TOS PROTOCOL AND PROCEDURE: SMALL MAMMAL SAMPLING

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B_DRAFT	01/24/2014	ECO-01181	Draft release. Will finalize in next rev.
C	03/31/2014	ECO-01671	Production release, template change, and other changes as detailed in Appendix C. Merged with rodent-borne pathogen sampling protocol.
D	04/10/2014	ECO-01792	Updated Appendix D with site-specific information. Updated References. Added Appendix D, Bleed Grid Designation.
E	12/08/2014	ECO-02530	Migration to new protocol template

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1 OVERVIEW

1.1 Background

Small mammals are widespread, sensitive to local environmental changes, and known to carry and transmit zoonotic agents; therefore they have been chosen as sentinel taxa for the Terrestrial Observation System (TOS) of NEON. From NEON's perspective, species-specific demography and population sizes, prevalence of pathogens important to public health, species richness, and relative abundances can be monitored simultaneously and ultimately linked to land use and climate changes, and therefore provide useful metrics of responses in biodiversity to these and other drivers (Kao et al. 2012). Small mammals as primary and secondary consumers interact significantly with plants and ground invertebrates (other NEON sentinel taxa), and generally represent size classes, life histories, and home range sizes that are distinct from the other NEON taxa (Kao et al. 2012). NEON will use mark-recapture methods to assess the dynamics of small mammal diversity and disease across time and space (Ostfeld and Parmenter 2008).

NEON small mammal sampling will assess the abundance and diversity of the nocturnal small mammal communities of North America, including the population dynamics of species that are competent reservoirs for infectious disease, at dozens of sites (depending on study design and associated logistics) throughout North America, Hawaii, and Puerto Rico for a period of 30 years. This represents a significant increase in the number and diversity of long-term datasets involving small mammals, and will also be unique in the degree of standardization across studies, as well as the availability of the data and archived samples to the scientific community and the public. This will allow NEON and the scientific community to address a diversity of questions, and the associated vouchering of specimens and tissue samples will provide critical resources for external PI-driven research to address an even wider range of questions.

1.2 Scope

This document provides a change-controlled version of Observatory protocols and procedures. Documentation of content changes (i.e. changes in particular tasks or safety practices) will occur via this change-controlled document, not through field manuals or training materials.

1.2.1 NEON Science Requirements and Data Products

This protocol fulfills Observatory science requirements that reside in NEON's Dynamic Object-Oriented Requirements System (DOORS). Copies of approved science requirements have been exported from DOORS and are available in NEON's document repository, or upon request.

Execution of this protocol procures samples and/or generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, and are documented in the NEON Scientific Data Products Catalog (RD[03]).

1.3 Acknowledgments

My thanks to Jesse Dulberger who wrote the first version of this protocol. Best practices are based on recommendations in Wilson et al. 1996, as well as the small mammal abundance and diversity working group (Guy Cameron, Bob McCleery, Bill McShea, Rebecca Rowe, Rob Swihart, Beatrice Van Horne).

2 RELATED DOCUMENTS AND ACRONYMS

2.1 Applicable Documents

Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

AD[01]	NEON.DOC.004300	EHS Safety Policy and Program Manual
AD[02]	NEON.DOC.004316	Operations Field Safety and Security Plan
AD[03]	NEON.DOC.000724	Domain Chemical Hygiene Plan and Biosafety Manual
AD[04]	NEON.DOC.001155	NEON Training Plan
AD[05]	NEON.DOC.050005	Field Operations Job Instruction Training Plan
AD[06]	NEON.DOC.000915	TOS Science Design for Small Mammal Abundance and Diversity
AD[07]	NEON.DOC.014051	Field Audit Plan
AD[08]	NEON.DOC.000824	Data and Data Product Quality Assurance and Control Plan
AD[09]	NEON.DOC.000911	TOS Science Design for Vectors and Pathogens

2.2 Reference Documents

Reference documents contain information that supports or complements the current document. Examples include related protocols, datasheets, or general-information references.

RD[01]	NEON.DOC.000008	NEON Acronym List
RD[02]	NEON.DOC.000243	NEON Glossary of Terms
RD[03]	NEON.DOC.005003	NEON Scientific Data Products Catalog
RD[04]	NEON.DOC.001271	NEON Protocol and Procedure: Manual Data Transcription
RD[05]	NEON.DOC.001585	Datasheets for TOS Protocol and Procedure: Small Mammal Sampling
RD[06]	NEON.DOC.001406	Raw Data Ingest Workbook for Small Mammal Abundance & Diversity
RD[07]	NEON.DOC.001402	Raw Data Ingest Workbook for TOS Rodent-borne Pathogen Sampling
RD[08]	NEON.DOC.001025	TOS Protocol and Procedure: Plot Establishment
RD[09]	NEON.DOC.001582	Lab Datasheet: 96-Well Plate

2.3 Acronyms

Acronym	Definition
CDC	Centers for Disease Control and Prevention
NIOSH	National Institute for Occupational Safety and Health
PIT	Passive Implant Transponder
PPE	Personal Protective Equipment
HEPA	High-efficiency particulate air

2.4 Definitions

Small mammal: Any mammal that is (1) nonvolant; (2) nocturnally active; (3) forages predominantly aboveground; and (4) is greater than 5 grams but less than approximately 600 g. In North America. This includes cricetids, heteromyids, small sciurids, and introduced murids. It does not include shrews, large squirrels, pocket gophers, rabbits, or weasels, despite the fact that individuals of these species may be incidentally captured.

Opportunistic vs. Non-target Species (terms and definitions modified from the National Park Service)

Non-Target species: Any non-study animals directly or indirectly affected by the research. Examples include the potential to live-capture or kill non-target individuals (e.g., loss of offspring due to taking of one or both parents) or disturb/harass other species during the research activity (e.g., during sampling that employs airplanes and/or boats).

Opportunistic species*:* Any animal whose capture is accidental or incidental, but whose capture can lead to valuable information. Examples include non-target species of small mammals that, if captured, will be marked and released or vouchered, etc.

Sampling bout: The three consecutive (or nearly so) nights of trapping per trapping grid intended to occur monthly or every other month around the new moon and includes all trapping grids.

3 METHOD

Standard Operating Procedures (SOPs), in Section 7 of this document, provide detailed step-by-step directions, contingency plans, sampling tips, and best practices for implementing this sampling procedure. To properly collect and process samples, field technicians **must** follow the protocol and associated SOPs. Use NEON's problem reporting system to resolve any field issues associated with implementing this protocol.

The value of NEON data hinges on consistent implementation of this protocol across all NEON domains, for the life of the project. It is therefore essential that field personnel carry out this protocol as outlined in this document. In the event that local conditions create uncertainty about carrying out these steps, it is critical that technicians document the problem and enter it in NEON's problem tracking system.

The procedures described in this protocol will be audited according to the Field Audit Plan (AD[07]). Additional quality assurance will be performed on data collected via these procedures according to the NEON Data and Data Product Quality Assurance and Control Plan (AD[08]).

3.1 Sampling Goals

NEON requires the study of live organisms in their natural setting to document the diversity, population sizes, and pathogen prevalence of small mammals through time, in relation to such critical drivers as climate change and land-use change. These parameters will be assessed through a mark-recapture live trapping study and associated tissue collection. The NEON sampling design allows for robust estimation of (1) population sizes and species diversity using standard mark-recapture techniques (AD[06]), and (2) inter- and intra-annual changes in pathogen prevalence (AD[09]). All methods conform to standard methods used in the study of wild small mammals (see Wilson et al. 1996, Sikes et al. 2011).

3.2 Trapping design

Sherman live traps (H. B. Sherman, Inc., Tallahassee, FL, folding or non-folding, 3" x 3.5" x 9" or, if kangaroo rats (*Dipodomys spp.*) are present, 3" x 3.75" x 12") will be used to capture animals for the study. Trapping grids will be laid out with 100 Sherman live traps (10 m spacing – 10 rows – 10 columns). Up to eight grids will be trapped during each sampling period, depending on the area of the site and associated logistics. The grids will be distributed proportionally across the dominant vegetation types, collocated with a subset of the TOS Distributed Plots (see TOS Science Design for Small Mammal Abundance and Diversity (AD[06]) for additional details).

3.3 Personnel

Four (2 teams of 2) or two personnel will conduct the sampling at each site, depending on the number of grids at a particular site. Each team will be comprised of at least one well-trained 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.

3.4 Timing of trapping

Each grid will be trapped for 3 consecutive nights within a sampling period, and sampling will occur year-round at a monthly (pathogen grids at core sites only) or every other month frequency, if resources are available and winter weather conditions permit (Table 1). 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). Within a sampling period, timing of trap setting is generally 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). Local conditions can necessitate deviations from this general plan; these deviations will be specified in the site-specific appendices for this document, if necessary. 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., 27°C (80°F) by 10:00 am) and all traps are not shaded, 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.

3.5 Bait

Sherman traps are to be set and baited with a seed mixture (sunflower seeds -35%- and millet – 65%) that has been sterilized to prevent germination; seeds are high quality resources utilized by most target small mammal species. In cold weather conditions (overnight lows < 18°C/65°F), polyester or wool batting for nesting will be placed in the traps, except at sites known to be dominated by rodents in the family Heteromyidae (e.g., Central Plains Experimental Range (CPER), Jornada Experimental Range (JORN)). These are burrowing rodents which are known to urinate on batting, thereby reducing its insulating properties, rather than building nests, and who will not consume peanut butter (see below). At sites at which shrews (*Soricomorpha: Soricidae*) comprise more than 20% of the captured individuals (e.g., Harvard Forest), a teaspoon of freeze-dried mealworms should also be added to the trap. Additional, high-calorie bait (e.g., peanut butter) can also be added in the event of extremely cold conditions at many sites, except where medium- to large- mammal (e.g., raccoons, bears) disturbance of

traps has occurred or fire ants occur (e.g., Jones Ecological Research Center (JERC), Ordway-Swisher Biological Station (OSBS)).

3.6 Processing

Upon capture, individual small mammals will be processed in one of three ways, according to their classification as target, opportunistic, or non-target (see section 2.4 and site-specific appendices for detailed species lists). 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. Handling time per individual will be 15 minutes or less. All non-target species will be released immediately at the point of capture, without handling. If possible, the species identification of these individuals will be recorded. All opportunistic species will be handled as described below for target species, except that no pathogen-related blood sampling will be conducted on these individuals.

Individuals will be transferred from the trap directly into a disposable plastic handling bag for processing (or a small mesh bag, if needed for efficient handling of larger and/or more aggressive species). If blood will not be collected or blood will be collected using the mandibular technique, the individual will not be anesthetized prior to handling, unless it is needed to safely collect blood (e.g., for individuals >100g). If blood will be collected from a particular individual using the retro-orbital technique, the animal will be anesthetized before any handling, to decrease stress and reduce chances for injury during the blood collection procedure. Note that only some species (i.e., cricetids) can be bled with the retro-orbital technique (Figure 1). No individual will spend more than 3-5 minutes in the animal handling bag and will be in hand for only brief time spans (< 15 minutes / animal) using techniques to reduce injury, stress, and pain. All plastic bags will be discarded after each use, and mesh bags must be laundered or placed under UV before re-use. Collected blood should be labeled and placed onto dry ice as soon as possible after collection.

All individuals will be identified to species, age, reproductive condition assessed by examination of external genitalia, and have standard measurements taken (i.e., hind foot length and weight (using a spring-scale)). Additional measurements (e.g., ear length, tail length, and/or total length) shall be taken when relevant to species identification; these are found in the site-specific dichotomous keys. All target and opportunistic species will be marked with either one numbered ear tag or a subcutaneous, RFID PIT tag (8 - 12mm), have tissue samples taken, and then released at the point of capture. Tissue samples to be collected from all individuals will include one ear tissue sample from the untagged ear for genetic analyses, and clipped whiskers and hair for potential isotopic analyses. Fecal samples will also be collected opportunistically from captured individuals, with a preference for fresh samples (vs. ones deposited in the trap at an unknown time during the night). Ear and fecal samples should be placed onto dry ice as soon as possible after collection, while hair and whiskers can be stored together at ambient temperatures.

DO use the mandibular technique, unless 5 attempts to use it on a particular species are unsuccessful.

DO collect blood from:

Cricetids (e.g., deer mice, voles, cotton rats) that are >10.0 grams
- If an individual's mass might be <= 10 g, be sure to weigh the individual
- If the mandibular technique proves unsuccessful for a particular species of cricetid, the retro-orbital technique should be used for that species if approved at that site.

Heteromyids (Pocket mice (*Chaetodipus, Perognathus spp.*), Kangaroo rats and mice (*Dipodomys* and *Microdipodops spp.*)) that are >10.0 grams
- Use only the mandibular bleeding technique on these species

Dipodids (jumping mice (*Zapus, Napeozapus spp.*)) that are >10.0 grams
- Use only the mandibular bleeding technique on these species

UNLESS individual has:
- Pronounced or physically debilitating injury, and/or
- Already been captured and bled during **current** sampling bout.
- UNLESS species is excluded based on permitting regulations (e.g., protected species)

DO NOT collect blood from:

Sciurids – chipmunks, squirrels, etc.
Soricids – shrews
Talpids – moles
Geomyids – pocket gophers
Murids – house mice (Mus musculus) and introduced rats (Rattus spp.)

Figure 1. Criteria for blood sampling

3.6.1 Marking

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 external pinnae for securing ear tags. Shrews will not be permanently marked. Individuals are marked to allow for the study of 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. All needles and PIT tags will be sterile. Disposable needles will be deposited in a sharps container immediately. 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). In rare cases, if tagging is deemed undesirable considering the condition of an animal (e.g., highly stressed), an animal may be released without tagging.

3.6.2 Animal Care

The condition of all captured individuals will be monitored closely throughout the handling period. Specifically, the rodent's rate of respiration, spontaneous movements, responses to tactile stimulation, and ability to retain sternal recumbency will be monitored, in order to gauge both the state of anesthetization (when relevant) and general condition of the animal. A small vial of 10% sugar water will always be available to revitalize stressed, dehydrated, hypothermic or heat-stressed captures; this solution should be replaced regularly to inhibit the growth of mold. Stressed individuals will be placed in a secure container containing bait and, in the case of hypothermia, a disposable hand warmer and batting. If conditions are particularly cold (< 65°C) overnight and into the morning, a hand warmer can be started while driving to the site and placed on the defroster vents of the work vehicle so that it is warm by the time of arrival. These individuals will be monitored every 15 minutes and released only when the animal exhibits normal behavior. If no signs of recovery are observed by the time processing a grid is complete, the animal will be euthanized with a lethal dose of isoflurane and the specimen tagged, bagged, and placed on ice as soon as possible, with ultimate disposition in a curated collection.

1. Identify to species
2. If bleeding, collect blood samples first
    - Anesthetize first, if the retro-orbital technique is needed
3. Mark individual
    - An ear tag in right ear OR
    - A subcutaneously implanted RFID tag
4. Assess age, sex, and reproductive condition
5. Measure:
    - Hind foot length – for all species
    - Ear length, tail length, and/or total length – if needed for species ID
6. Collect samples:
    - Fecal - Fresh (from animal in hand) or Old (from trap)
    - Hair – a small tuft from the back of the head
    - Whiskers – snip at base; one from each cheek towards the front of the nose
    - Ear punch from the outer margin of the untagged ear
7. Weigh
8. Put back in trap for transport back to point of capture for release
9. Clean all gloves, tools, and other items that have come in contact with a small mammal with quat before processing another individual or packing up the processing station

Figure 2. Summary of procedures per individual (see also Appendix A)

The plan includes:

- Hot checks in the field by HQ staff or contractor, if funding is made available.
- DNA barcoding of a subset of samples to quantify error rates in taxonomic IDs.

When unexpected field conditions require deviations from this protocol, the following field implementation guidance must be followed to ensure quality standards are met:

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. **Each mortality must be reported to the Field Operations Manager within 24 hours** of processing, to help ensure that all state- and site-specific permit requirements are followed. Please be aware that there are state- and site-specific permitting requirements detailed on permits provide by NEON EHS for reporting both live captures, as well as mortalities, of vertebrates captured, including either all species or, in some case, just the species with state status. It is imperative that the technician(s) leading the small mammal trapping is(are) familiar with the guidelines detailed in the permits for the sites and states in their jurisdiction.

If, for any reason, ≥ 5 individuals on any given trapping grid during a single bout die, either in the trap or in hand**, the trapping on that grid should be discontinued** until the next scheduled sampling bout, and a problem ticket should be issued detailing the locations, species, sex, and ages of the mortalities**.** If weather or any other circumstances cause one night of trapping within the sampling bout to be missed, trapping may be resumed and continued as normal within 5 days of the latest night of trapping. If this is not possible, sampling should be resumed as soon as possible, while maintaining the timing of trapping relative to the new moon for the sites.

Given acclimation and regional variation in the conditions that threaten the survival of trapped rodents, guidelines for preventing mass mortality in cold and hot conditions will be provided in the site-specific appendices, as necessary. The following guidelines are generally applicable across sites, and can also be found in Appendix A.

Cold conditions:

- Bedding (i.e., polyester or wool batting) should be used when low temperatures are expected to be < 18°C (65°F), except at sites where heteromyids dominate.
- Trapping should not occur on nights when **both** freezing temperatures (< 0°C (32°F)) and precipitation in the form of rain are expected (>20% chance at sites with bedding; >5% chance at sites that cannot use bedding).
- Due to the added logistical burden, trapping should not occur when snow cover exceeds 15 cm (6 inches) at the time of trap setting.

