CHAPTER 20

NONGAME MAMMALS

(Revised October 2013)

Nongame Mammal Program

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Introduction

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Of the 121 mammal species recognized in Wyoming, the Wyoming Game and Fish Department (WGFD) classifies 85 as nongame, including 43 Species of Greatest Conservation Need (SGCN; WGFD 2010, Orabona et al. 2012). Our ability to conserve nongame wildlife is enhanced by improving knowledge of these species' abundance and distribution (Oakleaf et al. 1996, WGFD 2010). Anthropogenic and natural habitat alterations (e.g., energy development, climate change, etc.), invasive species, and changes in land management practices all have the potential to impact populations of nongame mammals. Data obtained from standardized surveys are necessary to address these conservation challenges. The WGFD's Nongame Program also relies on standardized survey data to monitor populations and assess species' status in relation to objectives outlined by the State Wildlife Action Plan (WGFD 2010). Other uses include improving predictive distribution models, establishing programmatic priorities, documenting environmental reviews, assisting planning efforts, and responding to potential listings under the Endangered Species Act. All mammal observations, especially of SGCN with Native Species Status 4 or less, are potentially useful and should be recorded (Orabona et al. 2012, WGFD 2010). Record all sightings in the Wildlife Observation System (WOS) and submit a mammal observation record form to the Nongame Mammal Biologist at the Wyoming Game and Fish Department (WGFD) Lander Regional Office (refer to Attachment 1).

This chapter describes common survey techniques used to conduct inventories, document species presence, and estimate abundance or species richness of nongame mammals, and reflects the preferred or alternative techniques that should be used by WGFD personnel. The chapter is organized according to species or, where appropriate, major taxonomic group. Survey techniques for most mammals are covered in Subchapter 20.1; survey techniques for bats are described in Subchapter 20.2. In addition to survey techniques, each section addresses immobilization, handling, and marking methods; determination of sex and age; collection of biological samples; and common infectious diseases. We also discuss types of data that should be collected, basic analytical procedures, and handling and dissemination of information. We do not, however, provide direction on rigorous statistical design and analysis. For additional information and guidance, contact the Nongame Mammal Biologist at the WGFD Lander Regional Office.

Subchapter 20.1

Nongame Mammals Other Than Bats

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- I. SMALL MAMMALS (Families Soricidae, Talpidae, Sciuridae, Geomyidae, Heteromyidae, Cricetidae, Muridae, and Zapodidae)
 - A. Survey Techniques -
 - 1. <u>Trapping Transects</u>
 - a. <u>Rationale</u> Small mammals must be captured to reliably identify species and individuals, and to obtain morphometric measurements, demographic data, and biological samples. Individuals may also be marked with passive integrated transponder [PIT] tags or ear tags for unique identification and to estimate abundance. Transects are the simplest trap setup for basic inventories and to assess community assemblages; however, trapping grids are better suited to assess relative and absolute abundance (refer to Section I.A.2; Jones et al. 1996).
 - Application Transects should be at least 150 m long with traps spaced every 10 m. Set at least 2 traps per trapping station. Depending on the goal of the project and anticipated species assemblage, use a mixture of live box traps (such as Sherman or Tomahawk), Museum Special mouse or rat snap traps, and pitfall traps. Pitfall traps are used to collect shrews and other mammals weighing under 10 g, and work best if set in conjunction with drift fences that funnel passing animals into the traps. Supply all live traps with sufficient bait consisting of small grains, seeds, or oats mixed with peanut butter. Place poly fill bedding in each trap to sustain individuals until traps are checked. Set traps along habitat features, such as logs, trees, rocks, shrubs, runways, and burrows whenever possible. To increase probability of catching habitat specialists such as jumping mice (Zapus spp.), place transects along landscape features, such as riparian corridors where these species are more likely to be caught (USFWS 2004, Thompson 2011). Check all traps twice daily, preferably mid-morning and mid-afternoon, to document both diurnal and nocturnal species. Plan at least 500 trap nights per trapping session for preliminary inventories (refer to Section I.A.1.c; Jones et al. 1996). Capturing fossorial mammals such as moles (Family Talpidae) and pocket gophers (Family Geomyidae) may require specialized traps and methods (Baker and Williams 1972, Jones et al. 1996, Griscom et al. 2010).

- c. Analysis of Data Report total number of trap nights, number of species captured, number of individuals of each species captured per trap night, and minimum number alive (MNA) for each species. Trap nights are defined as the number of traps multiplied by number of nights the traps were set during a given trapping session. Traps that were triggered but failed to capture an individual are recorded as ½ trap night each; all other traps, including traps with captures, are recorded as full trap nights. MNA is defined as number of unique individuals of each species captured during an entire trapping session (Krebs 1966).
- d. <u>Disposition of Data</u> Send a report containing trapping records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office. The Nongame Program will incorporate data into the Threatened, Endangered, and Nongame Bird and Mammal Investigations annual completion report. Data will also be used to update wildlife distribution maps and databases, including the WGFD's Wildlife Observation System and Atlas of Birds, Mammals, Amphibians, and Reptiles in Wyoming.

2. <u>Trapping Grids</u> –

- a. <u>Rationale</u> Rational is similar to that provided for transects (Refer to Section I.A.1.a.). Trapping grids can be deployed in conjunction with mark-recapture techniques to estimate density and abundance (Parmenter et al. 2003).
- b. <u>Application</u> The application of trapping grids is identical to that of transects except traps are arrayed in a square grid instead of a linear transect (refer to Section I.A.1). Each grid is comprised of a 10 × 10 or larger array of trapping stations, with ≥ 2 traps placed every 10 m (Jones et al. 1996). Captured individuals must be marked prior to release to estimate recapture rates and abundance (refer to Section I.B). Record each capture location within the grid if data will be used to estimate density.
- c. <u>Analysis of Data</u> Report total number of trap nights, number of species captured, number of individuals of each species caught per trap night, and MNA for each species (refer to Section I.A.1.c). If conducting a mark-recapture study, report locations of each capture as well as the following statistics for each species (Chao and Huggins 2005:25):
 - Number of trap nights (*k*)
 - Number of individuals captured each trap night (n_i)
 - Number of unmarked individuals captured each trap night (u_i)
 - Number of marked individuals captured each trap night (m_i)
 - Minimum number of individuals alive and marked prior to each trap night (M_i ; refer to Section I.A.1.c)

- Numbers of individuals captured based on frequency of capture, i.e., 1, 2,..., *k* times (*f_i*)
- d. <u>Disposition of Data</u> Send a report containing capture records and associated data (refer to Sections I.A.1.c and I.A.2.c) to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

B. Immobilization, Handling, and Marking –

- 1. <u>Rationale</u> Small mammals must be handled or collected as voucher specimens to reliably identify species (Reynolds et al. 1996). In order to calculate relative or absolute abundance, individuals must be handled and marked prior to release.
- 2. Application Most handling and marking procedures can be accomplished with a re-sealable plastic or cloth handling bag. When it is necessary to chemically immobilize small mammals, for example to insert a PIT tag, use a cotton ball soaked in isoflurane. Enclose the animal and the cotton ball in an airtight container and monitor breathing. Smaller species can be transferred directly to a re-sealable plastic bag containing cotton balls. Limit isoflurane exposure to the time necessary to achieve immobilization, as prolonged exposure can lead to mortality. Individuals are properly immobilized when they display deep, regular breathing; lack of whisker movement; total body relaxation; and lack of response to external stimuli (Anstee and Needham 1998). Release individuals only after they display normal activity and movement.

Upon initial capture, transfer smaller species to a plastic Ziploc bag. Use one hand to immobilize individuals at the bottom of the bag. Slide your other hand inside the re-sealable plastic bag, grip the individual by the nape of the neck, and remove the individual from the bag. Identify species; record morphometric measurements; and determine sex, age, and reproductive status (refer to Section I.C). Use cloth handling cones to process larger species such as squirrels (Koprowski 2002).

Morphometric measurements are commonly used to identify species of small mammals. Record the following measurements for all captured individuals: total body length from tip of nose to tip of tail; tail length from base of tail to tip of tail; hind foot length from heel of foot to end of longest nail; ear length from base of ear to tip of ear; and weight.

To avoid recounting individuals, mark each captured animal by affixing numbered ear tags prior to release. Alternatively, use colored dye for short-term marking. PIT tags are a reliable method for long-term marking. Implant PIT tags subcutaneously in the scruff of the neck between the shoulder blades. Apply skin glue to close puncture holes when necessary to speed healing and prevent PIT tags from being excreted (Gannon et al. 2007).

Individuals must be euthanized if they will be collected as voucher specimens or are seriously injured during trapping. Individuals weighing over 200 g are euthanized with an overdose of isoflurane. Individuals weighing less than 200 g can be euthanized with an overdose of isoflurane, or chemical immobilization followed by cervical dislocation (Mills et al. 1995, AVMA 2007).

- 3. <u>Analysis of Data</u> Report the number of individuals of each species caught and information on sex, age, and morphometric measurements (refer to Section I.C). Report unique identification numbers, including ear tags and PIT tags assigned to each individual. If collecting voucher specimens or in the event of mortality, record pertinent information, affix a voucher specimen tag (refer to Attachment 2), and freeze the specimen (Gannon et al. 2007).
- 4. <u>Disposition of Data</u> Send a report containing capture records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d). Also note any trapping-related mortalities or voucher specimens collected.
- C. Assessment of Sex and Age Sex and age of small mammals, especially insectivores, are often difficult to determine. Body size and weight of males and females commonly overlap and are often poor characteristics for determining sex. During the breeding season, most males can be distinguished by descended testes. During the nonbreeding season, testes often retract into the abdominal cavity and different characteristics must be used. In rodents, the anal-genital distance of males is greater than that of than females. In insectivores, males and females can be distinguished by the number of openings in the perineal region males have 2 openings, and females have 3 openings. The presence of nipples can also be used to identify females, but these may not always be readily evident, especially if the female has not yet reproduced. Males of some species can also be distinguished by presence of scent glands (Kunz et al. 1996b).

Ages of small mammals are commonly classified according to categories rather than specific ages. Weight is a poor predictor of age. Some species exhibit distinct molt patterns on which age estimates up to adulthood can be based. Juveniles often have fur that is darker, longer, duller, and less dense than adult fur (Kunz et al. 1996b). Generally, small mammals are classified as juvenile or adult, often based on the timing of trapping in relation to the reproductive season.

D. <u>Collection of Biological Samples</u> –

1. <u>Rationale</u> – Biological samples may be needed to distinguish among individuals and species, or for diseases surveillance. Blood, tissue, or hair samples are typically collected. Disease analyses are based on blood samples, whereas all biological samples can be used for genetic analyses. In particular, the Preble's meadow jumping mouse (*Z. hudsonius preblei*), is distinguishable from the

sympatric western jumping mouse (*Z. princeps*) only through genetic analyses (King et al. 2006).

2. Application – The preferred method of collecting blood from individuals weighing less than 100 g is to perforate the submandibular area with a lancet point. After the individual has been chemically immobilized, (refer to Section I.B.2), locate the back of the jaw bone. Insert a 5.5-mm lancet point (Golden Rod Animal Lancet, Medipoint Inc., Mineola, NY) into the vascular bundle located at the rear of the jaw bone. Collect blood into small vial. After collection is completed, apply a clean compress with slight pressure to the wound to stop bleeding. Release the individual after it has resumed normal activity. Most species will self-groom and clean the wound area after release. Although drawing blood has little effect on survival, special techniques may be required to obtain samples from some species, including voles (*Microtus* spp.) and pocket mice (*Perognathus* spp.; Frase et al. 1990, Swann et al. 1997). Blood can also be collected for genetic sampling by pressing an FTA card to the wound created by an ear punch or tail snip (Thompson et al. 2011).

Use a 2-mm diameter ear punch to collect tissue samples from the external pinna of the ear. Disinfect the ear punch with 10% bleach solution to avoid cross-contamination between samples (Thompson et al. 2011). To sample species lacking external pinna, use canine nail clippers to cut a 1–2 mm segment from the tip of the tail. Apply an antibiotic cream to the wound when necessary (Antolin et al. 2001, Castro-Arellano 2005). Store tissue samples in individually labeled, 2.5-ml vials containing enough 95% ethyl alcohol to keep the tissue suspended.

- 3. <u>Analysis of Data</u> After analyses are completed, report results from each individual. Prior to beginning surveys, contact appropriate laboratories to obtain direction regarding proper storage and shipping protocol and to ensure timely completion of analyses. Biological samples collected for disease testing are sent to the WGFD Veterinary Laboratory in Laramie. Samples collected for genetic analyses must be sent to a qualified, independent laboratory.
- 4. <u>Disposition of Data</u> Forward a report containing capture records and disease or genetic results to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- E. <u>Diseases</u> Hantavirus pulmonary syndrome (HPS) is the primary human health hazard associated with trapping and handling small mammals, especially deer mice (*Peromyscus maniculatus*) and other rodents. Although HPS has little effect on small mammal populations, it can be deadly to humans. Only handle rodents in open air and hold the specimen and trap downwind. Wear masks, eye protection, and latex or nitrile gloves to minimize exposure to bites, scratches, and HPS. Disinfect all traps with a 10% solution of bleach and water after each trapping session and before they are transported in an enclosed vehicle (Mills et al. 1995, Kunz et al. 1996*a*, Mills and Childs 2001, Kelt et al. 2010).

PYGMY RABBIT (Sylvilagus idahoensis) -

F. Survey Techniques -

1. Presence –

- a. <u>Rationale</u> Presence of pygmy rabbits is readily detected through noninvasive survey techniques. Such surveys should be used when it is not necessary to collect population parameters.
- b. <u>Application</u> Searches for pygmy rabbit sign (i.e., burrows, runways, recently deposited fecal pellets) can be conducted year-round. However, searches in winter (> 2.5 cm of snow cover) tend to maximize return on effort (Green and Flinders 1980, Katzner 1994, Thimmayya 2010). At other times of year, identifying pellets of pygmy rabbits becomes problematic because pellets of juvenile cottontails (*Sylvilagus* spp.) overlap in size. In addition, detection probabilities can be low because pellets are cryptic and easily overlooked.

Search suitable habitats (i.e., sagebrush with more than 46% cover that is over 56 cm in height) for sign of pygmy rabbits for 30 minutes or until species is detected, whichever occurs first. Burrows are approximately 10-12 cm in diameter and located at the base of shrubs. Runways are formed in high use areas and result from compaction of snow. Fecal pellets are round, approximately 5 mm in diameter, and generally appear in small clusters of about a dozen or more.

- c. <u>Analysis of Data</u> Record location and type of sign at every site where pygmy rabbit sign is observed.
- d. <u>Disposition of Data</u> Send a report containing locations of observations and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

2. Live Capture –

- a. <u>Rationale</u> Pygmy rabbits are captured to obtain morphometric and demographic information, and to collect biological samples. Individuals may also be marked with PIT tags or ear tags for unique identification. Capture surveys may be combined with mark-recapture studies to estimate population size, survival rates, and to compare relative abundance among sites.
- b. <u>Application</u> Although pygmy rabbits can be captured all seasons, trapping is most effective during winter (Thimmayya 2010). To maximize

success, personnel should familiarize themselves with sign of pygmy rabbits (refer to Section II.A.1). Use Tomahawk collapsible single-door (Model #202) or double-door (Model #206) traps to capture pygmy rabbits. Place unbaited, double-door traps in runways. Place single-door traps near burrow entrances and at the base of shrubs where fecal pellets are observed. Leave traps open day and night and check once daily. Trapping success during summer months may be increased by using canned green beans as bait (Larrucea and Brussard 2007). Cover traps with 4-mil plastic in winter and burlap during other seasons to provide thermal cover.

- c. <u>Analysis of Data</u> Report total number of trap nights, number of individuals caught per trap night, and MNA for each area trapped (refer to Section I.A.1.c). If conducting a mark-recapture analysis, also report location and descriptive statistics from each capture (refer to Section I.A.2.c).
- d. <u>Disposition of Data</u> Send a report containing locations of traps and associated capture data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

G. <u>Immobilization, Handling, and Marking</u> –

- 1. <u>Rationale</u> Pygmy rabbits must be handled for marking, determination of sex, and to collect biological samples. In order to calculate relative or absolute abundance, individuals must be uniquely marked prior to release.
- 2. <u>Application</u> Immobilization can be accomplished by physical restraint and a cloth handling cone (Koprowski 2002). Chemical immobilization is generally not required to handle and mark pygmy rabbits.

Use a cloth handling cone to facilitate handling of captured rabbits. A general technique for carrying pygmy rabbits is to fold your arm across your sternum, grasp the individual by the scruff of the neck with your other hand, and tuck its head in the crook of the folded arm so the feet and body are supported by your hand. Ventral cradling is a similar method except the hind feet are held with your fingers.

Record the following morphometric measurements: weight; ear length from base of ear to tip of ear; length of each hind foot from heal to tip of foot, excluding nail; and total body length from tip of nose to tip of tail along contour of the back.

Use PIT tags for long-term identification. Implant PIT tags subcutaneously in the scruff of the neck between the shoulder blades (refer to Section I.B.2.paragraph 4). Ear tags can also be used to mark individuals.

Any rabbits seriously injured during trapping should be euthanized by lethal injection. Use isoflurane to anesthetize the individual and inject 2-3 cc of potassium chloride directly into the heart (T. Kreeger, WGFD Veterinarian, personal communication). After the rabbit is euthanized, record pertinent information, affix a voucher specimen tag (refer to Attachment 2), and freeze the specimen.

- 3. <u>Analysis of Data</u> Report number of individuals caught and information on sex, age, and morphometric measurements if collected (refer to Section II.C). Report unique identification numbers including ear and PIT tag numbers, from all marked individuals.
- 4. <u>Disposition of Data</u> Send a report containing capture records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d). Also note any trapping-related mortalities
- H. Assessment of Sex and Age Sex of pygmy rabbits can easily be determined. Cradle the individual in one hand, ventral side up, with head facing away. Place your index and middle fingers on either side of the vent area just in front of the anus and press down gently. Females will display a slit or central line running vertically. Both sides of the slit will be pink. Adult males will display a penis that looks like a pink tube with a pointed end that resembles a bullet. Juvenile males will display a slit with white sides.

Age determination is difficult in all lagomorphs. Typically, individuals are classified as either juvenile or adult; however, juveniles grow quickly and generally resemble adults in size and weight by around 80 days of age. When it is possible to determine age based on body size and weight, classify young of the year are as juveniles and all others as adults.

I. <u>Collection of Biological Samples</u> –

- Rationale Biological samples may be needed to distinguish among individuals
 or species. This typically involves collection of blood, tissue, and fecal samples.
 Disease analyses are based on blood samples, whereas all biological samples can
 be used for genetic analyses.
- 2. Application The simplest method for bleeding rabbits is by venipuncture of the marginal or central ear artery, although this commonly results in hematoma or bruising (Mader 2004). However, this method can easily be performed without chemical immobilization or shaving. It may help to have an assistant restrain individuals. Begin by cleaning the ear with alcohol, then warm the ear by wrapping in a warm cloth. Use a 25- or 27-gauge needle without a syringe to puncture the vessel; syringes or vacuum tubes generally collapse the artery. Allow blood to drip from the hub of the needle, and collect blood in a collection tube. For standard disease analysis, place blood into a red-top collection tube.

