# NEON Terrestrial Observation System Protocol: Small Mammal Sampling Protocol

| PREPARED BY | ORGANIZATION | DATE |
|---|---|---|
| Jessie Dulberger | TOS Consultant | March 2011 |
| Katherine Thibault | TOS | March 2012 |

| APPROVALS (Name) | ORGANIZATION | APPROVAL DATE |
|---|---|---|
| David Tazik | CCB PROJ SCI | 7/10/2012 |
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| RELEASED BY (Name) | ORGANIZATION | RELEASE DATE |
|---|---|---|
| Anne Balsley | CCB ADMIN/DCS | 7/11/2012 |



See Configuration Management System for approval history.

# Change Record

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# 1 INTRODUCTION

## 1.1 Purpose

The primary purpose of this document is to provide a change controlled version of Observatory protocols and is the version used for external review by subject-matter experts. Content changes (i.e. changes in particular tasks or safety practices) occur via this change controlled document, not through field manuals or training materials.

This document is a detailed description of the field data collection, relevant pre- and post-field tasks, and safety issues as they relate to this procedure and protocol.

## 1.2 Scope

This document relates the tasks for a specific field sampling or laboratory processing activity and directly associated activities and safety practices. This document does not describe:

- general safety practices (i.e. how to drive a boat)
- site-specific safety practices (e.g. how to safely walk in a stream)
- general maintenance (i.e. fill the car with gas)

It does identify procedure-specific safety requirements such as safe handling of small mammals or safe use of required chemicals and reagents.

## 1.3 Acknowledgements

I'd like to thank all the mammals out there for their patience, understanding, and undeniable charm.

# 2 RELATED DOCUMENTS AND ACRONYMS

## 2.1 Reference Documents

| | |
|---|---|
| RD[01] | NEON EHS Safety Policy and Program Manual |
| RD[02] | NEON Occupational Safety for Vertebrate Collection and Handling |
| RD[03] | NEON TOS Small Mammal Sampling Design Document, *in preparation* |
| RD[04] | NEON TOS Small Mammal Sampling Training Plan, *in preparation* |
| RD[05] | NEON TOS Small Mammal Sampling QA/QC Protocol, *in preparation* |
| RD[06] | NEON TOS Small Mammal-Borne Disease Protocol, *in preparation* |
| RD[07] | Methods for trapping and sampling small mammals for virologic testing, (Mills et al. 1995) |
| RD[08] | Basking in the moonlight? Effect of illumination on capture success of the endangered giant kangaroo rat (Prugh and Brashares 2010) |
| RD[09] | Guidelines of the American Society of Mammalogists for the use of wild mammals in research. (Sikes et al. 2011) |
| RD[10] | Species composition and abundance of mammalian communities (Thibault et al. 2011) |
| RD[11] | Measuring and Monitoring Biological Diversity: Standard Methods for Mammals (Biodiversity Handbook). (Wilson et al. 1996) |

## 2.2 Acronyms

| | |
|---|---|
| NEON | National Ecological Observatory Network |
| FSU | The NEON Fundamental Science Unit at Headquarters |
| TOS | The NEON Terrestrial Observation System |
| P&P | Procedure and Protocol |
| PPE | Personal Protective Equipment |
| IACUC | Institutional Animal Care and Use Committee |
| RMNP | Rocky Mountain National Park |
| SNV | Sin Nombre Virus, strain of Hantavirus |

# 3 BACKGROUND AND OBJECTIVES

## 3.1 Background

Small mammals are wide-spread, sensitive to local environmental changes, and known to carry and transmit zoonotic agents; therefore they have been chosen as sentinel taxa for the Terrestrial Ecology component of NEON. Data about small mammals (diversity, condition, demography) will be collected to better understand how environmental changes are affecting populations over time, including the occurrence of the diseases they carry. The foci of the mammal sampling efforts are the temporal dynamics in the demography, disease prevalence, structure, and composition of the small mammal community, as they relate to climate, productivity, and insect abundance.

This study is interested in both (1) the community dynamics of small mammals (i.e., shrews and rodents in the family's Cricetidae, Heteromyidae, and Sciuridae that weigh less than 500 grams), thus encompassing all species that occur at the study sites, and (2) the prevalence of mammal-borne diseases, including hantaviruses, particularly the Sin Nombre strain (SNV). While it has little if any effect on the fitness of infected rodents, SNV is an etiologic agent of Hantavirus pulmonary syndrome in humans, a condition which can cause extreme morbidity and is often fatal. The primary carrier of SNV is *Peromyscus maniculatus*, but other rodent species are also known to be carriers of this and other strains of hantavirus, a number of which are of public health concern.

The purpose of this document is to describe in detail the small mammal sampling protocol to be performed from May through October, 2012, in association with the National Ecological Observatory Network (NEON) for Domain 10. The activities described herein are intended to collect preliminary data only, and, as such, this protocol should be considered to be in draft form. Many of the methodological details presented in this protocol are intended for application only at this site and at this time. The 2012 surveys are to be conducted within the Rocky Mountain National Park (RMNP), as approved by the National Park Service Institutional Animal Care and Use Committee (IACUC). The goals of the 2012 field season are as follows:

1. derive a statistically robust baseline for small mammals within RMNP;
2. field test the proposed small mammal sampling protocol such that it can be modified, if necessary, and applied in the future within Domain 10 and at other NEON domains to be sampled in the future;
3. field test the training and QA/QC protocols for NEON technicians in the identification of small mammal species.

## 3.2 Science Requirements

This protocol fulfills the following Observatory science requirements:

| | | |
| --- | --- | --- |
| NEON.FSU.3.015 | Biodiversity | Small mammal species richness, relative abundance, and density shall be quantified using mark-recapture techniques. |
| NEON.FSU.3.041 | Biodiversity | FSU shall maintain mammal species lists and site-specific keys for identifying species. |

### 3.3 Data Products

Data products are currently being revised, but generally encompass the following areas of small mammal ecology:

- Abundance and diversity
- Mark-recapture density estimates
- *Peromyscus* demography, breeding activity
- Hantavirus prevalence in *Peromyscus*

## 4 PROTOCOL

**Trapping Summary 2012:**

- **8 trapping grids (10 x 10) distributed across the main habitat types at RMNP**
  - **Run traps for 2 consecutive nights per grid monthly from May through October**
- **22 trapping grids to assess distribution of mammal diversity and abundance in the Park**
  - **Run traps opportunistically, particularly for training purposes**
- **Trapping should coincide with the new moon, whenever possible**

NEON requires the study of live organisms in their natural setting to document the diversity, population sizes, and disease prevalence of small mammals through time, in relation to such critical drivers as climate change and land-use change. These parameters are only able to be assessed through a mark-recapture live trapping study and associated tissue collection. All methods conform to standard methods used in the study of wild small mammals (see Wilson et al. 1996, Sikes et al. 2011).

