

Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# **TOS PROTOCOL AND PROCEDURE:**

## **SMALL MAMMAL SAMPLING**

PREPARED BY	ORGANIZATION	DATE
Katherine M. Thibault	FSU	02/15/2015
Yuri Springer	FSU	01/15/2015
Liz Knapp	HR	12/31/2013

APPROVALS	ORGANIZATION	APPROVAL DATE
Andrea Thorpe	SCI	02/16/2015
Kirsten Ruiz	OPS	03/20/2015
Mike Stewart	SYS	03/23/2015

RELEASED BY	ORGANIZATION	RELEASE DATE
Judy Salazar	СМ	03/23/2015

See configuration management system for approval history.

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Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# **Change Record**

REVISION	DATE	ECO#	DESCRIPTION OF CHANGE
A_DRAFT	07/11/2012	ECO-00469	Draft release
B_DRAFT	01/24/2014	ECO-01181	Draft release. Will finalize in next rev.
С	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	12/05/2014	ECO-02530	Migration to new protocol template
F	03/23/2015	ECO-02644	Decreased sampling bout duration for abundance and diversity grids from three nights to one. Changed cold temperature thresholds for trapping. Removed retroorbital bleeding technique. Removed Heteromyidae from bleeding list and added Muridae. Added prioritization of processing steps. Added equipment and protocol modifications for D04, D19, D20. Added sp codes. Removed datasheet column references due to changes in datasheet; updated datasheet quick references.

Date: 03/23/2015

NEON Doc. #: NEON.DOC.000481

Author: K. Thibault

Revision: F

## **TABLE OF CONTENTS**

1	OVE	ERVIEW	1
	1.1	Background	1
	1.2	Scope	1
	1.2.	1 NEON Science Requirements and Data Products	1
	1.3	Acknowledgments	2
2	REL	ATED DOCUMENTS AND ACRONYMS	2
	2.1	Applicable Documents	2
	2.2	Reference Documents	2
	2.3	Acronyms	3
	2.4	Definitions	3
3	ME	тнор	4
	3.1	Sampling Goals	4
	3.2	Trapping design	4
	3.3	Personnel	5
	3.4	Timing of trapping	6
	3.5	Bait	6
	3.6	Processing	7
	3.6.	1 Marking	9
	3.6.	2 Animal Care	9
4	SAN	MPLING SCHEDULE	10
	4.1	Sampling Frequency and Timing	10
	4.2	Criteria for Determining Onset and Cessation of Sampling	11
	4.3	Timing for Laboratory Processing and Analysis	15
	4.4	Sampling Timing Contingencies	15
5	SAF	ETY	17
	5.1	Working with Small Mammals	17
6	PER	SONNEL AND EQUIPMENT	18
	6.1	Clothing and PPE	18
	6.2	Equipment	20



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

6	.3	Traini	ng Requirements	38
6	.4	Specia	alized Skills	39
	6.4.	1 T	rapping	39
	6.4.2	2 F	landling	39
6	.5	Estima	ated Time	39
7	STA	NDARE	O OPERATING PROCEDURES	.40
SOF	Α	FLAG	GING THE GRID	.40
SOF	В	PREPA	ARING FOR SAMPLING	.42
SOF	C	FIELD	SAMPLING	.44
SOF	D	EQUIF	PMENT CLEANING IN THE LABORATORY	.62
SOF	E	LABO	RATORY SAMPLING AND ANALYSIS	.63
SOF	F	PROC	ESSING FOR GENETIC ANALYSIS	.64
SOF	G	DATA	ENTRY AND VERIFICATION	.67
SOF	Н	SAMP	PLE SHIPMENT	.68
8	REF	ERENC	ES	.70
APF	PENDI	ХА	DATASHEETS	.74
APF	PENDI	ХВ	QUICK REFERENCES	.75
APF	PENDI	хс	REMINDERS	.79
APF	PENDI	X D	ESTIMATED DATES FOR ONSET AND CESSATION OF SAMPLING	.81
APF	PENDI	ΧE	SITE-SPECIFIC INFORMATION	.82
APF	PENDI	ΧF	BLEED GRID DESIGNATION1	41

Revision: F

Author: K. Thibault

## **LIST OF TABLES AND FIGURES**

NEON Doc. #: NEON.DOC.000481

Table 1. Summary of sampling frequencies by site and grid type*	11
Table 2. Descriptions and prescriptions of codes used in Notes field of small mammal datasheet	
Table 3. Contingent decisions	
Table 4. Equipment list – Flagging the grid, one bout	20
Table 5. Equipment list – Trapping, one bout	
Table 6. Equipment list – Checking traps and processing captures, one bout	
Table 7. Equipment list – Cleaning and sterilization, one bout	
Table 8. Equipment list – Cleaning traps, per bout	
Table 9. Equipment list – Preparing blood samples	34
Table 10. Equipment list – Shipping blood samples	35
Table 11. Equipment list – Genetic analysis	
Table 12. Possible scenarios encountered while checking traps in the morning	
Table 13. Approximate blood sample volumes for a range of body weights	
Table 14. List of codes for genus-level identifications	56
Table 15. Codes for identification qualifier entries	57
Table 16. Summary of non-blood samples to be collected	58
Table 17. Datasheets associated with this protocol	74
Table 18. Site-specific species list (HARV)	82
Table 19. Site-specific species list (BART)	84
Table 20. Site-specific species list (SCBI)	86
Table 21. Site-specific species list (SERC)	88
Table 22. Site-specific species list (BLAN)	90
Table 23. Site-specific species list (OSBS)	92
Table 24. Site-specific species list (DISN)	93
Table 25. Site-specific species list (JERC)	95
Table 26. Site-specific species list (GUAN)	97
Table 27. Site-specific species list (UNDE)	98
Table 28. Site-specific species list (STEI)	100
Table 29. Site-specific species list (TREE)	102
Table 30. Site-specific species list (KONZ)	104
Table 31. Site-specific species list (UKFS)	106
Table 32. Site-specific species list (ORNL)	108
Table 33. Site-specific species list (GRSM)	110
Table 34. Site-specific species list (TALL)	112
Table 35. Site-specific species list (DELA)	114
Table 36. Site-specific species list (LENO)	
Table 37. Site-specific species list (WOOD)	118
Table 38 Site-specific species list (CPFR)	120



1	Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
	NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Table 39. Site-specific species list (STER)	122
Table 40. Site-specific species list (RMNP)	124
Table 41. Site-specific species list (OAES)	126
Table 42. Site-specific species list (NIWO)	128
Table 43. Site-specific species list (MOAB)	129
Table 44. Site-specific species list (SRER)	131
Table 45. Site-specific species list (JORN)	133
Table 46. Site-specific species list (ONAQ)	135
Table 47. Site-specific species list (SJER)	137
Table 48. Site-specific species list (HEAL)	139
Figure 1. Schematic of trapping grids. Sherman traps, when used, are placed at all points in the grid	
whereas Tomahawk traps, when used, are placed at only the stations marked by a blue box	
Figure 3. Summary of procedures per individual (see also Appendix B)	
Figure 4. An example of a sampling schedule for a typical temperate site where weather conditions	
permit and technician resources are available only from April through October. The days of the moi	
are found at the top of the figure, with the months on the left. Within each month, each site in a do	
is listed: $C = \text{core site}$ ; $R1 = \text{one of the relocatable sites}$ ; $R2 = \text{the other relocatable site}$ . The new mo	
dates for 2015 are indicated by the dark blue cells, and the grey cells demarcate the available samp	
window each month that includes 10 days before and after the new moon. The sampling bout dura	_
for the core sites are indicated by the horizontal blue lines. These lines span 5 nights of trapping – a	
assumed duration to cover 3 nights of trapping on 3 grids and 1 night of trapping on the remaining	
grids. The lines for April and October are dashed, as only one of these bouts is needed to meet the	
bout minimum. The red and green horizontal lines span 4 nights of trapping— an assumed duration	
cover 3 nights of trapping on 3 grids and 1 night of trapping on the remaining 3 grids, where available	
Figure 5. The grid coordinate system, consisting of 100 trap stations 10 meters apart. Trapping grids	
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	
Figure 6. Schematic demonstrating where the facial vein and the submandibular vein meet at the re	
end of the mandibular bone on a lab mouse (Mus). The facial vein (lower branch) is typically the tar	
for blood collection, but the vascular bundle associated with the junction of these vessels can also	be
used. From http://www.medipoint.com	51
Figure 7. Schematic demonstrating the placement of an ear punch, an ear tag, and how the ear leng	gth
measurement should be made on a lab mouse (Mus)	53
Figure 8. Placing tissue in a well.	65



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

## 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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

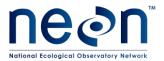
Applicable documents contain higher-level information that is implemented in the current document. Examples include designs, plans, or standards.

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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## 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
IACUC	Institutional Animal Care and Use Committee

## 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, the target species include 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 which, if captured, will be marked and released or vouchered, etc.

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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 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]).

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.

## 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.*) or rats (*Rattus spp.*) are common, 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). In D04 and D20, collapsible wire mesh traps (Tomahawk Live Trap, Hazelhurst, WI, 5" x 5" x 16") will also be used (50 per grid, at alternate stations starting with A1; see Figure 1).). Trapping will alternate between Tomahawk and Sherman traps; Tomahawks should be set during sampling bouts that occur in odd-numbered months, whereas Sherman traps (100 per grid) should be



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

set during even-numbered months. 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).

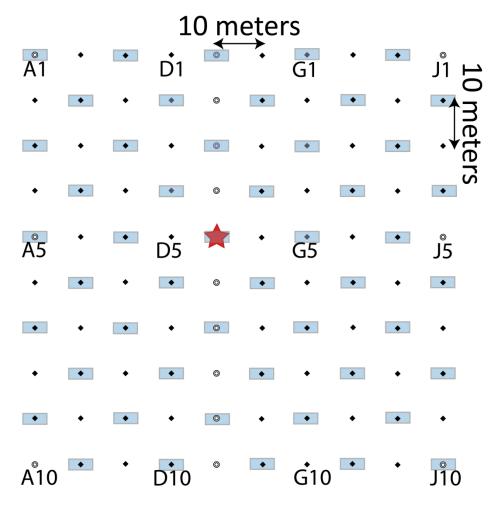


Figure 1. Schematic of trapping grids. Sherman traps, when used, are placed at all points in the grid, whereas Tomahawk traps, when used, are placed at only the stations marked by a blue box.

#### 3.3 Personnel

Four (2 teams of 2) or two personnel will conduct the trap checking at each site, depending on the number of grids at a particular site and the capture rates per grid. 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. Best practices in trapping include having the same person set and check specific traps, to decrease the



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

odds of missing any traps during the check. That said, logistics often dictate the use of distinct teams to perform these temporally disjunct tasks. Under these circumstances, traps shall be counted and recounted each morning to prevent traps from being missed.

## 3.4 Timing of trapping

Three grids at each site will be designated as pathogen grids (see Appendix F for bleed grid designation). Each pathogen grid will be trapped for 3 consecutive nights within a sampling period, while the remaining grids (i.e., abundance and diversity grids) will be sampled for only one night within a sampling period. Sampling will occur year-round at a monthly (at core sites only) or approximately every other month frequency (at relocatable sites), if resources are available and winter weather conditions permit (see section 4 for further details). 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

For all domains, except D04 and D20, 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 where shrews (*Soricomorpha: Soricidae*) are commonly captured (i.e., comprise more than 20% of the captured individuals on average (e.g., Harvard Forest)), a teaspoon of freeze-dried mealworms should also be added to the trap. Additional, high-calorie bait, such as peanut butter can also be added in the event of extremely cold conditions at many sites, except where not permitted (see Appendix E), where medium- to large- mammal (e.g., raccoons, bears) disturbance of



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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



• To minimize the mess associated with the opportunistic use of peanut butter, place peanut butter between 2 unbleached paper towels and cut into 1-inch squares (R. Rowe, pers. comm.)

For D04 and D20, Sherman traps and Tomahawk traps will be baited with "bait balls" -- a small (1/2 - 1 inch) ball of oatmeal, peanut butter, and vanilla extract. These balls should be encased in cheesecloth and cinched into the back of a Sherman or hung from a paperclip inside a Tomahawk (B. Patterson, pers. comm.). The cheesecloth is used to decrease the mess and to reduce attractability and access to ants. Although ants are of significant concern in both of these domains, previous small mammal sampling in both Puerto Rico and Hawaii successfully used peanut butter as bait (Engeman et al. 2005, Shiels et al. 2012).

- Use just enough peanut butter to hold the oats together.
- Place the bait ball in the center of a roughly 6"x6" square of cheesecloth. To seal the bait ball, draw the corners up around the bait and twist to make a tight tail around the bait clump.



Bait balls can be prepared in large quantities prior to sampling, and stored in the freezer until
needed (S. Hauser, pers. comm.). Insert the bait ball tail into the top of the back door on a
Sherman trap to hang the bait ball inside the trap. Use a paper clip to hang the bait ball toward
the rear of a wire trap.

## 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 2.4 and Site-Specific Information 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 (i.e., any species that are not designated as opportunistic or target), 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 an individual is of a target species (Figure 2), blood will be collected using the mandibular technique. These individuals will not be anesthetized prior to handling, unless it is needed to safely collect blood (e.g., for individuals >100g). 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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 from individuals belonging to the dominant genus at a site for potential isotopic analyses. Fresh fecal samples will also be collected opportunistically from captured individuals (i.e., fecal samples that are produced during handling rather than 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 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

Murids – house mice (Mus musculus) and introduced rats (Rattus spp.)

