



Shenandoah Salamander Monitoring Protocol

Narrative Version 1.0

Natural Resource Report NPS/SHEN/NRR—2021/2226



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ON THE COVER

The Shenandoah salamander (*Plethodon shenandoah*).

Photo by Ann and Rob Simpson

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Contents

	Page
Figures.....	v
Tables.....	vii
Abstract.....	ix
Revision History	xi
Background and Objectives	1
Introduction	1
Background and history.....	1
Rationale for selecting this resource to monitor.....	2
Information for management.....	2
Measurable objectives	3
Sampling Design.....	5
Rationale for selecting this sampling design over others	5
Site selection.....	6
Background; P. shenandoah range and habitat delineations; estimated occupancy statistics	6
Procedures for selecting sampling locations; stratification, spatial design	10
Recommended number and location of sampling sites	11
Sampling frequency, timing, and replication	12
Design summary.....	12
Level of change that can be detected for the amount/type of sampling being instituted	13
Field Methods	15
Field season preparations and equipment setup	15
Details of field sampling (i.e., taking measurements)	15
End-of-season procedures	16
Data Handling, Analysis, and Reporting	17

Contents (continued)

	Page
Metadata procedures.....	17
Overview of database design.....	17
Data entry, verification, and editing	17
Data certification and archiving	17
Recommendations for routine data summaries and statistical analyses to detect change.....	17
Recommended report format with examples of summary tables and figures	18
Personnel Requirements and Training	19
Roles and responsibilities	19
Qualifications and training	19
Operational Requirements	21
Annual workload and field schedule	21
Facility and equipment needs	21
Startup costs and budget considerations.....	21
Literature Cited	23

Figures

	Page
Figure 1. Estimates of annual occupancy of <i>Plethodon shenandoah</i> from 2008–2014 in each defined stratum with rates of colonization and extinction assumed to be constant over time at Shenandoah National Park.	9
Figure 2. Estimates of annual occupancy of <i>Plethodon shenandoah</i> in high-elevation habitat of Shenandoah National Park from 2008–2014 in each defined stratum with rates of colonization and extinction allowed to vary annually	9
Figure 3. A conceptual figure of the monitoring design for high-elevation habitat in Shenandoah National Park.....	11
Figure 4. Power to detect declines (using the two-point method of Guillera-Arroita and Lahoz-Monfort 2012) under different levels of annual effort, assuming constant rates and initial occupancies of a) the historic range (70% initial occupancy for 53 sites), b) the range edge (30% initial occupancy for 100 sites) and c) overall (40% initial occupancy for 153 sites).	14

Tables

	Page
Table 1. Estimates (and SE) for ψ , ε , γ , p and calculated τ using 2008–14 data (24 historic sites, 55 edge) collected across the high-elevation habitat in Shenandoah National Park, U.S.A.	8
Table 2. Stratification and number of sample sites in high-elevation habitat of Shenandoah National Park.	10
Table 3. Number of replicate visits needed (K) within a sampling window to detect the species at least once with desired probability (p^*), given the range of estimated per-visit detection probabilities (p) and given that the site is occupied.	13
Table 4. Annual cost of protocol implementation by Northeast Amphibian Research and Monitoring Initiative (NEARMI).....	22

Abstract

The Shenandoah salamander (*Plethodon shenandoah*) is a federally endangered salamander whose distribution is restricted to high-elevation habitats in Shenandoah National Park (SHEN). During the ranking of vital signs monitoring priorities for this park, “Federal Threatened and Endangered Species” received the 2nd highest ranking for terrestrial vital signs because of ecological, management, and policy criteria. The following protocol narrative describes the objectives and methods for monitoring the Shenandoah salamander. Because the range size and habitat occupancy are of principle interest, this protocol generates data suitable for estimating the annual proportion of occupied habitat across the species range. Objectives focus on describing the status and trends of the species distribution (i.e., spatial extent) and the proportion of the range that is occupied (i.e., density of occupied sites). To do so, monitoring sites in the core and the edge of the known range are surveyed for salamanders multiple times during both the spring and fall. The resulting data not only allow for an assessment of the status and trends of the species but also provide supporting data to assist in understanding causal drivers of population change.

This monitoring protocol consists of a protocol narrative and eight standard operating procedures (SOPs) which are listed below in the Background and Objectives and are available as separate documents at irma.nps.gov.

Revision History

The following table lists all changes that have been made to this Protocol Narrative since the original publication date. Rows will be added in the future as revisions occur. Any recommended or required changes added to the log must be complete and concise and promptly brought to the attention of the project leader. The project leader will review and incorporate all changes, officially complete the revision history log, and change the date and version number on the title page. For a complete set of instructions, please refer to SOP#8 — Revising the Protocol.

