Mobile Bat Acoustical Survey Protocol

U.S. Fish and Wildlife Service, Region 4

Division of Refuges

Introduction

The establishment of mobile bat acoustical surveys on National Wildlife Refuges within Region 4 is being undertaken for two central purposes. First, this work will establish a baseline inventory of the bat species which occur on or near national wildlife refuges. This information will then provide opportunities to explore more focused questions regarding habitat occupancy of threatened, endangered, or species of concern at the local, refuge level. Second, these efforts will contribute to regional and national efforts to provide population level estimates/ indices of bats. Through a collaborative effort of state, federal, and other conservation organizations, long-term monitoring is being undertaken to address population declines attributed to white-nose syndrome, wind energy development, habitat loss/modification, and urbanization. Acoustical detection equipment and automated software for species identification in conjunction with a mobile sampling methodology provides a robust, relatively unbiased measure of species abundance from which population trend analysis can be evaluated. Fundamental to this abundance measure is that the protocol used to collect data be standardized among sampling areas and be spatially and temporally repeated across the population of interest. Given the relatively low rates of bat detections/mile separated by species; potentially high variability of detections between sampling periods; and challenges with separating calls by species, it is critical that other factors which might increase variability in the data be minimized. Therefore, the development and adherence to a standardized collection protocol is imperative. Equally important is a process of selecting appropriate survey routes, actual use and selection of the bat echolocation data logger, survey route establishment, data management and storage, analysis of bat calls, and establishment of a project record. The following protocol provides direction for these actions and insures the overall quality and integrity of the data collection process.

Objectives

Project Objective *:* Establish mobile transects across refuges in the Gulf Coastal Plains and Ozarks and South Atlantic Landscape Conservation Cooperatives to inventory and monitoring bat communities.

Proposal Objective *:* The primary objective of the funded proposal is to perform a baseline inventory of bat species on refuges across the southeast region. This will provide presence/absence data sets and be the foundation for long term monitoring of bat populations. This project will inform what species are present, where they are present and may be the initial step towards determining whether these species need to be listed. Specific objectives of the project are:

1. Determine baseline occurrence of bat species on refuges in the southeast during the breeding season.
2. Index bat populations on a species by species basis to monitor population trends at the refuge, landscape and regional level using standardized protocol using acoustical detection data loggers.
3. Examine bat species occurrence based on habitat type classification.
4. Integrate indices of bat species abundance with other agencies to support Strategic Habitat Conservation initiatives for these species on a broader geographic area.

Protocol and Methods

Acoustical Bat Detectors

Presently there are a number of acoustical bat detection devices in the US commercial market (e.g., Anabat, Petterson, Avisoft, and Wildlife Acoustics). The detectors capture high frequency (mostly ultrasonic) bat echolocations and convert them to an audible tone we can hear (lower the frequency of the call to <20 kHz) and provide a means to digitally store and analyze the structural characteristics of the call so one can identify them to species. The three primary technologies for collecting and converting the bat calls involve **1) heterodyne, (2) frequency division,** and **(3) full spectrum**. The most simplistic is the heterodyne system which allows one to actively monitor bat calls by tuning the device to a narrow frequency which better defines potential species. Frequency divisions detectors (also referred to as zero-crossing analysis) cover the entire frequency range of bat echolocation calls but limit certain aspects of the call signature as a means of reducing data storage. Full spectrum (time-expansion) devices capture frequency division parameters of the calls and also amplitude and harmonic measures. Generally, full spectrum units collect more information about each call and therefore a greater ability to describe calls to species.

For the purpose of this survey design, it was decided to limit the bat detector to **the Anabat SD2** and the older **Anabat SD1** (Titley Scientific). These frequency division detectors are widely used in the USA and meet the standards necessary to efficiently capture and store bat echolocations. By defining the detection type, this provides a standardized unit that makes comparative data analysis easier. The ability to efficiently and accurately describe bat species from frequency division detections has also been developed through commercial and open source software (**see Data Analysis Section**). In addition, issues regarding firmware and sensitivity of microphones are eliminated by adhering to a subset of detectors on the market. Because directional microphones have some effects on data signal capture it is important to define the standard microphone for use (3 microphones types are available for the SD1 & SD2 units). If an external microphone is used with a roof-mounted bracket (preferred system), this should

be the GREEN unit. This microphone utilizes more power to address signal loss along the cable connector to the Anabat detector. **DO NOT USE** the Black microphone with the cable extension. If you do not have the roof-bracket, or the Green microphone should fail during a sampling period, data can still be collected by directly placing the Anabat unit on the roof with attached Black microphone and obtain the same quality of data. The microphone should be oriented vertical.