Dipodids (jumping mice (*Zapus, Napeozapus spp.*)) that are >10.0 grams UNLESS individual has:

Pronounced or physically debilitating injury or condition (e.g., advanced pregnancy or gave birth in trap), and/or

Already been captured and bled (whether the attempt was successful or not) during **current** sampling bout.

UNLESS species is excluded based on permitting regulations (e.g., protected species)

#### **DO NOT collect blood from:**

Heteromyids (Pocket mice (*Chaetodipus, Perognathus spp.*), Kangaroo rats and mice (*Dipodomys* and *Microdipodops spp.*))

Sciurids – chipmunks, squirrels, etc.

Soricids - shrews

Talpids – moles

Geomyids – pocket gophers

Figure 2. Criteria for blood sampling



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

## 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. Discretion can be used by field personnel to select the appropriate tagging method. For example, technicians have found that ear-tagging is effective in Myodes gapperi and Microtus pennsylvanicus. Jumping mice (Napeozapus spp., Zapus spp.) can be marked using either method, as technicians have reported varying success with both techniques. Shrews will not be permanently marked, but will be temporarily marked using a permanent marker. 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, and surgical glue can be used to seal the skin. 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

This protocol has been reviewed and approved by NEON's Institutional Animal Care and Use Committee (IACUC). Any deviations from this protocol that may impact the well-being of a capture are not permitted.

During the trapping period, it is important to do everything possible to ensure the well-being of all potential captures. This includes providing adequate bait and batting under cold conditions (outlined below) and keeping traps in the shade under hot conditions. In addition, animals should not be kept in the traps for excessive periods of time, not to exceed 16-18 hours. If capture rates are high and there is a risk of approaching this threshold, perform only the highest priority tasks during handling (see SOP C.4 for further details).

While handling, the condition of all captured individuals must be monitored closely. 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° F/ 18° 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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. Death will be confirmed via cervical dislocation. The specimen will then be tagged on the foot, bagged, and placed on ice as soon as possible, with ultimate disposition in a curated collection (see SOP C.9 for additional details).

## 1. Identify to species

- If the species is unknown or known but rare (as indicated by a federal or state designation of threatened or endangered), take a photograph of the individual as a digital voucher
- 2. If bleeding, collect blood samples first
  - Anesthetize first, if 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)
  - 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 3. Summary of procedures per individual (see also Appendix B)

## 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 pathogen grids and one night of trapping on the abundance and diversity grids. Sampling frequency, or the number of bouts per year, is determined by whether or not the trapping grid is located at a core or relocatable site (Table 1). Sampling shall occur year-round, where personnel resources and weather conditions permit, with a minimum of four bouts per year for all grids at



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

relocatable sites and a minimum of 6 bouts per year for all grids at core sites. (Table 1). Sampling shall occur year-round, where personnel resources and weather conditions permit, with a minimum of four bouts per year for all grids at relocatable sites and a minimum of 6 bouts per year for all grids at core sites.

Table 1. Summary of sampling frequencies by site and grid type\*

Site Type	Grid Type	Sampling Frequency	Number of nights per bout	Minimum bouts per year
Core	Pathogen	Monthly	3	6
Core	Abundance & Diversity	Monthly	1	6
Relocatable	Pathogen	Approximately Every Other Month	3	4
Relocatable	Abundance & Diversity	Approximately Every Other Month	1	4

<sup>\*</sup> Sites in Alaska will likely not be able to adhere to these guidelines given the limited sampling window. It is expected that each site (core and relocatable) will accomplish a minimum of 3 sampling bouts over a course of 3 months.

#### 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. If necessary, the sampling schedule can be modified significantly between the first and second years of operations, to account for lessons learned. Once a schedule is established, acceptable variation around this timing for the initiation of sampling from one sampling bout to the next (and year to year) is 1-5 days (relative to the new moon). Trapping at core and relocatable sites should occur within a 21 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. An example of a sampling schedule for an average temperate site is shown in Figure 4. 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

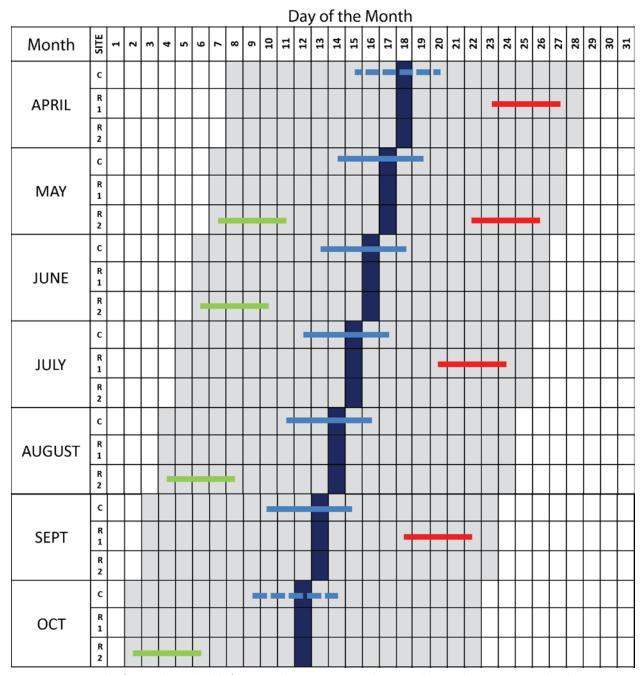


Figure 4. An example of a sampling schedule for a typical temperate site where weather conditions permit and technician resources are available only from April through October. The days of the month are found at the top of the figure, with the months on the left. Within each month, each site in a domain is listed: C = core site; R1 = one of the relocatable sites; R2 = the other relocatable site. The new moon dates for 2015 are indicated by the dark blue cells, and the grey cells demarcate the available sampling window each month that includes 10 days before and after the new moon. The sampling bout durations for the core sites are indicated by the horizontal blue lines. These lines span 5 nights of trapping — an assumed duration to cover 3 nights of trapping on 3 grids and 1 night of trapping on the remaining 3-5 grids. The lines for April and October are dashed, as only one of these bouts is needed to meet the 6-bout minimum. The red and green horizontal lines span 4 nights of trapping—an assumed duration to cover 3 nights of trapping on 3 grids and 1 night of trapping on the remaining 3 grids, where available.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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 provided 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 (regardless of species) on any given trapping grid during a single night 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, consult section 4.44.4..

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 as a Quick Reference in Appendix C.

#### **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.</li>
- Extra bait should be added to traps on nights when temperatures are expected to be < 7°C (45°F)
- Trapping should not occur on nights when **both** very cold temperatures (< 5.5°C (42°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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

• If you are unable to arrive at a site 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.

### Windy conditions:

If winds are very strong (> 35 mph) and there is little vegetation to protect the traps, trapping is not recommended.

#### **Predators:**

If predators destroy (i.e., damage beyond repair) >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.

If predators disturb (i.e., not damaging but closing trap doors, moving traps, etc.) > 30 traps on a single grid on any given night (or the combination of destroyed and disturbed traps > 30), 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 issued.

#### Cattle:

If cattle disturbance is significant, issue a problem ticket. A mechanism to secure traps could prove useful.

#### **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 Nontarget (N) option for the fate field.
- 2. If traps are not set on a particular grid as scheduled or if no individuals are captured, this should be recorded in the NOTES field (Table 2), 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 2), with any other known details described in the 'add'l notes' cell.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Table 2. 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 disturbed/door closed but empty	Used per trap coordinate, when necessary
3 – trap door open or closed with feces left behind	Used per trap coordinate, when necessary
4 – >1 capture per trap	Used per trap coordinate; in the rare event of multiple captures per trap; enter this note code for each individual
5 – single capture in trap	Used per trap coordinate, when necessary
6—no captures	Used ONLY at the per grid level; there is no need to write down every trap that has been set but is empty

## 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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 sampling on all plots impacts diversity metrics and target sample size.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 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]).



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

## 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
  - o Safety glasses with ventilation to minimize fogging up in humid environments
  - o 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
- o Optional: a disposable or reusable (cotton) laboratory coat or apron
- o 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



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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]).



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## 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.

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

Item No.	R/S	Description	Purpose	Quantity	Special Handling		
	Durable Items						
MX100322	R	Laser Rangefinder, ½ foot accuracy	Set Traplines	1	N		
MX100320	R	Compass with mirror and declination adjustment	Set and Follow Traplines	6	N		
MX100318	R	Measuring tape, 100 m	Set Traplines	4	N		
	R	Survey marking flag, wire, PVC or fiberglass stake	Set Traplines	100+	N		
MX100703	R	GPS receiver, recreational accuracy	Navigate to Traplines	2	N		
MX104361	S	Chaining pins or other suitable anchor	Anchor measuring tapes	10	N		
		Consumable Items					
	S	AA battery	Spare battery for GPS receiver	4	N		



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Quantity	Special Handling
	S	CR123A battery	Spare battery for laser rangefinder		
	R Permanent marker, chisel tip		Label flags	6	N

R/S=Required/Suggested

Table 5. Equipment list – Trapping, one bout

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
Durable Items						
MX100320	S	Compass with mirror and declination adjustment	Follow traplines	All domains	6	N
MX100703	S	GPS receiver, recreational accuracy	Navigate to traplines	All domains	2	N
MX100324	S	2-way radio	Communication	All domains	6	N
MX106060	MIXTUMUM I R I Sharman fran cmall folding 3"v3 5"vu"		Trap rodents (D01, D02, D03, D05, D06, D07, D08, D18, D19)	Domains D01-D08 (excluding D04), D18, D19	1200	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100702	R	Sherman trap, x-large folding, 3"x3.75"x12"	Trap rodents (D04, D09, D10, D11, D12, D13, D14, D15, D16, D17, D20)	Remaining domains (including D04 and D20)	1200	N
MX109264	R	Tomahawk trap, 16" x 5" x 5"	Trap rodents (D04 and D20 only)	Domains D04 and D20 only	300	N
MX109265	S	Tomahawk trap cover 16" x 5" x 5"	Trap rodents (D04 and D20 only)	Domains D04 and D20 only	300	N
MX101632	S	Headlamp	Hands-free lighting at dusk and dawn	All domains	6	N
MX105391	S	Storage bin, 26 gal rolling	Store seed	All domains	1200	N
MX104507	S	Tree planting bag	Carry traps	All domains	8	N
MX104510	S	Lumbar bag	Carry bait	All domains	8	N
MX100504	S	Storage bin	Store extra supplies in truck	All domains	2	N
		Consum	able Items			
MX105097	R	Seed, black oil sunflower	Bait traps (not D04 or D20)	All domains except D20 and D04	20 lb	N
MX100683	R	Seed, whole millet	Bait traps (not D04 or D20)	All domains except D04 and D20	2 lb	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Freeze-dried mealworms	Supplement bait for shrews	TBD	25 lb	N
	R	Peanut butter	Bait traps (D04 and D20 only)	D04 and D20		N
	R	Rolled oats	Bait traps (D04 and D20 only)	D04 and D20		N
	R	Unbleached cheesecloth	Bait traps (D04 and D20 only)	D04 and D20		N
	R	Paper clips, jumbo	Secure bait balls in mesh traps (D04 and D20 only)	D04 and D20	300	N
MX100673	R	Batting, polyester	Nesting material	All domains	500	N
MX104521	R	Gusseted plastic bag, 2 gal	Contain traps during collection for Sherman traps	All domains	1 lb	N
MX109412	R	Gusseted plastic bag, 5 gal	Contain traps during collection for Tomahawk traps (D04 and D20 only)	D04 and D20	1 lb	N
MX107194	R	Talstar EZ	Control fire ants (D02, D03, D04, D07, D08, D11)	Order for domains D02, D03, D04, D07, D08, D11	1	Υ
	S	Biohazard warning sticker	Label traps	All domains	1200	N
	S	Resealable plastic bag, minimum 1 gal	Carry batting, secure to lumbar bag with carabiner	All domains	6	N



Title: TOS Protocol and Procedure: S	Protocol and Procedure: Small Mammal Sampling		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Permanent marker, chisel tip	Label traps	All domains	6	N
	S	Wet erase markers, fine tip	Label traps	All domains	6	N
	S	Field notebook	Record field notes	All domains	1	N
	S	Pencils - #3	Label traps	All domains	6	N
	S	AA battery	Spare battery for GPS receiver	All domains	4	N
	S	Battery, AAA, Rechargeable	Headlamp or GPS	All domains	1200	N
	S	Bathtub crayons	Marking traps	All domains	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							
MX104715	R	Amber bottle, 30 ml with dropper	Administer isoflurane	All domains	2	N		



