FREQUENTLY ASKED QUESTIONS (FAQs)

Range-wide Indiana Bat Summer Survey Guidance

January 2013

The U.S. Fish and Wildlife Service (Service) received a tremendous level of interest during our preparation for proposing revised summer survey guidance for the Indiana bat. We appreciate the comments received during and after the peer-review stage of our guidance development. We noticed several common themes among comments received to date and hope the following FAQs will be helpful to understand some of the background and rationale for the proposed changes.

Introduction/General Topics:

1. Why is the Service revising the summer survey guidance?

   The Service is proposing revisions to the summer survey guidance because we believe there are new and improved ways to detect presence of Indiana bats in the summer. The proposed revised guidance has been developed based on our current understanding of Indiana bat life history and their habitat. Traditionally, surveys to determine the presence or probable absence of Indiana bats in summer habitats have focused on mist-netting techniques. In addition to providing researchers and managers with comparable data sets to estimate changes in spatial distribution over time, other benefits of mist-netting include the collection of demographic data and the possibility of transmitter attachment. However, there are several limitations that reduce the effectiveness of mist-netting for the documentation of Indiana bats at a particular site, especially in areas where bats occur in low densities.

   First, mist-nets cannot be deployed in all habitats used by Indiana bats, thereby leaving these habitats under-sampled. Additionally, some bats avoid capture in mist-nets. It is well documented that Indiana bats, even when we know they are present, can be difficult to capture using currently accepted mist-netting survey protocols. In response to this limitation, studies have been conducted to investigate the effectiveness 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). Murray et al. (1999) deployed mist-nets and acoustical monitoring equipment at the same locations on the same nights and found that ultrasonic detectors consistently detected bat species that mist-netting missed, including Indiana bats indicating a higher detection probability with less "effort" than can be realized with mist-netting.

   Additionally, white-nose syndrome (WNS) has dramatically reduced bat densities across an expanding area, thereby reducing the effectiveness of mist-netting to capture bats. In post-WNS areas, low-detection probabilities for mist-netting normally associated with Indiana bats when present have been further reduced. Thus, with all of these limitations on mist-netting, another technique is now

needed to more effectively survey for bats under these rapidly changing circumstances.

Ultrasonic detectors allow researchers to eavesdrop on the echolocation calls produced by bats as they forage and navigate in their surroundings without disturbing them. Although ultrasonic detectors have been used for decades, recent advances in the equipment and quantitative analysis now allow for automated analysis of echolocation call data (Britzke et al 2011). With these advancements and since many bat echolocation characteristics are species-specific, bat detectors are now more efficient at documenting individual species presence than the time-consuming and labor-intensive traditional capture techniques such as mist-netting (Murray et al. 1999). Thus, the Service's decision to use ultrasonic detectors to determine presence or probable absence of Indiana bats and to focus subsequent survey efforts is a logical use of this technology in the current environment.

2. What is the intent of our survey guidelines? To detect maternity colonies, males or both?

   The intent of the summer survey guidance is to document the presence/probable absence of any Indiana bats, not just maternity colonies.

3. Will the Service be developing survey guidance for documenting migrating Indiana bats in spring and fall? Does the Service plan to develop guidelines for assessing potential hibernacula?

   While there is no current timeline in place for the development of these documents, the Service believes they are important to complete.

4. Is the Service considering increasing the timeframe for acceptance of negative survey results under the newly revised range-wide summer survey guidelines?

   Yes, we are considering this. Under the current 2007 mist-netting-based survey protocols, negative survey results were valid for a minimum of 2 years. Our team set a goal to extend the validity period for negative acoustic-based surveys to a minimum of 5 years. We assume higher detection rates/confidence levels will be achieved under our proposed acoustic surveys than under the current mist-netting protocol. However, we also assume that there may be a significant learning curve associated with the new acoustic protocol as surveyors become more experienced with their acoustic equipment and in selecting good acoustic survey sites. Therefore, we will keep the survey validity period set at 2 years and revisit this issue after the first two years of field implementation.

5. How long will the Service accept negative results from previously conducted surveys (following the 2007 protocols)?

   Surveys with negative results completed prior to the beginning of the 2013 survey season and in compliance with the 2007 protocols will be accepted for two survey seasons from the date of completion (i.e., a survey completed on July 1st, 2012 will be accepted until May 15th, 2014). However, surveys completed for some project types (i.e., coal mining) or at some project sites may be considered valid for a longer period of time (i.e., 5 years) due to existing biological opinions or when considering other available information about bat populations (e.g., in states with severely reduced bat populations).