Hot conditions:

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

- Extra effort must be made to ensure that all traps containing captured individuals are processed or placed in the shade as soon as possible. Work can continue past 10 a.m. or when temperatures exceed 80 degrees Fahrenheit, but care should be taken to ensure the well-being of the captures.
- If you are unable to begin checking small mammal traps due to unforeseen circumstances 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 >15 traps on a single grid on any given night, traps should be removed from the grid and that sampling bout terminated prematurely for that grid. The Field Operations Manager shall be informed as soon as possible, and a problem ticket should be issued.

Documentation of Issues:

1. Data from animals that die during the course of handling or trapping should be recorded on the datasheet, with a 'D' marked in the appropriate column (fate). The 'D' supersedes the recapture options (i.e., 'N' or 'R') for the fate field.
2. If traps are not set on a particular grid as scheduled or if no individuals are captured, these should be recorded in the NOTES field (Table 1), as a line on the datasheet for that given date, grid, and bout combination. These notes can also be used on a trap by trap basis, if there is variation within the grid.
3. If traps are damaged or disturbed overnight, note trap locations and code the nature of the disturbance on the datasheet in the NOTES field (Table 1), with any other known details described in the 'add'l notes' cell.

Table 1. Descriptions and prescriptions of codes used in Notes field of small mammal datasheet.

Definitions	Application Rules
1 –traps not set	Used at the level of the entire trapping grid, or, if some traps on a grid were set and others were not, can be used to indicate which ones were not set
2 – trap door closed but empty	Used per trap coordinate, when necessary
3 – trap door open with feces left behind	Used per trap coordinate, when necessary
4 – trap disturbed	Used per trap coordinate; if entire trapping grid is disturbed, can be used at the grid level
5—trap ID suspect *	Used per trap coordinate, when necessary
6—no captures	Used ONLY at the per grid level

*indicates uncertainty in trap coordinate – e.g., if two traps have the same coordinate, or if smudging of marker reduces legibility

4 SAMPLING SCHEDULE

4.1 Sampling Frequency and Timing

Small mammal sampling shall occur in bouts, with a bout comprised of three consecutive (or nearly) nights of trapping on a particular grid. Sampling frequency, or the number of bouts per year, is determined by two factors: whether or not a particular trapping grid is being used to study pathogen prevalence and whether or not the trapping grid is located at a core or relocatable site (Table 2). Sampling shall occur year-round, where personnel resources and weather conditions permit, with a minimum of four bouts per year for all grids except the pathogen sampling grids at core sites, where a minimum of 6 bouts per year is needed.

Table 2. Summary of sampling frequencies by site and grid type

Site Type	Grid Type	Sampling Frequency	Minimum bouts per year
Core	Pathogen	Monthly	6
Core	Abundance & Diversity	Every Other Month	4
Relocatable	Pathogen	Every Other Month	4
Relocatable	Abundance & Diversity	Every Other Month	4

4.2 Criteria for Determining Onset and Cessation of Sampling

Sampling bouts should occur as close as possible to the new moon, and the timing of sampling at each site should remain as consistent as possible with respect to the new moon over the course of NEON operations. Acceptable variation around this timing for the initiation of sampling from one sampling bout to the next is 1-5 days (relative to the new moon). Trapping at core and relocatable sites should occur within a 20 day window centered around the new moon (i.e., 10 days before the new moon – 10 days after). This schedule is based on an assumption of a logistical constraint of trapping 3-4 grids at one site at one time. However, there is no scientific requirement to schedule the trapping in this way, if sufficient personnel are available to sample more grids simultaneously. These details are left to the discretion of the Field Operations Manager to be approved annually by Science Operations.

If weather or any other circumstances cause a night of trapping within the sampling bout to be missed, trapping may be resumed and continued as normal within 5 days of the latest night of trapping. If this is not possible, sampling should be resumed as soon as possible, while maintaining the timing of trapping relative to the new moon for the sites.

4.3 Timing for Laboratory Processing and Analysis

Process all frozen samples immediately upon returning to the lab. Once samples are frozen, they must remain frozen; plan accordingly. Please note that all subsequent instruction in this SOP pertain to the blood samples only; instructions will be added for the remaining samples whenever institutions for archiving those samples have been identified. A subset of the ear tissue samples will be used in SOP G.

4.4 Sampling Timing Contingencies

If weather or any other circumstances cause a night of trapping within the sampling bout to be missed, trapping may be resumed and continued as normal within 5 days of the latest night of trapping. If this is not possible, sampling should be resumed as soon as possible, while maintaining the timing of trapping relative to the new moon for the sites.

Table 3. Contingent decisions

Delay	Action	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 (within safety limitations) to prevent mortality of study animals.	Trapping-induced mortality violates the assumptions of the mark-recapture models that are used to estimate density. In addition, high mortality rates from trapping threaten the scientific and ethical integrity of the study.
1-10 days	Add additional days of sampling as soon as possible to sample all points.	Fewer sampling points could result in less precise estimation of richness, diversity, or density. Capture-recapture models require recapture data from >1 night of sampling per bout to estimate densities. If additional time is not available, fewer samples will be collected.
11 or more days	Do not sample. Resume sampling as scheduled during the next month.	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.

5 SAFETY

This document identifies procedure-specific safety hazards and associated safety requirements. It does not describe general safety practices or site-specific safety practices.

Personnel working at a NEON site must be compliant with safe field work practices as outlined in the Operations Field Safety and Security Plan (AD[02]) and EHS Safety Policy and Program Manual (AD[01]). Additional safety issues associated with this field procedure are outlined below. 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.

5.1 Working with Small Mammals

Personnel working with small mammals should familiarize themselves with the Zoonotic Diseases section of AD[02]. The incidence of these diseases in humans is extremely rare, with the exception of Lyme disease in certain regions of the country, and is typically associated with working outside in vegetated areas. Although rodents and other small mammals and their ectoparasites (e.g., ticks and fleas) are critical to the lifecycle of many of these disease-causing organisms, there is no evidence that the handling of small mammals significantly increases risk of exposure to many of these diseases beyond other types of field work. One possible exception to this is Hantavirus Pulmonary Syndrome (HPS), which is believed to result from inhalation of contaminated, aerosolized urine and feces, as well as through bites. Most of the known cases of HPS have resulted from inhalation of aerosolized virus present in cabins and other remote buildings in which small mammals are nesting (Kelt et al. 2007, 2010).

There are a number of Hantavirus species in North America, and most of the cricetid rodents in North America appear to be competent reservoirs for these pathogens. Of these cricetids, *Peromyscus maniculatus* is the primary reservoir for the Sin Nombre virus, the hantavirus most often linked to HPS. This species is widespread throughout North America, but, according to Centers for Disease Control and Prevention (CDC) data, HPS cases are more numerous in western states. The highest incidence of HPS is found in California, Arizona, New Mexico, and Colorado. Consequently, NEON is using a tiered approach to personal protective equipment (PPE) that reflects HPS incidence reported by the CDC (AD[02]).

6 PERSONNEL AND EQUIPMENT

6.1 Clothing and PPE

According to the NEON Operations Field Safety and Security Plan (AD[02]), whenever directly handling small mammals or working with equipment/supplies that have been in contact with small mammals, personnel are required to wear, at a minimum:

- Eye protection
  - Safety glasses with ventilation to minimize fogging up in humid environments
  - Intended to discourage rubbing of the eyes with potentially contaminated hands and protect from the unlikely occurrence of extreme splashes during blood collection
  - At all NEON sites
- Gloves
  - Latex and/or nitrile – disposable - to provide a barrier against infectious excreta and body fluids during small mammal handling
  - Optional: Reusable cloth gloves with rubber-tipped fingers may be worn for handling to provide additional protection from bites (latex/nitrile gloves should be worn over these to allow for effective decontamination)
  - Reusable cloth gloves with rubber-tipped fingers should be worn when checking traps
  - At all NEON sites
- Clothing
  - Long-sleeved shirt
  - Long pants
  - Close-toed shoes with socks
  - Optional: a disposable or reusable (cotton) laboratory coat or apron
  - At all NEON sites
- Respiratory protection
  - Disposable respirators with N95 (HEPA-equivalent) particulate filter
    - Required: all sites in CO, NM, AZ, CA
    - Recommended: all sites in KS, TX, UT, MT, NV, ID, OR, and WA
    - Optional at all other sites
  - Optional half-face respirator with P100 filters at all sites

Any items of clothing that have or may have been in contact with small mammal excreta or bodily fluids should be sterilized to ensure safety. According to the Centers for Disease Control and Prevention, decontamination can be accomplished by exposing contaminated clothing to UV (i.e., sunlight) for 4-6 hours or laundering in a washing machine using hot water and any commercially available laundry detergent. Spot treatment of personal attire or protective equipment using a spray bottle filled with quat cleaner should be performed in the field during sampling. Contaminated clothing should not be

washed with other personal or with family laundry. If decontamination cannot be performed immediately, potentially contaminated clothing should be stored in a closed bag.

Other personal protective equipment will be cleaned throughout each sampling day. Eye protection and shoes will be wiped down with disinfectant, quat cleaner or 70+% alcohol. Gloves will be cleaned and disinfected. Disposable gloves and respirators will be discarded if they become torn or damaged while sampling. At a minimum, gloves should be replaced after captures from each sampling grid have been processed. If used, half-face respirators will be fully cleaned in accordance with the NEON EHS Safety Policy and Program Manual (AD[03]).

6.2 Equipment

The following equipment is needed to implement the procedures in this document. Equipment lists are organized by task. They do not include standard field and laboratory supplies such as charging stations, first aid kits, drying ovens, ultra-low refrigerators, etc.

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. Please note that the current protocol does not apply to studying the invasive *Rattus spp.* in Puerto Rico or Hawaii, and therefore the equipment lists included herein should not be used in those domains. Future versions of this protocol will include SOPs and equipment applicable to those domains.

Table 4. Equipment list – Flagging the grid, one bout

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
MX100322	R	TruPulse 360R Laser Range Finder	Setting Traplines	1	N
	R	Magnetic Compass, Handheld, Mirror-sighting, Floating	Setting and Following Traplines	6	N
	R	Measuring Tape, Fiberglass, Metric gradation, 100 m length	Setting Traplines	4	N
	R	Survey Marking Flags, Vinyl Flag with Wire Stake, 18 inch wire, 2.5 inch x 3.5 inch flag, orange	Setting Traplines	100+	N
	R	GPS receiver, handheld, recreational accuracy	Navigating to Traplines	2	N
	R	Battery, AA, Rechargeable	GPS	4	N
	R	Permanent Markers, Chisel, Black; Sharpie or Equivalent	Marking flags	6	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	Chaining pins or similar	Anchor measuring tape while in use	10	N
Consumable Items					
(None)					

R/S=Required/Suggested

Table 5. Equipment list – Trapping, one bout

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	Magnetic Compass, Handheld, Mirror-sighting, Floating	Following traplines	All	6	N
	R	GPS receiver, handheld, recreational accuracy	Navigating to traplines	All	2	N
	R	Battery, AA, Rechargeable	GPS	All	4	N
	R	Battery, AAA, Rechargeable	Headlamp or GPS	All	1200	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX106060	R	Small Mammal Trap, Small, Folding, 3"x3.5"x9"	Trapping	Domains D01-D08 (excluding D04), D18, D19	1200	N
MX100702	R	Small Mammal Trap, X-Large, Folding, 3"x3.75"x12"	Trapping	Remaining domains (not D04 or D20)	10 lb	N
	R	Headlamp, LED, Wide and Narrow Beam, AAA or AA Battery Powered	Setting & checking traps	All	6	N
	S	Seed Storage Bin, Rolling, 26 Gallon Capacity	Seed storage	All domains except D04 and D20	1200	N
	S	Professional Model Tree Planting Bag with Split Bag	Carrying traps	All domains except D04 and D20	8	N
	S	Lumbar bags (or similar)	Carrying bait	All domains except D04 and D20	8	N
	S	>= gallon-sized bag secured to lumbar bag with carabiner	Carrying batting	All domains except D04 and D20	6	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Permanent Markers, Chisel; Sharpie or Equivalent	Marking traps	All domains except D04 and D20	6	N
	S	Markers, Wet Erase, Fine Tip	Marking traps	All domains except D04 and D20	6	N
	S	Storage Tote, Plastic, Double-walled Lid with Lockable Latches, 24 gallon Capacity	Storing extra supplies in truck	All	2	N
Consumable Items						
	R	Black Oil Sunflower Seed – shelled or unshelled	Bait	All domains except D20 and D04	20 lb	N
	R	White Proso Millet Seeds	Bait	All domains except D04 and D20	2 lbs.	N
	R	Freeze-dried mealworms	Bait for shrews	TBD	10 yd	N
	R	Batting, Wool or Synthetic, pillow type, For Small Mammal Trap bedding	Nesting material	All domains except D04 and D20	500	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	5 x 4 x 21" 1 Mil Gusseted Poly Bags	Trap collection bags	All domains except D04 and D20	1 lb	N
	R	0.20% Bifenthrin in granular form (Talstar EZ or equivalent)	Fire ant control	Order for domains D02, D03, D07, D08, D11	1	Y
	S	NEON trap stickers	Labeling traps	All domains except D04 and D20	6	N
	S	Pencils - #3	Marking traps	All domains except D04 and D20	6	N

R/S=Required/Suggested

Table 6. Equipment list – Checking traps and processing captures, one bout

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
	R	Site- or domain-specific dichotomous key	Identifying species	All	2	N
	R	1-3 oz. amber glass bottle with dropper in lid	Anesthesia/ Euthanasia	All domains except D04 and D20	2	N
	R	Spoon, Stainless Steel, Tea Infuser, Spring Loaded	Anesthesia/ Euthanasia	All domains except D04 and D20	2	N
	R	Small Animal Ear Tag Applicator, size Monel #1	Marking	All domains except D04 and D20	4	N
	R	PIT tag portable reader capable of reading ISO 11784/11787 FDX-B and HDX PIT tags, simple data retrieval via USB port, Bluetooth	Marking	All domains except D04 and D20	2	N
	R	Metric Ruler, Clear Plastic, mm gradation, 12 inch length	Measurements	All domains except D04 and D20	2	N
	R	Ruler, Clear Plastic, Flexible, 6 in	Measurements	All domains except D04 and D20	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Scale, Spring, Tareable, Capacity 30 g maximum, Accuracy ±0.3%	Measurements	All domains except D04 and D20	2	N
	R	Scale, Spring, Tareable, Capacity 100 g maximum, Accuracy ±0.3%	Measurements	All domains except D04 and D20	2	N
	R	Scale, Spring, Tareable, Capacity 1000 g maximum	Measurements	All domains except D04 and D20	2	N
	R	Microhematocrit Tube, Heparinized, Mylar Coated	Sample collection -- blood	All domains except D04 and D20	1000	N
	R	Storage Box, For Cryovial Tube, 81 Position, Deep Well, Plastic, With Lid	Sample collection -- blood	All domains except D04 and D20	4	N
	R	Tissue Biopsy Punch, 2000 micron ID, clicker style	Sample collection – ear punch	All domains except D04 and D20	2	N
	R	Pen, marker, black, permanent, ultra-fine (0.3mm) Sharpie or equivalent	Sample collection - labeling	All domains except D04 and D20	2	N
	R	Pen, marker, black, permanent, ethanol safe 12 ea/ pkg	Sample collection - labeling	All domains except D04 and D20	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Cooler, 16qt, Material HDPE, Insulation Ultratherm Foam	Sample collection - storage and transport	All	2	N
	R	Camera, Digital	Species IDs	All	2	N
	R	Carrying Case, Compact Digital Camera, 3.3 In. W x 2 In. D x 5.5 In. H, Brushed-Tricot Lining, Black; Lowepro Ridge 30 or Equivalent	Species IDs	All	2	N
	R	Field guide, mammals	Species IDs	All domains except D04 and D20	2	N
	S	Ventilated, folding cardboard carrier; approx. 10" x 6 3/4" x 5 1/2"; or equivalent reusable option	Animal care	All domains except D04 and D20	5	N
	S	Nalgene screw top bottle (<5oz)	Animal care – sugar solution	All domains except D04 and D20	2	N
	S	Shade/rain tent for processing rodents during inclement conditions, with ventilation and guy ropes and stakes*	Handling	All domains except D04 and D20	2	N
	S	Backpack	Handling - equipment organization	All domains	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Transparent organizer boxes with lid, plastic, adjustable, large, approx. 14.25" x 9.125" x 2.0"	Handling - equipment organization	All domains except D04 and D20	4	N
	S	Mesh wash bags, approx. 9 inches x 16 inches (mesh should be large enough to pull a woodrat ear through for tagging)	Handling larger species	All domains except D04 and D20	20	N
	S	Canvas holding bags, approx. 9 inches x 16 inches	Handling larger species	All	20	N
	S	Multi-Tool, with Needle-nose Pliers, Knife, and Scissors	Marking	All domains except D04 and D20	2	N
	S	Diagonal Plier, 4 in	Marking - cutting off ear tags	All	2	N
	S	Short (< 6 inches) piece of rope (550 paracord works well) for 1000 g scale	Measurements	All domains except D04 and D20	2	N
	S	Pipette Bulb, Microhematocrit Tube	Sample collection -- blood	All domains except D04 and D20	10	N
	S	Scissor, iris, straight, sharp / sharp, 3.5", 9cm	Sample collection – ear punch	All domains except D04 and D20	2	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Forcep, Cushing Dissecting, Delicate Thumb, Stainless Steel	Sample collection	All domains except D04 and D20	4	N
	S	Forcep, Dissecting Microforceps, Jeweler Style Curved, Stainless Steel	Sample collection	All domains except D04 and D20	4	N
	S	Cuticle clippers, 4" long, stainless-steel	Sample collection, ear tagging	All domains except D04 and D20	2	N
	S	Hand lens, 10x, 7/16". Coddington type double lens magnifier	Species IDs	All domains except D04 and D20	2	N
	S	Thermohygrometer Mini, Temp Range 14-122F, miniature	Monitoring trapping conditions	All	2	N
Consumable Items						
	R	Paper, Copy, All Weather, 8-1/2 inches W x 11 inches L, White; Rite in the Rain or Equivalent.	Datasheets	All	25 sheets	N
RD[05]	R	Small mammal field datasheets	Recording data	All	10	N
	R	Cotton Ball, Medium	Anesthesia/ Euthanasia	All domains except D04 and D20	150	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Isoflurane (Forane), Liquid, Halogenated Anesthesia,	Anesthesia/ Euthanasia	All domains except D04 and D20	25 mL	Y
	R	Hand Warmer, Single use	Animal care	All domains except D04 and D20	2	N
	R	Sugar packets, small mammals	Animal care	All domains except D04 and D20	2	N
	R	Freeze-dried mealworms	Animal care - shrews	TBD	2 lbs.	N
	R	Styptic Powder, 42g	Animal care (in case of open wound)	All domains except D04 and D20	1 pack, 42 g	N
	R	Cotton Swab, Dual-tipped	Animal care – applying styptic powder	All	50	N
MX100727	R	Small Animal Ear Tag, Approximately 0.5 in x 0.125 in, Laser-etched	Marking	All domains except D04 and D20	200	N
	R	PIT tags + implanter - 8 - 12mm (prefer 9mm)	Marking	All domains except D04 and D20	50	N
	R	Animal lancets, 4mm and 5mm	Sample collection -- blood	All domains except D04 and D20	1000	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Wipe, Alcohol Pad, Individually Packaged	Sample collection - blood	All domains except D04 and D20	1000	N
	R	Gauze Pad, Sterile, 2 in x 2in, 12-ply	Sample collection -- blood	All domains except D04 and D20	1000	N
	R	Tube, Microcentrifuge, 1.5ml Self Standing, Sterile, Clear Polypropylene, External Threads	Sample collection - storage of blood, fecal samples, ear punches	All domains except D04 and D20	1000	N
	R	Acid-free artifact identification tags	Sample collection - vouchers	All	10	N
	R	Thread, Cotton, Size 10, Crochet Thread, White	Sample collection - vouchers	All	2 rolls	N
	R	Archival coin envelopes, 2.25 x 3.5”	Sample collection - whiskers, hair	All domains except D04 and D20	150	N
	R	Dry Ice, Carbon Dioxide Solid, Pelletized	Sample collection - storage and transport	All domains except D04 and D20	50 lbs	N
	S	Freezer Bag, Resealable, 10" x 12" or 1 gal, 1.5 – 2 mil thickness	Handling	All domains except D04 and D20	200	N
	S	Freezer Bag, Resealable, 10" x 12" or 1 gal, 4 mil thickness	Handling	All domains except D04 and D20	100	N