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100729	R	Tea infuser spoon	Administer isoflurane	All domains	2	N
MX104506	R	Ear tag applicator, size monel #1	Affix ear tag	All domains	4	N
MX104821	Read PIT tag number (not D04 or D20)			All domains except D04 and D20	2	N
MX100326	R	Ruler, 12" plastic, mm gradation	Measure	All domains	2	N
MX100724	R	Ruler, 6", flexible clear plastic	Measure	All domains	2	N
MX104442	R	Spring scale, tareable, capacity 30 g max	Weigh individuals	All domains	2	N
MX100704	R	Spring scale, tareable, capacity 100 g maximum	Weigh individuals	All domains except D04 and D20	2	N
MX100705	R	Spring scale, tareable, capacity 1000 g maximum	Weigh individuals	All domains	2	N
MX106661	S	Storage box, 81 position with lid	Organize cryovials during blood collection	All domains	4	N
MX104724 MX106667	R	Cryovial freezer storage box with dividers	Organize samples	All domains	10	N
MX106990 MX100730	R	Ear tissue punch	Collect ear tissue	All domains	2	N
	R	Cooler, 16qt	Chill perishable samples in field	All domains	2	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100696	R	Digital camera, 12 megapixel	Capture images of rodents for species identification	All domains	2	N
MX101866	R	Camera bag	Protect digital camera	All domains	2	N
	S	Ventilated tupperware or cardboard container	Care for hypothermic animals	All domains	5	N
MX100574	S	Bottle, 250 mL wide-mouth HDPE	Store sugar/water solution for animal care	All domains	2	N
	S	Shelter/tent	Shade collected traps, provide shelter while processing	All domains	2	N
	S	Backpack	Transport field equipment	All domains	2	N
	S	Organizer boxes with lid	Organize equipment	All domains	4	N
Need mx#	S	Mesh wash bag	Restrain larger species	All domains	20	N
MX104448	S	Restraint bag	Restrain larger species	All domains	20	N
MX100723	S	Multi-tool	Marking	All domains	2	N
MX104462	S	Diagonal plier, 4 in	Remove ear tags	All domains	2	N
	S	Short (< 6 inches) piece of rope (550 paracord works well) for 1000 g scale	Attaching to scale, in place of alligator clip, to weigh larger animals in plastic bags	All domains	2	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	S	Iris scissors	Collect ear tissue	All domains	2	N
MX100661	S	Forceps, cushing dissecting	Collect sample	All domains	4	N
MX100659 MX100660	S	Forceps, dissecting microforceps	Collect sample	All domains	4	N
MX100717	S	Cuticle clippers	Remove ear tags	All domains	2	N
MX106656	S	Magnifier hand-lens, 10X	Aid in species identification (not D04 or D20)	All domains except D04 and D20	2	N
MX103931	S	Plastic tray	Alternative or additional mammal processing surface	All domains	2	N
MX106652	S	Thermohygrometer	Monitor weather conditions	All domains	2	N
	•	Consu	imable Items			
MX103942	R	All weather copy paper	Print datasheets	All domains	25 sheets	N
MX109413	S	Clear plastic disposable restraint cones for large rats (≤450g)	Restrain Rattus norvegicus (D04 and D20 only)	D04 and D20	100	N
MX109414	S	Clear plastic disposable restraint cones for rats (≤225g)	Restrain Rattus rattus and R. exulans (D04 and D20 only)	D04 and D20	300	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX100591	R	Cotton ball	Administer isoflurane	All domains	150	N
MX100672	R	Isoflurane	Anesthetize/euthanize rodents	All domains	25 mL	Υ
MX100707	S	Microhematocrit tube	Collect blood (not D04 or D20)	All domains except D04 and D20	100	N
MX100678	R	Hand warmer	Care for hypothermic animals	All domains	2	N
	R	Sugar	Care for hypothermic animals	All domains	2	N
	R	Freeze-dried mealworms	Care for stressed animals	TBD	2 lb	N
MX100688	R	Styptic Powder	Slow/stop bleeding (in case of open wound)	All domains	1 pack, 42 g	N
MX100676	R	Cotton swab	Apply styptic powder	All domains	50	N
MX100727	R	Ear tag, numbered	Tag captured individuals	All domains	200	N
MX104419	R	PIT tag and implanter	Tag captured individuals (not D04 or D20)	All domains except D04 and D20	50	N
MX109424	S	Veterinary tissue adhesive	Gluing tissue at PIT tag implant (not D04 or D20) location	All domains except D04 and D20	3 mL	?
	S	Permanent marker (>1/2 inch), red, blue, or green preferred	Mark–captured shrews (not D04 or D20)	All domains except D04 and D20	4	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX108572 MX108573	R	Lancet, 4mm and 5mm	Collect blood	All domains	1000	N
MX108574	R	Lancet, 5.5mm and 6mm	Collect blood	All domains	300	N
MX109415 MX109416	R	Lancet, 7mm and 8mm	Collect blood (D04 and D20 only)	D04 and D20	1000	N
MX109422	S	Sterile artificial tears ointment	Aid in blood collection	All domains	7 grams	N
MX100714	R	Alcohol wipe	Collect ear punches	All domains	1000	N
MX108576	R	Gauze pad	Apply pressure to bleeding	All domains	1000	N
MX103485	R	Microcentrifuge tube, 1.5 mL with external threads	Contain blood, fecal samples, ear punches	All domains	1000	N
MX107195	R	Artifact identification tag	Label vouchers	All domains	10	N
MX105073	R	Thread	Attach tags to voucher specimens	All domains	2 rolls	N
MX104801	R	Coin envelope, small	Contain whisker and hair samples	All domains	150	N
MX100212	R	Dry ice, pelletized	Freeze blood samples	All domains	50 lbs	N
MX104844	S	Resealable plastic bag, 1 gal, 2 mil	Restrain captured individuals	All domains	200	N
MX100592	S	Resealable plastic bag, 1 gal, 4 mil	Restrain captured individuals (not D04 or D20)	All domains except D04 and D20	100	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
MX109419	S	Resealable plastic bag, 12 x 15", 4 mil	Restrain Rattus spp. (D04 and D20 only)	D04 and D20	500	N
MX104766	S	Heat shrink tubing	Cover clamp teeth on spring scales	All domains	1 package	N
MX104432	S	Cryogenic label	Label sample	All domains	1000	N
MX100593	S	Resealable plastic bag, 1 qt, 4 mil	Organize collected samples (coin envelopes, filled vials, and voucher specimens)	All domains	20	N
	S	Battery for thermo-hygrometer	Spare battery for thermo- hygrometer	All domains	2	N
	S	Digital camera battery	Spare battery	All domains	2	N
MX109418	S	Plastic sheeting, 3 x 50ft, 4 mil, clear	Provide mammal processing surface	All domains	10	N
	R	Permanent marker, ultra-fine tip	Label sample	All domains	2	N
MX104422	R	Permanent marker, archival ethanol-safe	Label sample	All domains	2	N
Resources						
	R	Field guide, regional dichotomous key	Identify unknown species	All domains	2	N



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity	Special Handling
	R	Field guide, mammals	Identify unknown species (not D04 or D20)	All domains except D04 and D20	2	N
RD[05]	R	Small mammal field datasheets	Record data	All domains	10	N

Table 7. Equipment list – Cleaning and sterilization, one bout.

Item No.	R/S	Description	Purpose	Conditions Used	Quantity*	Special Handling
Durable Items						
MX103202	R	Butane lighter	Sterilize tools	All domains	2	N
MX104772	R	Spray bottle	Disinfect tools and work area	All domains	2	N
MX103240	R	Portable Sharps container	Contain Sharps waste	All domains	2	N
MX104741	S	Narrow mouth jug, 1 gal	Transport quaternary ammonia	All domains	2	N
MX104449	S	3 cup container	Contain quaternary ammonia for disinfecting tools	All domains	2	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Conditions Used	Quantity*	Special Handling
	S	Mason jar	Transport used quaternary ammonia	All domains	2	N
		Consu	imable Items			
	R	Alcohol wipe	Disinfect tools	All domains	50	N
MX100586	R	Quaternary Ammonia 5% solution	Disinfect tools, gloves and work surfaces	All domains	10 L	N
	R Trash bag, large Contain and transport waste		All domains	20	N	
	R	Resealable plastic bag	Contain Sharps container	All domains	2	N
	R	Paper towels	Dry tools	All domains	2 rolls	N

<sup>\*</sup> Quantities are generous estimates per bout per site



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Table 8. Equipment list – Cleaning traps, per bout

Item No.	R/S	Description	Purpose	Quantity	Special Handling		
	Durable Items						
MX104772	S	Spray bottle	Wash traps	1 pack, 6	Υ		
	S	Scrub brush, long and short handle	Scrub traps	6	Υ		
MX104429	S	Bottle brush, 19"	Scrub traps	1	Υ		
	S	Chemical-resistant glove	Protect hands	1	Υ		
MX100379	S	Graduated cylinder, 25 ml, plastic	Measuring quaternary ammonium for dilution	2	Υ		
MX100526	S	Plastic bucket, 5 gal	Wash traps	6	Υ		
MX100639	S	Carboy, 20 L	Wash traps	1	Υ		
	Consumable Items						
MX100586	R	Quaternary disinfectant	Disinfect traps	1 bottle, 1 gal	Υ		



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Table 9. Equipment list – Preparing blood samples

Item No.	R/S	Description	Purpose	Quantity	Special Handling
		Durable Items			
MX104724 MX106667	R	Cryovial freezer storage box with dividers	Organize samples	5	N
MX106668	R	Cryogenic gloves	Protect hands while handling dry ice	1 pair	N
		Consumable Items			
MX103942	R	All weather copy paper	Print datasheets	5 sheets	N
Resources					
RD[05]	R	Small mammal datasheet (lab portion)	Datasheets	5 sheets	N



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Table 10. Equipment list – Shipping blood samples

tem No.	R/S	Description	Purpose	Quantity*	Special Handling
		Durable Items			
MX106668	R	Cryogenic gloves	Protect hands while handling dry ice	1 pair	N
	Consumable Items				
	R	Corrugated fiberboard or insulated shipper, UN packing group III	Package samples for shipment	3	N
	R	Watertight inner shipping container, certified for UN3373	Package samples for shipment		N
	S	Dry ice shipping label	Label shipments containing dry ice	3	N
	S	Biological Substance Category B shipping label	Label shipments containing diagnostic specimens	3	N
MX100212	R	Dry ice, pelletized	Keep samples frozen during shipment	5 lbs	Y
	R	Packaging tape	Package samples for shipment		
		Absorbent material (i.e. cellulose wadding, cotton balls, superabsorbent packets or paper towels)	Package samples for shipment		
		Resources			



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

tem No.	R/S	Description	Purpose	Quantity*	Special Handling
	R	Shipping manifest	Inventory of specimens being shipped	1	N

R/S=Required/Suggested

Table 11. Equipment list – Genetic analysis

Item No.	R/S	Description	Purpose	Quantity	Special Handling			
	Durable Items							
MX100660 MX100659	R	Forceps, jewelers	Remove tissue	3	N			
MX103202	R	Butane lighter	Sterilize ear tissue punch	1	N			
MX103487	R	Chill block and microplate cooler	Prepare well plates	1	N			
Lab supplied	R	96-well microwell plates	Store tissue to be barcoded	3	N			
Lab supplied	R	96-well microwell caps	Cover microwell plates	285	N			
MX104772	S	Spray bottle for ethanol	Disinfect bench space and gloves	1	N			

<sup>\*</sup> Quantities are generous estimates per batch



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Item No.	R/S	Description	Purpose	Quantity	Special Handling
MX106668	R	Cryogenic gloves	Protect hands while handling dry ice	1 pair	N
		Consumable It	ems		
MX100213	R	Ethanol, 190 proof (95%)	Disinfect bench space and gloves	1 L	Υ
	S	Corrugated fiberboard or insulated shipper, UN packing group III	Package samples for shipment	1	N
	R	Watertight inner shipping container, certified for UN3373	Package samples for shipment	Variable	N
	S	Biological Substance Category B shipping label	Label shipments containing diagnostic specimens	Variable	N
	R	Packaging tape	Package samples for shipment	1 roll	N
	R	Nitrile gloves, powderless	Prevent contamination of samples	Variable	N
	R	Address labels	Address shipments	1	N
MX100212	R	Dry ice, pelletized	Preserve samples during shipment	2 lbs	Υ



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# **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.
- Within each domain, the lead mammal technician will provide a review of the sampling equipment and the small mammal species of each site within that domain.
- Domains in the first year of sampling (i.e., while in construction) will receive laboratory-based training in handling and bleeding techniques prior to the onset of sampling.
- Prior to the start of operational field sampling, 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)



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 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 handwriting, 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 30 - 60 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 45 – 90 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 45 – 90 minutes per trapping grid per experienced two-person team.



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 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 54). 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. If permitted, permanent markers suited to domain-specific conditions can be used in place of pin flags.

		Α	В	С	D	Ε	F	G	Н	I	J
	1	A1	B1	C1	D1	E1	F1	G1	H1	l1	J1
	2	A2	B2	C2	D2	E2	F2	G2	H2	12	J2
	3	А3	В3	C3	D3	E3	F3	G3	Н3	13	J3
•	4	A4	B4	C4	D4	E4	F4	G4	H4	14	J4
T NORTH	5	A5	B5	C5	D5	<b>E5</b>	F5	G5	H5	15	J5
NOKIH	6	A6	В6	C6	D6	E6	F6	G6	Н6	16	J6
	7	Α7	В7	<b>C7</b>	D7	E7	F7	G7	H7	17	J7
	8	A8	В8	C8	D8	E8	F8	G8	Н8	18	J8
	9	A9	В9	<b>C</b> 9	D9	E9	F9	G9	Н9	19	J9
	10	A10	B10	C10	D10	E10	F10	G10	H10	110	J10

Figure 5. 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 one of the high accuracy marked points (A1, J1, A10, J10 or preferably E5; see Figure 5) from plot establishment to begin flagging.