6. How long are positive survey detections/records valid?

   Positive detections of Indiana bats, whether by acoustics or mist-netting are valid until such time that significant follow-up surveys consistently produce negative results. This is because it is well documented that Indiana bats have high site fidelity for summer home ranges, returning to these areas year after year as long as adequate habitat is present. In general, the Service will not accept a single negative survey following this revised range-wide guidance as sufficient evidence to conclude that the species is absent from a previously occupied area. Although acoustic detections and/or capture of Indiana bats under these guidelines confirm their presence, failure to acoustically detect or capture them in mist nets does not absolutely confirm their absence. Since each documented "presence" is unique (e.g., data availability, timeline of documentation), you should coordinate with your local Service FO(s), if you are interested in evaluating an area with a previously documented record.

7. How many field seasons are needed to accomplish all phases of the draft guidance?

   If timed appropriately, all surveys can be conducted within one field season. Whether survey effort carries over from one year to the next is dependent upon when survey work is started and the progression of each subsequent phase. As a reminder, Phase 1 habitat surveys can be completed any time during the year.

8. Will the Service require some form of training for each of the survey phases? If so, when will this likely occur and where can one go to receive Service-approved training?

   The Service is planning to offer training courses on various aspects of the summer guidelines in the near future. We anticipate that these courses will serve to certify that individuals are proficient in the skills required in the guidance. The courses will not, however, replace the need for Federal/State permits for those activities

that may cause "take"[1] of Indiana bats. We hope to work with the Service's National Conservation Training Center to develop the training program. Individuals who are currently proficient in conducting such surveys may be able to opt out of certain courses if they can show adequate experience/competence.

Appendix A- Habitat Assessments:

9. How many sample sites are needed within a project area (i.e., Habitat Assessment Worksheets)?

   The worksheet refers to "sample sites". Sample sites for the purposes of the habitat assessment refer to areas within the boundary of a project representing discrete or different habitat types present. A separate datasheet should be completed for each discrete habitat type observed. The number of worksheets completed depends on the size of the project and whether a single worksheet is representative of all habitats present or whether there are multiple habitats or variation of one habitat type to document.

Appendix B- Acoustics:

10. What types of detectors can be used during acoustic surveys?

    Frequency-division and direct-recording units can be used. There are four major classes of ultrasonic detectors: heterodyne, time-expansion, frequency-division, and direct-recording. Frequency-division and direct-recording units are broadband detectors that sample 100% of the time and are suitable for this protocol. Heterodyne detectors are not suitable for this protocol, because they only sample a small part of the frequency range used by bats. The reduced duty cycle (i.e., the detector does not record while a call is being "processed") offered by time-expansion detectors means that these detectors are also not suitable for this protocol because the results cannot be acceptably compared to detector types that record 100% of the time.

11. Why does the Service recommend verification of proper equipment functioning?

    As is the case with any electronic field-sampling equipment, verification that acoustic detectors are functioning properly is important to ensure that high-quality

[1] **Take**- to harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect or attempt to engage in any such conduct. **Harm** is further defined by the Service to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering. **Harass** is defined by the Service as actions that create the likelihood of injury to listed species to such an extent as to significantly disrupt normal behavior patterns which include, but are not limited to breeding, feeding, or sheltering.

bat calls are being recorded (Larson and Hayes 2000). It is common practice for electronic field equipment to undergo an annual verification process, with perhaps variable interval testing depending on demonstrated reliability (or lack thereof).

12. Will annual testing by manufacturers of acoustic detectors be required or will this remain a recommendation?

    The Service currently recommends having acoustic detectors tested annually, or at a frequency recommended by the manufacturer, to ensure that they are operating to the original manufacturer's specifications. Tests should be completed by the original manufacturer or by a reputable third-party vender. We believe this is a critically important component of the process to ensure that accurate, consistent and comparable results are produced. This likely will be a required step in the near future.

13. Why does the acoustic protocol specify the use of directional microphones?

    Microphones available for different bat detectors vary in the size and shape of their detection cones (i.e., the area/volume of air space in which a sound will be detected). Microphones can be classified into two broad categories: directional and omnidirectional. Omnidirectional microphones can detect sound from any direction; while directional microphones have a narrower detection cone. The focused detection cone of a directional microphone allows for better detection of sound in the direction that the microphone is oriented from farther away. While both types of microphones have their uses, directional microphones allow greater flexibility in sampling a wider variety of habitats. Furthermore, specific instructions on the most appropriate/effective orientation and placement of omnidirectional microphones for the detection of Indiana bats is not as readily available as it is for directional microphones making it imprudent at the current time to develop guidance necessary to allow their use for these protocols. We currently have developed protocols for deployment of directional microphones and are interested in developing similar methods that would allow for omnidirectional microphones to be used in the future.

