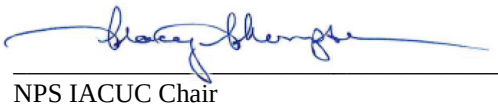
NPS Institutional Animal Care & Use Committee
Standard Operating Procedure for the Study of Bats in the Field

Effective Date:

Purpose: The purpose of this Standard Operating Procedure (SOP) is to describe methods the National Park Service Institutional Animal Care and Use Committee (NPS IACUC) has approved for field research of bats. This SOP covers the capture, handling, tagging, sampling, and collection of bats. It is not comprehensive; methods not described herein may be approved by the NPS IACUC upon further review.

Approved:



NPS IACUC Chair

May 10, 2016
Date

Contents

I. Introduction

There is a growing interest in bat research by the National Park Service (NPS). NPS units have become frequently involved in research activities of bat distribution and abundance, population dynamics, genetics and systematics, disease, habitat use, and impacts of contaminants and energy development. These activities sometimes require the capture, holding, marking, tissue sampling, or collection of bats. The objective of this Standard Operating Procedure (SOP) is to provide a suite of methods that have been approved by the NPS Institutional Animal Care and Use Committee (IACUC) for use in bat research activities. It may be referenced in individual study plans and NPS IACUC project submission forms and will help speed the review process for those submissions. It does not preclude the use of other methods that are not described in this SOP. However, those methods will require additional NPS IACUC review prior to approval. This SOP adheres to guidelines approved by the American Society of Mammalogists for use of mammals in research (Sikes et al. 2011).

In addition to NPS IACUC review of activities involving capture and handling of bats, other permits will be required prior to beginning field work. These include scientific and collecting permits from the parks through the NPS Research Permit and Reporting System (RPRS) , as well as United States Fish and Wildlife Service (USFWS) permits for any threatened or endgangered bat species included in the research activities.

II. Human Health Precautions

All field workers maintain up-to-date tetanus immunizations and practice common sense personal hygiene during bat studies, including hand-washing and prevention of contamination of food. There is a risk of rabies infection from bites or exposure to saliva incurred while handling bats. This risk is emphasized in written and verbal instructions to all field personnel. All personnel involved in handling bats receive pre-exposure rabies inoculations and biennial titer checks prior to each field season. Bats are handled only with leather-gloved hands and with surgical latex (or latex-free) gloves over the leather gloves. In the event personnel are bitten by bats during handling, they immediately wash the wound site with soap and water and are referred to seek advice from their physician or local health clinic. The bat may be retained and submitted for standard rabies testing by appropriate public health agencies, and the NPS Office of Public Health is notified under those circumstances. The use of ultraviolet (UV) light to screen bat wings presents certain health risks, which are minimized by wearing UVA-blocking safety glasses and following the guidelines described later in this document (see Section VI, E). Women who are pregnant do not work with isoflurane.

III. Methods of Capture

A. Mist nets

Mist nets may be set along watercourses, across small ponds and edges of lakes, across ravines or gullies, perpendicular to patches of trees and shrubs, across likely "flyways" or "corridors", at potential roosts under bridges, at buildings, cisterns, tree cavities, overhangs, in shallow grottos along cliffs, and cave and mine entrances. Mist nets (6-18 m in length) typically extend 2-3 m high with bottoms set at or near ground or water level, but may also use a portable pulley system (Gardner et al. 1994) to raise nets an additional 10-15 m above ground should bats be regularly observed in flight at greater heights. In forested areas, trees may be used to support vertical stacked nets, by rigging a natural pulley system over branches near the canopy (Hodgkison et al. 2002).

Nets are tended regularly by field biologists and technicians beginning just before sunset until nightly sampling is complete. In most instances, nets are checked at 5-10 minute intervals; on occasion widely separated nets may be tended at 10-15 minute intervals. Regular monitoring prevents undue entanglement and reduces potential for immersion of bats in sagging bottom strands of nets over water. It also reduces the likelihood of bats escaping from the net (e.g., chewing their way out) and prevents problems with potential predators on bats in nets. Nets are closed and/or taken down and sampling is discontinued in inclement weather conditions. All nets should be closed or taken down when sampling activities are completed so that no unattended nets are left open.

Additional details and illustrations on standard procedures for capturing bats by mist net are provided in the specific references found in Appendix A (Kunz and Kurta 1988, Kunz et al. 1996, 2009) and references therein. Use of mist nets is a procedure recommended for capturing bats by the American Society of Mammalogists (ASM 1998, Gannon et al. 2007, Sikes et al. 2011). Decontamination procedures for mist nets and other gear after surveys are implemented to reduce the risk of spreading the fungus, *Psuedogymnoascus destructans* (*Pd*), the cause of white-nose syndrome (WNS) in bats (Appendix E). Mist-nets or other gear used in areas known to be affected by WNS will not be used in presumed WNS-free areas.

B. Harp Traps

Harp traps may be employed at roost exits during emergence and at entrances to night roosts or along flyways with bat traffic. Harp traps (Tuttle 1974, Francis 1989) are recommended by the American Society of Mammalogists (ASM 1998, Gannon et al. 2007, Sikes et al. 2011) for use at roosts or other locations where concentrations of bats are expected (such as at roost entrances). This is because harp traps minimize periods of entanglement that would otherwise occur in situations when numerous bats are caught in nets over a brief period of time. In harp traps, bats in flight fall to a soft canvas bag after attempting to negotiate openings between offset strands of vertically oriented banks of fine monofilament line. They crawl up the sides of the bag to a seam with a clear plastic flap (allowing instant visibility by tenders) and typically rest quietly at the seam until removed by hand. Harp traps are monitored continuously when placed at roosts. Additional details on standard procedures for capturing

bats by harp trap are provided in Appendix A (Kunz and Kurta 1988, Kunz et al. 1996, 2009) and references therein. Decontamination procedures for harp traps and other gear after surveys are implemented to reduce the risk of spreading *Pd*. Harp traps or other gear used in areas known to be affected by WNS will not be used in presumed WNS-free areas.
(Appendix E).

C. Other Methods of Collection

Bats may be extracted from roosts with gloved hands, long-handled forceps with padded ends, or hand-held hoop ("butterfly") nets upon exit or during flight in confined situations (such as attics or tunnels). Flick netting may be used where bats are foraging in open areas and flyways (Kunz et al. 2009). Bucket and funnel traps may also be employed. These are also standard methods employed in bat studies as described and illustrated in the references provided in Appendix A. Many of these methods would be used in situations where bats must be sampled at the roost. Bats are captured almost instantaneously under such conditions and capture devices are regularly monitored by investigators. Decontamination procedures for equipment after surveys is implemented to reduce the risk of transmitting *Pd* and gear used in areas known to be affected by WNS will not be used in presumed WNS-free areas (Appendix E).

D. Alternatives

Mist nets and harp traps are the recommended methods of choice for capturing bats approved by the American Society of Mammalogists (ASM 1998, Gannon et al. 2007, Sikes et al. 2011), and by leading experts in capturing bats for scientific study (e.g., Kunz and Kurta 1988, Kunz et al. 1996, 2009). They are put into place temporarily, supervised, and are removed each night at the end of the evening capture session. Extracting bats from roosts by hand or long-handled forceps, use of hoop nets in confined spaces, and application of bucket traps or funnel traps at roosts are also long-established methods considered safe for bats and researchers. Acoustic devices (such as Anabat) may be used to non-invasively measure distribution and relative abundance of bat species.

Non-acceptable alternatives to mist-netting or harp traps are shooting bats in flight with firearms (e.g., shotguns and long guns with dust shot), or stringing fine wires just above the water surface at drinking places, then scooping the immersed bats from the water with dip nets as they try to swim to shore. Shooting does not allow non-destructive sampling and recapture, while the wire technique is less effective and more likely to cause harm or drowning. Chemicals or gases used to force bats out of roosts have potential to be lethal or debilitating and are not acceptable alternatives to the methods described for removal from roosts.

E. -addressed belowPotential Hazards

Injuries to bats captured using the methods described should be rare. There are occasionally minor injuries or broken blood vessels when individuals get entangled in the net. Bats caught in bottom strands of nets set over water could conceivably drown if left unattended for more than a few minutes.

F. Training and Personnel

The use of mist nets and harp traps for capture of bats must be performed by experienced personnel properly trained in these techniques and in safe work practices.

IV. Handling, Temporary Holding, and Transport of Live Bats

A. Methods of Handling

Purpose and Description

Bats may be handled for a number of research, teaching, training, or management purposes. These include species identification, determining reproductive condition and age class, obtaining mass and measurements, sampling tissues and ectoparasites, swabbing to sample microbes, marking and determining if previously marked, categorizing degree of disease progression as in the case for white-nose syndrome, and radio tagging.