Revision History Log:

New Version #	Previous Version #	Revision Date	Author (full name, title, affiliation)	Location in Document and Description of Change	Reason for Change
—	—	—	—	—	—

Background and Objectives

Introduction

This monitoring protocol consists of this protocol narrative and the following standard operating procedures (SOPs), available at <https://irma.nps.gov>.

- Standard Operating Procedure (SOP) #1 — Preparations for the Field Season
- Standard Operating Procedure (SOP) #2 — Training Observers
- Standard Operating Procedure (SOP) #3 — Occupancy Site Surveys
- Standard Operating Procedure (SOP) #4 — Data Management and Archiving
- Standard Operating Procedure (SOP) #5 — Data Analysis
- Standard Operating Procedure (SOP) #6 — Reporting
- Standard Operating Procedure (SOP) #7 — After the Field Season
- Standard Operating Procedure (SOP) #8 — Revising the Protocol

Background and history

The Shenandoah salamander (*Plethodon shenandoah*; Highton and Worthington 1967) is a federally endangered salamander whose distribution is restricted to high-elevation habitats in Shenandoah National Park (SHEN). Recent concerns for climate change-induced risk to high-elevation ecosystems sparked National Park Service (NPS) interest in assessing the extinction risk of *P. shenandoah*, developing a long-term monitoring plan, and considering potential management strategies to ensure persistence of this species. Potential habitat maps have been developed by the National Park Service and the Virginia Department of Game and Inland Fisheries (VDGIF), and though these maps differ in total area and method of mapping, both describe an extremely limited distribution for the species. These maps consider potential habitat to exist at elevations above 800m, on northerly aspects, and within forested talus habitat, based on early work on the species (e.g., Highton and Worthington 1967).

Competitive interactions with the congeneric eastern red-backed salamander (*Plethodon cinereus*) are typically cited as the primary determinant of range boundaries for *P. shenandoah* (Highton 1972). Previous studies have investigated multiple hypotheses for competition between these species, but support for a primary mechanism of competition has not been found (Griffis and Jaeger 1998, reviewed in Jaeger et al. 2016). In contrast, research on other montane salamanders has described climate as a more important determinant of species ranges, and of the lower elevational limit in particular (Arif et al. 2007, Gifford and Kozak 2012); recent surveys of *P. shenandoah* support this idea (Grant et al. 2018). Nevertheless, this monitoring program incorporates observations of *P. cinereus* in data collection and analyses to address competition as a potential contributor to population changes for *P. shenandoah*.

Based on the biology and status of *P. shenandoah*, there are a number of considerations for the development of a monitoring protocol for this species. First, measuring the abundance of terrestrial salamanders is inherently difficult, often requiring more creative monitoring designs and

extraordinary effort. *P. shenandoah* is a cryptic animal that spends only a small proportion of its time above ground where it is available for observation. The species also only utilizes surface habitats during climatically favorable periods (primarily spring and fall), thus constraining the possible sampling period. In addition, it has a highly restricted range with a small spatial extent. This small extent greatly limits the number of available sampling sites. Lastly, *P. shenandoah* is a federally endangered species, and the concern for the welfare of individual animals and the entirety of the species' habitat is perhaps higher than might be for other species. All of these constraints make the development of a monitoring protocol for the species difficult.

Rationale for selecting this resource to monitor

In a 2008 review of terrestrial “vital signs” in Shenandoah National Park, “Federal Threatened and Endangered Species” received the 2nd highest ranking because of ecological, management, and policy criteria (Comiskey and Callahan 2008). The Shenandoah salamander is a federally and state-listed endangered species, and its distribution has recently been found to be (at least in part) related to climate variables (Grant et al. 2018). As such, extinction under future climate change projections is a potential real outcome. As a federally endangered species, conservation of the Shenandoah salamander is supported by a number of laws and NPS policies.

Monitoring *P. shenandoah* provides information not only about this federally endangered species, but also about the high-elevation plethodon community as a whole, including the relatively common red-backed salamander (*P. cinereus*) and the relatively rare slimy salamander (*P. glutinosus*). Perhaps more importantly, the Shenandoah salamander may also serve as a sensitive biologic indicator for entire high-elevation ecosystems in SHEN — ecosystems threatened by a variety of factors.

Lastly, successful monitoring programs provide information essential to meeting agency mission and goals. This protocol describes a logical and highly science-based methodology to provide information for management decisions affecting the Shenandoah salamander.

Research on the species to date (2007–present) has been conducted primarily by the U.S. Geological Survey (USGS), and has been supported by USGS, FWS, VA state, and NPS-provided research funds. Nevertheless, without dedicated monitoring funds, a long-term monitoring program for the species is unsustainable despite the species rarity, federal endangered status, documented threats, and NPS desire for maintaining persistence of the species within SHEN.