14. Why does the Service recommend use of PVC elbows when weather proofing is desired?

    The Service recommends use of PVC elbows because they have been shown to have minimal interference with call quality. Surveyors are interested in protecting their acoustic detectors from unforeseen weather events that could damage the equipment, especially given the fact that detectors will be deployed in the field for extended, unattended monitoring (i.e., overnight). Unfortunately, these methods have the potential to affect the ability to detect bats. Currently, there are two popular types of weatherproofing options: (1) PVC elbow and (2) flat reflector systems. Both rely on reflective surfaces to guide sounds to the microphone.

Britzke et al. (2010) found that the use of a 45-degree PVC elbow resulted in performance similar to unprotected units. The PVC-elbow weatherproofing system allows for recording of suitable call quality and, therefore, is an approved method for weatherproofing detectors (see O'Farrell 1998; Britzke et al. 2010 for examples of this setup). Although the results of Britzke et al. (2010) suggested that reflector plates and PVC had the same ability to document the presence of Indiana bats at a given site, the differences in call quality, number of calls recorded, and species detected suggested there was a significant difference in detectability of Indiana bats between methods. Thus, other available weatherproofing, including the use of a flat reflector, is currently not accepted. As other after-market weatherproofing options become available, they may be compared to unprotected units to determine if they perform at an acceptable level.

15. Why is it important to closely evaluate the orientation and placement of detectors?

    Ultrasonic detectors can be deployed in a variety of orientations depending on the recording situation; however the orientation of the detector and relative position of the microphone may have a significant impact on the quality of recordings obtained (O'Farrell 1998). When deciding the orientation of the detector, the user should always keep in mind that the detection cone extends out from the unit. Horizontal orientations are not appropriate as they waste roughly half of the detection cone that is pointed to the ground. This serves to reduce call quality and detection (Britzke et al. 2010).

    Greater horizontal and/or vertical spatial variability within a sampling area increases the probability of missing bat species (Hayes 2000, Weller and Zabel 2002, Broders et al. 2004). The positioning of detectors can also create biases. The relative position of the microphone to the bats is also an important factor in call quality. If a microphone is above the bats (e.g., on a stream bank) call quality will be reduced.

16. Did the Service consider specifying the acceptable moon phase(s) for surveys?

    Yes; however, there is nothing in the literature beyond anecdotal accounts to suggest that increased moonlight has a negative impact on the activity level of Indiana bats or any insect-eating bat species. Reith (1982), Rydell (1991), Negraeff and Brigham (1995) and Hecker and Brigham (1999) strongly suggest that overall activity of insect-eating bats is not directly influenced by moonlight, in contrast to anecdotal reports (Fenton et al., 1977; Padgett and Rose, 1991).

17. Why does the guidance only address passive acoustic survey techniques instead of active acoustic survey techniques?

    The use of active acoustic surveys was considered in the development of this

guidance; however, we were not able to develop standardized methodologies that could be consistently applied across project types or all suitable Indiana bat habitats. Thus, passive acoustic survey is currently the only technique approved for use.

18. Are current federal permit holders considered "qualified" to conduct acoustic surveys?

    Yes. Current federal permit holders are considered qualified to conduct acoustic surveys due to their direct knowledge and experience working with Indiana bats. However, federal Section 10A(1)(a) permits are not required to conduct acoustic surveys because no take, as defined by the ESA, is expected to occur. The Service may develop a standardized training/certification process covering the various bat survey and identification techniques in the future.

19. Why has the survey effort been tripled for non-linear projects but not for linear ones?

    Most linear projects are narrow and as a result have small impact acreages (when compared to non-linear projects). Likewise, the area of potential impact within individual maternity colonies from linear projects is usually going to be much smaller than for a typical non-linear project. Our experiences with using acoustics within these narrow project corridors is that one site per kilometer of suitable habitat adequately samples the area of potential impact, while larger/wider project areas need more survey effort.

20. Did the Service consider requiring the use of mist-netting and acoustics in tandem? Isn't detection probability the highest when mist-netting and acoustic surveys are conducted in tandem?

    Yes, the Service considered this option. Numerous publications discuss the general advantages of using acoustics and mist-netting in tandem for inventorying bat communities (Kunz and Brock 1975, Kuenzi and Morrison 1998, Murray et al. 1999, O'Farrell and Gannon 1999, Flaquer et al. 2007). However, the primary purpose of this survey guidance is not to document the species complex present within a project area, but rather to document the presence or probable absence of Indiana bats within a specific project area. Acoustic surveys have proven to be a more efficient method of "capturing" or detecting Indiana bats. Detection probabilities for Indiana bats in post-WNS environments (where Indiana bats are still known to be present) are near zero using current mist-netting protocols. For example, preliminary work in 2012 at within a known maternity colony homerange at Fort Drum, New York shows that detection probabilities after 10, 15, 25, and 50 "detector-nights" were 0.67, 0.38, 0.48 and 0.45, respectively for 5 fixed-site acoustical recorders. Conversely, detection probabilities for mist-netting after 10, 15, 25, and 48 "net-nights" were 0, 0, 0, and 0.0192, respectively