The Anabat detector has 2 other on-board controls which need to be addressed. This is the sensitivity controller, and the Data DIV selector. The sensitivity controller acts as a pre-filter to obtaining ultrasonic noise and can be used to limit the detection distance of the unit. Higher values allow further detection but increase back ground noise while very low numbers will substantially reduce call files. **This controller should remain set at ~7. DO NOT CHANGE IT (see exception below for calibration).** The DATA DIV selector acts to subsample acoustical data information as a means of increasing storage capability on the CF CARD (compact flash memory card). **The DATA DIV selector should be set at 8.** Periodically check these setting while conducting the survey.

Data Logger Frequency Validation and Microphone Calibration

The potential exists for the calibration and sensitivity of the data logger to drift. These parameters should be evaluated before deployment to insure the unit is working correctly. The data logger needs to be able to correctly measure the frequency of a call. Small shifts of (> 2 kHz) can dramatically affect the classification of a calls. The sensitivity of the microphone is the ability to "hear" a high frequency pulse at a maximum distance from the unit based on an initial sensitivity setting. Damage to the microphone can occur in the field and may reduce detection distance. Therefore it is important to calibrate and validate the data logger and microphone before each use.

Frequency validation of the Anabat detector is done by using the Anabat Chirper. The chirper produces a set tone at 40 kHz. To validate the detector is correctly calibrated; turns the data loggers on with a CF Card installed and press the 40 kHz button several times for several seconds. Subsequently download the data through CFC Read and view the corresponding call file in AnalookW. A horizontal line at 40 kHz should be recorded. If a shift of > 2 kHz is detected above or below 40 kHz, the calibration will need to be adjusted (return to Titley distributor for evaluation). Do not collect data if the unit is not functioning properly; obtain an alternate detector.

To calibrate the microphone sensitivity, you need to set the controller of the data logger to 7 (seven). Orient the microphone horizontal and walk ~50 feet from the unit repeatedly pressing the CHIRP button directly at the microphone. Adjust the distance either direction until the observer with the data logger no longer hears the chirp. Measure the distance to the microphone; this establishes the baseline sensitivity reading for the surveys (record this distance for the baseline calibration). To subsequently calibrate any drift in the microphone sensitivity, one merely returns to the establish distance and checks that the unit is "hearing" at the upper limits of detection for the established distance. Small upward or downward adjustments of the sensitivity controller may be needed to insure similar detection sensitivity. Prior to each survey, this calibration should be done.

Geo-referencing Acoustical Data and Survey Routes

The ability to provide locational information regarding the survey route and subsequently overlay specific calls provides opportunities to explore habitat occupancy and better understand changes of bat detections among various habitat types. In some situations, changes in bat detection at the refuge level may directly correlate with significant habitat modifications (e.g., stand harvest, growth of reforested areas). In such circumstances, these local level changes may not reflect population changes.

Because the population index to monitor bats is based on a repeated survey of established routes, it is important to map the transect. This provides a distance measurement from which to gauge bat detections/mile surveyed. The transect only needs to be geo-referenced once. There also is the need to collect site specific call location information which can be used later to infer habitat selection using GIS modeling and adjust for probability of detection among habitat cover types.

Geo-referencing the survey route can be done with any Global Positioning Unit or via integrating the Anabat data logger, PDA and an external GPS unit. Regardless of the process, the survey route should be mapped using a standardized projection system. This will be established as: **(Latitude, Longitude, Datum = WGS 84).** The external GPS Mouse has predefined these parameters. Survey routes which are made of several segments or include back-tracked segments should be mapped individually and then joined as a single line feature (Shapefile). This will prevent accidentally increasing the actual distance surveyed (see below).

If you are using a hand held GPS attached to the Anabat detector, these settings must be set prior to collecting the information because the AnalookW software cannot directly process other projections to embed the location information of individual call files.

Survey Route

The survey route (i.e., transect) represents an important consideration for the mobile acoustical sampling design. Several factors should be considered when creating the route because once established, the baseline of any change is predicated on repeating the same transect and should not be modified in the future. Parameters to consider include: location on the refuge and adjoining lands, transect length, habitat types, road conditions, traffic volume, and repeatability over several years.

A transect should be constructed in a manner that samples representative habitat on the refuge based on roads which a car or truck can easily navigate. However, interior road systems may preclude a major portion of the route from occurring on the refuge and necessitate sampling adjacent to the refuge.

The route cannot resample across itself. However, this does not prevent a route from going down a dead-end road system. The portion of backtracking is not counted for sampling purposes and the data logger is turned off during this interval. Given the way refuge roads exist, this may occur frequently if you want to capture information mostly from the refuge. Even a good loop transect may encounter the need to backtrack some sections. Again, these sections are not included in the distance nor will the data logger be recording during this time.