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Tubing, heat shrink, 3:1 shrink ratio, black, 6" length, wire range 12-6 AWG, 6 per pkg	Handling - for covering clamp teeth on spring scales	All domains except D04 and D20	1 package	N
	S	Label, Cryogenic, fits 1.2 -2 ml vials	Sample collection - labeling	All domains except D04 and D20	1000	N
	S	Freezer Bag, Reclosable, 7" x 8" or 1 qt, 4 ml thickness	Sample collection – storage for coin envelopes, filled vials, and voucher specimens	All	20	N
	S	Battery, Alkaline, 1.5 v, Size 357	For thermo-hygrometer	All	2	N

R/S=Required/Suggested

Table 7. Equipment list – Cleaning traps, per bout

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	S	Spray bottle	Cleaning traps	1 pack, 6	Y
	S	Toilet bowl brush, stiff bristles	Cleaning traps	6	Y
	S	Scrub Brush, Polypropylene bristle	Cleaning traps	1	Y
	S	Graduated Cylinder, Polypropylene, 25 mL, 0.5 mL graduation	Cleaning traps	2	Y
	S	Plastic bucket, 5 gal	Cleaning traps	6	Y
	S	Carboy, Round, LDPE, Wide-mouth with Spigot, and Screw Cap, 20 L	Cleaning traps	1	Y
Consumable Items					
	R	Quaternary Disinfectant, Concentrated (or bleach)	Cleaning traps	1 bottle, 1 gal	Y
	S	Glove, Chemical-Resistant, Flock-Lined, various sizes	Cleaning traps	1	Y

R/S=Required/Suggested

Table 8. Equipment list – Preparing blood samples

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	R	Cryovial freezer storage box, cardboard, capacity 9x9, cell dividers included	Storing blood samples	5	N
Consumable Items					
	R	Paper, Copy, All Weather, 8-1/2 inches W x 11 inches L; Rite in the Rain or Equivalent.	Datasheets	5 sheets	N
RD[05]	R	Small mammal datasheet (lab portion)	Datasheets	5 sheets	N
	R	Gloves, Cryogenic, Waterproof, Mid-arm Length, Various Sizes	Handling dry ice	1 pair	N

R/S=Required/Suggested

Table 9. Equipment list – Shipping blood samples

Item No.	R/S	Description	Purpose	Quantity*	Special Handling
Durable Items					
	S	Box Set, Shipping Box With Insulated Polystyrene Foam, 12 x 10 x 9 Inch Inner Box, Reusable; Includes Outer Cardboard Box, Foam Container With Lid	Sample shipping	3	N
Consumable Items					
	S	Shipping sticker, up arrow	Sample shipping	3	N
	S	Shipping sticker, dry ice	Sample shipping	3	N
	S	Label, Biohazard, Shipping, 2 x 2 inch	Sample shipping	3	N
	R	Hard-copy shipping manifest	Sample shipping	1	N
	R	Dry Ice, Carbon Dioxide Solid, Pelletized	Sample shipping	5 lbs	Y
	R	Gloves, Cryogenic, Waterproof, Mid-arm Length, Various Sizes	Sample shipping	1 pair	N

R/S=Required/Suggested

* Quantities are generous estimates per batch

Table 10. Equipment list – Genetic analysis

Item No.	R/S	Description	Purpose	Quantity	Special Handling
Durable Items					
	R	Jewelers forceps (fine point)	Removing tissue	3	N
	R	96-well plate datasheets	Recording data	3	N
	R	Lighter, Butane, Refillable, Stainless Steel Extension, Adjustable Flame Control	Sterilizing forceps	1	N
	R	Lab-top cooling setup	Preparing plates	1	N
	R*	96-well microwell plates	Storing tissue to be barcoded	3	N
	R*	96-well microwell caps	Covering microwell plates	285	N
	S	Spray bottle for ethanol	Sterilizing bench space and gloves	1	N
	S	Box Set, Shipping Box With Insulated Polystyrene Foam, Includes Outer Cardboard Box, Foam Container With Lid	Shipping	1	N
Consumable Items					
	R	95% Ethanol	Sterilizing bench space and gloves	1 L	Y
	R	Packing tape	Shipping	1 roll	N

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	R	Nitrile gloves	Prevent contamination of samples	Variable	N
	R	Shipping labels	Shipping	1	N
	R	Dry Ice, Carbon Dioxide Solid, Pelletized	Shipping plates	2 lbs	Y
	R	Gloves, Cryogenic, Waterproof, Mid-arm Length, Various Sizes	Handling dry ice	1 pair	N
	S	Shipping sticker, dry ice	Shipping	1	N

R/S=Required/Suggested

* Provided by CCDB

Table 11. Equipment list – Cleaning and sterilization, one bout

Item No.	R/S	Description	Purpose	Conditions Used	Quantity*	Special Handling
Durable Items						
	R	Butane Lighter	Sample collection - sterilizing tools	All domains except D20, D04	2	N
	R	Plastic bottle with screw top + spray attachment (approx. 32 oz)	Cleaning - transporting quat	All domains except D20, D04	2	N
	R	Sharps container, portable, slip top, 1 qt, red	Waste disposal	All domains except D20, D04	2	N
	S	Jug, Polyethylene, 3 L or 1 gal Capacity, Narrow Mouth, F-style Container, With Cap	Cleaning - transporting quat in truck	All domains except D20, D04	2	N
	S	Container, Square, 3 Cup Capacity, 7-1/8 Inch L x 7-1/8 W x 2-1/2 H, Polycarbonate Plastic, Clear; Rubbermaid or Equivalent	Cleaning – tray for sterilizing tools	All domains except D20, D04	2	N
	S	Mason Jar, Polypropylene, with Screw Lid, 1 L	Transporting used quat	All domains except D20, D04	2	N
Consumable Items						

Item No.	R/S	Description	Purpose	Conditions Used	Quantity*	Special Handling
	R	Wipe, Alcohol Pad, Individually Packaged	Sample collection - sterilizing tools	All domains except D20, D04	50	N
	R	Quaternary Ammonia 5% solution	Cleaning - sterilizing	All domains except D20, D04	10 L	N
	R	Trash bags, large	Waste disposal	All domains except D20, D04	20	N
	R	Resealable plastic bag	Waste disposal – transporting sharps container	All domains except D20, D04	2	N
	R	Paper Towels, Basic	Cleaning - drying tools	All domains except D20, D04	2 rolls	N

R/S=Required/Suggested

* Quantities are generous estimates per bout per site

6.3 Training Requirements

All technicians must complete required safety training as defined in the NEON Training Plan (AD[04]). Additionally, technicians must complete protocol-specific training for safety and implementation of this protocol as required in Field Operations Job Instruction Training Plan (AD[05]).

The training plan for small mammal abundance and diversity will minimally include the following components:

- A classroom session will be conducted prior to the field sampling to provide an overview of the procedure and the goals of the sampling. Review of the sampling equipment and the small mammal species of each site within a domain will be included.
- Prior to the start of operational field sampling and after the laboratory-based training in the proposed invasive procedures (i.e., bleeding, PIT tagging), technicians new to small mammal trapping will gain experience with these techniques in the field with wild-captured mammals through a trapping exercise on one or two training grids (depending on availability). NEON HQ or domain staff or contractor will conduct this hands-on training in the field. All methods will be the same as proposed for operational sampling, but will occur off of the long-term trapping grids and does not need to be organized with respect to the new moon. Each grid will be sampled for training purposes for a maximum of four nights per month, yielding approximately 20 – 100 individuals (reflecting capture rates between 5 and 25%) per grid for training. The species and associated relative abundances are expected to be the same as provided in the species lists for the site. These training grids will be permitted throughout the duration of the study, to provide opportunities for training whenever a new technician joins the study. It is expected that the training grids will be sampled up to a maximum of three to four times per year.
- NEON HQ or domain staff or contractor will perform hot checks of species ID on the NEON technicians involved in the data collection, if funding is made available.

All of the technicians will have access to the following materials:

- Field guide to Mammals of North America
- Electronic field guide to mammals of each particular domain
- Dichotomous keys for small mammal species at each site
- Equipment lists
- Guidelines of the American Society of Mammalogists for the Use of Wild Mammals in Research (Sikes et al. 2011)

6.4 Specialized Skills

6.4.1 Trapping

Technicians responsible for setting and checking traps must be willing and able to carry traps in bundles up to 40 pounds and wear the mandatory personal protective equipment (PPE) described in the NEON Operations Field Safety and Security Plan (AD[02]). 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).

6.4.2 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 safely 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 for reference. 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.

6.5 Estimated Time

The time required to implement a protocol will vary depending on a number of factors, such as skill level, system diversity, environmental conditions, and distance between sample plots. The timeframe provided below is an estimate based on completion of a task by a skilled two-person team (i.e., not the time it takes at the beginning of the field season). Use this estimate as framework for assessing progress. If a task is taking significantly longer than the estimated time, a problem ticket should be submitted.

An experienced two-person team will require approximately 15 - 45 minutes to set and bait one trapping grid (100 traps), depending on the difficulty of the terrain and the visibility of trap markers, if allowed at a given site. Checking the grid for disturbance and captures and transporting these traps to the processing station will require approximately 30 – 60 minutes per trapping grid per experienced two-person team. Processing time per trapping grid will vary with the number of captures on a grid. Each capture must be processed in less than 15 minutes; however, most captures should be able to be processed in less than 5 minutes. The final step in the process is to return captures to the point of capture, which should require approximately 30 – 60 minutes per trapping grid per experienced two-person team.

7 STANDARD OPERATING PROCEDURES

SOP A Flagging the Grid

Small mammal traps are arrayed in a 10 x 10 grid, with 10 meter spacing (Figure 3). Plot establishment for small mammal trapping grids as described in RD[08] must be completed prior to or concurrently with this procedure. During plot establishment, a primary marker is placed at the E5 trapping station and secondary markers at the corners, if permitted. Placing pin flags marked with the corresponding trap coordinate at each trapping location immediately prior to each sampling season or bout (depending on permitting guidelines for each site) is critical to ensure timely and accurate trap setting and checking.

		A	B	C	D	E	F	G	H	I	J
↑ NORTH	**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

Figure 3. The grid coordinate system, consisting of 100 trap stations 10 meters apart. Trapping grids are permanently marked at the E5 trap station during plot establishment, at sites that allow permanent markers. Grids may also be marked with secondary markers at the corners, if also permitted.

A.1 Procedure

1. Using a recreational GPS, navigate to either the permanent plot marker at E5, or, if available, to a secondary marker at one of the corners.

    - **Remember** that foot traffic should be restricted to paths along the North – South lettered traplines and along traplines 1 and 10, as much as possible.

2. Stretch a 100 meter tape along the trapline from a previously marked point, heading due south or due north from the marked point. Use chaining pins or similar stake at each end to hold the tape in place.

    - Use the high accuracy marked points (A1, J1, A10, J10 or E5; see Figure 3) from plot establishment to begin flagging; this will increase the accuracy of the pin flag placement.
    - The TruPulse 360R Laser Range Finder can be used as an alternative to the measuring tape in dense habitats where stretching tape is onerous.

- See RD[08] for details on using the TruPulse rangefinder.
- Using the TruPulse to mete out the 10m spacing between traps in dense or steep environments requires measuring both azimuth and distance with the rangefinder to maintain the straight line measurement (RD[08]).

3. **Place a pin flag** every ten (10) meters along the trapline. Traps will be set within 1 m of these pin flags during each sampling bout.

- For easier navigation in low light conditions, alternate flag colors along trapline rows (e.g., use orange flags in Rows A, C, E, G, I and blue flags for the remaining rows).

4. **Write the trap coordinate** (e.g., A2) with a permanent maker on each pin flag.

SOP B Preparing for Sampling

B.1 Technician Readiness

Field technicians should be prepared to identify all small mammal species in the area, but should also carry the appropriate dichotomous keys, lists of state and federally listed species, and the specified field guide in the event of the capture of a rare species. Technicians also need to be very familiar with the grid coordinate system, so that capture locations are accurately documented.

B.2 Field 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. Please note that the current protocol does not apply to studying the invasive *Rattus spp.* in Puerto Rico or Hawaii, and therefore the equipment lists included herein should not be used in those domains. Future versions of this protocol will include SOPs and equipment applicable to those domains. See Section 6.1 (Equipment) for a list of equipment necessary to complete this SOP.

B.3 Equipment and Supplies Readiness

A checklist version of this list can be found in Appendix B.

- ☑ Ensure **traps** and sampling equipment are functioning and sanitized.
- ☑ Ensure safety gear (**PPE**) is available in sufficient quantities, clean, and functioning.
- ☑ Prepare pre-printed **labels** and materials for handwriting on the sample containers in the field.
- ☑ Prepare a small vial of **10% sugar** in water to revitalize stressed, hypothermic or heat-stressed captures. Change solution often to prevent mold growth.

A clean eye drop bottle works well to administer the sugar solution.

- ☑ Ensure that all necessary field **datasheets** (RD[08])**, permits, identification keys,** and equipment (use Domain Lab checklist) are packed.

Dichotomous keys are the fastest and most reliable means for in-the-field identification. Knowing how to use one is critical for ecological field work.

Copies of permits and dichotomous keys should be stored in a field datum and never removed.

- ☑ Sterilize and mix a sufficient amount of millet (65% of seed mix) and sunflower seeds (35%).

Sterilization prevents germination of these introduced species at NEON sites. Sterilization requires baking in a thin layer for 45 - 60 minutes at 300°F.

- ☑ Prepare and maintain a **list of individuals already bled** within the current sampling bout to ensure that no individuals are bled twice within a bout.
- ☑ Prepare **quat**: if necessary, mix a batch of quat stock solution in the lab (5% solution, or 1:20 dilution in tap water). Fill up the spray bottle(s) and field stock bottles.
- ☑ Prepare **isoflurane** in a fume hood or well ventilated outdoor location. Fill up the glass bottle with rubber dropper bulb and glass bottle with screw top with isoflurane.

