ERDC TN-EMRRP-SI-35
December 2010

Mammalian Survey Techniques for Level II Natural Resource Inventories on Corps of Engineers Projects (Part II – Bats)

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PURPOSE: This technical note is a product of the Ecosystem Management and Restoration Research Program (EMRRP) work unit titled "Natural Resource Inventories for Special Status Species on Corps Operating Projects." The objective of this note is to provide information on methods for conducting inventories of bat species to satisfy the requirements of Level II Natural Resources Inventories for Corps of Engineers operating projects. Survey methods for other mammalian orders are provided in Martin (2009). A variety of techniques for conducting bat surveys are described, with emphasis on broad-based methods that can be used to obtain occurrence/non-occurrence data for multiple species within a community (Martin et al. 2006). Step-by-step procedures are provided for conducting mist-net surveys, acoustic surveys, roost searches, hibernacula surveys, and emergence counts. General information is also provided for specialized techniques such as radiotelemetry, thermal infrared imagery, and collection of material for genetic analysis.

BACKGROUND: Forty-five species of bats are known to occur in the United States. Of these, seven species or subspecies are federally listed as threatened or endangered by the U.S. Fish and Wildlife Service (USFWS). These are the Indiana bat (*Myotis sodalis*), gray bat (*M. grisescens*), Virginia big-eared bat (*Corynorhinus townsendii virginianus*), Ozark big-eared bat (*C. t. ingens*), greater long-nosed bat (*Leptonycteris nivalis*), lesser long-nosed bat (*L. curasoae yerbabuenae*), and Hawaiian hoary bat (*Lasiurus cinereus semotus*). Twenty additional species or subspecies are considered to be of special concern and may be considered for future listing (Table 1, Harvey et al. 1999). Also, states may recognize other species as sensitive and designate them as protected. A preliminary list of "special status species" in the Corps Operations and Management Business Information Link (OMBIL) database includes 23 species of bats (Martin 2009). Thus, Corps offices need to be aware of bat species that occur or potentially occur on their projects (Martin 2000, Martin et al. 2002). Although bats are an important component of many ecosystems (Figure 1), they have historically been neglected in wildlife inventories because they are difficult to sample, require special handling skills, and their activity patterns require nocturnal surveys. Most sampling methods for bats can only result in occurrence/non-occurrence or relative abundance of some species because of their high mobility and cryptic behavior.

Figure 1. Bats are an important component of many ecosystems.

Table 1. United States Bat Species and Subspecies Federally Listed as Endangered, and Species Considered to be of Special Concern (after Harvey et al. 1999)

Endangered Species/Subspecies	
Lesser Long-nosed Bat (*Leptonycteris curasoae yerbabuenae*)	Greater Long-nosed Bat (*Leptonycteris nivalis*)
Hawaiian Hoary Bat (*Lasiurus cinereus semotus*)	Gray Bat (*Myotis grisescens*)
Indiana Bat (*Myotis sodalis*)	Virginia Big-eared Bat (*Corynorhinus townsendii virginianus*)
Ozark Big-eared Bat (*Corynorhinus townsendii ingens*)	
Species/Subspecies of Special Concern	
California Leaf-nose Bat (*Macrotus californicus*)	Mexican Long-tongued Bat (*Choeronycteris mexicana*)
Spotted Bat (*Euderma maculatum*)	Allen's Big-eared Bat (*Idionycteris phyllotis*)
Southeastern Bat (*Myotis austroriparius*)	Western Small-footed Bat (*Myotis ciliolabrum*)
Western Long-eared Bat (*Myotis evotis*)	Eastern Small-footed Bat (*Myotis leibii*)
Arizona Bat (*Myotis lucifugus occultus*)	Fringed Bat (*Myotis thysanodes*)
Cave Bat (*Myotis velifer*)	Long-legged Bat (*Myotis volans*)
Yuma Bat (*Myotis yumanensis*)	Rafinesque's Big-eared Bat (*Corynorhinus rafinesquii*)
Western Big-eared Bat (*Corynorhinus townsendii pallescens*)	Townsend's Big-eared Bat (*Corynorhinus townsendii townsendii*)
Florida Mastiff Bat (*Eumops glaucinus floridanus*)	Western Mastiff Bat (*Eumops perotis californicus*)
Underwood's Mastiff Bat (*Eumops underwoodi*)	Big Free-tailed Bat (*Nyctinomops macrotis*)

Threats to bat populations include natural events (e.g., flooding, cave-ins, freezing, disease) and anthropogenic causes (e.g., vandalism, cave commercialization, modification of cave entrances, deforestation, stream alteration, improper use of pesticides, and urbanization). Recently, two major threats have emerged for bats: (a) Construction of industrial-scale wind turbines has resulted in bat mortality in many regions (Johnson 2005; Arnett 2005, 2006; Cryan and Barclay 2009), and (b) White-nose Syndrome (WNS) has emerged as a disease that is responsible for killing hundreds of thousands of hibernating bats in the northeastern United States (Blehert et al. 2009) (see discussion below).

PERMITS AND PRECAUTIONS: Permits are required before sampling mammals in any location. The appropriate state agency (usually the state Game and Fish Office or Department of Natural Resources) should be contacted to procure the applicable permit well in advance of the sampling event. Federal permits are required if it is likely that a federally listed species will be captured. The applicant should be aware that obtaining permits can often take weeks or even months and may require a background check, proof of technical competence regarding knowledge of the species to be collected, and references from professional sources knowledgeable of the permitee's abilities. Some states even require completion of an extensive training program before granting a permit. Trapping and handling of mammals should be conducted in accordance with guidelines on marking, trapping, housing, and collecting mammals for research (Gannon et al. 2007).

The project manager must take proper precautions to ensure that field and laboratory personnel are properly protected. Rabies (*Lyssavirus*) is an acute viral infection of the central nervous system that occurs mostly in warm-blooded animals, including bats (Kunz et al. 1996). Contracting rabies from bats is an extremely rare occurrence in the United States, but personnel who will be conducting bat surveys must be inoculated with a rabies vaccine before conducting field or laboratory work. Animal handlers should have their titer (concentration of the vaccine in one's system) checked annually to ensure protection. This will require that blood be drawn and submitted to a certified laboratory for testing. Booster doses are advisable when the titer falls below acceptable levels. If a person is bitten while handling a bat, a physician should be contacted as soon as possible.

All persons conducting bat surveys need to be aware of the current situation regarding WNS. Bat mortality due to WNS was first observed in populations of hibernating bats in several caves around the Albany, New York area in 2007. The disease was named for the presence of a white fungal growth on the muzzle, wings, and tail membrane of affected bats. This fungus has been identified as a new species, *Geomyces destructans* (Gargas et al. 2009, Meteyer et al. 2009). Since its discovery, an estimated one million bats have died as a result of WNS. Additionally, WNS has spread from New York and the fungus has been found in 12 states (NY, VT, NH, CT, MA, NJ, PA, WV, VA, TN, MO, and OK) and there is no sign of the disease slowing its rate of spread. While it is known that bat-to-bat transmission is occurring, the potential for humans to act as a vector for spreading the disease is unknown. Therefore, the United States Fish and Wildlife Service (USFWS) has issued a cave advisory to limit caving activities. This has been followed up by many other groups closing or limiting access to caves in their jurisdictions. Additionally, there are disinfection guidelines that must be followed by people that are caving and/or capturing bats. In fact, concern over possible transmission within a night of sampling has led Region 3 of the USFWS to severely restrict the amount of sampling done during spring and fall surveys. Until more is known about WNS, land managers should re-evaluate bat work being conducted on their property to minimize the chance for additional transmission. It is important to note that guidelines from the USFWS are routinely modified as information emerges, thus it is a good idea to frequently check the FWS WNS web page (*http://www.fws.gov/northeast/white_nose.html*). If WNS is suspected at a site, the state wildlife agency or local USFWS office should be contacted immediately.

