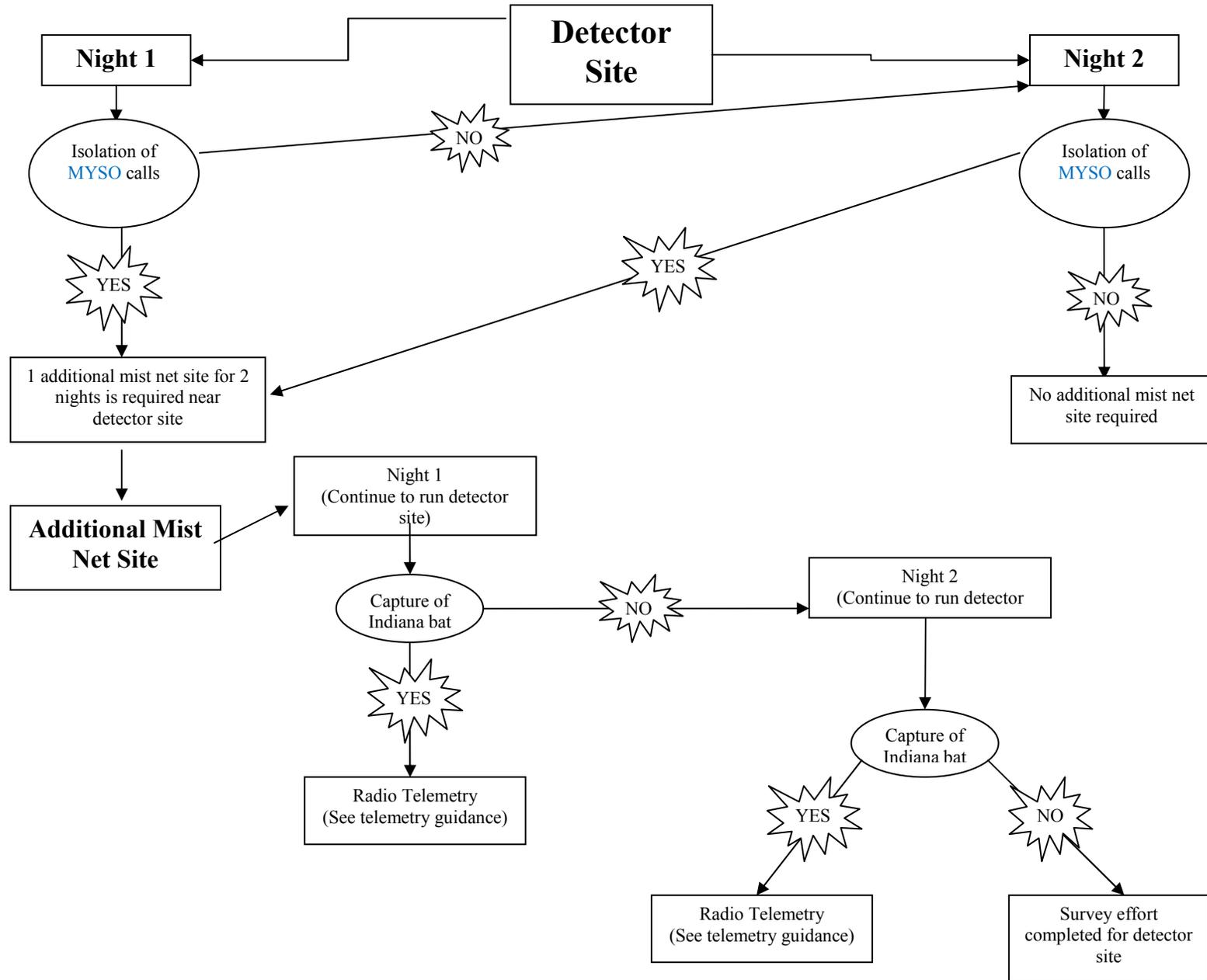
Appendix 3. Sample Data Sheets for Indiana Bat Surveys