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

- The tape-based grid and the corners established during plot establishment will only agree in a perfectly flat environment. Unlike in other protocols, the tape-based measurements should take priority over the established points, although variation between these will most often be within several meters.
- 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 metemeter 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.



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## **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. See Section 7.2 (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.
- Print the NEON template provided on the NEON intranet (in the FSU-FOPs folder) onto specimen tags in the event of voucher specimens.
- 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%), or, in D04 and D20, prepare bait balls.



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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** (including those in which an unsuccessful bleed attempt was made) 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 (follow manufacturer guidelines for dilution). 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 blood sample collection, if needed, 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.



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

## SOP C Field Sampling

## C.1 Setting traps

Trapping grids consist of 100 Sherman traps. However, in the event that conditions prevent setting of all traps on a given night, a minimum of 75 traps should be set to constitute a night of trapping. In D04 and D20, trapping grids will also include 50 Tomahawk wire mesh traps (see <a href="http://www.livetrap.com/images/instructions/2013">http://www.livetrap.com/images/instructions/2013</a> Instructions.pdf for instructions on setting these 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.
- TIPS & TRICKS
- 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. Consistency is the
  key; E-W travel can be used if strongly preferable for a given plot.
- 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. Be aware of drainage issues that may cause flooding of the localized area around the trap overnight.
- Use additional flagging if trap is hidden from view.
- Make sure trap is on level ground (using your foot to level an area, if necessary), and the door remains open after placement. If necessary, adjust trap sensitivity by gently pulling or pushing catch.
- Count and double count your traps, when setting or checking traps always know how many traps you brought out to the plot and how many you removed.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### **PROCEDURE**

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



When overnight lows will be  $<18^{\circ}$ 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.
  - a. In D04 and D20, place wire trap within a 2 meter radius around the marked point.
  - b. At sites with red imported fire ants (*Solenopsis* spp.), application of granulated insecticide (e.g., Talstar brand) is required, if permitted. Place immediately around the trap on flat ground or both under and around the trap when in a grassy area. Reapply after heavy rains.
- 3. Toss bait into trap.
  - a. Use about 1 TBSP 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).
    - 1) In D04 and D20, use a bait ball placed toward the back of the trap instead.
  - b. Use more bait if nighttime temperatures will be < 7°C/45°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 (where applicable).



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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F



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**

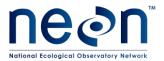
- Keep in mind that the liberal use of quat on tools and gloves, the one-time use of processing bags, and the bagging of traps prior to processing is critical to minimizing transmission of pathogens between small mammals, as well as to technicians.
- Keep in mind that very small individuals can crawl 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:
  - o Be sure to cross out any previous markings, if present.
  - o 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.
  - o If conditions are very wet, a #3 pencil or bath crayons 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	Grid coordinate	Processing station	Processing station
specimen of any species			
		Mark the belly with a	
Live capture of a shrew	Grid coordinate,	colored permanent	Drocossing station
Live capture of a sillew	species ID, sex, fate	marker, if not previously	Processing station
		marked, and release*	
Live capture of a non-target	Grid coordinate,	Immediate release	Drocossing station
species, e.g., a bird, weasel,	species (species ID, if	(fate = 'N' on PDA or	Processing station



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

or reptile	known)	datasheet)	
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



<sup>\*</sup>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:
  - o Handler: Handles captures, makes measurements, and collects blood and tissue samples.
  - o 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, plastic sheeting, or a plastic tray 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.



Title: TOS Protocol and Procedure: S	TOS Protocol and Procedure: Small Mammal Sampling		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

- 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", 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</li>
  - 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.
- o For Rattus spp. in D04 and D20, plastic restraint cones can be used to facilitate handling.
- o 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, perform cervical dislocation and collect the animal as a voucher specimen (see Section C.9).



• If there is no rigor and the animal may be overheated or dehydrated, wet the belly and administer sugar water via dropper.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

- 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.

#### PRIORITIZATION OF PROCESSING STEPS

Given the difficulties inherent to designing sampling to accommodate periods of both high and low capture rates, below is a prioritized list of processing steps to expedite processing during periods of high capture rates. The preference is to avoid situations where captures are released without any processing. The intent is that, by performing fewer processing steps on each individual, the total time spent on any given morning will be sufficiently decreased, when needed.

## The top priorities are:

- 1. Bleeding on the 3 bleeding grids (see Appendix FAppendix F Bleed Grid Designation for further guidance, if needed)
- 2. Species, sex, reproductive condition and tagID

The remaining steps in order of priority are:

- 3. Weight and hindfoot measurements
- 4. Fecal sample
- 5. Ear punch
- 6. Hair and whiskers

#### CHECKING FOR EXISTING MARKING AND BLEED HISTORY

#### Bleed animals that meet the following criteria:

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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

- 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 (whether the attempt was successful or not).
  - 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'.

### 3. Animals with lost tags:

- If an animal has clearly lost a tag, and:
  - o It is captured during the first night of trapping, go ahead and mark and bleed the animal.
  - It is captured during the second or third night of trapping, assess whether the loss of the tag appears recent. If so, mark the animal but do not bleed. Otherwise, mark and bleed the animal. When in doubt, err on the side of caution and do not bleed.

#### **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 introduced the mandibular blood sampling technique, which involves collection from the submandibular and/or facial vein or artery. This technique has proven successful across all species encountered in 2014, except for heteromyids. Therefore, the retro-orbital technique is no longer a recommended technique for NEON small mammal sampling.



The mandibular 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.



Title: TOS Protocol and Procedure: S	e: TOS Protocol and Procedure: Small Mammal Sampling	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## **Mandibular Bleeding**



 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) or by inexperienced personnel, if the technician cannot maintain a scruff.

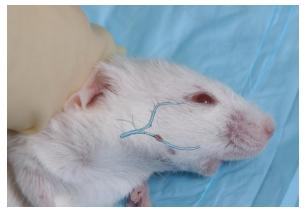


Figure 6. Schematic demonstrating where the facial vein and the submandibular vein meet at the rear end of the mandibular bone on a lab mouse (Mus). The facial vein (lower branch) is typically the target for blood collection, but the vascular bundle associated with the junction of these vessels can also be used. From http://www.medipoint.com

- 2. The 4 or 5 mm lancet sizes are appropriate for most species under 40 grams, 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, the 5 mm for those >20 grams, the 5.5 6mm for individuals >60 grams, and the 7 8mm for individuals >140 g.
- 3. Securely scruff the rodent between its shoulder blades in one hand.
- 4. 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).



- 7. 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.
- 8. In the event of an unsuccessful attempt, the other cheek can also be used. As a rule of thumb, do not try more than three attempts per cheek.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

- 9. Even if the first attempt was unsuccessful, bleeding of an individual should only be attempted once per bout.
- 10. Even small amounts of blood should be retained as a sample.
- 11. If blood is smearing into the fur, rather than forming drops, apply eye ointment to the area prior to lancing.
- 12. 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.
- 13. Seal the cryovial with a screw cap and apply label. See labeling guidelines in sample collection section below.
- 14. Dispose of lancet in sharps container, and spray used gauze with quat and place in trash bag.
- 15. Record the blood collection on the datasheet with an 'M' for mandibular.
- 16. 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 or cryovial storage box (cardboard preferred) 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 desired 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	.011014	.082105	.1114
25	1.37 - 1.75	.014018	.1013	.1418
30	1.65 - 2.10	.017021	.1216	.1721
35	1.93 - 2.45	.019025	.1418	.1925
40	2.20 - 2.80	.022028	.1621	.2228
125	6.88 - 8.75	.069088	.5266	.6988
150	8.25 - 10.50	.082105	.6279	.82 - 1.0
200	11.00 - 14.00	.1114	.82 - 1.05	1.1 - 1.4
250	13.75 - 17.50	.1418	1.0 - 1.3	1.4 - 1.8
300	16.50 - 21.00	.1721	1.2 - 1.6	1.7 - 2.1
350	19.25 - 24.50	.1925	1.4 - 1.8	1.9 - 2.5

<sup>\*</sup> Circulating blood volume

<sup>†</sup> Maximum sample volume for that sampling frequency



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## C.6 Collecting individual data



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. If measurements are not taken on a particular capture instance, do not enter measurements from a previous capture instance into that record.

#### 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).

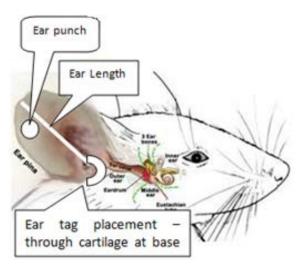


Figure 7. Schematic demonstrating the placement of an ear punch, an ear tag, and how the ear length measurement should be made on a lab mouse (Mus)...

- 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 (preferred) or left ear, if needed.
    - If an animal appears to have a torn ear due presumably to the loss of an ear tag, note which ear is torn in the replaced tag column of the datasheet and attached a new ear tag to the intact ear.
  - Use PIT tag if pinnae too small for ear tag.
    - This is the often the case with voles and pocket mice.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

- PIT tags should be inserted close to the rear of the animal, inserting the needle pointed towards the head.
- Suggested technique: Place animal flat on processing surface, with the two middle fingers of one hand securing the back of the head, while the fur is pinched with the thumb and forefinger of the same hand. Use the other hand to insert the needle into the tent of fur. Be careful not to pierce through the skin again (you should feel the tag being released from the applicator as it is inserted).
- The point of needle insertion can be sutured using veterinary tissue adhesive to reduce PIT tag loss rates.
- 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 PDA or datasheet in ear/PIT columns.
  - Format for ear tag is LXXXX for left ear or RXXXX for right ear (preferred).
  - If using a paper datasheet:
    - Write down last six digits of serial number for PIT tags and place one bar code sticker on the back of the datasheet.
      - Note that some PIT tags have two different serial numbers one labeled decimal and the other hexadecimal. Please confirm which one the reader reads and please write that one down on the datasheet.
    - Be sure to fill in the Recapture column 46, and whether there is evidence that a previous tag was lost. If a known tag is being replaced due to improper placement or any other reason, note the ID of the tag on the PDA or the back of the datasheet.
- 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 on PDA or datasheet (see also quick reference in Appendix B).
- 2. Note sex and reproductive condition and enter codes on PDA or datasheet.

#### Males

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

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Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### **Females**

- Nipples
  - o Enlarged (E)
  - o 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
  - o 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.
  - The full list of mammal species codes can be found on the NEON intranet in the <u>FSU-FOPs/taxonTables folder</u>.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

• Note that this list includes codes for instances where you cannot make an identification below genus (e.g., *Peromyscus sp.*):

Table 14. List of codes for genus-level identifications

taxonID	scientificName
AMSP	Ammospermophilus sp.
BLSP	Blarina sp.
CHSP	Chaetodipus sp.
CYSP	Cynomys sp.
DPSP	Dipodomys sp.
GESP	Geomys sp.
GLSP	Glaucomys sp.
LESP	Lemmus sp.
LPSP	Lepus sp.
MISP	Microtus sp.
MUSP	Mustela sp.
MYSP	Myodes sp.
NESP	Neotoma sp.
ONSP	Onychomys sp.
PGSP	Perognathus sp.
PESP	Peromyscus sp.
RASP	Rattus sp.
RESP	Reithrodontomys sp.
SNSP	Scapanus sp.
SCSP	Sciurus sp.
SISP	Sigmodon sp.
SOSP	Sorex sp.
SMSP	Spermophilus sp.
SLSP	Sylvilagus sp.
SYSP	Synaptomys sp.
TMSP	Tamias sp.
TSSP	Tamiasciurus sp.
THSP	Thomomys sp.
ZASP	Zapus sp.

- 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 15). 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

**Table 15.** 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

<sup>\*</sup> 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)

**EXAMPLE:** 

OSBS.20130714.R1357.B

• Sample Type (B for blood; E for ear; F for feces; H for hair and whiskers)

**Note:** It is recommended that cryovial labels are used, with some information pre-printed.

• Use fine point permanent marker (Sharpie or equivalent) for writing on cryovial labels, coin envelopes, or directly on the cryovials.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Table 16. 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	Write on	Ambient	Ambient
Whiskers	Once per bout; dominant genus	envelope	envelope	Ambient	Ambient
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.
  - Pluck 2 whiskers with forceps (or similar), or clip with cuticle scissors 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 if samples collected.
- 2. For all target and opportunistic species:
  - a. Collect any fresh, uncontaminated feces from the animal using either forceps or scooping the sample directly with the cryovial and label.
    - o Do not collect feces from the trap.
    - Store cryovial on dry ice for transport back to the lab.
    - Indicate on PDA or datasheet if sample was collected and condition ("F" for feces).
  - b. Collect one ear tissue sample one from near the edge of the untagged ear using a clicker-style 2000 Micron (2 mm) tissue biopsy punch or iris scissors. One ear tissue sample is to be taken per the lifetime of an individual, regardless of whether it has clearly lost a tag or not.
    - Place ear tissue in cryovial and label.
    - Indicate on PDA or datasheet if sample is collected.
  - c. Record the weight, to the nearest gram, using the appropriate, tared spring scale. Record on PDA or datasheet.
  - d. 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.
  - e. Put animal back into trap (trap is still in plastic bag) for transport back to point of capture.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

- Process all individuals on a grid before releasing animals at point of capture, unless extra personnel are available.
- 3. 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.
  - b. If an individual does not have a tag, assign a tagID with the format: 'O' + 3 digit number of plotID + trapCoordinate (e.g., O003A5).
  - c. Note that this tagID should be associated with all samples collected from a vouchered specimen, if the samples were collected prior to death.
  - d. If the trapCoordinate is from row 10, leave the 'O' off the front to meet the 6-character limit for the field.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

- 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.
- 4. Indicate that a voucher has been collected.