Information for management

Reducing uncertainty surrounding resource management decisions is a principle objective in a monitoring program, and monitoring protocol development often involves a research component (Fancy and Bennetts 2012). Two structured decision making (SDM) workshops focused on framing management decisions for this species revealed three major uncertainties: the current status and distribution of the species, the effect of competition with *P. cinereus* on extinction risk of *P. shenandoah*, and the relative importance of climate change (Grant et al. 2014). Moreover, NPS indicated that tolerance of risk to implement management actions which were not yet proven harmless to other aspects of the ecosystem was dependent on demonstration of near certain extinction

risk to *P. shenandoah* and that NPS would not support active management until three conditions were met: 1) high level of scientific agreement on the urgency for implementing active management, 2) clear understanding of the specifics of the threat to the population or habitat, and 3) high degree of confidence in our ability to predict the results of management action.

Although uncertainty was included in the SDM process, it was obvious from discussion that the NPS group was not comfortable with the high levels of uncertainty in many aspects of the decision (e.g., species ecology, climate change, and the interaction of these two factors) regardless of how it was included in the decision analysis. For that reason, we have designed this monitoring protocol to provide information that contributes to the estimation of extinction risk and related causes of distributional change, as this information will directly affect the preferred management actions and predicted outcomes of management actions for this species. For example, management actions directed at reducing competition will have limited effectiveness if competition has a relatively minor influence on the extinction risk for *P. shenandoah* (e.g., less than climate change). This management action would then be unsupported in the decision analysis if such estimates were available. This protocol provides information that can be directly incorporated into decision models utilized for management decision making.

Measurable objectives

Monitoring for the salamander describes annual variation in occupancy across the range of the Shenandoah salamander in Shenandoah National Park. Specific objectives include:

- Determine if the occupancy of *P. shenandoah* is increasing, decreasing, or is stable across the species' range. Assessments include changes in the range boundary (i.e., spatial extent of occupied sites) as well as the proportion of occupied sites (i.e., density of occupied sites) within that range.
- Identify regions of elevated turnover in population occupancy which may be early indicators of range reduction and associated extinction risk.

The core monitoring program outlined in this protocol is designed to address these two objectives. Though designed to detect changes in occupancy and range boundaries, data from this protocol cannot on their own explain the cause of a detected trend or range alteration. Nevertheless, in conjunction with targeted research efforts, this protocol provides information that will allow for an assessment of the contribution of biotic (i.e., competition with *P. cinereus*) and abiotic (e.g., climate change) drivers of population changes.

Sampling Design

Rationale for selecting this sampling design over others

Several sampling approaches have been developed to survey amphibians (e.g., Heyer et al. 1994). Long-term monitoring surveys are typically designed to record changes in occurrence or population sizes at particular sites over time within a particular area. Given the single-species target for this monitoring protocol and its endangered status, population size is of utmost importance.

Measuring amphibian abundance (i.e., population size) is inherently difficult, particularly for cryptic species. As such, the USGS's Amphibian Research and Monitoring Initiative (ARMI) has developed a site-occupancy metric (Percent Area Occupied — PAO), which has since been adopted by many other wildlife survey and monitoring programs. This approach focuses on estimating (from field data) the proportion of habitat occupied by one or more target species, rather than focusing on estimating population size. Still, it is rare that target species are always detected at occupied sites. Fortunately, the occupancy estimation methods developed by MacKenzie and colleagues are robust to variation in detectability due to species, habitats, and other biotic and abiotic variables (MacKenzie et al. 2005). In addition, these methods allow the incorporation of covariates to test specific hypotheses about factors influencing the distribution of amphibians by providing methods to estimate occupancy despite missed observations at a site (MacKenzie et al. 2005). The specific objective of analyses, when multiple years of data are collected at the same sites, is to provide annual estimates of change in species occupancy within an area of inference, which is defined prior to the initiation of the study and can include individual management areas, parks, or regions.

Estimating PAO and its variance requires recording detection/nondetection data for a species during each of multiple visits to sites (e.g., forest grid cells) within an area of inference during a specified time. During a survey, a species can be in one of three states: 1) present and detected, 2) present and not detected or 3) absent. Because the true state of a species cannot be accurately determined between states 2 and 3 from observations alone, researchers must estimate the likelihood of a species being present even when it is not detected. The approach of MacKenzie et al. (2005) therefore incorporates the detection probability, p (defined as the probability of detecting the species at an occupied site), within the estimation procedure, yielding unbiased estimates of the site-occupancy metric. The precision of an occupancy estimate can be increased by increasing the number of visits to a site. Despite the additional effort required to revisit sites multiple times in each season, the benefits of decreased bias in the estimates of occupancy, especially when analyzing long-term datasets on amphibian populations, cannot be matched with count data alone (Schmidt 2003).