Site No.			Project/Firm:					Date:							
Location:															
County:			State: KY		Quad:			Quadrant:							
Lat/Long (DMS):		N	W		Zone:			Observers:							
#	Time	Species	Age	Sex	Repro. Cond.*	RFA (mm)	Mass (g)	Net/Ht	Guano/Hair	Wing Score	Band # Type	Moon Phase:		%	
1													Rise	Set	
2													Moon:		
3													Sun:		
4															
										Time	Temp	Sky	Wind	# Bats	
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19															
20															
21															
22															
23															
24															
25															
26															
27															
28															
29															
30															
										Sky Code					
										0	Clear				
										1	Few Clouds				
										2	Partly Cloudy				
										3	Cloudy or overcast				
										4	Smoke or fog				
										5	Drizzle or light rain				
										6	Thunderstorm				
										Beauford Wind Code					
										0	Calm (0 mph)				
										1	Light wind (1-3 mph)				
										2	Light breeze (4-7 mph)				
										3	Gentle breeze (8-12 mph)				
										4	Moderate breeze (13-18 mph)				

*Repro. Cond (Reproductive Condition): (P) pregnant; (L) lactating; (PL) post-lactating; (NR) non-reproductive, (TD) testes descended

Net Site Diagram	Dominant Vegetation				
	1				
	2				
	3				
	4				
	5				
	Net Site(s) by Habitat				
	Habitat	A	B	C	
	River				
	Stream				
	Pond				
	Road Rut				
	Corridor				
	Cave/mine				
Total					
No. of Poles X Net length					
A	=		X		
B	=		X		
C	=		X		
D	=		X		
Other Species:					
Comments:					

Appendix 5. Acoustical Monitoring Site Flowchart



Appendix 6. Step-by-step instructions on the analysis of Anabat calls

- I. Open ANALOOK
- II. Hit any key to get to the main display (frequency-time display).
- III. Hit the F7 key for the appropriate display
- IV. Hit the spacebar until COMP shows up on the bottom of the screen
- V. Load an Anabat file by hitting Shift – L. Use the tab key to open up the directory key and use the right and left arrows to navigate through the tree. When you get to the directory of the files you want to analyze, hit the enter key. Use the home and end keys to note the start and stop recording times and record these times. Hit the enter key to select the first file and display it.
- VI. Delete the junk files
 - a. Press ALT – b to open up the filter menu
 - b. If the filter name noise is not listed in the menu Press O (letter o)
 - c. Type the name of the filter to open (noise) and hit ENTER
 - d. Hit A for apply
 - e. The screen will return to the main ANALOOK screen and the filter should be loaded (the name of the filter should be to the right of the FILT located on the bottom center of the screen).
 - f. Press ALT-F
 - g. Hit F key
 - i. The program will then run through all of the files in the correct directory and will mark all files that meet the criteria of the filter. So after this is completed the marked files will represent BAT calls.
 - h. Hit ALT-R to reverse the marks so that the trash files are marked.
 - i. Hit ALT-D to delete the marked files
 - j. Hit ALT-U to make sure all files are unmarked
 - k. Visually examine the calls on the screen. This process should remove most of the extraneous noise, but on some nights there might be too much noise. If you don't see definite lines on the screen, then it is probably ok to delete the files. Go through and mark the files with ALT-M. Once you have all of the files that need to be deleted hit ALT-D. Now you should just have files with bat calls in them remaining.
- VII. Hit ALT-R to reverse all marks (make sure all files are marked).
- VIII. Hit ALT-G. This opens up the text header for change. In this header you can enter in the location, date, and start and stop times. Try and keep the 2 SPEC fields empty).
- IX. At the bottom of the screen ANALOOK counts the # of files marked. This represents the # of files recorded (to be included in the report).
- X. Hit the enter key to make changes.
- XI. Use of the ID filter –
 - a. Press ALT – b to open up the filter menu
 - b. If the filter name ID is not listed in the menu Press O (letter o)
 - c. Type the name of the filter to open (ID) and hit ENTER
 - d. Hit A for apply

- e. The screen will return to the main ANALOOK screen and the filter should be loaded (the name of the filter should be to the right of the FILT located on the bottom center of the screen).
- f. Press ALT-Q
 - i. The program will then run through all of the files in the correct directory and will create parameter files containing data from calls that survive the cleaning process.
- XII. Double click the automated analysis program to start
 - a. Click the box next to 12 species.
 - b. Click browse and select the folder that houses all of the folders to be analyzed
 - c. Click run.
- XIII. The output file is generated automatically and is saved in the same folder as selected in b above.