1 – 3 ounces should suffice for both retro-orbital blood sample collection, if planned, and/or in the event that an animal needs to be euthanized due to a serious trapping-related injury.

Always wear gloves whenever handling isoflurane.

Please note that pregnant women may want to use a respirator when handling isoflurane.

- ☑ Pack **bleeding supplies**: bring enough supplies for processing at least twice as many individuals as you expect.
- ☑ Obtain **dry ice**: this should be done as close to departure for the field as possible and stored in a dry ice cooler (e.g., Yeti or Thermosafe) prior to use.

SOP C Field Sampling

C.1 Setting traps

TIMING

- Traps are set in the evening, not more than 2.5 to 3 hours before sunset.

Setting traps too early will increase the possibility of catching opportunistic, diurnal species such as chipmunks and ground squirrels; these captures could be subjected to heat stress if not shaded.

- Time sampling so that the last trap is set as the sun is setting.

TIPS & TRICKS

- Each person should carry enough traps at one time for at least two traplines (i.e., 20 traps plus a few extra), for efficiency.
- Always walk the traplines along the N-S axis, except when moving to the next trapline. This will constrain vegetation disturbance to narrow trails within the grids over time.
- Whenever possible, place traps near shrubs, downed logs, burrows, or other microsites that offer shelter or potential runways. When placing traps next to runways (e.g., rocks, downed logs), set trap parallel to the runway.
- Make sure trap is on level ground, and the door remains open after placement. If necessary, adjust trap sensitivity by gently pulling or pushing catch.

PROCEDURE

1. Upon arrival at a grid location, place at least 2” of batting in trap, if needed.

When overnight lows will be <18°C (65°F), place approximately 5 cm (2 in) of batting into trap (except at sites dominated by heteromyids - see site-specific appendices*).*

2. Place trap within a 1 meter radius around marked point.
3. Toss bait into trap.
   a. Use about 1 TBS of the seed mix (all sites) and 1TSP of freeze-dried mealworms (at select sites where shrews comprise more than 20% of the captured individuals; see site-specific appendices).
   b. Use more bait if nighttime temperatures will be < 10°C/50°F.
   c. Toss so as to distribute seed from front to back of trap.

C.2 Checking traps the following morning

TIMING

- Begin checking traps the following morning at dawn, within 30 minutes after civil twilight.

In very hot climates or if ants are causing significant disturbance to a trapping grid, trap checking can begin up to an hour before dawn.

- Check all traps in grid before processing captures.

Counting the traps along a trapline is the best way to ensure that all traps are checked.

At the end of the sampling bout, know precisely how many traps are being retrieved from the grid, as well as any extras that may have been brought to the processing area. A final count will then indicate whether all traps have been removed from the grid.

Any trap that is accidentally left open during the day or at the end of a sampling bout will likely result in the capture and subsequent death of an animal.

TIPS AND TRICKS

- Keep in mind that the liberal use of quat on tools and gloves, the one-time use of processing bags, and the bagging of traps prior to processing is critical to minimizing transmission of pathogens between small mammals, as well as to technicians.
- Keep in mind that very small individuals can crawl under the treadle at the back of the trap and therefore are hidden from view. Be careful handling any trap with a closed door until this possibility has been ruled out. Gentle shaking and tapping of trap should encourage the individual to surface.
- Marking traps:
  - Be sure to cross out any previous markings, if present.
  - If conditions are very dry, use a wet-erase marker directly on the trap.
  - Assigning different colors of marker to the technicians facilitates resolution of any legibility or other issues that may arise in trap marking.
  - Wet erase can be easily wiped off with alcohol wipes or during the trap cleaning process.
  - If conditions are damp, use a dry erase marker directly on the trap or a permanent marker on the plastic bag into which the trap is placed.
  - If conditions are very wet, a #3 pencil can be used directly on the trap.

PROCEDURE

1. Put on PPE as specified in NEON Operations Field Safety and Security Plan.
2. If trap door is closed, QUICKLY peek inside to verify there is a capture.
   - Be quick or the animal may escape.
3. There are a variety of possible scenarios involving a closed trap (Table 12).

Table 12. Possible scenarios encountered while checking traps in the morning

Trap contents	Marking the trap	Fate of the capture	Fate of the trap
Live capture of a target or opportunistic species or dead specimen of any species	Grid coordinate	Processing station	Processing station
Live capture of a shrew	Grid coordinate, species ID, sex, fate	Mark the belly with a colored permanent marker, if not previously marked, and release*	Processing station
Live capture of a non-target species, e.g., a bird, weasel, or reptile	Grid coordinate, species (species ID, if known)	Immediate release (fate = 'L' on datasheet)	Processing station
No capture, but feces present	Grid coordinate	NA	Processing station
No capture and no feces	NA, but grid coordinate should be recorded on datasheet with appropriate code in the Notes column	NA	Remain at trap station

*To minimize handling of *Blarina spp.,* particularly by inexperienced personnel, transfer individuals to a plastic bag and then reach into the bag with the permanent marker (rather than a hand) to mark the back or belly.

4. For all traps to be removed from grid, place trap in plastic bag and place in tree-planting bag.
   - Trap can be re-used if there is no evidence (i.e., no feces or other sign) that an animal visited.
   - If feces are present in an open trap, mark and bag trap for removal to the processing station.
5. Continue checking and bagging traps in the grid.
6. Bring bagged traps to processing station once tree-planting bag is full or all traps are checked.
7. Close empty traps for the day IF another day of sampling is scheduled.
8. Remove all traps if it is the last day of sampling in a bout.

C.3 Setting up processing station

- Divide tasks between two person team:
  - Handler: Handles rodents, makes measurements, and collects blood and tissue samples.
  - Recorder: Prepares equipment and consumables, processes samples, and records data.

WORKFLOW

1. Select location for processing station that is:
   a. Immediately adjacent to, but not within, the trapping grid
   b. Upwind of staging area for animal processing, where possible
   c. Shaded, for keeping sun off of the traps in hot weather
2. Unpack and set up processing station for data collection, animal marking/tagging, anesthetization, tissue and blood sampling, and sterilization.
3. If working directly on the ground, line the area with trash bags to allow for spraying with quat and wiping clean in between individuals.
4. Prepare a small tray filled with quat to sterilize instruments between individuals.
5. Put on the additional PPE specified in the NEON Operations Field Safety and Security Plan (AD[02]) for handling animals. Sample animals for population and pathogen data, as directed below.
6. Once processed, return captures to their respective traps and release at the sites of capture while wearing the PPE required for handling animals.
7. Replace dirty traps with clean traps, either prior to or during the trap setting effort in the evening. Bring all used traps back to the lab for cleaning.
8. Reset and re-bait all traps the following dusk. Clean traps that have been baited on previous trap nights will require a smaller amount of bait, particularly on the trap door. Please note that too much bait can cause the trap to malfunction.

C.4 Processing captured animals

The small mammal field datasheet is provided separately (RD[05]), but a key to the datasheet can be found in Appendix B.

TRANSFERRING CAPTURE FROM TRAP

1. Record grid ID and point ID on Datasheet and code the "Notes" (column 16), if appropriate (see Table 1).
2. Transfer capture to resealable plastic bag by slipping bag over end of trap that opens and then turning trap over.

   - Use standard thickness gallon resealable plastic bag for species < 100 g
   - Use 4 mil gallon resealable plastic bag for species > 100 g
   - For particularly large or aggressive individuals:
     - Cloth or mesh wash bags can be used instead of a resealable plastic bag, if desired. Keep in mind that these bags must be decontaminated, via laundering or UV, before re-use on another individual.
     - Provide a thick, cotton-gloved hand for the animal to bite on as distraction
     - Cover the animal's head with a piece of material to help calm the animal during the procedure

3. Immediately observe the animal for signs of stress, and continue to do so throughout the handling period.

   - 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 10).
   - 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, at the point of capture, when the animal exhibits normal behavior.

4. Place bag on handling surface and pin the animal behind the neck. Check capture for existing marking (ear tag or RFID tag).

If during processing you notice a hole in your glove, stop, put on a new glove, and discard the used glove.

CHECKING FOR EXISTING MARKING AND BLEED HISTORY

Bleed animals that meet the following criteria:

- Member of the Cricetidae, Heteromyidae, or Dipodidae families
- Weighs at least 10 g
- No pronounced or physically debilitating injury
- Has not yet been bled during the current sampling bout
- Not excluded based on permitting regulations (e.g., protected species)

1. **Marked animals:** Check if animal has already been bled during this sampling bout (not necessary if this is the first day of a sampling bout).
    - Blood sample is not required if animal has been bled.
    - If rodent has not been bled and meets bleed criteria listed above, proceed with bleeding.
2. **Unmarked animal:** Assess if animal meets bleed criteria (listed above).
    - Meets bleed criteria: Proceed with bleeding.
    - Does not meet bleed criteria: Remove the non-anesthetized individual from the handling bag, and proceed to the section below, 'Collecting Individual Data'.

C.5 Sampling for rodent-borne pathogens

Anesthetization and blood sampling should be done in a well-ventilated area and upwind of animal being processed.

PROPER INFECTION-CONTROL TECHNIQUES

- Disinfect all equipment that is used during processing of a capture before processing the next.
- Disposable items (e.g., gauze, microhematocrit tubes, lancets, paper towels used for clean-up) should be sprayed with quat and properly disposed of (e.g., trash bag, sharps container).

BLOOD COLLECTION TECHNIQUES

- In 2014, NEON is introducing the mandibular blood sampling technique, which involves collection from the submandibular and/or facial vein or artery.
- This technique is widely used on laboratory mice, but has limited application to date in the field. It is known to work well on Peromyscus spp., but no published evidence of utility on a diversity of wild-caught species. Consequently, the retro-orbital bleeding technique is being retained as an alternative for some species, if needed.
- Technicians are expected to attempt the mandibular technique first. If a technician is unsuccessful attempting to use this technique on five (5) consecutive individuals of a given species, a problem ticket should be issued. If there is an unsuccessful attempt to collect blood from an individual, enter a 'U' in the Blood sample column on the datasheet.
- Use of the retro-orbital technique is not permitted at UNDERC. At all other sites, it is only acceptable for use on cricetids and only after five unsuccessful attempts to use the mandibular technique. After a problem ticket is issued, the FSU Vertebrate Ecologist may determine that the retro-orbital technique should be used for all subsequent blood collection at a specific site for a specific species.

Mandibular Bleeding

1. The use of anesthesia is not required for the mandibular bleeding technique, but anesthesia is recommended for use on larger animals (e.g., >100 g).

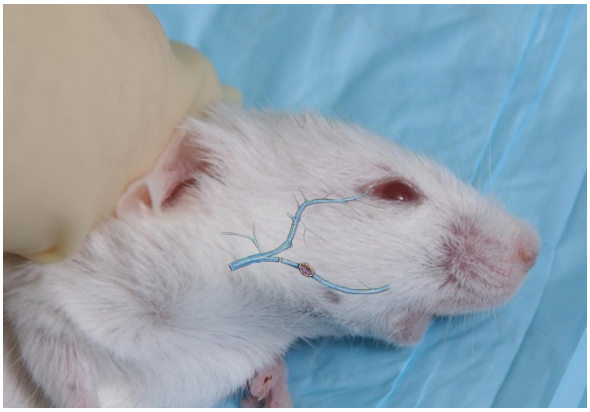

Figure 4. Schematic demonstrating where the facial vein and the submandibular vein meet at the rear end of the mandibular bone on a lab mouse (Mus). The facial vein (lower branch) is typically the target for blood collection, but the vascular bundle associated with the junction of these vessels can also be used. From http://www.medipoint.com

2. The 4 or 5 mm lancet sizes are appropriate for all species, except Rattus spp. The size affects the depth of the puncture, and so the selection of size is dependent on the force used by a

particular handler. As a rule of thumb, use the 4 mm for individuals < 20g and the 5 mm for those >20 grams.

3. Securely scruff the rodent between its shoulder blades in one hand.
4. Wipe the cheek area with an alcohol wipe and allow to dry.
5. Locate the back of the mandible using the blunt end of the lancet to determine appropriate placement of the lancet.

    - The optimal puncture point is at the back of the jaw of the mouse, very slightly behind the hinge of the jawbones, toward the ear, just behind the point at which the upper and lower jawbones meet (Golde et al. 2005).

6. Puncture the cheek with a quick, firm thrust with the lancet. Be ready with the cryovial. Tilt the head to facilitate blood flow into the cryovial. Withdraw a volume of blood less than 1% of the rodent's body weight (e.g., 0.2 mL of blood from a 20g mouse).

- Blood flow can be remarkably rapid and of high volume with this method. Familiarize yourself with the blood sample guidelines relative to the approximate fill levels in the given cryovial.
- If blood flow is too low, use the lancet to puncture the same spot with a bit more force.
- In the event of an unsuccessful attempt, the other cheek can also be used. As a rule of thumb, do not try more than two attempts per cheek.

7. When the desired amount of blood has been collected, place a fresh piece of sterile gauze over the puncture point and pinch closed for up to 30 seconds to stem further bleeding.
8. Seal the cryovial with a screw cap and apply label. See labeling guidelines in sample collection section below.
9. Dispose of lancet in sharps container, and spray used gauze with quat and place in trash bag.
10. Record the blood collection on the datasheet with an 'M' for mandibular in column # 49.
11. Proceed with animal processing, as described in next section. After processing for that individual is complete (but no more than 15 minutes after collection), place sample in 4 mil resealable plastic bag on dry ice and KEEP FROZEN until sample can be transferred to the -80°C freezer in the lab.

Blood Sample Volume Guidelines

- Hantavirus testing requires at least 0.02 mL
- Extra blood for archiving is also desirable (approximately 0.06 mL)
- Minimum sample volume = 0.08 mL
- A microhematocrit tube that has an internal opening of 1.15 mm and is 75 mm long can hold 0.075 mL of blood
- The National Institutes of Health Office of Animal Care and Use recommends a maximum sample volume of 10% of the circulating blood volume (CBV) of an animal:

Table 13. Approximate blood sample volumes for a range of body weights

Body Weight (g)	* CBV (ml)	1% CBV (ml) *every 24 hrs†*	7.5% CBV (ml) *every 7 days†*	10% CBV (ml) *every 2-4 wks†*
20	1.10 - 1.40	.011 - .014	.082 - .105	.11 - .14
25	1.37 - 1.75	.014 - .018	.10 - .13	.14 - .18
30	1.65 - 2.10	.017 - .021	.12 - .16	.17 - .21
35	1.93 - 2.45	.019 - .025	.14 - .18	.19 - .25
40	2.20 - 2.80	.022 - .028	.16 - .21	.22 - .28
125	6.88 - 8.75	.069 - .088	.52 - .66	.69 - .88
150	8.25 - 10.50	.082 - .105	.62 - .79	.82 - 1.0
200	11.00 - 14.00	.11 - .14	.82 - 1.05	1.1 - 1.4
250	13.75 - 17.50	.14 - .18	1.0 - 1.3	1.4 - 1.8
300	16.50 - 21.00	.17 - .21	1.2 - 1.6	1.7 - 2.1
350	19.25 - 24.50	.19 - .25	1.4 - 1.8	1.9 - 2.5

* Circulating blood volume

† Maximum sample volume for that sampling frequency

- For example, a 20 g individual can have approximately 1-2 microhematocrit tubes full of blood collected once a month.

Retro-Orbital Bleeding

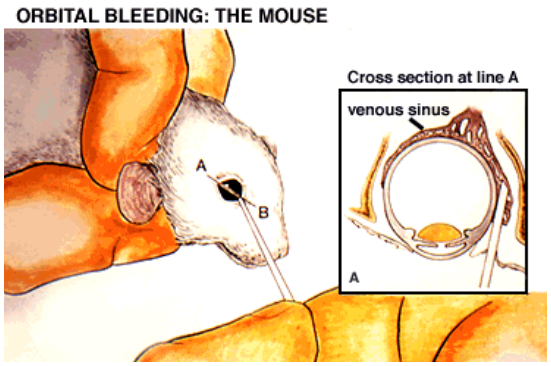

Figure 5. Schematic demonstrating the vessels that are targeted when using the retro-orbital bleeding technique on a lab mouse (Mus).

1. Anesthetize animal.
2. Hold rodent in one hand and a sterile microhematocrit tube in the other hand.
3. Place the tip of the tube at the medial canthus of the rodent's right eye underneath the nictitating membrane. Carefully push the tip of the tube around and behind the eye until slight resistance from the sinus membrane is encountered.
4. Apply gentle pressure and gently rotate the tube (back and forth, <1 rotation clockwise, <1 rotation counterclockwise) between thumb and forefinger to puncture the sinus membrane.

    - Blood will enter the microhematocrit tube as soon as the membrane is punctured.
    - Flow can be increased by withdrawing the tube slightly and gently rolling it between the thumb and forefinger.

5. Allow the blood to drain out of the back of the tube into the bottom of the cryovial. Withdraw a volume of blood less than 1% of the rodent's body weight (e.g., 0.2 mL of blood from a 20g mouse).
6. When the desired amount of blood has been collected, gently withdraw the microhematocrit tube from the eye.
7. Place a fresh piece of sterile gauze close to the eyelid and apply gentle downward for up to 30 seconds to stem further bleeding.

    - Take care not to scratch the cornea.