Data for an occupancy analysis must satisfy several basic assumptions; 1) data are collected using a probabilistic sampling frame with methods that are designed to sample the species of interest, 2) the occupancy of a set of habitats does not change over the survey period (i.e. a site is 'closed' to changes in occurrence), 3) a species is never falsely detected when absent, and may or may not be detected at occupied sites, and 4) the occupancy of each site and species detection is independent. The core sampling design addresses assumption one. For assumption two, resampling sites over a short time period will assist with meeting this assumption within seasons. In addition, we may

account for cross-seasonal violations of this assumption by analyzing single-season datasets as part of a multiple-season analysis (MacKenzie et al. 2005). Modeling techniques can be used to deal with assumption 3 by directly estimating the probability of these "false negatives." Though field protocols have been designed to minimize these errors, there are also protocols to collect additional data when necessary to inform estimation of these "false negatives." Lastly, violations of assumption 4 may be explored and corrected using goodness-of-fit tests (MacKenzie and Bailey 2004).

Inference to the entire distribution of the species is critical for assessing species-level responses to environmental change and shifts in species' occupancy over time. However, the periphery of the species' range ("range edge") may be the most responsive to environmental change and show trends that would otherwise go undetected if the range edge isn't specifically assessed. For this reason, a design that supports monitoring the range edge as well as the total range provides information to address two potential mechanisms of potential declines of *P. shenandoah* (i.e., due to competition with *P. cinereus* and/or due to abiotic environmental changes).

Data from this protocol will contribute to our overall understanding of the extinction risk of *P. shenandoah*. These data help identify whether the range edge is expanding or shrinking and whether the density of occupied sites within the range is changing. For example, it is possible that range extent remains the same while within the range the proportion of occupied sites is declining. This design also provides supporting information to assist with determining whether changes are related to abiotic (i.e., environmental) or biotic (i.e., competition with *P. cinereus*) drivers. The outcome of past decision-making workshops deems this additional information about causal relationships critical to satisfying information needs for future decision making about management of the species.

Site selection

Background; *P. shenandoah* range and habitat delineations; estimated occupancy statistics

Plethodon shenandoah occurs in three "populations" in Shenandoah National Park (defined here as Populations A, B, and C (see SOP 3 for details). Populations A and B are the largest in surface occupied area; the spatial extent of Population C is relatively small and may be more vulnerable to disturbance (very few monitoring sites can fit within the spatial extent of the population). Nevertheless, monitoring of Population C is included to help inform the overall species status. Given the smaller distribution of salamanders in Population C, it receives proportionally less monitoring effort relative to the two larger populations.

Population C was initially excluded from the monitoring design. There was some concern that a stochastic catastrophic event directly associated with the monitoring effort (e.g., disease introduction) could occur and therefore some portion of the species should be "unmonitored" and thus relieved of this risk. (Note: the probability of disease transmission is reduced to near zero via equipment cleaning and salamander handling protocols). After some debate, it was determined that excluding Population C was not prudent. The small risk of disease transmission (or other stochastic event occurring directly and solely as a result of the monitoring effort) was outweighed by the substantial and more immediate risk of population decline and extirpation due to climate change (due to its lower maximum elevation, Population C has less ability to move upslope and escape rising

temperatures compared to the other populations). Given this real and established risk, gathering some information about Population C and allowing that information to contribute to the full species assessment was deemed to be appropriate. Even though sample size constraints do not allow for direct statistical inference to individual populations, sampling in Population C will provide some information about the population. In addition, it relieves the assumption that population dynamics in the rest of the range were representative of the unsampled Population C. Therefore, all populations are to be sampled, roughly proportionately to their distributions.

The spatial extent where these populations are found can be further delineated into two range components (i.e., strata), the “historic range” for each population (i.e., locations where *P. shenandoah* is located as previously mapped by NPS) and the “range edge” (i.e., an area up to 300 m beyond the historic range). Together, these areas are expected to encompass the entire range of the salamander. Population change is characterized either by an expansion or contraction of the geographic area occupied by the species or by a change in the density of occupied sites within these range components.

We initially considered that inference may be desirable at three specific spatial scales:

- Historic range = locations within the mapped historic range of the species;
- Range edge = locations outside historic range and up to 300 m from the historic boundary;
- Overall range = overall area combining the historic range + range edge (as both are defined above).

We used data from 2008–2014 surveys for a related project (Sevin 2014, Grant et al. unpublished data) to estimate an average detection, occupancy, and rate parameters (colonization and extinction) at each of these three scales as a way of setting bounds on our expectations for each parameter value (Table 1). We also calculated another quantity (turnover), which includes both extinction and colonization processes and describes the probability that a randomly selected occupied site is occupied in time t :

$$\tau_t = \frac{\gamma_{t-1}(1 - \psi_{t-1})}{\gamma_{t-1}(1 - \psi_{t-1}) + (1 - \varepsilon_{t-1})\psi_{t-1}}$$

for $t=2, \dots, T$; where T is the number of seasons. ψ_{t-1} is the occupancy probability in $t-1$, $(1 - \varepsilon_{t-1})$ is the probability a site did not undergo an extinction, and γ_{t-1} is the probability a site was colonized in $t-1$. The parameter τ_t reaches a maximum value when all sites occupied in t were unoccupied in $t-1$, and a minimum when all occupied sites in t were occupied in $t-1$ (i.e., $(1 - \varepsilon_{t-1}) = 1$).