Given the time limit for completing a survey (2 hours after sunset), a transect length should be between 20-30 miles. The length only includes uniquely sampled portions (not back-tracked sections). Most routes will likely take 1.5 hours to complete.   If the transect needs to be smaller not a problem. It is merely ideal to be 20-30 miles and conducted within a 2 hour period.

The transect should be designed to go through the representative habitat types on the refuge **if possible**. One should limit major sections which consist of large expanses of agricultural fields that occur off the refuge. This may not be possible to exclude but the idea is not to have major portions of the transect off the refuge consisting of open agricultural fields

The road conditions and traffic volume are issues dealing with safety and the ability to maintain a relatively constant speed of **20 mph**. The speed is critical because it affects the quantity of calls obtained by predefined pulse constraints of the hardware and software. Faster speeds yield much fewer usable calls and slower speeds can greatly increase call files. Recognize that you will be going at well below normal speeds on certain roads during reduced periods of visibility. It is better to fragment a transect into smaller sections to avoid high speed roads than create an accident.

Finally, think about the repeatability of the survey within a season and among years.   Evaluate whether a particular road or route will be under water 1 in 5 years during the first part of the sampling period or will have other logistical constraints that makes repeatability within the season or among years difficult. Repeatability of the ENTIRE route is paramount and necessary for long-term monitoring. Conduct a dry run of your transect at night before committing to the design and adjust as necessary.

Sampling Period and Duration

The sampling period and duration describe the calendar months and time period after dark during which the sampling will be conducted. Within the southern and mid-Atlantic regions, the period needs to be limited to when only resident bats are present (i.e., excludes a large period when northern migrant bats might occur).   In addition, the sampling period needs to be narrow to increase the precision of detections along the sampling route through multiple surveys, and prevent sampling of volant young which would bias detections upwards. Within the Southeast, a sampling period of **June 1 – July 15** represents the appropriate sampling period. The starting period could be adjusted earlier for refuges along the coast and into Florida where migrant species are unlikely to occur. Surveys should be repeated in subsequent years within the same **10-day** window of the baseline survey.

The degree of variability of bat detections may be a function of habitat structure, height of bats flying, and multiple weather conditions. None of these are singularly or in combination well understood. Thus, these uncontrolled set of covariates may substantially increase the variance associated with sampling within and among years. Two approaches provide means of reducing the variability; increasing sample size (more unique transects on the landscape) or repeated sampling of the same transects (pseudo-replication-increases precision of the bat detection estimate on that transect). For refuge complexes, efforts should be made to conduct at least 1 transect for each refuge annually. This provides a means of monitoring bat populations and also a baseline inventory of the bat species (presence/apparent absence). All refuges should subsequently resample of each transect preferably 2 times/year. These pseudo-replications will be used to adjust for probability of detecting bats by species, and within and between habitat types, and

allow for other statistical considerations of the data. The resampling of transects needs to be done during the June-July 15 period.

The duration of the detection on a given night is important because bats tend to have a bimodal activity pattern with highest activity occurring the first few hours after dark and those immediately preceding sunrise.   For logistical considerations, the time period of sampling should begin **½ hour after sunset and be completed within 2 hours**. **Do NOT** start earlier than this time frame.   Subsequent surveys along the transect must be initiated within 10 minutes of the start time or delayed until a subsequent day.

(For the purpose of a more complete inventory on a refuge, alternative survey periods and locations should be undertaken to examine seasonal bat species occurrence and/or inventory at fixed sites with high bat activity potential).

Weather Conditions

Ambient conditions have a major effect on bat activity. Wind, rain, temperature, and fog reduce bat activity at varying degrees. To minimize these effects the survey should be conducted within the following weather parameters: wind speeds of less than 15 mph, no rain, and no fog. If winds become excessive during the survey, fog develops over major portions of the survey or constant rain occurs for >10 minutes) the survey should be terminated and conducted on an alternate night and the data NOT used for monitoring.

(*Data should be retained an used for baseline inventory at the refuge level*)

Data Download and Filter Parameters

During passive monitoring, high frequency noise is automatically recorded and stored within a single file (DATA.DAT) on the CF Card in the Anabat Detector or if so configured to the PDA. This includes high frequency insects, other environmental noise, and bat echolocations. In order to analyze the data, this file must be extracted into multiple files representing the raw data, individual call files, GPS data, and data logger status information. The **CFC Read** software determines what potentially is a bat call based on a set of parameters (**smooth-50, min line length-5, and MaxTBC-1**). If these parameters are modified, especially the MaxTBC, the number of uniquely exported Anabat Data files will be substantially altered. **Set these parameters as the default.** The exported Anabat Data files from **CFC Read** will be subsequently processed as a means of eliminating those without bat calls. The remaining files will then be automatically processed by software to identify them to species or functional/frequency groups. (*Therefore, the initial output files from **CFC Read** application may generate several hundred **potential** bat call files. However, through additional filters and analysis perhaps only 25% contain actual bat calls; and fewer yet can be identified to species with confidence*).