For genetic and other analyses, place blood into a purple-top collection tube. Label each collection tube with date, age, sex, and unique identification number. Keep blood containers in a cooler with ice. After blood is collected, apply pressure on the puncture site until the wound stops bleeding.

Techniques for collection of tissue and genetic samples are similar to those described for small mammals (refer to Section I.D.2, paragraph 2).

- 3. <u>Analysis of Data</u> –Within 72 hours, send biological samples to a genetic laboratory or the Wildlife Veterinary Laboratory in Laramie for genetic and disease analyses, respectively (refer to Section I.D.4). After analyses are completed, report results from each captured individual.
- 4. <u>Disposition of Data</u> –Send a report containing capture records and disease or genetic results to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- J. <u>Diseases</u> Pygmy rabbits are believed susceptible to the common diseases that affect other rabbits and hares (Family Leporidae), including Colorado tick fever, equine encephalitis, botfly infestations, papillomas, and tularemia (a.k.a., rabbit fever; Mörner and Addison 2001, Williams and Barker 2001). Unlike other Leporides, pygmy rabbit populations are not known to be cyclical or irruptive, and these diseases likely do not present significant threats to populations. Although major human health risks from handling pygmy rabbits are not known, rabbits often carry relatively high flea loads; consequently, we recommend using DEET-based insect repellent as a precautionary measure.

II. <u>BLACK-TAILED AND WHITE-TAILED PRAIRIE DOG (*Cynomys ludovicianus* and *C. leucurus*) –</u>

A. <u>Survey Techniques</u> –

1. <u>Ground Mapping</u> –

- a. <u>Rationale</u> Ground surveys are conducted to delineate the spatial extent of colony boundaries. Ground mapping provides an alternative technique to evaluate changes in distribution of prairie dog colonies and occupied area when counts of individuals or mark-recapture sampling are not feasible (Biggins et al. 1993, McDonald et al. 2011).
- a. <u>Application</u> Conduct surveys during summer months, preferably during the green-up period from May–July. To map colonies, circumnavigate the colony by walking from active burrow to active burrow along the outer periphery of each colony, and record UTM coordinates with a GPS unit every 5 m. Active burrows have openings over 7 cm dia., display evidence of use by prairie dogs such as fresh feces within 5 m, and are free

from obstructions, including clumps of dirt, vegetation, or spider webs. Exclude inactive burrows and burrows of other species when mapping prairie dog colonies. Black-tailed prairie dogs are unique in that colony boundaries are marked by a noticeable "clip line" where vegetation tends to be much shorter between active burrows and areas not used by prairie dogs. Stop recording locations once you have returned to the start point. Colonies are considered separate if the distance between active burrows is ≥ 200 m (Grenier et al. 2009b). Because prairie dog colonies are dynamic, we recommend surveys every 3 yrs to adjust boundaries and assess changes in distribution (McDonald et al. 2011).

- Analysis of Data Import waypoints into ArcGIS (Esri, Inc., Redlands, CA) and digitally connect them to create a polygon shapefile. Complexes and subcomplexes are defined based on proximity of colonies. The criterion for delineating a prairie dog complex is the maximum distance a black-footed ferret (Mustela nigripes) will move in a night (a.k.a., the 7km rule; Biggins et al. 1993). Accordingly, a complex is a group of prairie dog colonies in which the distance between colonies is ≤ 7 km. To determine whether individual colonies form a complex, draw a 3.5-km buffer around the exterior boundary of each colony; colonies with overlapping 3.5-km buffers constitute a complex. Recent research, however, has shown that ferrets most commonly move between colonies less than 1.5 km apart, and these are defined as a subcomplex (Biggins et al. 2004). Subcomplexes are determined by drawing 0.75-km buffers around each colony; colonies with overlapping 0.75-km buffers constitute a subcomplex. Size of a complex or subcomplex is determined by adding the total area (in ha) of colonies within the complex or subcomplex excluding interstitial spaces between colonies.
- c. <u>Disposition of Data</u> Send a report containing colony inventories and digital information to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d). Provide final shapefiles and report number of colonies and size (ha) of each colony, subcomplex, and complex.

2. Aerial Surveys –

- a. Rationale Aerial surveys can be flown over larger areas to determine presence and status of prairie dog colonies, although ground mapping is preferred to delineate colony boundaries (refer to Section III.A.1; Cudworth et al. 2012).
- Application Prairie dog colonies are easy to detect from the air.
 Locations of interest can be systematically searched, or incidental observations can be recorded during aerial surveys for other species.
 Conduct aerial surveys from a fixed-wing aircraft (e.g., Cessna 180, 210,

or SuperCub) flying approximately 150 m above ground level at a speed of 160 km per hr. Surveys should be flown during or shortly after spring green-up (typically mid-May through mid-Jul) to maximize detection rates. If the objective is to delineate boundaries, record locations of colonies with a GPS unit and return to the area on foot to map the colony (refer to Section III.A.1). Status of black-tailed prairie dog colonies is easily determined from the air. However, status of white-tailed prairie dog colonies can only be assessed from the ground (Cudworth et al. 2012). Colonies are classified as active if the following conditions are noted throughout at least 50% of the colony: recent excavation within and around most mounds, unobstructed burrow entrances, and vegetation absent from mounds. Inactive colonies do not meet the 50% criterion, and mounds will often appear old and 'crusty' or dilapidated (Grenier et al. 2004).

- c. Analysis of Data Record location and status of all observed colonies.
- d. <u>Disposition of Data</u> Send a report containing colony locations, status information, and flight paths to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- B. <u>Immobilization</u>, <u>Handling</u>, and <u>Marking</u> Not applicable.
- C. <u>Assessment of Sex and Age</u> Not applicable.
- D. Collection of Biological Samples Not applicable.
- E. <u>Diseases</u> Prairie dogs are known to susceptible to sylvatic plague caused by the bacterium *Yersinia pestis*, which is spread when fleas from infected individuals (typically rodents) bite other individuals of the same or different species (Gasper and Watson 2001, Orloski and Lathrop 2003). Mortality can approach 100% and can spread rapidly through a colony; (Orabona-Cerovski 1991, Antolin et al. 2002). Various insecticides and vaccines have been used with some success to control the spread of sylvatic plague (Antolin et al. 2002, Seery et al. 2003). Exercise caution when working in and around prairie dog colonies by avoiding sick or dead individuals and use DEET-based insect repellent to minimize risk of flea bites. Refer to Section I.E for a description of proper protective equipment to use when it is necessary to handle prairie dogs.

III. NORTHERN FLYING SQUIRREL (Glaucomys sabrinus) –

A. Survey Techniques -

1. Remote Cameras –

- a. Rationale Flying squirrels are easily detected through noninvasive survey techniques. Because flying squirrels are susceptible to capture myopathy, noninvasive surveys are recommended whenever it is unnecessary to handle them for data collection (Rosenberg and Anthony 1993). Remote infrared cameras are used to detect northern flying squirrels and can be deployed in structured surveys to meet specific monitoring objectives (e.g., occupancy modeling; Finley et al. 2005).
- Each grid will consist of 16 camera stations at 50-m spacing within a 4 × 4 square grid, and a 50-m buffer between the exterior stations and grid border (Meyer et al 2005). At each station, secure 1 remote infrared camera 1.5 m above ground on the trunk of a tree. Attach a 10-cm dia. polyvinyl chloride (PVC) pipe enclosure to another tree within 2 m from the camera (Van Fleet and Grenier 2012). Point the camera at the PVC enclosure and ensure the view is unobstructed. Use a mix of peanut butter, rolled oats, and bacon grease to bait the PVC pipe enclosure, and reapply bait at each station in the late afternoon for 5 consecutive days. Program cameras to activate between 1800 and 0600 to maximize detections of flying squirrels and avoid nuisance diurnal photos. Set cameras to take 3 photos every 10 seconds each time the camera is triggered. Deploy cameras for 5 consecutive nights. After the fifth night, retrieve cameras, download pictures, and erase each memory card.
- c. <u>Analysis of Data</u> Combine data from all cameras within the survey area. Report total number of camera nights, all species detected (species richness), total detections of each species, number of detections per camera night, and locations of cameras (refer to Section I.A.1.c).
- d. <u>Disposition of Data</u> Send a report containing photo records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- B. <u>Immobilization</u>, <u>Handling</u>, and <u>Marking</u> Not applicable.
- C. Assessment of Sex and Age Not applicable.
- D. Collection of Biological Samples Not applicable.

E. <u>Diseases</u> – Although individuals are not handled directly, humans may still come in contact with urine or feces from flying squirrels and other non-target species that may carry hantavirus particles. Use proper protective equipment and follow appropriate protocols when handling and disinfecting bait tubes (refer to Section I.E).

IV. <u>SWIFT FOX (Vulpes velox)</u> –

A. Survey Techniques –

1. Remote Cameras -

- a. <u>Rationale</u> Remote infrared cameras are used to detect presence of swift fox and can be deployed in structured surveys to meet specific monitoring objectives (e.g., occupancy modeling; Finley et al. 2005). This method is also effective for detecting other carnivores.
- Application Although survey areas can be any size, 31-km² grids are recommended to conform with statewide protocols (Cudworth et al. 2011). Alternatively, cameras can be placed at ≥ 0.8 km intervals along transects. Secure each camera to a rebar stake. Position a wooden surveyor's stake $(2.5 \times 5 \times 45.7 \text{ cm})$ 2.5 m from the camera for application of a lure and to focus the camera. Lure should consist of a skunk-based attractant of petroleum jelly and skunk essence mixture as well as a few sprays of fish oil (Knox and Grenier 2010). Time surveys to correspond with seasons of high movement and activity (e.g., juvenile dispersal during Sep–Nov; Olson et al. 2003, Finley et al. 2005). Program cameras to activate between 1800 and 0600 hrs to maximize detections of nocturnal canids and avoid nuisance diurnal photos, including moving vegetation. Set cameras to take 3 photos every 10 seconds each time the camera is triggered. Deploy cameras for at least 5 consecutive nights. Collect cameras and download pictures on 6th day. Cameras can be reset as soon as memory cards are erased (Cudworth et al. 2011).
- c. <u>Analysis of Data</u> –Combine data from all cameras within a survey area. Report total number of camera nights, all species detected (species richness), total number of detections of each species, number of detections per camera night, and locations of cameras (refer to Section I.A.1.c).
- d. <u>Disposition of Data</u> Send a report containing photo records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

2. Spotlight Surveys –

- a. <u>Rationale</u> Spotlight surveys are an effective method for detecting individuals and dens in locations where swift fox are known or suspected to occur.
- Application Spotlight surveys are most effective if a manageable area is searched within clearly defined boundaries. We recommend the survey be conducted by a single observer in most situations. Although surveys can be completed both from vehicle and on foot (refer to Section VI.A.1.b), vehicle surveys cover a larger area more efficiently. However, vehicles should be confined to existing roads and 2-track trails unless landowner authorizes off-road driving; consequently, observers may need to search inaccessible areas on foot with a portable spotlight. Refer to Section VI.A.1.b for a description of recommended spotlight equipment. Survey areas should be 404 ha or less depending on accessibility. Areas surveyed without vehicle access should be less than 129 ha. Complete at least 1 pass through the entire survey area per hr. Conduct surveys in blocks of 3 consecutive nights during May-Sep when foxes den and rear pups. Each survey session should span 4 hrs beginning at dusk. Do not conduct surveys during unsuitable weather (i.e., high winds over 40 km per hr, rain, or lightning storms). In some instances it may be necessary to observe individuals at least 30 min to locate dens. Extend surveys if necessary to identify dens in areas where swift fox have been detected. Use a GPS unit to record locations of dens.
- c. Analysis of Data Refer to Section VI.A.1.c.
- d. <u>Disposition of Data</u> Send a report containing all observation records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

3. Live Capture –

- a. <u>Rationale</u> Swift fox are typically captured for translocation and population recovery. Foxes are also captured to obtain morphometric and demographic information and to collect biological samples. Individuals may be marked with PIT tags or radio collars for unique identification and to estimate abundance or survival.
- b. Application Swift fox are most effectively captured during the pup dispersal period but may be captured at other times of year as well (refer to Section V.A.1.b). Set $28 \times 30 \times 82$ cm single door live traps (Model 608, Tomahawk Live Trap Company, Wisconsin, USA) along transects at spacing similar to that described for remote camera surveys (refer to Section V.A.1.b). Use rabbit quarters (*Lepus* or *Sylvilagus* spp.) or small

chunks of ungulates for bait and secure to back of trap with metal bailing wire. In Wyoming, baiting with dead game animal parts (e.g. obtained from road kills) must be authorized in a Chapter 33 permit issued to conduct scientific research. If live game animals such as cottontail rabbits (*Sylvilagus* spp.) will be killed and used for bait, a Chapter 56 permit is also required. Jackrabbits are classified as predatory animals and may be taken and used for bait without a permit or license.

Swift fox are cautious and delicate when approaching bait, and often succeed in removing bait without triggering the treadle if bait is not properly secured. Apply a long-range skunk based lure (refer to Section V.A.1.b) near traps. An alternative trap layout for mark-recapture studies is a grid pattern (refer to Section I.A.2.b; Finley et al. 2005).

- c. <u>Analysis of Data</u> Report the number of individuals caught and information on sex, age, and morphometric measurements if collected (refer to Section V.C). Refer to Section I.A.2.c. for information on mark-recapture techniques. Report unique identification numbers, including PIT tags and radio-collar frequencies assigned to each individual.
- d. <u>Disposition of Data</u> Send a report containing capture records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

B. <u>Immobilization, Handling, and Marking</u> –

- 1. <u>Rationale</u> Swift fox must be handled to uniquely mark individuals, affix radio-collars, and collect biological samples.
- 2. <u>Application</u> Swift fox can be physically restrained with a cloth handling bag. Generally, chemical immobilization is unnecessary.

Keep all captured individuals in covered traps and place them in a cool, dry location until they can be processed. Personnel should work in pairs. One individual is responsible for handling and restraining the swift fox while the other collects and records data. Wear leather gloves at all times while handling and restraining foxes. Transfer captured individuals to a large capture bag. Hold the open end of the bag off the ground and roll it down and over the fox, forcing the individual's head into a corner of the bag. If the head is not in one of the corners, gently feel the body of the swift fox to determine location of the head and repeat the previous steps. Pin the individual to the ground by placing a free hand outside the bag just behind the swift fox's head. Slowly unroll the capture bag. Carefully insert your other hand into the bag, grasp the swift fox by the scruff of the neck, and remove the individual from the bag. A properly restrained fox will be unable to turn its head and will have slightly bulging eyes. The handler should sit facing the processor, with the swift fox on the handler's lap and the fox's hind feet

restrained between the handler's legs. The processor should affix a small canid muzzle as soon the fox is secured. Once processing is completed, remove the muzzle and place the fox in a holding cage until it is released.

Record the following morphometric measurements on the handling form for swift fox (refer to Attachment 3): shoulder height from top of scapula to tip of outstretched foot; right upper canine width at gum line; and canine length from gum line to tip of tooth. Use a caliper to take tooth measurements. Also note and record observations about tooth wear and staining. Carefully examine individuals for past and current injuries and note these under "comments."

PIT tags provide a means for long-term identification of captured individuals. Implant PIT tags subcutaneously in the scruff of the neck between the shoulder blades (refer to Section I.B.2.paragraph 4). If affixing radio-collars, verify the collar is transmitting before beginning. When placing a collar on individuals less than 1 yr old, apply foam padding to the back of the collar and use a single layer of duct tape to temporarily tighten the fit. Padding will pack down and disintegrate over time as the fox grows. It is often helpful to lay a plastic bag between the shackle and the neck to avoid catching hair when tightening collar bolts. Double check to verify proper fit and ensure the fox's lower jaw cannot become caught under the collar. If in doubt, tighten the collar one notch. Spin the collar around the neck to free any hair from the shackle, and check that shackle nuts are tight. Record the collar frequency on the capture form.

Refer to Section II.B.2, paragraph 5 for euthanasia protocol in the event of a serious trapping injury.

- 3. <u>Analysis of Data</u> If several foxes are handled, complete a data summary table. Otherwise, no analysis is necessary.
- 4. <u>Disposition of Data</u> Send a report containing capture records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d). Also note any trapping-related mortalities.
- C. <u>Assessment of Sex and Age</u> Males are easily distinguished by presence of a penis; descended testes are also present in males over 6 months of age. Conversely, females have a noticeable vulva shaped like a pointed leaf anterior to the anus.

Generally only juvenile and adult age classes can be distinguished in the field. The exact age of swift fox can be determined from tooth cementum annuli (Richholt and Carbyn 2003). By September, juveniles resemble adults in size; juveniles over 6 months of age are difficult to distinguish from adults based on size alone. Juveniles have bright white canines that lack wear or staining and may not be fully erupted. Conversely, adults have stained, well-worn, and fully erupted canines.

D. <u>Collection of Biological Samples</u> –

1. <u>Rationale</u> – Biological samples are generally collected for disease analysis. Blood samples are preferred for determining prevalence of common wildlife diseases.

<u>Application</u> – Venipuncture of the cephalic vein is the preferred method for collecting blood. Begin by locating the cephalic vein on the front leg. Wet the area just above the knee with rubbing alcohol and press your thumb down on the vein to raise it above the point of insertion. Use a 25-gauge needle and 12-ml syringe to draw blood. Insert the needle parallel to the vein and pull back on the plunger slightly until blood fills the syringe, being careful not to collapse the vein. Blood flow may be slow and may take some time to fill the syringe. For standard disease analysis, place blood into a red-top collection tube. For genetic and other analyses, place blood into a purple-top collection tube. Label each collection tube with the date, a unique identification number, and the fox's age and sex. Keep blood containers in a cooler with ice.

- 2. <u>Analysis of Data</u> Report results from each captured individual.
- 3. <u>Disposition of Data</u> Send biological samples to a genetics laboratory or the WGFD Wildlife Veterinary Laboratory in Laramie for genetic or disease analyses, respectively, within 72 hrs (refer to Section I.D.4). Send a report containing capture records and results from disease or genetic analyses to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- E. <u>Diseases</u> Swift fox have been reported to carry many diseases common to wild canids, including canine distemper, sylvatic plague (refer to Section III.E), rabies, tularemia (refer to Section II.E), and mange (Williams and Barker 2001). None of these diseases are expected to pose serious population-level threats. Human health risks are minimized if researchers take precautions not to expose themselves to fluids (e.g., blood and saliva). Protective equipment is necessary when handling swift fox, particularly if biological samples are collected (refer to Section I.E). Use a DEET-based insect repellent as a precautionary measure against ectoparasites.