PRE-SURVEY RECOMMENDATIONS: A variety of factors must be considered before conducting inventories. Objectives of the inventory must first be determined to allow the selection of appropriate techniques. It is also critical to determine how the data collected will be stored, analyzed, and used for management purposes. Some important aspects of inventories are provided below.

Species Identification. Persons conducting surveys should be thoroughly familiar with species potentially occurring in the area. Regional guides and diagnostic keys are available for many areas, but descriptive information may be highly variable. Recent field guides for North American mammals include Kays and Wilson (2002) and Reid (2006). Additionally, descriptions and photographs of all North American bat species are included in Harvey et al. (1999). Regional guides may also be available and usually provide more descriptive information for species of interest. For example, a dichotomous key to bats in the southeastern and mid-Atlantic states is provided in Menzel et al. (2002). Area and state museums and universities should also be checked for availability of museum mounts that can be used to verify species identification. Development of high-quality digital cameras allows researchers to take photographs of diagnostic features of bats, which can be sent to species experts for confirmation.

Sample Site Selection. As with any inventory, site selection plays a critical role in results obtained. Water sources (streams, ponds, cattle tanks, and road ruts) and travel corridors (e.g., forested roads, dry creek beds) are areas that are heavily used by bats (Figure 2). Often bats at these sites are simply traveling from one place to another and thus may be more easily captured. Carroll et al. (2002) showed that it may also be possible to sample with some success in the middle of forested blocks. The main consideration when selecting sites is that they must be suitable for the methodology that will be deployed. For example, there might be a lot of bat activity over a lake, but trying to capture bats at these sites will be unproductive, while acoustic methods would be well suited to sample these areas. A first step to site selection should be to examine project maps and file information. Also, project managers and natural resources personnel will often have information on terrain features and habitat characteristics that may not appear on maps. It will be necessary to identify road access and areas that are off limits, especially where private lands are intermixed with Corps property. To maximize the detection of all species in the area, it is beneficial to use multiple techniques. Another factor to consider in developing a sampling protocol is that bat activity varies significantly from night to night at the same site. Thus, sampling should be conducted across multiple nights to get a true idea of bat use of a site.

Figure 2. Streams and ponds are heavily used as foraging sites by bats.

NATURAL HISTORY CONSIDERATIONS: Knowledge of the natural history of bat species in an area is important. This information will guide the timing of surveys and help with selection of sample sites for best determining species occurrence. General information on bat migration, hibernation, reproduction, and growth are provided below. Prior to conducting surveys, specific biological and ecological information should be obtained for each species potentially occurring in the area of interest.

Most bat species in the United States experience some form of food restriction during the year. The response of bats to this period of limited food availability ranges from migrating to warmer sites where food resources are available to hibernating in suitable sites until food items are again available. Migration distances vary by species and region. Some species may only undergo small local shifts from season to season whereas other species migrate long distances. For example, Brazilian free-tailed bats (*Tadarida brasiliensis*) in the southwestern United States may migrate up to 1,300 km to and from their winter roosts in Mexico (Harvey et al. 1999). Managers should be aware that information on the migratory movements of many species is limited due to infrequent band returns and limited survey work outside the summer maternity season. Bats that hibernate lower their body temperature to near ambient conditions to save energy. This period of hibernation is interrupted by bats raising their body temperature (arousals) every 10-14 days to active levels (Thomas et al. 1990). Reasons for arousals are unknown, but they represent about 90 percent of the total energy used during hibernation. Because of the energy used during these arousal periods, human disturbance at hibernation sites should be limited.

Most North American bats breed in the fall, and females store sperm until the following spring when fertilization takes place (Harvey et al. 1999); this is referred to as "delayed fertilization." After emerging from their winter roosts, females travel to their maternity range. Maternity sites vary by species and may include caves, trees (under exfoliating bark, in hollow trees, or in clumps of leaves), buildings, bridges, and other structures. Females of some bat species may be solitary roosters while other species may form maternity colonies of up to thousands of individuals or more. Typically females produce 1-2 offspring per year but foliage-roosting bats in the genus *Lasiurus* may bear 3 to 4 young. This low reproductive rate makes recovery of bat populations a long process. Typically bats are born after ~ 40 days gestation. Pups are born naked and quickly attach to their mothers for nursing. During the nursing phase mothers return to the maternity roosts throughout the night to allow the young to suckle. Young develop quickly and are able to fly on their own within 3-4 weeks. Due to the vulnerability of bats during the maternity season, bat researchers should exercise extreme caution when conducting roost surveys and examining captured bats during this period (Kunz 1982).

Information on the age and reproductive condition of bats can be determined when bats are captured and examined in-hand. Bats can be aged into young of the year and adult groups by the presence of an ephiphysal plate in the joints of the fingers (Anthony 1988). In juveniles, the growth plates of the long bones have not fused, and two light bands can be seen when a flashlight is shown through a wing (Figure 3). By the end of the summer the growth plates have fused and it can be very difficult to distinguish juveniles from adults. Reproductively, male bats are generally classified as either scrotal or non-scrotal depending on presence of sperm stored in their epididymis. This usually occurs during the summer to early fall, at which time the scrotal sacks become enlarged and are usually darker in color. Females are characterized as pregnant, lactating, post-lactating, or non-reproductive. Pregnant bats are captured early in the summer and can be verified by palpating the uterus to determine the presence of offspring, although this is difficult to do when the offspring are small and/or the stomach is full. Lactating bats are those that have their nipple areas free of hair, and milk can be expressed from the nipple. Post-lactating bats are bats where the nipples have started to grow replacement skin, and the hair is starting to grow back around the nipple.

Figure 3. Unfused growth plates in the wing of a juvenile bat.

INVENTORY METHODS: Most bat inventories consist of a combination of mist-netting, harp trapping, roost searches, and ultrasonic sound detection. Some species are easier to capture in mist-nets than with other methods, and field surveys based on mist-net and harp net captures allow the collection of biological and morphological data. Many species are difficult to capture in nets and are best surveyed using ultrasonic detectors, which also have the advantage of causing no stress to the bats. Radiotelemetry and thermal imagery methods are appropriate when detailed life history information is needed for special status species. While each method has its own advantages, a combination of techniques will allow a more complete picture of the bat community present at a site.

Mist-net Surveys. Mist-netting is the most common technique for capturing bats (Figure 4). Mist nets are relatively inexpensive, lightweight, compact, and easily transported and erected in the field (Kunz et al. 2009). Netting consists of placing a large rectangular net (having several tiers of a fine, hairnet-like material) in a position where it will be in a bat flyway. Typical mist nets have a black mesh size of 36 mm, are of 50 or 70 denier/2-ply nylon, have four shelves, and are 2.6 m high. They come in a variety of lengths ranging from 2.6 m to 18 m wide and greater with 6-, 9-, 12-m-wide mist nets being most commonly used. Some mist net sets consist of a single net, while other sites require mist nets stacked on top of each other. While numerous systems have been developed to stack mist nets on a frame, most use a pulley system based on a method described by Gardner et al. (1989). Weather conditions should be suitable for general bat activity. Netting should be avoided on cold nights, nights with strong wind that moves the nets and makes them more visible to bats, and nights with precipitation.