Sherman live traps (folding or non-folding) will be used to capture animals for the study. Trapping grids will be laid out with 100 Sherman live traps (15 m spacing – 10 rows – 10 columns). Eight grids will be trapped during each sampling period. In RMNP, plant biodiversity plots are distributed throughout two dominant habitat types, closed evergreen canopy and mixed open canopy forests. In 2012, grids will placed throughout these dominant habitats, as well as in each of two subordinate habitat types, riparian and shrubland habitats. An additional 22 grids have been permitted by the Park to allow for opportunistic sampling for training purposes and to characterize the distribution of small mammals to inform future sampling efforts.

Each grid will be trapped for 2 consecutive nights within a sampling period, and sampling will occur monthly at each site, starting in May, as the requisite permits are in place. If possible, trapping should occur as close as possible to the new moon, as small mammal activity is thought to be restricted under high light conditions when predation risk is presumed higher (but see Prugh and Brashares 2010). In 2012, sampling will continue through September or October, depending on weather conditions. This trapping effort attempts to maximize documented small mammal diversity in each habitat, and allow for robust estimation of (1) population sizes using standard mark-recapture techniques and (2) intra-annual changes in disease prevalence. Four personnel will conduct the sampling at each site, working in 2 teams of 2. Each team will be comprised of at least one well-trained, experienced field technician who will be responsible for all of the handling procedures. The other technician will assist in trap setting and checking and data recording, and will assist in handling only if the technician is experienced in handling small mammals and has received all necessary training (see associated Training Manual for details).

Within a sampling period, timing of the setting of traps is managed so that the last trap is set as the sun is setting and the first trap is checked the following morning as soon as it is light enough to process animals (i.e., within 30 minutes of civil twilight). The animals to be studied are nocturnal, and this routine ensures that traps are not set longer than necessary – particularly during the hottest parts of the year. Where possible, between consecutive nights of trapping, traps will remain *in situ* but closed during the day to prevent diurnal animals entering and becoming heat-stressed. Furthermore, when temperatures will be extremely high even in the early morning (i.e., >80 °F by 09:00), one team will focus solely on checking traps and placing traps with captured individuals under the protection of shade and a heat shield, until they can be processed by the second team. This procedure reduces the amount of time animals spend in direct sunlight which can result in lethal heat stress. Sherman traps are set and baited with a seed mixture (milo and millet), a high quality resource utilized by all resident small mammal species. In cold weather conditions, a ball of polyester or cotton batting for nesting will be placed in the traps at all sites, except at sites known to be dominated by rodents in the family *Heteromyidae* (e.g., Central Plains Experimental Range (CPER)). These are burrowing rodents who tend to urinate on batting, thereby reducing its insulating properties, rather than building nests, and who will not consume peanut butter. Further, at such sites, it is important to avoid high salt content bait, such as sunflower seeds and peanut butter, as this will impair these species' abilities to maintain water balance in their arid habitats. Additional, high-calorie bait (e.g., peanut butter) should also be added in the event of cold conditions, except at sites where large mammal disturbance of traps has occurred or fire ants occur (e.g., Ordway-Swisher Biological Station (OSBS)).

Upon capture, individual small mammals will be processed in one of two ways, depending on whether or not blood samples will be taken (see criteria for sampling below). All processing will take place in the field, to allow for quick release of captured individuals at the point of capture and to provide adequate ventilation for the technicians. Individuals will be transferred from the trap directly into a 4 mil, disposable plastic handling bag for processing. If blood will be collected from a particular individual, the animal will be anesthetized before any handling, to decrease stress and reduce chances for injury during the blood collection procedure (see Mammal-Borne Disease Sampling Protocol for details). If blood will not be collected, the individual will not be anesthetized prior to handling. No individual will spend more than 30-60 seconds in the plastic bag and will be in hand for only brief time spans (e.g. < 5 minutes / animal) using techniques to reduce injury, stress, and pain.

**Criteria for blood sampling:**

**DO collect blood from:**

1. All cricetids that are >10.0 grams
    - If an individual's mass might be <= 10 g, be sure to weigh the individual prior to anesthetizing
2. *Chaetodipus spp*.

**DO NOT collect blood from:**

1. Sciurids – chipmunks, squirrels, etc.
2. Soricids – shrews
3. Talpids – moles
4. Geomyids – pocket gophers
5. Dipodids – jumping mice (*Zapus spp.)*
6. Kangaroo rats – *Dipodomys spp.*

All individuals will be identified to species, reproductive condition assessed, have standard measurements taken (i.e., hind foot length, weight), be marked with either one numbered ear tag in each ear or a subcutaneous, RFID, PIT tag, have tissue samples taken, and then released at the point of capture. Additional measurements (e.g., ear length, tail length, and/or total length) may be taken when relevant to species identification. For 2012 sampling at RMNP, ear length will be measured for all *Peromyscus spp.*, while tail length will be measured for all vole species (*Phenacomys, Microtus spp.*).

Mode of tagging will be based on the length of the external pinnae, as some species, such as voles (e.g., *Microtus spp., Myodes spp.*) and pocket mice (e.g., *Chaetodipus spp., Perognathus spp.*), do not have sufficient pinnae for securing ear tags. Shrews will not be marked. Individuals are marked to allow us to assess and study population dynamics, reproductive condition, timing of colonization of new individuals, longevity and movement, and a variety of other aspects of life history that require permanent individual marking. PIT tags are injected under the skin on the back of the animal. This placement does not impede movement, and tags do not migrate from this location. All needles and tags will be sterilized prior to use. Disposable needles with be deposited in a sharps container immediately. In rare cases, if PIT tagging is deemed undesirable considering the condition of an animal (e.g., highly stressed), an animal may be released without tagging. Ear tags are small, metal, and uniquely numbered. Ear tags do not cause unnecessary pain when applied quickly and with sharp and correct application (using provided tool and placed in cartilage at base of external pinnae). Tissue samples to be collected from all individuals will include two ear tissue samples (one from each ear) using a sterile 2000 Micron (2 mm) tissue biopsy punch, and clipped toenails and hair for isotopic analyses to assess changes in diet and metabolic condition.