for 10 areas netted in the same general area. Because current mist-netting detection probabilities are so low, the combination of netting with acoustics in the post-WNS environment is unlikely to increase the surveyor's efficiency and effectiveness for determining the presence of Indiana bats. Furthermore, after 16 nights (52 "net-nights") only one Indiana bat was captured during mist-netting in the same area referenced above in the summer of 2012 (U.S. Army- Fort Drum, unpublished data, 2012). For acoustics to be used in this way, Indiana bats have to be easy to detect and they must produce calls that are easy to identify. With the recent developments and ongoing improvements to automated acoustic ID programs, we have confidence that acoustics are suitable for use in the capacity recommended.

21. If Indiana bats are detected on the first night of acoustic surveys can the surveyors stop and/or begin mist-netting efforts?

    Yes. A surveyor may stop acoustic surveys within a given 30-acre section of the project area and begin mist netting once an Indiana bat(s) has been detected at an acoustic survey site. The suggested mist-netting minimum level of effort is based on the total number of acoustic survey sites with positive acoustic detections; thus, the number of nights or number of individual calls where Indiana bats are detected (at a given site) has no direct bearing on the next phase of the survey process. Although, a surveyor would typically want to locate mist nets closer to an acoustic site that had lots of positive detections rather than near one that had few hits.

22. Can a project proponent opt to complete acoustic and mist-netting phases concurrently/in tandem?

    Yes; however, project proponents may need to complete additional mist-netting surveys based on the final acoustic results (e.g., number of sites with positive detections, location of detections) if Indiana bats are detected.

23. Is the Service concerned about the lack of physical evidence of Indiana bat presence by shifting to acoustics for presence/absence?

    No. While it is true that acoustic records do not provide physical evidence (e.g., tissue samples) supporting a bat species' presence, such evidence has rarely been collected during recent mist netting surveys. The reliance on acoustics in this protocol is predicated on the fact that properly deployed acoustic detectors are better able to detect Indiana bats on the landscape than mist-netting. Thus, when using results from a program that has been validated, we will increase our survey effectiveness through the use of acoustic surveys. This increase in detection rate and efficiency is deemed more important than the need for physical proof gained through capture efforts. Additionally, assumed presence of Indiana bats can be corroborated and colony type discerned (e.g., maternity colony vs. adult males)

via physical captures during follow-up mist netting efforts in Phase 3.

24. Within automated acoustic ID programs, what is the number of pulses allowed in the discriminate analysis?

    This is a specific decision that will be made by programmers for each analysis program. However, to account for variation within a call sequence, a minimum of three pulses will be required.

25. What is a maximum-likelihood estimate and how are they used to determine species presence in this protocol?

    In statistics, maximum-likelihood estimation (MLE) is a method of estimating the parameters of a statistical model. When applied to a data set and given a statistical model, MLE provides estimates for the model's parameters. In general, for a fixed set of data and underlying statistical model, the method of maximum likelihood selects the set of values of the model parameters that maximizes the likelihood function. Intuitively, this maximizes the "agreement" of the selected model with the observed data, and for discrete random variables it indeed maximizes the probability of the observed data under the resulting distribution. MLE gives a unified approach to estimation, which is well-defined in the case of the normal distribution and many other problems.

    For our purposes, the MLE is a statistical method that can be used to determine species presence or probable absence at a particular site on a particular night by means of a classification matrix. This matrix is developed within the analysis programs used for the acoustic identification as well as the number of calls identified as each species. While this method relies on the classification matrix of all of the species included in the species model, it can be improved upon by developing regional models limited to the species that are incorporated in the analysis. However, the species model is robust to species being absent from the area as it also relies on the number of files identified as each species. This serves to allow its use in places where there might be a slight change in the members of the species community.

26. Can the Service clarify the interpretation of Maximum-Likelihood Analysis?

    While it is well recognized that *p*-value thresholds such as 0.05 are arbitrary benchmarks, a *p*-value “provides a measure of the strength of the evidence against a specified null hypothesis” (Cherry 1998), e.g., that the bat recorded is not an Indiana bat. *P*-values are not the probability of falsely rejecting the null hypothesis. In other words, it is incorrect to assume with a *p*-value 0.01 that one will be incorrectly identified out of 100 samples. Additionally, the probability values provided in the guidelines of 99%, 95% and 90% from the maximum-likelihood indicator relate not to the probability of a “missed” bat but rather a

measure of the probability that the call parameters recorded matches the distribution of the known or established call parameters of that species as determined by Britzke et al. (2002).