Figure 4. Mist-netting is a common technique used in bat surveys.

Net placement is critical to successful mist-netting efforts. To maximize capture efficiency, nets should be placed in areas where bats concentrate for feeding and/or drinking, or across trails that are used as travel corridors. Nets set over streams and ponds should have the lower shelf-cord set near enough to the water to prevent bats from flying under the net, but should be high enough so that the lowest net-pocket does not touch the water even with a bat in the net. It is best to select sites where the water is calm. As bats vary in their use of the habitats available to them, it is prudent to pick net sites in a variety of habitat types to get a more complete representation of the bat community present in the area.

Bats usually have a primary peak in activity shortly after sunset and a secondary peak shortly before sunrise. Nets should be opened at first sight of a bat or at sunset. They should be checked every 15 minutes unless bat activity is high, in which case nets should be checked more frequently. Bats captured in nets may become stressed if left in the net too long. Additionally, bats may get tangled in the net, thereby making it more difficult to remove them. Finally, bats may be able to chew their way out of the net, resulting in loss of data and damage to nets. Personnel should take care not to injure bats, thus experienced researchers should be present to remove bats from the nets. Additionally, birds may become entrapped in the nets, especially if nets are opened too early in the evening.

Capturing bats with mist nets provides useful data on the bat species' presence, reproductive condition, and sex and age ratios at sampling locations. Additionally, because mist net surveys are widespread and are the standard method for sampling, comparisons can be made across studies. However, there are some limitations of the use of this system. Some species, such as hoary bats (*Lasiurus cinereus*), fly too high and are thus underrepresented during normal capture activities. Additionally, some individuals may detect the net and avoid capture at the site. This avoidance behavior may increase on successive nights as bats learn the location of the net site.

The following steps should be taken for mist net surveys:

- Ensure that all personnel who will be handling bats are properly trained, have sufficient species recognition skills, have up-to-date rabies inoculations, and wear gloves when handling animals.
- Design an appropriate data sheet to be used by all recorders. A sample data sheet is provided in Appendix A.
- Select sample sites based on location and survey objectives. Sample stations will usually consist of multiple nets set at least 30 m apart that are sampling as many different habitat features as possible.
- Describe ecological conditions at each sample site. Also record climatic conditions (temperature, relative humidity, cloud cover, moon phase, and wind). It is a good idea to draw a sketch of the site on the back of the data sheet showing net locations and major habitat features.
- Erect poles and connect mist-nets prior to dusk.
- Open nets just before dusk or at first sign of bat activity. In most cases nets should be left open for 4 to 5 hours.
- Check nets every 15 minutes, or sooner if large numbers of bats are being captured.
- Carefully retrieve bats from nets and carry them (usually in cloth bags) to a central point for identification and data recording. Data recorded should include species, sex, age (adult or juvenile), reproductive condition (pregnant, lactating, post-lactating, scrotal),

weight, and forearm length. Digital calipers gauged to 0.01 mm are usually used for measurements, but clear plastic millimeter rules may also be used. Scales should be precise to 0.1 g.

- Additional measurements may also need to be taken for species of questionable identification. Recording length of ear, tragus, and hind foot may be necessary for some species. Any unusual marks or characteristics (e.g., hair loss, old injuries, bands) should be recorded on the data form. Photographs should be taken of unusual specimens or species that are difficult to identify. These pictures should focus on the identifiable characters to ensure correct identification.
- All data sheets should be filed for further analysis. State and federal agencies generally require that data sheets be submitted annually as a requirement of state collecting permits.

Harp Traps. Bats may also be sampled using a specialized trapping device referred to as a harp trap (Figure 5). The harp trap consists of two frames each with a set of closely spaced vertical strands of line (Constantine 1958, Tuttle 1974, Kunz and Kurta 1988, Francis 1989). The strands are closely spaced and kept taut so that if a bat avoids the first set of strings it does not have sufficient room to avoid the second set of strings, hits the strings, and slides down into a bag at the bottom of the frame. The bag is lined with plastic to minimize the likelihood that bats can escape. Harp traps are most effective when partially hidden by the natural terrain and placed adjacent to objects that form natural flyways, such as canyon walls and forest trails with overhanging tree branches (Kunz and Kurta 1988). Because this technique only requires researchers to reach in and remove bats from the bag, it is the most effective method for sampling bats at large colonies. As with mist nets, capture success decreases when traps are repeatedly deployed at the same site.

Figure 5. Bats may be sampled in some locations using harp traps.

Acoustic Surveys. Ultrasonic detectors can be used to investigate questions of bat ecology that cannot be addressed using other techniques. Typically researchers have more net sites than they can sample, so detectors are often used to determine areas of high activity for selecting netting sites. Additionally, numerous studies have employed ultrasonic detectors to examine habitat use of bats (Krusic et al. 1996, Menzel et al. 2001). Species exhibit differing behavioral patterns that should be taken into account when developing sampling approaches. Active sampling involves an observer attempting to aim the detector microphone at bats as they fly. Passive sampling refers to the practice of leaving a detector in a set position and recording any ultrasound that enters the zone of detection. Detector systems placed in the field for remote, passive sampling are often housed in waterproof detectors with an aperture through which the microphone can be fitted (Figure 6). Recent work has shown that placing detector systems a few feet above the ground (e.g., on a tripod) reduces the ultrasonic clutter from insects and improves recording quality and yield (Weller and Zabel 2002). At streams and other corridor sites, detectors angled parallel to the flight path obtain higher quality recordings than those angled perpendicular to corridors (Law et al. 1998).

Figure 6. Anabat II acoustic detector field mounted for passive sampling.

Echolocation Overview. Echolocation is the term used to describe the process of sound production, detection, and interpretation of returning echoes that some bats use to navigate in their surroundings and capture prey. With respect to bat echolocation, a call refers to a single sound emitted by an individual whereas a call sequence refers to a continuous series of pulses from a single individual (Fenton 1999, O'Farrell et al. 1999). Echolocation calls are commonly classified into three phases: search, approach, and terminal (Griffin et al. 1960) (Figure 7). These are not distinct phases of the call sequences but describe a continuum of calls within a sequence. Search phase calls are produced as a bat searches for prey. Approach phase calls develop from search phase calls once a potential prey item is detected. Initially, frequency range (bandwidth) covered by the call increases to gain detailed information on the potential prey item detected. As a bat continues toward a potential prey item, bandwidth decreases, duration of calls decreases, and time between calls decreases. Immediately prior to prey capture, bats produce terminal phase echolocation calls. In this phase, calls continue to decrease in bandwidth and duration until prey capture (feeding buzz). As bats proceed from search phase calls to feeding buzz the repetition rate of calls changes from 10 calls/second in the search phase to 200 calls/second at the end of the terminal phase. Search phase calls are useful in the study of bat echolocation because they constitute a majority (ca. 90 percent) of calls produced by bats, exhibit consistency in structure throughout the call sequence, and may possess species-specific characteristics (Betts 1998, Fenton and Bell 1981, O'Farrell et al. 1999).