8. Jiggle the microhematocrit tube in the cryovial to dislodge as much remaining blood into the cryovial.

    - Use the microhematocrit tube pipette bulb to dislodge any remaining blood, only if necessary to obtain a sufficient sample volume. Apply gentle pressure to the bulb so as not to forcibly eject and potentially aerosolize infected blood.

- Attempt to expel blood into the bottom of the cryovial (rather than on the sides) and avoid getting blood on the mouth or threads of the cryovial.

9. Seal the cryovial with a screw cap and apply label. See labeling guidelines in sample collection section below.
10. After spraying with quat, dispose of microhematocrit tube in sharps container and used gauze in trash bag.
11. Record the blood collection on the datasheet with an 'R' for retro-orbital in column # 49.
12. Proceed with animal processing, as described in next section. After processing for that individual is complete (but no more than 15 minutes after collection), place sample in 4 mil resealable plastic bag on dry ice and KEEP FROZEN until sample can be transferred to the -80°C freezer in the lab.

C.6 Collecting individual data

TIPS & TRICKS

Recaptures are processed the same way as new captures. Repeated measurements of the same individual are valuable. However, if time is limited during periods of unusually high capture rates, repeated size-related measurements of recaptures can be skipped.

MARKING ANIMAL

1. **Check** capture for existing marking (ear tag or RFID tag).

   - Ear tags that do not have NEON laser-etched on them should be indicated by adding an 'O' for Other in front of the ear tag id (e.g., OL1001).

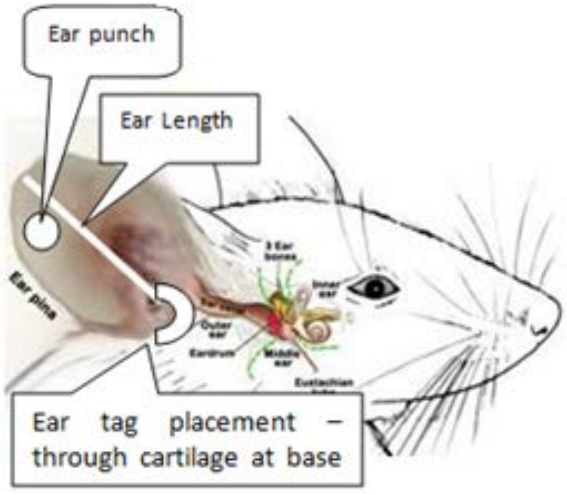

Figure 6. Schematic demonstrating the placement of an ear punch, an ear tag, and how the ear length measurement should be made on a lab mouse (Mus).

2. **Mark** the individual (if needed) with ear tag or PIT tag.

    - Use ear tag if pinnae are of sufficient size. Attach to right ear.
    - Use PIT tag if pinnae too small for ear tag. (This is the often the case with voles and pocket mice).
    - Shrews should not be marked with these methods.
    - After spraying with quat, dispose of used PIT tag needle in sharps container. To prevent needle sticks, needles should not be recapped prior to discarding into the sharps container.
    - PIT tag reader should be kept in a sealed plastic bag, to facilitate disinfection (i.e., the bag can be sprayed with quat and wiped clean after each use).

3. **Record** the tag number on datasheet in ear/PIT columns (#41 – 46).

    - Format for ear tag is LXXXX for left ear or RXXXX for right ear (preferred).
    - Use last six digits of serial number for PIT tags and place one bar code sticker on the back of the datasheet.
    - Be sure to fill in the Fate column (48), and whether there is evidence that a previous tag was lost (47).

4. Once the individual is successfully marked, **label** all sample vials with the unique individual ID, as described below.

ASSESSING AGE, SEX, AND REPRODUCTIVE CONDITION

1. Note animal age: juvenile, sub-adult, or adult in column 22 of datasheet (see also quick reference in Appendix A).
2. Note sex and reproductive condition and enter codes in columns 21 and 23 – 26 of datasheet.

    Males

    - Scrotal (S): testes may be descended (obvious bulging under the tail)
    - Non-scrotal (N): testes not descended (abdominal)

    Females

    - Nipples
        - Enlarged (E)
        - Not enlarged (N)
    - Pregnant (P)
        - Pregnancy can be determined by palpating the abdomen for fetuses or by assessing the width of the pubic symphysis

- Vagina
  - Swollen (S) – indicates estrous
  - Plugged (P) – (some use the term Perforate)
  - Neither (N) – (some use the term Non-perforate)

TAKING MEASUREMENTS

Take and record standard measurements to the nearest millimeter, using standard rounding guidelines:

1. 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).
2. Take additional measurements, at your discretion, if useful for species discrimination. Refer to the dichomotous key for guidance.

- **Ear length:** 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.
- **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.
- **Total length**: 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.

IDENTIFYING TO SPECIES

1. Once all measurements have been taken, the individual should be identified to species, and the corresponding species code (listed on the site-specific datasheet) entered on the datasheet.
2. If there is any uncertainty in the species identification, please note this in the idQ (i.e., identification qualifier) column on the datasheet – using one of the codes below (Table 11). Leave blank if there is not uncertainty.
3. Use the digital camera to take pictures of species for which the identification is uncertain (except in very common cases like Peromyscus maniculatus vs. P. leucopus), the species is very rare or has state or federal status, or if there is something unusual or noteworthy about a particular individual.

Table 14. Codes for identification qualifier entries

idQ	Identification Qualifier Description*
CS	cf. species
AS	aff. species
CG	cf. genus
AG	aff. genus
CF	cf. family
AF	aff. family

* cf. roughly equals "not sure"; aff. roughly equals "similar to, but is not"

C.7 Collecting samples

If possible, use different forceps for each sample. Forceps and scissors should be cleaned with quat and the biopsy punch cleaned with an alcohol wipe and then flamed with lighter in between processing individuals.

SPECIMEN LABEL AND STORAGE REQUIREMENTS

- Site Code (e.g., RMNP)
- Date (Year, month, day)
- Tag ID (RXXXX or last 6 digits of PIT tag)
- Sample Type (B for blood; E for ear; F for feces; H for hair and whiskers)

EXAMPLE:
OSBS.20130714.R1357.B

Note: Some information may be pre-printed.

- Use ethanol-safe pen for writing on cryovial labels, coin envelopes, and voucher tags.
- Use fine point permanent marker (Sharpie or equivalent) for writing directly on the cryovials. Can also be used for writing on coin envelopes.

Table 15. Summary of non-blood samples to be collected.

Sample	Frequency	Storage container	Label	Field storage	Long-term storage
Hair	Once per bout; dominant genus	Archival coin envelope	Write on envelope	Ambient	Ambient
Whiskers	Once per bout; dominant genus				
Fecal	Every capture event	Vial rated to -80°C	Label rated to -80°C	Dry ice	-80 °C Ultralow freezer
Ear punch	Once per life of individual	Vial rated to -80°C	Label rated to -80°C	Dry ice	-80°C Ultralow freezer
Vouchers	Opportunistically	Resealable plastic bag	Acid-free, archival tag	Dry ice	standard freezer

1. For individuals belonging to the site-specific dominant genus (see site-specific appendices):

    - Collect a tuft (approximately 5 hairs) of hair from the scruff of the individual with forceps.
    - Clip off 2 whiskers with cuticle scissors (or similar). Clip as close to the base as possible without injury – one from each cheek – from the front half of the nose.
    - Place hair and whiskers in archival coin envelope together.
    - Indicate on datasheet (columns # 52, 53) if samples collected.

For all target and opportunistic species:

2. Collect any fresh, uncontaminated feces from the animal using either forceps or scooping the sample directly with the cryovial and label.

    - If fresh feces are not available, collect feces from the trap if only one individual was captured in the trap.

    

    Fresh feces are preferred, as immediate freezing will preserve more genetic material for sequencing.

    - Store cryovial on dry ice for transport back to the lab.
    - Indicate on datasheet (column # 50) if sample was collected and condition ("F" for fresh feces and "O" for older feces collected from the trap).

3. Collect one ear tissue sample – one from near the edge of the right, untagged ear - using a clicker-style 2000 Micron (2 mm) tissue biopsy punch or iris scissors.

    - Place ear tissue in cryovial and label.
    - Indicate on datasheet (column # 51) if sample is collected.

4. Record the weight, to the nearest gram, using the appropriate, tared spring scale. Record on datasheet in column #37 -40.
5. Make sure all cryovials are labeled correctly, put in cooler with dry ice, and entered on the datasheet. Hair and whiskers are stored at ambient temperature.
6. Put animal back into trap (trap is still in plastic bag) for transport back to point of capture.

    - Process all individuals on a grid before releasing animals at point of capture, unless extra personnel are available.

7. Follow clean up procedures described in next section before processing the next animal to avoid cross contamination.

C.8 Cleanup between processing of individuals

Equipment is cleaned and sterilized in the field between processing of individuals to prevent cross contamination. The recorder is typically responsible for these tasks.

1. After spraying with quat, place all contaminated consumables (e.g., paper towels, plastic bags, gauze, and cotton) in the trash bag.
2. Place all used small instruments in the tray filled with quat for at least 5 full seconds (e.g., clip of spring scale only, forceps, biopsy punch, tea infuser spoon, scissors).

    - Dry instruments with paper towel before use.

3. Spray quat on larger reusable equipment (e.g., insulated cooler, squirt bottle containing quat). Use paper towels to carefully and thoroughly wipe the surfaces.
4. Spray down the processing surface with quat. Wipe processing surface with paper towels.
5. Always spray contaminated areas of clothing with quat as soon as possible.
6. Spray quat on gloved hands of animal handler and then own hands.

    - Wipe hands together and dry with paper towels as needed.
    - Sterilized gloves can be reused with the next animal but should be changed if a hole develops and after each grid is completed.

7. You are now ready to process the next animal.

C.9 Voucher specimens

Dead animals in good condition should be collected as voucher specimens. Dead animals should be processed only after all the live ones have been processed. Blood, whisker, hair, and ear tissue samples do not need to be collected from dead individuals.

1. Label a specimen tag with the site ID, tag ID, sex, species, and date of capture.
   a. Use archival quality Pigma pen or, if this is not available, a hard lead pencil (#3) to write information on tag.
2. Securely affix the tag to the right hind foot above the ankle using cotton thread. Since long threads tend to become tangled during storage, tie the tag as close to the leg as possible.
3. Place the animal in a resealable plastic bag and immediately place in the cooler. Avoid placing directly on dry ice.

Note: If carcasses are not saved for voucher specimens (e.g., if they are in poor condition), they should be sprayed liberally with disinfectant, double-bagged, and placed in the trash bag.

C.10 End of the sampling day

SAMPLES AND SPECIMENS

1. Keep all samples stored in cryovials on dry ice for transport back to the lab.
   - Frozen samples must remain frozen at all times. Repeated freeze-thaw cycles will compromise the integrity of the sample.
2. Transfer frozen samples to ultralow freezer (-80°C).
3. Store voucher specimens in a standard freezer (≤ 0° C but ≥ -20° C).

EQUIPMENT – IN THE FIELD

When all animals are processed and released, clean up as described in previous section, plus do the following.

1. Spray used batting with quat and dispose of in trash bag.
2. Pack up all supplies and equipment, once they are clean and dry.
3. Clean PPE as directed by EHS Safety Policy and Program Manual and dispose final round of nitrile gloves and wipes.
4. Close and tie the trash bag(s). Place bag in bed of pickup truck for transport back to the lab
5. If another night of sampling is scheduled, be sure that all traps are closed until dusk.
6. Be sure to replace dirty traps with clean ones.
7. If another night is not scheduled, collapse all traps and place in large plastic bag.

- Place bag in bed of pickup truck for transport back to the lab or outside of passenger compartment of vehicle to prevent exposure to any remaining pathogens, if present.
- Be sure to keep clean and dirty traps separate, as dirty traps should not be reused until they have been cleaned and sterilized.

8. Be sure that all soiled disposable sharps (e.g., PIT tag needles, heparinized microhematocrit tubes) have been disposed of in a 1 quart, slip-top, OSHA NIOSH/CDC compliant sharps container in the field, and is in a sealed plastic bag when not in use.

SOP D Equipment Cleaning in the Laboratory

D.1 Timing

Cleaning of dirty traps occurs at the Domain Support Facility, at the earliest convenience of the domain staff. Cleaning should follow the guidelines provided in the NEON Operations Field Safety and Security Plan (AD[02]). Traps must be cleaned before re-deployment in the field. If stored, dirty traps must be stored in plastic bags in a well-ventilated area posted with Biohazard per Biosafety Level 2 requirements.

D.2 Equipment Cleaning and Disposal Procedures

To reduce the risk of disease transmission to technicians and among captured small mammals, thorough cleaning and disinfection of equipment that is known to have been in contact with small mammals is required. Small mammal traps that have contained trapped animals will be removed from the trapping grid, transported back to the domain lab in a plastic bag, where dirty traps will be thoroughly cleaned, as described below. These traps will be replaced with clean traps before the next night of trapping. The bags containing dirty traps will then be placed in the bed of a pickup truck, which is separated from the passenger compartment. Back at the laboratory, a solution of quaternary ammonia (follow manufacturer guidelines for dilution) or a 10% bleach solution (i.e., 1:10 dilution with water) should be mixed in a carboy, or similar container. Cleaning is best performed in a well-ventilated area, while wearing the full PPE required for handling small mammals at a given site. Chemical-resistant rubber gloves should be worn in place of nitrile gloves. Traps must then be soaked in the quaternary ammonia solution for a minimum of 10 minutes (as specified by the manufacturer guidelines) or the bleach solution for 10 seconds, and scrubbed out with stiff bristled brushes. Traps will be rinsed thoroughly with water, to avoid damage and to remove as much of the disinfectant scent as possible.

SOP E Laboratory Sampling and Analysis

E.1 Sample processing timing

Process all frozen samples immediately upon returning to the lab. Once samples are frozen, they must remain frozen; plan accordingly. Please note that all subsequent instruction in this SOP pertain to the blood samples only; instructions will be added for the remaining samples whenever institutions for archiving those samples have been identified. A subset of the ear tissue samples will be used in SOP G.

E.2 Preparation

1. Print out a rodent-borne pathogen laboratory datasheet (RD[05]).
2. Be sure there are enough cryovial storage boxes to accommodate all collected blood samples. Label new boxes as necessary.

E.3 Blood sample processing in the lab

1. Transfer each cryovial into a well in a labeled cryovial storage box.
2. Transfer cryovial storage boxes containing cryovials into an ultralow (-80°C) freezer.
3. For each blood sample, record relevant information on the rodent-borne pathogen laboratory datasheet (RD[05]) and then enter into a computer using the NEON Raw Data Ingest Workbook for TOS Rodent-borne Pathogen Sampling (RD[07]).

E.4 Sample preservation

1. Store samples in an ultralow (-80°C) freezer until they are sent to an external facility for processing.
2. Samples must remain frozen at all times

SOP F Processing for Genetic Analysis

F.1 Sample Processing Timing

A subset of ear tissue samples will be prepared for DNA barcoding, to provide quality assurance of species identification. Selection of samples for barcoding should not occur prior to the end of field sampling or October, whichever is earlier. DNA barcode samples must be submitted (shipped to a barcoding facility such as the Canadian Centre for DNA Barcoding - CCDB) by January of the following calendar year in which they were collected.

F.2 Preparation

1. Select the small mammal ear tissue samples to be barcoded.
    a. Select up to 285 individuals for 3 DNA barcode plates per domain (95 per plate). The following rationale should be used to select specimens based on the confidence in the species identification:
        1) A minimum of 5 individuals per species recorded in a domain should be sent for barcoding annually.

            - Where possible, select samples from a variety of sampling dates and locations within a domain.
            - Additionally, if there is obvious morphological variation within a species, select specimens that represent that variation.

        2) Individuals with species identifications that have uncertainty associated with them are the priority for the remaining wells.

            - Uncertainty should have been recorded in the identificationQualifier field on the small mammal field datasheet (RD[05]).
            - For these species, submit either 20 individuals or 21% of the individuals sampled (whichever is higher, if space is available). If species are present at multiple sites in the domain, include representatives from all possible sites.

2. Print one 96-well datasheet per plate (RD[09]).
3. Prepare a workspace with shipping box, 96-well microplate with row caps loosely attached, forceps, lighter, pencil or ethanol-safe pen, and gloves.

F.3 Sample Processing in the Lab

Fill each microplate completely (95 specimens) before shipping.

1. Wipe down the work area with 95% ethanol.
2. Prepare 95 specimens for barcoding.
   a. Work with a single microplate at a time and enter all data before proceeding to the next microplate.
   b. To keep samples frozen, stage cryovials containing samples using a lab-top cooling device, dry ice bed, or similar.
   c. Fill out the 96 well datasheet with the plate number, sample location in the plate, barcode lab and tag ID.
   d. DO NOT place any foreign objects (e.g. labels) into sampling wells. If something falls into a well (e.g., eyelash), note it and do not place a sample into that well. Move to the next well.
   e. Cover wells that are not in use with row caps. Note that strip caps are numbered and correspond to numbering on plates.
   f. Prior to beginning, and between each specimen, flame-sterilize forceps for at least 2 seconds to ensure that no residual tissue is present.
   g. Place the ear tissue sample in the well (Figure 7).
      1) Static from the plate and on the forceps can make it difficult to get the tissue into the well.
      2) Be gentle so that the sample does not end up in a different well.
      3) It can be helpful to gently tap the forceps to shake the tissue into the well. If it has gone into a different well, do not remove it.