Estimates were generated with a single-species multi-season model.

Table 1. Estimates (and SE) for ψ , ε , γ , p and calculated τ using 2008–14 data (24 historic sites, 55 edge) collected across the high-elevation habitat in Shenandoah National Park, U.S.A.

Part of range	Occupancy (ψ)	Extinction (ε)	Colonization (γ)	Detection (p)	Turnover (τ)
Historic	0.74 (0.10)	0.06 (0.04)	0.23 (0.13)	0.48 (0.03)	0.08
Edge	0.27 (0.07)	0.22 (0.08)	0.11 (0.04)	0.30 (0.03)	0.28
Overall	0.41 (0.06)	0.17 (0.05)	0.12 (0.04)	0.40 (0.02)	0.17

As expected, sites near the edge of the range of the species are estimated to have had higher turnover than those within the historic range. Both biotic and abiotic factors may be responsible for the differences in extinction and colonization rates across different parts of the range. In the future, increased warming within the historic range may change these rates, independent of intraspecific competition with the red-backed salamander, *P. cinereus* (Dallalio et al. 2017).

If we assume estimated extinction and colonization rates are constant over time (Figure 1), the result is an expected equilibrium occupancy of 0.79 in the historic range, 0.33 in the edge, and 0.41 overall by:

$$\psi^{eq} = \frac{\gamma}{\gamma + \varepsilon}.$$

Occupancy in $t+1$ may be calculated from the initial occupancy and the dynamic rates by:

$$\psi_{t+1} = (1 - \varepsilon)\psi_t + \gamma(1 - \psi_t).$$

Calculations show that these equilibrium occupancy rates will be achieved in approximately 10 years (ignoring stochasticity and assuming constancy in the rate parameters; see Figure 1 for plot of occupancy over the monitoring period assuming constant rates). However, rates are unlikely to be constant over time, and are likely to respond to annual variation in weather, fluctuating densities of the presumed competitor, *P. cinereus*, or other biotic and abiotic factors. Allowing for annual variation in extinction and colonization rates results in larger inter-annual dynamics in occupancy for *P. shenandoah* (Figure 2), and a more realistic characterization of the system. Further, as any management action will act on the colonization and extinction rates, describing relationships between abiotic, biotic and occupancy dynamic rates will not only help to understand trends in the population, but what management actions may help to avoid extinction.

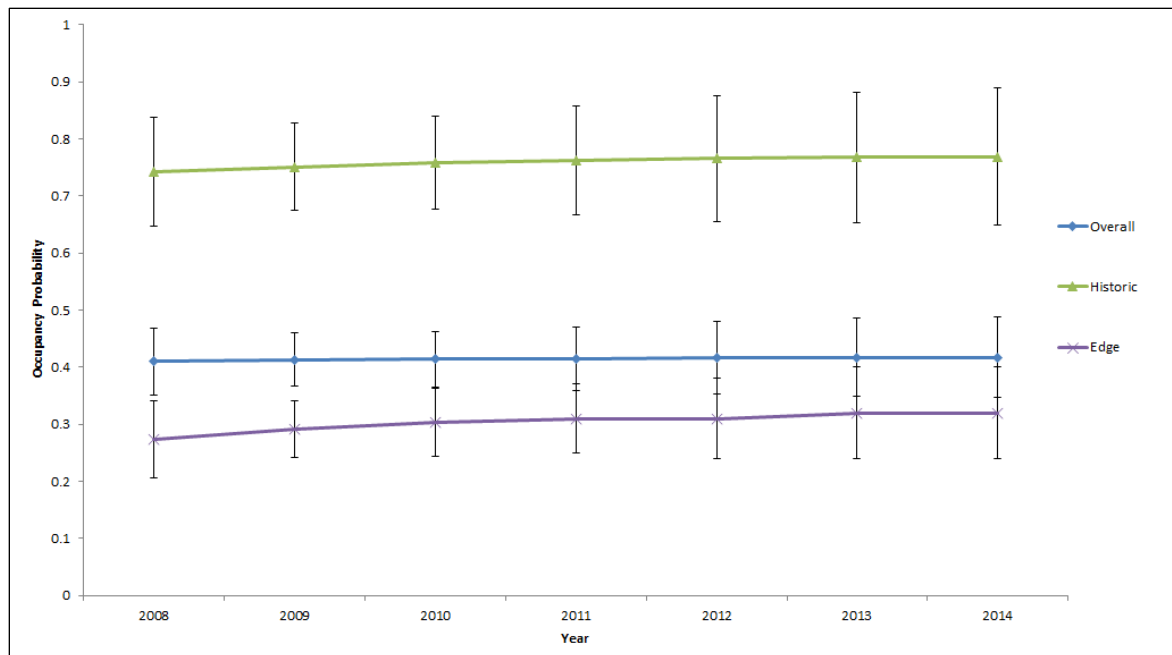


Figure 1. Estimates of annual occupancy of *Plethodon shenandoah* from 2008–2014 in each defined stratum with rates of colonization and extinction assumed to be constant over time at Shenandoah National Park.