**Summary of procedures per individual - 2012:**

- Identify to species
- **If bleeding, anesthetize and collect blood samples first**
- Assess reproductive condition
- Measure:
    - hind foot length – for all species
    - ear length – for *Peromyscus spp.*
    - tail length – for voles
    - total length –if needed for species ID
    - weight – for all species
- Mark individual
    - An ear tag in each ear OR
    - A subcutaneously implanted RFID tag
- Collect samples:
    - Fecal
    - Toenail
    - Hair
    - Ear punches

## 5 QUALITY ASSURANCE AND QUALITY CONTROL

The QA/QC plan is in development, and all details will be found in the associated document.
The plan will include:

- Double-entry of all data
- Hot checks in the field by HQ staff or contractor
- DNA bar-coding of subset of samples to quantify error rates in taxonomic IDs

## 6 DECISION TREE

| Delay | Action | Adverse Outcome | Outcome for Data Products |
|---|---|---|---|
| **Hours** | If traps are set, the traps must be checked and any captured individuals processed or released without processing AS SOON AS POSSIBLE. Do whatever it takes to prevent mass die-off of our study animals. | Death of study animals violates the scientific and ethical integrity of our protocol. | Trapping-induced mortality violates the assumptions of the mark-recapture models that are used to estimate density. |
| **1-10 days** | Add additional days of sampling as soon as possible to sample all points. | If additional time is not available, fewer samples will be collected. | Fewer sampling points could result in less precise estimation of richness, diversity, or density. |
| **11 or more days** | Do not sample. Resume sampling as scheduled during the next month. | Miss target sampling window. | 1. Species richness or demography due to changes in seasonal phenology could be influenced by significant changes in temporal sampling window. 2. Not completing all plots impacts diversity metrics and target sample size. |

## 7 SAFETY

Personnel working at a NEON site shall be familiar with and practice safe field work as outlined in the EHS Safety Policy and Program Manual. Small mammals are reservoirs for the Sin Nombre virus (SNV) and plague, both of which occur in Colorado. Therefore, there is potential that field technicians handling small mammals may be exposed to these and other zoonotic diseases. To mitigate these risks, field technicians shall also follow the safety procedures outlined in the NEON Occupational Safety for Vertebrate Collection and Handling. The Field Operations Manager and the Lead Field Technician have primary authority to stop work activities based on unsafe field conditions; however, all employees have the responsibility and right to stop their work in unsafe conditions.

# 8 PERSONNEL REQUIREMENTS

**Trapping:**
Technicians responsible for setting and checking traps must be willing and able to carry traps in bundles up to 50 pounds and wear the mandatory personal protective equipment (PPE) described in the NEON Occupational Safety for Vertebrate Collection and Handling. Technicians who are responsible for checking traps but not handling captured animals must also be willing and able to (1) record data in neat, legible hand-writing, and (2) assist the mammal handler in any way (e.g., preparing tags for marking, handling or labeling blood samples after collection).

**Handling:**
The technicians employed to handle and process the small mammals must have prior experience handling wild small mammals or receive sufficient training prior to sampling, and must be able to correctly identify and process all small mammals potentially found within the domain. An identification key based on external features, and summary description of species expected in the study area, will be available for technicians in the field. Also, all personnel shall thoroughly review the Guidelines of the American Society of Mammalogists for the use of wild mammals in research (*Sikes et al. 2011*) prior to field sampling.

# 9 TRAINING REQUIREMENTS

The NEON training plan associated with these activities is under development and TBD.

The training plan for 2012 will minimally include the following components:

1. A half-day workshop will be conducted prior to the field sampling to provide an overview of the procedure and the goals of the sampling. Hands-on work with the sampling equipment and review of the small mammal species of RMNP will be included.
2. NEON HQ staff or contractor will conduct hands-on training in the field throughout the first sampling bout. NEON HQ staff or contractor will perform hot checks of species ID on the NEON technicians involved in the data collection.

# 10 FIELD STANDARD OPERATING PROCEDURE

## 10.1 Sampling Frequency and Timing

Four technicians working in pairs will sample each grid monthly, from May 2012-October 2012, for two consecutive nights per grid. Sampling bouts should occur as close as possible to the new moon. In the difficult terrain that characterizes RMNP, it is recommended that only one set of grids (i.e., 4 grids) be trapped on any given night of trapping during each bout. This effort of 400 traps is likely to yield about 40 individuals per day. This should be a logistically reasonable number of individuals for 4 technicians to process in a safe and timely fashion. [This estimate is based on a meta-analysis of several hundred studies of small mammal communities (Thibault et al. 2011 *Ecology*) which revealed an average capture rate of about 10%]. If capture rates prove to be higher at any of our study sites, we will reduce sampling accordingly to allow for efficient handling of all captured individuals. If this schedule is followed, each sampling bout of the core 8 grids should take 4 nights and 5 days. Sampling of the remaining 22 grids

should be done opportunistically, as frequently as time allows (at the discretion of the Field Operations Domain Manager).

**Table 1. The approximate sample dates for small mammal sampling at RMNP, 2012**

| Date of New Moon | Range of Possible Sampling Dates |
|---|---|
| May 20 | May 13 - May 27 |
| June 19 | June 11 - June 25 |
| July 18 | July 9 - July 23 |
| August 17 | August 12 - August 25 |
| September 15 | Sept 9 - Sept 23 |
| October 14 | Oct 7 - 21 |

### 10.2 Contingent decisions

Small mammal sampling can be performed under a variety of weather conditions. Care must be taken, however, to prevent conditions such that the trapped individual cannot thermoregulate properly, either in hot or cold conditions. Such conditions will result in the death of the trapped individuals.

- **Cold conditions:**
  - Traps shall not be set if there is a blanket of snow on the ground, or if nighttime lows are expected to reach freezing temperatures AND there is a significant chance of rain or snow for the site (i.e., >25%).
  - If freezing temperatures are expected, but no precipitation:
    - placed a ball of bedding material approximately 1.5” x 1.5” and a 1 inch ball of unsalted peanut butter in the back of the trap.
    - Bait with 2 tbsp seed from lumbar bag. Toss seed into trap such that it is evenly distributed from the front edge of the trap back.
  - If low temperatures (32 – 50 degrees F):
    - placed a ball of bedding material approximately 1.5” x 1.5” at the back of the trap
    - Bait with 2 tbsp of seed from lumbar bag. Toss seed into trap such that it is evenly distributed from the front edge of the trap back.
  - If low temperatures > 45 degrees F:
    - Bait with approximately 1 tablespoon of seed from lumbar bag. Toss seed into trap such that it is evenly distributed from the front edge of the trap back.

- **Hot conditions:**

  When ambient temperatures are expected to exceed 80 degrees Fahrenheit by 10 a.m.:

  1. extra effort must be made to ensure that all traps containing captured individuals are processed or placed in the shade as soon as possible.
  2. If you are unable to begin checking small mammal traps prior to 8 - 9 am, field technicians should open all traps to release animals and avoid heat-induced mortality. Work should start over that night by resetting the traps.

- **All conditions:**
  If there is any chance (i.e., >0%) that traps cannot be checked the following morning at dawn, traps shall not be set. For example, if trap locations are accessed via dirt roads that become impassable when wet, do not set traps if there is a possibility of rain overnight.