Sample Directory Structure

```

11BXXXX (USFWS project number that will be provided)
  Site 1
    19May11
    20May11
  Site 2

```

Commonly Used Analoook Commands

- F (1-10)** – adjusts the total amount of time displayed on a single computer screen. This also alters the amount of time represented by each tick on the X –axis of the Analoook display.
- #0- 9** and **A-F** – moves the selected location (1-9, A-F on the X-axis) to the left-hand margin of the screen.
- ESC** – returns the display to real time and changes the total time displayed on the Y- axis to 15 second (F1 value)
- SPACEBAR** – toggles between real compressed times.
- RETURN** – redraws the graph of the currently loaded Anabat file
- RIGHT/LEFT ARROW** keys – moves to next/ previous pulse in the currently displayed Anabat file
- HOME key** – return the initial part of the file to the left hand side of the screen.
- CONTROL - B** adjusts the size of the pixels used to display the current file. Three different pixel sizes are available, one with each subsequent depression of these keys. Adjusting the pixel size enhances the image of the individual calls within a sequence.
- PAGE UP** – applies the currently opened filter
- PAGE DOWN** – unapplies the currently opened filter to return the file to an unclean state
- ALT – O** opens up the options menu which contains the following options:
- Colors – bring up a submenu that allows for the colors of different aspects of the display (background, axes, echolocation calls, etc.) to be customized. There are also several defined color schemes for the Analoook display.
- ALT- M** marks the file. When a file is marked a shaded box appears to the right of the filename located at the lower left corner of the Analoook screen. This command can also

be use to unmark a previously selected file. Several functions can be applied to marked files including:

ALT – U unmarks all selected files

ALT – G allows for the global change of the text headers for all marked files

ALT – C copies all marked files to another directory.

ALT – V moves all marked files to be moved to another directory. There is the option of deleting for the old directory all of the files that are moved.

ALT – R reverses the selection of the files. All marked files are unmarked and vice versa.

ALT – D deletes all marked files. Before the files are actually deleted, there is an option for the user to confirm the deletion by pressing Y or cancel the deletion by pressing N.

ALT- T saves copy of the currently displayed sequence of a particular file with the .TIF extension in the current directory. This extension indicates that the file is a picture of the displayed sequence that can be imported into a variety of graphic computer programs to generate reports or slide presentations.

SHIFT – L lists files in the selected directory for loading into Analook.

TAB moves among directories

LEFT AND RIGHT ARROW – moves cursor to select a directory or file

ENTER shows files in selected directory

ENTER opens highlighted file into Analook

M – allows for the creation of a new subdirectory

[- when viewing a file on the frequency – time scale, this key loads the previous file in the current directory into Analook.

] - when viewing a file on the frequency – time scale, this key loads the next file in the current directory into Analook.

CONTROL – D deletes the currently loaded Anabat file. Before the file is deleted, there is an option for the user to confirm the deletion by pressing Y or canceling the deletion by pressing N.

ALT – F opens the file menu.

Scanfiles (F) – scans through all of the files and marks files that meet the criteria set forth in the currently loaded filter

CountScan (C) – this option counts the number of pulses in each file that make it through the filter process.

ALT – B opens the new filter menu. This menu allows for the construction of a customized filter for the quantified cleaning of echolocation calls.

Appendix 7: Other Relevant Information to Indiana Bat Consultations

Things to Remember:

- Indiana bat survey study plans are no longer required to be submitted for approval by the Kentucky Field Office but must be submitted to KDFWR as part of their permit requirements.
- Summer portal surveys are no longer necessary.
- Indiana bat survey reports emailed to the Kentucky Field Office will no longer be accepted due to email size restraints.
- Make sure all corresponding permit numbers (e.g., coal) are included with the survey report.