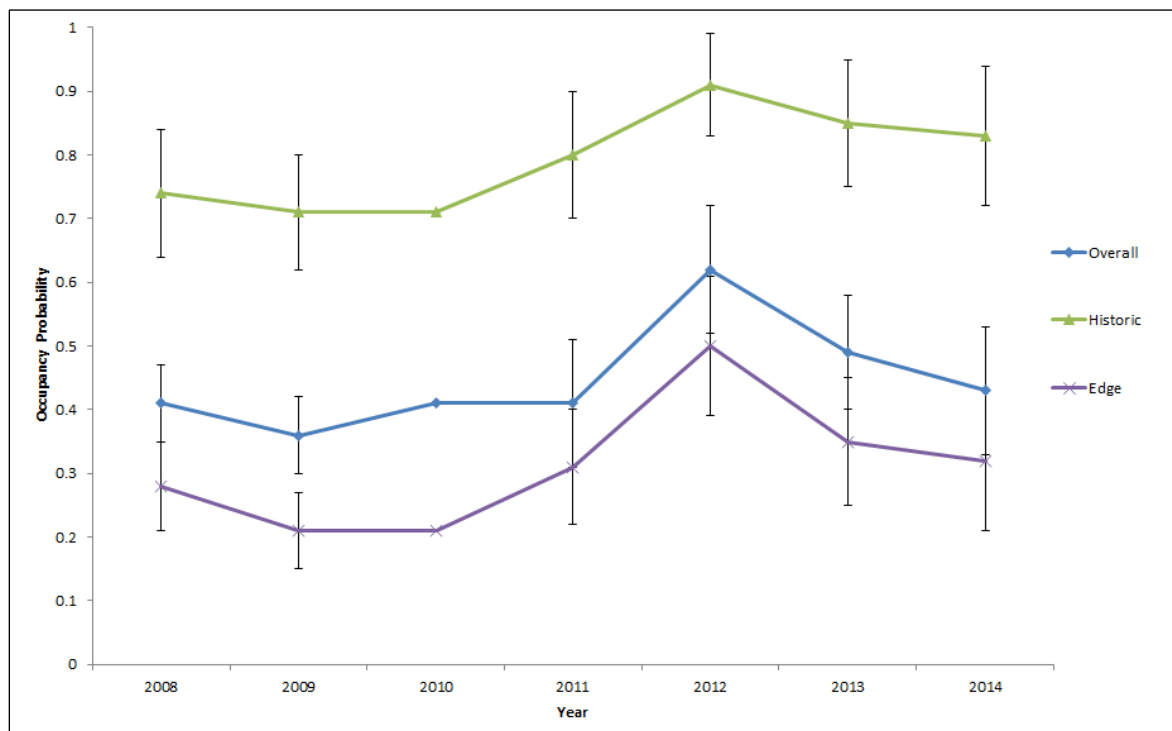


Figure 2. Estimates of annual occupancy of *Plethodon shenandoah* in high-elevation habitat of Shenandoah National Park from 2008–2014 in each defined stratum with rates of colonization and extinction allowed to vary annually (SEs in 2010 inestimable — because we did not sample this year, estimates are interpolated from the estimated dynamic parameters of years with data).

Procedures for selecting sampling locations; stratification, spatial design

Occupancy sites:

The overall sampling design consists of seasonal surveys at selected sites within each of three populations, stratified by range scale (i.e., historic or edge; Table 2, see next section below for information on number of monitoring sites), and roughly proportional to population distributions.

Table 2. Stratification and number of sample sites in high-elevation habitat of Shenandoah National Park.

Population and total number of sites	Number of sites in the historic or edge of the historic range
Population A - Overall range (65 sites)	Historic range (25 sites) Range edge (40 sites)
Population B - Overall range (65 sites)	Historic Range (25 sites) Range edge (40 sites)
Population C - Overall range (23 sites)	Historic Range (3 sites) Range edge (20 sites)

Sites are defined as randomly selected 100 m grid cells, which ensures independence among sites within a given sampling season. Sampling in a site occurs with paired, perpendicular 32 m x 2 m transects. This transect size provides an optimal site sampling design for maximizing the potential to detect *P. shenandoah* when it is present (Sevin 2014); the perpendicular replicate helps account for intra-site variation in detection probability. Sites are located at the centroid of selected grids and the orientation of the transects are established randomly during the first site visit. Following initial site selection, sites and associated transects remain fixed for the life of the monitoring protocol. A conceptual figure of the sampling design is shown in Figure 3.

