

<i>Title:</i> TOS Science Design for Small Mammal Abundance and Diversity		<i>Date:</i> 04/16/2018
<i>NEON Doc. #:</i> NEON.DOC.000915	<i>Author:</i> K. Thibault	<i>Revision:</i> B

## TOS SCIENCE DESIGN FOR SMALL MAMMAL ABUNDANCE AND DIVERSITY

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## Change Record

<b>REVISION</b>	<b>DATE</b>	<b>ECO #</b>	<b>DESCRIPTION OF CHANGE</b>
A	08/11/2014	ECO-02131	Initial release
B	06/15/2018	ECO-05596	Minor format and text edits; update to exclude D04 and D20 from the design; added seed sterilization; updated marking for shrews; updated section of temporal frequency of sampling to reflect reductions in number of nights on diversity grids and number of bouts at all sites; added number of grids per site and preliminary capture data by site

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## 1 DESCRIPTION

### 1.1 Purpose

NEON design documents are required to define the scientific strategy leading to high-level protocols for NEON subsystem components, linking NEON Grand Challenges and science questions to specific measurements. Many NEON *in situ* measurements can be made in specific ways to enable continental-scale science rather than in ways that limit their use to more local or ecosystem-specific questions. NEON strives to make measurements in ways that enable continental-scale science to address the Grand Challenges. Design Documents flow from questions and goals defined in the NEON Science Strategy document AD[01] and inform the more detailed procedures described in Level 0 (L0; raw data) protocol and procedure documents, algorithm specifications, and Calibration/Validation (CalVal) and maintenance plans.

### 1.2 Scope

This document defines the rationale and requirements for sampling small mammal abundance and diversity in the NEON Science Design.

### 1.3 Acknowledgements

The design of the small mammal abundance and diversity sampling for NEON described herein is the result of invaluable input from the original Small Mammal Abundance and Diversity Technical Working Group, including Guy Cameron, Bob McCleery, Bill McShea, Rebecca Rowe, Rob Swihart, and Beatrice Van Horne, the leaders of the NEON Tiger team for small mammals, Bob Parmenter and Rick Ostfeld, as well as the decades of effort and dedication of countless field mammalogists. Thanks to Tanya Chesney for her thorough copy-editing and formatting of the document.

## 2 RELATED DOCUMENTS AND ACRONYMS

### 2.1 Applicable Documents

Applicable documents contain information that shall be applied in the current document. Examples are higher level requirements documents, standards, rules and regulations.

AD[01]	NEON.DOC.000001	NEON Observatory Design
AD[02]	NEON.DOC.001282	Introduction to the TOS Science Designs
AD[03]	NEON.DOC.000913	TOS Science Design for Spatial Sampling Design
AD[04]	NEON.DOC.002652	NEON Level 1, Level 2 and Level 3 Data Products Catalog
AD[05]	NEON.DOC.000481	TOS Protocol and Procedure: Small Mammal Sampling
AD[06]	NEON.DOC.000911	TOS Science Design for Vectors and Pathogens

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## 2.2 Reference Documents

Reference documents contain information complementing, explaining, detailing, or otherwise supporting the information included in the current document.

RD [01]	NEON.DOC.000008	NEON Acronym List
RD [02]	NEON.DOC.000243	NEON Glossary of Terms

## 2.3 Acronyms

CDC	Centers for Disease Control and Prevention
CPER	Central Plains Experimental Range
LTER	Long-term Ecological Research
LTREB	Long-term Research in Environmental Biology
MCDB	Mammal Community Database
MNKA	Minimum Number Known Alive
SECR	Spatially-Explicit Capture-Recapture
TN	Trap Nights

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### 3 INTRODUCTION

#### 3.1 Overview of the Observatory

The National Ecological Observatory Network (NEON) is a continental-scale ecological observation platform for understanding and forecasting the impacts of climate change, land use change, and invasive species on ecological systems. NEON is designed to enable users, including scientists, planners and policy makers, educators, and the general public, to address the major areas in environmental sciences, known as the Grand Challenges (**Figure 1**). NEON infrastructure and data products are strategically aimed at those aspects of the Grand Challenges for which a coordinated national program of standardized observations and experiments is particularly effective. The open access approach to the Observatory’s data and information products will enable users to explore NEON data in order to map, understand, and predict the effects of humans on the earth and understand and effectively address critical ecological questions and issues. Detailed information on the NEON design can be found in AD[01], AD[02].

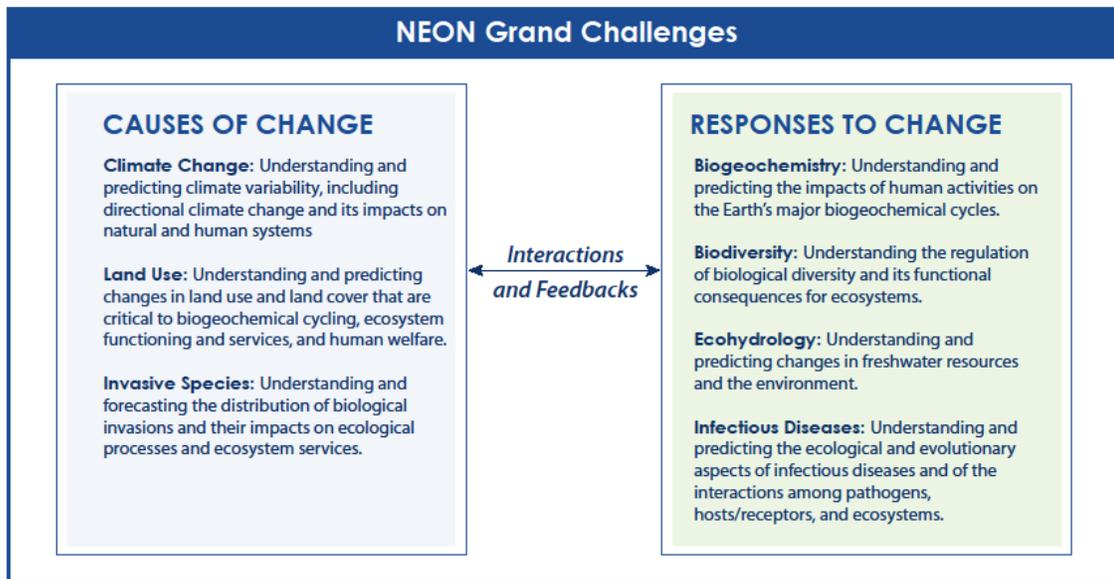


Figure 1. The seven Grand Challenges defined by the National Research Council (2001)

#### 3.2 Components of the Observatory

There are five components of the Observatory, the Airborne Observation Platform (AOP), Terrestrial Instrument System (TIS), Aquatic Observation System (AOS), Aquatic Instrument System (AIS), and Terrestrial Observation System (TOS). Collocation of measurements associated with each of these components will allow for linkage and comparison of data products. For example, remote sensing data provided by the Airborne Observation Platform (AOP) will link diversity and productivity data collected on individual plants and stands by the Terrestrial Observation System (TOS) and flux data captured by

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instruments on the tower (TIS) to that of satellite-based remote sensing. For additional information on these systems, see Keller et al. 2008, Schimel et al. 2011.

**3.3 The Terrestrial Observation System (TOS)**

The NEON TOS will quantify the impacts of climate change, land use, and biological invasions on terrestrial populations and processes by sampling key groups of organisms (sentinel taxa), infectious disease, soil, and nutrient fluxes across system interfaces (air, land, and water) (AD[01], AD[02]). The sentinel taxa were selected to include organisms with varying life spans and generation times and wide geographic distributions to allow for standardized comparisons across the continent. Many of the biological measurements will enable inference at regional and continental scales using statistical or process-based modeling approaches. The TOS sampling design captures heterogeneity representative of each site to facilitate this inference when possible. Plot and organism-scale measurements will also be coordinated with the larger-scale airborne measurements, which provide a set of synergistic biological data products at the regional scale. Details of these design elements and algorithms can be found in individual design documents available through the NEON website ([www.neonscience.org](http://www.neonscience.org)).

The standardization of protocols across all sites is key to the success of NEON and must be maintained at all sites through time. Thus, although specific techniques may be required at some sites (e.g., due to different vegetation types), protocols have been developed to ensure data comparability. These details can also be found in individual design documents available through the NEON website ([www.neonscience.org](http://www.neonscience.org)).

The TOS Science Designs define the scientific strategies leading to high-level sampling designs for NEON sentinel taxa, terrestrial biogeochemistry, and infectious disease, linking NEON Grand Challenges and science questions to specific measurements (AD[02]). The TOS Spatial Sampling Design document describes the sampling design that collocates observations of the components of the TOS (AD[03]). TOS Science Design documents were developed following input from the scientific community, including discipline-specific Technical Working Groups, and the National Science Foundation (AD[02]). Science Designs will be reviewed periodically to ensure that the data collected by NEON are those best suited to meet the requirements of the observatory (AD[01]), are (to the extent possible) consistent with standards used by the scientific community, and fit within the scope of NEON. Additional information on the development and review process can be found in AD[02].

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#### 4 INTRODUCTION TO THE SMALL MAMMAL SAMPLING DESIGN



The foci of the small mammal sampling efforts are the dynamics of

- **demography, density, and pathogen prevalence**
- **small mammal community structure & composition**

as they relate to climate, plant biomass, & insect abundance.

**Figure 2.** *Peromyscus maniculatus* (Rodentia: Cricetidae), the North American deer mouse, a widespread and abundant species throughout temperate North America. Photo courtesy of the National Park Service via Wikimedia Commons.

##### 4.1 Background

Small mammal field studies have played a key role throughout the history and development of the field of ecology, particularly in the subdisciplines of behavioral, population, and community ecology (Stapp 2010). Their scientific utility results from a number of important characteristics, including the relative ease of handling imbued by their small size and trapability. Small mammals are also abundant in virtually all ecosystems, from harsh deserts to arctic and alpine tundra (Merritt 2010; Box 1). These characteristics lend this group to studies of fundamental autecological issues, such as intraspecific interactions and behavior (e.g., Cameron 1995, Cameron and Spencer 2008, Torregrossa and Dearing 2009), resource selection (e.g., Kelt et al. 2004), habitat selection (e.g., Stapp and Van Horne 1997), and distribution (e.g., Orrock et al. 2000, Anderson et al. 2002). In the temperate zones in which they have been most intensively studied, small mammal communities vary in diversity, from monotypic assemblages to those including more than a dozen species. This variation combined with the relatively low, and thus scientifically tractable, levels of local richness (compared to insects or birds, for example) have led ecologists studying small mammals to important insights into patterns of diversity (e.g., McCain 2005), community assembly, and interspecific interactions (e.g., Bowers and Brown 1982, Meserve et al. 1996, Kelt et al. 1999).