Captured bats are disentangled from nets or removed from harp traps by hand and placed individually in separate cloth or paper bags (e.g., 4" x 6 ") that are fastened closed. Once removed from nets or traps and placed in bags, bats typically become quiet and do not outwardly appear to be in stress; often they will enter torpor under such conditions. Bags are marked with information on individual bat and time of collection. Bags with bats are kept individually in well-ventilated, quiet, dark areas and placed where accidental damage from inadvertent placement of objects or feet on bags in the dark cannot occur. Because capture and marking operations take place after dark, no precautions to keep bags from direct sunlight are necessary. Once a bat is placed in a cloth bag, the bag must be cleaned prior to use again in order to reduce the risk of transmitting *Pd*, the cause of WNS in bats, or other infectious diseases between individuals (Appendix E). A paper bag must be used only once to hold a bat, then discarded. Bats are not held in cloth or paperbags under circumstances when they can be processed immediately after disentanglement (i.e., no other bats to disentangle at the time and no other bats to be processed from prior captures). Bats are not housed in groups in cages. Group holding can result in bats biting one another, damaging teeth on the cage, and transmitting diseases among individuals and species that may not otherwise contact each other.

For determination of species, sex, relative age, reproductive condition, body mass, and morphometrics, bats are gently held in gloved hands for about 5-10 minutes (as illustrated in Kunz et al. 1996, 2009), then released by allowing them to launch from an outstretched palm. Sex is determined based on simple inspection of external genitalia. Categorization to relative age class (adult or young-of-the-year) is based on visual inspection of degree of ossification of phalangeal epiphyses when the wing is held outstretched and illuminated from behind with a light (Anthony 1988, Brunet-Rossinni and Wilkinson 2009).
The season in which captures are conducted should be considered when preparing to engage in bat research activities. Pregnant and lactating female bats are commonly and, in some activities, intentionally captured . Handling techniques for these females should be well-established to minimize

the handling time and to reduce stress from the procedures. Reproductive condition is observed visually in males (distended saccula or scrota) and visually or by gentle palpation in females (lactation or pregnancy) (Racey 1988, 2009).

Body mass is determined with a Pesola scale or portable balance while the bat is in the cloth or paper bag, and external morphometrics are obtained with dial calipers or ruler while gently holding the bat in a gloved hand.

Bats captured for telemetry studies are handled longer but released during the night of capture, usually within 1-2 hours of initial capture. They are subject to clipping approximately 8-12 mm diameter circle of fur at the interscapular area using fine dissection scissors, followed by fingertip restraint of the transmitter in place over this trimmed circle of fur for about 20 minutes while the surgical cement dries. The head may remain covered in the cloth bag during tag attachment to minimize stress (bats with heads covered during this process are typically calm).

Potential Hazards

Injuries to bats handled in the manner described in this document should be rare. However, the most likely source of injury would be pressure from holding a bat too firmly, or inadvertently placing an object over a bat in a cloth or paper bag. Should an injury occur where survival of the bat in the wild would be unlikely (e.g., fractured wing), the individual will be euthanized following the protocol described in Section VII, C.

Length of Restraint

Bats are typically restrained due to net entanglement for 5-10 minutes or less. In species identification surveys, bats are then restrained by hand for 5-10 minutes and released, or held in cloth or paper bags (where they are free to crawl but usually reach a corner and rest or become torpid) for up to one hour prior to processing. Bats held for tissue sampling or marking other than radiotagging may be restrained by hand for an additional 10 minutes (15-20 minutes total hand restraint plus time held in bag). Individuals that are candidates for radiotagging are restrained for about 30 minutes further (10 minutes hair-clipping, 20 minutes as non-toxic surgical cement dries) during application of radiotransmitters. Total time of restraint for bats does not exceed 2 hours.

Monitoring to Prevent Overt Risk or Stress

Bats are continuously observed while hand-held and while being disentangled from nets. Once placed in cloth or paper bags there are typically no outward signs of stress (e.g., excessive vocalization or movement within the bag). Any bats that display outward signs of stress over an extended period of time due to prolonged net entanglement or other factors are immediately released .

B. Transport

Purpose

Bats are usually only transported short walking distances from nets to processing stations…typically a small field table that can be easily disinfected. Normally, they are released at the point of capture. On rare occasions bats may need to be transported by motor vehicle to experts to confirm identifications, diagnose or treat injuries or illness, or to a laboratory to collect samples such as saliva. Projects that require tranport during the day or to a laboratory/clinic require additional detail in specific project plans.

Method of Transportation and Restraint During Transport

For routine processing, bats are normally hand-carried in small cloth or paper bags, one bat per bag, as described elsewhere in this document. Under rare circumstances when transport is necessary over longer distances, bats in individual bags are placed in cool dark places within solid but ventilated containers and transported by motor vehicle.

Monitoring During Transportation

Bats in bags are assessed for evidence of distress at least every hour when transported by motor vehicle.

C. Anesthesia

The application of anesthesia for any of the procedures described in this SOP is not normally necessary. However, it may be judged useful and appropriate to apply anesthesia on occasions when multiple procedures are performed on an individual animal (e.g., blood sampling, saliva sampling, PIT tag injection, wing membrane biopsy, and radiotagging). Procedures for anethesia are described in this SOP, but additional detail will likely be needed in specific project plans.

Anesthetic, dosages, and methods of administration

Inhalant anesthesia (isofluorane) employing a gas anesthetic machine is the method of choice in situations where manual restraint is insufficient. Under this procedure, a portable gas anesthetic machine is used to deliver isofluorane at about 4-5% and oxygen at 2 L/min by mask. Once induced, each bat is maintained on about 1-3% isoflurane and 1.5 L/min O_2. Isoflurane amounts will vary based on species and individual response. All procedures last 10 minutes or less to minimize hypothermia, and external heat sources are supplied if indicated (i.e., to prevent a drop in body temperature of more than 2 ^{o}C). Bats are recovered under direct observation until awake. The bats are then held in cloth or paper bags, as already described, for an observation period prior to release.

Personnel Administering Anesthetic and Training

Anesthesia is administered according to approved protocols and agency guidelines. It is only administered by cooperating veterinarians experienced in these techniques or field workers that have been trained in these techniques by cooperating veterinarians and judged competent by the veterinarian.

V. Marking and Tagging of Bats

A. Radio Transmitters

Purposes and Description

Radio tags may be used to discover locations of roost sites and to gather short-term information on movements of individual bats. Temporary attachment of miniaturized radio transmitters to locate bat roosts is the standard procedure utilized in the U.S. and Canada (e.g., Brigham et al. 1997, Kalcounis and Brigham 1998, Ormsbee and McComb 1998, Rabe et al. 1998, Wilkinson and Bradbury 1988). It is non-invasive, involves temporary adhesive approved for human use (e.g., surgical cement or colostomy bag adhesive), and is applied during gentle restraint. A small (approximately 8-12 mm diameter) patch of hair over the interscapular region is trimmed with dissecting scissors. Transmitters are attached at the trimmed site using surgical cement. The transmitter is held in place over this trimmed circle of fur by fingertip restraint for approximately 20 minutes while the surgical cement dries. Transmitters that are attached in this manner typically fall off in less than two weeks after application, which provides sufficient time to locate colonies and determine nightly foraging areas but keeps the period of attachment minimal.

Temporary radio transmitters that are attached to bats must be $\leq$ 5 % of body weight (the 5% rule; typically 0.53 - 0.78 g), as recommended in well-established guidelines for bat telemetry studies (Aldridge and Brigham 1988). Follow-up studies on radio-tagged bats of various species have shown no effect of radiotags at $\leq$ 5 % of body mass on success at prey capture (Hickey 1992) or foraging time budgets (Hickey and Fenton 1990). Neubaum et al. (2005) tested the 5% rule for the ratio of radiotransmitter mass to body mass on female big brown bats (*Eptesicus fuscus*) and found that all bats examined 1 year after radiotagging were reproductively active and had body masses similar to bats not radiotagged.

Additional information on radiotagging bats is available in Appendix B (Amelon et al. 2009, Barclay and Bell 1988, Wilkinson and Bradbury 1988). The method of attachment is recommended by the American Society of Mammalogists (1998, Gannon et al. 2007, Sikes et al. 2011).

Alternatives

There are no practicable alternatives for determining real-time locations of bats across large geographic distances. Chemical light-tags have been applied to bats to observe flight paths visually, but typically

persist for only minutes before bats are lost from view. Locating roosts by searching by eye can be nearly impossible in many areas because of countless possibilities of potential roosts under bark, in trees, in rock crevices, or in buildings. Bats also frequently move to alternate roosts from day to day within a season (Ellison et al. 2007a, Lewis 1995) and roosts can be several km apart, requiring radiotelemetry for locating alternate sites. Similarly, foraging areas can be many km from roosts, also requiring the power of radiofrequency transmitters for location. PIT or RFID tags can provide presence/absence information at roosts if readers are available, but bats must be within 6" of readers. The use of PIT tags does not allow detection without disturbance, except in specific situations where logistics allow leaving PIT tag readers in place at roost entrances and downloading data later. However, this method does not allow real-time as well as remote monitoring, and is applicable only in select situations. RFID tags may be used to allow real-time and remote monitoring for specific locations where receiving attennas and data storage devices have been installed.