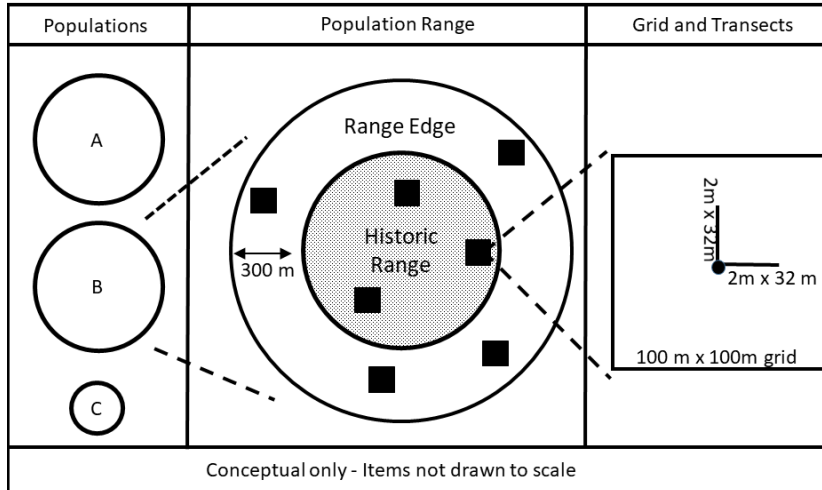


Figure 3. A conceptual figure of the monitoring design for high-elevation habitat in Shenandoah National Park. Each population was overlaid with a 100 m x 100 m grid. In each population a set of sites (forest patches of 100 m x 100 m area — represented as black boxes above) from the historic range and from the range edge were randomly selected. Within each selected grid cell, two 2 m x 32 m perpendicular transects are established at the centroid for resampling.

Data from these sites will allow us to estimate the occupancy status (probability of site occupancy by *P. shenandoah*) and the local extinction and colonization rates (i.e., occupancy dynamics). We considered a spatial design including stratification by two major physiographic features known to be important to *P. shenandoah* occurrence (elevation and aspect) but were unable to distribute sites evenly among all categories due to the limited high-elevation habitat. Instead, all analyses will incorporate elevation and aspect as covariates that influence occupancy. Additional site-level and remotely-sensed covariates (e.g., percent microhabitat cover categories, forest type) will be related to estimates of occupancy and dynamics, as robust relationships with environmental covariates will be valuable for developing effective management strategies for the species.

Recommended number and location of sampling sites

It is important to understand that the small range of the species limits the number of sampling locations available. Sites are chosen within randomly selected 100m grid cells, which ensures independence among sites within a sampling season. Given the $\sim 1 \text{ km}^2$ historic range of the species across all three populations, this results in only 95 sites available for sampling in the historic range. Considering that a proportion of those are inaccessible for sampling and that sampling the full entirety of the species' range would be undesirable (from a disturbance perspective), a maximum of only ~ 50 sites may be available for sampling. As a frame of reference, data for Figures 1 and 2 come from 24 established occupancy sites in the historic range. Sampling outside of the historic range (i.e., in the range edge) allows for more possible sampling locations and an ability to detect range expansion or contraction (via $\gamma < \text{or} > \epsilon$ in the range edge). Nevertheless, the number of possible site visits are also constrained by sampling window (spring and fall, when salamanders are surface active and available for sampling) and revisit interval considerations (> 7 days between sampling events; see “Sampling frequency” below).

Increased precision is desirable in describing changes in the range edge, as this is where we have observed the greatest turnover. Therefore, increased allocation of the overall effort to the range edge is necessary. Given that a maximum of ~50 sites are possible in the historic range, we suggest an additional 100 sites be selected within the range edge. This number of field sites allows for reasonable power at detecting trend (see “Level of change that can be detected” section below; at least 77 sites required for each range component for a $SE < 0.05$) and is feasible given sampling constraints. Once sites are established, they will remain fixed for the life of the monitoring protocol.

A map of sampling sites and specific site location information can be found in SOP #3 — Occupancy Site Surveys.

Sampling frequency, timing, and replication

Each site will be sampled multiple times during the spring and fall seasons on an annual basis (for six revisits per site per year).

Sampling frequency, timing, and replication are constrained by a variety of factors. First, visiting a site repeatedly over a short time frame is required to meet the “closure” assumption in the occupancy state of a site. However, habitat disturbance without a period of recovery is of concern given the endangered status of the species and the potential to bias observations and salamander surface occupancy dynamics. We generally considered visiting a single site no more than once every ~7 days (based on guidance in Otto et al. 2013), which constrains the maximum number of revisits which may be conducted within a limited temporal sampling window.