**Box 1. What is a small mammal?**

While there is no established definition of the term ‘small mammal’ (Merritt 2010), it is generally used to refer to small rodents (voles and mice in the order Rodentia) and insectivores (shrews and moles in the order Soricomorpha), and sometimes squirrels (Rodentia: Sciuridae) and rabbits (order Lagomorpha), all with body masses less than 120g – 2kg, depending on the source. Here small mammals are defined based on a combination of behavioral, dietary, and size constraints, as the NEON design is limited to species sampled by box traps, due to logistical constraints. This definition includes any mammal that is (1) nonvolant; (2) nocturnally active; (3) forages predominantly aboveground; and (4) is greater than 5 grams but less than approximately 500 g (one exception to this includes the bushy-tailed woodrat, *Neotoma cinerea*, males of which can weigh up to 600 grams). In North America, this includes cricetids, heteromyids, small sciurids, and introduced murids. It does not include shrews, large squirrels, rabbits, or weasels, despite the fact that individuals of these species may be incidentally captured.

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Small mammals are primary and secondary consumers and, due to their often high densities, can significantly impact plant communities (e.g., Weltzin et al. 1997, Bricker et al. 2010). Further, a number of studies have documented important interactions between small mammal consumers and invasive plant species (McMurray et al. 1997, Valone and Schutzenhofer 2007). Small mammals are a critical prey base for a diversity of consumers, making this group a suitable model system for evaluating roles of resource limitation and predation in population dynamics (e.g., Meserve et al. 2003a, Letnic et al. 2011). In addition, the relatively high reproductive rates of many species of small mammals can result in significant fluctuations in population sizes, facilitating understanding of ecological drivers of these systems. Consequently, small mammals are known to respond to changes in climate, land use, and habitat (e.g., Rowe 2007, Urban and Swihart 2009, 2011, Merritt 2010).

Moreover, small mammals serve as reservoirs for a number of zoonotic pathogens. Throughout North America, small mammals, particularly those species in the genus *Peromyscus* (Family: Cricetidae) collectively referred to as deermice, serve as reservoirs of hantavirus and the bacteria associated with Lyme disease, plague, and tularemia (Ostfeld and Parmenter 2008, Mills et al. 2010). *Peromyscus maniculatus*, the North American deermouse, and *P. leucopus*, the white-footed mouse, are the most widespread and abundant species in this genus in North America, and consequently most studies of pathogen dynamics in small mammals have focused on these species (Yates et al. 2002). However, there are many additional species in the family Cricetidae that are reservoirs for infectious pathogens, as well as a diversity of shrews (Order Soricomorpha: Soricidae; Dearing et al. 1998, Mills et al. 1998, Arai et al. 2008).

Consequently, small mammals have long been considered suitable candidates for long-term studies. Long-term studies of small mammal communities have proven essential to garnering understanding of a number of important aspects of ecological dynamics (Brown et al. 2001b), including: responses to extreme climatic events (Thibault and Brown 2008); responses to climatic cycles (Lima et al. 1999); population variability and periodicity (Brady and Slade 2004); density-dependence of habitat selection (Shenbrot et al. 2010); density-dependence of recruitment and fecundity (Reed and Slade 2008); interspecific trends in survival (Reed and Slade 2007); resource limitation (Meserve et al. 2003); the fundamental relationships between species richness and community structure (Ernest et al. 2008); changes in prevalence, geographic spread of novel pathogens, and the resulting impacts on public health (Mills et al. 1999, Douglass et al. 2001, Previtali et al. 2010); and impacts of invasive species (Valone and Schutzenhofer 2007). These processes operate on longer time scales or occur in unpredictable ways.

A single long-term study of desert rodents in southeastern Arizona, the Portal Project (funded through the National Science Foundation’s Long-term Research in Environmental Biology (LTREB) program), has alone provided insights into many of these ecological questions, with the exception of pathogen-related questions (e.g., Valone and Brown 1996, Brown 1998, Ernest et al. 2008). The project has resulted in over 110 publications since its inception in 1977, in high-visibility journals, such as *Science*, *PNAS*, and *Ecology*. A recent analysis of citation data using Google Scholar determined that, if Portal were an

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individual “community ecologist,” she would be the 6<sup>th</sup> most cited one (S.K.M Ernest, pers. comm., 2012). These results emphasize the value of such studies and demonstrate the importance of starting with a good design and then sticking with it for the long-term. The small mammal portion of NEON hopes to learn from and build upon the success of long-term studies such as this.

**4.2 NEON’s Contribution**

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 pathogens 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 pathogens, at up to 47 sites (depending on study design and associated logistics) throughout North America for a period of 30 years (Hawaii and Puerto Rico are currently not included in this design due to permitting constraints). 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 (Table 1), 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.

**Table 1.** Examples of science questions that could be addressed with NEON data

How do small mammal communities vary both within core sites and across land use types and ecoregions?
Which bioclimatic and habitat factors best predict the species composition of small mammal communities?
How do climate-driven plant and insect resources determine the population growth, fecundity, and density of small mammal populations?
How do changes in biodiversity affect resource use and infectious disease dynamics?
What are the specific local habitat traits (e.g., vegetation, slope, soil moisture, insect abundance etc.) favored by <i>Peromyscus spp.</i> that constitute refugia for hantavirus dynamics?
What is the time frame for the response of small mammal host populations to climate driven resource pulses and what is the threshold density for increased hantavirus transmission?

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### 4.3 Purpose and Scope

The purpose of sampling small mammals is to capture interannual variation in abundance, diversity, density, species distribution, and prevalence of select pathogens (e.g., Hantavirus). The pathogen component of this work will be covered in the TOS Science Design for Vectors and Pathogens (AD[06]), but it is introduced here, as the rodent-borne pathogen sampling will occur in conjunction with the small mammal abundance and diversity work. This document details the approach used to derive a scientifically rigorous, logistically feasible sampling design that meets the goals of the Observatory.

## 5 SAMPLING FRAMEWORK

### 5.1 Science Requirements

This science design is based on 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.

### 5.2 Data Products

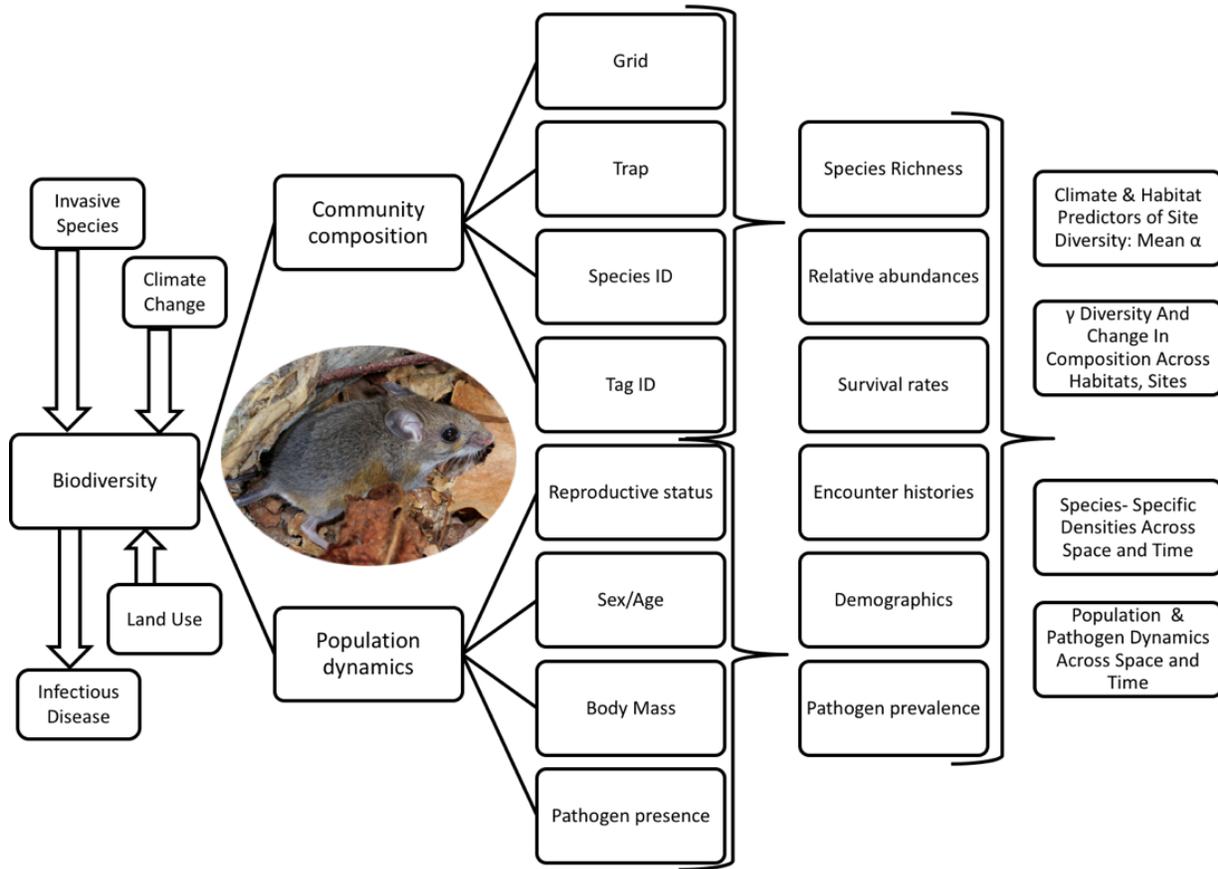
Execution of the protocols that stem from this science design procures samples and generates raw data satisfying NEON Observatory scientific requirements. These data and samples are used to create NEON data products, as documented in the NEON Data Products Catalog (AD[04]; <http://data.neonscience.org/data-product-catalog>; Figure 3).

Community and population-level data will be collected to better understand and predict responses to changes in climate and land use. Key measurements include relative abundance, size, and age-structure of populations; these data are necessary to model changes in species abundances through time.

### 5.3 Priorities and Challenges for Small Mammal Abundance and Diversity

The sampling design for the small mammal component of the NEON TOS must meet the following criteria:

1. **Must** be able to be employed at most, if not all, NEON sites, within the existing budgetary and logistical constraints.
2. **Must** be standardized across the Observatory.
3. **Must** yield robust estimates of community diversity and population density for a diversity of species at each site.



**Figure 3.** Conceptual map from Grand Challenges to several key small mammal data products.

## 6 SAMPLING DESIGN FOR SMALL MAMMAL ABUNDANCE AND DIVERSITY

### 6.1 Sampling Design for Small Mammal Abundance and Diversity

Small mammal populations can be sampled in a number of ways (Wilson et al. 1996), and the best method depends on study objectives and the species of interest. Since the target species for NEON are nocturnal and cryptic, the most common methods used to sample them are passive, involving kill- or live-traps, baited or unbaited (e.g., Wilson et al. 1996, Evangelista et al. 2008). For long-term studies of small mammal community, population, and pathogen dynamics, mark-recapture methods are the most commonly deployed (e.g., Mills et al. 1999, Douglass et al. 2001, Meserve et al. 2003, Ernest et al. 2009). A number of analytical methods have been developed to use mark-recapture data to derive estimates of density and diversity, given that many animal species, including small mammals, cannot be observed perfectly (White and Burnham 1999, Efford et al. 2009, Royle et al. 2009). Moreover, pathogen studies often employ live-trapping (even for removal studies), in order to ensure the integrity of the tissue

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samples (Mills et al. 1995). For these reasons, NEON will employ a mark-recapture approach to studying small mammals and rodent-borne pathogens. There remain many design options within the context of a mark-recapture approach, and these are discussed below.