27. Is the Service requiring a minimum size for call libraries used by acoustic identification program developers?

    No. Acoustic identification programs rely on a library of known bat calls for the correct classification of species. While there is no magic number of calls needed to adequately represent the call repertoire of a given species, in practice, a call library must be extensive enough to include the normally accepted sources of variation (i.e., intra-individual, inter-individual, and geographic). Sufficiency and utility of a program's associated call library will be assessed by the accuracy rates achieved by the developers and ultimately by the Service during our validation testing.

28. How will the Service determine which software programs are capable of accurately identifying Indiana bat calls recorded in the field?

    Each acoustic identification program submitted to the Service for approval and official use with the range-wide Indiana bat summer survey guidance will be independently evaluated by U.S. Geological Survey biologists, who will conduct a standardized test of the programs to determine whether a program meets the Service's approval standards and passes the testing criteria (e.g., accuracy rates). The Service's draft standards and testing criteria are available online (http://www.fws.gov/midwest/Endangered/mammals/inba/inbasummersurveyguidance.html). Once finalized, the standards and testing criteria will ensure that each program approved for use has met a set of minimum standards and, therefore, provides a consistent analysis and results. A list of candidate and approved programs will be maintained on the Service's Indiana bat summer survey guidance website: http://www.fws.gov/midwest/Endangered/mammals/inba/inbasummersurveyguidance.html

29. How will the Service address new analyses of existing acoustic data with improved software over time?

    The Service is required to use the best available information during our decision-making processes. If new analytical tools/programs prove to have a higher confidence in accurately identifying species, then we may recommend reanalyzing existing acoustic data for various sites on a case-by-case basis.

30. Have any acoustic data analysis software/programs been peer-reviewed and field-tested?

    To our knowledge, no automated software programs for identifying Indiana bats (or other eastern U.S. bat species) have been peer-reviewed and published in the scientific literature yet. However, several programs have been informally used and "beta-tested" using field-collected calls recorded by interested users throughout the species' range. Nonetheless, acoustic analysis through the earlier discriminate function analysis (DFA) has been used for several years as a research tool that has been accepted as authoritative and accurate for assigning habitat use/preference relative to bat activity as identified by species (Ford et al. 2011, Corcoran 2007, Schirmacher et al. 2007, Britzke et al. 2002, Parsons and Jones 2000). Even though DFA is often considered as "best science" at present, the change of its use as an investigative tool to a regulatory assessment tool requires additional scrutiny to better understand its accuracy rates, risk, and probabilities in this new context. The Service will be independently evaluating each program submitted for approval and use with the range-wide summer survey guidance to determine if submitted programs meet our standards and testing criteria (See FAQ #28). The Service/USGS will test each program using a standardized test and call library, which will include 'raw' field recordings that were collected following the Service's proposed acoustic protocols and 'known' species' calls submitted from trusted sources throughout the Indiana bat's range.

31. Are there options for researchers that have their own call libraries to develop their own ID programs?

    If a researcher develops a program meeting all the standards set forth by the Service and her/his program passes the Service's standardized test, then it may be used for analysis of acoustic bat calls during official summer surveys for Indiana bats.

32. Who do you contact if you have questions regarding which species set to select for the ID program for the area you are sampling?

    Individual automated programs will provide instructions on which sets of bat calls are most suited to your particular project area. Please contact your local Service FO(s) if there are questions regarding which set of calls to use in the analysis for your project.

33. What will the Service do if no acoustic ID programs pass the validation testing process in time for the 2013 field season?

    We believe that it is essential to survey for Indiana bats using protocols that will maximize the likelihood of their detection and employ tools that are accurate and efficient to use. Therefore, if we do not have a Service-approved automated

acoustic identification program(s) available for use in 2013, then Indiana bat surveyors would follow a contingency plan. The Service's proposed 2013 Contingency Plan is available for review on the Indiana bat summer survey guidance website: http://www.fws.gov/midwest/Endangered/mammals/inba/inbasummersurveyguidance.html

Appendix C- Mist-netting:

34. Between what dates does the Service accept captures as summer records of Indiana bats?

    May 15 – August 15 are the acceptable limits for capturing summer populations of Indiana bats, especially maternity colonies. However, when possible, we encourage mist-netting to be completed prior to August 1st to allow more time for radio-tracking efforts prior to the onset of fall migration. Because presence of Indiana bats will be established by acoustic detection under the new survey guidance, the primary purpose of mist-netting will be to capture individual bats for radio-tracking and documentation of summer roosts.