- **Predators:**
  If predators destroy > 2 traps on a single grid on any given night, traps should be removed from the site and that sampling bout terminated prematurely. Consult with FSU staff scientist on future tactics to avoid predator damage.

- **Shrews:**
  If > 3 dead shrews are found in the traps on a single grid on any given night, traps should be removed from the site and that sampling bout terminated prematurely. Consult with FSU staff scientist on future tactics to avoid shrew capture.

## 10.3 Field Procedure

### 10.3.1 Plot Location

Sampling will occur throughout the site. FSU is responsible for providing plot locations.

### 10.3.2 Plot Establishment

1. Locate provided plot locations using a GPS and study area maps.

2. Mark capture stations as permitted by RMNP, preferably with pin flags marked with grid coordinate.

   a. NOTE: When permanent plot locations are determined in future years, permanent markers will be used where permitted.

**Table 2. The grid coordinate system.**

| ↑ NORTH | A | B | C | D | E | F | G | H | I | J |
|---|---|---|---|---|---|---|---|---|---|---|
| **1** | A1 | B1 | C1 | D1 | E1 | F1 | G1 | H1 | I1 | J1 |
| **2** | A2 | B2 | C2 | D2 | E2 | F2 | G2 | H2 | I2 | J2 |
| **3** | A3 | B3 | C3 | D3 | E3 | F3 | G3 | H3 | I3 | J3 |
| **4** | A4 | B4 | C4 | D4 | E4 | F4 | G4 | H4 | I4 | J4 |
| **5** | A5 | B5 | C5 | D5 | E5 | F5 | G5 | H5 | I5 | J5 |
| **6** | A6 | B6 | C6 | D6 | E6 | F6 | G6 | H6 | I6 | J6 |
| **7** | A7 | B7 | C7 | D7 | E7 | F7 | G7 | H7 | I7 | J7 |
| **8** | A8 | B8 | C8 | D8 | E8 | F8 | G8 | H8 | I8 | J8 |
| **9** | A9 | B9 | C9 | D9 | E9 | F9 | G9 | H9 | I9 | J9 |
| **10** | A10 | B10 | C10 | D10 | E10 | F10 | G10 | H10 | I10 | J10 |

### 10.3.3 Equipment and Materials

A significant amount of specialized equipment is required to conduct surveys of small mammals. Therefore all field personnel must be familiar and comfortable with using all the equipment before heading into the field.

#### 10.3.3.1 General equipment

1. GPS
2. Compass
3. Necessary permits

#### 10.3.3.2 Mammal Population Sampling Equipment

| Item | General Purpose |
|---|---|
| 4 oz. amber glass bottle with dropper in lid | Anesthesia/Euthanasia |
| Cotton balls, sterile | Anesthesia/Euthanasia |
| Isoflurane, halogenated anesthesia | Anesthesia/Euthanasia |
| Spoon, tea infuser, spring loaded, stainless steel | Anesthesia/Euthanasia |
| Bottle, spray, 480ml, PE bottle, PP spray | Cleaning traps |
| Brush, scrub, bar brush | Cleaning traps |
| Brush, toilet bowl, stiff-bristled | Cleaning traps |
| Bucket, 5 gal | Cleaning traps |
| Carboy, 20L, PP, with spigot | Cleaning - quat preparation |
| Glove, heavy duty, rubber, cotton lining | Cleaning traps |
| Plastic graduated cylinder, 25ml, PP | Cleaning - quat preparation |
| Quatcleaner disinfectant, concentrate 1:256 | Cleaning - quat preparation |
| Instrument sterilization tray | Handling – sterilizing between individuals |
| Large tray (approx. 18” x 18”) with sterilizable surface | Handling surface |
| 4 mil, gallon size 'ziplocs' | Handling |
| Sugar packets, small mammals | Handling - animal care for stress |
| Warmers, small mammals | Handling - animal care for cold stress |
| Styptic powder | Handling - animal care for open wounds |
| Ruler, 12", plastic, rigid, transparent | Handling - body and tail measurements |
| Paper towel, all purpose, roll | Handling - cleaning between individuals |
| Paper, all weather copier | Handling - data entry |
| Pencil, #3 lead | Handling - data entry |
| Sharps container, portable, slip top, 1 qt, red | Handling - disposal of PIT tag needles |
| Digital Camera | Handling - documenting species IDs |
| Ruler, 6inch, plastic, flexible, clear | Handling - hind foot and ear measurements |
| Multi-tool with needle-nose pliers, knife, and scissors | Handling - marking (for manipulating ear tags when necessary) |
| Self-piercing, small animal ear tag - monel - 1/2 inch long; 1/8 inches wide; laser-etched- NEON on one side, 4 digit number on the other | Handling - individual marking (one per ear) |

| | |
|---|---|
| Ear tag applicator | Handling - individual marking (one per technician) |
| Spring scale, tare-able, 30 grams max | Handling - mass measurements |
| Spring scale, tare-able, 100 grams max | Handling - mass measurements |
| Spring scale, tare-able, 1000 grams max | Handling - mass measurements |
| Scissor-style biopsy punch, 2000 micron | Handling - sample collection |
| Clipper, toenail, small | Handling - sample collection |
| Dissecting forceps | Handling – fecal sample collection |
| Standard dressing forceps, 9cm, straight | Handling – hair plucking |
| Tube, glass sample vial with rubber-lined caps | Handling - sample collection |
| Pen, marker, black, permanent, ethanol safe | Handling - sample collection - marking vials |
| Pre-printed labels on ethanol safe paper | Handling – sample vial labeling |
| Tube, microcentrifuge, 1.5ml, with attached caps | Handling - sample collection - storage - toenails |
| Ethanol, 95% Reagant grade | Handling - sample collection - storage - fecal samples |
| 1.2 mL cryogenic storage vials | Handling - sample collection - storage - fecal samples and ear punches in ethanol; hair samples dry |
| Pre-printed cryovial labels | Handling – sample vial labeling |
| Hand lens, 10x, 7/16". Coddington type double lens magnifier | Handling - species IDs |
| Biohazard bags and sealing tape | Handling – waste disposal |
| Field guide, mammals | Handling - reference material |
| Bait, mixed sterilized seed (no sunflower seeds) - white millet and(milo or rolled oats) | Trapping |
| Small mammal traps | Trapping |
| Plastic bags (e.g., Uline 10 x 16” 2 mil bags on a roll) | Trapping – trap transport for processing |
| Tree planting bags | Trapping – carrying traps |
| lumbar bags (or similar, i.e., fanny packs) | Trapping - bait carrying |
| cotton or synthetic batting - by the yard | Trapping - nesting material in cold temps |
| Peanut butter | Trapping - bait supplement in cold temps |
| Wax paper | Trapping - bait supplement in cold temps |
| Wet erase markers | Trapping – trap numbering |
| Non-mechanical pencils | Trapping – trap numbering |
| Archival quality specimen tag (100% rag paper, 110 lb. weight) with strong, white, cotton, mercerized thread, size 10-12 | Trapping – labeling voucher specimens prior to long-term archiving |

#### 10.3.3.3 Safety equipment

See the NEON Occupational Safety for Vertebrate Collection and Handling for list of safety-related equipment.