**6.1.1 Sampling Methods**

**6.1.1.1 Mark-Recapture Design: Trap Type**

Small mammals are typically live-trapped using 3 general trap types: box traps (e.g., Sherman traps, Longworth traps, Elliott traps) that are placed on the ground or in trees and typically baited; cage traps (e.g., Tomahawk, Havahart traps) that are set like box traps; and pitfall traps, which are buckets buried in the ground and are not intentionally baited (Wilson et al. 1996). Within these categories, a diversity of sizes is available as well, as no single trap type or size is appropriate for all species. For NEON’s target small mammal species, box traps are the most commonly used trap type, and Sherman traps (H.B. Sherman Inc., Tallahassee, FL) have been the standard in mammalogy for decades, particularly in the U.S. Longworth traps (Penlon Ltd., Oxford, U.K.) are distinguished from Sherman traps by an additional, padded compartment that provides greater insulation under very cold conditions. Cage traps are more often used for larger mammals such as mesocarnivores, due to their relative sturdiness and greater camouflage in the environment, whereas pitfall traps are the standard for capturing shrews, given their distinctive behaviors and extremely small sizes that are often not sufficient to set off the trigger mechanism in box traps. Pitfalls have been shown to be more efficient than box traps for capturing individuals less than 20 grams (Francl et al. 2002).

Box traps are particularly efficient at capturing deermice (*Peromyscus spp.*), a NEON target taxon. Within the box trap, there are a number of additional design options available, including Sherman vs. Longworth, size options, and whether or not the traps are folding or non-folding. Shermans and Longworths have not been compared in a diversity of studies, but one study found differences in species composition of captures and increased mortality rates in Longworth relative to large (7.7 x 9.1 x 23 cm/ 3 x 3.5 x 9 in) Sherman traps (Anthony et al. 2005). For studies in which traps are able to remain in place on a semi-permanent basis, storage is abundant, and travel times are relatively limited, the non-folding option is very practical. However, none of these features are likely to apply at NEON sites, with traps having to rotate among the core and relocatable sites within each domain.

**NEON plan:** H.B. Sherman folding traps will be used to facilitate movement of traps among sites; however, whether folding or non-folding traps are used should not have a significant scientific impact. Logistically, the folding traps do allow more water inside the trap, which can lead to mortality of captured individuals in cold and wet conditions. Site-specific recommendations for covering of traps will be included in the field sampling protocol at sites where necessary. In terms of trap size, most species in North America can be successfully and humanely captured in traps that measure 3 x 3.5 x 9 inches (7.7 x 9.1 x 23 cm). However, many domains have jumping mice, kangaroo rats, and/or large woodrats whose

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tails have a higher incidence of being injured in the door of a trap this size. For these domains (namely Domains 10 through 17), a longer trap (3 x 3.75 x 12 inches; 7.7 x 9.5 x 30 cm) will be used.

**6.1.1.2 Mark-Recapture Design: Bait**

Bait significantly increases capture success, but few significant effects of bait type have been documented for the NEON target species (Woodman et al. 1996, Ashe 2007, Manville et al. 2011 but see Oswald and Flake 1994). Bait preferences presumably vary across species according to diet (Oswald and Flake 1994), and may also vary with region and/or time of year. Additional bait-related considerations include attractiveness to non-target species that will negatively impact the traps or the captured individuals (e.g., bears, mesocarnivores, slugs, and fire ants), the nutritional content of the bait, including the salt content in arid regions, and the amount of energy required to sustain a captured individual under the existing weather conditions. Peanut butter and oats are one of the most commonly used bait formulations, as it is aromatic, attractive to a diversity of small mammal species, and high in calories. However, peanut butter is often not allowed where bear or fire ant densities are high (e.g., Florida Fish and Wildlife Conservation Commission guidelines for scientific research).

**NEON plan:** A seed mixture of millet and sunflower seeds will be used as bait at all sites, as it is cleaner, easier to transport and put in traps than peanut butter, and presumably less likely to attract unwanted and destructive visitors. A mixture that includes sunflower seeds will attract a greater diversity of species, with particular utility for squirrels. Seeds will be sterilized in drying ovens prior to use to prevent germination of non-native plants at NEON sites. At sites where capture rates of shrews exceed 20%, 4 grams of freeze-dried mealworms will be added to the seed mix (Do et al. 2013).

**6.1.1.3 Mark-Recapture Design: Marking Method**

Currently, the NEON budget includes a line item for ear tags, which are approximately \$0.10 a piece. PIT tags are orders of magnitude more expensive than ear tags (\$3-9 each), but the advantages afforded by them warrant directing resources to provision them (Schooley et al. 1993, Gibbons and Andrews 2004), particularly in high tick areas where ear tagging can increase tick infestation rates (Ostfeld et al. 1993). PIT tags offer bar-code technology that could be integrated into the NEON data management system; the serial numbers are >10 characters long, making duplicate tag numbers through the spatial and temporal extent of the entire Observatory improbable; and PIT tags can be used by the community to conduct behavioral studies (e.g., Harper and Batzli 1996). However, one study did find that failure rates of PIT tags in deermice and cotton rats were higher than ear tag loss rates (Fokidis et al. 2006), but PIT tag loss rates can be decreased significantly with the use of surgical glue (Lebl and Ruf 2010).

**Emerging technologies:** A new technology, electronic p-Chips (PharmaSeq, Inc., Monmouth Junction, NJ), has been developed recently for tagging laboratory mice. Although development of a field ready reader and testing in wild mammals has yet to be done, the technology holds promise for providing a

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lower cost (relative to PIT tags; \$2-3 per tag vs. \$6-10 per tag), more reliable tagging method in the future.

**NEON plan:** The characteristics of ears that are best-suited for ear tagging include a combination of conspicuousness, length of external pinna, and robustness of cartilage at the base of the ear. Given the qualitative nature of this assessment, expert opinion is applied here to designate those species lacking sufficiently large pinnae for ear-tagging (i.e., members of the subfamilies Arvicolinae (family Cricetidae) and Perognathinae (family Heteromyidae). For these species, PIT tags will be used. For species with sufficiently long and robust ears, each individual will be marked with one ear tag in the right ear (Self-piercing, small animal ear tag; laser-etched with NEON on one side, 4 or 5 digit number on the other; e.g., National Band and Tag 1005-1). The laser-etching significantly improves the readability of the number, even long numbers (i.e., 4-5 digits), and the NEON etching is to ensure uniqueness of the tag ID at sites when other small mammal trapping is occurring. If a marked individual is recaptured but is missing its tag, a replacement will be attached on the alternate ear. Since shrews (Soricomorpha: Soricidae) are not targets of the NEON sampling and pose unique challenges for marking, shrews will not be permanently marked but will be temporarily marked using a waterproof marker to assess recaptures within a trapping bout (see section 6.1.3.2 below for additional details regarding the temporal distribution of trapping).

**6.1.1.4 Samples and Specimens for Analysis and Archiving**

**Voucher specimens:** As a complement to the mark-recapture data, voucher specimens of all resident species will be collected from each domain, with a target minimum of 5 vouchers per species collected every 5 years. Incidental losses due to trapping and handling will all be vouchered as well. Ideally, these specimens will include skin, skeleton, and frozen tissues (e.g., liver, heart). These specimens minimally provide a long-term record of the taxonomy of the species being studied, as a reference for verification or evidence to support taxonomic changes that occur as the science advances. The costs associated with the extensive NEON archive planned for both the terrestrial and aquatic systems remain a significant unknown at this point. Therefore, it is a challenge to estimate how many voucher specimens could be accommodated by the current operations budget, as a per-sample cost structure negotiated with existing collections is anticipated.

**Samples opportunistically collected from released individuals:** As discussed above, the costs associated with the NEON archive remain a significant unknown, creating large uncertainties in estimates of how many samples and specimens can be accommodated. Moreover, logistical limitations prevent technicians from collecting all of the possible samples (see Box 3 below). The goal of many of these samples is to provide an archive that external scientists can take advantage of to ask interesting questions not provided for in the scope of NEON. Here the advantages and utility of several additional samples that could be collected in the course of the mark-recapture study are presented.

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#### 6.1.1.4.1 Ear Punches

- Uses
  - If stored in ethanol, punches can provide tissue for population genetics, DNA barcoding, etc.
  - If frozen (-80°C; and not stored in ethanol), punches can be used for pathogen analyses, in addition to population genetics and DNA barcoding.
- Cost: vial + storage cost (ethanol < -80°C freezer)
- Cons: time consuming – adds approximately 1 minute to processing time per individual
- Analyses: Most will have to be planned, funded, and executed by external PIs, except for some of the DNA barcoding component used to confirm species identifications in the field.
- **NEON plan:** Include these samples stored in -80°C freezer, but only one per individual to ameliorate processing time constraints. Use a subset for DNA barcoding.

#### 6.1.1.4.2 Ectoparasites

- Uses
  - Enable parasite load and diversity studies
  - Assess pathogen prevalence from ticks feeding on mammals to complement analyses currently planned for ticks collected independently
- Cost = vial + storage cost (ethanol < -80°C freezer)
- Cons:
  - time consuming – adds > 1 minute to processing time per individual
  - scientific utility limited by the ability of and variability in technicians to see and collect them – bias towards large size
- Analyses: would have to be planned, funded, and executed by external PIs
- **NEON plan:** Ectoparasites will not be collected, as the original intent of this design recommendation was to enable tick-borne pathogen analyses. Ticks for pathogen analyses are to be collected via alternative methods (see AD[06]).

#### 6.1.1.4.3 Feces

- Uses
  - Enable diet, pathogen, hormone, isotopic, genetic, and microbial studies
- Cost = vial + storage cost (ethanol < -80°C freezer)
- Cons:
  - Opportunistic sampling only
  - Samples are relatively susceptible to contamination
- Analyses: will have to be planned, funded, and executed by external PIs
- **NEON plan:** Include these samples stored in -80°C freezer for NEON-supported archiving only. Analyses will have to be planned, funded, and executed by external PIs.

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#### 6.1.1.4.4 Hair

- Uses: DNA barcoding and isotopic analyses
- Cost = storage container + storage space (ambient, dry storage <<< frozen)
- Very easy to collect (<10 seconds + labeling time) and minimally invasive
- Cons:
  - Samples are relatively susceptible to contamination
  - Analysis require additional processing to extract DNA
  - Failure rates for acquiring a bar-code compliant sequence are higher than other tissue samples
  - For isotopic analyses, molting patterns are not well understood
- Analyses: If selected for DNA barcoding, NEON would fund a subset of the analyses.
- **NEON plan:** Include these samples – hair and whiskers - for NEON-supported archiving. Isotopic analyses will have to be planned, funded, and executed by external PIs.