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 ( $\leq 0^{\circ}$  C but  $\geq -20^{\circ}$  C) and record on freezer log, if available.

#### **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.
  - a. Clean batting can be reused.
- 2. Pack up all supplies and equipment, once they are clean and dry.
- Clean PPE as directed by EHS Safety Policy and Program Manual and dispose final round of nitrile gloves and wipes.
- 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

## 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. Cleaning of traps that are extremely soiled, full of bait, contaminated by ants, slugs, etc., or to be transported for use at another site is recommended. 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# 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. A subset of the ear tissue samples will be used in SOP F.

### **E.2** Preparation

- 1. Print out a sample shipping manifest (RD[05]).
- 2. If the cryovial storage box does not have labeled well coordinates, print out the cryovial well datasheet, cut, and place into the bottom of the box (RD[05]).
- 3. Frozen samples should be stored in the cryovial storage boxes that they were placed in in the field. Label the outside of the boxes with the included plotIDs and collection dates.

# E.3 Sample processing in the lab

- For each frozen sample stored in a cryovial, record relevant information on the sample shipping manifest (RD[05]) and then enter into a computer using either the NEON Raw Data Ingest Workbook for Small Mammal Abundance & Diversity (for ear and fecal samples) or the NEON Raw Data Ingest Workbook for TOS Rodent-borne Pathogen Sampling (for blood samples; RD[07]).
- 2. Voucher specimens contained in individual plastic bags should be placed in a -20°C freezer until ready to ship (see SOP H).

## 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## **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 February 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 95 individuals for 1 DNA barcode plate per domain. The following rationale should be used to select specimens based on the confidence in the species identification:
    - 1) A minimum of 3 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 identification
         Qualifier field on the small mammal field datasheet (RD[05]).
      - For these species, submit either 10 individuals or 11% 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

# 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 8).
    - 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.

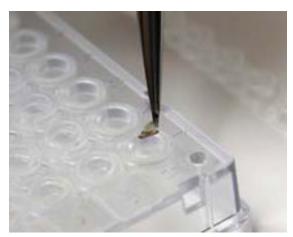


Figure 8. Placing tissue in a well.

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).



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

- 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		mall Mammal Sampling	Date: 03/23/2015	
	NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

### **SOP G** Data Entry and Verification

As a best practice, field data collected on paper datasheets should be digitally transcribed within 7 days of collection or the end of a sampling bout (where applicable). However, given logistical constraints, the maximum timeline for entering data is within 14 days of collection or the end of a sampling bout (where applicable). See RD[04] for complete instructions regarding manual data transcription.

Protocol-specific instructions and the associated data ingest workbook for entering small mammal data can be found on The NEON intranet in the <u>FSU-FOPs folder</u>. Be sure to enter data even if traps were not set as scheduled, due to unforeseen circumstances.



Title: TOS Protocol and Procedure: Small Mammal Sampling  Date: 03		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

### 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 <u>CLA shipping document</u> on <u>CLA's NEON intranet site</u>.

Other shipping details (e.g., acceptable days of the week for shipping, ship to address, manner of shipping etc.) will be specified by the external facilities and communicated to FOPS by CLA.

Samples should be shipped frozen on dry ice via overnight delivery service to a pathogen testingthe external facility. **Samples must remain frozen at all times.** 

#### H.1 Handling Hazardous Material

Dry ice is a Class 9 regulated material and must be shipped according to CFR 49 Subchapter C, Hazardous Materials Regulations.

Dry ice releases carbon dioxide gas which can build up pressure and rupture packaging. Ensure the packaging used allows the release of this pressure to prevent rupturing the package. Dry ice must be packaged using **UN packing group III** compliant materials. The maximum amount of dry ice per package is **200 kg**.

Any human or animal material including, but not limited to, excreta, secreta, blood and its components, issue and tissue fluids, being transported for diagnostic or investigational purposes are considered diagnostic specimens for shipping. These materials are shipped as **Biological Substance**, **Category B** (UN3373).

Refer to Chemical Hygiene Plan and Biosafety Manual (AD[03]) for additional requirements on commercial shipment of hazardous or dangerous materials.

Dry ice should be handled according to the guidelines in the EHS Safety Policy and Program Manual (AD[01]) and the Domain Chemical Hygiene Plan and Biosafety Manual (AD[03]).

#### H.2 Supplies/Containers

Samples in vials should be stored dry at -80°C until shipped to an external facility. Samples should be stored and shipped in cardboard vial storage boxes packed within insulated shipping containers containing dry ice. Vouchers (i.e., whole body specimens) should be stored dry at -20°C until shipped to an external facility. Vouchers should be stored individually in resealable plastic bags, with siteID, date, and species code written on the bag in permanent marker. For shipping, place vouchers in and insulated shipping container containing dry ice. Layer dry ice, thin cardboard, and plastic bags containing voucher specimens; use dry packaging to fill any headspace.



Title: TOS Protocol and Procedure: S	: TOS Protocol and Procedure: Small Mammal Sampling  Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### H.3 Timelines

Samples stored consistently at -80°C will retain their integrity for many months to years. Frozen samples must be shipped via overnight service on dry ice.

#### H.4 Conditions

Blood samples will be shipped on dry ice.

#### H.5 Grouping/Splitting Samples

All samples collected during any given site/bout combination should be shipped together and placed in a continuous sequence within the sample storage box in which they are shipped (e.g., wells A1, A2, A3, A4, A5, etc.). Samples from multiple site/bout combinations can be shipped together in the same sample storage box so long as samples from each site/bout combination are stored in a separate, continuous sequence in the storage box. This method of storage and shipping allows samples to be quickly inventoried sorted at the pathogen testing facility. Expediting these processes reduces the probability of thawing events that reduce sample quality.

#### H.6 Shipping Inventory

Whenever a batch of 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 or csv file) emailed to the testing facility (RD[05]; see SOP E). The hard-copy shipping manifest lists every sample vial or voucher specimen in the shipped batch. SampleIDs should be listed in ascending order by storageContainerID and then by wellCoordinate on the hard-copy shipping manifest. Do not sort the list according to sampleID or any other variable(s). Storage Container IDs should follow the format DXX.YYYY.#.(sample type letter from sampleID - e.g., B for blood), where # indicates the number of the box (1-n). The electronic manifest is an excel or csv file that should be emailed to the testing facility as soon as possible after a batch of samples has been shipped.

#### H.7 Laboratory Contact Information and Shipping/Receipt Days

See the <u>CLA shipping document</u> on <u>CLA's NEON intranet site</u>.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 8 REFERENCES

- Adams, J., C. Edmondson, D. Willis, and R. Carter. 2013. Effects of prescribed burning on small mammal, reptile, and tick populations on the Talladega National Forest, Alabama. Pages 123–126 *in* J. M. Guldin, editor. Proceedings of the 15th Biennial Southern Silvicultural Research Conference. e-Gen. Tech. Rep. SRS-GTR-175. U.S. Department of Agriculture, Forest Service, Southern Research Station See more at: http://www.srs.fs.usda.gov/pubs/43491#sthash.l0CCuJHG.dpuf, Asheville, NC.
- Annotated Mammal Species List of the Ordway-Swisher Biological Station. (n.d.). . http://ordway-swisher.ufl.edu/species/os-mammalia.htm.
- Armstrong, D. M. 2008. Rocky Mountain mammals: a handbook of mammals of Rocky Mountain National Park and vicinity. Page 264. Third edition. University Press of Colorado.
- Baker, M. F., and N. C. Frischknecht. 1973. Small mammals increase on recently cleared and seeded juniper rangeland. Journal of Range Management 26:101–103.
- Bock, C. E., and J. H. Bock. 1978. Response of birds, small mammals, and vegetation to burning sacaton grasslands in southeastern Arizona. Journal of Range Management 31:296–300.
- Cardoza, J. E., G. S. Jones, and T. W. French. 2009. Massachussetts State Mammal List. http://www.mass.gov/dfwele/dfw/wildlife/facts/mammals/mammal\_list.htm.
- Clevenger, G. A. 1977. The effects of campgrounds on small mammals in Canyonlands and Arches National Parks, Utah. Utah State University.
- Cook, J. A., S. O. MacDonald, and A. Runck. 2002. Denali National Park mammal checklist. http://www.nps.gov/dena/naturescience/mammalchecklist.htm.
- Degrassi, A., and A. M. Ellison. 2013. A Mark-Recapture of the Rodent and Shrew Populations in a Declining Hemlock Stand. http://harvardforest.fas.harvard.edu:8080/exist/xquery/data.xq?id=hf208.
- Discover Life in America All Taxa Biodiversity Inventory. 2008. The ATBI Database. http://tremont22.campus.utk.edu/ATBI\_start.cfm.
- Engeman, R., D. Whisson, J. Quinn, F. Cano, P. Quiñones, and T. H. White Jr. 2005. Monitoring invasive mammalian predator populations sharing habitat with the Critically Endangered Puerto Rican parrot Amazona vittata. Oryx 39:95.
- Evangelista, P. H., D. T. Barnett, T. J. Stohlgren, P. Stapp, C. Jarnevich, S. Kumar, and S. Rauth. 2008. Field and costs sssessment for the Fundamental Sentinel Unit (FSU) at the Central Plains Experimental Range, Colorado. Page 98.



Title: TOS Protocol and Procedure: Small Mammal Sampling  Date: 03		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

- Fitch, H. S., and N. A. Slade. 2014. Checklist of Field Station Mammals. http://kufs.ku.edu/resources/mammals-list/.
- Giffen, N. R., R. S. Reasor, and C. A. Campbell. 2011. Small Mammal Abundance and Distribution Survey. Page 38. Oak Ridge National Laboratory, Oak Ridge, TN.
- Golde, W., P. Gollobin, and L. Rodriguez. 2005. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. Lab Animal 34:39–43.
- Gómez, A., a M. Kilpatrick, L. D. Kramer, A. P. Dupuis, J. G. Maffei, S. J. Goetz, P. P. Marra, P. Daszak, and a A. Aguirre. 2008. Land use and west nile virus seroprevalence in wild mammals. Emerging infectious diseases 14:962–5.
- Halfpenny, J. 2010. Small mammal herbivore trapping in alpine tundra, 1981-1990. http://niwot.colorado.edu/exec/.extracttoolA?smammals.jh.
- Kao, R. H., C. M. Gibson, R. E. Gallery, C. L. Meier, D. T. Barnett, K. M. Docherty, K. K. Blevins, P. D. Travers, E. Azuaje, Y. P. Springer, K. M. Thibault, V. J. McKenzie, M. Keller, L. F. Alves, E. S. Hinckley, J. Parnell, and D. S. Schimel. 2012. NEON terrestrial field observations: designing continental-scale, standardized sampling. Ecosphere 3:115.
- Kays, R. W., and D. E. Wilson. 2011. Mammals of North America. Page 248. Second Edi. Princeton University Press.
- Kelt, D. A., M. S. Hafner, and The Committee for Guidelines on Handling Rodents in the Field of the American Society of Mammalogists. 2010. Updated guidelines for protection of mammalogists and wildlife researchers from hantavirus pulmonary syndrome (HPS). Journal of Mammalogy 91:1524–1527.
- Kelt, D. A., D. H. Van Vuren, M. S. Hafner, B. J. Danielson, and M. J. Kelly. 2007. Threat of hantavirus pulmonary syndrome to field biologists working with small mammals. Emerging Infectious Diseases 13:1285–7.
- Kilcline, B. 2003. Trap Biases in Surveying the Small Mammals of UNDERC.
- Leis, S. A., D. M. Leslie, D. M. Engle, and J. S. Fehmi. 2008. Small mammals as indicators of short-term and long-term disturbance in mixed prairie. Environmental Monitoring and Assessment 137:75–84.
- Maher, S. 2010. Spatial dynamics of mammals and their pathogens and parasites. University of Kansas, United States -- Kansas.
- Mammals of Florida | American Society of Mammalogists. (n.d.). . http://www.mammalsociety.org/mammals-florida.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