Need for Anesthesia or Restraint

Anesthetics are not required. Temporary restraint is to be used as noted elsewhere in this document.

B. Passive Integrated Transponders (PIT tags)

Purposes and Description

PIT tags are used to permanently mark animals in captivity, for identification of pets, and for ecological research on fish and wildlife. They are injectable microchips (8-12 mm in size) that do not emit signals except momentarily (less than 0.04 seconds) when activated by an electronic reader. The chip then emits a unique identification code at a frequency of 125 or 134.2 kHz. This is substantially above the echolocation frequencies and hearing thresholds of most U.S. bats. PIT tags have been used with minimal harm on a variety of terrestrial small mammals, for identification of captive bats, and in a limited number of ongoing field studies of bats. PIT tag methodology was recommended as a tool to improve studies of bat populations at an expert workshop in 1999 (O'Shea and Bogan 2000, O'Shea and Bogan 2003).

PIT tags may be applied to individuals captured at selected colony sites and while foraging. PIT tags are applied to adults and to young of the year that are fully furred and capable of directed flight. PIT tagging enables estimation of population attributes through mark-recapture and Cormack-Jolly-Seber models and tracking other biological traits, such as serological evidence of infection rates, reproduction, etc., as marked bats are followed over the course of long-term studies. Before PIT tags are applied, a small amount of hair is trimmed at the injection site, and the site is treated with Betasept or similar non-irritating antiseptic. The pit tag is then injected subdermally with a sterile 12 or 16-gauge disposable needle applicator on the dorsum at the midline just above (cranial to) the uropatagium. Medical tissue adhesive or surgical cement is used to seal the entry site. Tag readers of different configurations may be used for bats held by hand, positioned remotely at entrances to roosts, and passed over groups of roosting bats.

Alternatives

Bats can be difficult subjects for other permanent marking procedures. Radiotagging is temporary, and entails a greater amount of handling and restraint. Banding with numbered metal (butt-end) bird bands is not approved because of potential injury and improper healing of wounds at banding sites, as well as hampered recognition of numbers from chewing on bands by the bats (see review by Ellison 2008). Newer, less injurious wing bands are now available (see Section IV, Part C below). Dye marks in fur are lost after the molt, and U. S. bats can live as long as 30 years, undergoing many molts in a lifetime. Freeze branding, which results in growth of unpigmented hair, is used by some investigators but definitive studies on its usefulness are not yet available (see section D below). Application of tattoo punch marks (without ink) results in healed numbered scars in the wing membranes, but these become amorphous within weeks of application (Bonnacorso and Smythe 1972, O'Shea 1975) (see section D below).

Potential hazards

Use of sterile instruments and microchips on each individual, along with antiseptic skin preparations minimizes the potential for infection or for transmission of disease when multiple bats are sampled. There is the potential for tumors to develop around the site of implantation of the PIT tag. Recent research has addressed tissue reactions to PIT tags in laboratory mice and dogs. In six citations, it was reported that between 0.8% and 10.2% of laboratory mice and rats developed malignant tumors around or adjacent to implanted PIT tags. Two additional citations reported microchip-related cancer in dogs. See *http://www.chipmenot.org/scientificevidence.htm* for a review of the literature.

Need for Anesthesia or Restraint

Anesthetics are not required. Temporary restraint is used as noted elsewhere in this document and pain is momentary and minimal.

C. Wing Bands

Purposes and Description

The need to mark bats individually in order to assess life history parameters and movements is especially important as threats from WNS continue to negatively impact bat populations. Responses to this threat at the population level can only be discerned through measurements made possible through individually marking bats. Several new types of lightweight wing bands have been specifically developed to reduce injury to bats. These bands come in various diameters and can be applied over the forearms of bats to enable individual recognition by researchers for mark-recapture studies. Bands can either be colored split celluloid rings or special lipped bat bands of soft aluminum alloy or harder, more durable incoloy (a nickel-chromium alloy, Porzana *http://www.porzana.co.uk/bat_rings.html)* (Kunz and Weise 2009). Lipped bat bands are designed to minimize the risk of damage to the bat's wing membrane, which is known to occur when standard metal (butt-end) bird bands are used (see review by

Ellison 2008). There are no standardized wing bands currently being used in mark-recapture studies; however, results from ongoing studies will be incorporated in future versions of this SOP.

Use of wing bands is described in Appendix B (Barclay and Bell 1988, Kunz and Weise 2009). The open band is slipped over the forearm of the hand-held bat proximal to the wrist. The band is then closed so that a pre-set gap remains between the two lips of the band. An appropriately sized band is critical for reducing the potential for injury. For lipped bands, the gap is large enough to allow the band to move freely along the bat's forearm without abraiding the underlying wing membrane, yet not so large that it falls off. The procedure typically takes less than 30 seconds. Unique color-coding and numbering of individual bands allow researchers to gather information on social behavior and population biology.

Alternatives

Metal (butt-end) bird bands are not approved because they cannot be read without handling the bat, are associated with injury, are susceptible to chewing, and are not easily removed if injury is noted (Barclay and Bell 1988, Ellison 2008, Kunz and Weise 2009). PIT tags are the best alternative, but require handling bats or greater disturbance at roosts. PIT tags also require the bats to pass in close proximity to a PIT tag reader, which may not be feasible at certain roost types (e.g., in caves or mines with large or multiple entrances or when bats are roosting out of reach of hand-held tag readers.) Determination of individual identity through videotaping or simple visual observation at colony sites without handling or very close approach is not possible with PIT tags, metal wing bands, necklaces, punch marks, or dyes. Bats in colonies pack tightly into crevices or among each other, leaving only heads and forearms near the wrist (where bands are applied) visible from below. Temporary identification methods and metal bands do not allow recognition at roosts without capture across years.

Potential Hazards

The plastic split-ring bands result in less wounding than metal bird bands, but have the potential to result in abnormal healing around application sites. There are no statistically-based evaluations of the impact of wing bands on long-term survival of bats, but field and zoo or captive animal studies that have deployed plastic split-ring bands assert that impacts are minimal.

Need for Anesthesia or Restraint

Anesthetics are not required. Temporary restraint is used as noted elsewhere in this document.

D. Wing Punch Marks and Freeze Branding

Purposes and Description

Small tattoo punches used to identify domestic pets have been successfully applied to wing membranes of bats for temporary marking for a few weeks duration (Bonnacorso and Smythe 1972). One person

extends the wing with one hand and holds the body with the other, while a second person applies the punch tool instantaneously after aseptic preparation (3 swipes with Betasept swabs or similar disinfectant). The punch marks leave the outline of a number formed by a series of pin-prick holes, which are legible as white scar tissue for several weeks after marking (O'Shea 1975, Bonnacorso et al. 1976, Kleiman and Davis 1974). Wing punches can be used in situations where information on short-term identification of individual bats is needed, but not in studies of long duration. Wings of bats heal very rapidly (see discussion under biopsy sampling below).

Freeze marking of bats is a fast, non-invasive means of permanently marking portions of the pelage of bats. It has long been used as a marking technique in mammals (Hadow 1972, Russell 1981). A coolant (e.g. dry ice and alcohol, or canned refrigerants such as freon) is applied to a small patch of clipped hair for about 5-20 seconds in a well-ventilated area. The coolant is not directly applied to the skin of the bat. The resultant regrowth of hair is permanently unpigmented. This has been noted as a potential marking technique for bats (Barclay and Bell 1988, Appendix B) but only recently applied. A suggested application of this technique on bats is to use a small cardboard template to a single small circular spot < 5 mm in diameter to the lower lumbar region of the dorsum when needed to permanently mark bats as previously captured. Specific applications may include double-marking studies to estimate tag loss rates of PIT tags. Use of freeze patterns in pelage of mammals has been used on a wide range of species (three species of mongoose, coati mundi, grass mice, house mice, two species of rats, two species of squirrels, duck-billed platypus, horses, ungulates, and other taxa, Hadow, 1972, Russell 1981, Rood and Nellis 1980, Grant and Whittington 1991, Carroll and Wilson 1984).

Alternatives

Wing punching may be useful to determine if individual bats captured by hand over a few weeks period have been previously marked and sampled. However, it is a non-permanent marking technique and, while wing punches heal very quickly, there is a risk for infection and other techniqes are less invasive. Justification for using wing punching will need to be evaluated by the IACUC.

Freeze brand patterns are also rapidly applied with little handling and allow some individual marking, but are most useful for a permanent record of a past capture event. Marking with other techniques (e.g. PIT tags, radio tags) can take longer to apply or involve more invasive handling.