#### 6.1.1.4.5 Toenails

- Use: isotopic analyses
- Cost = storage container + storage space for dry, ambient storage
- Cons:
  - Time consuming – can be up > 1 minute of processing due to extremely small size of toenails
  - Literature involving small mammal toenail analyses is limited
- Analyses: If selected for isotopic analyses, NEON would archive the necessary samples. Isotopic analyses would have to be planned, funded, and executed by external PIs.
- **NEON plan:** Replace toenails with whiskers, as toenail collection is unduly time-consuming and prone to error (as toenail samples are extremely small and difficult to manipulate).

#### 6.1.1.4.6 FTA Cards

- Uses: population genetics and DNA barcoding
- Cost = US\$3-4 per micro card (1 sample); Storage is dry ambient
- Adds minimal processing time for individuals from whom blood is being collected for pathogens
- Cons:
  - Cost
  - Not efficient for individuals that are not having blood collected for pathogen analyses
  - Duration of sample stability unclear
- **NEON plan:** Will not include, as these samples were not supported by members of the technical working group

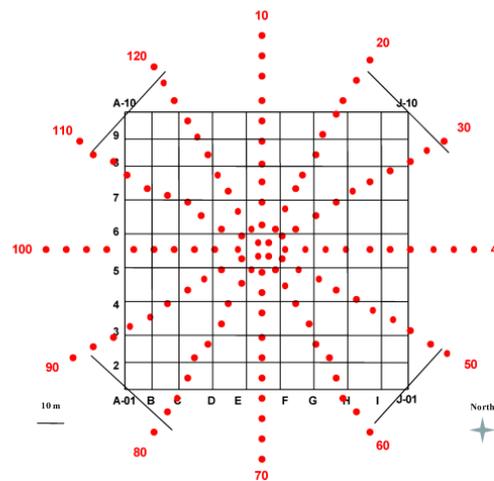
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## 6.1.2 Spatial Distribution of Sampling

### 6.1.2.1 Mark-Recapture Design: Trap Array

The next design issue that arises is how to array the traps on the landscape to (a) capture sufficient numbers of individuals to characterize community diversity and seroprevalence, and (b) provide insights into local densities and population dynamics for common species. Different geometrical arrangements of traps coupled with spacing between traps yield different effective sampling areas, which in turn impacts the number of individuals whose home ranges overlap with the trapping array (Pearson and Ruggiero 2003). For density estimation, grids and trapping webs are the most-commonly used methods, although transects are used by researchers at some Long-term Ecological Research (LTER) sites, such as the Shortgrass Steppe and Konza Prairie LTERs (Evangelista et al. 2008). Transects can be an efficient method for increasing capture rates in low density habitats (Read et al. 1988, Pearson and Ruggiero 2003; but see results below). As such, the NEON Tiger team (a group of small mammal biologists) recommended the use of the trapping web or the grid (Ostfeld and Parmenter 2008).

A trapping grid is a simple square of traps arrayed with equal spacing, whereas trapping webs comprise a series of traplines radiating from a central cluster of traps, like the spokes of a wheel, with distances increasing from proximal to distal points on each line (Anderson et al. 1983, Mills et al. 1999, Parmenter et al. 2003; Figure 4). A trapping web is a form of distance sampling (Buckland et al. 2001), which enables the use of statistical algorithms to generate density estimates that account for variation among species in trapability or detectability (Anderson et al. 1983, Parmenter et al. 2003). Such algorithms are not used for grid data, but mark-recapture methods can be used as an alternative approach to generate density estimates (White and Burnham 1999, Efford et al. 2009, Royle et al. 2009).



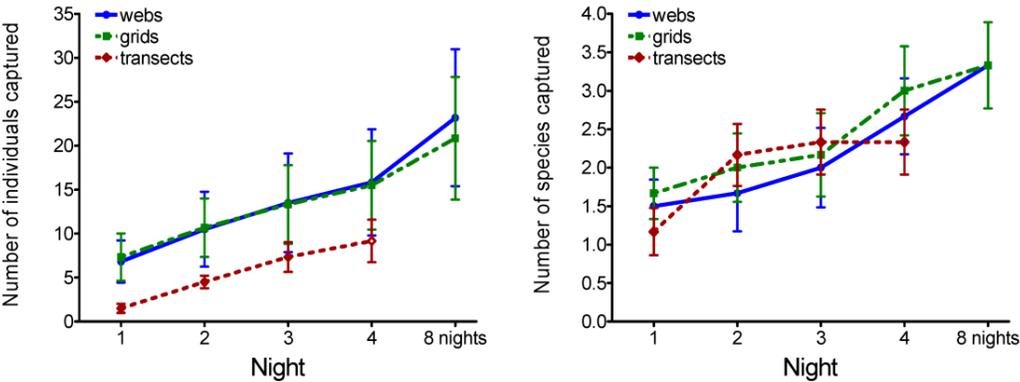
**Figure 4.** Schematic depicting grid, web, and transect trapping arrays. Figure by NEON contractor, Paul Stapp (Evangelista et al. 2008).

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Trapping webs are used at several LTER sites (e.g., Sevilleta, Shortgrass Steppe), and some have argued that they are statistically superior to grids (Parmenter et al. 2003). Logistically, however, trapping webs present unique challenges. By design, they cover 3.14 hectares, whereas most grids cover an average of 1 hectare. Therefore, fewer independent samples on a landscape can be taken. Moreover, trapping webs are more labor intensive to deploy. This latter concern can be minimized at sites where each individual trap location can be permanently marked. However, at most NEON sites, this is not possible.

**Analyses of Prototype Data and Results:** NEON contractors conducted field trials in September 2008 comparing these approaches at the NEON Central Plains Experimental Range (CPER) site in northeastern Colorado (Evangelista et al. 2008). Six square 1.82-ha grids (10 x 10 stations, with 15-m spacing, and a single Sherman trap at each station) were centered directly on top of six existing sampling webs (Figure 4), and paired transects were also associated with each web and grid location. Transects were 285 m long, with 20 stations placed 15 m apart and two Sherman traps per station. The two transects were >100 m from the edge of the grids and were perpendicular to one another. Webs were trapped from 8 through 18 September 2008, and grids and transects were trapped concurrently from 22 September through 2 October 2008.

Total trapping effort was 6,336 trap nights (TN) (6,238 TN, adjusting for sprung traps). A total of 187 different individuals of six species was captured: Ord’s kangaroo rat (*Dipodomys ordii*; 133 individuals, 71.1% of total); northern grasshopper mouse (*Onychomys leucogaster*; 30, 16.0%); deer mouse (*Peromyscus maniculatus*; 12, 6.4%); western harvest mouse (*Reithrodontomys megalotis*; 7, 3.7%); silky pocket mouse (*Perognathus flavus*; 4, 2.1%); and plains harvest mouse (*R. montanus*; 1, 0.5%). The rate of accumulation of new individuals over the four nights of trapping was similar for all three arrays (Figure 5). The capture rates were highest on the grids: 95 individuals were captured on webs (3.25/100TN), 93 individuals on grids (3.92/100TN), and 55 individuals on transects (2.89/100TN). Moreover, the proportion of individuals recaptured was also highest on grids (28.7±0.3%), followed by webs (25.7±1.6%) and transects (25.6±3.3%).

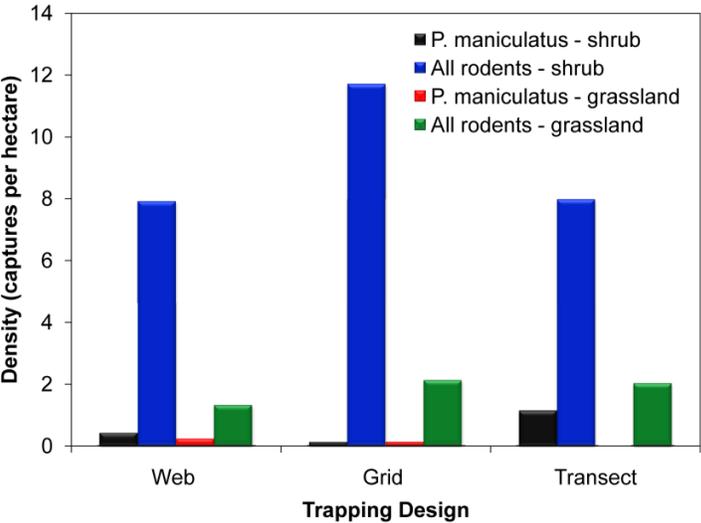


**Figure 5.** Means ± STD of individuals & species accumulated across nights, among trapping designs, from the NEON core site, CPER. Figures by NEON contractor, Paul Stapp (Evangelista et al. 2008).

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Assuming all individuals that were captured over eight nights at a given site were available for capture on both the web and grid (omitting individuals captured only on outer web rings, outside the grid area), grids missed fewer individuals (24%) than webs (34%) on shrub sites, whereas similar fractions were missed by grids and webs on grassland sites (31% and 29%, respectively). The cumulative number of species captured also increased over the number of nights of trapping (Figure 5), reaching a plateau on transects on the second night but continuing to increase on grids and webs through the fourth night. On any given night, more species were captured on grids than on webs (Figure 5). Assuming all species captured at a given site could have been caught using all three trapping designs, the fewest species were missed on grids (0% for grassland sites, 17% for shrub sites), followed by webs (11%, 24%) and transects (28%, 24%).

Density estimates were calculated using Schnabel estimates of population size ( $\hat{n}$ ) and the programs CAPTURE and DISTANCE (Otis et al. 1978, Thomas et al. 2010). On both shrub and grassland sites, mean Schnabel density estimates were higher on grids (shrub:  $12.31 \pm 1.4/\text{ha}$ ; grassland:  $3.19 \pm 1.54/\text{ha}$ ) than on transects ( $10.19 \pm 1.21/\text{ha}$ ;  $2.73 \pm 0.60/\text{ha}$ ) or webs ( $9.29 \pm 2.95/\text{ha}$ ;  $1.69 \pm 0.97/\text{ha}$ ) (Figure 6). Because of the low number (<10) of captures in grasslands, densities could only be modeled for shrub webs and grids. As with the Schnabel estimates, modeled density estimates for shrub sites were higher and less variable on grids ( $12.94 \pm 0.28/\text{ha}$ ) than on webs ( $7.89 \pm 2.15/\text{ha}$ ;  $n = 3$  sites per type). For modeled density estimates on shrub sites, the average coefficient of variation was 14.0% for grids, compared to 20.6% for webs. Considering only shrub sites, naive density values underestimated Schnabel-based density estimates by 4.2% for grids and 11.3% for webs, and modeled density estimates by 9.8% for grids. Modeled density estimates from shrub webs were equivalent to naive density estimates because the model and data used (uniform function, no truncation) estimated density as the number of individuals caught divided by the effective web area (detection probability = 1.0 for a web with a 105-m radius). Schnabel-based density values were lower than modeled estimates by 5.3% for grids, but higher on webs by 13.9%.