- Martin, S. C., and H. G. Reynolds. 1973. The Santa Rita Experimental Range: Your Facility for Research on Semidesert Ecosystems. Journal of the Arizona Academy of Science 8:56–67.
- Matter, S. F., J. F. Zawacki, and M. A. Bowers. 1996. Habitat fragmentation and the perceived and actual risk of predation. Virginia Journal of Science 47:19–27.
- McMillan, B. R., D. W. Kaufman, G. A. Kaufman, and R. S. Matlack. 1997. . . Mammals of Konza Prairie: new observations and an updated species list. , : The Prairie Naturalist 29:263–271.
- McShea, W. J., John Pagels, John Orrock, Elizabeth Harper, and Kevin Koy. 2003. Mesic deciduous forest as patches of small-mammal richness within an Appalachian Mountain Forest. Journal of Mammalogy 84:627–643.
- Mitchell, J. C., S. C. Rinehart, J. F. Pagels, K. A. Buhlmann, and C. A. Pague. 1997. Factors influencing amphibian and small mammal assemblages in central Appalachian forests. Forest Ecology and Management 96:65–76.
- Osbourne, J. D., J. T. Anderson, and A. B. Spurgeon. 2005. Effects of habitat on small-mammal diversity and abundance in West Virginia. Wildlife Society Bulletin 33:814–822.
- Ostfeld, R. S., and R. R. Parmenter. 2008. Small mammals, Fundamental Sentinel Units TIGR team report.
- Perry, M. C., P. C. Osenton, and C. S. Stoll. 1998. Biological diversity of created forested wetlands in comparison to reference forested wetlands in the bay watershed. Pages 261–268. Maryland Department of Natural Resources, Annapolis, Maryland.
- Price, M. V. 1978. The role of microhabitat in structuring desert rodent communities. Ecology 59:910–921.
- Prugh, L., and J. Brashares. 2010. Basking in the moonlight? Effect of illumination on capture success of the endangered giant kangaroo rat. Journal of Mammalogy 91:1205–1212.
- Purcell, K. L., D. A. Drynan, and K. M. Mazzocco. 2007. Vertebrate Fauna of the San Joaquin Experimental Range, California: an annotated checklist based on 70 years of observations.
- Rexstad, E. A., and E. Debevec. (n.d.). Dynamics of Small Mammal Populations in the Rock Creek Watershed of Denali. http://www.nps.gov/articles/aps-v6-i2-c16.htm.
- Reynolds, H. G. 1950. Relation of Merriam kangaroo rats to range vegetation in southern Arizona. Ecology 31:456–463.
- Shiels, A. B., C. A. Flores, A. Khamsing, P. D. Krushelnycky, S. M. Mosher, and D. R. Drake. 2012. Dietary niche differentiation among three species of invasive rodents (Rattus rattus, R. exulans, Mus musculus). Biological Invasions 15:1037–1048.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
	NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

- Sikes, R. S., W. L. Gannon, and The Animal Care And Use Committee of The American Society Of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.
- Smith, C. B., and P. J. Urness. 1984. Small mammal abundance on native and improved foothill ranges, Utah. Journal of Range Management 37:353–357.
- Sureda, M., and M. L. Morrison. 1998. Habitat use by small mammals in southeastern Utah, with reference to Mexican spotted owl management. Western North American Naturalist 58:76–81.
- Wiewel, A. S., W. R. Clark, and M. A. Sovada. 2007. Assessing small mammal abundance with track-tube indices and mark-recapture population estimates. Journal of Mammalogy 88:250–260.
- Wilson, D. E., F. R. Cole, J. D. Nichols, R. Rudran, and M. S. Foster (Eds.). 1996. Measuring and Monitoring Biological Diversity: Standard Methods for Mammals. Page 409 Biological Diversity Handbook Series. Smithsonian Institution Press, Washington, D.C.



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### APPENDIX A DATASHEETS

The following datasheets are associated with this protocol:

Table 17. 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.



Title: TOS Protocol and Procedure: Small Mammal Sampling  Date: 03/2		Date: 03/23/2015	
	NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

#### APPENDIX B QUICK REFERENCES

# Quick Reference: Checking Traps and Processing Captures - I

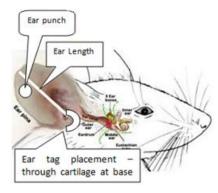
#### STEP 1 - Check ALL traps in grid for captures.

IF trap door CLOSED	IF trap door OPEN
Peek inside to see if there is a capture or feces.	Clean trap can be reused.
Immediately release non-target captures.	Traps with feces only should be marked, bagged, and taken to processing station.
Write grid coordinate on trap.	
	If mid-bout, leave empty trap in place and <b>close</b> door.
Place trap in plastic bag, for transport to processing station (even if capture has been released)	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: Sciurids - chipmunks, squirrels, etc. Soricids – shrews Cricetids that are > 10 g Talpids - moles Dipodids that are > 10 g Geomyids - pocket gophers Murids that are > 10 g Heteromyids – kangaroo rats, pocket mice Any protected species listed on state or **UNLESS** individual has: federal permit Pronounced or physically debilitating injury, and/or Already been captured and bled during current sampling bout.

**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.



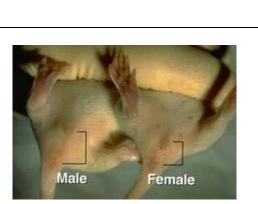
Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

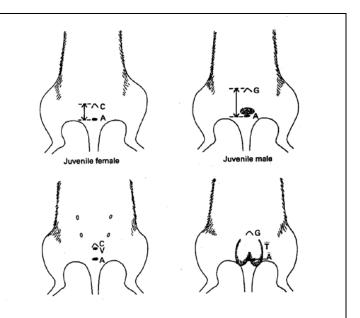
# **Quick Reference: Checking Traps and Processing Captures-II**

**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	, , , , , , , , , , , , , , , , , , ,	Write on envelope	Ambient	
Whiskers	pluck or snip at base, once per bout; one from each cheek dominant genus	• •	Archivar com envelope	write on envelope	Ambient
Fecal	fresh only	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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# **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.</li>
- Trapping should not occur on nights when both very cold temperatures (< 5.5°C (42°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.



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# **Quick Reference: Small Mammal Sampling Datasheet**

Column Number(s)	Data Field	Description/What to Enter
1-3	plotID	Enter number only (Use leading zeros where applicable.)
4-6	trapCoordinate	Indicate point of capture with grid coordinate e.g., B5
7	Notes	Information on trap condition and quality. Use codes (1 through 6) from top left corner of datasheet. See below.
8-11	taxonID	Use 4 letter species code (examples listed on top of datasheet). Create own code if captured species not listed and record full name on back of Datasheet.
12	idQ – identification Qualifier	CS – cf. species; cf. = "not sure"; AS – aff. species; aff. = "similar to, but is not"
13	Sex	Male (M) or female (F)
14-19	Ear/PIT	Unique tag ID, format: Ear tags: RXXXX or LXXXX; PIT tags: <b>last</b> 6 digits of serial number
20-24	Age and reproductive status	Use uppercase letter codes from datasheet
25-26	HFL	Hind foot length (mm) – to nearest millimeter
27-28	Ear	Right ear length (mm) – to nearest millimeter
29-31	Tail	Tail length (mm) – round to nearest millimeter
32-34	TTL	Total length (head + body) (mm)
35-37	WGT	Weight (g) – round to nearest gram
38	Recapture	Yes/No – indicates whether an individual is a recapture
39	Ear tag replaced	Indicate from which ear tag was presumably lost (if applicable). Record 'N' in the Recapture field.
40	Fate	Indicate history/condition of capture. Use uppercase code on datasheet. Dead supercedes the Non-target option.
41-46	Samples	Indicate type of sample collected.
47	Comments	Indicate 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 on the back of the datasheet.

#### **NOTES codes**:

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 disturbed/door closed but empty	Used per trap coordinate, when necessary
3 – trap door open or closed with feces left behind	Used per trap coordinate, when necessary
4 – >1 capture per trap	Used per trap coordinate; in the rare event of multiple captures per trap; enter this note code for each individual
5 – single capture in trap	Used per trap coordinate, when necessary
6—no captures	Used ONLY at the per grid level; there is no need to write down every trap that has been set but is empty



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 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

	А	В	C	D	Ł	ŀ	G	Н		J
1	A1	B1	C1	D1	E1	F1	G1	H1	11	J1
2	A2	B2	C2	D2	E2	F2	G2	H2	12	J2
3	A3	В3	C3	D3	E3	F3	G3	Н3	13	J3
4	A4	B4	C4	D4	E4	F4	G4	H4	14	J4
5	A5	B5	C5	D5	E5	F5	G5	H5	15	J5
6	A6	В6	C6	D6	E6	F6	G6	H6	16	J6
7	A7	В7	C7	D7	E7	F7	G7	H7	17	J7
8	A8	B8	C8	D8	E8	F8	G8	Н8	18	J8
9	A9	В9	C9	D9	E9	F9	G9	H9	19	J9
10	A10	B10	C10	D10	E10	F10	G10	H10	110	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 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.
- 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).

#### **Setting 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



Title: TOS Protocol and Procedure: S	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# **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.
- ☑ Ensure pit tag reader and camera batteries are charged. Bring spare batteries for headlamps.
- Print the NEON template provided on <u>the NEON intranet</u> (in the FSU-FOPs folder) onto specimen tags in the event of voucher specimens.
- 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%), or, in D04 and D20, prepare bait balls.
- ☑ 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 ultralow freezer (-80°C) prior to use.

#### **Personal Protective Equipment (PPE)**

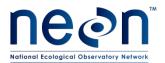
- Eye protection (safety glasses to discourage rubbing of the eyes and protect from extreme splashes)
- Disposable respirators with N95 (HEPA-equivalent) particulate filter
- Required: all sites in CO, NM, AZ, CA
- Recommended: all sites in KS, TX, UT, MT, NV, ID, OR, and WA
- Latex and/or nitrile gloves
- 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 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 F).



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

#### APPENDIX E SITE-SPECIFIC INFORMATION

#### E.1 D01 – CORE – 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 very cold temperatures (< 5.5°C (42°F)) 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 18. Site-specific species list (HARV)

TARGE (Scientific and	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
OPPORTUNI (Scientific and		
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



Title: TOS Protocol and Procedure: S	Date: 03/23/2015		
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

NON-TARGE (Scientific and C		
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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### 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 very cold temperatures (< 5.5°C (42°F)) 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 19. Site-specific species list (BART)

TARGET: (Scientific and C		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
OPPORTUNIS <sup>-</sup> (Scientific and C		
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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

	RGET ANIMALS nd 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### 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 very cold temperatures (< 5.5°C (42°F)) 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 20. Site-specific species list (SCBI)

	T SPECIES 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Sorex longirostris	Southeastern shrew	0-5
Tamias striatus	Eastern Chipmunk	0-5
Tamiasciurus hudsonicus	Red Squirrel	0-5
	ARGET ANIMALS 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.4 D02 – RELOCATABLE – SERC (Smithsonian Environmental Research Center)

#### **Trapping Issues**

- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 Perry et al. 1998 and Gómez et al. 2008..

Table 21. Site-specific species list (SERC)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Myodes gapperi	Southern Red-backed Vole	0-20
Microtus pennsylvanicus	Meadow Vole	100-500
Microtus pinetorum	Woodland Vole	0-50
Mus musculus	House mouse	0-50
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	0-5
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	50-100
Cryptotis parva	North American Least Shrew	0-5
Sorex cinereus	Cinereus Shrew	0-20
Sorex fumeus	Smoky shrew	0-20
Sorex hoyi	American Pygmy Shrew	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Sorex longirostris	Southeastern shrew	0-5
Tamias striatus	Eastern Chipmunk	0-5
Tamiasciurus hudsonicus	Red Squirrel	0-5
	ARGET ANIMALS and Common Name)	
Condylura cristata	Star-nosed Mole	0-5
Glaucomys volans	Southern Flying Squirrel	0-5
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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# E.5 D02 – RELOCATABLE – BLAN (Blandy Experimental Farm)

### **Trapping Issues**

- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 Matter et al. 1996 and Mitchell et al. 1997..

Table 22. Site-specific species list (BLAN)

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
Microtus pinetorum	Woodland Vole	0-50
Mus 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	0-5
Sorex fumeus	Smoky shrew	0-5
Sorex hoyi	American Pygmy Shrew	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Sorex longirostris	Southeastern shrew	0-5
Tamias striatus	Eastern Chipmunk	0-5
Tamiasciurus hudsonicus	Red Squirrel	0-5
	ARGET ANIMALS and Common Name)	
Condylura cristata	Star-nosed Mole	0-5
Glaucomys volans	Southern Flying Squirrel	0-5
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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.6 D03 – CORE – OSBS (Ordway-Swisher Biological Station)

### **Trapping Issues**

- Fire ants
  - o 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 very cold temperatures (< 5.5°C (42°F)) 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 23. Site-specific species list (OSBS)

TARGET species		Number to be	
(Scientific and Cor	mmon Name)	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	
OPPORTUNISTI	CANIMALS		
(Scientific and Co	mmon 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 Co			
Geomys pinetis floridanus	Florida pocket gopher	0 - 5	
Scalopus aquaticus australis	Southern mole	0 - 5	
Glaucomys volans	Southern Flying Squirrel	0 - 20	



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.7 D03 – RELOCATABLE – DISN (Disney Wilderness Preserve)

### **Trapping Issues**

- Fire ants
  - o 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
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 24. 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

	RGET ANIMALS d Common Name)	
Geomys pinetis	Southeastern Pocket Gopher	0 - 5
Scalopus aquaticus	Eastern Mole	0 - 5
Glaucomys volans	Southern Flying Squirrel	0 - 20



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.8 D03 – RELOCATABLE – JERC (Jones Ecological Research Center)

### **Trapping Issues**

- Fire ants
  - o 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 very cold temperatures (< 5.5°C (42°F)) are coupled with precipitation.</li>

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.