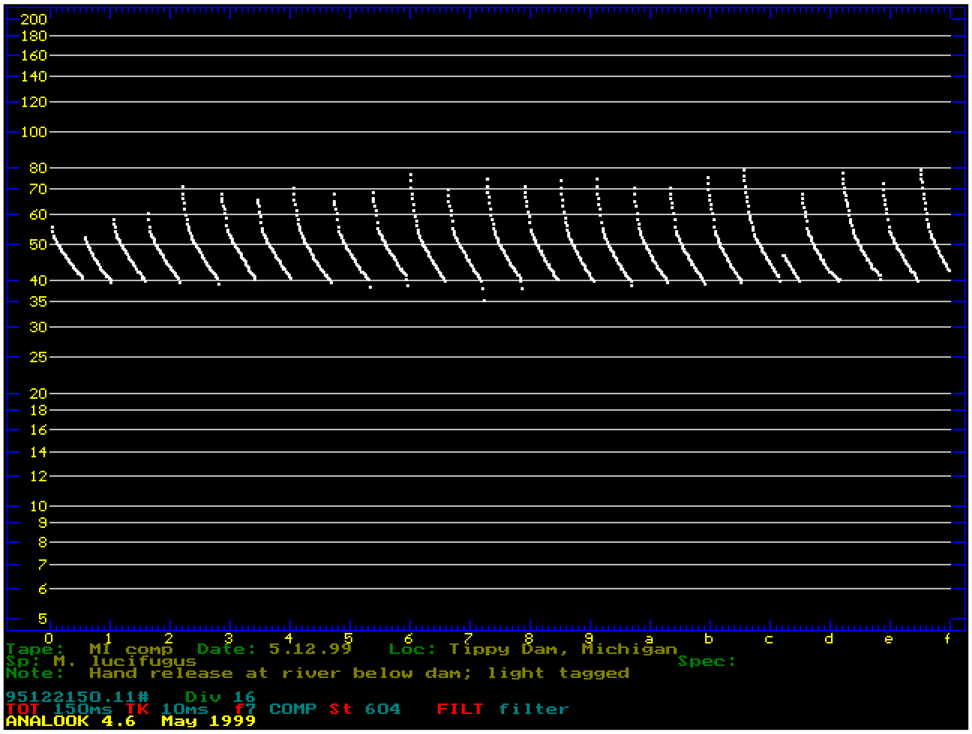

Figure 7. Example of search phase echolocation calls.

Types of Ultrasonic Detectors. There are four major types of ultrasonic bat detectors: heterodyne, frequency division, time expansion, and real time recording. These detectors differ in their ease of use, the method used to extract information from the echolocation calls, and the resources required to record. Because each type of detector has its own advantages and disadvantages, choosing the appropriate detector for the study depends on the effective design of the sampling scheme to incorporate the objectives of the study and matching the design with the advantages and disadvantages inherent in each system.

Call Analysis. Before any acoustic identification can be accomplished, a library that includes examples of calls made by bats of known identity must be recorded. Without a call library, the researcher has no idea of the structure of the echolocation calls of the species included. While early studies showed the presence of geographic variation (Barclay et al. 1999, Brigham et al. 1989), recent work with large libraries has shown that geographic variation is a small source of variation (and thereby a small source of error in acoustic identification) (Murray et al. 2001, O'Farrell et al. 2000). Acoustic identification can be conducted either through visual comparison of unknown calls to a known call library (i.e., qualitative) or statistical comparison of unknown calls to a known call library (quantitative). Qualitative identification is quick, and with an experienced researcher can be effective, but accuracy rates are unknown and highly variable. Quantitative identification involves statistical comparison of unknown calls to a known call library. Quantitative identification requires additional time for data analysis, but provides the user with an objective method that can be compared across studies with known accuracy rates that can be used to statistically determine species presence at a site (Britzke et al. 2002).

Radiotelemetry. Construction of radio transmitters small enough to attach to bats has allowed a vast amount of information to be gathered on bat ecology. Transmitters are commonly attached to the back of captured bats using nontoxic surgical glue. The combined weight of the transmitter and glue should ideally be less than 5 percent of the body weight of the bat (Aldridge and Brigham 1988). However, researchers sometimes use transmitters that are less than 10 percent of the body weight when dealing with bats that are too small for available transmitters; in this case radiotelemetry should only be used to examine roosting habitat. Once transmitters are applied in the field, bats are generally tracked using a directional antenna and radio receiver to examine roosting and/or foraging ecology. Different individuals can be tagged with transmitters that emit signals at different frequencies to allow multiple individuals to be tracked during a single event or project. If foraging data are to be collected, there should be 3-4 teams of researchers spread out over the anticipated study area. The teams should be able to maintain contact and should move to locate the bats while they are foraging. This effort is difficult for all but the most experienced researchers. As transmitters continue getting smaller, the utility of these transmitters will likely continue to grow.

Roost Searches. Roost site detection is important for determining the distribution and habitat preferences of bats in the area of interest. Bats generally choose different roost sites seasonally and there are often specific criteria for selecting maternity roosts and hibernacula. Additionally, many species are known to switch roosts, especially during the maternity period. Knowledge of local bat species and their ecology is important when conducting manual roost searches, and understanding the roosting habitat of target species will aid in the effectiveness of roost searches. Some species roost only in caves or mines during all seasons, whereas other species (e.g., Indiana bat) winter in caves but generally form maternity colonies in tree cavities or beneath tree bark. Numerous species roost in tree cavities year-round, and foliage-roosting species in the genus *Lasiurus* primarily roost by hanging singly or in small clusters on tree branches, vines, and moss.

Timing of roost searches is an important factor in their success. If a roost is known, then one can simply conduct exit counts or hibernacula counts (depending on the time of year). When searching for potential roost sites, it is sometimes possible to observe bats directly, but often it is necessary to use presence of guano, stains, or insect parts as clues to infer bat use. While this only provides evidence of some bat use, genetic assays of guano allow species identification. If the site is used by bats during the winter, there should be a scattering of guano near the entrance to the site. If it is used during the summer season, there should be a pile of guano present under the roost site that allows estimation of the number of bats present.

Bats have been found using a large number of artificial structures. Some species have adapted to roosting in structures such as bridges, abandoned houses, wells, cisterns, and mines (e.g., Keeley and Tuttle 1999, Adam and Hayes 2000, Trousdale and Beckett 2004), and some species readily use structures specifically designed as artificial roosts (e.g., bat houses). Kiser et al. (2002) found that checking bridges for night-roosting bats can compliment traditional mist netting surveys. As bridges have been used by a wide variety of species, survey work should include checks of these potential roost sites to maximize the detection of all species within an area. Bridges may serve as both day and night roosts. Accumulation of fecal material and/or staining of the bridge structure may be used to document bat use. Bat houses have been very effective in providing roosting habitats for some species. The success of bat houses can be maximized if a colony is present in the area when it is erected and there is sufficient sun exposure. Bat box surveys are useful for providing data on bat communities in some areas. For more information on artificial structures for bats see *www.batcon.org*.