**Figure 6.** Mean naive densities (number of unique individuals/trapping area) of *P. maniculatus* and all species across trapping designs and habitats, from prototype small mammal trapping efforts at CPER (Evangelista et al. 2008).

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**NEON plan:** NEON will employ trapping grids as the unit of sampling, due to (1) the logistical advantages, (2) the fact that they are more commonly adopted in small mammal studies (which facilitates comparison with a greater number of data sets; e.g., Steele et al. 1984, Read et al. 1988, Pearson and Ruggiero 2003), (3) the additional modeling demands associated with webs in heterogeneous environments where the probability of capture varies across microhabitats, and (4) the recent advances in spatially-explicit capture-recapture (SECR) models that can effectively model grid data to estimate densities (e.g., Efford 2004).

### 6.1.2.2 Mark-Recapture Design: Size and Density of Trap Array

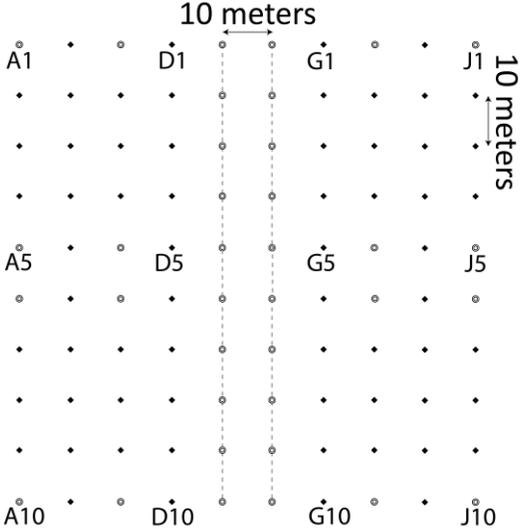
Webs are of a standard design in terms of trap spacing and size (see above). Grids are more variable but are often 10 x 10. Traps are typically spaced 10 or 15 m apart, with a 10 m spacing yielding a 1-hectare grid area. The trade-offs between these two spacing options include (a) the probability that more individuals' home ranges will be covered by the trapping grid and (b) the ease with which the trapper can locate trap stations and navigate the grid in challenging environments. Recent developments in capture-recapture models use the relative capture locations of marked individuals within a trapping array to estimate the effective sampling area of the array, as well as population sizes (i.e., SECR models; e.g., Efford 2004, Royle and Young 2008). A diversity of computational approaches can be used to fit these models, including maximum likelihood (Borchers and Efford 2008) and Bayesian methods (Royle and Young 2008), but the algorithms all require that some individuals are captured at more than one location within the trapping array (Efford and Fewster 2012). Appropriate spacing to realize this requirement therefore varies by species (with home range size), with 10 meters more appropriate for shrews and some voles, but 15 meters is likely more appropriate for *Peromyscus* and *Tamias*, and even larger might be appropriate for *Glaucomys* or *Neotoma* (Swihart, pers. comm.). However, SECR models have been shown to be robust to variation in spatial sampling designs and do not place stringent requirements on the size of trapping arrays relative to the home range of the target species (Sollmann et al. 2012).

At each of the 100 trap stations in a trapping grid, it can be desirable to place two traps per station. This is the case particularly in deciduous forest environments where *Peromyscus leucopus* exhibits arboreal behavior (e.g., Klein and Cameron 2012) or when densities are extremely high, to avoid trap saturation. A proposed guideline for avoiding trap saturation is that >20% of the traps should remain empty following a night of trapping (Southern 1973, Gurnell 1976). Capture rates >80% are extremely rare; a meta-analysis of several hundred studies of small mammal communities (Thibault et al. 2011) revealed an average capture rate of about 10%, suggesting that trap saturation is indeed a rare occurrence. Given the added logistical and budgetary pressures conferred by doubling the number of traps per grid, the low probability of trap saturation does not seem to warrant this approach. However, a consistently applied adaptive approach (e.g., place additional traps in a grid whenever capture rates exceed a specified threshold) may be a viable option, as the varying effort can be accounted for in analyses if

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necessary. An alternative is the placement of a second trap consistently at 20% of stations, using vertical (>1 m above the ground) placement in deciduous forest habitats.

**NEON plan:** NEON will employ 10 x 10 trapping grids, with 10 m spacing and 1 trap per station (Figure 7). This design yields an approximate sampling area of 1 ha; allows for consistent sampling across NEON sites of deer mice and voles, the most common reservoirs of the NEON target rodent-borne pathogens (AD[06]); is logistically desirable to facilitate the setting of as many grids as possible within the time available; and would yield sample sizes of approximately 10 individuals per grid (assuming a mean capture rate of 10%), on average. However, initial years of trapping have yielded dramatically higher rates of capture than expected at most NEON sites (see 7APPENDIX B). Consequently, the resources required to perform this sampling as originally designed quickly exceeded those available, and reductions to the sampling were implemented as described below to meet budget constraints.



**Figure 7.** Schematic of NEON small mammal 10 x 10 trapping grid.

**6.1.2.3 Mark-Recapture Design: Number and Distribution of Arrays in the Landscape**

Logistical limitations in terms of labor, equipment costs, and, for some sites, area available to sample, typically prevent small mammal studies from being able to meet minimum sample size requirements as determined from traditional power analyses and similar statistical approaches. Ostfeld and Parmenter (2008) initially recommended that NEON deploy three trapping webs or grids at each site. Some studies are based on only one array (e.g., Merritt 1999), and others on up to 10 or more (e.g., Letnic et al. 2005, Ernest et al. 2009), with pathogen studies typically sampling on only 2-3 arrays (e.g., Mills et al. 1998, Douglass et al. 2001). This aspect of the design will be determined largely by budgetary and logistical constraints, with the understanding that the greater the replication across habitat types and the greater

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the collocated number of samples, the more robust the NEON data products will be. Specific constraints include area of the site, capture rates, and handling times (Box 2).

**Analyses of Existing Data and Results:** Existing long-term data, such as those from the Portal Project, can be used to assess the impact of varying trapping array sample size on species accumulation in a small mammal community.

**Portal LTREB:** These data come from a long-term study established in 1977 by J. H. Brown and colleagues in the Chihuahuan Desert, in the foothills of the Chiricahua Mountains (elevation 1,330 m, 6.5 km east and 2 km north of Portal, Cochise County, Arizona) (Brown 1998, Ernest et al. 2009). On each plot rodents were trapped monthly since 1977, during the new moon phase, using Sherman traps (model LFA; H. B. Sherman Traps, Tallahassee, Florida) baited with millet. Traps were placed at each of 49 permanently staked locations (7 x 7 grid, with 6.25 m between stakes) per plot for 1 night. Captured rodents were measured, weighed, identified to species and sex, and uniquely marked with a passive integrated transponder tag (Biomark, Boise, Idaho) or ear tag (National Band and Tag Co., Newport, Kentucky), and their reproductive condition was assessed.

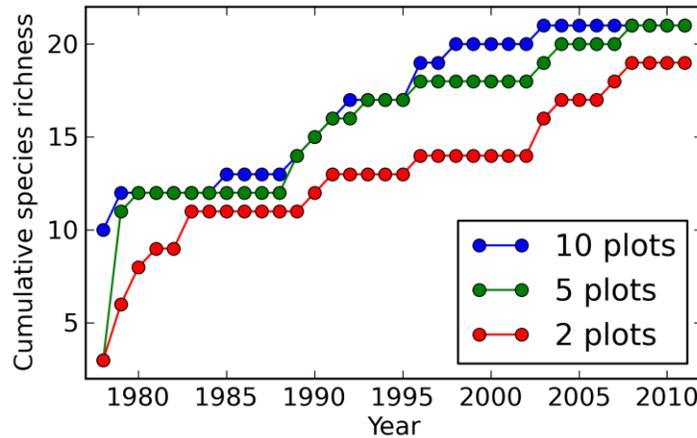
**Box 2. Pilot study 2012: logistical constraints at Rocky Mountain National Park**

The current budget for the small mammal sampling allows for 3-4 technicians to conduct the sampling for all three sites within a domain. The number of plots that can be trapped and processed on any given morning depends on travel times between plots, capture rates, and handling times. Handling times are in turn impacted by the number and types of samples and measurements being taken from each individual. In 2012, NEON conducted a pilot field season at Rocky Mountain National Park with 4 technicians, one of whom had significant experience handling and bleeding small mammals. The field crew attempted to sample 8 grids for 2 consecutive nights per month from May through October, while keeping track of time needed to complete tasks. The use of anesthesia (isoflurane) prior to bleeding cost approximately 3 minutes per individual, with other sampling (ear punches, toenail clips, hair samples, and fecal samples) costing another 3 minutes per individual. In combination with standard measurements of hindfoot, ear length, tail length, total length, and body mass, as well as species, sex, and reproductive state assessment and marking (2 ear tags or 1 PIT tag), the technicians were frequently handling individuals for over 10 minutes. In July and August, capture success was 30-50%, due to high rates of reproduction and high densities of *Peromyscus maniculatus*. Under these conditions and given the often-challenging terrain at the Park, the technicians were unable to collect all the samples from all of the individuals captured on 8 plots in a reasonable amount of time (i.e., before noon).

The differences in annual species accumulation over time at the study site are depicted in Figure 8. The number of species detected increased over the course of the entire study for all sample sizes, presumably due to concomitant regional habitat changes. The number of species detected increased at a faster rate and to a greater absolute number of species when 5 or 10 plots were sampled versus only 2

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plots. More species were detected sooner with 10 plots than with 5 plots as well, but the differences are only substantial in the first year.



**Figure 8.** Cumulative number of desert rodent species detected through time with varying sample size of trapping grids at a 20-ha site near Portal, AZ, USA (Ernest et al. 2009).

### NEON plan: Collocation and plot selection

Given the range in sizes of NEON sites (as the boundaries are currently defined) and the current understanding of budget and logistical constraints (see Box 2 above), it is feasible to sample 3 – 8 grids at each NEON site (except in HI and PR; see APPENDIX A below). Given the increased logistical hurdle of trapping at relocatable sites due to the typically greater distance from the Domain Support Facility (which houses the staff and equipment), the number of grids at these sites is capped at 6. Many core sites have also been limited to 6 grids, given relatively high capture rates (>20%) that increase processing times per grid. To accommodate the goal of the rodent-borne pathogen sampling to track infection status in individuals through time, blood sampling is concentrated on only 3 of the trapping grids, given logistical constraints (i.e., blood sample collection is time-intensive; AD[06]). At sites with extremely low capture rates (e.g., TALL - Talladega National Forest), blood sample collection occurs on all grids to achieve greater sample sizes.