V. <u>BLACK-FOOTED FERRET (Mustela nigripes)</u> –

A. Survey Techniques –

- 1. Spotlight Surveys
 - a. Rationale Spotlight surveys are the most common and effective method used to locate ferrets (Campbell et al. 1985, Grenier et al. 2009*a*). Spotlight surveys may also be combined with capture methods to identify individuals or to collect biological samples.

b. Application – Schedule surveys to coincide with kit emergence (15 Aug – 15 Sept) or dispersal (16 Sept – 1 Nov) to detect presence of ferrets. Surveys to estimate abundance or document production of kits are competed during the emergence phase. Survey coverage and specific routes depend on available resources, personnel, and availability of roads within prairie dog colonies. Contact landowners for permission to access their land prior to initiation of surveys. If surveys are conducted on foot, survey areas should be under 120 ha. Areas with adequate vehicular access can be up to 240 ha. Actual size of the survey area will depend on size and configuration of prairie dog colonies as well as geographic boundaries (Grenier 2008). It is more effective to survey smaller areas multiple times during a single night than to survey a larger area once.

Conduct spotlight surveys from 2000-2400 hrs and 0200-0600 hrs, in blocks of 3 consecutive nights (Grenier 2008, Grenier et al. 2009). Equip vehicles with roof- or window-mounted spotlights (Model RM 240 Blitz, Lightforce Professional Lighting Systems, Orofino, ID). Portions of the colony that cannot be surveyed from a vehicle should be traversed on foot by personnel wearing a backpack spotlight unit (Walkabout Kit, Lightforce Professional Lighting Systems, Orofino, ID). Sweep spotlights back and forth to provide constant illumination. In most situations, observers are able to detect eye shine up to 400 m depending on experience, topography, and vegetation, although ferrets have been detected at distances up to 1,200 m. All Mustelids including ferrets display green eye shine; Leporids have red eye shine; Canids and Felids have yellow eye shine; and pronghorn (Antilocapra americana) have turquoise eye shine. Record observations and associated information on the ferret spotlighting record form (Attachment 4). Use a GPS unit to record locations of all ferret observations and burrow entrances. Once ferrets are located, personnel may need to observe individuals for extended periods of at least 30 min or multiple times to accurately count the number of kits.

- c. <u>Analysis of Data</u> Report total number of ferrets observed and minimum number alive (MNA). MNA is determined by spatially and temporally distinguishing among observations (Grenier 2008). Also report survey dates and total hours spotlighting. Include individual ferret locations, date and time of observation, and the observer's name.
- d. <u>Disposition of Data</u> Send a report containing records of observations and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d). A report will also be submitted annually to the U.S. Fish and Wildlife Service's Black-footed Ferret Recovery Coordinator.

2. <u>Live Capture</u> –

- a. <u>Rationale</u> Ferrets are captured to obtain morphometric and demographic information and to collect biological samples. Individuals may also be marked with PIT tags and hair dye to provide a means of uniquely identifying individuals and to estimate abundance.
- b. Application After a ferret is located during spotlight surveys (refer to VI.A.1), place an unbaited live trap in the burrow entrance where the ferret was observed (Sheets 1972). Traps should be wrapped in burlap from the trap entrance to approximately 15 cm from the end of the trap and secured in 2 places with nylon cord. Place a reflector pole near the burrow entrance to easily relocate the trap, and record the location with a GPS unit. Use metal or plastic cups, wood, or grass to plug entrances of connected burrows and prevent the ferret from escaping. Record from trapping sessions on the ferret spotlighting record form (Attachment 4). Check traps hourly and remove all traps and burrow plugs at sunrise. Leave reflector poles in place until the end of the spotlight survey.
- c. <u>Analysis of Data</u> Enter capture data on a spreadsheet at the conclusion of the survey.
- d. <u>Disposition of Data</u> Send a report containing capture records, associated data, and spreadsheets to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Sections I.A.1.d and VI.A.1.d).

B. <u>Immobilization, Handling, and Marking</u> –

- <u>Rationale</u> Ferrets must be chemically immobilized to collect biological samples, record morphometric measurements or mark individuals for recapture analyses. Ferrets must also be marked to derive a population estimate. Individuals are typically marked with hair dye for short-term identification. Long-term marking methods are used to estimate demographic parameters.
- 2. Application Transfer captured ferrets from live traps to transfer tubes constructed of 10-cm diameter, perforated, corrugated black pipe. To do this, place the trap and transfer tube on the ground with the open end of the transfer tube next to the end of trap. Open the trap and place the transfer tube as close as possible. Gently coax the ferret into the transfer tube by removing the burlap cover from the trap. In most cases, captured individuals will run into transfer tubes with little difficulty. To encourage hesitant individuals, crinkle a piece of plastic repeatedly near the trap. When the ferret enters the transfer tube, quickly insert the partition and secure it with a cotter pin. Be sure both ends of the tube are secured. Hold the tube with both hands when carrying it. Transport captured individuals to a mobile processing trailer for chemical immobilization. While

transporting a ferret, keep the cab of the truck at a moderate temperature and lower the volume of the radio.

Only trained and qualified WGFD personnel should process ferrets. Refer to Attachment 5 for set up of immobilization equipment including oxygen tank, isoflurane vaporizer, and an overview of the processing trailer. All personnel present during immobilization should wear masks, and the anesthesiologist should wear nitrile gloves while handling ferrets. To chemically immobilize a captured individual, connect the transfer tube to the immobilization chamber (refer to Attachment 5). Cover the clear portal of the chamber with a cloth. Remove both partitions of the transfer tube, beginning with the partition between the tube and chamber. While wearing a welding glove, insert a fist into the other end of the transfer tube to force the ferret into the chamber. Once the ferret is in the chamber, slowly close the door taking care not to pinch appendages.

Record processing data onto the ferret chemical immobilization form (refer to Attachment 6). Follow the numerical order outlined on the form to ensure the ferret reaches a deep and consistent state of immobilization before procedures are conducted. Turn on oxygen and set to 3.5 Lpm. Set vaporizer unit to 4.0 percent by volume (Vol %). Wait approximately 3 min for the drug to take effect. Ferrets can be removed from the chamber when they are nonresponsive; exhibit deep, regular breathing; and the eyes have rolled back slightly, exposing their white portion. Remove the immobilized ferret from the chamber and lay the individual on its ventral side on a towel. Straighten all appendages. Remove the tube connecting the vaporizer to the chamber and insert a gas mask on the exposed end. Insert the entire head of the ferret inside the mask (refer to Attachment 5). Adjust the oxygen setting to 2.0 Lpm and vaporizer to 2.5 Vol %. Processing time should take approximately 10–15 min. Begin by applying eye drops, taking the ferret's rectal temperature, and counting breaths for 15 sec. Multiply the breath count by 4 to calculate respiration rate. Normal vital measurements are 98° - 105° F (37° - 41° C) body temperature and 12 - 24 breaths per min.

Place the individual on its back to obtain morphometric measurements. Record the following measurements: total body length from tip of nose to tip of tail; body length from tip of nose to anterior point of anus; and upper canine width at gum line. Use a caliper to take tooth measurements. Record tooth wear and note broken teeth on the ferret chemical immobilization form (refer to Attachment 6). Carefully examine each individual for past and current injuries, paying special attention to mouth and chin areas. If extensive wounds are apparent, administer an additional 1cc of penicillin.

To avoid double counting and re-processing of previously captured individuals, mark the underside of the neck just below jaw line using hair dye (e.g., Clairol Nice N' Easy). Dark colors including black, red, and brown work best. Dye marks typically last 4 weeks (Grenier et al. 2009a). Implant PIT tags

subcutaneously in the scruff of the neck between the shoulder blades (refer to Section I.B.2.paragraph 4).

When processing is complete, turn the vaporizer and oxygen off. Wrap individuals in towels and move to a "pet taxi" for recovery. Lay the ferret on a towel, on its side, and ensure the face and nose are not covered. Most ferrets recover quickly, will shake and shiver profusely within minutes, and are able to metabolize residual isoflurane within 15 min. Once ferrets are alert and standing on all 4 legs, they can be removed from the processing trailer and returned to burrows for release. Use Nolvalsan Otic cleaning solution to disinfect all equipment, including the chamber, mask, and tools, after each ferret is processed.

Refer to Section II.B.2, paragraph 5 for euthanasia protocol in the event of a serious trapping injury.

- 3. <u>Analysis of Data</u> Report the number of individuals captured and information on sex, age, and morphometric measurements if collected (refer to Section VI.C). Report unique identification numbers (e.g., dye marks and PIT tags) from each individual.
- 4. <u>Disposition of Data</u> Send a report containing capture records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d and Section VI.A.1.d). Also note any trapping-related mortalities.
- C. <u>Assessment of Sex and Age</u> Sex of ferrets can be readily determined throughout the year. The anal-genital distance of male ferrets is greater than that of females (the same criterion used to determine sex of rodents). These characteristics can be examined while the ferret is in the trap and without handling it. Sex can also be determined in the field based on skull shape. Male ferrets have a broad head that appears generally large and square whereas females have a much narrower, slender skull.

Most individuals can be classified as adult or juvenile based on upper canine width measured during Aug and Sep. Adults have a fully erupted upper canine that measures over 4.0 mm wide in males and over 3.7 mm in females. Nipples are also visible on adult females, as most adult female reproduce annually (Grenier 2008). Nipples are not visible in juvenile females.

D. Collection of Biological Samples –

1. <u>Rationale</u> – Biological samples are required to perform genetic and disease analyses. This typically entails collection of blood and hair samples. Blood samples are collected to test for, and monitor prevalence of diseases; hair is collected for genetic analysis.

2. Application – The preferred method to obtain blood samples is venipuncture of the anterior vena cava (Quesenberry and Orcutt 2004; refer to Attachment 7). Ferrets must be chemically immobilized to perform this procedure. Position the immobilized ferret on its back while keeping its head firmly inside the anesthesia mask (refer to Section VI.B.2). Disinfect the puncture area by swabbing with alcohol. Insert a 25-gauge needle attached to a 3-ml syringe at a 45° angle between the first rib and manubrium. Direct the needle toward the opposite hind leg and insert it almost to the hub. Pull back on the plunger slightly until blood fills the syringe. It is possible to collect up to 4 ml of blood from an individual of average weight (i.e., >750 g). In most applications, 3 ml should suffice for standard disease testing (refer to Section VI.E). For standard disease analysis, place blood into a red-top collection tube. For genetic and other analyses, place blood into a purple-top collection tube. Label each collection tube with the date and the ferret's age, sex, and unique identification number. Keep blood samples upright in a cooler. After a blood draw is completed, administer 20 ml of lactated ringer solution (LRS) subcutaneously between the shoulders.

Use tweezers to collect hair samples. Collect one small tuft of hair from the rump and another from the side. Place hair in a 6.4×8.3 cm manila envelope and store in a cool dry place. Record stud book number, date, and age and sex of the ferret on all biological samples. The stud book number is a unique 4-digit identification number assigned to each ferret when it is first captured. The number reflects the year the individual was first captured as well as the order in which it was captured.

- 3. Analysis of Data Report analytical results from each captured individual.
- 4. <u>Disposition of Data</u> Deliver all blood samples to the WGFD laboratory in Laramie, Wyoming within 72 hrs (refer to Section I.D.4). Send a report containing capture records and results of disease analyses and all hair samples to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Sections I.A.1.d and VI.A.1.d).
- E. <u>Diseases</u> Black-footed ferrets are susceptible to a number of infectious diseases including canine distemper, Sylvatic plague (refer to Section III.E), and tularemia (refer to Section II.E; Williams and Barker 2001). Tularemia presents little threat to the persistence of ferret populations, but both canine distemper and Sylvatic plague are 100% fatal to ferrets. Plague and tularemia also pose potential risks to humans. Apply DEET-based insect repellent as a precautionary measure and use proper protective equipment when handling ferrets (refer to Section I.E), and check yourself and others for ticks every 2–3 hrs.

VI. <u>WOLVERINE (Gulo gulo)</u> –

A. Survey Techniques -

1. Remote Cameras –

- a. <u>Rationale</u> –Noninvasive survey techniques, including remote cameras, can be used to detect presence of wolverines (refer to Section IV.A.1.a) and to identify individuals (Magoun et al. 2011).
- Application Divide the study area into 3.2×3.2 km survey grids (Kucera et al. 1996). Attach cameras to tree trunks 2 m above ground and approximately 4 m from a bait tree. Cameras can also be positioned to capture images of the ventral side of wolverines in order to identify individuals. Ventral photos display unique pelage patterns of individuals and can also be used to identify sex (Magoun et al. 2011). Locate camera sites in travel corridors within preferred habitat. Distance between camera sites can vary depending on terrain features and survey grid size (Magoun et al. 2011). Wire a whole beaver carcass to the bait tree and apply a longrange skunk-based lure as an attractant. Program cameras to take 3 photos every 10 seconds each time the camera is triggered. Program a sleep period of at 10-min or more intervals between triggers. Check cameras bimonthly to download memory cards and re-bait sites (Bradbury and Fisher 2007, Nielsen and McCollough 2009). Because of potential for conflicts with bears, use proper precautions when selecting time of year and locations for camera setups (Nielsen and McCollough 2009).
- c. <u>Analysis of Data</u> Combine data from all cameras within the survey area. Report the following information from each study area: total number of camera nights, all species detected (species richness), total detections of each species, number of detections per camera night, and locations of cameras (refer to Section I.A.1.c).
- d. <u>Disposition of Data</u> Send a report containing photo records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

2. <u>Snow Tracking (Aerial)</u> –

a. <u>Rationale</u> –Track surveys conducted from a low-flying aircraft are an effective method to detect wolverines when weather and snow conditions are conducive. Large areas can be surveyed efficiently from fixed-wing aircraft or helicopters (Magoun et al. 2007). Probability of occurrence can also be estimated for the entire survey area (Koen et al. 2008).

- Application Conduct snow tracking surveys in late winter beginning after mid-February. Partition the survey area into hexagon grids of at least 100 km² – the approximate average minimum size of the home range of a female wolverine (Inman et al, 2009, Magoun et al. 2007). Plan flight paths in advance to minimize ferry time and avoid densely forested areas. To maximize coverage, conduct fewer repeated surveys and fly more survey grids (Koen et al. 2008). Use aircrafts such as PA-18 Super Cub, from which the observer can see out both sides. Fly surveys at 110–140 km per hr and 100 m above ground level. Conduct surveys 24 hrs or more following widespread snowfall of at least 3 cm, or after windstorms with average gusts exceeding 50 km per hr. Ideal survey conditions are sunny or lightly overcast days with wind conditions that are safe for operating aircraft at slow speeds. Favorable lighting conditions are typically from 1000-1500 hrs depending on location, weather, and time of year. Wolverine tracks are identified based on a combination of track size, shape, depth, and 3-lope gait (refer to Attachment 8; Halfpenny et al. 1996, Magoun et al. 2007, Koen et al. 2008). Exclude tracks if a positive identification cannot be made (Magoun et al. 2007).
- c. <u>Analysis of Data</u> Record the flight path and all tracks and observations of carnivores on a wolverine survey form (refer to Attachment 9; Koen et al. 2008).
- d. <u>Disposition of Data</u> Send a report identifying areas surveyed, track locations, and species detected to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

3. Snow Tracking (Ground) –

- a. <u>Rationale</u> –Track surveys conducted from the ground can also be effective to detect presence of wolverines. This approach provides an inexpensive alternative to aerial surveys, however ground surveys are most effectively done in smaller areas of less than 100,000 km² (Koen et al. 2008). Track identification can be verified by collecting and analyzing biological samples (Ulizio et al. 2006).
- b. Application Partition the survey area into 8×8 km grids and select random grids to survey. The number of grids depends on project objectives, amount of personnel time available, and budgets. When feasible, conduct track surveys from a snowmobile at 15–20 km per hr (refer to Section VII.A.2.b), or use snowshoes or skis if snowmobile access is not possible (Squires et al. 2004, Ulizio et al. 2006). Survey routes should be 10 km long within each grid and should focus on preferred habitat. Survey each grid at least 3 times per winter or until wolverines are detected (Halfpenny et al. 1996). Record locations of

- survey routes and all wolverine tracks encountered. Back trail along each set of tracks to collect biological samples (refer to Section VII.D).
- c. <u>Analysis of Data</u> Record all tracks and observations of carnivores on a wolverine survey form (refer to Attachment 9; Koen et al. 2008).
- d. <u>Disposition of Data</u> Send a report containing survey data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- B. Immobilization, Handling, and Marking Not applicable.
- C. <u>Assessment of Sex and Age</u> Not applicable, although sex can be determined from biological samples (refer to Section VII.D).
- D. Collection of Biological Samples -
 - 1. <u>Rationale</u> Collection of biological samples may be necessary to monitor diseases, identify individuals, or confirm species identification. This typically entails collecting fecal or hair samples during snow tracking.
 - 2. Application After intersecting a set of tracks while conducting ground surveys (refer to Section VII.A.3.), back trail the tracks at least 2 km or until biological samples, including feces or hair, are encountered. Fecal and hair samples may be found in tracks or temporary resting places such as daybeds. Scan from multiple angles approximately 30 cm above the snow surface to locate hair samples in suitable locations including daybeds, foraging sites, tracks, tree boles, and woody debris along the animal's trail. Fecal samples are more visible and may be encountered while back trailing an individual. Place fecal samples in a vial with 10–18 mesh silica desiccant to inhibit enzyme activity from degrading samples. Place hair samples in a small manila envelope (i.e., 6.4 × 8.3 cm) and store in a cool dry place (Ulizio et al. 2006).
 - 3. <u>Analysis of Data</u> Report results from genetic analyses, including number and sex of unique individuals documented throughout the survey area.
 - 4. <u>Disposition of Data</u> Deliver samples collected for genetic analysis to the University of Montana, U.S. Forest Service Rocky Mountain Research Station in Missoula. Send a report containing sample records, including location and unique identification numbers, and results from genetic analyses, to the Nongame Mammal Biologist in the WGFD Lander Regional Office (refer to Section I.A.1.d).
- E. <u>Diseases</u> Wolverines are known to harbor many parasites, such as flukes, tapeworms, roundworms, trematodes, nematodes, cestodes, heartworms, ticks, fleas, and ear canker mites (Pasitschnaik-Arts and Larivière 1995). Because researchers

normally do not directly handle individuals, human health risk is minimal. However, we recommend use of gloves and other personal protective equipment when collecting and handling biological samples (refer to Section I.E).

VII. NORTHERN RIVER OTTER (Lontra canadensis) –

A. Survey Techniques -

1. Latrine Surveys –

- a. <u>Rationale</u> Indirect, noninvasive survey methods can be used effectively to detect river otters. Fecal deposition rates can provide an index of river otter abundance per site, per river mile, or per unit time. Population attributes including sex or genetics can be evaluated from biological samples (Ben-David et al. 1998, Ben-David and Golden 2007).
- Application Walk along river banks to locate latrine sites. Some terrain may necessitate use of either a small boat or raft to access the river bank. Sites actively used by river otters are often characterized by wellestablished trails or slides, low slopes, and vegetation (Bowyer et al. 1994, 1995). Once a latrine is located, use a GPS device to record its location. Mark all latrine sites with forestry flagging for short-term identification and metal tags attached to tree trunks for long-term identification. Visit each latrine site at least twice during the survey year and separate individual visits by 2-3 days. Search latrines thoroughly (sites can range from 10-500 m²). To index river otter abundance, count and remove all fecal deposits or mark them to prevent recounting on subsequent visits. Do not mark samples that will be used for genetic analyses. Record all fecal deposits distinguished as fresh or old (i.e., >12 hr). To determine density and other population attributes, collect all fresh feces and anal gland excretions at each site (refer to Section VIII.D.2). Conduct surveys every 3 yrs to evaluate changes in abundance and every 6 yrs to evaluate changes in density (Ben-David and Golden 2007).
- c. <u>Analysis of Data</u> –Tally numbers of fresh and old fecal samples counted at each latrine site visit.
- d. <u>Disposition of Data</u> Send a report containing latrine locations and collection records to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- B. <u>Immobilization</u>, <u>Handling</u>, and <u>Marking</u> Not applicable.
- C. <u>Assessment of Sex and Age</u> Not applicable, although sex can be determined from biological samples (refer to Section VIII.D).