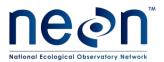
Table 25. Site-specific species list (JERC)

	ET SPECIES d 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	20-200
	NISTIC ANIMALS d 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
	GET ANIMALS d Common Name)	
Geomys pinetis	Southeastern Pocket Gopher	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481 Author: K. Thibault		Revision: F

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



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# E.9 D04 – CORE – GUAN (Guanica Forest)

## **Trapping Issues**

• The community here is dominated by invasive rats (*Rattus spp.*). Please note that these species can be quite aggressive; use of anesthesia is recommended.

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

Dominant genus for hair & whisker sampling purposes: Rattus

### **Species List and Abundance Estimates**

This species list is based on Kays and Wilson 2011.

Table 26. Site-specific species list (GUAN)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually	
Mus musculus	House mouse	0-50	
Rattus norvegicus	Norway rat	0-50	
Rattus rattus	Black rat	100-200	
Rattus exulans	Roof rat	0-50	



Title: TOS Protocol and Procedure: Small Mammal Sampling		mall Mammal Sampling	Date: 03/23/2015
	NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.10 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 very cold temperatures (< 5.5°C (42°F)) 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 and data collected by NEON.

Table 27. 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	50-300
Peromyscus maniculatus	North American Deermouse	50-500
Synaptomys cooperi	Southern Bog Lemming	0-50
Napaeozapus insignis	Woodland Jumping Mouse	20-200
Zapus hudsonius	Meadow Jumping Mouse	0-50
OPPORTUNISTIC ANIMALS (Scientific and Common Name)		
Blarina brevicauda	Northern Short-tailed Shrew	20-100
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	0-10
Tamias striatus	Eastern Chipmunk	0-50
Tamiasciurus hudsonicus	Red Squirrel	0-10
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.11 D05 – RELOCATABLE – STEI (Steigerwaldt)

### **Trapping Issues**

- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 28. Site-specific species list (STEI)

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



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# E.12 D05 – RELOCATABLE – TREE (Treehaven)

### **Trapping Issues**

- Large (i.e., bear) and medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 29. Site-specific species list (TREE)

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	
	NISTIC ANIMALS d 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	



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

NON	I-TARGET ANIMALS	
(Scientif	ic 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# E.13 D06 – CORE – KONZ (Konza Prairie Biological Station)

## **Trapping Issues**

- Medium sized carnivore (e.g., raccoons, coyotes) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 McMillan et al. 1997.

Table 30. Site-specific species list (KONZ)

	ET SPECIES d Common Name)	Number to be Used Annually
Chaetodipus hispidus	Hispid Pocket Mouse	50-200
Microtus ochrogaster	Prairie Vole	0-20
Microtus pinetorum	Woodland Vole	50-200
Mus musculus	House mouse	20-100
Neotoma floridana	Eastern Woodrat	0-20
Onychomys leucogaster	Northern Grasshopper Mouse	20-100
Peromyscus leucopus	White-footed Deermouse	20-100
Peromyscus maniculatus	North American Deermouse	0-50
Reithrodontomys megalotis	Western Harvest Mouse	50-200
Reithrodontomys montanus	Plains Harvest Mouse	100-500
Sigmodon hispidus	Hispid Cotton Rat	20-100
Synaptomys cooperi	Southern Bog Lemming	0-5
Zapus hudsonius	Meadow Jumping Mouse	0-20
	IISTIC ANIMALS d Common Name)	
Blarina brevicauda	Northern Short-tailed Shrew	0-20
Blarina hylophaga	Elliot's Short-tailed Shrew	0-50
Cryptotis parva	North American Least Shrew	0-50
Tamias striatus	Eastern Chipmunk	0-20
Spermophilus franklinii	Franklin's Ground Squirrel	0-5
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

	TARGET ANIMALS c and Common Name)	
Geomys bursarius	Plains Pocket Gopher	0-5
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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# E.14 D06 – RELOCATABLE – UKFS (University of Kansas Field Station)

### **Trapping Issues**

- Medium sized carnivore (e.g., raccoons, coyotes) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 Fitch and Slade 2014..

Table 31. Site-specific species list (UKFS)

	ET SPECIES d Common Name)	Number to be Used Annually
Chaetodipus hispidus	Hispid Pocket Mouse	50-200
Microtus ochrogaster	Prairie Vole	0-20
Microtus pinetorum	Woodland Vole	50-200
Mus musculus	House mouse	20-100
Neotoma floridana	Eastern Woodrat	0-20
Onychomys leucogaster	Northern Grasshopper Mouse	20-100
Peromyscus leucopus	White-footed Deermouse	20-100
Peromyscus maniculatus	North American Deermouse	0-50
Reithrodontomys megalotis	Western Harvest Mouse	50-200
Reithrodontomys montanus	Plains Harvest Mouse	100-500
Sigmodon hispidus	Hispid Cotton Rat	20-100
Synaptomys cooperi	Southern Bog Lemming	0-5
Zapus hudsonius	Meadow Jumping Mouse	0-20
	IISTIC ANIMALS d Common Name)	
Blarina brevicauda	Northern Short-tailed Shrew	0-20
Blarina hylophaga	Elliot's Short-tailed Shrew	0-50
Cryptotis parva	North American Least Shrew	0-50
Tamias striatus	Eastern Chipmunk	0-20
Spermophilus franklinii	Franklin's Ground Squirrel	0-5
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

	TARGET ANIMALS and Common Name)	
Geomys bursarius	Plains Pocket Gopher	0-5
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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.15 D07 – CORE – ORNL (Oak Ridge National Laboratory)

## **Trapping Issues**

- Fire ants
  - o 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 very cold temperatures (< 5.5°C (42°F)) 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 32. Site-specific species list (ORNL)

	ET SPECIES d 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

	TUNISTIC ANIMALS 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
	ARGET ANIMALS 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

#### E.16 D07 – RELOCATABLE – GRSM (Great Smoky Mountains National Park)

### **Trapping Issues**

- Fire ants
  - o 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, coyotes) disturbance
  - Due to the high density of bears in the Park, if ONE or more traps are disturbed, traps
    must be removed from the disturbed grid and any other grid within one mile. The
    sampling bout must be terminated prematurely for these grids; trapping can resume
    after a month.
  - o If bears disturb the same grid twice, then trapping cannot recur for one year.
  - The Park and the Field Operations Manager shall be informed as soon as possible, and a problem ticket should be issued.
  - o To reduce the probability of bear disturbance, every effort should be made to collect all seed for disposal off site.
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 Discover Life in America - All Taxa Biodiversity Inventory 2008.

Table 33. Site-specific species list (GRSM)

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
Myodes gapperi	Southern red-backed 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



		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

Γ		1
Oryzomys palustris	Marsh Oryzomys	0-20
Peromyscus gossypinus	Cotton Mouse	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
OPPORT	UNISTIC ANIMALS	
(Scientific a	and Common Name)	
Blarina brevicauda	Northern Short-tailed Shrew	50-200
Cryptotis parva	North American Least Shrew	0-5
Glaucomys sabrinus	Northern Flying Squirrel	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
Sorex palustris	American Water Shrew	0-5
Tamias striatus	Eastern Chipmunk	50-200
Tamiasciurus hudsonicus	Red Squirrel	0 - 20
NON-TA	ARGET ANIMALS	
(Scientific a	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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.17 D08 – CORE – TALL (Talladega National Forest)

## **Trapping Issues**

- Fire ants
  - o 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 very cold temperatures (< 5.5°C (42°F)) 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 34. Site-specific species list (TALL)

	ET SPECIES d 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
	NISTIC ANIMALS d 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
	RGET ANIMALS d Common Name)	
Glaucomys volans	Southern Flying Squirrel	0-5
Mustela frenata	Long-tailed Weasel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling  D		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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



		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# E.18 D08 – RELOCATABLE – DELA (Dead Lake)

### **Trapping Issues**

- Fire ants
  - o 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 very cold temperatures (< 5.5°C (42°F)) 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 35. Site-specific species list (DELA)

	GET SPECIES nd 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
	JNISTIC ANIMALS nd 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
	ARGET ANIMALS nd Common Name)	
Glaucomys volans	Southern Flying Squirrel	0-5
Mustela frenata	Long-tailed Weasel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.19 D08 – RELOCATABLE – LENO (Lenoir Landing)

### **Trapping Issues**

- Fire ants
  - o 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 very cold temperatures (< 5.5°C (42°F)) 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 36. Site-specific species list (LENO)

	GET SPECIES 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
	JNISTIC ANIMALS 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
	ARGET ANIMALS and Common Name)	
Glaucomys volans	Southern Flying Squirrel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.20 D09 – CORE – WOOD (Woodworth Field Station)

## **Trapping Issues**

- Medium sized carnivore (e.g., raccoons) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 and data collected by NEON.

Table 37. Site-specific species list (WOOD)

	ET SPECIES I 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	50-200
Zapus hudsonius	Meadow Jumping Mouse	5-50
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
	IISTIC ANIMALS I 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	10-100
Sorex haydeni	Prairie Shrew	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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-TAI	RGET ANIMALS	
(Scientific ar	d 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

## E.21 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
- **Do not trap** when very cold temperatures (< 5.5°C (42°F)) 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 (<a href="http://sgs.cnr.colostate.edu/species">http://sgs.cnr.colostate.edu/species</a> 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 and data collected by NEON.

Table 38. Site-specific species list (CPER)

	Г SPECIES Common Name)	Number to be Used Annually
Chaetodipus hispidus	Hispid Pocket Mouse	0 - 50
Chaetodipus intermedius	Rock Pocket Mouse	0 - 20
Dipodomys ordii	Ord's Kangaroo Rat	50 - 100
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	5 - 40
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	5 - 100
Reithrodontomys megalotis	Western Harvest Mouse	5 - 200
Reithrodontomys montanus	Plains Harvest Mouse	5 - 100
	STIC ANIMALS Common Name)	
Cryptotis parva	North American Least Shrew	0 - 20



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

Spermophilus spilosoma	Spotted Ground Squirrel	0 - 20
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0 - 20
NON-TARGET ANIMALS (Scientific and Common Name)		
(Scientific and C	Common Name)	
(Scientific and C	Common Name)  Plains Pocket Gopher	0 - 20



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

## E.22 D10 – RELOCATABLE – STER (Sterling)

## **Trapping Issues**

- Coyote disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 (<a href="http://www.parks.state.co.us/parks/northsterling/Pages/NorthSterling.aspx">http://www.parks.state.co.us/parks/northsterling/Pages/NorthSterling.aspx</a>, accessed 2012). Abundance estimates were based on an average capture rate of 10%, and relative abundances in data collected by NEON.

Table 39. Site-specific species list (STER)

	species 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	20 - 300
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
Mus musculus	House Mouse	0 - 50
	TIC ANIMALS Common Name)	
Cryptotis parva	Least Shrew	0 - 20
Sorex cinereus	Masked Shrew	0 - 20
Spermophilus spilosoma	Spotted Ground Squirrel	0 - 20
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0 - 20



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

NON-TARGET ANIMALS (Scientific and Common Name)		
Geomys bursarius	Plains Pocket Gopher	0 - 20



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.23 D10 – RELOCATABLE – RMNP (Rocky Mountain National Park)

## **Trapping Issues**

- Cold temperatures, wind, and snow
  - Do not trap when very cold temperatures (< 5.5°C (42°F)) 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 40. Site-specific species list (RMNP)

TARGET SPECIES (So	ientific 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
OPPORT	JNISTIC ANIMALS	
(Scientific a	ind 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



Title: TOS Protocol and Procedure: S	mall Mammal Sampling	Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

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



Title: TOS Protocol and Procedure: Small Mammal Sampling  Date: 03/23/201		Date: 03/23/2015		
	NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

# E.24 D11 – CORE – OAES (Klemme Range Research Station)

#### **Trapping Issues**

- Medium sized carnivore (e.g., raccoons, coyotes) disturbance
- Do not trap when very cold temperatures (< 5.5°C (42°F)) are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

*Use of bedding:* Pending capture rates of heteromyids; 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 Leis et al. 2008..

Table 41. Site-specific species list (OAES)

	ET SPECIES d Common Name)	Number to be Used Annually
Chaetodipus hispidus	Hispid Pocket Mouse	0-20
Dipodomys ordii	Ord's Kangaroo Rat	0-20
Neotoma micropus	Southern Plains Woodrat	0-20
Onychomys leucogaster	Northern Grasshopper Mouse	0-20
Perognathus flavescens	Plains Pocket Mouse	0-20
Perognathus flavus	Silky Pocket Mouse	0-20
Peromyscus attwateri	Texas Deermouse	0-50
Peromyscus leucopus	White-footed Deermouse	100-500
Peromyscus maniculatus	North American Deermouse	100-500
Reithrodontomys fulvescens	Fulvous Harvest Mouse	0-20
Reithrodontomys montanus	Plains Harvest Mouse	50-200
Sigmodon hispidus	Hispid Cotton Rat	50-200
	IISTIC ANIMALS d Common Name)	
Cryptotis parva	North American Least Shrew	0-50
Spermophilus spilosoma	Spotted Ground Squirrel	0-5
Spermophilus tridecemlineatus	Thirteen-lined Ground Squirrel	0-5
Tamias striatus	Eastern Chipmunk	0-20



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

NON-TARGET ANIMALS (Scientific and Common Name)		
Geomys bursarius	Plains Pocket Gopher	0-5
Mustela frenata	Long-tailed Weasel	0-5
Scalopus aquaticus	Eastern Mole	0-5
Sciurus niger	Eastern Fox Squirrel	0-5
Sylvilagus audubonii	Desert Cottontail	0-5
Sylvilagus floridanus	Eastern Cottontail	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.25 D13 – CORE – NIWO (Niwot Ridge Mountain Research Station)

## **Trapping Issues**

- Cold temperatures, wind, and snow
  - Do not trap when very cold temperatures (< 5.5°C (42°F)) 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

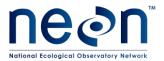
Dominant genus for hair & whisker sampling purposes: Peromyscus

## **Species List and Abundance Estimates**

This species list is based on Armstrong et al., 2001. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Halfpenny 2010%...