The primary goals in selecting pathogen grids are: 1) long-term sampling of target species, which requires moderate to high average capture rates (i.e., >10%), and 2) representation of the site’s dominant vegetation type. If there are more or fewer than 3 trapping grids that occur within the dominant vegetation type(s) and trapping data from previous years are available, the selected grids will be those with the highest abundances of target species. Otherwise, pathogen grids will be chosen at random or based on an educated assessment of habitat quality for small mammals. Once a grid has been designated as a pathogen grid (after an initial assessment period of two years), that classification will apply for all subsequent trapping seasons for consistent, long-term data collection. If the majority of captures in the dominant vegetation type at a site are heteromyids (not approved for blood collection),

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then an alternate habitat type may be preferred (e.g., Moab, UT). Abundance will vary by season and year, so pathogen grids will not always have the highest abundance relative to the other grids but should offer consistent long-term sampling.

The distribution of mammal plots is currently intended to be collocated to the extent possible with the TOS Distributed plots (these are the plots at which the greatest diversity of sampling is planned to occur, including plants, soils, microbes, beetles). There will be 5 – 35 of these plots at each site distributed via a stratified random design based on vegetation type (AD[03]). The procedure for identifying trapping grid locations is described in Box 3, and an example of selected grid locations at one NEON site is shown in Figure 9. Mammal grids are distributed accordingly to the stratified-random design used for all TOS sampling.

**Box 3. Summary of procedure used to select plot locations for small mammal sampling**

- Grid locations are selected by using a random number generator to select a compass direction from the collocated TOS plot, and the grid centers are then placed 150 meters from the edge of the biodiversity plot to avoid disturbance to the other sampling
- 100 x 100 m (to accommodate 10 x 10 grids of 10 m spacing)
- 25 m buffer from paved or large (> 10m wide) dirt roads
- Maximum distance from road of 300 meters, due to equipment and logistical constraints; at sites with extremely dense vegetation, the maximum distance to road is 100 m
- Distribute mammal grids by proportional habitat availability, according to National Land Cover Database (NLCD) classifications
- Provide extra grids for training, voucher collection, and contingency, if possible
- Assume that spatial independence requires at least the width of the grid between grids
- 3 – 8 grids per site

**6.1.3 Temporal Distribution of Sampling**

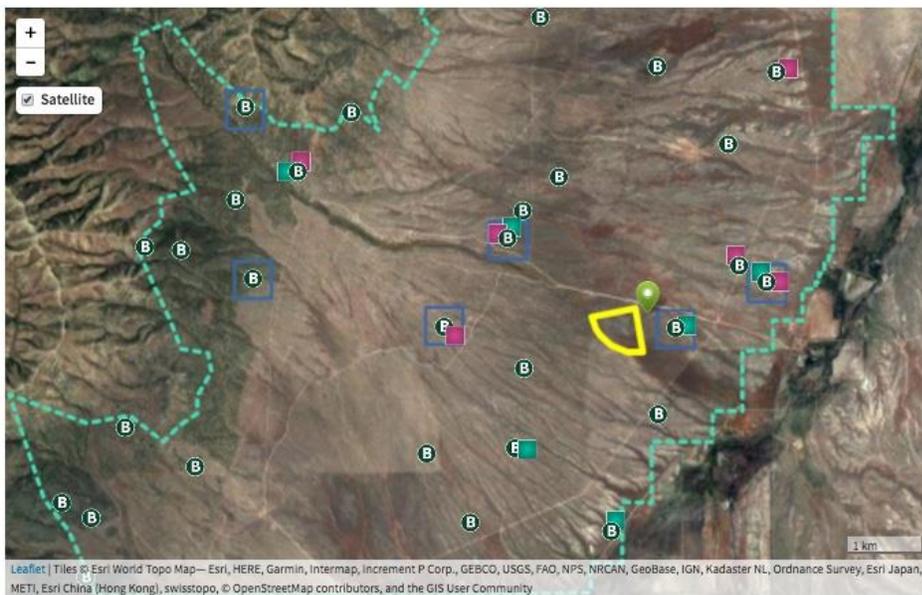
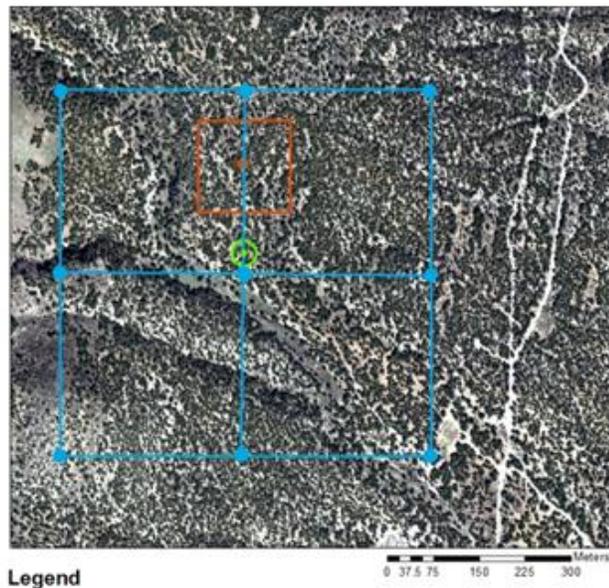
**6.1.3.1 Mark-Recapture Design: Number of Sampling Periods per Year**

In addition to spatial intensity of sampling, the temporal intensity of sampling required to produce robust estimates of density, vital rates, reproductive phenology, and, where relevant, pathogen prevalence is also an important consideration. Ideally, NEON would capture the seasonal variation in these parameters as well, as they are known to vary on shorter than annual time scales (Wilson et al. 1996, Dearing et al. 2009). As with spatial intensity, the temporal intensity of sampling is typically dictated more by budgetary and logistical constraints than through a statistical assessment (such as a power analysis). Only one known study to date has evaluated the impact of sampling frequency on metrics of small mammal community dynamics, including abundance and prevalence of hantavirus

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(Carver et al. 2010). In this study, the authors used an existing 15-year dataset to compare estimates of abundance (minimum number known alive (MNKA)) of and hantavirus prevalence in deermice derived from sampling monthly, bimonthly sampling, quarterly, semiannually (twice per year), and annually (once per year). When sampling occurred less often than monthly in this Montana ecosystem, deermouse abundance was underestimated 10-20%, while Sin Nombre Virus prevalence was overestimated when prevalence was high (i.e., >15%; Carver et al. 2010).

**Figure 9.** Example maps of collocated mammal sampling locations. A) Map of a bird (large blue box) and mammal grid (smaller orange box) collocated with each other and with a TOS distributed base plot (green circle); B) Map of sampling locations at Onaqui, stratified by the two dominant habitats (NLCD classes) at the site, evergreen forest and shrub scrub.



**Map Legend**

Click the check boxes to filter results on the map

- NEON Sampling Boundaries
- Tower Airshed Boundary
- Tower Location

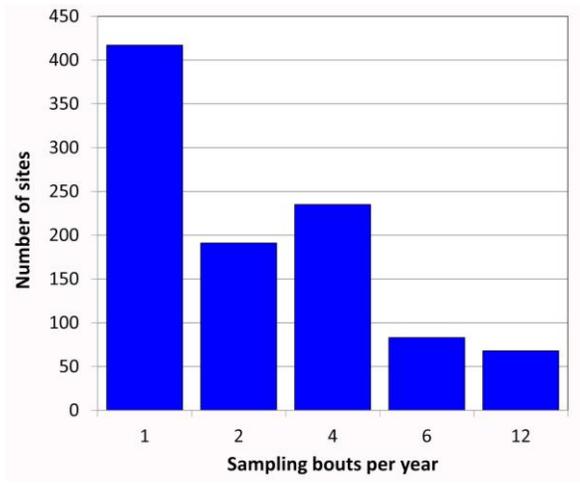
Distributed Plot Types

- Distributed Base Plot
- Distributed Bird Grid
- Distributed Mammal Grid
- Distributed Mosquito Plot
- Distributed Tick Plot

From:  
<http://www.neonscience.org/field-sites/field-sites-map/ONAQ>

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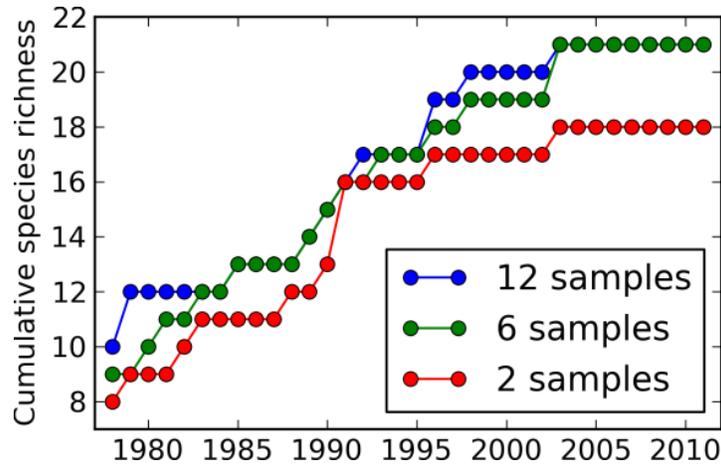
**Analyses of Existing Data and Results:** Here two existing datasets are used to (1) describe temporal sampling intensities reported in published studies, and (2) apply Carver et al.’s (2010) method to another long-term dataset of small mammal communities that includes monthly data, the Portal Project, described above. The Mammal Community Database (MCDB; Thibault et al. 2011) is a compilation of data from the literature that includes species lists for 1,000 mammal communities, excluding bats, with species-level abundances available for 940 of these communities. Site-level data are generally limited to species groups that are sampled using a single technique (e.g., small mammals sampled with Sherman traps). These data show that the vast majority of studies in the MCDB sample four or fewer times a year (Figure 10).



**Figure 10.** Number of site x small mammal trapping study combinations that used the specified number of sampling bouts per year - data from Thibault et al. 2011

The Portal Project does not include pathogen data; therefore, Carver et al. (2010) analyses were conducted to compare abundance (MNKA) and species richness estimates across the specified levels of sampling frequency, as well as species accumulation curves, as performed for the spatial analysis. The number of species continued to accumulate over the first 20 years of the study (Figure 11), with rates of accumulation greatest when all monthly samples were used and lowest when only 2 samples (spring and fall) were included. Two samples a year also failed to document three of the 21 species found at the site (Figure 11). Further, six samples a year (bimonthly sampling) required a six-year study period to document the same number of species documented by year 2 with a monthly sampling regime. Accordingly, abundance and species richness were underestimated with decreasing frequency of