Roost surveys can be conducted in the winter when bats are hibernating (hibernacula surveys) or during the spring/summer maternity period. The success of surveys depends on the species of interest, location of roost sites, and capability of the survey team. For some migratory species (e.g., Brazilian free-tailed bats that migrate into Mexico) counts can only be made during the warmer months. However, during the maternity period bats are very susceptible to disturbance and have been known to abandon traditional roosts (Jones and Suttkus 1975, Kunz 1982). In fact, some species may even abandon their young if the disturbance is great enough. Roost counts made in artificial structures (e.g., beneath bridges and in abandoned buildings) and in natural features such as basal cavities of trees (Figure 8) should be made as quickly as possible. Cave and mine roosts should generally not be entered during the reproductive period. Instead, it is best to attempt counting bats as they emerge from their roost sites at dusk. Exit counts are also most appropriate for colonies exiting from the upper levels of trees. General procedures for hibernacula surveys and exit counts are described below.

Figure 8. Systematic searches should be made of potential natural and artificial roost sites.

Hibernacula Surveys. For species that hibernate in caves and mines, winter surveys in hibernacula provide the best opportunity to monitor population levels. Hibernacula are difficult to identify because of the lack of guano accumulation, so it is only during the winter that bat use of the sites can be assessed. While each species has its own hibernating preferences, hibernacula typically have air flow through them to lower the cave temperature during the winter. Thus, depending on the target species of the search, certain portions of the cave should be a higher priority for survey. A single trip should be planned to conduct the bat survey every 1-3 years. Survey trips should be as short as possible and include only the number of people required to safely conduct the survey. These efforts are undertaken to limit arousals of bats from hibernation. While arousals are a natural occurrence in hibernating bats, additional arousals prompted by disturbance have been implicated for population declines at some hibernacula. If possible, a hibernation survey should be led by a person experienced with the cave and knowledge of typical roost sites in order to minimize disturbance to the bats.

Some species are encountered as solitary individuals and are counted throughout the survey, while other species form dense clusters including over 100,000 bats. Counts of clustered bats have historically focused on estimating the area covered by the cluster, then multiplying the area by the clustering index (the number of individuals per unit area) to determine the total estimate. Kunz (2003) stated that censusing hibernating bats is best achieved by counting each individual bat or group of bats as they are encountered, or by estimating the mean density of bats in several representative clusters, and extrapolating this density to the total area of the cave wall or ceiling covered by bats. Recently, bat researchers have started to utilize digital photography to document clusters of bats. Pictures are then taken back to the office and displayed on a computer screen where the researchers simply count bats in the pictures. This technique has the advantage of being more repeatable than other methods, but is of limited use under some roosting conditions in the hibernaculum (e.g., bats spread out over a large area or bats roosting in layers). For hibernacula surveys to be as good as possible, the same researchers should visit the sites each time they are surveyed to make sure all sections are covered.

Exit Counts. A common method for surveying populations of some species of bats consists of counting individuals as they emerge from a roost site. A variety of vision-based techniques have been used, including direct visual counts, sometimes aided by near-infrared (NIR) illuminators (invisible to bats) and night vision scopes, and manually counting bats recorded on video collected with NIR cameras. For exit counts, researchers generally try to identify the site where bats are going to exit and place themselves so that their line of sight is perpendicular to the flight of the bats. Additionally, if researchers can adjust their location so that the bats are being viewed with the sky as a background it is easier to detect the bats. With some sites, it is preferable to use night vision equipment to assist in detecting bats after it becomes too dark. The researcher notes the start and stop time and may use a counter to assist in keeping track of large numbers of bats. While this method is useful in estimating the population size, accuracy of the counts using visual methods varies with the experience of the researcher, the site characteristics (open area for viewing), the species (bat species vary in their emergence times and thus may emerge well after it is too dark to see), and the number of bats. Because bats may change roosts throughout the summer, exit counts should be conducted multiple times to ensure an accurate representation of the colony size. Additionally, counts should be done before the young become volant so that counts are comparable among years.

Availability of thermal infrared (TIR) cameras and development of methods appropriate for imaging bats has improved the ability to make accurate emergence counts (Sabol and Hudson 1995). TIR imaging equipment measures the heat-based radiant energy emitted from objects so no external light source is necessary. The primary limitation is that an object in a thermal image must be warmer or cooler than the ambient background. Since the body temperature of bats is generally much warmer than the ambient sky, bats are easily detected using this method. For exit counts, the thermal camera (typically a low-cost uncalibrated camera used for surveillance applications) is set up in a position such that all bats emerging from the roost will fly through the field of view. The entire emergence is recorded and taken back to the lab for analysis using automated counting software (Sabol and Hudson 1995, Melton et al. 2005). This technique allows for increased objectivity in counts on populations of up to millions of bats (Sabol and Hudson 1995, Betke et al. 2008).

Collection of Fecal and Tissue Samples. When bats are in hand, there is also the opportunity to collect other types of samples that can be used for various analyses. Captured bats are commonly placed in individual holding bags until they are processed. During this time, bats produce guano, which can easily be collected from the bag, placed in an appropriate container, and frozen for later analysis of prey items (Whitaker et al. 2009) and provision of DNA for genetic analyses. Hair samples commonly are collected from the back of bats to allow for stable isotope analysis to investigate foraging ecology or migration, or to test for the presence of heavy metals. Hair samples remain viable for long periods of time so they can be collected and stored for future use. A protocol for hair collection for stable isotope analysis is provided in Appendix B. Additionally, tissue samples are commonly collected from the wings of bats as a method to obtain DNA for genetic examination. Sites where tissue has been removed generally heal within 3-4 weeks (Worthington and Barratt 1996). A protocol for the collection of wing biopsy punches is provided in Appendix C.

OTHER CONSIDERATIONS

Central Repository. Until recently there was no central repository for the collection of bat information. To fill this data gap, the Southeastern Bat Diversity Network and the Northeast Bat Working Group jointly created and are administering a bat database. The inclusion of bat data in this database is completely voluntary, but the two groups are hopeful that researchers will see the benefits of this repository and will participate. Information can be entered in the database on capture data, exit counts, or hibernacula surveys. While there is the opportunity to add data from banding, the users do not have to band in order to provide valuable information for the conservation of bats. The database and instructions for its use can be found at *http://www.sbdn.org/Bat_DB2006.html*.

Sources of Information. Corps natural resources personnel should be aware of professional organizations and other sources that can readily provide information on bats occurring in their region. Regional bat working groups include the Western Bat Working Group (WBWG; *http://www.wbwg.org*), Northeast Bat Working Group (NEBWG), and Southeastern Bat Diversity Network (SBDN; *http://www.sbdn.org*). Additionally, some states have formed separate working groups linked to the regional working groups.

SUMMARY AND CONCLUSIONS: Corps of Engineers projects support a diversity of bat species, many of which are federally or state-protected or otherwise considered to be species of concern. Thus, Corps operational projects need to be aware of bat species that occur or potentially occur on their project lands. However, few Corps projects have conducted even preliminary bat surveys. This technical note provides basic information needed to develop and implement a plan for surveying bat populations on project lands. Additionally, information is included on permits, health concerns, and natural history considerations for personnel conducting bat surveys.

A variety of methods are available to sample bat populations, and inventories generally consist of a combination of techniques. Methods commonly used include mist-netting, harp traps, ultrasonic sound detectors, and roost searches. Mist-netting consists of placing specially designed nets in areas where bats concentrate for feeding and/or drinking, or across trails used as travel corridors. Bats can also be captured using a specialized trapping device referred to as a harp trap.

Harp traps are very effective for sampling large numbers of bats as encountered at cave entrances or other roost sites. Many species of bats are difficult to capture with mist nets and are best inventoried using ultrasonic detectors. Each system of ultrasonic detector has its own advantages and disadvantages, and choosing the appropriate system depends on the effective design of a sampling scheme that incorporates the objectives of the study.