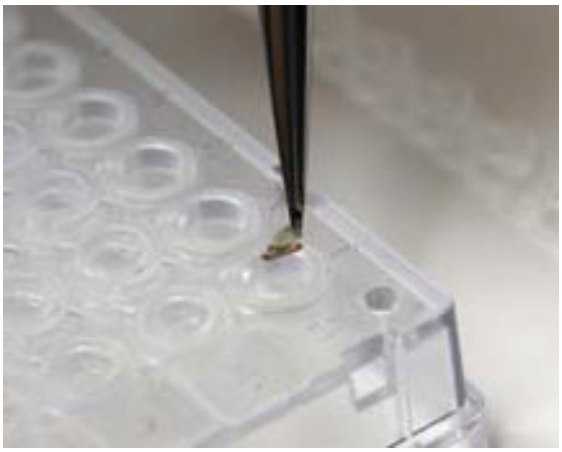

Figure 7. Placing tissue in a well.

   h. Repeat until you have filled all wells in the microplate, **EXCEPT** for well H12 which always remains empty as a negative control (fill 95 wells).
   i. Cover plate.
   j. Complete data entry prior to filling a new plate.

F.4 Sample Preservation

Store plates in a standard (-20°C) freezer until shipped.

F.5 Data Handling

STOP after each plate and enter the Plate number and Sample location into the Barcode Plate datasheet. Plate number is the unique plate number recorded on the 96-well datasheet. Sample location refers to the position each sample occupies in the plate (A01-H12). Have another technician double-check data entry.

See SOP G for further details on data handling.

F.6 Sample Shipping

Once the data have been quality checked, ship the plates. Ship 96-well microplates overnight with dry ice to external facilities along with a hard copy of the 96-well datasheet for each plate (RD[09]).

See SOP H for further details on sample shipment.

SOP G Data Entry and Verification

1. Enter information from datasheet into NEON CI digital system. This should be done at the end of the sampling day. If not possible, then as soon as possible, but no more 14 days after the end of the sampling bout.
   a. Enter data in format provided in Excel file (RD[06]). Follow QA/QC procedures for ensuring accurate transcription of data (RD[04]).
   b. Include notes on all deviations from procedures. Indicate what was done and why.
   c. Mam_capturedata Table:

      - Enter the data from the rows on the datasheet, including only entries for traps with capture data, traps with any values in the Notes field, or plots with no captures indicated in the Notes field. Be sure that every record has a value in the date and plot fields. If a trap coordinate is not written down, but the record applies at the trap-level (not the plot-level), enter a 'XX' into the trap coordinate field. All other fields can be left blank, if they are blank on the datasheet.

   d. Mam_pernight Table:

      - Enter a record for each combination of plotID and date when trapping was scheduled to occur. If weather or other circumstances prevent trapping when scheduled, enter a '0' in the trapsSet field and provide the details of the circumstance in the remarks field.
      - Enter the employee ID of the technicians involved into the measuredBy and recordedBy fields. If more than one technician was involved, add the IDs into additional columns., naming the fields aMeasuredBY, bMeasuredBy, etc.

   e. Mam_perbout Table

      - Enter a record for each sampling bout at each site, indicating the startDate of the bout and the versions of the sampling protocol and dichotomous keys used.

2. Scan and then file hard copy of datasheet, as described in RD[06].

SOP H Sample Shipment

Information included in this SOP conveys science-based shipping and handling requirements, not lab-specific or logistical demands. For that information, reference the CLA shipping document on CLA's NEON intranet site.

Shipping details are TBD and will be added in a later revision of this document (as of Rev E).

H.1 Handling Hazardous Material

TBD

H.2 Supplies/Containers

TBD

H.3 Timelines

Blood samples will be shipped via overnight service.

H.4 Conditions

Blood samples will be shipped on dry ice.

H.5 Grouping/Splitting Samples

TBD

H.6 Return of Materials or Containers

TBD

H.7 Shipping Inventory

Whenever a batch of blood samples is shipped, the batch must be accompanied by a hard-copy shipping manifest enclosed within the shipping container AND a corresponding electronic version of the manifest (excel file) emailed to the testing facility.

The hard-copy shipping manifest lists every blood sample in the shipped batch. Blood samples should be listed in ascending order by storageBoxLabel and then by storageBoxWell on the hard-copy shipping manifest. Do not sort the list according to bloodSampleID or any other variable(s). Include the following fields from the NEON Raw Data Ingest Workbook for TOS Rodent-borne Pathogen Sampling (RD[07]): bloodSampleID, storageBoxLabel, storageBoxWell, senderID, dateSent, and receiverID. An example of a populated hard-copy manifest is provided in Figure 7.

bloodSampleID	storageBoxLabel	storageBoxWell	senderID	dateSent	receiverID
OSBS.20130714.R1357.B	1	A1	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130714.R1366.B	1	A2	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130714.R1298.B	1	A3	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130714.R1101.B	1	A4	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130714.R1109.B	1	B1	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130714.R2286.B	1	B2	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130714.R2183.B	1	B3	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130714.R1632.B	1	B4	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1777.B	1	C1	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R2483.B	1	C2	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1072.B	1	C3	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1384.B	1	C4	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1350.B	2	A1	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1200.B	2	A2	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1091.B	2	A3	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1375.B	2	A4	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R1998.B	2	A5	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico
OSBS.20130715.R2981.B	2	A6	R. Nelson, D03	20131001	B. Hjelle, U. New Mexico

Figure 8. Example of a hard-copy shipping manifest

The electronic manifest is an excel file that should be emailed to the testing facility as soon as possible after a batch of samples has been shipped. It is an electronic version of the corresponding hard-copy manifest that additionally contains all of the remaining data columns in the full NEON Raw Data Ingest Workbook for TOS Rodent-borne Pathogen Sampling (RD[07]). These remaining data columns, which are blank, will be filled in with testing data and metadata by the testing facility. The order of samples in the electronic manifest should be the same as the order in the corresponding hard-copy shipping manifest.

H.8 Laboratory Contact Information and Shipping/Receipt Days

See the CLA shipping document on CLA's NEON intranet site.

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APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 16. Datasheets associated with this protocol

NEON Doc. #	Title
NEON.DOC.001585	Datasheets for TOS Protocol and Procedure: Small Mammal Sampling
NEON.DOC.001582	Lab Datasheet: 96-Well Plate

These datasheets can be found in Agile or the NEON Document Warehouse.

APPENDIX B QUICK REFERENCES

Quick Reference: Checking Traps and Processing Captures–I

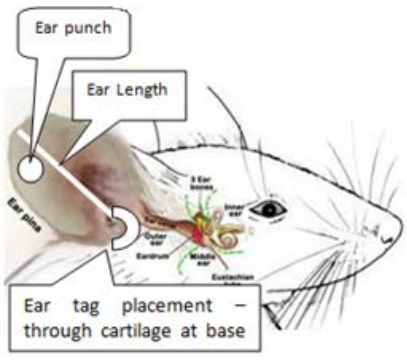
STEP 1 - Check ALL traps in grid for captures.

IF trap door CLOSED	**IF trap door OPEN**
Peek inside to see if there is a capture or feces. Immediately release non-target captures. Write grid coordinate on trap. Place trap in plastic bag, for transport to processing station (even if capture has been released)	Clean trap can be reused. Traps with feces only should be marked, bagged, and taken to processing station. If mid-bout, leave empty trap in place and **close** door. On last day of sampling, remove trap.

STEP 2 – Set up processing station.
STEP 3 – Transfer capture to plastic bag (use stronger 4 mil bags if capture > 100g).
STEP 4 – Assess animal for signs of stress. Treat/release as needed.
STEP 5 – Check for existing marking, identify species, and assess if blood sampling is required.

CRITERIA FOR BLOOD SAMPLING	**DO NOT** collect blood from:
COLLECT blood from: • Cricetids that are > 10 g • Dipodids that are > 10 g – mandibular technique only • Heteromyids that are > 10 g – mandibular technique only **UNLESS** individual has: • Pronounced or physically debilitating injury, and/or • Already been captured and bled during **current** sampling bout.	• Sciurids - chipmunks, squirrels, etc. • Soricids – shrews • Talpids – moles • Geomyids – pocket gophers • Murids – house mice and introduced rats Any protected species listed on state or federal permit

STEP 6 – Proceed with bleeding, if required.
STEP 7 – Mark Individual (if needed)



MARKING GUIDELINES

- Do not tag shrews or non-target species.

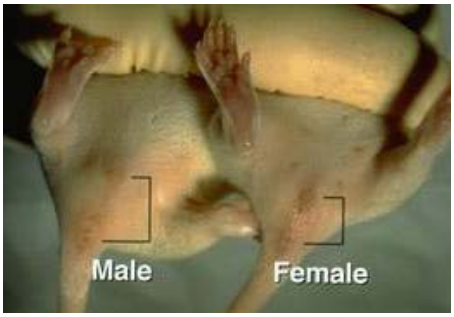
Ear Tag:
- Use if pinnae are of sufficient size.
- Right (R) ear preferred.
- Record ear (L or R) and ID number on datasheet.
- For recaptures, ensure that NEON is on one side of the tag. If not, record 'O' for other and the tag number.

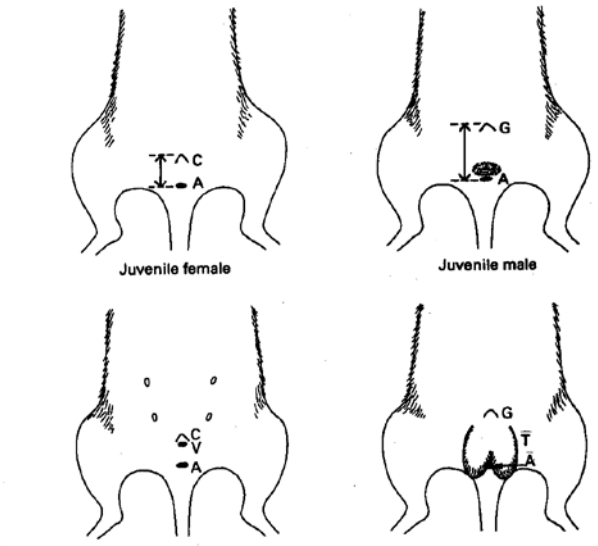
PIT Tag:
- Use if pinnae are of insufficient size (e.g., voles and pocket mice)
- Record last 6 digits of tag # on datasheet.
- Dispose of needles in Sharps container.
- Place bar-code sticker on back of datasheet.

Quick Reference: Checking Traps and Processing Captures–II

STEP 8 – Assess age, sex and reproductive condition.

Most juvenile cricetids have a uniform steely grey.





External sex difference in rodents. A = anus; C = clitoris; G = genital papilla; V = vaginal opening; T = testes. The A-C distance in female rodents is less than the A-G distance in males. From Watts and Aslin 1981.

STEP 9 – Measure hind foot length (all species).

STEP 10 – Measure ear length/tail length, and/or total length (if needed for species ID)

STEP 11 – Collect remaining samples

Sample	Description	Frequency	Storage container	Label	Field storage
Hair	tuft (~5 hairs) from back of neck	once per bout; dominant genus	Archival coin envelope	Write on envelope	Ambient
Whiskers	snip at base, one from each cheek	once per bout; dominant genus			
Fecal	fresh (preferred) or from trap	every capture event	vial rated to -80°C	Label rated to -80°C	Dry ice
Ear punch	punch from outer margin of right untagged ear	once per life of individual	vial rated to -80°C	Label rated to -80°C	Dry ice
Vouchers	entire specimens	opportunistically	resealable plastic bag	Acid-free, archival tag	Dry ice

STEP 12 – Weigh

STEP 13 – Put back in trap for transport back to point of capture and release.

STEP 14 – Clean all gloves and tools with quat before using on the next individual.

Label all containers→

Site ID
YR, MO, DAY
Tag #
Sample Type
MO/DAY

TAG ID

Quick Reference: Decision Tree for Challenging Conditions

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.

MORTALITIES

- **Each mortality must be (a) reported to the Field Operations Manager and (b) reported in a problem ticket within 24 hours** of processing, in accordance with all state- and site-specific permit requirements.
- **If ≥ 5 individuals** on any given trapping grid during a single night of trapping **die, the trapping** on that grid should be **discontinued** until the next scheduled sampling bout, and a **problem ticket should be issued** detailing the locations, species, sex, and ages of the mortalities.

LOGISTICAL HURDLES

- If there is any chance (i.e., >0%) that traps cannot be checked the following morning at dawn, traps shall not be set (e.g., impassable roads).
- If one night of trapping within the sampling bout is missed, **trapping may be resumed** and continued as normal at any time **within 5 days** of the latest night of trapping.
- If **predators** destroy **>15** traps on a single grid on any given night, traps should be removed from the site and that sampling bout terminated prematurely. The Field Operations Manager shall be informed as soon as possible, and a problem ticket should be issued.

COLD WEATHER

- **Bedding** should be used when low temperatures are expected to be **< 18°C (65°F), except** at sites where heteromyids dominate.
- **Trapping should not occur** on nights when both (2) **freezing** temperatures (< 0°C (32°F)) and precipitation in the form of **rain** are expected (>20% chance at sites with bedding; >5% chance at sites that cannot use bedding).

HOT WEATHER

- When ambient temperatures are expected to reach 27°C (80°F), by 10 a.m., extra effort must be made to **ensure that all traps** containing captured **individuals are processed or placed in the shade as soon as possible**. Initiation of trap checking before sunrise may be necessary in extremely hot environments, especially where shade is lacking.
- If trap checking **does not begin** prior to 8 - 9 am and **all traps are not shaded**, all traps should be opened to release animals and avoid heat-induced mortality. Work should start over that night by resetting the traps.

Quick Reference: Small Mammal Sampling Datasheet

Column Number(s)	Data Field	Description/What to Enter
1 – 8	Year, Month, Day	Format YYYYMMDD e.g., 20130728
9 – 13	plotID	Enter number only (There may be extra spaces in this field.)
13-14	PT	Indicate point of capture with grid coordinate e.g., B5
13	Notes	Information on trap condition and quality. Use codes (1 through 6) from top left corner of datasheet. See below.
16-19	Genus and Species	Use 4 letter codes listed on top of datasheet. Create own code if captured species not listed and record full name in Add'l notes section or on back of Datasheet.
20	idQ – identification Qualifier	CS – cf. species; cf. = "not sure"; AS – aff. species; aff. = "similar to, but is not"
21	Sex	Male (M) or female (F)
22-26	Age and reproductive status	Use uppercase letter codes from datasheet
27-28	HFL	Hind foot length (mm) – to nearest millimeter
29-30	Ear	Right ear length (mm) – to nearest millimeter
31-33	Tail	Tail length (mm) – round to nearest millimeter
34-36	TTL	Total length (head + body) (mm)
37-40	WGT	Weight (g) – round to nearest gram
41 – 46	Ear/PIT	Unique tag ID, format: Ear tags: RXXXX or LXXXX; PIT tags: **last** 6 digits of serial number
47	Ear tag replaced	Indicate to which ear new tag was attached (if applicable). Enter 'New' into fate field (48).
48	Dead, New, Recap, Release	Indicate history/condition of capture. Use uppercase code on datasheet. Dead supercedes the other option.
49 -53	Samples	Indicate type of sample collected.
54	Esc, Comments	Indicate if the individual escaped before all data could be collected ("E") or if there are comments on back of datasheet ("C").

- If traps are not set on a particular grid as scheduled or if no individuals are captured, these should be recorded in the NOTES field as a line on the datasheet for that given date, grid, and bout combination.
- If traps are damaged or disturbed overnight, note trap locations and code the nature of the disturbance on the datasheet in the NOTES field, with any other known details described in the 'add'l notes' cell.

NOTES codes:

Definitions	Application Rules
1 – Traps not set	can be used at the level of the entire trapping grid, or, if some traps on a grid were set and others were not, can be used to indicate which ones were not set.
2 – trap door closed but empty	used per trap coordinate, when necessary
3 – trap door open with feces left behind	used per trap coordinate, when necessary
4 – trap disturbed	used per trap coordinate; if entire trapping grid is disturbed, can be used at the grid level
5—trap ID suspect *	used per trap coordinate, when necessary
6—no captures	used ONLY at the per grid level

*(indicates uncertainty in trap coordinate – e.g., if two traps have the same coordinate, or if smudging of marker reduces legibility)

APPENDIX C REMINDERS

Trapping Small Mammals

Always have on hand:

- ☑ Copy of IACUC protocol form and IACUC approval letter
- ☑ Copy of state collection permit
- ☑ Copy of site-specific research permit
- ☑ Personal ID
- ☑ GPS with grid locations
- ☑ Compass to aid in following trap lines

Layout of Small Mammal Sampling Grid

	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

Setting Traps:

- ☑ Each person should carry enough traps at one time for at least two traplines (i.e., 20 traps plus a few extra), for efficiency.
- ☑ Always walk the traplines along the N-S axis, except when moving to the next trapline. This will constraint vegetation disturbance to narrow trails within the grids over time.
- ☑ Whenever possible, place traps near shrubs, downed logs, burrows, or other microsites that offer shelter.
- ☑ Place trap, making sure trap is on level ground and the door remains open. If necessary, adjust trap sensitivity by gently pulling or pushing catch.
- ☑ Bait trap, by distributing a teaspoon up to a tablespoon (use more in colder conditions) of seed mix throughout the trap, including near the entrance to the trap.
- ☑ When overnight lows will be <18°C (65°F), place approximately 5 cm (2 in) of batting into trap (except at sites dominated by heteromyids).