Potential Hazards

There are no known hazards to wing-tattooing. Wing tissue in bats heals very rapidly (see discussion under biopsy sampling below). Freeze-branding, if applied directly to the skin of the bat, can cause local necrosis of the skin, but recovery occurs and detrimental long-term effects on other mammals are not known to have been reported. Use of sterile instruments and aseptic procedures will minimize potential for infection or for transmission of disease when multiple bats are sampled.

Need for Anesthesia or Restraint

Anesthetics are not required. Temporary restraint is used as noted elsewhere in this document.

VI. Sampling Tissues from Bats

A. Wing Membranes

Purposes and Description

Individual bats can be sampled for genetics studies and disease surveillance (e.g., WNS) using wing punch biopsies. Punching small holes in wing membranes of bats is a procedure that has been used widely for at least 30 years for temporary marking of bats in the field (Barclay and Bell 1988, Bonnacorso and Smythe 1972). Recently wing punches have also become a preferred method for obtaining biopsy tissue samples of wild bats for population genetics and taxonomic studies (e.g., Burland et al. 1998, Rossiter et al. 1999, Worthington Wilmer et al. 1999). Punched holes as large as 14-17 mm diameter in wing membranes of pallid bats have been shown to heal rapidly (Davis and Doster 1972). This SOP approves wing-punch biopsy methods detailed by Worthington et al. (1996) and the USGS Wildlife Health Center (2014/2015) (Appendix C) to sample a circular area of 3-5 mm diameter. This is only about 8 % of the maximum area noted by Davis and Doster (1972) as healing rapidly in pallid bats. No irreversible changes to the biopsy site or decreases in bat survival are known to result from these procedures, which have been used in studies of multiple species of bats (Burland et al. 1998, Rossiter et al. 1999, Worthington et al. 1996, Worthington et al. 1999). Sampling is from the distal third of the plagiopatagium or the uropatagium and is limited to 2 punch biopsies per bat (from different wings or one from the plagiopatagium and one from the uropatagium of the same wing). Antiseptic surgical preparation of the sample area (3 wipes with Betasept swabs or similar antiseptic) are made, followed by a 3-5 mm round punch biopsy of the wing taken with a previously sterilized and sterile-packaged skin biopsy punch. Wing biopsies are either destroyed in the analysis or deposited at accredited collections that meet NPS collection standards and standards of the American Society of Mammalogists Hafner et al. 1997).

Alternatives

Wing-punch biopsies are fast and heal rapidly Blood samples are an alternative for genetic sampling. However, drawing blood from bats is more difficult and time-consuming, and has a greater potential for injury than wing membrane sampling. Wing biopsies have thus become a preferred method for genetics-based studies of bats.

Potential Hazards

Use of sterile instruments on each bat and aseptic skin preparation minimizes potential for infection or for transmission of disease when multiple bats are sampled. Excessive bleeding could occur if a large blood vessel is severed, but careful choice of the biopsy site minimizes this possibility. If excessive bleeding occurs, direct pressure is applied to the site using sterile gauze until bleeding stops. No other potential hazards are known.

Need for Anesthesia or Restraint

Anesthesia or restraint other than by hand is not required.

B. Blood Sampling

Purposes and Description

Blood sampling may be necessary for studies of diseases in bats as well as for studies of genetics, physiology, hematology, and blood chemistry. Blood is collected by venipuncture of blood vessels in the wing membranes as described in Appendix D (Kunz and Nagy 1988). Vessels of choice are the antebrachial vein near the forearm or the uropatagial vein that runs parallel to the tail in the interfemoral membrane. Obtaining blood from bats by venipuncture in these areas is the procedure recommended by the American Society of Mammalogists (1998, Gannon et al. 2007, Sikes et al. 2011). A volume of blood no greater than the equivalent of 1% of the body mass of the bat is drawn at no shorter than one-week intervals. This follows general veterinary guidelines of 1 % of body mass for blood sampling of mammals (mass-specific blood volumes in bats are similar to other mammals, Bassett and Studier 1988). Bats in torpor should be allowed to arouse to a warm body temperature (a supplemental heat source may be used to facilitate this process) prior to blood collection to ensure adequate peripheral blood circulation. The area where blood is drawn is aseptically prepared with alcohol prior to blood extraction. Depending on the size of the bat, the vein is either lanced with a 25-30 ga needle and pooled blood drawn into capillary tubes or other blood collection device, or blood is drawn directly from the vein using a tuberculin syringe with 25-30 ga needles. Careful hemostasis is applied to minimize hematoma formation. Blood sampling of bats in the field using wing veins (easily viewed at night under transillumination with a small portable light source) follows well-established techniques (e.g., Appendix D, McCracken and Wilkinson 1988, Kunz and Nagy 1988, Wimsatt et al. 2005). In certain circumstances, such as studies of blood chemistry, the volume of sample necessary for analysis requires humanely euthanizing the bat and then collecting blood by cardiac puncture and/or decapitation. In such situations, bats are euthanized immediately prior to cardiac puncture or decapitation using methods described below in Section VII, Part C (Euthanasia).

Alternatives

There are no non-lethal alternatives to blood sampling for serological studies, and few as standard or simple for many other kinds of research.

Potential Hazards

Development of hematomas is the major hazard. Excessive bleeding could occur if a large blood vessel is severed. If excessive bleeding occurs, direct pressure is applied to the site using sterile gauze, until bleeding stops. Silver nitrate (Striptik) or coagulating powder (QuickStop) may also be used to stem excessive bleeding. Sterile procedures minimize potential for infection or for transmission of disease when multiple bats are sampled.

Need for Anesthesia or Restraint

In a study by Wimsatt et al. (2005), there was no difference in short-term survival and 1-yr return rates of bats bled with anesthesia when compared to bats bled without anesthesia suggesting that the use of anesthesia during sampling of blood has no advantages in terms of enhancement of survival in big brown bats (Ellison et al. 2006).

C. Saliva Swab Sampling

Purposes and Description

Saliva swab samples are obtained by sterile calcium alginate swabs or by plastic-tip pipettes in hand-held bats (Dominguez et al. 2007, Osborne et al. 2011). They are used for PCR-based determination of the presence of rabies and for screening for other viruses of potential importance in bats that may also be shed through saliva and spread by oral contact. To collect oral swabs, the mouth is gently kept open (typically passively as the hand-held bat bites down at the edge of a tongue depressor or similar soft disposable object) using safe work practices to avoid human contact with saliva and a brief contact is made on the mouth with a sample swab or plastic-tip pipette. In the field, samples are kept in a cooler or liquid Nitrogen and later stored at 4°C (Dominguez et al. 2007, Osborne et al. 2011).

Alternatives

There are no known non-invasive alternatives to swabs or micropipettes of the oral cavities of hand-held bats for obtaining saliva samples.

Potential Hazards

Disease could spread if swabs, pipettes, or other objects that come into contact with the mouth were used among bats repeatedly, but they are to be employed only for one-time use. There is potential for tooth or jaw damage if the swab or pipette is not gently applied to the bat's mouth.

Need for Anesthesia or Restraint

No anesthesia is required. The bats are held by hand briefly and the swab is taken instantaneously.

D. Hair Sampling

Purposes and Description

Hair sampling may be necessary at times for studies of genetics, hair structure, contaminants, food habits, geographic origins, and assessment of migratory pathways using isotope analyses. In many cases, previously preserved specimens of bats can be used, but it may be necessary to obtain hair from live animals in some cases. Typically only one or a few (up to 12) hairs are needed for these analyses

and they are usually obtained by simply clipping with fine scissors. Hair from the mid-dorsal region is usually used. Gentle restraint with the head of the bat covered with a soft cloth or bag is sufficient for this process. In some cases, hair clipped from bats prior to attaching radiotransmitters or PIT tagging can be used.

Alternatives

Other samples, such as tissue, saliva, blood, and feces, may be used for analysis of genetics or relatedness of individuals. Degradation of these types of samples can occur and scope of information provided by the analyses may vary so make certain to consult with reference laboratories on preferred samples recommended to meet your research objectives.

Potential Hazards

This technique presents no known hazards. It is possible that cutting hair too close to the skin could cause skin injuries, but care to avoid the dermal surface with the scissors would prevent potential injuries. Relative to the total number of hairs in the pelage a truly miniscule sample is removed for these purposes.

Need for Anesthesia or Restraint

No anesthesia is required. The bats are held by hand briefly and the hair is clipped free from the skin.

E. Longwave Ultraviolet Florescence Screening of Wings

Purpose and Description

Longwave ultraviolet (UV) light is used as a non-invasive technique to screen bat wings for lesions indicative of WNS (Turner et al. 2014). Bats infected with *Pd* often display little to no visible fungal growth under white light. However, examination with longwave UV light (wavelength 366–385 nm) has been shown to elicit a yellow-orange fluorescence in wing membranes that corresponds directly with the fungal cupping erosions in histologic sections of skin currently used for diagnosis of WNS (Turner et al. 2014). Although histopathology is needed to confirm WNS in bats, UV light is useful as a preliminary screening technique and can guide targeted non-lethal biopsy sampling for histopathology (Turner et al. 2014).