D. <u>Collection of Biological Samples</u> –

- 1. <u>Rationale</u> Genetic analysis of biological samples provides a means to distinguish individuals. Identification of unique individuals is necessary to estimate population size.
- 2. Application Refer to Section VIII.1.b for selection of sampling locations in the field. Collect all fresh fecal deposits at each latrine site. Fresh deposits are whole and intact with a glossy appearance and strong smell, often with visible crayfish carapaces (Bowyer et al. 1994, Hansen et al. 2007). Older fecal deposits may have a glossy appearance when they are wet, but lack the characteristic smell. Whenever possible, collect anal gland secretions (a.k.a., anal jellies) or feces with this material attached, as these samples provide more DNA than fecal samples alone. Use a clean stick or twig to pick up each sample and place it into an individual 50-ml vial. Be sure to use a different stick for each sample. Add enough ethyl alcohol to completely cover the sample and shake the vial lightly to ensure the sample was collected and a unique identification number. Store vials in coolers with ice packs (Ben-David and Golden 2007).
- 3. <u>Analysis of Data</u> Report results from genetic analyses including number and sex of unique individuals documented at each latrine site and throughout the survey area.
- 4. <u>Disposition of Data</u> Send fecal samples to a genetics laboratory within 72 hrs (refer to Section I.D.4). Send a report containing sample records including number of individuals recorded at each latrine site, locations, unique identification numbers, and results from genetic analyses to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- E. <u>Diseases</u> River otters are susceptible to a number of diseases, including canine distemper, rabies, respiratory tract disease, urinary infection, jaundice, hepatitis, feline panleucopenia, and pneumonia (Larivière and Walton 1998, Williams and Barker 2001). None of these diseases are expected to have severe population-level effects. Because researchers do not directly handle otters, human health risks are minimal. However, river otters are known to carry various endoparasites including nematodes, cestodes, trematodes, the sporozoan *Isopora*, and acanthocephalans (Larivière and Walton 1998). Consequently, we recommend use of gloves and other personal protective equipment when collecting and handling biological samples (refer to Section I.E).

VIII. <u>CANADA LYNX (Lynx canadensis)</u> –

A. <u>Survey Techniques</u> –

1. <u>Snow Tracking (Ground)</u> –

- a. Rationale Track surveys are an effective method to detect lynx when weather and snow conditions are suitable. Biological samples can also be collected and analyzed to obtain additional information about population attributes including estimates of population size.
- b. <u>Application</u> Snow track surveys for lynx follow the same methods used for wolverines (refer to Section VII.A.3.b.). Characteristic of Felids, lynx forepaws leave larger tracks than hind paws and claw prints are usually not visible. Toe pads are often indistinguishable due to the dense hair on the bottom of a lynx's feet (Halfpenny et al. 1996).
- c. Analysis of Data Refer to Section VII.A.3.c.
- d. <u>Disposition of Data</u> Send a report containing track locations, observation records of all carnivores, and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

2. Live Capture -

- a. Rationale Lynx must be captured to obtain morphometric and demographic information, and to collect biological samples. Individuals may also be marked with PIT tags or radiocollars for unique identification and to estimate abundance.
- b. Application Lynx are most effectively captured during winter when snow cover is extensive. Kolbe et al. (2003) designed a lynx trap made of PVC pipe and chicken wire. The Nongame Program has several of these traps on-hand. Contact the Nongame Mammal Biologist at the WGFD Lander Regional Office to determine availability. Assemble traps on-site and conceal them among existing vegetation, usually pine trees. Cover traps with pine boughs for camouflage and to provide thermal protection. Bait traps with ≤5 lbs of deer and apply a long-range lure such as beaver castor. Use fishing line to hang a visual attractant such as a grouse wing, pie plate, or CD, within 50 m of traps. Check traps every 24–36 hrs and re-bait as necessary. In Wyoming, baiting with dead game animal parts (e.g. from road kills) must be authorized by a Chapter 33 permit issued to conduct scientific research. If live game animals such as cottontail rabbits will be killed and used for bait, a Chapter 56 permit is also required.

- c. <u>Analysis of Data</u> Report number of individuals caught and information on sex, age, and morphometric measurements if collected (refer to Section IX.C). Record unique identification numbers including PIT tags and radio-collar frequencies from each previously marked individual captured.
- d. <u>Disposition of Data</u> Send a report containing capture records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).

B. Immobilization, Handling, and Marking –

- <u>Rationale</u> Lynx must be handled in order to obtain morphometric measurements and tissue samples or to mark individuals. Chemical immobilization is required to handle lynx.
- 2. <u>Application</u> Immobilization and handling recommendations were provided by J. Squires and J. Kolbe (personal communication, USDA Rocky Mountain Research Station). Calmly approach traps being careful not to startle the lynx. In most situations, individuals will remain relatively docile and are easy to inject. A second person can distract the lynx, if necessary, during the injection. Use a syringe pole and 20-gauge × 2.5-cm needle to administer drugs in the front shoulder or ham while the lynx is in the trap. To immobilize lynx, administer Ketamine at 8 milligrams per kilogram body weight plus Xylazine at 3 milligrams per kilogram body weight. Record time of injection on the lynx chemical immobilization form (refer to Attachment 10).

Although unusual reactions to drugs are rare in healthy individuals, malnourished or severely dehydrated lynx may display unstable vital signs. Normal vital signs are: respiration: 20-40 breaths per min; heart rate: 80-120 beats per min; and body temperature: 37 °-39° C. If unusual symptoms are observed, do not antagonize the Xylazine as this can cause a convulsive reaction. Allow the individual to metabolize the dosage without the reversal agent. This process can be facilitated by keeping individuals as warm as possible.

Use the lynx chemical immobilization form (refer to Attachment 10) to record data during processing. The form is designed to be followed in numerical order to ensure an appropriate level of immobilization is achieved before procedures are conducted. Lynx can usually be handled within 10 min of injection. In rare cases, including young, agitated, or very large individuals, a booster dose may be necessary. Wait at least 15 min after the initial injection before administering a booster. A booster dosage should never exceed $^{1}/_{3}$ of the initial capture dose. While waiting for the drug to take effect, place a handling tarp on top of a sleeping bag on the ground. Lynx are properly immobilized when breathing becomes deep and regular and individuals are unresponsive to stimuli. Remove the lynx from the trap. Position the lynx on its side on the tarp and immediately

apply ophthalmic lotion to eyes, rubbing gently. Cover the head and eyes, being careful not to obstruct the nasal passage.

Record the following morphometric measurements from the individual lying in a natural position: shoulder to hip length from front point of shoulder to ball of hip; shoulder height from top of scapula to tip of outstretched foot; total body length from tip of nose to tip of tail along the contour of back; right upper canine width at the gum line; canine length from gum line to tip; and ear tuft length from base of ear to end of longest hair. Use a caliper to take tooth measurements. Note tooth wear and broken teeth on the lynx chemical immobilization form (refer to Attachment 10). Carefully examine the lynx for past and current injuries, paying special attention to condition of its feet. Manually extend each claw and check the pad condition. Apply triple antibiotic ointment to any open wounds or abrasions.

Insert PIT tags for long-term identification of captured individuals. Implant PIT tags subcutaneously in the scruff of the neck between the shoulder blades (refer to Section I.B.2.paragraph 4). Refer to Section V.B.2, paragraph 2 for the procedure to affix radio-collars.

To ensure individuals are released in good condition, place lynx back in the trap for 3-3.5 hrs after capture. Before placing individuals in traps, remove the trigger wire or bury it underneath the treadle so individuals will not snare themselves during recovery. If the ambient temperature is less than 0° C, lay traps with lynx inside on the sleeping bag. Check the lynx often to assure the head and airway are clear and to look for signs of heightened consciousness. Administer Yohimbine (0.10 mg per kg) when individuals begin to move on their own, usually 75–90 min after the initial injection. Release the lynx only after it is fully recovered.

Refer to Section II.B.2, paragraph 5 for euthanasia protocol in the event of a serious trapping injury.

- 3. <u>Analysis of Data</u> If multiple individuals are handled, complete a summary table containing data from each lynx. Otherwise, no data analysis is necessary.
- 4. <u>Disposition of Data</u> Send a report containing capture records and associated data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d). Also note any trapping-related mortalities.
- C. <u>Assessment of Sex and Age</u> Determining sex of Felids is generally more difficult than for other carnivores, especially in young individuals (Rolley 1987). This can usually be accomplished by palpitation of genitals. In some cases, males can be distinguished from females by presence of testes. Replacement patterns of teeth can be a useful method to distinguish between adults and juveniles up to 240 days of age

(McCord and Cardoza 1982). Ages of older adults can be determined based on cementum annuli in tooth cross-sections (Crowe 1972).

D. <u>Collection of Biological Samples</u> –

- Rationale Biological samples are collected to detect and monitor diseases and to distinguish individuals through genetic analysis. Blood samples are typically obtained during capture, and scat or hair samples can be collected during snow tracking surveys.
- 2. Application The method for collecting blood from lynx is identical to that described for swift fox (refer to Section V.D.2). Use a 22-gauge needle and 12-or 20-ml syringe to draw blood, depending upon how much is needed for analysis. Generally, fecal and hair samples can be found near temporary resting places, day beds, or ambush beds. Day beds are sites where individuals lay to rest and are characteristically oval-shaped depressions, usually with crusty or icy snow. Ambush beds lack crusty or icy snow; depressions formed by the front legs are often visible in the snow. Refer to Section VII.D.2 for information on how to collect and store fecal and hair samples.
- 3. <u>Analysis of Data</u> Report analysis results from each captured individual.
- 4. <u>Disposition of Data</u> Deliver all blood samples to the WGFD Laboratory in Laramie, Wyoming within 72 hrs. Send a report containing sample records including locations and unique identification numbers, and results from genetic analyses, to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section I.A.1.d).
- E. <u>Disease Precautions</u> Felids including lynx, are highly susceptible to sylvatic plague (refer to Section III.E). Wear proper protective equipment when handling lynx, particularly if collecting biological samples (refer to Section I.E), and use appropriate protective measures to minimize exposure risk while handling or transporting dead individuals.

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ATTACHMENT 1: MAMMAL OBSERVATION RECORD FORM

Rationale – The Nongame Program relies on Mammal Observation Record Forms to track nongame mammalian observations of interest and validate their identification. This enables Nongame personnel to plan follow up surveys as necessary. Many mammalian species are easily confused. Information provided on the form helps Nongame Program personnel determine whether observers used appropriate criteria to consider and eliminate similar species. The form is routinely sent to WGFD personnel and the general public to request additional information about observations.

<u>Species / Observations of Interest</u> – The following list identifies species and geographic regions for which observations are of particular interest to the Nongame Mammal Program.

<u>Small mammals and Bats</u> – Generally, most small mammals and bats must be captured for proper identification. Consequently, a mammal observation form is not the appropriate instrument to record information from encounters. Voucher specimens are usually collected and cataloged to document new records of species and locations.

American pika – Eastern $\frac{1}{3}$ of Wyoming only

Black-tailed jackrabbit – Southwest corner of Wyoming only

<u>Abert's squirrel</u> – All observations

<u>Eastern gray squirrel</u> – All observations

Northern flying squirrel – Eastern Wyoming only

Swift fox – Western ½ of Wyoming only

Gray fox – All observations

Ringtail— All observations

Fisher – All observations

Least weasel – All observations except Sheridan County

Wolverine – All observations and tracks

Spotted skunk (eastern and western) – All observations and tracks

<u>Canada lynx</u> – All observations and track

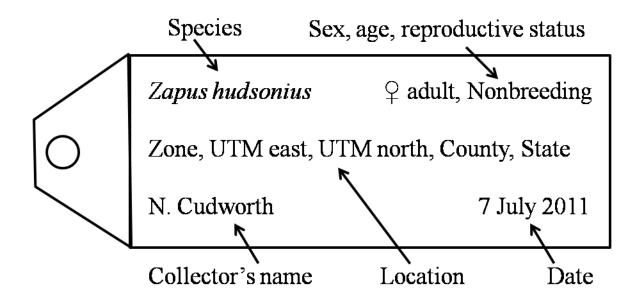
MAMMAL OBSERVATION RECORD

PLEASE RETURN TO: Nongame Mammal Biologist
Wyoming Game and Fish Department

260 Buena Vista Drive Lander, Wyoming 82520

Species Observed:			
Number Observed:		Photograph Ta	ken:
Observer's Name:			
Address:			
Occupation:			
Name of Other Observers:			
Address of Other Observers:			
Agency/Organization:			
Reporting Date:			
Location (direction and distance from the no	earest town or iden	tifiable landmark and le	egal description of the site):
UTM Coordinates:	E	N Zone_	Datum (e.g. NAD83)
Latitude/Longitude Coordinates:			
Latitude/Longitude Coordinates:	tion	Township	Range
Describe Site (details about land use, habita	it type, etc.):		
COLLECTION OF DEAD ANIMAL: Currently Held Where?			
How Was Specimen Acquired?			
OBSERVATION OF LIVE ANIMAL:			
Time and Duration of Sighting:			
Weather Conditions:			
Distance of Observation:		Castina Casas Has 40	
Binoculars Used?		Spotting Scope Used?	
Description of Animal(s) (color, size, marki	-		
Body:			
Legs and Feet:			
Tail:			
Head and Face:			
Other Comments:			
Behavior (describe in observer's words):			
behavior (describe in observer's words).			
Cimilar Caraina and Ham Obsama Elimina	4 - 4 Th		
Similar Species and How Observer Elimina	ted I nem:		
PLEASE DO NOT WRITE IN THIS SPAC	Œ:		
Classification:			
Confirmed: Probable:	Unconfirme	ed:	

ATTACHMENT 2: VOUCHER SPECIMEN TAG



ATTACHMENT 3: HANDLING RECORD FORM FOR SWIFT FOX

Date:	_ Survey Area:	Field Collector:	
Trap Location:	Handler:	Processor:	
Swift Fox ID #	Sex:	Verified age:	
1. Handling sta	art time:		
2. Gross weigh	t:Kg		
3. Bag weight:	Kg		
4. Calculated A	Animal Weight (GW – TW	' = CAW)	Kg
5. Current coll	lar frequency:	Khz	
6. Turn on and	l verify new VHF collar fro	equency:	Khz
7. Affix new ra	ıdio collar		
8. Sex: Male	eFema	le	
9. Estimated a	ge: Juvenile	Adult	
10. Check vitals	s. Body temperature	°F Time	
11. Test and im	plant transponder chips. I	Head chip#	_•
12. Hair sample	e: Yes No		
13. Draw Blood	: (Purple Top)	cc (Red Top)	_ cc
14. Measuremen	nts (Shoulder Height):	cm	
15. Right Upper	r canine measurement (Wie	dth) mm (Le	ength) mm
16. Time handli	ing complete:		
17. Time anima	l released: Da	ate Released:	

18. **Comments** (*dye mark, teeth, body condition, wounds, irregularities*):

ATTACHMENT 4: SPOTLIGHTING RECORD FORM FOR FERRETS

Survey Information Start End Time Species R Method: (24hr) (24hr) Code		OBSERVER	ER:			PD COLONY #:	1 # X		
urvey Informat Start End Time Time	rvey began	(v	Datum	: NADI	Datum: NAD1927 zone 13		ROUTE #:		
Start Time (24hr)	ion								
	Species	Reflector Pole#	Obs. Time (24hr)	UTM Easting	UTM	1* Observation: Marked Y/N/Un² Color/Symbol	Traps Plugs Set	Capture Time (24hr)	Color and Symbol Assigned & Comments
				St.	46				
				8	46				
				B	46				
				8	46				
				B	46				
				B	46				
		2,		8	46				
				8	46				
				8	46				
				B	46				
				B	46				
				8	46	0			
				B	46				
Total Hours:	Truck		Backpack:	ack:			BFF:	BFF: Total Obs.	s. uals
1- Method = T (Truck), EP (Eackpack) 2 If you observe a ferret but can't determine if it has a symbol or not use "UN" (unknown). FOR BFF ORS ONLY	BP (Backpac) ret but can't d	c) letermine if	it has a s	ymbol or no	r not use "UN" (unka	cnown).			
Species Code	Species	Eve Color	, io	1. Rec	ord species cod	e, reflector pole m	umber, ob	servation	1. Record species code, reflector pole number, observation time, UTM's, and if trap was
	Black-footed ferret	Green	-	set	record number	set, record number of plugs and traps at burrow	s at burro	w.	
	Burre wing Owl	Yellow	>	2.Reco	nd if ferret is n	Record if ferret is marked, unmarked or unknown for first observation.	or unkno	wn for firs	t observation.
	Badger	Green		3. Wh	en a litter is loc	 When a litter is located, record each ferret separately. 	ferret sep	arately.	
GHOW Great H	Great Homed Owl	Yellow	> >	4. Alor	ng with letter(s)	designating a ferr	ret (BFF),	include th	4. Along with letter(s) designating a ferret (BFF), include the survey night number and the
	Long-tailed Weasel	Green		nbes	ence number (f	sequence number (for each ferret). For example:	For examp	ole: 1BFF2	
MOUP Mount	Mountain Player	ANA	,			1= th	e first nig	1 = the first night of the survey	nvey
	Swift Fox	Yellow					dicates se	cond sight	2 = indicates second sighting of a ferret that night.

Color and Symbol Assigned & Comments																								
Capture Time (24hr)																								
Traps/ Plugs Set																								
1** Observation Marked Y/N/Un Color(Symbol ²																				land li				
UTM	46	46	46	46	46	46	46	46	94	46	46	46	46	46	46	46	46	46	46	46	94	46	46	46
UTM	8	B	g	B	8	20	94	8	90	8	B	90	B	94	8	04	8	40	90	94	84	90	B	97
Obs. Time (24hr)							0		65.														4 9	
Refector																								
Species																								
End Time (24hr)									500															
Start Time (24hr)																								
Method'																								

ATTACHMENT 5: EQUIPMENT SET-UP FOR PROCESSING FERRETS



Fig. 5.1. Oxygen tank and gauges for chemical immobilization of black-footed ferrets. Gauge no. 1 represents pressure (kPa) remaining in oxygen tank. Generally, a full D-size oxygen tank will show 14,000 kPa. Replace tank if under 3,000 kPa. Gauge no. 2 represents pressure to oxygen flow meter, which is connected to vaporizer (refer to Fig. 5.2). Maintain gauge no. 2 at 300 kPa and adjust using brass adjustment for gauge no. 2.