#### 10.3.3.4 Mammal-borne Disease sampling equipment

See the Mammal-Borne Diseases Sampling Protocol for list of disease sampling equipment.

### 10.3.4 Preparation

#### 10.3.4.1 Pre-field Preparation

The following steps should be completed by field technicians before each sampling session begins.

1. Make sure all traps and sampling equipment are functioning and sanitized.
2. Check functionality and cleanliness of all safety gear.
3. Prepare labels on ethanol safe paper with Site ID and year, and space for writing Rodent ID and MO/DAY:

| RMNP 2012<br>MO/DAY<br>TAG ID |
| --- |

4. Field technicians should be prepared to identify all small mammal species in the area.
5. Prepare a small vial of 10% sugar in water. This is used to revitalize stressed, hypothermic or heat-stressed captures. A clean eye drop bottle works well to administer the sugar solution. Always carry the bottle and remember to change the solution often.
6. Prepare a small vial of anesthesia (isoflurane) to carry into the field, for blood sample collection (see Mammal-borne disease protocol for details), if planned, and in the event that an animal needs to be euthanized due to a serious trapping-caused injury. Perform the transfer in a fume hood or well-ventilated location. Wear gloves whenever handling the anesthesia.
7. Fill 100 cryovials with ethanol for the collection of fecal samples and ear punches.
8. Make sure a sufficient quantity of dry ice is on hand for preservation of blood samples, if applicable.
9. Sterilize a sufficient amount of white millet, and mix with equal parts rolled oats or sterilized milo to prepare trap bait.
    a. Sterilization requires baking in a thin layer on a baking sheet for 30 minutes at 300°F.
10. If blood samples will be taken, prepare a bleeding history record per individual tag ID, to be able to determine in the field whether or not a previously marked individual should have blood samples collected, based on the criteria established in the Mammal-borne Diseases protocol.

#### 10.3.4.2 Additional preparation requirements

Scientific collecting permits have been obtained from the Colorado Division of Wildlife and the U.S Fish and Wildlife Service. Permission to conduct research on live vertebrates is also obtained from the affiliated Institutional Animal Care and Use Committee (IACUC). The IACUC must approve the research protocols for animal capture and handling. Specific study sites, such as RMNP, often require permits to conduct research on the property; be sure to carry these at all times.

**State Permit requirements**

1) Sampling of Blood is only authorized for animals greater than 8 grams in weight and less than 500 grams in weight.
2) All animals are to be released immediately after processing is completed (3 hours maximum holding at capture site).
3) In the unlikely event of a Preble's Meadow Jumping Mouse capture, the mouse must be released immediately. EHS must be notified within 1 business days. In the further unlikely event of a mortality, work with EHS to determine the disposition of the animal and notification is required immediately.
5) The following subpermittees are authorized: Kate Thibault, Kali Blevins, Tracey Baldwin, Nick Schroeter, David Durden, Sean Hauser, Kevin Barrett, Isabel Gottlieb, Scott Severs, Jessie Dulberger, Carron Meaney.
6) Contacts for required pre-collection notifications:
Larimer County (RMNP) is AWM Larry Rogstad, (larry.rogstad@state.co.us; 970 472-4461); DWM Rick Spowart (rick.spowart@state.co.us)

Collection Manner and Location:
The use of Sherman live traps is authorized.

Standard Stipulations:
1) PRINT A COPY OF THE LICENSE AND CARRY IT WITH YOU IN THE FIELD.
2) As a condition of this license, and prior to field work, the license holder must contact the Area Wildlife Manager (AWM) and District Wildlife Manager (DWM) in the wildlife office(s) nearest the locality(ies) of the field work. (SEE Regulation No. 1316 B.2.)
3) Salvage is authorized for inadvertent kills and/or dead or moribund non-T&E specimens found in the field.

Rocky Mountain National Park permit stipulations must be followed.

### 10.3.5 Sample Collection in the Field

#### 10.3.5.1 Basic Small Mammal Trapping Protocol Overview

##### 10.3.5.1.1 Trap Setting on First Night of Sampling Bout

1. Time sampling so that the last trap is being set as the sun is setting. Avoid setting traps > 2.5 – 3 hours before sunset, as this will increase the possibility of catching non-target species such as chipmunks and ground squirrels.

2. At each grid station, place one Sherman trap.

    a. Adjust treadle **very carefully** (it will break otherwise) to reach the setting required to close the trap by tapping firmly with at least two fingers on the top of the trap.

3. Toss bait into trap.
    a. If freezing temperatures are expected, placed a ball of bedding material approximately 1.5" x 1.5" and a 1 inch ball of unsalted peanut butter mixed with seed and/or rolled oats in the back of the trap. Proceed with baiting with seed, as normal.
    b. Grab approximately 1 tablespoon (or more if nighttime temperatures will be < 50 degrees Fahrenheit) of seed from lumbar bag. Toss seed into trap such that it is evenly distributed from the front edge of the trap back.

#### 10.3.5.1.2 Trap checking

1. Two technicians work as a pair to check traps the following morning at dawn (i.e., within 30 minutes of civil twilight).

2. Be sure to wear PPE as specified in the NEON Occupational Safety for Vertebrate Collection and Handling when checking traps and handling animals.

3. Check all traps in the sampling grid.

    a. If the door of the trap is closed, DO NOT PEEK inside. Many animals can escape surprisingly quickly.

    b. If the door of the trap is closed, mark trap with grid coordinate.

        i. Suggested method of marking: wet erase marker

        ii. Alternative marking method when wet: pencil

    c. Place trap in plastic bag to prevent disease transmission, and then transport bagged traps with captures back to processing station for the grid.

    d. If it is:

        i. The last morning of sampling, remove and box all traps from the grid.

        ii. Not the last day of sampling, close empty traps for the day.

    e. Check all traps in the grid before processing captures, so that all traps with captures can be placed in the shade in a timely fashion.

4. Sample animals for population and disease data, as directed below.

5. Once processed and awake (if anesthesia has been used for disease sampling), return the individual to its respective trap and transport the trap to release the individual at the site of capture while wearing all PPE.