Detailed methods are described by Turner et al. (2014) and USGS Wildlife Health Center Bat Submission Guidelines (see *http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/USGS_NWHC_Bat_WNS_submission_protocol.pdf*). The bat is examined in complete darkness by shining the UV flashlight facing down approximately 7.5-12.5 cm above the extended

ventral surface of the flight membranes. If a UV box is used, the bat is placed on its back, and the wing and corresponding foot are extended over the UV light source. The wing is examined for circular areas of yellow-orange florescence. A digital photograph of the wing may be taken when using a UV box, as visualization of florescence is greatly enhanced by examining the photograph when using the box. Photography does not improve visualization with the UV flashlight. If live-sampling techniques are used, paired wing punch biopsies 3-5 mm in diameter are taken that incorporate areas of UV florescence and follow procedures described in Section VI, A. If the bat is to be euthanized. a permanent marker is used to circle representative areas of florescence on the wing membrane. Euthanization follows procedures described in Section VII, Part C.

To minimize exposure to UV light, the following precautions are also implemented:

- Field personnel wear UV protective eyewear when illuminating bats and work as far away from the UV source as feasible.
- Time the UV light is in use is limited. This is accomplished by turning off or covering the light between bats and by enhancing the efficiency of procedures so the processing time per bat is minimized.
- Decreasing both the exposure time and dose by minimizing reflective surfaces on the UV light source and on processing equipment (e.g., table surface).
- Holding the bat's head inside a holding bag or otherwise covered while viewing the outstreatched wing. The UV light is OFF or completely covered when a bat's head is outside the bag.

Alternatives

Non-lethal swabbing of bat skin (see F below) can be used to detect the presence of *Pd*, although it is not a useful technique for targeting areas of skin for biopsy sampling. Like UV light screening, it does not confirm WNS and should not be used as the sole sampling methodology.

Potential Hazards

UV radiation is known to pose health risks to humans, including sunburn, premature aging, cataracts, and skin cancer. Those hazards are minimized by following the guidelines previously described .

Need for Anesthesia or Restraint

No need for anesthesia. Bats are held gently by hand during the procedure.

F. Miscellaneous Sampling (Feces, Urine, Milk, Parasites, Microbes)

Purposes and Description

Capture and handling of bats provides opportunities to collect additional materials that may be useful in some directed studies. For example, bats frequently defecate and urinate during handling and thus fecal

pellets (which can provide information on diet, some endoparasites, and virus infection), and urine (which provides information on kidney function) often can be easily collected as an adjunct to the capture and handling process. Food passage time in most bats is quite rapid (e.g., 35-170 min for *Myotis lucifugus*, Buchler 1975) and since mist-net capture of bats typically occurs while bats are foraging, important information on diet can be obtained. Although fecal pellets can simply be scavenged as a part of the handling process, the usual process to obtain a fecal sample is to place the bat in a sealable (e.g., Ziplock) plastic bag for 5-10 min with air holes and cover the bag with a soft cloth to prevent stress. Bats almost always defecate in the bag and when the bat is removed for processing, the plastic bag can be resealed, labeled with the pertinent data, and conveniently stored until analysis. Whitaker (1988a) and Whitaker et al. (2009b) provide additional information on such studies. As a precaution for investigator safety, fecal boluses are not to be handled directly but are to be manipulated with forceps while wearing disposable gloves. Forceps are to be cleaned and rinsed in ethanol immediately after sampling.

Collecting urine samples is easily done by turning the bat on its back momentarily, before placing it in a bag, and holding a glass capillary tube to the urethra. Tubes are sealed with critocaps, labeled, and stored at –20C until analyzed. Milk can be obtained from lactating females in the same manner by applying gentle pressure around the edges of the mammary glands and collecting the expelled milk in a capillary tube. Milk is stored similar to urine samples.

It is possible to remove some ectoparasites from bats during handling by using fine forceps, with the aide of 10X jeweler's glass or other form of optical visor that enhances magnification (Whitaker et al. 2009a). The obvious ectoparasites amenable to such removal are wingless flies (Nycteribiidae and Streblidae), batbugs (Cimicidae), fleas (Ischnopsyllidae), ticks (Argasidae), and larger wing (Spinturnicidae) and fur (Macronyssidae) mites belonging to Acari.

However, in-depth studies of ectoparasites, including microscopic individuals, require systematic and thorough searching of the entire external surface of the bat's body and removal of ectoparasites to preservative fluids. Such searches can be done while bats are anesthetized for other purposes or prior to preparation of voucher specimens (Whitaker 1988b, Whitaker et al. 2009a). Microscopic examination of hosts are effective following Whitaker et al. (2009a). Washing vouchered specimens is another method, as described by Henry and McKeever (1971) and reviewed by Whitaker et al. (2009), of collecting ectoparasites at the community level. Caution is warranted as handling of bats during capture and preservation may have allowed cross-contamination of ectoparasites.

The skin of bats may be swabbed to detect *Pd* (see USGS Wildlife Health Center Bat Submission Guidelines at *http://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/USGS_NWHC_Bat_WNS_submission_protocol.pdf*) or other microbes. The tip of a sterile polyester-tipped swab is first dipped into a sampling tube of sterile water and then gently rolled several times across the surface of the skin along the foream and muzzle. The swab is placed in a storage tube, sealed, labeled, and then chilled (4C) or frozen (-20C) until analyzed.

Alternatives

Information on food habits can be obtained in other ways, including sacrificing the bat or collecting fresh carcasses and directly examining stomach contents. Fecal pellets are can be used for analyzing

prey sources and nutrient content.. Fecal pellets can be used for surveys of coronaviruses, but swabs are more efficient in determining prevalence of infection and not all bats produce feces when captured without extensive holding times Depending on the focus of the study, information on physiological parameters involved in water balance also can be obtained by using other body fluids, including blood. However, data that are directly relevant to kidney function are best obtained from urine. These same comments apply to analyses of milk and its constituents. Collection of endoparasites by examining internal organs, nasal cavities, and soft-body parts (e.g., eyes, scrotum) of fresh hosts follow Gardner and Jimenez-Ruiz (2009). Some endoparasites, such as coccidian parasites, can be obtained from the collection of fresh guano following Scott and Duszynski (1997).

Potential Hazards

No hazards are known for collection of feces, urine, milk, ectoparasites, or microbes as described above other than possible slight extension of holding time. As long as bats are kept in bags by themselves with no pressure other than a cloth covering the bag, bats remain calm and usually move into a corner of the bag. Investigators should be cognizant of the effect of collecting additional data on the total holding time of bats (not to exceed 2 hours).

Need for Anesthesia or Restraint

No anesthesia is required for the collection of these materials. The bats are held gently and briefly by hand as described elsewhere and rectal swabs, urine, and milk quickly collected. Ectoparasites are quickly plucked from the body surface when handling them shortly after capture.

VI. Remotely Observing Bats

A. Video monitoring

Purposes and Description

Over the past 20 years, both the equipment and technology available for video monitoring of bats have undergone many changes and advances. Near infrared video monitoring, active infrared night vision video monitoring, and thermal (or ‘far’) infrared imaging now make it possible to monitor bats even in closed cavities with narrow openings and no ambient light (Kerth and Dechman 2009). Thermal infrared cameras that visualize the infrared radiation emitted by the body heat of animals can also be used to document bat behavior without the need for a source of illumination (Sandel et al. 2004). Near-infrared video cameras image reflected infrared light and require an external source of infrared illumination.

Infrared video surveillance systems may be used in caves and mines where bats are hibernating or at maternity or other roosting sites in order to remotely observe the behavior of bats under natural conditions. Video surveillance systems consist of an array of infrared cameras (both thermal and near-infrared) situated at strategic locations. Cameras are situated at least 2 m away from clusters of bats. It is recommended that they are networked to a digital control and storage device situated outside of the hibernacula or roost, along with power system equipment. The video control unit is situated in a

location where accessing it to download video data does not disturb hibernating or roosting bats. Image data may be downloaded from the control unit and backed up to portable digital storage devices at various intervals. Alternatively, image data may be connected to servers or dataloggers accessed via the internet for remote download or real-time viewing and camera controls (e.g., a webcam). It is not anticipated that the physical presence of cameras on hibernacula floors or roosting sites > 2 m away from clusters of bats will affect their behavior. Camera units do not create light (visible), noise, or heat that could disturb roosting bats. Light levels can be assessed visually, because the human visual spectrum (about 380-750 nm) is more sensitive to infrared light (> 750 nm) than the visual spectrum of echolocating bats (approx. 350-700 nm; Hope and Bahtnagar 1979; Winter et al. 2003). Noise levels around camera units may be measured with full-spectrum bat detectors that are sensitive to high-frequency audible noises and ultrasound (> 10 kHz). Air temperature is measured around cameras prior to installation in caves to ensure that they are not capable of warming large chambers (>20 m^3) of hibernacula where they are operating. Surveillance systems are deployed in hibernacula before bats return for hibernation in autumn and left undisturbed until bats leave hibernacula in spring. If cameras or lighting units deployed within caves malfunction during the hibernation period, they are not retrieved for repair or replacement until the end of the hibernation period. Dates when bats are most likely to enter and leave hibernation sites during autumn and spring are determined by consultation with local wildlife professionals that have winter survey experience and relevant occurrence data. There is not any disturbance to bats from the surveillance cameras or associated installation. Use of camera equipment follows the National White-nose Syndrome Decontamination Protocol (Appendix E).