Trapping Equipment

- Traps (100 per grid + extras)
- Tree planting bags (1 per person or per 40-50 traps)
- Bait: Sterilized millet & sunflower seed (mealworms, where needed).
- Lumbar bags of bait (1 per person)
- Batting and bag for carrying batting
- Replacement pin flags and sharpies

Checking Equipment

- Replacement traps
- Trap-sized plastic bags
- Work gloves
- Tree planting bags (1 per person or per 40-50 traps)
- Plastic bags to line tree planting bags
- Wet or dry erase markers & sharpies

Getting Ready for Small Mammal Sampling

- ☑ Ensure all **traps** and sampling equipment is functioning and sanitized.
- ☑ Ensure safety gear (**PPE**) is available in sufficient quantities, clean, and functioning.
- ☑ Prepare pre-printed **labels** and materials for handwriting on the sample containers in the field.
- ☑ Prepare a small vial of **10% sugar** in water to revitalize stressed, hypothermic or heat-stressed captures. Change solution often to prevent mold growth.
- ☑ Sterilize and mix a sufficient amount of millet (65% of **seed** mix) and sunflower seeds (35%).
- ☑ Ensure that all necessary **datasheets, identification keys,** and equipment (use Domain Lab checklist) are packed.
- ☑ Prepare and maintain a **list of individuals already bled** within the current sampling bout to ensure that no individuals are bled twice within a bout.
- ☑ Prepare **quat**: if necessary, mix a batch of quat stock solution in the lab (5% solution, or 1:20 dilution in tap water). Fill up the spray bottle(s) and field stock bottles.
- ☑ Prepare **isoflurane**: in a fume hood or well ventilated outdoor location fill up the glass bottle with rubber dropper bulb and glass bottle with screw top with isoflurane.
- ☑ Pack **supplies**: bring enough supplies for processing at least twice as many individuals as you expect.
- ☑ Obtain **dry ice**: this should be done as close to departure for the field as possible and stored in an ultra-low freezer (-80°C) prior to use.

Personal Protective Equipment (PPE)

- Eye protection (safety glasses to discourage rubbing of the eyes and protect from extreme splashes)
- Disposable respirators with N95 (HEPA-equivalent) particulate filter
- Required: all sites in CO, NM, AZ, CA
- Recommended: all sites in KS, TX, UT, MT, NV, ID, OR, and WA
- Latex and/or nitrile gloves
- Long-sleeved shirt
- Long pants
- Close-toed shoes with socks
- a disposable or reusable (cotton) laboratory coat or apron
- Special cloth gloves with rubber-tipped fingers may be worn to facilitate the handling (latex/nitrile gloves should be worn over these

APPENDIX D ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING

Specific dates are TBD, as small mammals can be trapped year-round at most locations. Site-specific details may be added in a future revision of this document (as of Rev E).

APPENDIX E SITE-SPECIFIC INFORMATION

E.1 D01 – HARV – Harvard Forest

Trapping Issues

- Slugs after rainfall events may fill traps
- Large (i.e., bear) and medium sized carnivore (e.g., fishers, raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Recommended when low temperatures are expected to be <18°C/65°F.

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Cardoza, Jones, & French, 2009. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported in Degrassi & Ellison, 2013**.**

Table 17. Site-specific species list (HARV)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Myodes gapperi	Gapper's Red-backed Vole	150 - 500
Microtus pennsylvanicus	Meadow Vole	0 - 50
Napaeozapus insignis	Woodland Jumping mouse	0 - 50
Peromyscus leucopus	White footed mouse	50 - 300
Peromyscus maniculatus	N. American deer mouse	200 - 600
Microtus pinetorum	Woodland Vole	0 - 20
Synaptomys cooperi	Southern Bog Lemming	0 - 20
Zapus hudsonius	Meadow Jumping Mouse	0 - 20
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina brevicauda	Northern Short-tailed Shrew	10 - 100
Sorex cinereus	Masked Shrew	0 - 30
Sorex fumeus	Smoky Shrew	0 - 30
Tamias striatus	Eastern Chipmunk	10 - 100
Sorex dispar	Long-tailed Shrew	0 - 20
Sorex hoyi	American Pygmy Shrew	0 - 20
Sorex palustris	American Water Shrew	0 - 20
Tamiasciurus hudsonicus	Red Squirrel	0 - 20

NON-TARGET ANIMALS (Scientific and Common Name)		
Parascalops breweri	Hairy-tailed Mole	0 - 20
Scalopus aquaticus	Eastern Mole	0- 20
Glaucomys volans	Southern Flying Squirrel	0 – 20
Glaucomys sabrinus	Northern Flying Squirrel	0 - 20

E.2 D01 – RELOCATABLE - BART (Barlett Experimental Forest)

Trapping Issues

- Large (i.e., bear) and medium sized carnivore (e.g., fishers, raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Recommended when low temperatures are expected to be <18°C/65°F.

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Kays and Wilson 2011. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Ryan B. Stephens (pers. comm.) based on 2013 trapping efforts.

Table 18. Site-specific species list (BART)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Microtus chrotorrhinus	Rock Vole	0-20
Microtus pennsylvanicus	Meadow Vole	0-20
Microtus pinetorum	Woodland Vole	0-20
Myodes gapperi	Southern Red-backed Vole	50-200
Peromyscus leucopus	White-footed Deermouse	50-200
Peromyscus maniculatus	North American Deermouse	50-200
Synaptomys borealis	Northern Bog Lemming	0-5
Synaptomys cooperi	Southern Bog Lemming	0-5
Napaeozapus insignis	Woodland Jumping Mouse	100-500
Zapus hudsonius	Meadow Jumping Mouse	0-20
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina brevicauda	Northern Short-tailed Shrew	50-200
Sorex cinereus	Cinereus Shrew	50-200
Sorex dispar	Long-tailed Shrew	0-5
Sorex fumeus	Smoky Shrew	50-200
Sorex hoyi	American Pygmy Shrew	0-5
Sorex palustris	American Water Shrew	0-5
Tamias striatus	Eastern Chipmunk	0-50
Tamiasciurus hudsonicus	Red Squirrel	0-50

NON-TARGET ANIMALS (Scientific and Common Name)		
Condylura cristata	Star-nosed Mole	0-5
Glaucomys sabrinus	Northern Flying Squirrel	0-5
Glaucomys volans	Southern Flying Squirrel	0-50
Martes americana	American Marten	0-5
Martes pennanti	Fisher	0-5
Mustela erminea	Ermine	0-5
Mustela frenata	Long-tailed Weasel	0-5
Neovison vison	American Mink	0-5
Parascalops breweri	Hairy-tailed Mole	0-5
Scalopus aquaticus	Eastern Mole	0-5
Sciurus carolinensis	Eastern Gray Squirrel	0-5
Sylvilagus floridanus	Eastern Cottontail	0-5
Sylvilagus transitionalis	New England Cottontail	0-5

E.3 D02 – CORE – SCBI (Smithsonian Conservation Biology Institute)

Trapping Issues

- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Kays and Wilson 2011. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by McShea et al. 2003 and Osbourne et al. 2005.

Table 19. Site-specific species list (SCBI)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Myodes gapperi	Southern Red-backed Vole	0-50
Microtus pennsylvanicus	Meadow Vole	0-50
Microtus pinetorum	Woodland Vole	0-50
Mus musculus musculus	House mouse	0-20
Neotoma magister	Allegheny Woodrat	0-20
Peromyscus leucopus	Northern white-footed mouse	100-500
Peromyscus maniculatus	North American deer mouse	100-500
Rattus norvegicus	Norway rat	0-5
Rattus rattus	Black rat	0-5
Reithrodontomys humulis	Eastern Harvest Mouse	0-20
Synaptomys cooperi	Southern Bog Lemming	0-50
Napaeozapus insignis	Woodland Jumping Mouse	50-200
Zapus hudsonius	Meadow Jumping Mouse	50-200
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina brevicauda	Northern Short-tailed Shrew	50-200
Cryptotis parva	North American Least Shrew	0-5
Sorex cinereus	Cinereus Shrew	50-200
Sorex fumeus	Smoky shrew	50-200
Sorex hoyi	American Pygmy Shrew	0-50

Sorex longirostris	Southeastern shrew	0-5
Tamias striatus	Eastern Chipmunk	0-5
Tamiasciurus hudsonicus	Red Squirrel	0-5
NON-TARGET ANIMALS (Scientific and Common Name)		
Condylura cristata	Star-nosed Mole	0-5
Glaucomys volans	Southern Flying Squirrel	0-50
Mustela frenata	Long-tailed weasel	0-5
Mustela nivalis	Least weasel	0-5
Mustela vison	Common mink	0-5
Parascalops breweri	Hairy-tailed Mole	0-5
Scalopus aquaticus	Southern mole	0-5
Sciurus carolinensis	Eastern Gray Squirrel	0-5

E.4 D03 – CORE – OSBS (Ordway-Swisher Biological Station)

Trapping Issues

- Fire ants
  - If fire ant activity is present in the immediate vicinity of a trapping station, be sure to dust the ground under and around the trap with Talstar granules.
  - Traps can also be set closer to sunset and checked earlier, to minimize traps being open when ants are most active.
- Medium sized carnivore (e.g., coyotes, raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or traps are in any danger of becoming water-logged overnight.

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on the Annotated Mammal Species List of the Ordway-Swisher Biological Station. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Dr. Bob McCleery at the University of Florida.

Table 20. Site-specific species list (OSBS)

TARGET species (Scientific and Common Name)		Number to be Used Annually
Neotoma floridana floridana	Florida wood rat	50 - 250
Peromyscus gossypinus gossypinus	Cotton mouse	200 - 1000
Peromyscus polionotus subgriseus	Oldfield mouse	100 - 500
Podomys floridanus	Florida Deermouse	100 - 500
Sigmodon hispidus hispidus	Hispid Cotton Rat	0 - 50
Ochrotomys nuttalli	Golden Mouse	0 - 5
Oryzomys palustris natator	Marsh rice rat	0 - 50
Reithrodontomys humulis	Eastern Harvest Mouse	0 - 50
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina carolinensis	Southern Short-tailed Shrew	0 - 20
Cryptotis parva floridana	Least shrew	0 - 20
Sorex longirostris	Southeastern Shrew	0 - 20
NON-TARGET ANIMALS (Scientific and Common Name)		
Geomys pinetis floridanus	Florida pocket gopher	0 - 5
Scalopus aquaticus australis	Southern mole	0 - 5
Glaucomys volans	Southern Flying Squirrel	0 - 20

E.5 D03 – RELOCATABLE – DISN (Disney Wilderness Preserve)

Trapping Issues

- Fire ants
  - If fire ant activity is present in the immediate vicinity of a trapping station, be sure to dust the ground under and around the trap with Talstar granules.
- Medium sized carnivore (e.g., coyotes, raccoons) disturbance
- Cattle disturbance – square croquet wickets can be used to secure the traps in place to minimize disturbance.
- Do not trap when freezing temperatures are coupled with precipitation, or traps are in any danger of becoming water-logged overnight.

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on the Mammals of Florida | American Society of Mammalogists. The abundance estimates were based on an average capture rate of 10%, and, in the absence of existing data, estimates of captures are based on data for Ordway Swisher.

Table 21. Site-specific species list (DISN)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Neotoma floridana	Eastern Woodrat	50 - 250
Peromyscus gossypinus	Cotton Deermouse	200 - 1000
Peromyscus polionotus	Oldfield Deermouse	100 - 500
Podomys floridanus	Florida Deermouse	100 - 500
Sigmodon hispidus	Hispid Cotton Rat	0 - 50
Ochrotomys nuttalli	Golden Mouse	0 - 5
Oryzomys palustris	Marsh Oryzomys	0 - 50
Mus musculus	House mouse	0 - 50
Rattus norvegicus	Norway rat	0 - 50
Rattus rattus	Black rat	0 - 50
Reithrodontomys humulis	Eastern Harvest Mouse	0 - 50
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina carolinensis	Southern Short-tailed Shrew	0 - 20
Cryptotis parva floridana	Least shrew	0 - 20
Sorex longirostris	Southeastern Shrew	0 - 20

NON-TARGET ANIMALS (Scientific and Common Name)		
Geomys pinetis	Southeastern Pocket Gopher	0 - 5
Scalopus aquaticus	Eastern Mole	0 - 5
Glaucomys volans	Southern Flying Squirrel	0 - 20

E.6 D03 – RELOCATABLE – JERC (Jones Ecological Research Center)

Trapping Issues

- Fire ants
  - If fire ant activity is present in the immediate vicinity of a trapping station, be sure to dust the ground under and around the trap with Talstar granules.
- Medium sized carnivore (e.g., coyotes, raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation.

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Kays & Wilson, 2011 and Smith et al., 2006. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported in Ford et al. 1994.

Table 22. Site-specific species list (JERC)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Microtus pinetorum	Woodland Vole	0-50
Mus musculus	House mouse	0-20
Neotoma floridana	Eastern Woodrat	0-20
Ochrotomys nuttalli	Golden Mouse	0-50
Oryzomys palustris	Marsh Oryzomys	0-20
Peromyscus gossypinus	Cotton Deermouse	0-20
Peromyscus polionotus	Oldfield Deermouse	0-20
Rattus norvegicus	Norway rat	0-20
Reithrodontomys humulis	Eastern Harvest Mouse	0-20
Sigmodon hispidus	Hispid Cotton Rat	0-20
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina carolinensis	Southern Short-tailed Shrew	0-5
Cryptotis parva	North American Least Shrew	0-50
Sorex longirostris	Southeastern Shrew	0-50
Tamias striatus	Eastern Chipmunk	0-50
NON-TARGET ANIMALS (Scientific and Common Name)		
Geomys pinetis	Southeastern Pocket Gopher	0-5

Glaucomys volans	Southern Flying Squirrel	0-5
Mustela frenata	Long-tailed Weasel	0-5
Mustela vison	Mink	0-5
Scalopus aquaticus	Eastern Mole	0-50
Sciurus carolinensis	Eastern Gray Squirrel	0-5
Sciurus niger shermani	Sherman's fox squirrel	0-5
Sylvilagus floridanus	Eastern Cottontail	0-5
Sylvilagus palustris	Marsh Rabbit	0-5

E.7 D05 – CORE – UNDE (University of Notre Dame Environmental Research Center)

Trapping Issues

- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Kays and Wilson 2011. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Kilcline 2003.

Table 23. Site-specific species list (UNDE)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Myodes gapperi	Southern Red-backed Vole	0-20
Microtus pennsylvanicus	Meadow Vole	0-50
Mus musculus	House mouse	0-20
Peromyscus leucopus	White-footed Deermouse	0-20
Peromyscus maniculatus	North American Deermouse	0-50
Synaptomys cooperi	Southern Bog Lemming	0-50
Napaeozapus insignis	Woodland Jumping Mouse	0-20
Zapus hudsonius	Meadow Jumping Mouse	0-50
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina brevicauda	Northern Short-tailed Shrew	0-50
Sorex arcticus	Arctic Shrew	0-50
Sorex cinereus	Cinereus Shrew	0-50
Sorex hoyi	American Pygmy Shrew	0-5
Sorex palustris	American Water Shrew	0-5
Tamias minimus	Least Chipmunk	0-50
Tamias striatus	Eastern Chipmunk	0-5
Tamiasciurus hudsonicus	Red Squirrel	0-5
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0-5
NON-TARGET ANIMALS (Scientific and Common Name)		
Condylura cristata	Star-nosed Mole	0-5

Glaucomys sabrinus	Northern Flying Squirrel	0-5
Glaucomys volans	Southern Flying Squirrel	0-5
Lepus americanus	Snowshoe Hare	0-5
Sylvilagus floridanus	Eastern Cottontail	0-5
Sciurus carolinensis	Eastern Gray Squirrel	0-5
Martes pennanti	Fisher	0-5
Mustela erminea	Ermine	0-5
Mustela frenata	Long-tailed Weasel	0-5
Mustela nivalis	Least Weasel	0-5

E.8 D07 – CORE – ORNL (Oak Ridge National Laboratory)

Trapping Issues

- Fire ants
  - If fire ant activity is present in the immediate vicinity of a trapping station, be sure to dust the ground under and around the trap with Talstar granules.
- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Kays and Wilson 2011. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Giffen et al. 2011.