Table 42. Site-specific species list (NIWO)

	RGET SPECIES and Common Name)	Number to be Used Annually
Myodes gapperi	Red-backed vole	50 - 200
Phenacomys intermedius	Heather Vole	20 - 100
Microtus longicaudus	Long-tailed Vole	20 - 200
Microtus montanus	Montane Vole	20 - 200
Neotoma cinerea	Bushy-tailed Woodrat	0 - 50
Peromyscus maniculatus	N. American Deermouse	100 - 1000
Zapus princeps	Western Jumping Mouse	5 – 100
	UNISTIC ANIMALS and Common Name)	
Sorex cinereus	Cinereus Shrew	0 - 5
Sorex monticolus	Montane Shrew	0 – 5
Sorex nanus	Dwarf Shrew	0 - 5
Spermophilus lateralis	Golden-mantled Ground Squirrel	0 - 50
Tamias minimus	Least Chipmunk	0-50
	ARGET ANIMALS and Common Name)	
Thomomys talpoides	Northern Pocket Gopher	0 - 10
Mustela ermine	Short-tailed weasel	0-5
Mustela frenata	Long-tailed weasel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

### E.26 D13 – RELOCATABLE – MOAB (Moab)

### **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 very cold temperatures (< 5.5°C (42°F)) 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 Clevenger 1977 and Sureda and Morrison 1998..

Table 43. Site-specific species list (MOAB)

	GET SPECIES nd Common Name)	Number to be Used Annually
Dipodomys ordii	Ord's Kangaroo Rat	0-100
Microtus longicaudus	Long-tailed Vole	0-20
Microtus montanus	Montane Vole	0-50
Neotoma albigula	White-throated Woodrat	0-20
Neotoma cinerea	Bushy-tailed Woodrat	0-20
Neotoma lepida	Desert Woodrat	0-20
Neotoma mexicana	Mexican Woodrat	0-20
Onychomys leucogaster	Northern Grasshopper Mouse	0-50
Perognathus flavescens	Plains Pocket Mouse	50-200
Perognathus parvus	Great Basin Pocket Mouse	50-200
Peromyscus boylii	Brush Deermouse	0-20
Peromyscus crinitus	Canyon Deermouse	0-20
Peromyscus maniculatus	North American Deermouse	400-1000
Peromyscus truei	Pinon Deermouse	0-50
Reithrodontomys megalotis	Western Harvest Mouse	50-200
Zapus princeps	Western Jumping Mouse	0-20



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

	NISTIC ANIMALS d Common Name)	
Notiosorex crawfordi	Crawford's Gray Shrew	0-5
Sorex merriami	Merriam's Shrew	0-5
Sorex monticolus	Dusky Shrew	0-5
Sorex nanus	Dwarf Shrew	0-5
Ammospermophilus leucurus	White-tailed Antelope Squirrel	0-5
Spermophilus lateralis	Golden-mantled Ground Squirrel	0-5
Tamiasciurus hudsonicus	Red Squirrel	0-5
Tamias minimus	Least Chipmunk	0-5
Tamias quadrivittatus	Colorado Chipmunk	0-5
Tamias rufus	Hopi Chipmunk	0-5
	RGET ANIMALS d Common Name)	
Spermophilus variegatus	Rock Squirrel	0-5
Sylvilagus audubonii	Desert Cottontail	0-5
Sylvilagus nuttallii	Mountain Cottontail	0-5
Thomomys bottae	Botta's Pocket Gopher	0-5
Mustela erminea	Ermine	0-5
Mustela frenata	Long-tailed Weasel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.27 D14 – CORE – SRER (Santa Rita 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
- **Do not trap** when very cold temperatures (< 5.5°C (42°F)) 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 Martin and Reynolds 1973 and 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 Reynolds 1950, Price 1978, and Bock and Bock 1978).

Table 44. Site-specific species list (SRER)

	GET SPECIES nd Common Name)	Number to be Used Annually
Baiomys taylori	Northern Pygmy Mouse	0-20
Chaetodipus baileyi	Bailey's Pocket Mouse	50-200
Chaetodipus hispidus	Hispid Pocket Mouse	0-50
Chaetodipus intermedius	Rock Pocket Mouse	0-20
Chaetodipus penicillatus	Desert Pocket Mouse	0-50
Dipodomys merriami	Merriam's kangarro rat	100-500
Dipodomys ordii	Ord's Kangaroo Rat	20-100
Dipodomys spectabilis	Banner-tailed Kangaroo Rat	0-50
Neotoma albigula	White-throated Woodrat	0-50
Neotoma mexicana	Mexican Woodrat	0-20
Onychomys leucogaster	Northern Grasshopper Mouse	0-50
Onychomys torridus	Southern Grasshopper Mouse	0-50
Perognathus amplus	Arizona Pocket Mouse	0-20
Perognathus flavus	Silky Pocket Mouse	0-20
Peromyscus boylii	Brush Deermouse	0-20
Peromyscus eremicus	Cactus Deermouse	0-50
Peromyscus leucopus	White-footed Deermouse	0-20
Peromyscus maniculatus	North American Deermouse	400-1000



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

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Title: TOS Protocol and Procedure: Small Mammal Sampling  Do		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

### E.28 D14 – RELOCATABLE – JORN (Jornada 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
- **Do not trap** when very cold temperatures (< 5.5°C (42°F)) 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**

Data sets were provided by the Jornada Basin Long-Term Ecological Research (LTER) project. Funding for these data was provided by the U.S. National Science Foundation (Grant DEB-1235828). The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported by Bestelmeyer and Lightfoot (<a href="http://jornada-nation.org/lightfoot/">http://jornada-nation.org/lightfoot/</a>

www.nmsu.edu/studies/lter/projects/smlmamex.prj, accessed 5 March 2015).

Table 45. Site-specific species list (JORN)

	RGET SPECIES and Common Name)	Number to be Used Annually
Chaetodipus intermedius	Rock pocket mouse	0-20
Chaetodipus penicillatus	Desert pocket mouse	20-200
Dipodomys merriami	Merriam's kangaroo rat	100-500
Dipodomys ordii	Ord's kangaroo rat	100-500
Dipodomys spectabilis	Banner-tailed kangaroo rat	0-50
Mus musculus	House mouse	0-5
Neotoma albigula	White-throated woodrat	0-50
Neotoma micropus	Southern plains woodrat	0-20
Onychomys arenicola	Mearns' grasshopper mouse	20-200
Onychomys leucogaster	Northern grasshopper mouse	20-200
Perognathus flavus	Silky pocket mouse	0-50
Peromyscus boylii	Brush mouse	0-20
Peromyscus eremicus	Cactus mouse	0-20
Peromyscus leucopus	White-footed mouse	0-50
Peromyscus maniculatus	Deer mouse	0-20



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

	NISTIC ANIMALS nd Common Name)	
Notiosorex crawfordi	Desert shrew	0-5
Spermophilus spilosoma	Spotted ground squirrel	0-50
Spermophilus variegatus	Rock squirrel	0-5
NON-TARGET ANIMALS (Scientific and Common Name)		
Lepus californicus	Black-tailed jackrabbit	0-5
Sylvilagus audubonii	Desert cottontail	0-5
Thomomys bottae	Botta's pocket gopher	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.29 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 very cold temperatures (< 5.5°C (42°F)) are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Not recommended, as the relative abundance of heteromyids at the site is ~50%

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 Baker and Frischknecht 1973 and Smith and Urness 1984 and data collected by NEON.

Table 46. Site-specific species list (ONAQ)

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



		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

	NISTIC ANIMALS nd 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
	RGET ANIMALS nd 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



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

# E.30 D17 - CORE - SJER (San Joaquin 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
- **Do not trap** when very cold temperatures (< 5.5°C (42°F)) are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).

Use of bedding: Not recommended

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 Purcell et al. 2007.

Table 47. Site-specific species list (SJER)

TARGET SPECIES (Scientific and Common Name)		Number to be Used Annually
Chaetodipus californicus	California pocket mouse	50-200
Dipodomys heermanni	Heermann's Kangaroo Rat	0-50
Microtus californicus	California Vole	0-20
Microtus longicaudus	Long-tailed Vole	50-200
Microtus montanus	Montane Vole	50-200
Mus musculus	house mouse	0-20
Neotoma cinerea	Bushy-tailed Woodrat	0-20
Neotoma fuscipes	Dusky-footed Woodrat	0-50
Neotoma macrotis	big-eared woodrat	0-20
Onychomys torridus	Southern Grasshopper Mouse	0-50
Perognathus inornatus	San Joaquin pocket mouse	0-50
Peromyscus boylii	brush deermouse	100-500
Peromyscus californicus	California Deermouse	0-20
Peromyscus maniculatus	North American Deermouse	100-500
Peromyscus truei	Pinon Deermouse	100-500
Reithrodontomys megalotis	Western Harvest Mouse	50-200
Zapus princeps	Western Jumping Mouse	0-20



		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

	TUNISTIC ANIMALS and Common Name)	
Sorex lyelli	Mt. Lyell Shrew	0-5
Sorex ornatus	Ornate Shrew	0-5
Sorex palustris	American Water Shrew	0-5
Scapanus latimanus	Broad-footed Mole	0-5
Glaucomys sabrinus	Northern Flying Squirrel	0-5
Spermophilus beecheyi	California Ground Squirrel	0-5
Spermophilus lateralis	Golden-mantled Ground Squirrel	0-5
Tamias merriami	Merriam's chipmunk	0-5
Tamiasciurus douglasii	Douglas's Squirrel	0-5
	TARGET ANIMALS and Common Name)	
Sciurus griseus	Western Gray Squirrel	0-5
Sylvilagus audubonii	Desert Cottontail	0-5
Sylvilagus bachmani	Brush Rabbit	0-5
Thomomys bottae	Botta's Pocket Gopher	0-5
Mustela frenata	Long-tailed Weasel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F

## E.31 D19 – RELOCATABLE – HEAL (Healy)

### **Trapping Issues**

- Summers are cool and humid with 24 hours of daylight from mid-May through early August.
   Trapping can be done continuously, but, due to logistical constraints, NEON will set traps by 2000h and check and close traps at 0600h, regardless of light conditions.
- Bear disturbance
- **Do not trap** when very cold temperatures (< 5.5°C (42°F)) are coupled with precipitation, or when there is sufficient snow on the ground (i.e., > 15 cm (6 inches)).
- **Shrews are particularly abundant**; mealworms should be added to the bait. Shrew mortality may become prohibitively high at certain sites.

Use of bedding: Recommended under all conditions

Dominant genus for hair & whisker sampling purposes: TBD

#### **Species List and Abundance Estimates**

This species list is based on Kays and Wilson 2011 and Cook et al. 2002. The abundance estimates were based on an average capture rate of 10%, as well as the species-specific relative abundances reported for Denali National Park by Rexstad and Debevec n.d.

Table 48. Site-specific species list (HEAL)

TARGET SPECIES		Number to be
(Scientific a	and Common Name)	Used Annually
Lemmus trimucronatus	Nearctic Brown Lemming	0-5
Microtus miurus	Singing Vole	0-100
Microtus oeconomus	Root Vole	0-50
Microtus pennsylvanicus	Meadow Vole	0-20
Microtus xanthognathus	Taiga Vole	0-20
Myodes rutilus	Northern Red-backed Vole	0-200
Synaptomys borealis	Northern Bog Lemming	0-5
Zapus hudsonius	Meadow Jumping Mouse	0-20
OPPORT	UNISTIC ANIMALS	
(Scientific a	and Common Name)	
Sorex cinereus	Cinereus Shrew	50-200
Sorex hoyi	American Pygmy Shrew	0-20
Sorex monticolus	Dusky Shrew	0-50
Sorex palustris	American Water Shrew	0-50
Sorex tundrensis	Tundra Shrew	0-50
Sorex yukonicus	Tiny shrew	0-50
Glaucomys sabrinus	Northern Flying Squirrel	0-20
Spermophilus parryii	Arctic Ground Squirrel	0-50
Tamiasciurus hudsonicus	Red Squirrel	0-50



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015		
	NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

NON-TARGET ANIMALS (Scientific and Common Name)		
Mustela erminea	Short-tailed weasel	0-5
Mustela nivalis	Least Weasel	0-5



Title: TOS Protocol and Procedure: Small Mammal Sampling		Date: 03/23/2015	
NEON Doc. #: NEON.DOC.000481	Author: K. Thibault	Revision: F	

#### 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. The specific lists of plots by site designated as bleed grids are managed in the FSU-FOPS folder on the NEON intranet.

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.
- 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.
- 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. Blood samples MUST be collected on long-term bleed grids for all remaining sampling at the site unless otherwise directed.