35. Is the Service concerned about losing bat population data that is currently gathered during mist-netting surveys?

    Most Indiana bat mist-netting surveys to date have been conducted to fulfill Endangered Species Act-related requirements. They have not been strategically or evenly conducted across the species' geographic range nor routinely conducted through time. They have mostly occurred on a haphazard, project-by-project basis and yield relatively low-quality data for the purposes of monitoring bat populations through time and space. In fact, during a recent bat population-monitoring workshop, it was quickly decided that randomly deployed mist netting is not an effective method for monitoring bat populations (S. Loeb, U.S. Forest Service, pers. comm., 2012). The existing and currently proposed Indiana bat summer survey guidance is not designed to provide monitoring data. It is designed to determine the presence or probable absence of Indiana bats in a project area during the summer maternity season. While we recognize that the collection of data on non-target bat species has been a very tangible benefit of the traditional Indiana bat mist net surveys and has produced a lot of data that otherwise would not have been collected, this fact alone was not reason enough for us to not to switch to a more efficient and effective means of detecting our focal species on the landscape. When examining presence/absence, numerous studies have shown the effectiveness of acoustics (and this effectiveness gap appears to be increasing in a post-WNS world). As each project is independent in its determination of presence/absence, there is no benefit to overlapping methods.

36. Are surveyors required to use the example mist-netting data sheets provided in the guidance?

    No. The example datasheets provided are not required to be used for mist-netting surveys. Surveyors should, however, ensure that all of the data fields listed on the example data sheet are included in whatever datasheet they opt to use.

37. Is the 1-mile radius buffer circle a permanent Indiana bat acoustic 'capture' area? Is a maternity colony assumed within the 1-mile radius buffer circle?

    The 1-mile radius is an area used to focus mist-netting in the vicinity of where acoustic detections occurred and has no bearing on the geographic size of the assumed maternity colony. An assumed maternity colony based solely on an acoustic detection should receive a 5-mile buffer as we traditionally have done for mist net captures of reproductive adult females and/or juveniles without associated roost tree data.

38. If a "net night" is now a single mist net set operated for a single night, how many individual mist-net sets (or net nights) can one federally permitted biologist operate per night?

    Traditionally, a permitted biologist would run a pair of mist net "sets"/site/night for two calendar nights. As currently proposed, a single permitted biologist could only operate as many net set-ups as he/she could reasonably manage while maintaining the 10-minute net check timing while **walking** (not driving) between nets.

39. What was the Service's thought process leading to the minimum level of survey effort for mist-netting within a 1-mile radius around positive acoustic hits?

    The following is an explanation of the sampling scheme with mist-netting (Phase 3) following acoustic surveys (Phase 2) after each group of detectors in which an Indiana bat was detected are grouped to form 1-mile circles.

    Within a circle, the sampling effort varies with the number of Indiana bat hits.

    1. 10 net nights for 1 + detector site
    2. 14 net nights for 2 + detector sites
    3. 18 net nights for 3 + detector sites
    4. 20 net nights for 4 or more + detector sites

The following 3 examples of different scenarios are provided for clarity:

Example A. There is a single 1-mile circle with a single positive detection within it. This scenario would require 10 mist-nets nights of survey effort as a minimum (unless Indiana bats were captured with less effort).

Example B. There is single 1-mile circle with 5 positive detections within it. This scenario would require 20 mist-net nights of survey effort (unless Indiana bats were captured with less effort).

Example C. There are now 5 independent (i.e., non-overlapping) circles. In each circle there is a single positive detection. This scenario would require a total of 10 mist-net nights in each circle for a total of 50 mist-net nights (unless Indiana bats were captured with less effort).

The logic behind this sampling scheme is as follows:

1. When a single positive detection occurs, 10 mist-net nights typically represents enough sampling effort to capture Indiana bats in an area.

2. If positive detections are spread across the landscape, then each hit or small group of hits should be treated as individual colonies and the effort should be separate for each.

3. When looking within a single 1-mile circle, the sampling effort increases with the number of positive acoustic detections. However, as the number of acoustic sites with positive detections increases, we assume it will become easier to capture the bats with mist nets. Thus, the number of mist-net nights decreases as the number of acoustic detections increases. If we look at the sampling effort in terms of the number of sites with detections, then the rate actually does decrease:

    a. A single site with a positive detection prompts 10 mist-net nights.

    b. Five sites with positive detections prompts 20 mist-net nights, which translates to a much lower survey effort of 4 mist-net nights per acoustic survey site with a positive detection.

40. What happens when approved acoustic identification programs indicate (with high confidence levels) that Indiana bats are present, but none are captured during follow-up mist-netting efforts?