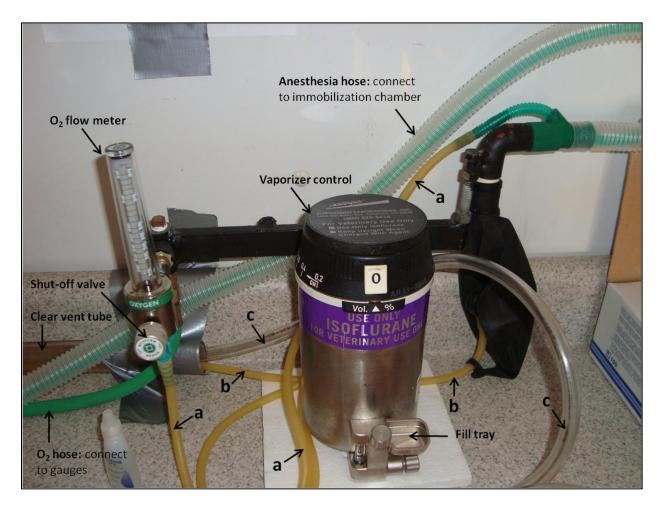


Fig. 5.2. Isoflurane vaporizer and associated connections needed for chemical immobilization of black-footed ferrets. Connect vent tube to exterior of processing trailer via existing roof vents in trailer. Secure hoses b and c and insert into clear vent tube. Connect hose c to top of immobilization chamber. To fill vaporizer with isoflurane, turn vaporizer control counterclockwise and pour liquid into fill tray. Connect anesthesia hose, which transports vaporized isoflurane mixed with oxygen to immobilization chamber or anesthesia mask (refer to Figs. 5.3 and 5.4). Turn shut-off valve of oxygen flow meter counter-clockwise to open and increase flow of oxygen. Proper setting of Lpm is achieved when green float is suspended at desired flow in meter. Turn valve clockwise to close. Turn vaporizer control counter-clockwise to set concentration level. Proper setting of Vol. % is achieved when concentration levels are aligned with white indicator triangle on front of vaporizer; turn clockwise to shut off. Drain all liquid from vaporizer before storing by opening drain plug below fill tray.

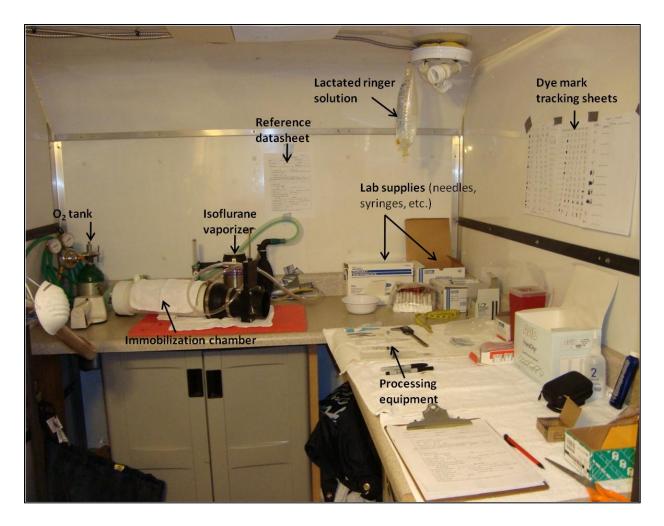


Fig. 5.3. Overview of processing trailer and equipment needed to chemically immobilize black-footed ferrets. Corrugated transfer tube connects to right side of immobilization chamber. Lay out all necessary processing equipment, including the thermometer, calipers, tweezers, syringes, etc., near edge of counter to facilitate handling.



Fig. 5.4. Chemically immobilized black-footed ferret with head inserted fully into anesthesia mask. Place individual ventrally, extend limbs fully, and ensure breathing is deep and consistent before initiating any procedures.

ATTACHMENT 6: CHEMICAL IMMOBILIZATION FORM FOR FERRETS

Date:	Survey Area:	Field Collector:									
Trap Location:	Anesthetist:	Recorder:									
Ferret ID #	Sex:	Verified age:									
20. Time of transference 21. Sex: Male 22. Estimated age:		(ISO 4.0 and O ₂ 3.5) (ISO 2.0 and O ₂ 2.5) [blood (I	SO 2.5 / 0 ₂ 2.0]								
, , , , , , , , , , , , , , , , , , ,											
Time Respiration r	ate										
7. Apply eye drop	s.										
8. Scan for existing	ng transponder.										
None	Head:	Hip:									
9. Hair sample: Y	9. Hair sample: Yes No										
10. Ticks: none	1-10 10-25 25 +, if	> 25, count Fleas: <i>none</i>	1-10 10-25								
25+	,	, <u> </u>									
	11. Blood drawn: Yes No If yes, ml drawn										
12. Upper canine width measurement:mm											
	ant transponder chips.										
_	-	Hip chip#									
	_	L and >1,000 g use 0.4 mL) Yes									
15. Fluids given: Y	/es No If y	es, cc given (Lacted Ringer	Solution - LRS)								
_	-	Time O ₂ turned off:	·								
17. Body length (h	ead to anus):	mm (head to tail):	mm								
18. Dye mark: Col	lor Symbol										
19. Body weight:											

20. Comments (swollen teats, teeth, body condition, wounds, irregularities):

ATTACHMENT 7: BLOOD COLLECTION FOR FERRETS



Fig. 7.1. The preferred method for bleeding black-footed ferrets is venipuncture of the anterior vena cava (Quesenberry and Orcutt 2004). Conduct this procedure while individual is chemically immobilized.

ATTACHMENT 8: GAIT AND SNOW TRACKS FOR WOLVERINE

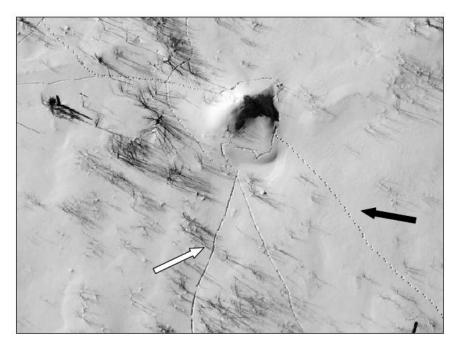


Fig. 8.1. Tracks of a sliding river otter (white arrow) and a wolverine (black arrow).

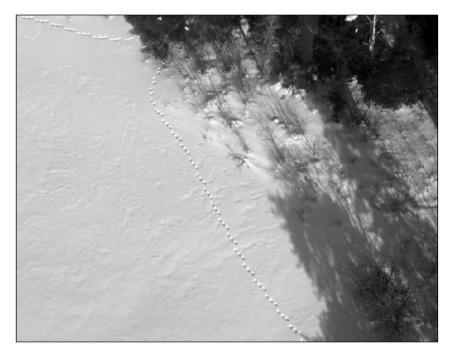


Fig. 8.2. Wolverine tracks, showing three by three pattern. Figs. 8.1 and 8.2 from Koen et al. (2008), Appendix 2, pgs 99, 101.



Fig. 8.3. Right front foot of a wolverine. Note the 1-3-1 spacing of toes, chevron-shaped interdigital pad, and metacarpal pad. (Utah) Photograph by D. Hall. Figure from Halfpenny et al. (1996), pg 137.

ATTACHMENT 9: EXAMPLE OF SURVEY FORM FOR WOLVERINE

Ontario V 2008	Ontario Wolverine Aerial Survey Data Sheet 2008	erial St	Irvey D	ata She	et	Page 1 of 8		Temp (°C): -22	: -22	Last sno ing, 5 cm	Last snowfall: Yesterday moming, 5 cm	esterday	-шош-	V= Visual observation
Date: Feb 16 2008	16 2008	Start : 0920	0920			Pilot: Smith		Tracking Conditions (circle): (Excellent) Fair Poor	Condition	ıs (circle	e): (Exce		Good	F= Fresh tracks O= Old tracks
Route #:	5	Finish: 1400	1400			Observer: Johnson		Sky (circle): Clear Heavy overcast	e): Clear rcast		Partly cloudy	Light overcast	rercast	Wind: Light, no turbulence
Target Hex	Distance	Latii	Latitude	Long	Longitude	Wolverine	Wolf	Caribou Moose		Lynx	Fisher	Other	Comments: (hat human sign, etc.)	Comments: (habitat, activity, human sign, etc.)
S-296c														
596	98.7								F		`		A lot of m burns	A lot of moose in cuts and burns
	81.3								Ь				A lot of m burns	A lot of moose in cuts and burns
	80.5	51	12.90	94	19.00		F						~6 on Peisk Lake	isk Lake
	71.8	51	15.36	94	22.95			F/O				7	Caribou tr on lakes (Caribou tracks and cratering on lakes (marten tracks)
	70.3	51	15.80	94	25.35			F/0					Caribou track on lakes; mat around lakes	Caribou tracks and cratering on lakes; mature forest around lakes
	27.6												Snowmot	Snowmobile tracks
470	42.2	51	26.09	94	30.82		F~2	٨3			7		Caribou tr ing on all spruce	Caribou tracks and crater- ing on all lakes; mature pine/ spruce
	20.4	51	27.16	94	32.40			ш					Caribou tr mature pii	Caribou tracks on all lakes; mature pine/spruce
	11.5	51	31.75	94	30.58		F~2		ч				~2, into a bum	purn
688	11.0										>	>	Hares in burn	pnm

Data sheet from Koen et al. (2008), Appendix 1, pg 87.

ATTACHMENT 10: CHEMICAL IMMOBILIZATION FORM FOR LYNX

Date:	Survey Area:	Field Collect	tor:	
Trap Location:	Anesthetist:	Record	ler:	
Lynx ID #	Sex:	Verified age:_		
 Time of Hand Apply eye dro Current collar 	art time: lling: ops and cover head. r frequency: verify new VHF collar t	(0.5 mL Ketamine a	and 0.2 mL Xylazine)	
6. Affix new rad7. Sex: Male_8. Estimated age9. Check vitals.	io collar Fen Kitten Body temperature	nale Adult °F Time _		
Time Respiration	rate Heart rate	Booster (Dosage)	Booster (Time)	
Head 11. Hair sample: 12. Gross weight: 13. Tarp Weight: 14. Calculated An 15. Draw Blood: 16. Body length (17. Right Upper (18. Ear Tuft leng 19. Check feet an 20. Time handling	ant transponder chips. d chip#	Hip chip# W = CAW (Red Top) cm (Shown of the come of	Kg Ilder Height): Im (Length)	em mm

22. Comments (dye mark, teeth, body condition, wounds, irregularities):

Subchapter 20.2

Bats (Order Chiroptera: Families Vespertilionidae and Molossidae)

Becky L. Abel and Martin B. Grenier

I. INTRODUCTION –

A. <u>Life History</u> – Bats are the only mammal capable of true powered flight. The ability to fly has enabled bats to become widely distributed and undoubtedly contributed to their diverse foraging and roosting habits and other behaviors (Hinman and Snow 2003). Indeed, 1,232 living species of bats occupy diverse ecological niches worldwide (Kunz et al. 2011). Forty-five species are found in the United States (Wilson and Ruff 1999) including 18 species documented in Wyoming (Table 1; Luce 1998). Bats inhabit all areas of Wyoming and account for 15% of mammalian species, thus contributing extensively to the State's biological diversity.

All bats that inhabit Wyoming are nocturnal insectivores, feeding exclusively on flying insects which they detect by echolocation. In their natural habitats, bats are capable of consuming up to 100% of their body weight per night (Kurta et al. 1989, Kunz et al. 2011). As the primary predator of nocturnal insects, bats likely play an important role in top-down regulation of insect populations (Kunz et al. 1995, Boyles et al. 2011). A large proportion of the insects bats eat are among North America's most costly agricultural and forest pests (Hester and Grenier 2005, Kunz et al. 2011). Bats have evolved a variety of strategies to capture and eat insect prey, for example "gleaning" and "aerial hawking." Bats typically forage throughout the night, with most activity around sunset when insects are most active.

During May through September, bats roost in locations affording protection from predation and weather. Roosts are important habitat for mating, pup-rearing, and energy conservation (Kunz and Lumsden 2003). Bats in Wyoming can be divided into 2 groupings based on roosting ecology: bats that roost in tree cavities, under bark, and among foliage; and bats that roost in caves, rock crevices, and manmade structures. Bats occupy day roosts between sunrise and sunset and often select a different roost for resting between foraging bouts at night. Reproductive females of several species congregate at maternity roosts to gestate and care for their pups. Maternity roosts are usually separate from roosts used by males and non-reproductive females. Lactating females return to the maternity roost frequently to nurse throughout the night rather than using different night roosts.

Species incapable of long distance migration hibernate during winter within areas that cannot supply enough insects for sustenance. Bats select hibernacula with suitable stable conditions: cool temperature, high humidity, and air flow. Additionally, hibernacula must provide security from predators and other threats. These conditions are usually found in underground sites such as caves or abandoned mines, or in cavities deep within old trees (Richardson 2002, Adams 2003, Hinman and Snow 2003).

Bats, unlike other small mammals, are extremely long-lived. Many Wyoming species live up to 30 years (O'Shea et al. 2003) and most raise only 1 pup annually. Under normal circumstances, low reproductive rates are offset by long life spans. However, bat populations are very vulnerable to declines in adult survival. Declines in many bat populations at both continental and local scales have led to concern about the future of migratory and resident bats in Wyoming. Reasons for declines are many: habitat loss, modification, and fragmentation; roost site disturbances; collisions with wind turbines; pesticides; and emerging pathogens have all been implicated (Hester and Grenier 2005).

Table 1. Resident (res), peripheral (per), and accidental (acc) species of bats that occur in Wyoming. Species of Greatest Conservation Need (SGCN) are indicated at the far right (Orabona et al. 2009).

Common Name	Scientific Name	Status	SGCN
Western small-footed myotis	Myotis ciliolabrum	res	у
Long-eared myotis	Myotis evotis	res	y
Northern myotis	Myotis septentrionalis	res	y
Little brown myotis	Myotis lucifugus	res	y
Fringed myotis	Myotis thysanodes	res	y
Long-legged myotis	Myotis volans	res	y
Hoary bat	Lasiurus cinereus	res	y
Silver-haired bat	Lasionycteris noctivagans	res	y
Big brown bat	Eptesicus fuscus	res	y
Spotted bat	Euderma maculatum	res	y
Townsend's big-eared bat	Corynorhinus townsendii	res	y
Pallid bat	Antrozous pallidus	res	y
California myotis	Myotis californicus	per	n
Yuma myotis	Myotis yumanensis	per	n
Eastern red bat	Lasiurus borealis	per	n
Brazilian free-tailed bat ^a	Tadarida brasiliensis	per	n
Eastern pipistrelle	Perimyotis subflavus	acc	n
Big free-tailed bat ^a	Nyctinomops macrotis	acc	n

^a Family Molossidae; all other species of bats found in Wyoming are members of the family Vespertilionidae

B. <u>Bat Conservation</u> – Bat conservation efforts in Wyoming began in 1994 when the Game and Fish Commission approved a nongame wildlife regulation that classified several wildlife species, including bats, as protected (Hester and Grenier 2005). Bats are currently protected against intentional take except permitted scientific collection or when control measures are deemed necessary for reasons of public health and approved by the Wyoming Game and Fish Department (WGFD).

The Department's Nongame Program began conducting bat surveys in caves and abandoned mines throughout the state from 1994-1997. Those surveys provided baseline data on roost distribution and bat abundance. Since then, the Section has continued to survey caves and abandoned mines known to be important roosts. Nongame conducted additional inventories of bats with mist nets and acoustic detectors in forested habitats during summer from 2007-2011.

The Department's Nongame Program, in cooperation with the Wyoming Bat Working Group (WYBWG), drafted the "Conservation Plan for Bats in Wyoming" in 2005. The conservation plan provides a technical framework to facilitate bat conservation by summarizing the most current literature on bat ecology and life history, and recommending conservation actions (Hester and Grenier 2005). In a similar cooperative venture, Nongame Section developed a strategic plan to coordinate a statewide response to the threat of White-Nose Syndrome in Wyoming (Abel and Grenier 2011).

II. <u>SURVEY TECHNIQUES</u> – To conduct bat surveys successfully, investigators must have an awareness of activity patterns including when and where bats roost and forage. A multitude of variables can drastically alter activity patterns between survey nights or even within a single survey. If a comprehensive inventory is the goal, more than one survey must be conducted at each site in order to maximize probability of detecting all species.

Although bats are primarily nocturnal, individual bats and certain species may emerge as early as ½ hour before sunset. On the other hand, some bat species are considered late fliers and typically emerge well after sunset. Under normal conditions, bat activity is highest during the first half of the night. In most cases surveys should be initiated a minimum of ½ hour before sunset and continue a minimum of 3 hours after sunset to maximize detection of all species.

If surveys are intended to document a particular species, focus near suitable roosting habitat of that species. If the objective is to document a range or diversity of species, focus on locations that concentrate large numbers of different species. For example, water bodies provide important foraging habitat and drinking water that attract many bat species. Additionally, bats use travel corridors such as forest edges and riparian areas to commute between roosts and foraging areas. When selecting a water feature to survey, choose an area where water is limited, because bats will often concentrate there. Large water features are not necessary to attract bats; suitable survey sites can include stock ponds, watering tanks, and even puddles in 2-track roads. Try to select sites with vegetation or landscape features that naturally funnel bats into a small area. Always survey a variety of sites throughout the entire area of interest. Bats often use specific or traditional foraging areas or roosts. Therefore, negative survey results from a single location are insufficient evidence to conclude bats are not present; they may be concentrated elsewhere nearby.

Multiple survey methods are most effective to survey bats in a given location because some species are difficult to detect with standard equipment (Kunz et al. 2009). For example, calls of Townsend's big-eared bat are quiet and those of the spotted bat are low in frequency, both of which may often be difficult to record and accurately identify. Conversely, certain species of bats are difficult to capture in nets because they fly high or are adept and nimble flyers that can easily avoid the net. To maximize the number of species identified, we recommend acoustic surveys in conjunction with capture methods whenever possible.

A. <u>Acoustic Surveys</u> – Acoustic surveys are used to document presence of bats at specific locations and to identify roost sites. They can also identify potentially productive sites for mist-netting. Advanced ultrasonic survey systems such as those developed by

Anabat, Petterson, and Wildlife Acoustics can be used to identify species from a recorded bat pass, and to determine composition of species within the community. A "pass" is a discrete event wherein a bat is heard or seen in the vicinity of the observer. Under most circumstances it is impossible to distinguish between 1 bat that passes by the detector many times and several bats each passing by the detector once.

- 1. <u>Audible Bat Surveys</u> –Surveys of species that vocalize in frequencies of 20 kHz or lower can be done without specialized equipment. In Wyoming, the spotted bat can be heard without specialized acoustic detectors.
 - a. <u>Rationale</u> Audible surveys are an inexpensive, effective method to document presence of spotted bats.
 - b. <u>Application</u> Observers who are unfamiliar with behavior and ecology of spotted bats should review the species account in the Conservation Plan for Bats in Wyoming and must have good hearing in high frequency ranges (Hester and Grenier 2005). Inexperienced observers should receive formal training or be accompanied by an experienced observer before conducting surveys alone. Avoid surveying areas when background noise is excessive because this can inhibit the observer's ability to detect bats. If multiple calls are detected simultaneously during a pass, record only the number of unique individuals. Audible surveys can be conducted during mist netting or while recorded acoustic surveys are conducted, or they can be conducted independently using stationary count or transect methods.