Appendix 8: KDFWR Project Proposal Form

Contact Information:

Permittee Name: _____ KY SC Permit #: _____
Institution/Company Name: _____
Address: _____
City: _____ State: _____ Zip: _____
Email address: _____
Phone #: _____

Proposed Project or Activity Information:

County: _____ Quad: _____
Project location: latitude: _____ longitude: _____
(Please include an **8.5" x 11" topo or aerial map** with project/activity location)

Project or Activity Description (include size of project/activity area): _____

Acres of forested habitat within project/activity area: _____

Is the project/activity linear? Yes: No:

If yes, indicate length of forested area in km (mi): _____

Please provide the project specific permit# (SMCRA, USACE, etc.): _____

Are caves or portals present? Yes: No:

Methodology & Survey Effort:

Check **all** that apply:

Mist nets: Harp traps: Acoustic sampling:

Other (please specify): _____

Are methods & survey effort consistent with the Indiana bat Survey Guidance for KY dated [5/10/2011](#) Yes: No:

If no, please explain: _____

of mist net and/or harp trap sites: _____ # of acoustic sites: _____

Signature

Date

Appendix 9: Bat Handling/Disinfection Protocol for Researchers

At this time WNS has been confirmed or is suspected in sixteen states (see <http://www.fws.gov/WhiteNoseSyndrome/> for an updated list), including Kentucky). To minimize the potential transmission of WNS while handling bats (both handler-to bat and bat to bat), cautionary procedures are warranted. Any equipment that comes in contact with bats has the potential to be a vector for spread of WNS (for example, mist nets, harp traps, bat bags, wing punches, weighing tubes, rulers/calipers, gloves). The USFWS has developed guidelines for researchers titled “Supporting Decontamination Documentation for Researchers (WNS Decontamination Supplement 2 of 2) U.S. Fish and Wildlife Service Version 01.25.2011. This document can be found at the following weblink:

<http://www.fws.gov/WhiteNoseSyndrome/research.html>

Caves, mine portals and hibernacula

A temporary, voluntary moratorium has been placed on entering any caves/mines in Kentucky. All research conducted in Kentucky caves/mines should be coordinated with the Kentucky Department of Fish and Wildlife Resources or U.S. Fish and Wildlife Service – Kentucky Field Office personnel via the email addresses listed below prior to initiation. Alternative guidance on assessing potential summer roosting caves (i.e., Virginia big-eared and gray bats) and/or hibernacula (i.e., Indiana, Virginia big-eared, and/or gray bats) to determine presence/absence of federally listed species is provided below.

A Phase I Habitat Assessment (see 2011 Indiana Bat Survey Guidance) is still an acceptable first step determining the potential use of a cave or mine portal by bats. If this assessment concludes that the cave/mine portal has potential to be used by bats, then the consultant should notify the Kentucky Department of Fish and Wildlife Resources and/or U.S. Fish and Wildlife Service – Kentucky Field Office personnel via the email addresses listed below to determine proper methods and obtain site specific guidance.

Notification of Signs of WNS

As a reminder, the white fungus is only one of the signs of WNS, and we do not expect to find bats with fungus on them during the summer (once they are active and grooming). However, other abnormal characteristics may be indicative of WNS. Abnormal characteristics observed in summer may include: extremely underweight bats; flaky, dehydrated or wrinkled wing/tail membranes; wing lesions; discolored spots /scarring of flight and tail membranes; multiple small to medium sized holes in wing membranes; torn or necrotic areas at the trailing edge of wing and tail membranes. If you should capture a bat that exhibits signs of WNS or abnormal characteristics, inform the Kentucky Department of Fish and Wildlife Resources or U.S. Fish and Wildlife Service – Kentucky Field Office personnel via the email addresses listed below, and:

- Photograph all suspicious bats

- Record a wing score for each bat using the Wing Damage Index, found on the Northeastern USFWS page:

<http://www.fws.gov/whitenosesyndrome/>

- Report suspicious bats (and send photos) within 24 hr to: brooke.slack@ky.gov or mike_armstrong@fws.gov

Appendix 10: Recommended Weatherproofing System

The weatherproofing system is comprised of three main components: (1) a 6.5 quart Rubbermaid container; (2) a 2" diameter, 45° PVC Elbow; and (3) a tripod that extends a minimum of 36 inches.



Figure 1. Container with 2 in. diameter male adapter.



Figure 2. Open-ended cap which screws onto the male adapter.



Figure 3. Two in. diameter 45 degree angle PVC elbow with drip-holes.



Figure 4. Close-up view of adapter inserted in container with silicone sealant.



Figure 5. Assembled container with Anabat II system.



Figure 6. Complete weatherproofing set-up.