6. For each trapping station from which a trap has been removed due to animal capture, replace that trap with a sanitized new trap. Bring used trap back to the lab for cleaning.

7. When traps are not in use during the day, keep them closed.
8. Reset and re-bait all traps the following dusk.

#### 10.3.5.2 Individual data collection

The following field methods are written in the order they should be completed for population and, if directed for the site, disease sampling. Species that do not need to be sampled for diseases do not need to be anesthetized (see Section 4 for bleeding criteria for species). Blood samples should be collected according to the sample sizes and guidelines laid out in the Mammal-Borne Diseases Sampling Protocol.

1. All sampling is performed by two technicians, one data recorder and one animal handler.
2. Set up all processing equipment in a small tray filled with quat, to sterilize instruments between individuals.
3. Record trap number (i.e., grid coordinate).
4. The handler should very carefully peek inside the trap to assess whether the animal should be handled or not. Without attempting to handle, immediately release any squirrels (including chipmunks), birds, and reptiles (possible, but extremely unlikely).
5. If the capture is of a target small mammal species, place 4 mil, gallon-sized 'ziploc' bag over the end of the trap that opens, and turn trap over to deposit capture in bag.
    a. If Sherman traps are of the folding variety, be sure to place the bag over the end without the pin sticking out to avoid snagging.
6. Place bag on the handling surface, and pin the animal in the bag behind the neck.
7. Immediately observe the animal for signs of stress, and continue to do so throughout the handling period.
    a. In the event that the animal shows signs of stress at any time:

    If the animal appears dead, check for rigor and for respiration within the bag. If the animal is clearly dead, collect the animal as a voucher specimen (see section 15.). If there is no rigor and the animal may be overheated or dehydrated, wet the belly and administer sugar water via dropper. If the animal appears hypothermic, place the animal in a secure container containing a disposable hand warmer, batting and bait. Check the animal every 15 minutes if possible, and release when the animal exhibits normal behavior.
8. Check capture for existing marking (ear tags or RFID tag).
    a. If the animal is both unmarked and meets the species-, size-, and condition-criteria described in the Mammal-Borne Diseases Sampling Protocol, continue to the instructions provided in that protocol. Once the blood sampling is complete, proceed with the following steps of this protocol.
    b. If the animal is marked, check the bleeding history list prepared as part of the Pre-field Preparation, and proceed with bleeding as described in the Mammal-Borne Diseases Sampling Protocol if individual has not been bled previously.

c. If the animal does not meet the criteria for bleeding, remove the non-anesthetized individual from the handling bag, and proceed with the following steps of this protocol.

d. NOTE: all steps of the following protocol should be followed for all individuals, including recaptures. Repeated measurements of the same individual are valuable.

9. If needed, mark the individual using ear tags (one per ear) or PIT (=RFID) tag.

    a. Mode of tagging will be based on the length of the external pinnae, as some species, such as voles (e.g., *Microtus spp., Myodes spp.*) and pocket mice (e.g., *Chaetodipus spp., Perognathus spp.*), do not have sufficient pinnae for securing ear tags. Shrews will not be marked.

    b. Individual IDS will be of the form LXXXXRXXXX, with L and R designating the left and right ears, respectively, and the Xs the unique tag numbers, when ear tags are used. If using a PIT tag, place one of the bar code stickers on the back of the datasheet., and record the LAST 10 digits of the serial number in the tag spaces provided on the front of the datasheet. Be sure to indicate on the datasheet that the tag is new.

    c. Once the individual is successfully marked, label all sample vials with the unique ear tag number from the right ear (of the form RXXXX) or the PIT tag number.

10. Note the age of the animal:

    a. juvenile (J)

    b. sub-adult (S)

    c. adult (A)

11. Note the sex and reproductive condition of the animal.

    a. Males

        i. Scrotal (S): testes may be descended (obvious bulging under the tail)

        ii. Non-scrotal (N): testes not descended (abdominal)

    b. Females

        i. Vagina:

            1. Swollen (S) – indicates estrous

            2. Plugged (P) (some use the term Perforate)

            3. Neither (N) - (some use the term Non-perforate)

        ii. Nipples

            1. Enlarged (E)

            2. Not enlarged (N)

12. Take standard measurements:

    a. Right hind foot: Using the 6 inch flexible ruler, measure the distance from the back of the heel to the end of the longest claw (beyond the fleshy toe).

b. Other measurements to be taken if useful for species discrimination (e.g., ear length for *Peromyscus spp.*, tail length for voles):

i. Ear: Insert the end of the ruler in the notch at the base of the ear and measure the maximum length to the distal portion of the pinna (medial aspect), excluding hairs that project beyond the fleshy portion.

ii. Tail length: Pin the animal onto the handling surface, belly down. Bend the tail up at a right angle. Use the 6 inch ruler to measure from the bend on the back at the base of the tail to the tip of the fleshy part of the tail, excluding projecting hairs.

iii. Total length:

1. If anesthetized: Place the animal flat on its back on the 12 inch rigid plastic ruler and hold it so that the body and tail are straight and taut, but not stretched. Measure the distance from the tip of the nose to the tip of the fleshy part of the tail, excluding any hairs which project beyond the tip.

2. If not anesthetized: Place animal, belly down, on the 12 inch rigid plastic ruler and hold it so that the body and tail are straight and taut, but not stretched. Measure the distance from the tip of the nose to the tip of the fleshy part of the tail, excluding any hairs which project beyond the tip.

13. Collect samples:

a. Use nail clippers or sharp cuticle scissors to clip off 1-2 mm of toenail from one front foot and one hind foot of the first 15 individuals of each species. Place toenails in 1.5 ml microcentrifuge tube, and label with tag ID (RXXX or PIT tag – last 6 digits) and date. Use pre-printed labels, if available.

b. Collect any fresh, uncontaminated feces from the animal using either forceps or scooping the sample directly with the cryovial. Put on dry ice until you have returned to the lab. Only collect fecal samples from up to 20 individuals per species per sampling bout. Use pre-printed cryovial labels, if available.

c. Collect two ear tissue samples – one from near the edge of each ear - using a scissor-style 2000 Micron (2 mm) tissue biopsy punch, and place in cryovial containing ethanol. Use pre-printed labels on ethanol-safe paper, if available. Dunk biopsy punch in quat bath to sterilize and to dislodge any remaining tissue.