Record time and location of each bat pass and habitat type (refer to Attachment 11: Acoustic Form).

- c. <u>Analysis of Data</u> –From stationary counts, report total number of passes and passes per minute. From transect counts, report total number of passes only (refer to Attachment 11).
- d. <u>Disposition of Data</u> Send a report containing detection totals to the Nongame Mammal Biologist at the WGFD Lander Regional Office. The Nongame Program will incorporate data into the Threatened, Endangered, and Nongame Bird and Mammal Investigations annual completion report. Data will also be used to update distribution maps and databases including the Department's Wildlife Observation System and the Atlas of Birds, Mammals, Amphibians, and Reptiles in Wyoming.
- 2. <u>Heterodyne Acoustic Surveys</u> Heterodyne detectors are used to document presence of bats and level of bat activity before capture surveys are conducted. This type of detector uses a tunable constant internal frequency that combines with the incoming bat call to produce sum and difference frequency sounds that are audible through speakers or headphones. The resulting audible sounds indicate a bat pass. Several manufacturers market a wide range of heterodyne detectors. Although all models of heterodyne detectors operate similarly, they vary from under \$100 to several hundred dollars and come with a variety of

feature options such as digital displays and frequency scanning. Detectors with a tunable frequency dial or digital scanner are preferable.

- a. <u>Rationale</u> Heterodyne detectors are used to document presence of bats at
 a given location. This is a valuable and cost effective method to identify
 areas used by bats.
- and 40 kHz, as most bats in Wyoming echolocate within this frequency range. Some detectors can be tuned to a specific frequency and may be used to document presence of a target species; however, species identification is generally difficult with heterodyne detectors because many species of *Myotis* emit calls with overlapping frequencies. Generally, the acoustic survey is conducted at a single location such as near a water source or roost portal. However, it is also possible to survey by walking systematically through a survey area. Survey intensity should ensure adequate coverage of the survey area.

Record time and location of each bat pass, and habitat type (Attachment 11).

- c. Analysis of Data Refer to Section II.A.1.c.
- d. <u>Disposition of Data</u> Send a report containing acoustic detection totals to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d).
- 3. Advanced Bat Detection Systems Bat detection systems that rely on advanced acoustic technologies such as frequency division, zero crossings analysis, time expansion, and full spectrum provide the capability to accurately identify species. Some of the systems using these technologies include AnaBat, Petterson, Binary Acoustics, and Wildlife Acoustics. Although AnaBat will detect vocalizations of all Wyoming bat species simultaneously, species identification is time-consuming and can be difficult for the *Myotis* species. Petterson, Binary Acoustics, and Wildlife Acoustics detectors are full spectrum and offer enhanced species identification by creating a high-resolution call diagram and incorporating additional call parameters such as harmonics and amplitude.
 - a. <u>Rationale</u> Acoustic surveys with advanced detectors are used to document species presence and composition of bat communities.
 - b. Application Acoustic surveys can be conducted actively or passively. During active surveys, the observer tracks bats in flight with a microphone to obtain more complete recordings and fewer fragmentary call sequences. The observer also records field notes to assist with data analysis. Passive surveys are conducted with acoustic recording devices in stationary locations and do not require an observer to be present. Passive surveys allow an area to be covered with multiple acoustic units or multiple areas can be surveyed simultaneously. Both active and passive surveys can be

completed in conjunction with other types of surveys (refer to Section II.B.1). If passive surveys are not associated with capture surveys, program detectors to operate the entire night whenever possible.

When detectors are set up, record location, habitat type, and weather conditions (refer to Attachment 11). Also record detector settings, memory card identification number, and equipment number. After the survey, download files containing call recordings. Call files are stored on a network drive or external hard drive and organized in separate directories corresponding to each survey site or date. Call files recorded with AnaBat detectors are qualitatively identified to species based on known call parameters of Wyoming bats. This method is time consuming, as each individual call needs to be identified by an experienced observer. When calls cannot be identified to species, they should be assigned to species groupings such as 40-kHz bats, 50-kHz bats, and so forth. The analysis of call files recorded with full spectrum detectors, such as Petterson, Binary Acoustics, or Wildlife Acoustics models, may be automated using SonoBat version 3.02 or later. Calls that cannot be identified by the automatic process will also need to be identified manually.

Record length of survey, number of species present, number of passes recorded for each species, and total number of passes.

- c. <u>Analysis of Data</u> Convert the number of passes by each species and total number of passes to passes per hour (refer to Attachment 11).
- d. <u>Disposition of Data</u> Send a report summarizing acoustic detection totals and if possible, provide acoustic files to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d).
- B. <u>Capture Surveys</u> A Chapter 33 Permit must be obtained from the Department prior to capturing and handling bats.

A wide variety of techniques have been developed to capture bats. They range from simple techniques such as hand capture to more complex techniques requiring highly specialized equipment such as harp traps. Capture method varies depending on target species, accessibility, and survey objectives. Only mist nets and harp traps are discussed in this section because the simpler techniques have only specialized applications and are not widely utilized.

Identify potentially productive capture sites by conducting acoustic surveys (refer to Section II.A) beforehand. Both mist nets and harp traps are suitable capture methods to deploy near roosts, however special care should be taken to avoid harming young bats or causing roost abandonment.

Capture probability varies among bat species and some may go undetected at a given site. For example, high-flying species such as hoary and silver-haired bats are more difficult to capture than lower-flying species such as little brown bat. Some less

abundant species may not be represented in capture samples obtained during a single survey period. To effectively detect all species present in the area, the observer should survey multiple nights and utilize a detector to supplement capture data. If multiple surveys are planned, avoid trapping on consecutive nights and change net locations and configurations to improve success (Kunz and Brock 1975).

If possible, sample survey sites at least twice each year and during two different seasons. Schedule the first sampling period in early summer to assess the community of adult bats. Schedule the second sampling period during August, after young are volant (i.e., capable of flying), to assess reproduction.

- 1. <u>Mist Net</u> Mist nets are the most commonly used equipment for capturing bats because they are lightweight, compact, relatively inexpensive, and easy to transport and erect in the field (Kunz et al. 2009). Mist nets can be deployed virtually anywhere.
 - a. <u>Rationale</u> Mist nets are used to determine species composition and relative abundance. Additional information such as sex, reproductive status, and health can also be obtained (Hester and Grenier 2005).
 - b. Application Mist nets can be deployed successfully in almost any location bats are expected to fly, and are effective for capturing bats at ground, sub-canopy, and canopy levels. Capture success is usually highest near water sources and flyways such as forest gaps, trails, and mountain ridges. Avoid large bodies of water where flight patterns are not concentrated enough to funnel bats into the nets. Choose smaller ponds that are less than 1.2 m deep, enabling personnel to wade and reach the upper pocket of the net. When netting over streams, choose streams with slow-moving water or large pools. Set the lowest shelf cord close enough to the water surface so bats do not fly beneath the net; however, keep the net pocket high enough to ensure captured bats do not contact the water.

The number of mist nets often depends on size of the area being surveyed. Larger areas require multiple nets. Nets can be deployed in many patterns, including H, T, V, W, X, Y, and Z configurations. Triple-high canopy nets can also be utilized where appropriate. Properly set, mist nets have distinct pockets formed by the netting and shelf cords. Avoid sagging nets. Stabilize net poles by anchoring guy ropes to other objects such as stakes, tree limbs, or large rocks.

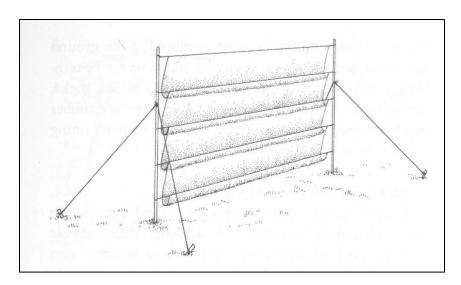


Fig. 1. Typical set-up of a mist net. Figure adapted from Kunz et al. (2009).

Nets may be installed any time during the day, but should be kept closed until ½ hour prior to sunset to ensure birds and other non-target species are not accidentally captured. Once nets are opened, monitor them continuously for at least 2.5 hours and remove captured bats as soon as possible. Bats in hand can be identified to species using a dichotomous key (refer to Attachment 18: Dichotomous Key to the Bats of Wyoming). At the end of the survey period, close all nets before dismantling sets. Refer to Section III.B.2.a for information on removing bats from nets.

Record location, date, weather conditions, and habitat type at each capture site (refer to Attachment 12: Netting and Acoustic Capture Form). Record the number and configuration of nets on data sheets. Also record the time of each capture and net number. Record the total number of captures, captures of each species, and total survey time (refer to Attachment 12).

- c. <u>Analysis of Data</u> Report capture per unit effort, such as number of captures per hour of survey time (refer to Attachment 12).
- d. <u>Disposition of Data</u> Send a report containing capture records and other data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d).
- 2. <u>Harp Trap</u> Harp traps are advantageous in locations where large numbers of bats are expected because captured bats can be removed relatively easily and quickly (Kunz et al. 2009). Harp traps are suitable for such locations as inside roosts or at entrances to caves and abandoned mines. Additionally, harp traps may be more effective than mist nets in certain habitats such as the forest understory, roosts, or across flight corridors (Kunz et al. 2009).
 - a. <u>Rationale</u> Harp traps are used to collect data on species composition and relative abundance. Additional information such as sex, reproductive status, and health can also be obtained.

b. <u>Application</u> – Harp traps can be deployed in almost any location bats are expected to fly and are effective for capturing bats at ground and subcanopy levels. Harp traps can also be deployed in many different situations including locations where the trap must be suspended. Because of the relatively small capture area, effectiveness of harp traps can be increased by using mist nets to funnel bats into the trap (Kunz et al. 2009). Record number of harp traps, mist nets, and their configuration on data sheets for future reference.

Harp traps may be set anytime during the day up to ½ hour prior to sunset. Once installed, harp traps can be monitored continuously or periodically, such as every 15 minutes, for a minimum of 2.5 hours. Captured bats should be removed regularly during monitoring intervals. Refer to Section III.A for additional information on handling bats after capture and see Attachment 18 for species identification.

Record the location, date, weather conditions, and habitat (refer to Attachment 12). Also record the time of each capture and net number. Bats in hand can be identified to species using a dichotomous key (refer to Attachment 18).

- c. Analysis of Data Refer to Section II.B.1.c
- d. <u>Disposition of Data</u> Send a report containing capture records and other data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d).
- C. Roost Surveys Surveys of known or suspected bat roosts are an effective means to document occupancy, species presence, and seasonal use. However, surveys conducted by poorly-trained but well intentioned individuals may negatively impact a roost site, individual bats, and populations, and may place the surveyor at risk of injury or death. Only trained personnel should conduct roost surveys. Hazards exist on the surface, around openings, and inside roosts and potential roosts. Several excellent resources including Altenbach et al. (2002) and Sherwin et al. (2000) provide specific guidelines for conducting roost surveys. The Nongame Program maintains a database of known and potential roosts in Wyoming. Personnel planning to conduct roost surveys should contact the Nongame Mammal Biologist for assistance and to obtain copies of protocols and additional information.
 - 1. Interior Roost Surveys
 - a. Rationale Interior surveys are done primarily to assess a site's potential to serve as a roost for bats and to census bats. Data are used to determine whether the site is currently used by bats and if so, the species and number present and seasonality of use.
 - b. <u>Application</u> Conduct interior surveys at least once during November-April and once during June-August. Conduct surveys of roosts known to be occupied by bats only once every three years during the season of

occupancy. Avoid entering known maternity roosts before August to minimize impacts. If abundance estimates are required, conduct exit count surveys during summer (refer to Section II.C.3.a).

Record the survey date, dimensions of the roost entrance, location and number of bats, and signs of bat use. Signs of bat use include guano, staining, and insect remains. Note locations of additional openings and noticeable airflow. Also map the interior of the site including interior dimensions, lengths of passages and chambers, and record ambient temperatures and humidity within each chamber and passage (refer to Attachment 13: Interior Roost Form).

- c. <u>Analysis of Data</u> –This type of survey is done for inventory purposes and there is no data analysis.
- d. <u>Disposition of Data</u> Send a report containing roost data and any bat observations to the Nongame Mammal Biologist at the WGFD Lander Regional Office. Roost data will be incorporated into the Wyoming bat roost database (refer to Section II.A.1.d)
- 2. <u>Diurnal Exterior Roost Surveys</u> Exterior surveys can be conducted with minimal impact to roosts and bats. Risk to the observer is also greatly reduced.
 - a. <u>Rationale</u> Diurnal exterior surveys are done primarily to document roost locations and physical features. Data are useful to identify potential roost sites and other survey priorities.
 - b. <u>Application</u> Diurnal exterior roost surveys can be performed year-round, however it is preferable to conduct them when snow cover is absent to ensure potential hazards are visible to the surveyor. It is usually not possible to determine with certainty whether bats are currently using the roost.

Record date and time of survey, potential hazards surrounding the site, substrate, habitat type, actual or approximate size of the portal or shaft, compass bearing of the opening, slope aspect, and GPS location. At mine shafts, if possible, record whether a horizontal passage exists (Pierson et al. 1999; refer to Attachment 14: Exterior Roost Form).

- c. <u>Analysis of Data</u> –This type of survey is done for inventory purposes and there is no data analysis.
- d. <u>Disposition of Data</u> Send a report containing roost data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d and II.C.1.c).

3. Nocturnal Exterior Roost Surveys –

a. Exit count surveys -

- i. Rationale Exit counts are conducted to document bat presence and count the numbers individuals at roost sites. These surveys cause minimal impact and require no special training. Exit surveys are particularly useful at sites that cannot be safely entered. Species are difficult to identify in flight, so identification of species is not a priority for this survey.
- ii. Application Visually count the number of bats exiting a roost at dusk. The observer should be positioned so exiting bats are backlit against the sky to enhance their visibility. Terminate surveys 1 hour after sunset, when no additional bats are observed exiting the roost, or when it is too dark to accurately count. If possible, repeat exit surveys at least twice during a season. A tally counter is recommended to minimize errors.

Record the roost location and type, presence and condition of any gates, and other physical features. Also record survey start and end times, number of bats observed, time of first exit, and time of last exit (refer to Attachment 15: Exit Count Form).

- iii. <u>Analysis of Data</u> Convert the total number of bats observed to bats exiting per hour (refer to Attachment 15).
- iv. <u>Disposition of Data</u> Send a report containing exit count data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d).

b. Acoustic roost surveys –

- v. <u>Rationale</u> Acoustic surveys are useful to document summer use and presence of bats at potential roost sites. This method is not suitable for identification of most species because bats exiting a roost do not emit foraging calls, which are diagnostic for species identification. Acoustic surveys of roosts are particularly useful at roosts that cannot be entered safely.
- vi. Application Surveys should be conducted June September. Depending on the model of detector used (refer to Section II.A), surveys can be conducted either actively (i.e., observer is present and operating the detector) or passively (i.e., detectors record data while observer is absent). Initialize surveys ½ hour before sunset and monitor the roost entrance a minimum of 2.5 hours. Locate survey stations 30-50 m from the roost entrance to reduce risk to the surveyor and maximize quality of call sequences. If possible, repeat surveys at least twice during a season.

Record the date and time of survey, length of survey, habitat type, distance from detector to roost, number of total passes, number of passes by each species, time of first pass, and time of last pass. If actively surveying, also note whether bats are flying into or out of the roost (refer to Attachment 11).

- vii. <u>Analysis of Data</u> Convert both the total number of passes and passes by each species to passes per hour (refer to Attachment 11).
- viii. <u>Disposition of Data</u> Send a report containing roost data and acoustic detection totals to the Nongame Mammal Biologist at the WGFD Lander Regional Office (Section II.A.1.d and II.C.1.c). If possible, also include the acoustic files.

b. Roost capture surveys –

- i. <u>Rationale</u> Capture surveys are commonly done to survey for bats using a roost and to identify species, sex, and reproductive status.
- ii. Application Prior to conducting capture surveys, confirm presence of bats through use of acoustic equipment (refer to Section II.C.3.a) or by conducting an exit count (refer to Section II.C.3.b). The size of the capture device will vary depending on the opening (refer to Section II.B). Once capture devices are deployed, monitor them continuously a minimum of 2.5 hrs and remove bats as soon as possible after each capture. Use plastic polysheeting to seal portions of the portal not covered by the capture device to prevent bats from evading capture. If mist nets are used, place nets a few feet in front of the portal so the surveyor has access to both sides to remove both incoming and outgoing bats.

Record physical conditions and measurements (refer to Section II.C.2.d), date and time of survey, weather conditions, capture device locations and configurations, and bat data (refer to Section II.B.1.c; Attachment 16: Roost Capture Form). Record the total number of captures, total captures of each species, and total survey time.

- iii. <u>Analysis of Data</u> Report captures per unit effort, such as number of captures per hour of survey time (refer to Attachment 16).
- iv. <u>Disposition of Data</u> Send a report containing roost data and capture records to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d and II.C.1.c).

III. IMMOBILIZATION, HANDLING, AND MARKING -

A. <u>Immobilization</u> – Bats are small and easy to handle, therefore chemical immobilization is unnecessary.

B. Handling -

- 1. <u>Rationale</u> –Bats must be handled to collect biological samples, demographic data (e.g., age, sex), and determine reproductive status.
- 2. Application Use care when handling bats to avoid harming yourself or the bats. Bats are delicate animals and can easily suffer broken limbs, torn wing membranes, abortion, or other physical harm if not handled properly. Bats may bite thereby exposing handlers to injury or disease (review health concerns in Section VIII). Surveyors should receive training in capture techniques before conducting surveys. Handlers should also be familiar with bat anatomy. The following sections provide direction on removal of captured bats, temporary holding devices, and processing and release of captured bats. Additional information is provided by Kunz et al. (2009).
 - a. Removing bats from mist nets Upon capture, bats usually drop into the pocket of the mist net. Always remove bats from the net as soon as possible. Determine which side of net the bat entered and work from that side. Carefully grasp the bat with one gloved hand and work the net away from its body with the other. Start with the portion of the bat that entered the net last, usually the posterior end. Bats seldom need to be cut free. However, in the event a bat becomes severely entangled cut the netting to free the bat. This should only be done as a last resort.
 - b. <u>Holding devices</u> After the bat is removed from the net, place each individual in a cloth or disposable paper holding bag and securely close the bag. Use a single bag to hold each individual bat. Keep bats in a warm and safe location until you can process them.
 - c. <u>Handling captured bats</u> Remove the bat from the holding bag and restrain it gently but firmly in the palm of a gloved hand with fingers wrapped around the body. The head of the bat can be examined by allowing it to protrude from either the lateral or medial side of the hand. To examine wings, gently clasp the humerus and extend the limb.

i. Common Measurements –

- Forearm length (mm) With the wing of the bat folded, hold the shaft of the forearm between thumb and forefinger. Carefully measure from the elbow to the carpals with a digital caliper. Be sure to orient the sharp points of a caliper away from the head and body of the bat.
- <u>Pinnae length</u> (mm) Hold the end of a small, clear ruler at the base of the pinna and measure to the tip.
- <u>Tragus length</u> (mm) Measure the tragus in the same manner as pinnae.