Because acoustic surveys tend to have higher detection rates than traditional mist net surveys, we would expect this situation to occur at some sites. Unless there is

site-specific information to suggest otherwise, the Service will take a conservative approach and consider the acoustic record to be valid and assume that it was from an Indiana bat associated with a maternity colony. Similar to mist-net-only captures now, the Service will place a 5-mile buffer around the positive detection and consider suitable habitat within that areas as presumed maternity colony habitat until such time that future survey effort can further define the type of use.

41. Will the Service ever assume that an Indiana bat maternity colony is NOT likely to be present based upon results of mist-netting and/or radio-tracking and emergence counts? If so, under what scenarios?

    The Service will rely on mist-netting and radio-tracking efforts to confirm that an Indiana bat maternity colony is NOT present. For example, if a male (or non-reproductive female) is captured and tracked to a tree where it or only a few bats emerge for multiple days and that bat never leads to a tree with numerous bats emerging (e.g., ≥5 bats), then the Service would likely concur that a maternity colony is not present. As currently is the case, any capture of a reproductively active adult female (i.e., pregnant, lactating, or post-lactating) or juvenile Indiana bat between 15 May and 15 August will confirm the presence of a maternity colony.

42. Is the Service requiring that a standard number of mist-net tiers (stacked nets) be used for each mist-net set up?

    No. Bat flight corridors come in many heights and widths and thus, optimal mist-netting sites within a project area are seldom at a consistent height. So, it would not be prudent to require a standard mist-net height to be used. The best mist-netting locations should be netted based on a suite of factors and not be limited to sites where a high net can be placed.

43. Why doesn't the Service require fecal DNA analysis be conducted for all questionable Indiana bats?

    While we still encourage the opportunistic collection of guano specimens from all captured Indiana bats, the Service does not require that fecal DNA analysis be conducted on questionable bats due to the simple fact that, currently, we are not aware of any genetics labs willing to provide this service on an ongoing basis.

Appendix D- Radio-tracking:

44. Has the Service considered setting a weight limit on radio-transmitters?

    Any transmitter attached to a bat has the potential to influence its behavior – no matter how small or light. The purpose for attaching transmitters to captured

Indiana bats is to document roosting locations, although foraging data may also be a project-specific goal, depending upon the proposed action. A heavier transmitter generally will have more effect on foraging studies than on documenting roosting locations. For most studies, especially foraging studies, transmitters should weigh less than or equal to 5% of body weight (American Society of Mammalogists 1998). If project-specific goals (e.g., roosting studies) justify the use of heavier transmitters, then transmitters weighing up to 10% of body weight may be used (O'Donnell 2006).

Also, it is important to consider percent body weight in terms of normal animal weight. Indiana bats average about 8 grams, but a pregnant female can weigh up to 10 grams. Adding a 1 gram (10%) transmitter to a pregnant Indiana bat would be unacceptable no matter the research question (i.e., equivalent to a bat carrying greater than 30% of its weight) (Hayssen and Kunz 1996). With pregnant bats, researchers should always use the lightest transmitter possible but no more than 5% of their normal weight as opposed to pregnant weight. The guidelines also state that no bats under 6.0 grams should be transmittered, which helps account for the health of the bat and juveniles that are of sufficient size to carry a transmitter.

45. Why is radio-tracking of all Indiana bats being required? What justification can the Service provide for imposing this additional regulatory burden?

Under the draft revised range-wide guidance, mist-netting techniques are no longer used to determine presence or absence of Indiana bats from a specific area. Instead, mist-netting is being used for the primary purpose of capturing bats to conduct radio-tracking. Radio-tracking is used to locate summer roost trees (and possibly for foraging studies) for Indiana bats so that these very important habitats can be identified and protected. If a project proponent is not interested in radio-tracking, then they should not opt to mist-net.

The Service has "encouraged" radio-tracking of captured Indiana bats for some time, but the focus has typically been on reproductively active females that may lead biologists to maternity roost trees. If radio-tracking is conducted, the protocol now requires that all (a maximum of 5 individuals per potential colony) Indiana bats (i.e., males, females, and juveniles) that meet the weight requirements be transmittered and radio-tracked, with discretion given at the Service FO level for males. This change is the result of numerous studies over the last decade documenting that males and non-reproductive females may, in fact, roost within maternity colonies, and thus, by tracking these individuals, there is a reasonable chance of locating a maternity colony. Furthermore, all individuals are afforded protection under the ESA from take. Information gained from radio-tracking any Indiana bats captured will aid in selecting appropriate avoidance and minimization measures necessary at the project level.

Literature Cited

American Society of Mammalogists. 1998. Guidelines for the capture, handling, and care of mammals. Journal of Mammalogy 79:1416-1431.