14. Record the weight in grams using the appropriate, tared spring scale.

15. Make sure all samples are put in cooler with dry ice, labeled correctly, and entered on the datasheet.

16. Continue to the clean up protocol, before processing the next trap, so no contamination occurs.

### 10.3.6 Sample Preservation

#### 10.3.6.1 Sample storage, labeling, disposition

**Table 3. Sample collection and storage specifications.**

| Sample | Storage container | Label | Field storage | Long-term storage |
|---|---|---|---|---|
| Toenail | vial rated to 25°C | Label rated to 25°C | Ambient | 25°C |
| Fecal | vial rated to -80°C | Label rated to -80°C | Dry ice | -80°C |
| Hair | vial rated to -20°C | Label rated to -20°C | Dry ice | -20°C |
| Ear punches | 95% ethanol-filled vial | Ethanol-safe | Ethanol; ambient | Ethanol; 25°C or dried |

### 10.4 Voucher Specimens

- If the carcass is to be retained, label a specimen tag with the animal ID number, sex, species, and date of capture.
- Tags should be made of 100% rag paper of about 110 lb. weight. They should be attached using strong, white, cotton, mercerized thread, size 10-12.
- Data on tags should be written with archival quality, permanent black India ink (e.g., a Pigma pen) or, if this is not available, a hard lead pencil may be used.
- Tags may be inscribed and threads looped once, prior to beginning processing
- Securely affix the tag to the right hind foot above the ankle. Since long threads tend to become tangled during storage, tie the tag as close to the leg as possible.
- Place the animal in a gallon 'ziploc' bag and immediately place in the cooler. Avoid placing directly on dry ice.

Note: If carcasses are not saved for voucher specimens, they should be sprayed with disinfectant, placed in double biohazard bags, and disposed of in accordance with hazardous waste biohazard removal procedures.

#### 10.4.1 Sample Shipping

- *Provide detail on shipping specifics (e.g. wrap the sample containers in a plastic bag, seal the top. Place containers upright in cooler labeled "Elephant Samples", include four reusable ice packs. Seal the container.*
- *In addition to the shipping label, the following hazmat labels are required:*
- *Check with EHS for label requirements*

The fate of the above samples and specimens is contingent on negotiations with natural history institutions that have not yet taken place. In 2012, all samples shall be provided to the FSU staff scientist for interim storage.

#### 10.4.2 Data Handling

At the end of each field day, all information from field data sheets must be scanned, typed up and saved to the NEON server as directed by the Field Operations Manager. Data should be entered into the spreadsheet provided by the FSU staff scientist. New data should be entered twice, and the versions compared for accuracy before adding to the existing spreadsheet. Data should be made available to the FSU staff scientist within a week of collection.

### 10.4.3 Refreshing the Field Sampling Kit

Prior to sampling make sure that all items are not only restocked, but functional and clean. Please see the section 10.3.3 for full equipment list.

### 10.4.4 Equipment Maintenance, Cleaning, and Storage

#### 10.4.4.1 Field Clean Up

Cleanup should be done in the field to avoid contamination of laboratory facilities. If conditions are not conducive for this then transport potentially contaminated equipment back to the lab in one or more biohazard bags and clean them there.

##### 10.4.4.1.1 In between processing of individuals:

1. When an animal has been processed and returned to its trap, place all contaminated paper towels, plastic bags, gauze, and cotton in the biohazard bag. Place all used instruments in the tray filled with quat. Dry with paper towel before use, if necessary. Use quat and paper towels to carefully and thoroughly clean the larger reusable equipment (e.g., insulated cooler, squirt bottle containing quat).
2. Always spray contaminated areas of clothing with quat as soon as possible.

##### 10.4.4.1.2 At the end of processing a grid:

1. Should there be any mortality, process these individuals after all the live animals have been processed. After all dead rodents are sampled, follow the instructions above for the preparation of voucher specimens (section 10.3.6.2). Transport dead animals back to the Domain Support Facility as soon as possible, and place in standard freezer until the disposition of the specimen is determined.
2. Close and seal the biohazard bag with autoclave tape.

##### 10.4.4.1.3 At the end of a sampling day:

1. Dirty traps should not be reused until they have been cleaned. All traps, clean and dirty, should be cleaned minimally at the end of each sampling bout. Cleaning should follow the guidelines provided in the NEON Occupational Safety for Vertebrate Collection and Handling.

NOTE: Modern molecular techniques, such as polymerase chain reaction, are so sensitive that a minute amount of viral or host nucleic acid can result in false-positive results if carried over to subsequent samples.

#### 10.4.4.2 Equipment maintenance

All equipment, including traps and safety equipment, should be checked for cleanliness and function prior to each sampling bout, and fixed before heading to the field. Extra equipment and tools should be brought into the field in case of equipment failure.

## 11 LAB STANDARD OPERATING PROCEDURE

*Write the lab procedures as if a PDA were not available. Use the same sections as the field protocol, above.*

*Separate the lab procedures into multiple sections and add on to the above title. For example:*
*11 Lab Standard Operating Procedure - Plant identification and drying*
*12 Lab Standard Operating Procedure - Plant mounting*

### 11.1 Timing

*Provide details on preferred timing of sample processing at the domain labs AND the maximum time between field collection and lab processing If the procedure involves multiple sampling events, include the sampling frequency and timing for each measurement. You may wish to summarize in a table.*

### 11.2 Lab Procedure

#### 11.2.1 Equipment and Materials

#### 11.2.2 Preparation

#### 11.2.3 Sample Processing in the Lab

After the field work is complete, the following steps must be performed to process samples in the lab:

1. Double check that all samples are properly labeled.
2. Fecal samples should be immediately placed in a -80°C freezer, organized in double freezer zip lock bags.
3. Voucher specimens should be kept in their plastic bags and placed in a standard freezer (<0°C).
4. Toenail and ear punch samples should be given to FSU HQ personnel for storage at room temperature.
5. See the Mammal-Borne Diseases Protocol for instructions on processing blood samples.

#### 11.2.4 Sample Preservation

See above.

### 11.2.5 Sample Shipping

In 2012, only blood samples will be shipped to an external facility. See the Mammal-Borne Disease Protocol for instructions on shipping blood samples.

### 11.2.6 Data Handling

### 11.2.7 Refreshing the Laboratory Supplies

### 11.2.8 Laboratory Maintenance, Cleaning, Storage

## 12 DEFINITIONS

**12.1 Small mammals = shrews and rodents in the family's Cricetidae, Heteromyidae, and Sciuridae that weigh less than 500 grams.**

## 13 REFERENCES

Mills, J. N., J. E. Childs, T. G. Ksiazek, C. J. Peters, and W. M. Velleca. 1995. Methods for Trapping and Sampling Small Mammals for Virologic Testing. 54 pp. Centers for Disease Control and Prevention.

Prugh, L., and J. Brashares. 2010. Basking in the moonlight? Effect of illumination on capture success of the endangered giant kangaroo rat. Journal of Mammalogy 91:1205-1212.