To embed GPS coordinate information into each Anabat Call file, the GPS data needs to be date and time synchronized with the bat detector. GPS date and time information is based on Greenwich mean time. When downloading data through CFC Read, you will need to adjust the time zone value (central daylight savings time (-5), central standard time (-6); eastern daylight savings (-4), eastern standard time (-5)). If you do not adjust the time zone, the coordinate information will not be embedded in each Anabat File.

Data Management and Storage

The management of acoustical data and subsequent long-term storage is critical for monitoring and difficult if not properly addressed. As such, Region 4 will be developing a centralized data storage location. This will serve both to archive the data and also serve as a means to redistribute the data for regional and national analysis. In addition to a regional storage location, original raw data sets should be archived at each refuge.

The data manager for the Inventory and Monitoring Network will be developing a sharepoint site or database application for data upload by field personnel. Each refuge will have designated folders/database profiles and appropriate permission to upload field data. Files to be uploaded to the central site will consist of the following: xxxx.dat, xxxx.status, GPS.txt, and/or GPS.abg, Survey data sheet (txt or pdf file), Survey.shp. The xxxx.dat file contains all the raw data concerning the acoustical transect prior to any filter parameters. Because the xxxx.dat file is large (may exceed 60 MB), this file may need to be uploaded separately to avoid maximum file size limitations or be zipped. The xxx.status file provides information regarding parameters and settings of the data logger; GPS.txt or GPS.abg provide coordinate information along the transect and is used to geo-reference locations within each call file; surveyt.txt/pdf is a copy of the field survey data sheet; and the Survey line shapefile defines the survey route (upload all 4 files representing the shapefile; .shp, .shx, .dbf, and .prj). Each refuge will upload their data annually by August.

Data Summary and Species Classification

The high frequency data recorded by the Anabat Detector contains embedded bat echolocation calls along with other environmental and mechanical noise. For the purposes of analysis and interpretation, these data are visually displayed on a frequency spectrogram. Until recently, calls of bats were manually reviewed by scrolling through the data and using multiple characteristics of the call structure (e.g., frequency, slope, shape) to identify them to species or frequency groups. This has been a laborious process and subject to observer bias or error from lack of training or adherence to predefined parameters which "identify" bat calls based on multiple structure characteristics. More recently, commercial and open-use software packages have been developed to automate this process. These programs use statistically generated filtering processes to "best place" calls to species. These more robust programs also allow the classification of species with probability of correct speciation.

Several factors may affect the call classification to species correctly and thus necessitates a uniform data analysis process. The automated software packages (BCID ver 2.4.11, Bat Call Identification Inc.; and Eric Britzke ver 1.0 – to be released through the U.S. Army Engineer Research Development Center) rely on set fixed processes such that calls can be reliably and repeatedly identified. However, because each software program uses different filter constraints, algorithms, and adjustments for probability of correct classification; they may disagree on calls which have overlapping characteristics among species. As a data standard, only calls of **five (5)** or **more** pulses will be analyzed to species. This will be further constrained by looking at each call from the Anabat data files which are exported through the **CFC Read** application. Each call file is limited to ≤ 15 seconds and in conjunction with a filter of 5 or more pulses determines the relative abundance of bats detected (index of bats; detections/species/mile surveyed).

For the purpose of this monitoring project, data analysis will be done using the Britzke ver 1.0 software. (U.S. Army Engineer Research and Development Center 2012). Validation of "outlier" species (species is not expected from the geographic range, or is considered a migrant during a period when it should not be found on the summer range) will be done using a call library and a determination made to retain the software classification or denote them as unknown. Presently, the software classifies all bats of eastern North America with the exception of the Brazilian free-tailed bat, Northern Yellow bat, Florida Bonneted bat, and Pallas' Mastiff bat.

Monitoring data analysis will be done by the Inventory and Monitoring Network in collaboration with other scientists. Refuges will have access to the software to assist in bat inventory determination.

Project Record

The project record is a functional summary of field work, analysis, and future action items. It provides an understanding of the objectives, level and role of involvement by individuals, financial investments, and planning. The project record will be developed and updated on an annual basis or more frequently as needed by the Inventory and Monitoring Network and submitted to the participants annually.