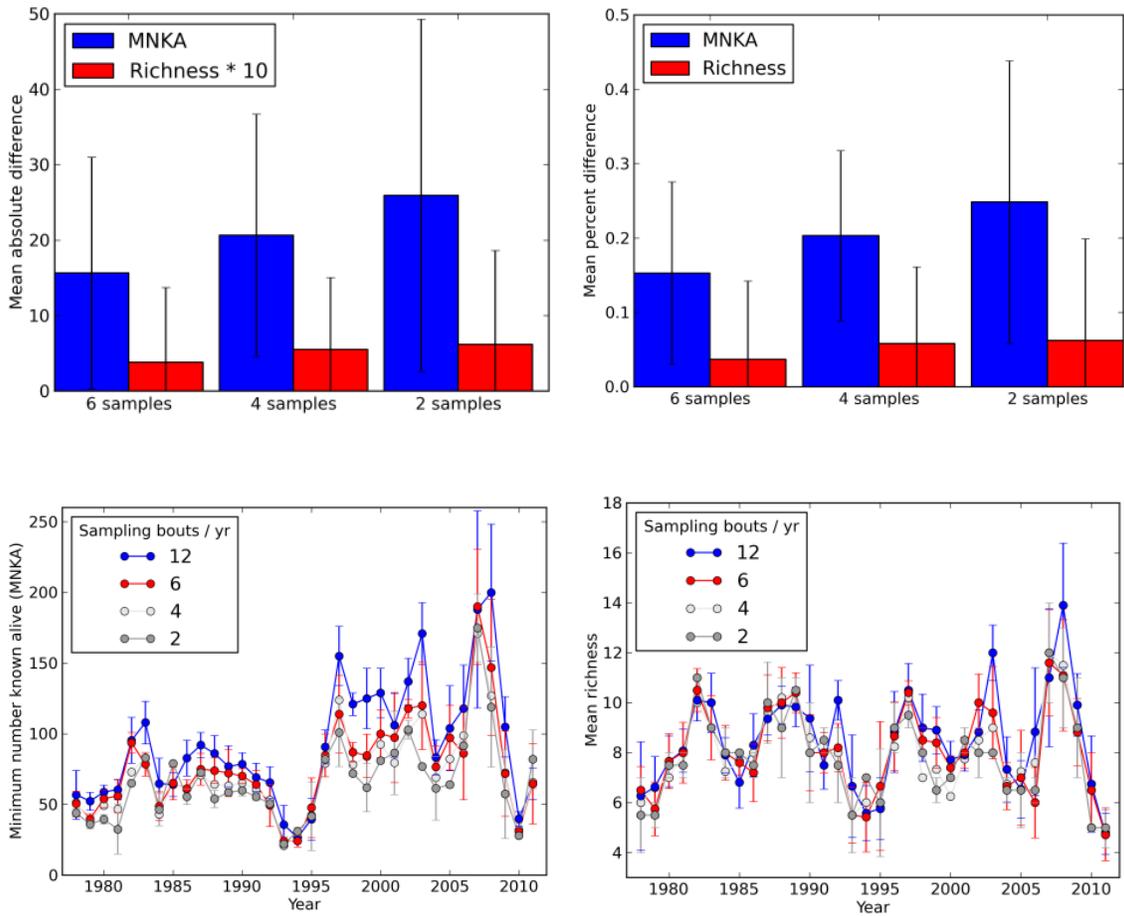
sampling, with the semiannual sampling (two samples per year) estimate of abundance 25% lower, corresponding to approximately 20 fewer individuals detected (Table 2, Figure 12).



**Figure 11.** Cumulative number of desert rodent species detected through time with varying samples per year at a 20-ha site near Portal, AZ, USA (Ernest et al. 2009)

**Table 2.** Percent and absolute differences between estimates of abundance and species richness based on monthly sampling and less frequent sampling

	MNKA (%)	S (%)	MNKA (abs)	S (abs)
Bi-monthly	0.14 (-0.2 – 0.33)	0.015 (-0.16 – 0.32)	10.2 (-8.20 – 53)	0.13 (-1.17 – 2.84)
Quarterly	0.22 (-0.07 – 0.41)	0.04 (-0.12 – 0.26)	18.1 (-2.80 – 73)	0.32 (-1 – 3)
Semiannually	0.25 (-0.27 – 0.55)	0.09 (-0.25 – 0.33)	19.5 (-17.5 – 94)	0.79 (-1.42 – 4)



**Figure 12.** Effects of sampling frequency on species richness and density estimates, from long-term monthly data collected on 10 controls plots at J. H. Brown’s LTREB site near Portal, AZ, USA. The top panels depict the mean differences between abundance and richness estimates across sampling frequencies; the bottom panels depict the differences on an annual basis throughout the course of the study.

**NEON Plan:** Although monthly, year-round sampling is supported by Carver et al. (2010) and the analyses herein, (a) NEON cannot provide sufficient resources to accomplish this due to the high costs, and (b) conditions at many sites over the winter months makes trapping logistically challenging due to deep snow and/or high rates of mortality of trapped individuals. NEON will therefore conduct six bouts of trapping at core sites and four bouts of trapping at relocatable sites each year. The four to six bouts of trapping will be scheduled monthly during the growing season at north temperate sites and as logistically feasible at southern or desert sites. Trapping will be limited to a window of  $\pm 10$  days around the new moon, due to the potential for moonlight to reduce small mammal activity (e.g., Price et al. 1984). This plan represents a compromise between the demand for increased spatial replication for estimating abundance and diversity across a site and the demand for increased temporal replication for tracking intra-annual dynamics of pathogen in individual small mammals. Despite this reduced effort,

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there will be some sites where even 4-6 months of trapping is impractical and risky, due to access and cold, wet weather that require additional precautionary measures to prevent mortality in trapped individuals (e.g., Alaska). The site-specific portion of the TOS Protocol and Procedure for Small Mammal Sampling (AD[05]) details the guidelines and limitations for trapping under a variety of conditions.

**6.1.3.2 Mark-Recapture design: Number of Consecutive Nights per Sampling Period**

The number of consecutive nights of trapping within a sampling period is yet another important consideration in the design of a small mammal mark-recapture study. The logistical trade-offs among longer sampling periods, sampling frequency, and sample size of trapping arrays are significant, and so this decision is not independent of previous discussion sections. The primary considerations are: (1) sufficient number of captures and recaptures within a short period of time to use density estimation models, particularly those with an assumption of closure; (2) sufficient time for trap-shy species to acclimate to the traps and therefore get captured; and (3) sufficiently short sampling duration to minimize edge effects and discovery by meso- and large carnivores.

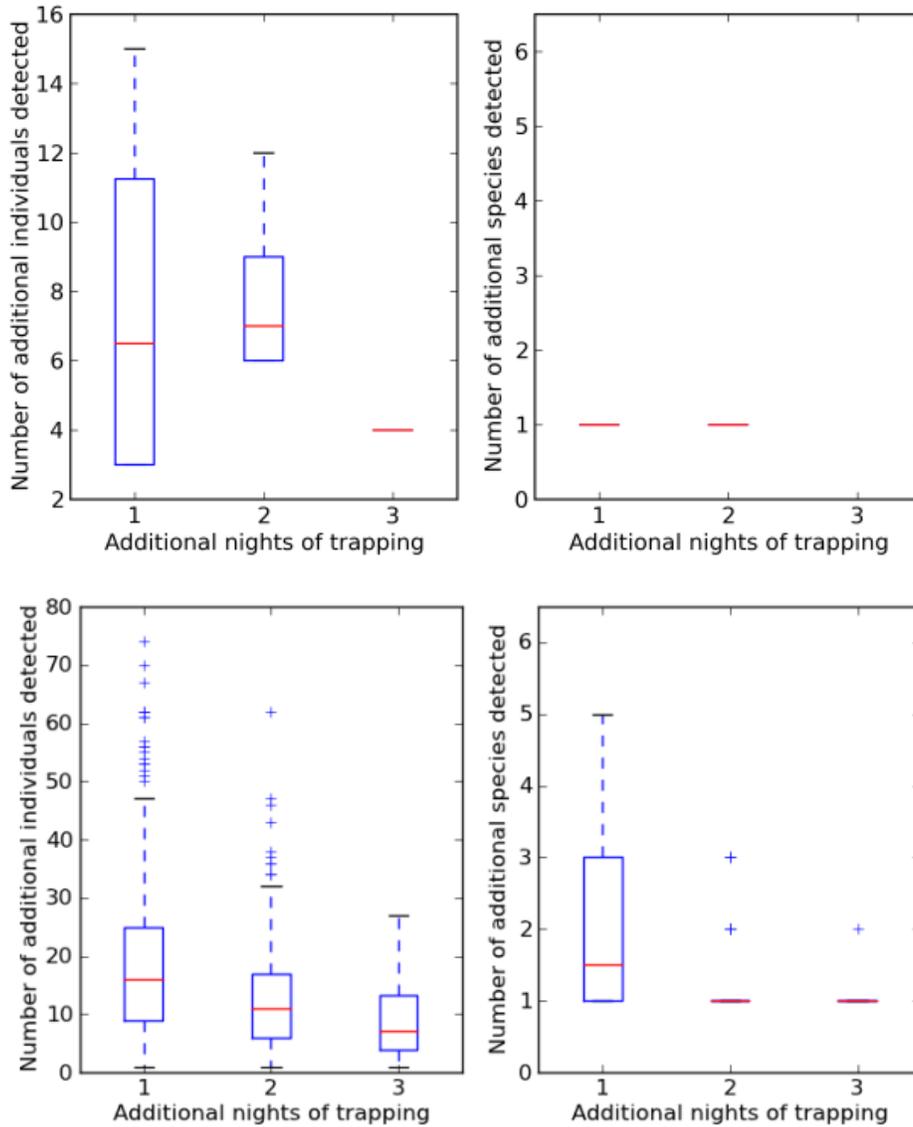
Rigorous studies of the impacts of varying nights of trapping within a sampling period (typically between 1 and 10, with an estimated median of 3 - 4) appear to be lacking in the literature. Here, analyses of existing data and NEON prototype data are presented to examine the effects of consecutive nights of trapping on the number of individuals and the number of species detected within a sampling period.

**Existing Data from Powdermill:** Small mammal data were collected at the Powdermill Biological Station, the field station of the Carnegie Museum of Natural History in Pennsylvania, from 1979 – 1999 (Merritt 1999). Data were collected from a 1-ha 10 X 10 grid of trap stations, with two large Sherman live-traps (7.6 X 8.9 X 30.5 cm) containing synthetic fiber nesting material and sunflower seeds as bait at each station. The grid was typically sampled for four consecutive nights, twice per month, and all individuals were uniquely marked.

**NEON Prototype Data:** In 2012, NEON technicians conducted two nights of consecutive trapping per sampling period on the standardized grids for small mammal sampling at Rocky Mountain National Park. However, two other areas were sampled for one 3-night period followed within a two-week window by one 4-night period; only the data from this targeted sampling effort are used for these analyses. All individuals were uniquely marked.

**Analyses and results:** To evaluate the impact of additional nights of trapping, the number of new individuals and species detected on each night of trapping within a sampling period were calculated for all periods in each dataset. The distributions of these differences are depicted in Figure 13. In both of these systems, new individuals and species were consistently detected on all nights of trapping, but with generally diminishing returns with each additional night. In terms of species richness, only one additional species was added, on average, by the second night of trapping in both systems. At Powdermill, this continued into the third night as well. Additional datasets are needed to further inform

this aspect of the design. Moreover, additional comparative analyses of the composition of the captures on each night, such as sex ratios and age classes, would provide valuable information.



**Figure 13.** Number of additional individuals (left panels) and species (right panels) detected with each additional night of trapping within a sampling bout at Rocky Mountain National Park in 2012 (top panels) and at Powdermill Biological Station (Merritt 1999) from 1979-1999.