Bat inventories should also include systematic surveys of roost sites. Roosts may be located by conducting habitat surveys to identify potential roosting habitat, making visual observations of bats leaving roost sites at dusk, or using radiotelemetry to track captured bats to roost sites. Roost surveys can be conducted in the winter when bats are hibernating or during the spring/summer maternity period. Success of surveys depends on the species of interest, location of roost sites, and capability of the survey team. Preferred roosting habitat varies by species and location and may include natural features (e.g., caves, mines, snags, tree cavities, beneath tree bark) or artificial structures (e.g., attics, abandoned homes and barns, bridges, wells, cisterns, bat houses). Hibernacula surveys in caves and mines require specialized techniques and should only be performed by trained personnel. Exit counts have historically been conducted using visual techniques, but the recent development of thermal infrared imagery technology suitable for bat surveys has improved the ability to make accurate emergence counts.

Bat surveyors should also consider collecting guano, tissue, and/or hair samples from specimens captured in the field. These data can be sent to the appropriate laboratory and analyzed to provide important information on bat movements, feeding habits, and habitat preferences. The samples can also be tested for heavy metals and pesticide levels. Additionally, persons conducting bat inventories in the eastern United States should consider submitting survey data to the central repository maintained by the Southeastern Bat Diversity Network and Northeast Bat Working Group. This need has become increasingly important due to recent concerns about the population status of many species of bats resulting from casualties associated with White-nose Syndrome and wind energy development. Finally, project Corps personnel responsible for natural resource inventories should be aware of state, regional, and national organizations and working groups that can provide information and expertise on bats in their area of interest.

ACKNOWLEDGEMENTS: The authors wish to thank the Ecosystem Management and Restoration Research Program (EMRRP) and the Stewardship Advisory Team for supporting this work. Funding was provided by the EMRRP under the work unit titled "Natural Resource Inventories for Special Status Species on Corps Operating Projects." Manuscript review was provided by Drs. Richard A. Fisher, William M. Ford, and Richard F. Lance. Photographs were provided by Dr. Eric R. Britzke and Chester O. Martin.

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Martin, C. O., and E. R. Britzke. 2010. *Mammalian survey techniques for Level II natural resource inventories on Corps of Engineers projects (Part II – Bats).* EMRRP Technical Notes Collection. ERDC TN-EMRRP-SI-35. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

REFERENCES

Adam, M., and J. Hayes. 2000. Use of bridges as night roosts by bats in the Oregon Coast Range. *Journal of Mammalogy* 81:402-407.

Aldridge, D. H., and R. M. Brigham. 1988. Load carrying and maneuverability in an insectivorous bat: A test of the 5% rule of radio-telemetry. *Journal of Mammalogy* 69: 379-382.

Anthony, E. L. P. 1988. Age determination in bats. In *Ecological and Behavioral Methods for the study of bats*, ed. T.H. Kunz, 47-58. Washington, DC: Smithsonian Institution Press.

Arnett, E. B., ed. 2005. *Relationships between bats and wind turbines in Pennsylvania and West Virginia: An assessment of bat fatality search protocols, patterns of fatality, and behavioral interactions with wind turbines*. A Final Report submitted to the Bats and Wind Energy Cooperative. Austin, TX: Bat Conservation International.

Arnett, E. B. 2006. A preliminary evaluation of the use of dogs to recover bat fatalities at wind energy facilities. *Wildlife Society Bulletin* 34:1440-1445.

Barclay, R. M. R., J. H. Fullard, and D.S. Jacobs. 1999. Variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*): Influence of body size, habitat structure, and geographic variation. *Canadian Journal of Zoology* 77:530-534.

Betke, M., D.E. Hirsh, N.C. Makris, G.F.McCracken, M Procopio, N.I. Hristov, S. Tang, A. Bagchi, J.D. Reichard, J.W. Horn, S. Crampton, C.J. Cleveland, and T.H. Kunz. 2008. Thermal imaging reveals significantly smaller Brazilian free-tailed bat colonies than previously estimated. *Journal of Mammalogy* 89:18-24.

Betts, B. J. 1998. Effect of interindividual variation in echolocation calls on identification of big brown and silver-haired bats. *Journal of Wildlife Management* 62:1003-1010.

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckels, J. T. H. Coleman, S. R. Darling, A Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat White-Nose Syndrome: An emerging fungal pathogen? *Science* 323:227.

Brigham, R. M., J. E. Creek, and M. B. C. Hickey. 1989. Intraspecific variation in the echolocation calls of two species of insectivorous bats. *Journal of Mammalogy* 70:426-428.

Britzke, E. R., K. L. Murray, J. S. Heywood, and L. W. Robbins. 2002. Acoustic identification. In *The Indiana bat: Biology and management of an endangered species,* ed. A. Kurta and J. Kennedy, 220-224. Austin, TX: Bat Conservation International.

Carroll, S. K., T. C. Carter, and G. A. Feldhamer. 2002. Placement of nets for bats: Effects on perceived fauna. *Southeastern Naturalist* 1:193-198.

Constantine, D. G. 1958. An automatic bat-collecting device. *Journal of Wildlife Management* 22:17-22.

Cryan, P. M., and R. M. R. Barclay. 2009. Causes of bat fatalities at wind turbines: Hypotheses and predictions. *Journal of Mammalogy* 90:1330-1340.

Fenton, M. B. 1999. Describing the echolocation calls and behaviour of bats. *Acta Chiropterologica* 1:127-136.

Fenton, M. B., and G. P. Bell. 1981. Recognition of species of insectivorous bats by their echolocation calls. *Journal of Mammalogy* 62:233-243.

Francis, C. M. 1989. A comparison of mist nets and two designs of harp traps for capturing bats. *Journal of Mammalogy* 70:865-870.

Gannon, W. L., R. S. Sikes, and the Animal Care and Use Committee of the American Society of Mammalogists. 2007. *Journal of Mammalogy* 88:809-823.

Gardner, J. E., J. D. Gamer, and J. E. Hoffman. 1989. A portable mist netting system for capturing bats with emphasis on *Myotis sodalis* (Indiana bat*). Bat Research News* 30: 1-8.

Gargas, A., M. T. Trest, M. Christensen, T. J. Volk, and D. S. Blehert. 2009. *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. *Mycotaxon* 108:147-154.

Griffin, D. R., F. A.Webster, and C. R. Michael. 1960. The echolocation of flying insects by bats. *Animal Behaviour* 8:141-154.

Harvey, M. J., J. S. Altenbach, and T. L. Best. 1999. *Bats of the United States*. Arkansas Game and Fish Commission.

Johnson, G. D. 2005. A review of bat mortality at wind-energy developments in the United States. *Bat Research News* 46:45-49.

Jones, C., and R. D. Suttkus. 1975. Notes on the natural history of *Plecotus rafinesquii*. *Occasional Papers of the Museum of Zoology, Louisiana State University* 47:1-14.

Kays, R. W., and D. E. Wilson. 2002. *Mammals of North America*. Princeton, MA, and Oxford, UK: Princeton University Press.

Keeley, B. W., and M. D. Tuttle. 1999. *Bats in American bridges*. Bat Conservation International, Inc. Resource Publication No. 4.