Britzke, E.R., K.L. Murray, J.S. Heywood, and L.W. Robbins. 2002. Acoustic identification. Pages 221-225 *In* A. Kurta, and J. Kennedy, eds. The Indiana Bat: Biology and Management of an Endangered Species, Bat Conservation International, Inc., Austin, Texas.

Britzke, E.R, B.A. Slack, M.P. Armstrong, and S.C. Loeb. 2010. Effects of orientation and weatherproofing on the detection of bat echolocation calls. Journal of Fish and Wildlife Management 1(2):136-141.

Britzke, E.B., J.E. Duchamp, K.L. Murray, and L.W. Robbins. 2011. Acoustic Identification of Bats in the Eastern United States: A Comparison of Parametric and Nonparametric methods. Journal of Wildlife Management 75(3): 660-667.

Broders, H.G., C.S. Findlay, and L. Zheng. 2004. Effects of clutter on echolocation call structure of *Myotis septentrionalis* and *M. lucifugus*. Journal of Mammalogy 85:273-281.

Cherry, S. 1998. Statistical tests in publications of The Wildlife Society. Wildlife Society Bulletin 26:947-953.

Corcoran, A.J. 2007. Automated acoustic identification of nine bat species of the eastern United States. MS Thesis, Humboldt State University. Arcata, CA.

Ford, W.M., E.R. Britzke, C.A. Dobony, J.L. Rodrigue and J.B. Johnson. 2011. Patterns of acoustical activity of bats prior to and following White-nose Syndrome occurrence. Journal of Fish and Wildlife Management 2:125-134

Fenton, M.B., N.G.H. Boyle, T.M. Harrison, and D.J. Oxley. 1977. Activity patterns, habitat use and prey selection by some African insectivorous bats. Biotropica 9:73-85.

Flaquer, C., I. Torre, and A. Arrizabalaga. 2007. Comparison of sampling methods for inventory of bat communities. Journal of Mammalogy 88:526-563.

Hayes, J. P. 2000. Assumption and practical considerations in the design and interpretation of echolocation-monitoring studies. Acta Chiropterologica 2:225-236.

Hayssen, V., and T.H. Kunz. 1996. Allometry of litter mass in bats: comparisons with respect to maternal size, wing morphology, and phylogeny. Journal of Mammalogy 77:476-490.

Hecker, K.R., and Brigham, R.M., 1999, Does moonlight change vertical stratification of activity by forest-dwelling insectivorous bats? Journal of Mammalogy 80: 1196–1201.

Kuenzi, A.J., and M.L. Morrison. 1998. Detection of bats by mist-nets and ultrasonic sensors. Wildlife Society Bulletin 26(2):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.

Larson, D. and J.P. Hayes. 2000. Variability in sensitivity of Anabat II bat detectors and a method of calibration. Acta Chiropterologica 2(2):209-213.

Murray, K.L., E.R. Britzke, B. Hadley, and L.W. Robbins. 1999. Surveying bat communities: a comparison between mist nets and the Anabat II bat detector system. Acta Chiropterologica 1(1):105-111.

Negraeff, O.E. and R.M. Brigham. 1995. The influence of moonlight on the activity of little brown bats (Myotis lucifugus). Zeitschrift für Säugetierkunde 60:330-336.

O'Donnell, C.F.J. 2006. Personal communication. Southern Regional Science Centre, Department of Conservation, Christchurch, New Zealand *in* Ecological and behavioral methods for the study of bats/ edited by T.H. Kunz and S. Parsons – 2nd ed. 2009.

O'Farrell, M.J. 1998. A passive monitoring system for Anabat II using a laptop computer. Bat Research News 39:147-150.

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(1):24-30.

Padgett, T. M. and R. K. Rose. 1991. Bats (Chiroptera: Vespertilionidae) of the Great Dismal Swamp of Virginia and North Carolina. Brimleyana 17:17- 25.

Parsons, S. and G. Jones. 2000. Acoustic identification of twelve species of echolocating bat by discriminant function analysis and artificial neural networks. Journal of Experimental Biology 203:2641-2656.

Reith, C.C., 1982. Insectivorous bats fly in shadow to avoid moonlight: Journal of Mammalogy 63: 685–688.

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 15:275-28.

Rydell, J. 1991. Seasonal use of illuminated areas by foraging northern bats, *Eptesicus nilssoni*. Holarctic Ecology 14:203-207.

Schirmacher, M.R., S.B. Castleberry, W.M. Ford and K.V. Miller. 2007. Species-specific habitat association models for bats in South-central West Virginia. Proceedings of the Annual Conference of the Southeastern Fish and Wildlife Agencies 61:46-53.

Weller, T.J., and C.J. Zabel. 2002. Variation in bat detections due to detector orientation in a forest. Wildlife Society Bulletin 30:922-930.