Alternatives

Using remote video monitoring of bats is the only way to gather information on bat behavior within their roosts without directly disturbing the bats during critical time periods (e.g., hibernation, maternity). Chronic disturbance of hibernating bats (e.g., inappropriately applied wing bands, human visitation of hibernacula) is known to cause high rates of winter mortality through increased activity and subsequent fat loss of the bats.

Potential Hazards

No disturbance to bats should occur from the surveillance cameras or associated installation.

Need for Anesthesia or Restraint

There is no need for anesthesia or restraint of bats using remote video monitoring.

B. Acoustic Surveys

Purposes and Description

Through the detection, recording, and analysis of bat vocalizations, researchers can learn much about the ecology, behavior, and biology of bats (Parsons and Szewczak 2009). Acoustic surveys are used to determine bat activity and provide a way to develop balanced field studies addressing patterns of

foraging activity or occupancy, and distribution on a larger scale than conventional trapping techniques (e.g., mist netting). A variety of acoustic detectors exist and currently are undergoing rapid evolution. Acoustic species identification of bats depends on a comprehensive accounting of the echolocation call characteristics for all species likely to be encountered at a given study area (Parsons and Szewczak 2009). This can be established through the compilation and analysis of a comprehensive reference library of the echolocation call repertoires for those species.

A combination of methods may be used to develop a representative call database. (Methods used to capture individual bats are described in Section II.) Recordings are made from hand-released bats in open areas far enough away from water sources or flyways to mitigate interference from other bats. Ideally, three people are used to secure the best recording from a hand-released bat: a recorder, a spotlighter, and a releaser. The releaser stands about 20-25 meters away from the recorder. The spotlighter should stand about 5 meters away from the releaser (with the releaser between the recorder and spotlighter). Releaser should then raise the bat above their head and release after it has been spotlighted. The recorder should aim the detector microphone toward the flying bat, saving files every 5-10 seconds or so.

An attached light tag will greatly facilitate tracking and diminish the possibility of recording another bat (Parsons and Szewczak 2009). Light tagging entails using a nontoxic school glue stick or surgical adhesive (e.g., Skinbond) to temporarily attach a miniature Cyalume light stick (<5% of bat's body weight) to a captured bat (Kunz and Weise 2009). The bat that is then released and tracked with the detector microphone. Attaching the light tag to the ventral surface of the bat optimizes visibility of the bat, and enables the bat to remove the tag when it returns to its roost. Light tagging is perhaps the best method for acquiring standard reference calls because the recordings are acquired from bats foraging naturally; however, the recovery of light-tagged bats is low.

The low recovery rate of light-tagged bats prompted the development of the tethered zipline method for acquiring reference calls (Parsons and Szewczak 2009, Szewczak 2000, 2004). Captured bats are tethered to a zipline with a 1.5-2 meter length of elastic sewing thread by a loose-fitting fixed loop in the elastic pulled over the bat's head. The other end of the elastic thread is attached via a small snap-swivel to the zipline consisting of 30-50 meters of taut monofilament line about 1 meter above the ground (see figures in Parsons and Szewczak 2009). The advantage of the zipline tether method over hand-released recordings is that the bat's flight will occur at a predictable distance from the microphone, and it provides the opportunity for repeated flights to record satisfactory calls. Bats may also be recorded inside temporary or fixed enclosures (Parsons and Szewczak 2009).

Alternatives

The least stressful method to record bat reference calls is by hand-releasing the bat after capture in an open area away from the capture site. Ziplined bats could suffer from the additional stress of attaching the zipline and flying the bat along the line; however, the resulting recordings are a more accurate reflection of their standard calls than recordings from hand-released bats.

Potential Hazards

Potential hazards or injuries to captured bats were described in Section III, Part A, and are rare. The most likely source of injury would be pressure from holding a bat too firmly, or inadvertently placing an object over a bat in a cloth or paper bag. If bats are tethered for collecting acoustic calls, care is to be taken to assure bats are not released with tethers still attached.

Need for Anesthesia or Restraint

There is no need to anesthetize bats released for echolocation recording. Bats are typically restrained by capture in mist nets or other capture devices for 5-10 minutes or less. In species identification surveys, bats are then restrained by hand for an addtional 5-10 minutes and released, or held in cloth or paper bags (where they are free to crawl but usually reach a corner and rest or become torpid) for up to one hour prior to a 5 to 10-minute hand restraint and release. If the bat is light-tagged or tethered on a zipline, restraint times will be increased, but not exceed 15 minutes.

VII. Disposition of Bats After Study, Collection of Specimens, and Euthanasia

A. Disposition After Completion of Study

Bats that are captured for species identification and morphological inspection, biopsy sampling, or marking and tagging are released on site after handling as described elsewhere in this document. Radiotransmitters typically drop off tagged bats within two weeks of attachment due to loss of adhesive properties of surgical cement. Two weeks is also an expected transmitting life of these units. Similarly, light tags usually fall off within 24 hours. Bats typically are not recaptured for removal of PIT tags or wing bands. Carcasses of bats that die during handling, or those that are intentionally collected for contaminants analyses, or for pathological, physiological, or morphological study are to be disposed of by incineration at veterinary facilities or by other approved protocols of cooperating biological research institutions after the study is over. Carcasses and remains may be stored in sealed plastic bags in freezers prior to disposal.

B. Collection of Bats

Bats may be collected for study purposes such as whole body or organ analysis for contaminants, pathological, morphological, or physiological study. Sample size requirements and methods of sample preparation for such collections are to be provided by the investigator in greater detail in other supporting documents pertaining to such study. Bats may need to be euthanized if potential human rabies exposure occurs or if the animal is injured during processing.

C. Euthanasia

In most circumstances, euthanasia is carried out by an overdose of the inhalant anesthetic isoflurane, a method accepted by the American Veterinary Medical Association (AVMA Guidelines for the Euthanasia of Animals: 2013 Edition). This is performed by trained personnel only and adheres to

agency guidelines on the use of pharmaceuticals. Bats are handled with leather-gloved hands and with surgical latex (or latex-free) gloves over the leather gloves during the entire euthanasia process. The bat is placed in a sealed container with a cotton ball soaked with isoflurane. The chamber is at least 2-4 times the volume of the animal (but not excessively large) to ensure adequate oxygen prior to death. When soaking the cotton ball with isoflurane, personnel are in a well ventilated area. Pregnant women do not handle isoflurane. A small circular piece of screen or other material is placed between the cotton ball and the bat to ensure that the animal does not come into direct contact with the isoflurane liquid anesthetic agent. Prior to placement of the bat in the chamber, sufficient time is allotted for the chamber to prime with the volatile anesthetic at the appropriate concentration to induce rapid loss of consciousness and subsequent death. After the bat is placed in the container, and the lid is secured, it is monitored to watch for signs of death (such as cessation of breathing). The bat should lose consciousness within 10-15 seconds, followed by respiratory arrest and cardiovascular collapse within 30-40 seconds of being exposed to the volatile anesthetic agent. When the bat appears to have died, it is removed from the container to verify that it is dead (absent corneal reflex). If the bat has not died, it is returned to the container, and the process is repeated.

Cervical dislocation is an appropriate alternative to isoflorane when isoflorane fails to euthanize the bat after successive attempts, or in circumstances when the study involves collecting physiological data that might be affected by chemical euthanizing agents. Cervical dislocation is recommended for small mammals in the field by the American Society of Mammalogists (2000), and for bats by Handley (1988). Cervical dislocation should be avoided, if possible, if the bat is being euthanized for rabies testing purposes. Field workers must be trained in this technique prior to implementing it.

Any unplanned mortality due to capture, holding, marking, tissue sampling, or other methods employed by the project investigator will be reported to the NPS IACUC within 48 hours. Field work will be immediately halted should two or more unplanned mortalities occur during any 24 hour period.

References Cited

Aldridge, H. D., 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.