**NEON Plan:** Based on (a) the need for sufficient recaptures within sampling periods to estimate detectability and parameterize capture-recapture models, (b) the study goal to collect demographic data, and (c) the consensus among experts in the community, three nights of sampling per sampling period will be conducted. Three nights, relative to two or one, also allow for greater flexibility to ensure

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consistency in number of nights of trapping through time, given unforeseen weather and other logistical events. Moreover, three nights is considered a minimum for spatially-explicit capture-recapture models, and 3-4 nights are the community standard, given that new individuals typically continue to be captured. After the first two years of trapping at a diversity of NEON sites (2013-2014), the resources required to conduct 3 nights of trapping on all NEON mammal grids exceeded those available. From 2015 onward, only 3 grids at each site designated for blood collection ('pathogen grids') continued to be sampled for 3 nights during each bout, while the remaining grids ('diversity grids') are being sampled for only one night per bout. This plan is intended to be revisited in 2018, once additional data have been collected from all NEON sites.

### 6.1.4 Logistics and Adaptability

The design for sampling small mammal abundance, diversity, and demography presented herein is summarized in Box 4, with comparisons to the Centers for Disease Control and Prevention (CDC) recommended approach for sampling small mammals for virologic testing (Mills et al. 1995) and the design originally envisioned by the NEON Tiger team (Ostfeld and Parmenter 2008). The design proposed herein is more intensive than originally envisioned, including greater replication across sites, increased sampling frequency in terms of more bouts per year, but shorter bouts.

**Box 4. Comparison of small mammal sampling designs**

Design Parameters	CDC	Tiger Team	neon
	Mills et al. 1995	2008	Current Design
<ul style="list-style-type: none"> <li>• Array size &amp; type</li> <li>• Traps per point</li> <li>• Point spacing</li> <li>• Arrays per site</li> <li>• Nights per bout</li> <li>• Bouts per year</li> <li>• Marking method</li> <li>• Sampling approach</li> </ul> 	<ul style="list-style-type: none"> <li>• transects, 50-100m</li> <li>• 1 Sherman</li> <li>• 5m</li> <li>• 5 - 20</li> <li>• 1-2</li> <li>• not specified</li> <li>• n/a - removal</li> <li>• not specified</li> </ul>	<ul style="list-style-type: none"> <li>• 3 ha web /grid</li> <li>• 1-2 Shermans</li> <li>• 10m, variable</li> <li>• 3</li> <li>• 4-5</li> <li>• 2-3</li> <li>• PIT tags</li> <li>• not specified</li> </ul>	<ul style="list-style-type: none"> <li>• 1 ha grid</li> <li>• 1 Sherman</li> <li>• 10m</li> <li>• 3 - 8</li> <li>• 1 (diversity) or 3 (pathogen)</li> <li>• 4 - 6</li> <li>• Ear + PIT</li> <li>• Random, spatially balanced, by vegetation type</li> </ul>

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Best practice for planning scientific studies includes identifying the statistical analyses intended as part of the design process (Gitzen et al. 2012), although NEON’s goal is to identify a sampling design that will prove sufficiently robust for the greatest diversity of models. This flexibility is particularly important in light of the fact that all of the raw data derived from the TOS field sampling efforts will be provided to the community, to enable scientists to conduct analyses as they see fit. The mark-recapture models to which the NEON sampling has been designed include Bayesian hierarchical models with covariates (Conn et al. 2006, Royle et al. 2009) and spatially explicit models (Efford 2004, Royle and Dorazio 2008, Efford et al. 2009). These models are powerful means to estimate densities and vital rates, such as survivorship, and are also demographic models, especially as applied to open populations. That is, they allow for estimation of population parameters such as density, apparent survival, and recruitment. The Bayesian approach can also produce derived parameters such as population growth rate. Depending on the spacing of grids, emigration can also be estimated using multi-state models in which grid ID is the state variable. Multi-state models will be important to model pathogen dynamics, where-in state refers to a categorical (or continuous) variable to which an individual can be assigned each time it is captured (e.g., parasitism status (e.g., for botflies or ticks), reproductive status, age class). Multi-state models enable state-specific estimates of demographic parameters to be computed as a means of testing the effect of state on demography.

An important caveat is that the design in its current form is intended to represent the ideal. These sampling frequencies are not going to be feasible at all sites, since weather and road conditions will prevent sampling at many sites during particular times of the year. For example, the sites in Alaska are unlikely to be accessible for most of the year, necessitating an alternate sampling design. The overarching goal of all sampling designs will be to be able to produce comparable estimates of abundance, diversity, and demography over time and space. This will require an iterative approach, in which the efficacy of the design is regularly evaluated at each site, given the data being collected. The Small Mammal Technical Working Group will continue to advise on approaches to sampling design evaluation, potential modifications to the design as needed, and issues affecting data quality. Moreover, new technologies and analytical methods are likely to emerge over the course of NEON, necessitating modifications to the design while maintaining the comparability and integrity of the data stream through time.

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## APPENDIX A INITIAL CONFIGURATION OF NEON SITES

**Table A1.** Number of mammal grids by site, 2018 (may have been reduced from the original number of plots established).

Domain	Domain Name	Site ID	Site Name	Site Type	No. Mammal Grids
D01	Northeast	BART	Bartlett Experimental Forest	Relocatable	6
D01	Northeast	HARV	Harvard Forest	Core	6
D02	Mid-Atlantic	SERC	Smithsonian Environmental Research Center	Relocatable	6
D02	Mid-Atlantic	BLAN	Blandy Experimental Farm	Relocatable	4
D02	Mid-Atlantic	SCBI	Smithsonian Conservation Biology Institute	Core	6
D03	Southeast	DSNY	Disney Wilderness Preserve	Relocatable	6
D03	Southeast	JERC	Jones Ecological Research Center	Relocatable	6
D03	Southeast	OSBS	Ordway-Swisher Biological Station	Core	6
D05	Great Lakes	STEI	Steigerwaldt Land Services	Relocatable	6
D05	Great Lakes	TREE	Treehaven	Relocatable	6
D05	Great Lakes	UNDE	UNotre Dame Environmental Research Center	Core	8
D06	Prairie Peninsula	KONZ	Konza Prairie Biological Station	Core	8
D06	Prairie Peninsula	UKFS	The University of Kansas Field Station	Relocatable	6
D06	Prairie Peninsula	KONA	Konza Prairie Biological Station	Relocatable	6
D07	Appalachians & Cumberland	GRSM	Great Smoky Mountains National Park	Relocatable	6
D07	Appalachians & Cumberland	MLBS	Mountain Lake Biological Station	Relocatable	6
D07	Appalachians & Cumberland	ORNL	Oak Ridge National Laboratory	Core	6
D08	Ozarks Complex	LENO	Choctaw National Wildlife Refuge (Lenoir)	Relocatable	6
D08	Ozarks Complex	DELA	Dead Lake	Relocatable	3
D08	Ozarks Complex	TALL	Talladega National Forest	Core	8
D09	Northern Plains	DCFS	Dakota Coteau Field School	Relocatable	6
D09	Northern Plains	NOGP	Northern Great Plains Research Laboratory	Relocatable	6
D09	Northern Plains	WOOD	Woodworth	Core	6
D10	Central Plains	STER	North Sterling, Co	Relocatable	6
D10	Central Plains	CPER	Central Plains Experimental Range	Core	8
D10	Central Plains	RMNP	Rocky Mountain National Park, CASTNET	Relocatable	6
D11	Southern Plains	CLBJ	CLBJ National Grassland	Core	8
D11	Southern Plains	OAES	Oklahoma Agricultural Experimental Station	Relocatable	6
D12	Northern Rockies	YELL	Yellowstone National Park	Core	6
D13	Southern Rockies & Colorado	NIWO	Niwot Ridge Mountain Research Station	Core	6
D13	Southern Rockies & Colorado	MOAB	Moab	Relocatable	6
D14	Desert Southwest	JORN	Jornada LTER	Relocatable	6
D14	Desert Southwest	SRER	Santa Rita Experimental Range	Core	6
D15	Great Basin	ONAQ	Onaqui	Core	6
D16	Pacific Northwest	ABBY	Abby Road	Relocatable	6
D16	Pacific Northwest	WREF	Wind River Experimental Forest	Core	8

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Domain	Domain Name	Site ID	Site Name	Site Type	No. Mammal Grids
D17	Pacific Southwest	SJER	San Joaquin Experimental Range	Core	8
D17	Pacific Southwest	SOAP	Soaproot Saddle	Relocatable	6
D17	Pacific Southwest	TEAK	Lower Teakettle	Relocatable	6
D18	Tundra	BARR	Barrow Environmental Observatory	Relocatable	6
D18	Tundra	TOOL	Toolik Lake	Core	8
D19	Taiga	DEJU	Delta Junction	Relocatable	6
D19	Taiga	BONA	Caribou - Poker Creeks Research Watershed	Core	7
D19	Taiga	HEAL	Healy	Relocatable	6

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**APPENDIX B PRELIMINARY CAPTURE RATES PER PLOT BY SITE BY YEAR**

Mean number of captures per plot per night (100 trap-nights) by site by year. Null values represent years in which sampling did not occur. See Appendix A for explanation of siteIDs.

siteID	2013	2014	2015	2016	2017
ABBY				11.2	18.5
BARR					1.0
BART		22.0	10.4	22.7	6.8
BLAN			10.2	7.3	7.1
BONA					15.6
CLBJ				10.2	19.1
CPER	1.3	5.4	10.4	2.6	1.6
DCFS					11.4
DEJU				1.9	12.8
DELA			4.9	4.3	4.6
DSNY	2.1	1.7	1.5	5.1	6.9
GRSM			15.5	5.5	6.5
GUAN				4.6	2.6
HARV	8.7	22.5	16.7	29.2	17.0
HEAL			4.0	4.1	13.0
JERC		12.0	16.6	10.6	8.4
JORN			35.9	19.3	17.1
KONA					19.2
KONZ			18.1	19.7	21.1
LENO				2.2	1.6
MLBS					12.9
MOAB			9.9	24.6	9.3
NIWO			10.9	4.4	5.5
NOGP				8.8	5.6
OAES			21.6	28.8	8.7
ONAQ		25.6	29.6	20.3	16.3
ORNL		2.5	10.9	9.6	10.2
OSBS	3.3	3.3	3.3	4.2	4.6
RMNP					9.9
SCBI		4.6	15.5	13.0	7.6
SERC			3.0	7.3	5.6
SJER				1.5	9.2

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<b>SRER</b>				34.6	35.9
<b>STEI</b>			16.2	18.6	13.2
<b>STER</b>		11.0	22.8	7.0	12.4
<b>TALL</b>		3.1	5.4	4.3	3.5
<b>TOOL</b>					1.7
<b>TREE</b>			7.5	9.5	10.1
<b>UKFS</b>			22.4	15.7	11.0
<b>UNDE</b>		24.4	11.0	11.6	12.6
<b>WOOD</b>		24.1	7.5	11.7	11.6
<b>WREF</b>					30.9