• Weight (g) – Using a 50-60 g spring scale, weigh the holding bag containing each bat. Later subtract the weight of each empty bag.

ii. Common examinations -

- <u>Calcar assessment</u> Examine the calcar located on the posterior edge of the uropatagium (i.e., tail membrane). Report whether or not a keel is present.
- <u>Determination of sex and species</u> –Identify the sex and species
 of the bat based on criteria outlined in the Wyoming
 dichotomous key for bats (refer to Attachment 18).
- Determination of age Refer to Section IV.A.
- Determination of sex Refer to Section IV.B.
- Reproductive assessment Refer to Section IV.C.

iii. Other examinations -

- Wing damage index (WDI) Examine both wing membranes for physical damage as well as damage resulting from whitenose syndrome infection (refer to Section VIII). Refer to Attachment 12 for reporting guidelines.
- d. Releasing Captured Bats To release a bat, first be sure it is warm and not in a state of torpor. In most cases the bat will launch itself from the observer's open hand after a brief reorientation. Watch the bat fly away to ensure it doesn't drop to the ground where it is likely to be injured.
 - Record the following information from each individual captured: time of capture, weight, forearm length, pinnae length, and presence or absence of a keeled calcar. Once the species is identified, record sex, age, reproductive status, condition of wing membranes, and any additional notes. Finally, report the time the bat was released from captivity (refer to Attachment 12).
- 3. <u>Analysis of Data</u> Calculate the number of bats captured per hour of survey (refer to Attachment 12).
- 4. <u>Disposition of Data</u> Send a report with capture records and data to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d).
- C. <u>Marking</u> –Marking bats is not recommended due to their small size and susceptibility to injury (Ellison 2008, Kunz and Weise 2009).

IV. ASSESSMENT OF AGE, SEX, AND REPRODUCTIVE STATUS –

A. <u>Age</u> – It is possible to distinguish between adults and juveniles in hand. No methods are available for determining precise ages of bats. Examine the epiphyseal plate in the joints of long phalanges. Transilluminate the joint with a flashlight to reveal whether the plate has mineralized (adult) or is composed of cartilage (juvenile). The cartilaginous region of the joint will allow light to pass through (Figs. 2A and 2B; Brunet-Rossinni and Wilkinson 2009).

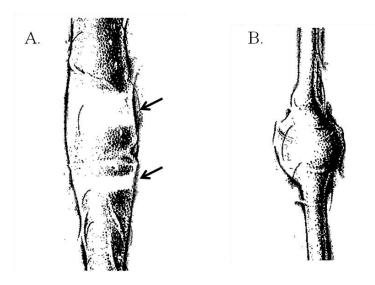


Fig. 2.A) Transilluminated joint of a long phalange in a juvenile bat. Black arrows indicate the cartilaginous epiphyseal plates. B) Transilluminated joint of a long phalange in an adult bat (Brunet-Rossinni and Wilkinson 2009).

- B. <u>Sex</u> –Sex of bats is easy to distinguish based on primary sexual characteristics. Males have a conspicuous penis at all life stages. Females have a conspicuous vulva.
- C. <u>Reproductive status</u> Although it is difficult to diagnose early pregnancy in bats, late pregnancy is easy to discern by careful palpation of the abdomen. Use extreme caution as the risk of miscarriage is high (Heideman 2000). Careful examination of the nipples can indicate whether female bats are nulliparous or parous. The nipples of nulliparous females remain tiny and display body hair, while the nipples of parous females are often larger and keratinized with short or no hair.

Since bats are seasonal breeders, the size and location of the testes in male bats can provide clues to reproductive status. The testes of most vespertilionid bats are lateral to the base of the penis and without a scrotum. The testes are covered with a layer of peritoneum, the tunica vaginalis, which is densely pigmented only in immature males (Racey 2009). In addition, testes of immature males are smaller than those of mature males. To assess whether mollosid bats are reproductively active, examine the gular gland located superior to the sternum. Gular glands in reproductive males will often be enlarged and secreting (Wilkins 1989).

V. COLLECTION OF BIOLOGICAL SAMPLES –

- A. <u>Rationale</u> Biological samples may be necessary for disease or genetic analysis and are typically obtained through a biopsy punch of one or both wing membranes. Of particular concern are the species susceptible to white-nose syndrome fungus: little brown bat; big brown bat; northern long-eared myotis; and eastern pipistrelle.
- B. <u>Application</u> Assemble the following supplies prior to performing wing punch biopsies:
 - Sterile 3.0 mm disposable biopsy punches
 - Cryovials filled with 0.75 ml of 95-100% ethanol
 - Latex gloves
 - Forceps
 - Small vial with 95-100% ethanol to flame-sterilize forceps
 - Lighter
 - Clean biopsy board and unused cards
 - Permanent marker for labeling vials
 - Cooler with ice

Place a clean biopsy card on top of the biopsy board. Place the bat ventral side up on the biopsy card with the board underneath. Carefully extend a wing and place the biopsy punch on the medial half of the wing membrane, avoiding major blood vessels, bones, and nerve fibers (Fig. 3). Press the punch firmly through membrane and twist slightly. Lift the bat off the board and locate the biopsy sample with forceps. Place tissue in a cryovial containing ethanol. If bleeding occurs, apply pressure to the wound for several minutes or until bleeding stops. Repeat this procedure on the other wing with same biopsy punch. Sterilize forceps in a flame before collecting samples from another bat.



Fig. 3. Ideal location of biopsy on wing of a bat. Note the biopsy was taken from the membrane between the blood vessels. Photo taken from AMNH (2011).

Record the date, location, and capture information (Section III.B) on the netting and acoustic capture form (Attachment 12). Be sure to label the cryovial with a unique identification number to cross-reference the biopsy sample with capture data from the same bat.

- C. <u>Analysis of Data</u> There is no data analysis associated with this type of survey.
- D. <u>Disposition of Data</u> Notify the Nongame Mammal Biologist that tissue samples are being shipped. Send samples to:

Nongame Mammal Biologist Wyoming Game and Fish Department 260 Buena Vista Dr. Lander, Wyoming, 82520

VI. EUTHANASIA –

- A. <u>Rationale</u> Bats with serious injuries or disease should be humanely euthanized. Occasionally, a biological voucher specimen may be necessary to test for rabies or WNS fungus, or may be needed to positively identify species.
- B. <u>Application</u> Only experienced personnel should euthanize bats. We recommend the following three methods: inhalants, cervical dislocation, or thoracic compression (Simmons and Voss 2009).
 - 1. <u>Liquid inhalant anesthetics:</u> <u>Isoflurane</u> Soak a cotton ball with isoflurane. Place the soaked cotton ball and holding bag containing the bat inside a heavy-duty plastic ziplock bag. Seal the plastic bag and allow sufficient time for the anesthetic gas to euthanize the bat (MIRWG 2008).
 - Cervical dislocation Euthanize small bats weighing ≤60 g by cervical dislocation. Hold the bat in one hand with the index finger across the throat and thumbnail on the back of the neck. Quickly pull backward on the hind limbs with the other hand so pressure from the thumbnail causes separation of the cervical vertebrae (Simmons and Voss 2009).
 - 3. Thoracic compression Euthanize small bats weighing ≤50 g by thoracic compression. Quickly and firmly compress the bat's chest between your thumb and forefinger. Force all air out of the lungs and maintain compression for at least 2 minutes until the heart stops beating (Simmons and Voss 2009).

Fill out a specimen tag (refer to Chapter 20.1, Attachment 2: Voucher Specimen Tag). Record notes including whether the specimen was found dead or was euthanized, the method by which it was euthanized, and a unique reference number to match any photos taken. Attach the specimen tag to the carcass. Place each carcass in its own plastic bag; close and seal the bag with tape.

If multiple specimens are processed at a time, place all individually bagged specimens inside a larger second bag and seal. Mark second bag with:

- Number of animals and species
- Date
- Location (Lat/long, UTM, County, State, etc.)
- Collector(s) (name, address, phone)

Line a hard-sided cooler with a third plastic bag and place absorbent material inside. Place enough frozen ice packs (sealed) inside the third bag to keep carcasses cold. Do not use dry ice. Seal the third bag securely. Mark package with appropriate information: "Tissue samples from dead animals; Biological Substance, Category B, UN3373".

- C. <u>Analysis of Data</u> There is no data analysis associated with this type of survey.
- D. <u>Disposition of Data</u> Notify the Nongame Mammal Biologist that specimens are being shipped. If photographs were taken, mark them with the same unique reference number assigned to the specimens. Include photos of the specimen that are not fuzzy or blurry in the package with specimens or email them to the WGFD Lander Regional Office. Send samples to:

Nongame Mammal Biologist Wyoming Game and Fish Department 260 Buena Vista Dr. Lander, Wyoming, 82520

VII. HUMAN-BAT INTERACTIONS — Over half the bat species found in the US are known to roost in or on buildings at least occasionally, however only two species are known to roost in buildings in Wyoming (Adams 2003, Kunz and Reynolds 2003). Unfortunately, this sometimes places them in conflict with humans (Fenton 2003). In many cases, owners are not bothered by or even aware of bats roosting in or on their houses and buildings (Olson 1991). Large concentrations of bats can cause odors and accumulations of guano. However many roosts are small and do not cause problems other than deposition of droppings (Brown and Berry 1991, Luce 1998, Tigner 2002). Although people are occasionally concerned about transmission of rabies and other diseases, bats pose little risk when roosting outside the living space of humans, such as in attics or on the exterior of buildings. Nonetheless, direct contact with bats should be avoided and any bites should be treated as potential rabies transmission cases (refer to Section VI for more details).

Many bats are loyal to specific roosts and studies have shown bats that are excluded from roosts in buildings often do not survive (Humphrey 1982, Neilson and Fenton 1994, Brittingham and Williams 2000). Because the vast majority of bat colonies occupying buildings do not cause problems, they should be allowed to remain in place wherever possible (Luce 1998).

A. <u>Bat Evictions and Exclusions</u> – If it is necessary to discourage bats from roosting in a building, eviction and exclusion is the most effective and permanent method (Barclay

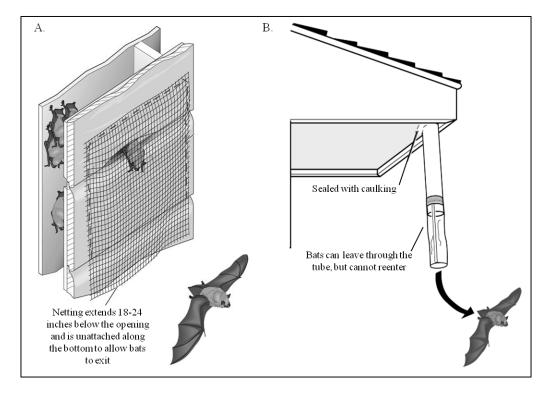
et al. 1980, Greenhall 1982, Humphrey 1982, Olson 1991). In addition, bats that are excluded from returning to a building have the opportunity to locate an alternative roost site. The Bat Conservation International website provides an excellent overview of humane bat exclusion methods (BCI 2009).

- 1. <u>Rationale</u> Bat exclusion devices are installed to physically displace bats from a structure and prevent them from returning. This is the best method to effectively and humanely rid the structure of bats and is best be done by trained personnel.
- Application If WGFD personnel become aware of a potential conflict with bats, they should contact the Nongame Mammal Biologist or encourage the owner of the structure to do so. The Nongame Mammal Biologist will provide further direction and advice as necessary. Information recorded on Attachment 17 (Batoccupied Building Form) will assist with identifying appropriate exclusion protocol.

The method of exclusion depends largely on type and construction of the structure. Avoid bat-proofing buildings while bats are present. The best time to do this is during winter, October 1 - April 1, when the bats are roosting elsewhere (Brown and Berry 1991, Luce 1998, Tigner 2002). If entrances are sealed while bats are present, they may be trapped and killed or they may seek alternative exits and inadvertently enter the building's living space (Constantine 1982, Luce 1998, Tigner 2002). Young bats unable to fly are at particular risk of mortality if exclusion occurs during the maternity period (Constantine 1982, Tigner 2002). If the exclusion must be completed while bats are present, exclude bats in April or early May before females give birth, or wait until late August when young are volant.

To complete the exclusion process while bats are present, it is necessary to establish one-way exits enabling bats to naturally leave roost at night before entry points are permanently sealed.

- Seal all possible points of entry from the roost area to the building's interior.
 Only the entry points from the exterior of building are left open. Water-based caulking, steel wool, or screening all work well to seal openings to the interior of building.
- Construct one-way exits with tubes or netting and affix them to entry points on the exterior of building (see designs in Figs. 4.A and 4.B)



<u>Fig. 4.</u> A) One-way netting affixed to an entry point on a building. The netting is attached securely on three sides, and bottom is left open enabling bats to exit but not reenter. B) A one-way exit using a smooth plastic tube with a flexible plastic sleeve attached to the end. The plastic sleeve collapses on itself and prevents bats from entering the tube from the exterior. Adapted from BCI (2009).

- Leave one-way exits in place for 5-7 days and carefully observe to ensure all bats have left the structure (BCI 2009). After all bats have exited building, remove one-way exits and permanently seal openings with caulking, steel wool, or screening. All possible entrances as small as 1 cm must be completely sealed to permanently and effectively exclude bats (Luce 1998, Olson 1991).
- Conduct a follow-up inspection to evaluate effectiveness of the exclusion measures.

Record the address of the structure and owner contact information, structure and roost data, additional comments, and any follow-up information (Attachment 17).

- 3. Analysis of Data There is no data analysis associated with this type of survey.
- 4. <u>Disposition of Data</u> Please contact the Nongame Mammal Biologist for advice before attempting to exclude bats from a structure. After inspecting the building and/or completing the exclusion process, send a report to the Nongame Mammal Biologist at the WGFD Lander Regional Office (refer to Section II.A.1.d).

VIII. <u>DISEASES</u> – Rabies and White-nose Syndrome (WNS) are among the relatively few documented diseases of bats. WNS is named after a conspicuous white fungus, *Geomyces destructans*, which invades and erodes the skin of hibernating bats. The fungus causes hibernating bats to arouse more frequently and deplete fat stores. *G. destructans* growth causes a loss of dermal integrity and disrupts skin's capability to regulate fluid balance. WNS-affected bats are known to leave hibernacula mid-day during winter presumably to forage or drink, and to roost in unusual areas of the hibernacula. *G. destructans* infection is the ultimate cause of death. However, resulting proximal causes can include starvation, dehydration, and exposure to cold temperatures (Abel and Grenier 2011, USFWS 2011). WNS is only known to affect bats and is not a known risk to humans (Abel and Grenier 2011).

Transmission of diseases from bats to humans is rare (Tuttle and Kern 1981). Only two diseases, rabies and histoplasmosis, are known to be transmissible from bats to humans. Exposure risks are easy to avoid (Keeley and Tuttle 1999). Anyone handling bats is considered at risk of contracting rabies and should receive a rabies prophylaxis immunization (CDC 2011a) prior to handling bats. Histoplasmosis is rare in northern latitudes and dry western states. Although it is possible for it to develop in environments such as warm, moist caves, it is rare in Wyoming and has only been documented in one cave (Luce 1998). As a precautionary measure, wear masks when entering caves.

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SM2 ACOUSTIC SURVEY FORM

SITE INFORMATION	ON	J.V.2 / 100	700110		•						
Site ID:	WP#:			(e.g. Place name/drainage	2):						
Property owner:	Elevation (m):		Observe	rs (circle recorder):							
Picture # (if applicable):			_								
Confirm Datum: NAD83	GPS EPE (m):										
GPS Location of Waypoint	t (UTM): Zone (circle):	12 or 13 ; E :		; N :							
SESSION INFORM	IATION										
Date (mm/dd/yy):		Time of civil suns	et (24hr):		Phase of Moon:						
Time Activated (24 hr):		Time Deactivated	d (24 hr):		Survey Length:						
Detector #: S	D Card #:	Gain:	SNR:		Height:						
Site type (Check): 🗌 Lake	/reservoir \square Pond \square] Marsh	Stream	Habitat type (Check):	☐ Grassland ☐ Riparian [☐ Aspen/Decid. ☐					
☐ Spring ☐ Flight corr	idor Cave/mine	☐ Roost;		Cliff/Cave/Canyon/Rocl	k-outcrop \Box Montane/suba	lpine forest Shrub					
☐ Other:		,		steppe ☐ Lower montane forest ☐ Pinyon-juniper ☐							
SM2 Configuration: Sketcl	h (grid cell size :	_)		Notes (Placement, veg.	species, etc. During active sur	veys at roost sites, note					
				whether bats observed	flying into or out of the roost e	ntrance):					
			ļļ								
			ļļ	 							
			<u> </u>	:							
			<u> </u>								
			ļļ	1							
			ļļ								
				1 1 1							
WEATHER DATA	Temperature	Barometric		Wind		Cloud Cover					
Survey Start:	(C)	(inH	g)	(mph)	Relative Humidity (%)	(%)					
Survey End:											
Precip in last 24 hrs? (Y/N)	NOTES:									
	,										
TOTALS											
	asses Pass/Hr	# Passes Pass	<u>/Hr</u>	# Passes Pass/Hr							
ANPA	MYCA		МҮТІ		Total Species	_					
сото	MYCI		MYV		Total # Bat Passes	_					
EPFU	MYE\		MYYU	J	Total Pass/Hour	_					
EUMA	MYLU		PIHE		-						
LABO	MYSE		NYN		-						
LACI	LANO	' —— —	TABR		_						

ACOUSTIC AND NETTING CAPTURE FORM

SITE	INFORMATIC	N											
Site II	D (R = replacement)	BLM Map	Locality (e.g., Dra	ainage, HWY,	reference	e pts):							
Roost	t Type (Cave, Mine, O	ther, n/a):	Elevation (m):					Observ	rers (full	name; c	ircle recorder)		
Prope	erty Owner:		Contact:										
Confi	rm Datum : NAD 83		GPS EPE (m):	\	NP #:								
GPS L	ocation of capture si		; Nort	hing									
SES	SION INFORM	ATION											
Date	(mm/dd/yy):		1	Time of civi	il sunset (24hr):					Phase of Moon:		
Time	Nets Open (24hr):		Time Nets Close	ed (24hr):			Total hrs	(to Qtr)	:		No. of Net Sets:		
Net C	onfiguration and Bat	Detector (BD) Plac	cements: Sketch (g	rid cell size: _		m) and	include ne	et #'s			# 2.6m:		
											# 6m:		
į					·								
											# 9m:		
									# 12m:				
									<u> </u>		# 18m:		
											Triple-high Net:		
											2.6m, 6m, 9m		
											Harp Trap:		
						<u> </u>					Total net (m):		
	at type (Check):		Riparian	☐ Asper			type (Ch					_	arsh
	Cliff/Cave/Canyon/Ro	ock-outcrop] Lower montan		ne/subalpine			Stream	□ :	Spring		Flight corridor	☐ Cave/m	nine
	Shrub steppe	i Lower montan	e Torest	□ Piliyoli	-juniper	∏ R	Other:						
	r plant/animal spp ob	served:					<u> </u>						
Dista	Distance to nearest water/ type of water:												
	WEATHER DATA	Temperatur (C)		Wind Relative Humid (mph) (%)					Cloud (
	Survey Start:	(0)		inHg)		μημ	,		(%)	<u> </u>	(70	<i>,</i>	
	Survey End:											·	
	Precin in last 24 hrs	2 (V/N)	NOTES:		<u> </u>						L		1

BAT DETECTOR SETUP

Capture Site Detector (A)		D; Detector ID:		Acoustic Site Detector (B)		:;	
UTM (Zone, Easting, Northing)	T	m E	m N	UТM	Т	m E	m N
		Time Activated				Time Activated	
Elevation (m)		Time Deactivated		Elevation (m)		Time Deactivated	
Signal-Noise Ratio (SNR)		Gain		SNR		Gain	
Height above Ground		Distance to nets		Height above Ground		Distance to Nets	
Habitat				Habitat			
Notes (Detector placement, habitat notes, etc)				Notes			

CAPTURE DATA

Bat ID Net# SPECIES (4 letter code)	TOC (24hr)	Sex (M/F)	Age (J/A)	Repro	FA (mm)	E (mm)	W	/t (g) 2 3	Keel	WDI	TOR	Notes (color, dentition, fringe, fur,
						(,	1	2 3	(y/n)		(24hr)	wing biopsy, etc)
	+											
											ļ j	I

TOC = Time of Capture; Repro = Males: N (Non-reproductive), D (descended); Females: N (non-reproductive), P (pregnant), L (lactating), PL (post-lactating); FA = Forearm Length; E = ear length; Wt = Weight in grams 1) weight of bat in bag, 2) bag weight, 3) bat weight; WDI (Reichard Wing damage index) = 0 (No damage), 1 (Light damage), 2 (Moderate damage), 3 (Heavy damage); Add "-P" to score if there is physical damage to wings without signs of splotching or necrotic tissue (0-P, 1-P, 2-P, or 3-P); TOR = Time of Release; include voucher number in notes, if collected.