Amelon, S.K., D.C. Dalton, J.J. Millspaugh, and S.A. Wolf. 2009. Radiotelemetry: techniques and analysis. Pp. 57-77 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

American Society of Mammalogists. 1998. Guidelines for the capture, handling, and care of mammals as approved by the American Society of Mammalogists. Available online at *http://www.mammalogy.org.*

Anthony, E. P. 1988. Age determination in bats. Pp. 47-58 in Ecological and behavioral methods for the study of bats (T.H. Kunz, ed.). Smithsonian Institution Press, Washington, D.C.

Bassett, J. E. and E. H. Studier. 1988. Methods for determining water balance in bats. Pp. 373-386 in Ecological and behavioral methods for the study of bats (T.H. Kunz, ed.). Smithsonian Institution Press, Washington, D.C.

Barclay, R. M., and G. P. Bell. 1988. Marking and observational techniques. Pages 59-76 in T. H. Kunz (ed). Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C.

Bonaccorso, F. J., and N. Smythe. 1972. Punch-marking bats: an alternative to banding. Journal of Mammalogy 53:389-390.

Bonaccorso, F. J., N. Smythe, and S. R. Humphrey. 1976. Improved techniques for marking bats. Journal of Mammalogy 57:181-182.

Brigham, R. M., M. J. Vonhof, R. M. R. Barclay, and J. C. Gwiliam. 1997. Roosting behavior and roost-site preferences of forest-dwelling California bats (*Myotis californicus*). Journal of Mammalogy 78:1231-1239.

Brunet-Rossinni, A.K. and G.S. Wilkinson. 2009. Methods for age estimation and the study of senescence in bats. Pp. 315-328 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Buchler, E.R. 1975. Food transit time in *Myotis lucifugus* (Chiroptera: Vespertilionidae). Journal of Mammalogy 56: 252-255.

Burland, T. M., E. M. Barratt, and P. A. Racey. 1998. Isolation and characterization of microsatellite loci in the brown long-eared bat, *Plecotus auritus*, and cross-species amplification within the family Vespertilionidae. Molecular Ecology 7:133-140.

Carroll, B. K., and D. E. Wilson. 1984. A comparison of liquid nitrogen and dry ice as coolants for freeze branding white-tailed deer fawns. Pages 122-123 in P. R. Krausman and N. S. Smith (eds). Deer in the southwest: a workshop. Arizona Cooperative Wildlife Research Unit and School of Renewable Natural Resources, University of Arizona, Tucson.

Davis, R., and S. E. Doster. 1972. Wing repair in pallid bats. Journal of Mammalogy 53:377-378.

Dominguez SR, T. J. O'Shea, L. M. Oko, K. V. Holmes. 2007. Detection of group 1 coronaviruses in bats in North America. Emerging Infectious Diseases 13: 1295–1300.

Ellison, L.E. 2008. Summary and analysis of the U.S. government Bat Banding Program. U.S. Geological Survey Open-File Report 2008-1363. 117 pp.

Ellison, L.E., T.J. O'Shea, J. Wimsatt, R.D. Pearce, D.J. Neubaum, M.A. Neubaum, and R.A. Bowen. 2006. Sampling blood from big brown bats (*Eptesicus fuscus*) in the field with and without anesthesia: impacts on survival. Journal of Wildlife Diseases 42:849-852.

Ellison, L.E., T.J. O'Shea, D.J. Neubaum, and R.A. Bowen. 2007a. Factors influencing movement probabilities of big brown bats (*Eptesicus fuscus*) in buildings. Ecological Applications 17:620-627.

Ellison, L.E., T.J. O'Shea, D.J. Neubaum, M.A. Neubaum, R.D. Pearce, and R.A. Bowen. 2007b. A comparison of conventional capture versus PIT reader techniques for estimating survival and capture probabilities of big brown bats (*Eptesicus fuscus*). Acta Chiropterologica 9:149-160.

Faure, P.A., D.E. Re, and E.L. Clare. 2009. Would healing in the flight membranes of big brown bats. Journal of Mammalogy 90:1148-1156.

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. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 88:809-823.

Gardner, J. E, J. D. Garner and J. E. Hofmann. 1994. A portable mist netting system for capturing bats with emphasis on Myotis sodalis. Bat Research News, 30:1-7.

Gardner, S. L., and F. A. Jimenez-Ruiz. 2009. Methods for the study of bat endoparasites. Pp. 795-805 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Grant, T. R., and R. J. Whittington. 1991. The use of freeze-branding and implanted transponder tags as a permanent marking method for platypuses, Ornithorhynchus anatus (Monotremata: Ornithorhynchidae). Australian Mammalogy 14:147-150.

Hadow, H. H. 1972. Freeze-branding: a permanent marking technique for pigmented animals. Journal of Wildlife Management 36:645-649.

Hafner, M. S., W. L. Gannon, J. Salazar-Bravo, and S. T. Alvarez-Castaneda. 1997. Mammal collections in the western hemisphere. American Society of Mammalogists, Allen Press, Lawrence, KS.93 pp. Available online at http://www.mammalsociety.org/committees/commsyscollection/collsurvey.pdf

Henry, L.G. and S. McKeever. 1971. A modification of the wasing technique for quantitative evaluation of the ecotoparasite load of small mammals. J. Med. Entomol. 8:504-505.

Hickey, M. B. C. 1992. Effect of radiotransmitters on the attack success of hoary bats, *Lasiurus cinereus*. Journal of Mammalogy 73: 344-346.

Hickey, M. B. C., and M. B. Fenton. 1990. Foraging by red bats (*Lasiurus borealis*): do intraspecific chases mean territoriality ? Canadian Journal of Zoology 68: 2477-2482.

Hodgkison, R., D. Ahmad, S. Balding, T. Kingston, A. Zubaid, and T. H. Kunz. 2002. Capturing bats (Chiroptera) in tropical forest canopies. Pp. 160-167 in The Global Canopy Programme Handbook: Techniques of access and study in the forest roof (A. W. Mitchell, K. Secoy, and T. Jackson, eds.). Global Canopy Programme, Oxford, United Kingdom.

Hope, G.M., and K.P. Bhatnagar. 1979. Electrical response of bat retina to spectral stimulation: comparison of four microchiropteran species. Experientia 35:1189-1191.

Kalcounis, M. C. and R. M. Brigham. 1998. Secondary use of aspen cavities by tree-roosting big brown bats. Journal of Wildlife Management 62: 603-611.

Kerth, G. and D.K.N. Dechmann. 2009. Field-based observations and experimental studies of bat behavior. Pp. 393-406 in Kunz, T.H. and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Kleiman, D. G., and T. M. Davis. 1974. Punch-mark renewal in bats of the genus *Carollia*. Bat Research News 15:29-30.

Kunz, T. H. and A. Kurta. 1988. Capture methods and holding devices. Pp. 1-30 in T.H. Kunz (ed) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C. 533 pp.

Kunz, T. H. and K. G. Nagy. 1988. Methods of energy budget analysis. Pp. 277-302 in T. H. Kunz (editor), Ecological and behavioral methods for the study of bats. Smithsonian Press, Washington, D.C.

Kunz, T.H. and C.D. Weise. 2009. Methods and devices for marking bats. Pp. 36-56 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Kunz, T.H. and S. Parsons. 2009. Ecological and behavioral methods for the study of bats. 2nd edition. John Hopkins University Press. Baltimore, Maryland. 901 pp.

Kunz, T.H., C. R. Tidemann, and G. C. Richards. 1996. Capturing mammals: small volant mammals. Pp. 122-145 in Wilson, D. E. et al. (eds). Measuring and monitoring biological diversity: standard methods for mammals. Smithsonian Institution Press, Washington, D.C. 409 pp.

Kunz, T. H., R. Hodgkinson, and C. D. Weise. 2009. Methods of capturing and handling bats. Pp. 3-35 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Lewis, S. E. 1995. Roost fidelity in bats: a review. Journal of Mammalogy 76: 481-496.

McCracken and Wilkinson. 1988. Allozyme techniques and kinship assessment in bats. Pp. 141-156 in T. H. Kunz (editor), Ecological and behavioral methods for the study of bats. Smithsonian Press, Washington, D.C.

Neubaum, D.J., M.A. Neubaum, L.E. Ellison, and T.J. O'Shea. 2005. Survival and condition of big brown bats (*Eptesicus fuscus*) after radiotagging. Journal of Mammalogy 86:95-98.

Ormsbee, P. C. And W. C. McComb. 1998. Selection of day roosts by female long-legged myotis in the central Oregon Cascade range. Journal of Wildlife Management 62: 596-603.

Osborne, C., P. M. Cryan, T. J. O'Shea, L. M. Oko, C. Ndaluka, C. H. Calisher, A. D. Berglund, M. L. Klavetter, R. A. Bowen, K. V. Holmes, and S. R. Dominguez. 2011. Alphacoronaviruses in New World Bats: Prevalence, persistence, phylogeny, and potential for interaction with humans. PLoS ONE 6(5): e19156. doi:10.1371/journal.pone.0019156

O'Shea, T. J. 1975. Further comments on punch-marking bats. Bat Research News 16: 37-38.