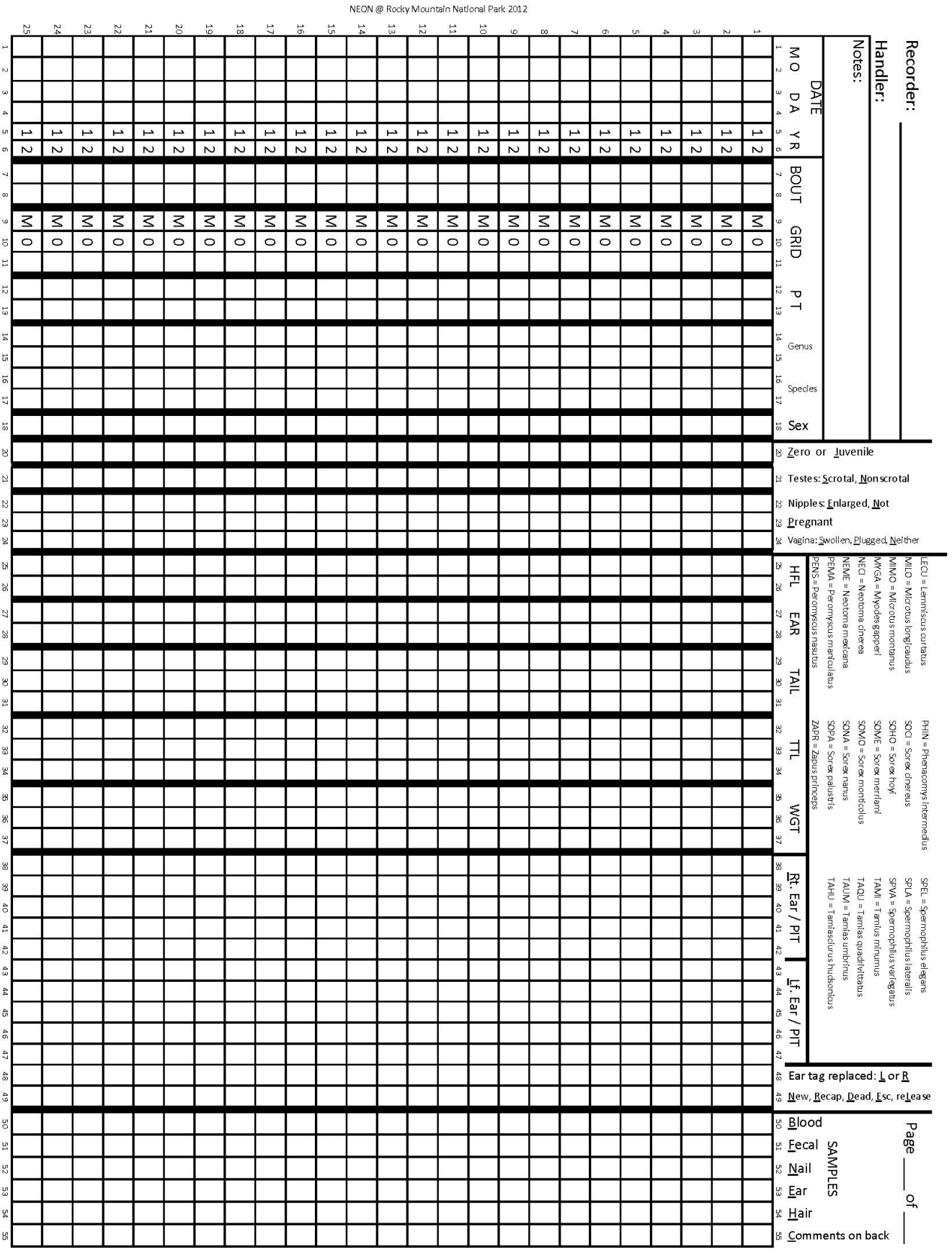
Sikes, R. S., and W. L. Gannon. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235-253.

Thibault, K. M., S. R. Supp, M. Giffin, E. P. White, and S. K. M. Ernest. 2011. Species composition and abundance of mammalian communities. Ecology 92:2316.

Wilson, D. E., F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster. 1996. Measuring and Monitoring Biological Diversity: Standard Methods for Mammals. 409 pp. (M. S. Foster, Ed.) Biological Diversity Handbook Series. Smithsonian Institution Press.

## APPENDIX A Field Data Sheets

The following field data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.


NEON @ Rocky Mountain National Park 2012
Recorder:
Handler:
Notes:
DATE
MO DA YR
BOUT
GRID
PT
Genus
Species
Sex
Zero or Juvenile
Testes: Scrotal, Nonscrotal
Nipples: Enlarged, Not
Pregnant
Vagina: Swollen, Plugged, Neither
HFL
EAR
TAIL
TTL
WGT
Rt. Ear / PIT
Lf. Ear / PIT
Ear tag replaced: L or R
New, Recap, Dead, Esc, rel ease
Blood
Fecal
Nail
Ear
Hair
SAMPLES
Comments on back
Page ____ of ____
LECU = Lemmiscus curtatus
MILO = Microtus longicaudus
MIMO = Microtus montanus
MYGA = Myodes gapperi
NEOT = Neotoma cinerea
NEME = Neotoma mexicana
PEMA = Peromyscus maniculatus
PENS = Peromyscus nasutus
PHIN = Phenacomys intermedius
SOCI = Sorex cinereus
SOHO = Sorex hoyi
SOME = Sorex merriami
SOMO = Sorex monticolus
SONA = Sorex nanus
SOPA = Sorex palustris
ZAPR = Zapus princeps
SPEL = Spermophilus elegans
SPLA = Spermophilus lateralis
SPVA = Spermophilus variegatus
TAMI = Tamias minimus
TAQU = Tamias quadrivittatus
TAUM = Tamias umbrinus
TAHU = Tamiasciurus hudsonicus

**Lab Data Sheets**

The following data sheets serve as a backup procedure for times when electronic data collection devices (PDA) are not available.

*Include copies of all data sheets – jpg format*
*(data sheets are useful for CI to define PDA and data ingest requirements)*

## APPENDIX B Considerations for implementation

*Indicate activities that could result in equipment damage, degradation of sample, or possible invalidation of results; listed here and at the critical steps in the procedure.*

*Describe any component of the process that may interfere with the accuracy of the final product.*
*Discuss how to avoid common errors in sampling or common ways samples can be contaminated.*

*Clearly flag things that might impact their work or the scientific data that aren't covered in the procedural pieces (stupid examples: "We're measuring nitrates, if you are exposed to or using nitrates at home on your lawn, trace amounts might contaminate our data"; "If it's raining, sky water getting into the samples before you seal them could alter results" )... i.e. call out weird issues and folklore explicitly. See: http://en.wikipedia.org/wiki/Phantom_of_Heilbronn*

## APPENDIX C Procedure Checklist

## 14 TABLES

## 15 FIGURES