Table 24. Site-specific species list (ORNL)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Microtus chrotorrhinus	Rock Vole	0-20
Microtus ochrogaster	Prairie Vole	0-20
Microtus pennsylvanicus	Meadow Vole	0-20
Microtus pinetorum	Woodland Vole	0-20
Mus musculus	House mouse	0-20
Neotoma floridana	Eastern Woodrat	0-20
Neotoma magister	Allegheny Woodrat	0-20
Ochrotomys nuttalli	Golden Mouse	0-20
Oryzomys palustris	Marsh Oryzomys	0-20
Peromyscus leucopus	White-footed Deermouse	400-1000
Peromyscus maniculatus	North American Deermouse	0-20
Rattus norvegicus	Norway rat	0-20
Reithrodontomys humulis	Eastern Harvest Mouse	0-20
Sigmodon hispidus	Hispid Cotton Rat	0-20
Synaptomys cooperi	Southern Bog Lemming	0-5
Napaeozapus insignis	Woodland Jumping Mouse	0-20
Zapus hudsonius	Meadow Jumping Mouse	0-20

OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina brevicauda	Northern Short-tailed Shrew	50-200
Cryptotis parva	North American Least Shrew	0-5
Glaucomys volans	Southern Flying Squirrel	0-50
Sorex cinereus	Cinereus Shrew	0-5
Sorex dispar	Long-tailed Shrew	0-5
Sorex fumeus	Smoky Shrew	0-5
Sorex hoyi	American Pygmy Shrew	0-5
Sorex longirostris	Southeastern Shrew	0-5
Tamias striatus	Eastern Chipmunk	50-200
NON-TARGET ANIMALS (Scientific and Common Name)		
Mustela frenata	Long-tailed Weasel	0-5
Neovison vison	Mink	0-5
Scalopus aquaticus	Eastern Mole	0-5
Sciurus carolinensis	Eastern Gray Squirrel	0-5
Sciurus niger	Eastern Fox Squirrel	0-5
Sylvilagus floridanus	Eastern Cottontail	0-5

E.9 D08 – CORE – TALL (Talladega National Forest)

Trapping Issues

- Fire ants
  - If fire ant activity is present in the immediate vicinity of a trapping station, be sure to dust the ground under and around the trap with Talstar granules.
- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation.

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Kays and Wilson 2011. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Adams et al. 2013.

Table 25. Site-specific species list (TALL)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Microtus pinetorum	Woodland Vole	0-20
Neotoma floridana	Eastern Woodrat	0-20
Ochrotomys nuttalli	Golden Mouse	50-200
Oryzomys palustris	Marsh Oryzomys	0-20
Peromyscus gossypinus	Cotton Deermouse	0-20
Peromyscus leucopus	White-footed Deermouse	400-1000
Peromyscus polionotus	Oldfield Deermouse	0-20
Reithrodontomys humulis	Eastern Harvest Mouse	0-20
Sigmodon hispidus	Hispid Cotton Rat	50-200
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina carolinensis	Southern Short-tailed Shrew	0-5
Cryptotis parva	North American Least Shrew	0-5
Sorex longirostris	Southeastern Shrew	0-5
Tamias striatus	Eastern Chipmunk	50-200
Mus musculus	house mouse	0-50
NON-TARGET ANIMALS (Scientific and Common Name)		
Glaucomys volans	Southern Flying Squirrel	0-5
Mustela frenata	Long-tailed Weasel	0-5

Geomys pinetis	Southeastern Pocket Gopher	0-5
Neovison vison	American Mink	0-5
Scalopus aquaticus	Eastern Mole	0-5
Sciurus carolinensis	Eastern Gray Squirrel	0-5
Sciurus niger	Eastern Fox Squirrel	0-5
Sylvilagus aquaticus	Swamp Rabbit	0-5
Sylvilagus floridanus	Eastern Cottontail	0-5

E.10 D09 – CORE – WOOD (Woodworth Field Station)

Trapping Issues

- Medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Microtus

Species List and Abundance Estimates

This species list is based on Kays and Wilson 2011. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Wiewel et al. 2007.

Table 26. Site-specific species list (WOOD)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Onychomys leucogaster	Northern Grasshopper Mouse	0-20
Peromyscus maniculatus	North American Deermouse	50-200
Reithrodontomys megalotis	Western Harvest Mouse	0-50
Microtus pennsylvanicus	Meadow Vole	400-1000
Zapus princeps	Western Jumping Mouse	0-20
Zapus hudsonius	Meadow Jumping Mouse	50-200
Microtus ochrogaster	Prairie Vole	0-20
Myodes gapperi	Southern Red-backed Vole	0-20
Perognathus fasciatus	Olive-backed Pocket Mouse	0-20
Perognathus flavescens	Plains Pocket Mouse	0-20
Peromyscus leucopus	White-footed Deermouse	0-20
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina brevicauda	Northern Short-tailed Shrew	0-50
Sorex arcticus	Arctic Shrew	0-5
Sorex cinereus	Cinereus Shrew	0-50
Spermophilus franklinii	Franklin's Ground Squirrel	0-50
Spermophilus richardsonii	Richardson's Ground Squirrel	0-5
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0-50
Sorex haydeni	Prairie Shrew	0-5
Tamiasciurus hudsonicus	Red Squirrel	0-5
Tamias striatus	Eastern Chipmunk	0-5

Rattus norvegicus	Norway rat	0-5
Mus musculus	House mouse	0-5
NON-TARGET ANIMALS (Scientific and Common Name)		
Mustela frenata	Long-tailed Weasel	0-5
Mustela nivalis	Least Weasel	0-5
Mustela vison	Mink	0-5
Sciurus niger	Eastern Fox Squirrel	0-5
Thomomys talpoides	Northern Pocket Gopher	0-5
Sylvilagus nuttallii	Mountain Cottontail	0-5
Mustela erminea	Ermine	0-5
Sylvilagus floridanus	Eastern Cottontail	0-5
Sciurus carolinensis	Eastern Gray Squirrel	0-5

E.11 D10 – CORE – CPER (Central Plains Experimental Range)

Trapping Issues

- Kangaroo rats require larger traps (12" long rather than 9"), to minimize trap-related injuries to their long tails.
- Coyote disturbance
- Cattle disturbance – square croquet wickets can be used to secure the traps in place to minimize disturbance.
- **Do not trap** when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: NOT recommended under any circumstances.

Dominant genus for hair & whisker sampling purposes: Dipodomys

Species List and Abundance Estimates

This species list is based on the Shortgrass Steppe LTER website (http://sgs.cnr.colostate.edu/species_list.aspx, accessed 2012). Abundance estimates were based on an average capture rate of 10%, and the species-specific abundances reported in Evangelista et al., 2008.

Table 27. Site-specific species list (CPER)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Chaetodipus hispidus	Hispid Pocket Mouse	0 - 50
Dipodomys ordii	Ord's Kangaroo Rat	300 - 1000
Microtus longicaudus	Long-tailed Vole	0 - 50
Microtus pennsylvanicus	Meadow Vole	0 - 50
Neotoma cinerea	Bushy-tailed Woodrat	0 - 50
Onychomys leucogaster	Northern Grasshopper Mouse	50 - 400
Perognathus fasciatus	Olive-backed Pocket Mouse	0 - 50
Perognathus flavescens	Plains Pocket Mouse	0 - 50
Perognathus flavus	Silky Pocket Mouse	5 - 100
Peromyscus maniculatus	N. American Deer Mouse	25 - 300
Reithrodontomys megalotis	Western Harvest Mouse	5 - 200
Reithrodontomys montanus	Plains Harvest Mouse	5 - 100
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Cryptotis parva	North American Least Shrew	0 - 20
Spermophilus spilosoma	Spotted Ground Squirrel	0 - 20
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0 - 20

NON-TARGET ANIMALS (Scientific and Common Name)		
Geomys bursarius	Plains Pocket Gopher	0 - 20
Mustela frenata	Long-tailed Weasel	0 - 5

E.12 D10 – RELOCATABLE – STER (Sterling)

Trapping Issues

- Coyote disturbance
- Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Recommended only when low temperatures are expected to be <18°C/65°F; depends on the relative abundance (RA) of heteromyid species – stop use if RA of heteromyids exceeds 50%

Dominant genus for hair & whisker sampling purposes: Onychomys

Species List and Abundance Estimates

This species list is based on the North Sterling State Park website (http://www.parks.state.co.us/parks/northsterling/Pages/NorthSterling.aspx, accessed 2012). Abundance estimates were based on an average capture rate of 10%, and provided relative abundances.

Table 28. Site-specific species list (STER)

TARGET species (Scientific and Common Name)		Number to be Used Annually
Chaetodipus hispidus	Hispid Pocket Mouse	0 - 50
Dipodomys ordii	Ord's Kangaroo Rat	0 - 50
Microtus ochrogaster	Prairie Vole	0 - 50
Microtus pennsylvanicus	Meadow Vole	0 - 50
Neotoma cinerea	Bushy-tailed Woodrat	0 - 5
Onychomys leucogaster	Northern Grasshopper Mouse	0 - 50
Perognathus flavescens	Plains Pocket Mouse	0 - 50
Perognathus flavus	Silky Pocket Mouse	0 - 50
Peromyscus maniculatus	Deer Mouse	5 - 500
Reithrodontomys megalotis	Western Harvest Mouse	5 - 100
Reithrodontomys montanus	Plains Harvest Mouse	0 - 50
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Cryptotis parva	Least Shrew	0 - 20
Mus musculus	House Mouse	0 - 50
Sorex cinereus	Masked Shrew	0 - 20
Spermophilus spilosoma	Spotted Ground Squirrel	0 - 20
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0 - 20
NON-TARGET ANIMALS (Scientific and Common Name)		
Geomys bursarius	Plains Pocket Gopher	0 - 20

E.13 D10 – RELOCATABLE – RMNP (Rocky Mountain National Park)

Trapping Issues

- Cold temperatures, wind, and snow
  - Do not trap when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).
- Bear and elk disturbance

Use of bedding: Recommended when low temperatures are expected to be <18°C/65°F

Dominant genus for hair & whisker sampling purposes: Peromyscus

Species List and Abundance Estimates

This species list is based on Armstrong, 2008, as well as the NEON 2012 capture data. The abundance estimates were based on an average capture rate of 10%, with declining returns of unique individuals over the consecutive nights of a sampling bout, as well as the species-specific relative abundances reported in Maher, 2010.

Table 29. Site-specific species list (RMNP)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Myodes gapperi	Red-backed vole	50 - 200
Lemmiscus curtatus	Sagebrush Vole	20 - 100
Microtus longicaudus	Long-tailed Vole	20 - 200
Microtus montanus	Montane Vole	20 - 200
Neotoma cinerea	Bushy-tailed Woodrat	0 - 50
Neotoma mexicana	Mexican Woodrat	0 - 5
Peromyscus maniculatus	N. American Deermouse	500 - 2000
Peromyscus nasutus	Northern Rock Deermouse	0 - 50
Zapus princeps	Western Jumping Mouse	5 – 100
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Sorex cinereus	Cinereus Shrew	0 - 25
Sorex hoyi	American Pygmy Shrew	0 - 25
Sorex merriami	Merriam's Shrew	0 - 25
Sorex monticolus	Dusky Shrew	0 - 50
Sorex nanus	Dwarf Shrew	0 - 25
Sorex palustris	American Water Shrew	0 – 10
Spermophilus elegans	Wyoming Ground Squirrel	0 - 50
Spermophilus lateralis	Golden-mantled Ground Squirrel	5 - 50
Spermophilus variegatus	Rock Squirrel	0 - 10
Tamias minimus	Least Chipmunk	100 - 500
Tamias quadrivittatus	Colorado Chipmunk	0 - 20

Tamias umbrinus	Uinta Chipmunk	20 - 100
Tamiasciurus hudsonicus	Red Squirrel	0 - 50
NON-TARGET ANIMALS (Scientific and Common Name)		
Thomomys talpoides	Northern Pocket Gopher	0 - 50

E.14 D15 – CORE – ONAQ (Onaqui-Benmore)

Trapping Issues

- Kangaroo rats require larger traps (12" long rather than 9"), to minimize trap-related injuries to their long tails.
- Coyote disturbance
- **Do not trap** when freezing temperatures are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: TBD – depends on the relative abundance of heteromyids

Dominant genus for hair & whisker sampling purposes: TBD

Species List and Abundance Estimates

This species list is based on Kays and Wilson 2011. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Baker and Frischknecht 1973 and Smith and Urness 1984.

Table 30. Site-specific species list (ONAQ)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Onychomys leucogaster	Northern Grasshopper Mouse	0-50
Peromyscus truei	Pinon Deermouse	0-50
Peromyscus maniculatus	North American Deermouse	400-1000
Reithrodontomys megalotis	Western Harvest Mouse	50-200
Lemmiscus curtatus	Sagebrush Vole	50-200
Microtus pennsylvanicus	Meadow Vole	0-20
Microtus longicaudus	Long-tailed Vole	0-20
Microtus montanus	Montane Vole	0-20
Neotoma cinerea	Bushy-tailed Woodrat	0-20
Peromyscus boylii	Brush Deermouse	0-20
Neotoma lepida	Desert Woodrat	0-20
Peromyscus crinitus	Canyon Deermouse	0-20
Dipodomys microps	Chisel-toothed Kangaroo Rat	0-50
Dipodomys ordii	Ord's Kangaroo Rat	100-500
Perognathus parvus	Great Basin Pocket Mouse	400-1000
Zapus princeps	Western Jumping Mouse	0-20
Chaetodipus formosus	Long-tailed Pocket Mouse	0-20
Microdipodops megacephalus	Dark Kangaroo Mouse	0-20

OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Sorex cinereus	Cinereus Shrew	0-5
Sorex palustris	American Water Shrew	0-5
Sorex vagrans	Vagrant Shrew	0-5
Sorex merriami	Merriam's Shrew	0-5
Ammospermophilus leucurus	White-tailed Antelope Squirrel	0-50
Tamiasciurus hudsonicus	Red Squirrel	0-5
Tamias dorsalis	Cliff Chipmunk	0-5
NON-TARGET ANIMALS (Scientific and Common Name)		
Spermophilus variegatus	Rock Squirrel	0-5
Sylvilagus audubonii	Desert Cottontail	0-5
Sylvilagus nuttallii	Mountain Cottontail	0-5
Brachylagus idahoensis	Pygmy Rabbit	0-5

APPENDIX F BLEED GRID DESIGNATION

At each Observatory site, NEON will conduct small mammal abundance and diversity sampling on a set of trapping grids distributed among the major vegetation types (AD[06]). There will generally be between three and eight grids per site. According to the NEON science design for vectors and pathogens (AD[09]), blood samples to be used in rodent-borne pathogen testing will be collected on one or more of these grids following their designation as bleed grids. There will generally be three long-term bleed grids per site.

Long-term bleed grids will be designated such that they collectively span the range of small mammal communities (i.e. levels of diversity and abundance) present at a site to the greatest degree possible. The designation of bleed grids thus requires sufficient grid-level data on variation in small mammal abundance and diversity for each site. At least one and sometimes two years of operational sampling will be required to generate these data. During this period, provisional bleed grids may be designated. Instructions for bleed grid designation during the first, second, and subsequent years of operational sampling at a site are provided below.

Instructions related to bleed grid designation:

1. **Sites in the first year of operations:** no grid-level data on small mammal abundance and diversity are available, so neither long-term nor provisional bleed grids can be identified. During the first year of operations, bleeding should be performed on a minimum of three sampling grids. These grids should be those with the highest abundance of target species based on the first bout of small mammal abundance and diversity sampling. In the event that absolute capture rates on these grids are low, blood samples can be collected on as many additional grids as logistics will allow. Expanding the number of grids on which blood samples are collected will increase sample sizes for rodent-borne pathogen testing and allow for additional opportunity to gain experience with associated methods. Once bleeding on a given grid is initiated it should continue for the remainder of the first year of operations. This condition is particularly important for the three high abundance grids and is desirable but not critical for additional grids.
2. **Sites in the second year of operations:** grid-level data on small mammal abundance and diversity should be available for designation of bleed grids, but the quality and/or amount of these data may not be adequate to designate long-term bleed grids with confidence. In the interim, FSU will designate provisional bleed grids in this appendix. In the event that absolute capture rates on these grids are low, blood samples can be collected on as many additional grids as logistics will allow. If additional grids are being considered, inform FSU (via JIRA) so that input on grid selection can be provided.

Blood samples MUST be collected on provisional bleed grids for the duration of the second year of operational sampling. Similar continuity is desirable but not required for any additional grids on which blood samples are collected. At the conclusion of the second year of operations, domain staff should reference this appendix to track any changes in bleed grid designation (e.g., changes in the identification of provisional bleed grids, selection of long-term bleed grids).

3. **Sites for which long-term bleed grids have been designated:** Following the second year of operational sampling, sufficient data should be available to designate long-term bleed grids. FSU will designate these grids in this appendix. Blood samples MUST be collected on long-term bleed grids for all remaining sampling at the site unless FSU provides instructions to the contrary.

   As above, blood samples can be collected on additional grids if absolute catch rates on long-term bleed grids are low and logistics allow for blood collection on additional grids. If additional grids are being considered, inform FSU (via JIRA) so that input on grid selection can be provided. Continuity of sampling on additional bleed grids is highly desirable. Thus, these should be selected so that they can be sampled during all bouts in a particular year, ideally over multiple years.

Table 31. Bleed grid designations, field season 2014

Domain	Site	First year ops	Provisional bleed grids	Long-term bleed grids
D01	BART	X		
D01	HARV		HARV_008	
D01	HARV		HARV_016	
D01	HARV		HARV_021	
D02	SCBI	X		
D03	DSNY		DSNY_001	
D03	DSNY		DSNY_004	
D03	DSNY		DSNY_005	
D03	JERC	X		
D03	OSBS		OSBS_003	
D03	OSBS		OSBS_004	
D03	OSBS		OSBS_023	
D05	UNDE	X		
D07	ORNL	X		
D08	TALL	X		
D09	WOOD	X		
D10	CPER		CPER_005	
D10	CPER		CPER_011	
D10	CPER		CPER_015	
D10	STER		STER_001	
D10	STER		STER_002	

Domain	Site	First year ops	Provisional bleed grids	Long-term bleed grids
D10	STER		STER_003	
D15	ONAQ	X		