O'Shea, T. J. and M. A. Bogan (editors). 2000. Interim report of the workshop on monitoring trends in U.S. bat populations: problems and prospects. U. S. Geological Survey, Midcontinent Ecological Science Center, Fort Collins, Colorado. 124 pp.

O'Shea, T.J., and M.A. Bogan (editors). 2003. Monitoring trends in bat populations of the United States and territories: problems and prospects. U.S. Geological Survey Information and Technology Report 2003-0003. 274 pp. Available online: *http://www.fort.usgs.gov/Products/Publications/pub_abstract.asp?PubId=21329*

Parsons, S. and J.M. Szewczak. 2009. Detecting, recording, and analyzing the vocalization of bats. Pp. 91-111 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Rabe, M.J., T.E. Morrell, H. Green, J.C. de Vos, Jr., and C.R. Miller. 1998. Characteristics of ponderosa pine snag roosts used by reproductive bats in northern Arizona. Journal of Wildlife Management 62: 612-621.

Racey, P. A. 1988. Reproductive assessment in bats. Pp. 31-46 in T. H. Kunz (editor), Ecological and behavioral methods for the study of bats. Smithsonian Press, Washington, D.C.

Racey, P.A. 2009. Reproductive assessment of bats. Pp. 249-264 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Rood, J. P., and D. W. Nellis. 1980. Freeze marking mongooses. Journal of Wildlife Management 44: 500-501.

Rossiter, S. J., T. M. Burland, G. Jones, and E.M. Barratt. 1999. Characterization of microsatellite loci in the greater horseshoe bat *Rhinolophus ferrumequinum*. Molecular Ecology 8:1957-1969.

Russell, J. K. 1981. Patterned freeze-brands with canned freon. Journal of Wildlife Management 45: 1078.

Sandel, U., A. Kiefer, R. Prinzinger, and S. Hilsberg. 2004. Behavioural thermoregulation in greater mouse-eared bats, *Myotis myotis*, studied by infrared thermography. Myotis 41-42:129-142.

Scott, D.T. and D.W. Duszynski. 1997. Eimera from bats of the world: two new species from Myotis spp. (Chiroptera: Vespertilionidae). J. Parisitol. 83:495-501.

Sikes, R.S., W.L. Gannon, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235-253.

Szewczak, J.M. 2000. A tethered zip-line arrangement for reliably collecting bat echolocation reference calls. Bat Research News 41:142.

Szewczak, J.M. 2004. Advanced analysis techniques for identifying bat species. Pp. 121-126 in R.M. Brigham, E.K.V. Kalko, G. Jones, S. Parsons, and H.J.G.A. Limpens (eds.) Bat Echolocation Research: Tools, Techniques, and Analysis. Bat Conservation International, Austin, Texas.

Turner, G.G, C.U. Meteyer, H. Barton, J.F. Gumbs, D.M. Reeder, B. Overton, H. Bandouchova, T. Bartonicka, N. Martinkova, J. Pikula, J. Zukal, and D.S. Blehert. 2014. Nonlethal screening of bat-wing skin with the use of ultraviolet fluorescence to detect lesions indicative of white-nose syndrome. Journal of Wildlife Diseases 50: 566-573.

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

Whitaker, J.O., Jr. 1988a. Food habits analysis of insectivorous bats. Pp. 171-189 in Ecological and behavioral methods for the study of bats (T.H. Kunz, ed.). Smithsonian Institution Press, Washington, D.C.

Whitaker, J.O., Jr. 1988b. Collecting and preserving ectoparasites for ecological study. Pp. 459-474 in T.H. Kunz (ed) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C. 533 pp.

Whitaker, J. O., Jr., C. M. Ritzi, and C. W. Dick. 2009. Collecting and preserving bat ectoparasites for ecological study. Pp. 806-827 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Whitaker, J. O., Jr., G. F. McCracken, and B. M. Siemers. 2009. Food habits analysis of insectivorous bats. Pp. 567-609 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Wilkinson, G. S. and J. W. Bradbury. 1988. Radiotelemetry: techniques and analysis. Pp. 105-124 in T.H. Kunz (ed) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C. 533 pp.

Wimsatt, J., T.J. O'Shea, L.E. Ellison, R.D. Pearce, and V.R. Price. 2005. Anesthesia and blood sampling of wild big brown bats (*Eptesicus fuscus*) with an assessment of impacts on survival. Journal of Wildlife Diseases 41:87-95.

Winter, Y., J. Lopez, and O. von Helverson. 2003. Ultraviolet vision in a bat. Nature 425:475-477.

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

Worthington Wilmer, J., L. Hall, E. Barratt, and C. Moritz. 1999. Genetic structure and male-mediated gene flow in the ghost bat (*Macroderma gigas*). Evolution 53:1582-1591.

Appendixes

APPENDIX A. Detailed Descriptions of Methods for Collecting Bats

Kunz, T. H. and A. Kurta. 1988. Capture methods and holding devices. Pp. 1-30 in T.H. Kunz (ed) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C. 533 pp.

Kunz, T.H. and S. Parsons. 2009. Ecological and behavioral methods for the study of bats. 2nd edition. John Hopkins University Press. Baltimore, Maryland. 901 pp.

Kunz, T.H., C. R. Tidemann, and G. C. Richards. 1996. Capturing mammals: small volant mammals. Pp. 122-145 in Wilson, D. E. et al. (eds). Measuring and monitoring biological diversity: standard methods for mammals. Smithsonian Institution Press, Washington, D.C. 409 pp.

Kunz, T.H., R. Hodgkinson, and C. D. Weise. 2009. Methods of capturing and handling bats. Pp. 3-35 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Whitaker, J. O., Jr., G. F. McCracken, and B. M. Siemers. 2009. Food habits analysis of insectivorous bats. Pp. 567-609 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Whitaker, J. O., Jr., C. M. Ritzi, and C. W. Dick. 2009. Collecting and preserving bat ectoparasites for ecological study. Pp. 806-827 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

APPENDIX B. Detailed Descriptions of Methods for Marking and Radiotagging Bats

Amelon, S.K., D.C. Dalton, J.J. Millspaugh, and S.A. Wolf. 2009. Radiotelemetry: techniques and analysis. Pp. 57-77 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Barclay, R. M. R., and G. P. Bell. 1988. Marking and observational techniques. Pp. 59-76 in T.H. Kunz (ed) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C. 533 pp.

Kunz, T.H. and C.D. Weise. 2009. Methods and devices for marking bats. Pp. 36-56 in T. H. Kunz and S. Parsons (eds.) Ecological and behavioral methods for the study of bats (2nd ed.). The Johns Hopkins University Press, Baltimore. 901 p.

Wilkinson, G. S. and J. W. Bradbury. 1988. Radiotelemetry: techniques and analysis. Pp. 105-124 in T.H. Kunz (ed) Ecological and behavioral methods for the study of bats. Smithsonian Institution Press, Washington, D.C. 533 pp.

APPENDIX C. Detailed Description of Wing Biopsy Sampling

USGS Wildlife Health Center. 2015 Bat Submission Guidelines, Appendix D: Instructions for taking a wing biopsy. Can be downloaded at h*ttp://www.nwhc.usgs.gov/disease_information/white-nose_syndrome/USGS_NWHC_Bat_WNS_submission_protocol.pdf*

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

APPENDIX D. Descriptions of Techniques for Sampling Blood in Bats

Ellison, L.E., T.J. O'Shea, J. Wimsatt, R.D. Pearce, D.J. Neubaum, M.A. Neubaum, and R.A. Bowen. 2006. Sampling blood from big brown bats (*Eptesicus fuscus*) in the field with and without anesthesia: impacts on survival. Journal of Wildlife Diseases 42:849-852.

Kunz, T. H. and K. G. Nagy. 1988. Methods of energy budget analysis. Pp. 277-302 in T. H. Kunz (editor), Ecological and behavioral methods for the study of bats. Smithsonian Press, Washington, D.C. See pages 283-285

McCracken and Wilkinson. 1988. Allozyme techniques and kinship assessment in bats. Pp. 141-156 in T. H. Kunz (editor), Ecological and behavioral methods for the study of bats. Smithsonian Press, Washington, D.C. See page 142.

Wimsatt, J., T.J. O'Shea, L.E. Ellison, R.D. Pearce, and V.R. Price. 2005. Anesthesia and blood sampling of wild big brown bats (*Eptesicus fuscus*) with an assessment of impacts on survival. Journal of Wildlife Diseases 41:87-95.

APPENDIX E. National WNS Decontamination Recommendations (Attached)

Hard copy attached.

Can be downloaded at

http://www.whitenosesyndrome.org/sites/default/files/resource/national_wns_revise_final_6.25.12.pdf