Kiser, J. D., J. R. MacGregor, H. D. Bryan, and A. Howard. 2002. Use of concrete bridges as nightroosts. In *The Indiana Bat: Biology and Management of an Endangered Species,* ed. A. Kurta and J. Kennedy, 208-215. Austin, TX: Bat Conservation International, Inc.

Krusic, R. A., M. Yamasaki, C. D. Neefus, and P. J. Pekins. 1996. Bat habitat use in the White Mountain National Forest. *Journal of Wildlife Management* 60:625-631.

Kunz, T. H. 1982. Roosting ecology of bats. In *Ecology of Bats,* ed. T.H. Kunz, 1-55. New York: Plenum Publishing Corporation.

Kunz, T. H. 2003. Censusing bats: Challenges, solutions, and sampling biases. In *Monitoring Trends in Bat Populations of the United States and Territories: Problems and Prospects*, ed. T.J. O'Shea and M.A. Bogan, 9-19. U.S. Geological Survey, Biological Resources Discipline, Information and Technology Report, USGS/BRD/ITR-2003-0003.

Kunz, T. H., and A. Kurta. 1988. Capture methods and holding devices. In *Ecological and Behavioral Methods for the Study of Bats,* ed. T.H. Kunz, 1-29. Washington, DC: Smithsonian Institution Press.

Kunz, T. H., R. Hodgkison, and C. D. Weise. 2009. Methods of Capturing and handling bats. In *Ecological and behavioral methods for the study of bats, 2nd ed*., ed. T.H. Kunz and S. Parsons, 3-35. Baltimore, MD: Johns Hopkins University Press.

Kunz, T. H., R. Rudran, and G. Gurri-Glass. 1996. Human health concerns. In *Measuring and monitoring biological diversity: Standard methods for mammals,* ed. D.E. Wilson, F.R. Cole, J.D. Nichols, R. Rudran, and M.S. Foster. Washington, DC, and London: Smithsonian Institution Press.

Law, B. S., J. Anderson, and M. Chidel. 1998. A bat survey in state forests on the southwest slopes region of New South Wales with suggestions for improvements of field surveys. *Australian Zoologist* 30:467-479.

Martin, C. O. 2000. *Bat habitat restoration and management opportunities on Corps of Engineers projects*. EMRRP Technical Notes Collection. ERDC TN-EMRRP-SI-18. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Martin, C. O. 2009. *Mammalian survey techniques for Level II natural resource inventories on Corps of Engineers projects) (Part I).* EMRRP Technical Notes Collection. ERDC TN-EMMRP-S1-34. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Martin, C. O., W. A. Mitchell, and M. S. Wolters. 2002. *Eastern cave- and crevice-dwelling bats potentially impacted by USACE reservoir operations.* EMRRP Technical Notes Collection. ERDC TN EMRRP-SI-24. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Martin, C. O., J. Krause, and D. N. Weise. 2006. *Natural resource level one inventories: What are the needs and process for Corps projects?* EMRRP Technical Notes Collection. ERDC TN-EMRRP-EM-04. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Melton, R. E., B. M. Sabol, and A. Sherman. 2005. Poor man's missile tracking technology: Thermal IR detection and tracking of bats in flight. *Proceedings International Society of Optical Engineering* 5811:24-33.

Menzel, M. A., J. M. Menzel, S. B. Castleberry, J. Ozier, W. M. Ford, and J. W. Edwards. 2002. *Illustrated key to skins and skulls of bats in the southeastern and mid-Atlantic states*. Research Note NE-376. Newtown Square, PA: USDA Forest Service Northeastern Research Station.

Menzel, M. A., J. M. Menzel, W. M. Ford, J. W. Edwards, T. C. Carter, J. B. Churchill, and J. C. Kilgo. 2001. Home range and habitat use of male Rafinesue's big-eared bats (*Corynorhinus rafinesquii*). *American Midland Naturalist* 145:402-408.

Meteyer, C. U., E. L. Buckles, D. S. Blehert, A. C. Hicks, D. E. Green, V. Shearn-Bochsler, N. J. Thomas, et al. 2009. Histopathologic criteria to confirm White-nose Syndrome in bats. *Journal of Veterinary Diagnostic Investigations* 21:411-414.

Murray, K. L., E. R. Britzke, and L. W. Robbins. 2001. Variation in search-phase calls of bats. *Journal of Mammalogy* 82:728-737.

O'Farrell, M. J., B. W. Miller, and W. L. Gannon. 1999. Qualitative identification of free-flying bats using the Anabat detector. *Journal of Mammalogy* 80:11-23.

O'Farrell, M. J., C. Corben, and W. L. Gannon. 2000. Geographic variation in the echolocation calls of the hoary bat (*Lasiurus cinereus*). *Acta Chiropterologica* 2:185-197.

Reid, F. A. 2006. *A field guide to the mammals of North America, 4th edition*. The Peterson Field Guide Series. Boston, MA: Houghton Mifflin Company.

Sabol, B. M., and M. K. Hudson. 1995. Technique using thermal infrared-imaging for estimating populations of gray bats. *Journal of Mammalogy* 76:1242-1248.

Thomas, D. W., M. Dorais, and J. Bergeron. 1990. Winter energy budgets and cost of arousals for hibernating little brown bats, *Myotis lucifugus*. *Journal of Mammalogy* 71:475-479.

Trousdale, A. W., and D. C. Beckett. 2004. Seasonal use of bridges by Rafinesque's big-eared bat, *Corynorhinus rafinesquii*, in southern Mississippi. *Southeastern Naturalist* 3:103-112.