Bat ID	Net#	NT 12: NE SPECIES (4 letter code)	TOC (24hr)	Sex (M/F)	Age (J/A)	Repro	FA (mm)	E (mm)	V	Vt (g 2	g) 3	Keel (y/n)	WDI	TOR (24hr)	Notes (color, dentition, fringe fur, wing biopsy etc)
									1						

TOC = Time of Capture; Repro = Males: N (Non-reproductive), D (descended); Females: N (non-reproductive), P (pregnant), L (lactating), PL (post-lactating); FA = Forearm Length; E = ear length; Wt = Weight in grams 1) weight of bat in bag, 2) bag weight, 3) bat weight; WDI (Reichard Wing damage index) = 0 (No damage), 1 (Light damage), 2 (Moderate damage), 3 (Heavy damage); Add "-P" to score if there is physical damage to wings without signs of splotching or necrotic tissue (0-P, 1-P, 2-P, or 3-P); TOR = Time of Release; include voucher number in notes, if collected.

ATTACHMENT 12: NETTING AND ACOUSTIC CAPTURE FORM

LACI

LANO

CAPTURE TOTALS M | F A | J M | F A | J M | F A | J **ANPA** _____ MYCA MYTH **Total Species** сото MYCI MYVO **Total Adults** ____ MYEV **EPFU** MYYU **Total Juveniles EUMA** ____ MYLU __ PISU **Total Males** ____ MYSE ___ NYMA **LABO Total Females** LACI ____ LANO **Total Bats** _____ TABR **ACOUSTIC TOTALS** CAPTURE SITE; SITE ID: ____ # Files Files/Hr # Files File/Hr # Files File/Hr **ANPA MYCA** _____ MYTH **Total Species** сото MYCI MYVO **Total # Bat Files** ____ **EPFU** MYEV MYYU **Total File/Hour EUMA** MYLU PIHE ____ **LABO** MYSE NYMA LACI LANO **TABR** ACOUSTIC SITE; SITE ID: _____ # Files File/Hr # Files File/Hr # Files File/Hr ANPA **MYCA** MYTH **Total Species** сото MYCI MYVO **Total # Bat Files EPFU** MYEV ____ MYYU **Total File/Hour** MYLU **EUMA** PIHE **LABO** MYSE ____ NYMA

TABR

INTERIOR ROOST SURVEY FORM

SITE INFORMATION	1				Date (r	nm/dd/yy):				
Site ID:	W	/P#:		Locality (e.g. Place name/	drainage):					
Property owner:	C	ontact:		Observers (Full name; circ	le recorder):					
Picture # (if applicable):	1									
Confirm Datum: NAD83	GPS EPE	(m):	Elev. (m):							
GPS Location of Waypoin	t (UTM): Z	one (circle)	: 12 or 13 ; E :		; N:					
Route from known location	on:									
ROOST TYPE										
☐ Mine Adit (Horizontal	opening)	□ N	l ine Shaft (Vertical	opening) Cave	☐ Other:					
GATE										
Gate Present?	□No	Describe	Gate:							
EXTERNAL FEATUR	ES									
Slope:			Aspect:		Entrance Substrate	:				
Entrance Width (m or in):			Entrance Height (m or in):	Entrance length/de	epth (m):				
Airflow? (Yes/No/Unk)			Airflow Direction	(in/out):	Airflow speed (mpl	ո)։				
Habitat description (Chec		odland; [Scrub; Car	nyon; Alpine meadow;	☐ Rocky; ☐ Agri	culture;	asture;			
INTERNAL FEATURI	ES									
Width (m):			Height:		Substrate:					
Dark zone present? (Yes/	No/Unk)		Depth to dark zor	ne (m):	Survey Length:					
Timbered Adit? (Yes/No)			Condition of Timb							
Water Present? (Yes/No)			Location and dept	th of water:						
Number of Entrances:			Previously mappe	ed? (Yes/No/Unk)	Map Location:					
HAZARDS										
Physical hazards present?	○ □ Yes	No	Describe Hazard	s:						
OBSERVED BATS										
ANPA	MYCA			BAT SIGN PRI	SENT? (circle)	Yes	No			
сото	MYCI		MYVO	_						
EPFU	MYEV			TYPE (Guano, wrappings, etc):						
EUMA	MYLU		PIHE	If no bats or bat	sign observed, evalua	te suitability fo	r bats based on			
LABO	MYSE		NYMA	habitat characte	ristics: (HIGH	LOW	NIL)			
LACI LANO TABR										

ATTACHMENT 13: INTERIOR ROOST FORM

OBSERVED BATS DATA

ODSLINALD D								T
SPECIES (4 letter code)	QTY	STATUS*	LOCATION (room or corridor)	DEPTH (m)	HEIGHT (m)	TEMP (°C)	HUMIDITY (%)	NOTES
			g) R (roosting					

* Status = T (torpid), F (flying), R (roosting and alert)								
Previously mapped? (Yes/No/Unk)	Map Location:							
INTERIOR MAP (Draw if not mapped an	d plot locations for: bats, sign, hazards, temperature, and h	umidity)						

EXTERIOR ROOST SURVEY FORM

SITE INFORMATION Date (mm/dd/yy): Site ID: WP#: Locality (e.g. Place name/drainage): Observers (Full name; circle recorder): Property owner: Contact: Picture # (if applicable): Elev. (m): Confirm Datum: NAD83 GPS EPE (m): GPS Location of Waypoint (UTM): Zone (circle): 12 or 13 ; E: ; N: Route from known location: **ROOST TYPE** \square Cave ☐ Other: ☐ Mine Adit (Horizontal opening) ☐ Mine Shaft (Vertical opening) **GATE Describe Gate: Gate Present?** ☐ Yes ☐ No **EXTERNAL FEATURES** Aspect: **Entrance Substrate:** Dark zone present? (Yes/No/Unk) Approx. depth to dark zone (m): **Survey Length:** Entrance Width (m or in): Entrance Height (m or in): **Entrance length/depth** (m): Habitat description (Check): ☐ Woodland; ☐ Scrub; ☐ Canyon; ☐ Alpine meadow; ☐ Rocky; ☐ Agriculture; ☐ Pasture; ☐ Riparian; ☐ Other: **HAZARDS** Describe Hazards: Physical hazards present? \Box Yes □No **COMMENTS**

EXIT COUNT SURVEY FORM

SITE INFORMATION	J				Date (mm/dd/yy):
Site ID:	V	VP#:		Locality (e.g. Place name/	drainage):
Property owner:	C	Contact:		Observers (Full name; circ	le recorder):
Picture # (if applicable):					
Confirm Datum: NAD83	GPS EPE	(m):	Elev. (m):		
GPS Location of Waypoin		Zone (circle)	: 12 or 13 ; E :		; N:
Route from known location	on:				
ROOST TYPE					
☐ Mine Adit (Horizontal	opening)	□м	ine Shaft (Vertical o	ppening) 🗌 Cave	☐ Other:
Entrance width (m or in):				Entrance Height (m	or in):
GATE					
Gate Present? ☐ Yes	□No	Describe	Gate:		
SURVEY INFORMAT	ΓΙΟΝ				
Time of civil sunset:		<u> </u>	C	Moon phase:	Comment and the trade
Survey start (24 hr) Distance to roost:			Survey end (24 hr):	Time of 1 st exit:	Survey Length (hr): Time of last exit:
Number of bats observed	exiting ro	oost:		Time of 1 exit.	Bats/hr:
Habitat description (Chec			Scrub; Cany	yon; Alpine meadow;	☐ Rocky; ☐ Agriculture; ☐ Pasture;
☐ Riparian; ☐ Other:					
COMMENTS					

ROOST CAPTURE FORM

SITE INFORMATION

Site II	D:			WP	#:			Locality (e	e.g. [g. Drainage):				
Prope	erty owner:		Elevation (m):		Obser	vers (circl	e recorder):						
Roost	Survey: Y	or N	Roost Type	:										
Confi	rm Datum: NA	AD83	GPS EPE (m	n):										
		l			e): 12 or 13	· F·				; N:				
GP3 L	ocation or wa	aypoint (C	Tivij. Zone	(circie	:). 12 01 13	, E.				, IN.				
ROO	ST TYPE													
□ v	line Adit (Hor	izontal op	ening)		/line Shaft (Ver	tical openi	ng) l	☐ Cave [□ R	Rock Shelter	Other:			
Gate	Present?	Yes	□No	Descri	ibe Gate:									
Slope	:				Aspect:				Eı	ntrance Substrate:				
	zone present	? (Yes/No/	'Unk)		Approx. depti	h to dark z	one (m):			urvey Length:				
	nce Width (m		,		Entrance Heig					ntrance length/dep	th (m):			
	at type (Chec	_	ff/Cave/Cany e tundra;				ŕ			forest 🗌 Montan	e/subalpine forest;			
SESS	L Aspen/Decid. L Alpine tundra; L Grassland; L Riparian Notes:													
	(mm/dd/yy):				Time of civi	l sunset (2	24hr):			Phase of Moon:				
	nets open (24	l hr):			Time nets c					Survey Length:				
			draw diagrai	m on l	back if needed)	•				, ,				
		•	· ·		•									
Descr	ibe location o	of addition	nal nets (w/ ı	numb	ers):									
	WEATHE	R	Temperatur	p	Baromet	ric	w	ind	Re	elative Humidity	Cloud Cover			
	DATA		(C)		Pressure (ii			ph)	I	(%)	(%)			
	Survey Start:	:	(6)		11033410 (11			P11)		(70)	(70)			
	Survey End:													
	Precip in last 24 hrs? (Y/N) NOTES:													
CAP	TURE TOT	ALS <u>M_ _</u> F	<u>A J</u>		<u>M F</u>	A J	<u>N</u>	<u> F </u>	J					
	ANPA		M	IYCA		M				Total Spec	cies			
										·				
	COTO EPFU			IYCI IYEV			IYVO IYYU		_	Total Adu Total Juve				
														
	EUMA			IYLU			HE		_	Total Mal				
	LABO MYSE NYM				YMA		_	Total Fem	ales					
	LACI LANO		TABR					Total Bats						

Bat ID	Net #	IN/ OUT	SPECIES (4 letter code)	TOC (24hr)	Sex (M/F)	Age (J/A)	Repro	FA (mm)	E (mm)	1	Nt (g	g) 3	Keel (y/n)	WDI	TOR (24hr)	Notes (color, dentition, fringe, fur, wing biopsy, etc)

IN/OUT = Indicate whether bat was attempting to fly into or out of roost, if net is blocking entrance; **TOC** = Time of Capture; **Repro** = Males: **N** (Non-reproductive), **D** (descended); Females: **N** (non-reproductive), **P** (pregnant), **L** (lactating), **PL** (post-lactating); **FA** = Forearm Length; **E** = ear length; **Wt** = Weight in grams 1) weight of bat in bag, 2) bag weight, 3) bat weight; **WDI** (Reichard Wing damage index) = **0** (No damage), **1** (Light damage), **2** (Moderate damage), **3** (Heavy damage); Add "-P" to score if there is physical damage to wings without signs of splotching or necrotic tissue (0-P, 1-P, 2-P, or 3-P); **TOR** = Time of Release; include voucher number in notes, if collected.

BAT-OCCUPIED BUILDING CONTACT FORM

CONTACT INFORMATION Date of 1st call: WGFD Contact: Date of 1st Visit: Name of Occupant: Phone: City/Town: Zip Code: **Street Address:** Address of occupied building (If different than above): **STRUCTURE & ROOST INFORMATION** Type of Building: ☐ Residence ☐ Commercial ☐ Abandoned ☐ Outbuilding near residence ☐ Other: **Check options:** Bats are ☐ **Entering/** ☐ **Leaving** from the ☐ **Inside/** ☐ **Outside** of the building **Comments:** Location of entry/exit point: What time of day/night are bats observed entering/leaving the building? Date bats were first observed: Do bats have access to attic? \square Yes \square No Can we get access to the attic? \square Yes \square No Comments:

FOLLOW-UP

Date of Follow-up:	Observer:
Recommendations:	
	,
☐ Completed "WGFD Bat-Occupied Building Record"	□ wof′D

DICHOTOMOUS KEY TO THE BATS OF WYOMING

The criteria listed only apply to adult animals in which the metacarpal-phalangeal joint on the right second finger is not bulbous and appears solid with no open spaces when viewed against a bright light. Revised June 2005.

1a. Tail fully within the interfemoral membrane or extending a few millimeters beyond the edge of the interfemoral

membrane (FAMILY VESPERTILIONIDAE) 1b. Approximately 50% of the tail extends beyond the trailing edge of the interfemoral membrane (FAMILY MOLLOSSIDAE) 17 **FAMILY VESPERTILIONIDAE** 2a. Black dorsal fur; conspicuous white spot on each shoulder, one white spot on rump; ears 45 to 50 mm Spotted Bat (Euderma maculatum) 2b. Lacks white spots on rump and shoulders 3 3a. At least the anterior half of the dorsal surface of the interfemoral membrane is well-furred 4 Dorsal surface of the interfemoral membrane naked or sparsely-furred 6 4a. Uniform black dorsal fur with silver tips; black face Silver-haired Bat (*Lasionycteris noctivagans*) 4b. Dorsal fur color variable but not uniformly black; face not black 5 5a. Dorsal fur dark gray and tipped with band of white (hoary appearance); forearm length 46 to 58 mm; light-colored ears distinctively edged in black Hoary Bat (Lasiurus cinereus) Red Bat (Lasiurus borealis) 5b. Dorsal fur bright reddish-orange to yellow

6a. Ear length 25 mm or more; ear color translucent or paler than pelage	7
6b. Ear length 25 mm or less; ear color variable, ranging from same as pelage to black	8

- 7a. Pale yellow-brown dorsal fur, lighter at base than tip; blunt snout; light-colored translucent ears 25 to 33 mm long; forearm 50 to 55 mm long
- Pallid Bat (*Antrozous pallidus*)
 7b. Slate gray or brown fur; prominent fleshy lumps above nose; ears 30 to 39 mm long
- 7b. Slate gray or brown fur; prominent fleshy lumps above nose; ears 30 to 39 mm long

 <u>Townsend's Big-eared Bat</u> (*Corynorhinus townsendii*)

8a. Tri-colored dorsal hairs, brown at tip and base, yellow between; forearm length 30 to 35 mm; pink forearm	
	Eastern Pipistrelle (Pipistrellus subflavus)
8b. Dorsal fur uniformly medium brown to pale brown	9

9a. Keel on calcar visible to the naked eye	10
9b. Keel on calcar absent	13

ATTACHMENT 18. DICHOTOMOUS KEY TO THE BATS OF WYOMING

10a. Wingspan 325 to 350 mm; tragus round; forearm length > 42 mm	
	Big Brown Bat (Eptesicus fuscus)
10b. Wingspan < 300 mm; forearm length < 42 mm	11

- 11a. Underside of wing furred from side of body to the elbow; wingspan 250 to 270 mm; forearm length 35 to 41 mm <u>Long-legged Myotis</u> (*Myotis volans*)
- 11b. Underside of wing not furred from side of body to the elbow
- 12a. Tail extends slightly beyond the interfemoral membrane; black mask visible; no distinct rise in the braincase profile; length of bare snout approx. 1.5 times the width across nostrils; forearm length 30 to 36 mm

Western Small-footed Myotis (Myotis ciliolabrum)

12b. Tail does not extend beyond the interfemoral membrane; black mask absent; distinct rise in the braincase profile; length of bare snout approx. equal to the width across nostrils; forearm length 32 to 35 mm

California Myotis (Myotis californicus)

13a. Distinct fringe of hair on trailing edge of interfemoral membrane visible to naked eye; ears 16 to 20 mm; forearm length 39 to 46 mm

Fringed Myotis (Myotis thysanodes)

13b. Some hairs may be present but lacks distinct fringe on trailing edge

14

12

- 14a. Ears 19 to 25 mm long; ears extend up to 7 mm beyond nose when laid forward; tragus long and thin Long-eared Myotis (Myotis evotis)
- 14b. Ears < 19 mm long
- 15a. Ears 17 to 19 mm; ears extend < 2 mm beyond nose when laid forward; tragus long, thin, pointed, and > 50% of ear height

Northern Long-eared Myotis (Myotis septentrionalis)

15a. Ears < 16 mm

16a. Ears generally darker than dorsal fur; forearm length 36 to 41 mm; usually 1 upper premolar; foot hairs usually extend past toes; pelage dark brown with silky sheen

<u>Little Brown Bat</u> (Myotis lucifugus)

16b. Ears pale and nearly same color as dorsal fur; forearm length 32 to 38 mm; always 2 upper premolars; foot hairs do not extend past toes; pelage lacks silky sheen

Yuma Myotis (Myotis yumanensis)

FAMILY MOLLOSSIDAE

- 17a. Ears connected and joined at base before reaching top of nose; forearm length 44 to 50 mm

 Big Free-tailed Bat (*Nyctinomops macrotis*)
- 17b. Ears not connected, although occasionally meeting before reaching top of nose; forearm length 36 to 46 mm <u>Brazilian Free-tailed Bat</u> (*Tadarida brasiliensis*)