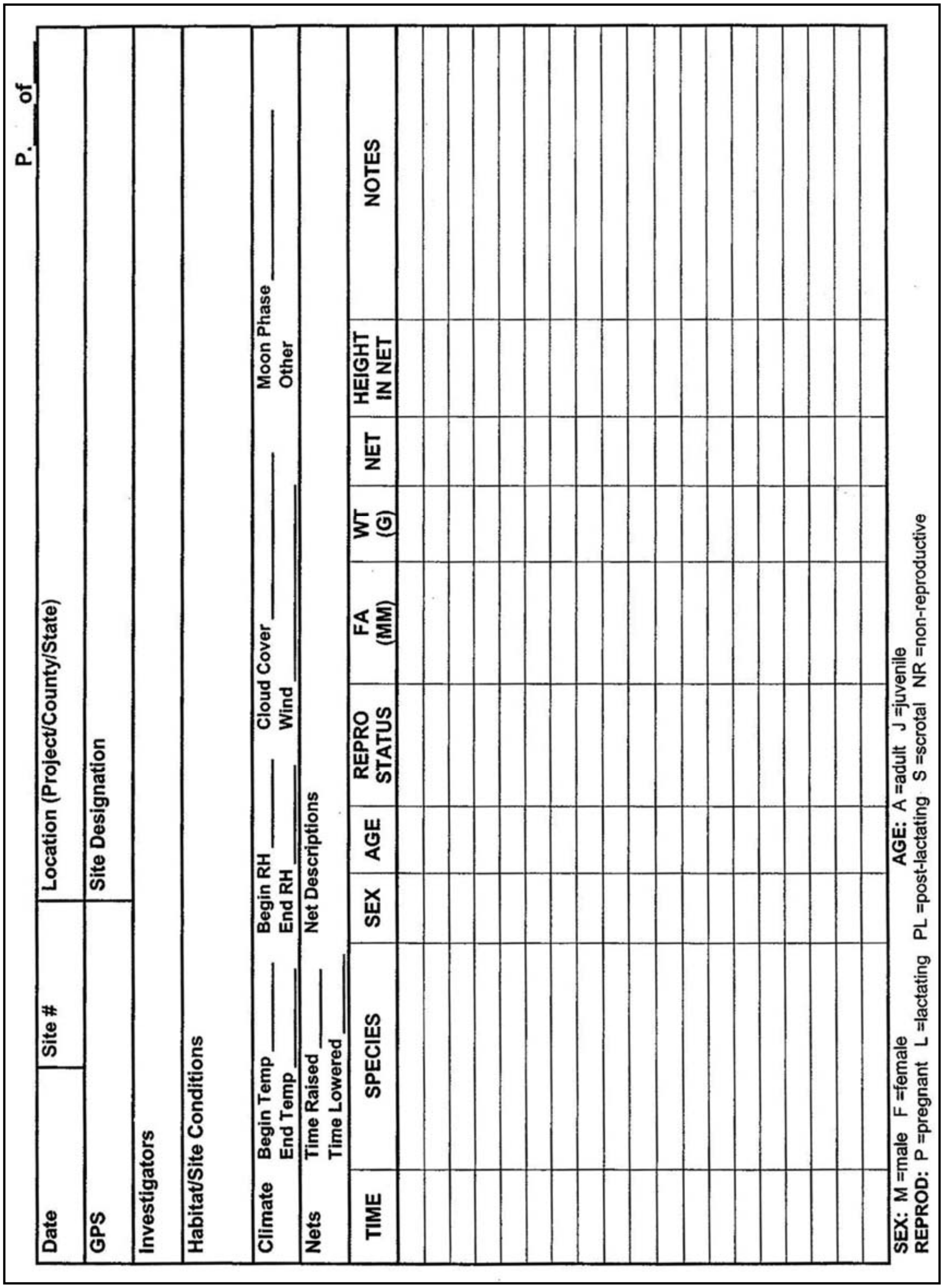
Tuttle, M. D. 1974. An improved trap for bats. *Journal of Mammalogy* 55: 475-477.

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.

Whitaker, J. O., Jr., G. F. McCracken, and B. M. Siemers. 2009. Food habits analysis of insectivorous bats. In *Ecological and behavioral methods for the study of bats, 2nd ed,* ed. T.H. Kunz and S. Parsons, 567-592. Baltimore, MD: Johns Hopkins University Press.

Worthington, W. J., and E. Barratt. 1996. A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Research News* 37:1-3.

Appendix A: Sample Data Form for Recording Information on Captured Bats



P.___ of___

Date | Site # | Location (Project/County/State)

GPS | Site Designation

Investigators

Habitat/Site Conditions

Climate: Begin Temp ___ End Temp ___ Begin RH ___ End RH ___ Cloud Cover ___ Wind ___ Moon Phase ___ Other ___

Nets: Time Raised ___ Time Lowered ___ Net Descriptions

TIME	SPECIES	SEX	AGE	REPRO STATUS	FA (MM)	WT (G)	NET	HEIGHT IN NET	NOTES

SEX: M =male F =female AGE: A =adult J =juvenile
REPROD: P =pregnant L =lactating PL =post-lactating S =scrotal NR =non-reproductive

Appendix B: Example Protocol for Hair Sampling

1. Clip a small amount of fur (1.5-cm × 1.5-cm area) from the area between the scapulae using scissors. Get as much of the length of the hair as possible, without necessarily cutting down to the base. There should be a sizeable amount of hair in the tube. While the analysis does not require a lot of hair, there must be a sufficient amount for all analyses.

2. Store the hair in one of the empty vials provided. Label the vial with the date (with the month written out, e.g., 12/Aug/2001, or Aug/12/2001), bat species (use the 4-letter species code (e.g., MYSO, MULU, etc.)), sex, age, unique identifier for that bat (e.g., band number), and the location. Leave room for a second identifier on the vial. Be careful when choosing the marking pen to write on the vial, as some will rub off in handling. Ultra fine point Sharpies provide a point small enough to permit writing on the tubes, while still providing permanence of the data on the sample.

3. Once finished, wipe any remaining hair from the scissors with an alcohol swab. Be very careful to avoid cross-contamination.

4. Fill out the datasheet completely as each sample is collected.

Appendix C: Example Protocol for Tissue Sampling

When taking tissue from the wing membranes, stay close to the body (between the leg and the fifth digit in the wing) so as not to greatly affect flight performance. Do not punch areas with large blood vessels. Based on recaptures of sampled bats, the hole in the membrane usually grows back within 2-3 weeks, so there are no long-term effects. Bats are commonly captured with holes much larger than those resulting from tissue sampling, and these holes don't appear to result in a loss of flight ability.

Membrane Sampling Protocol:

1. Flame the instruments (punch, forceps) thoroughly to sterilize them, and to ensure that no tissue or hair from the last bat remains. The instruments should get hot.

2. LET THE INSTRUMENTS COOL by placing them on the vial box in such a way that the business ends do not touch anything, and remain sterile. If the instruments are not cooled, the hole will be cauterized, and it won't grow back. Wipe the instruments with an alcohol swab to remove any residue from the flaming, and then let the instruments dry for a few seconds.

3. Stretch the wing or tail membrane over a flat, hard or semi-hard surface (cutting board, clipboard, binder, cardboard, etc.). While stretching the membrane, press the punch down onto the membrane of one wing close to the legs (between the legs and the fifth digit), and twist and/or rock the punch slightly until it is apparent that the punch has gone through the membrane on all sides. Be sure to avoid major blood vessels. There is no need to hammer the punch down through the membrane, and doing so will decrease the life of the punch. Each punch can be reused multiple times (5-40, depending on wear and tear). Some judgment must be used in determining how well the punch is cutting. Dispose of punches as soon as they start to dull.

4. The cut tissue should now be sitting on the punched surface and can be easily picked up with forceps. If not, the membrane may be in the hollow portion of the punch, and can be dug out with the forceps. Store the punches in the O-ring vials containing salt/DMSO solution (clear liquid). The tissue tends to stick to the forceps, so the forceps may need to be shaken semi-vigorously in the solution in the vial to dislodge it, or it can be wiped off onto the side of the vial.

5. Repeat for the other wing. Place both pieces of membrane from an individual into the same vial containing salt/DMSO solution. When finished, verify that both pieces of tissue are sitting in the solution; the vial may have to be shaken (with the cap on!) to dislodge the pieces of tissue from the sides of the vial.

6. Label all vials with a unique identifier for that bat, the date (with the month written out, e.g., 12/Aug/2001, or Aug/12/2001), location, bat species, sex, reproductive condition, and age. Also fill out the data sheet provided with the necessary information.

7. Between bats, clean the punching surface well, either by flushing with a spray bottle containing alcohol (isopropyl, 70-95 percent ethanol) or wiping down the surface well with an alcohol swab. The goal is to minimize the chances of contaminating future samples.

8. If collecting from dead bats, be sure to collect a significant amount of membrane from each wing (1-cm × 1-cm area) and drop it into a vial with DMSO. Also take some muscle tissue (it is easiest to take it from the pectoral muscles) and store it in a separate vial containing salt/DMSO. At a minimum, take a 2-mm^3 piece of tissue (a small cube), but collect as much as will fit into the vial.