Spring and fall are optimal for sampling as the detection probability of *P. shenandoah* is at its maximum during the cool, humid conditions during these seasons (Grant, unpublished data). For the purposes of this protocol, spring is defined as mid-April through mid-June and fall as mid-September through mid-October. Sampling should be conducted during seasons of maximal availability, though annual variation in weather will affect the surface activity (and therefore availability) of the salamander.

Likely no more than six visits (four in the spring and two in the fall) are possible to a site each year given these constraints; however, utilizing multiple independent observers during a single visit can greatly improve efficiency within short sampling windows. Therefore, it is recommended that all site surveys are conducted with two observers, who each survey 1 replicate transect at each site. Because the probability of detection may vary within a site, two perpendicular transects are sampled in each site, thus allowing for intra-site variation in detection probability to be included in estimates of occupancy rates.

Design summary

In summary, of the three known populations of *P. shenandoah*, the two larger populations are targeted for monitoring with the third, smaller, population receiving proportionally less effort. The range of each population is conceptually split into the “historic range” and the “range edge”. The historic range is defined by existing NPS maps outlining the range of the species, and the range edge is defined by including a 300 m buffer around the historic range. In combination, these are termed the

“overall range.” A grid of 100 m x 100 m cells was established across the overall range, with a total of 669 available cells spread across the three populations. Cells were stratified by historic (n=95) and edge (n=574) range location. Sixty-five 100 m x 100 m grid cells (forest patches) were chosen via simple random selection for each of the two larger populations individually, with 25 patches in the historic range and 40 patches in the range edge. A proportionate number of cells (relative to the total available) were selected in the third, smaller population, with 3 cells in the historic range and 20 in the range edge. Therefore, in combination, 153 forest cells, stratified by historic and range edge, will be monitored across all populations. All cells in each strata were considered equally suitable for selection, although logistic constraints in the field when the sampling is implemented may limit accessibility to some sites (e.g., proximity to cliffs). Within each selected cell, two perpendicular transects of 32 m x 2 m will be established at the centroid of the grid cell with an initial random transect orientation. These transects compose “a site” and are then fixed for the life of the monitoring protocol. Transects will be sampled during the spring and fall with a maximum of six visits per year.

Level of change that can be detected for the amount/type of sampling being instituted

The precision of the occupancy estimate increases with greater sampling effort, both in the number of sampling sites or the number of visits. It can also vary as a function of the detection probability associated with each species, and as a function of the relationship between detection and habitat covariates. A principle step in determining the number of sampling sites is to estimate the detection probabilities for the species of interest. Data from 2008–2014 surveys from a project using similar methods indicate that detection ranges from ~0.3–0.5 on average across all visits to *P. shenandoah* sites in SHEN (Table 1).

The number of replicate visits (K) that would be needed within a sampling window to detect presence of the species can be calculated based on the estimated detection probability using: $K = (\log(1-p^*) / (\log(1-p)))$ where p is the estimated detection probability and p* is the desired power to detect the species at an occupied site on at least one visit within a sampling season (Table 3).

Table 3. Number of replicate visits needed (K) within a sampling window to detect the species at least once with desired probability (p*), given the range of estimated per-visit detection probabilities (p) and given that the site is occupied.

p	p*	K
0.3	0.9	6.5
0.3	0.95	8.4
0.3	0.99	12.9
0.4	0.9	4.5
0.4	0.95	5.8
0.4	0.99	9.0
0.5	0.9	3.5
0.5	0.95	4.6
0.5	0.99	7.0

The sampling effort proposed here combined with dynamic site-occupancy models will allow unbiased estimates of local colonization and extinction rates. Precision in estimating colonization and extinction rates is reliant on precise estimates of annual occupancy probabilities.

Calculating the number of sites needed to achieve an occupancy estimate with specified precision (as a standard error (SE) of the estimate) is achieved by (MacKenzie et al. 2005):

$$s = \frac{\psi}{SE(\psi)^2} \left[(1 - \psi) + \frac{(1 - p^*)}{p^* - Kp(1 - p)^{K-1}} \right]$$

To achieve annual estimates of occupancy with a $SE < 0.05$, 96 sites need to be surveyed across the range, and to achieve precision ($SE < 0.05$) in estimating edge and historic range occupancy rates, 77 sites need to be surveyed in each range group. Because of the limited number of sites that can be located within the historic range of the species, only ~50 sites may be surveyed, this results in a minimum $SE(\psi) \sim 0.06$ within the historic range.

Under this level of effort ($100 + 53 = 153$ sites, visited 6 times annually), we will have an 80% probability to detect a decline in occupancy across both populations (inclusive) of approximately 40% within the historic range, a decline of approximately 60% in the range edge, and a decline of approximately 40% for the overall range (Figure 4).

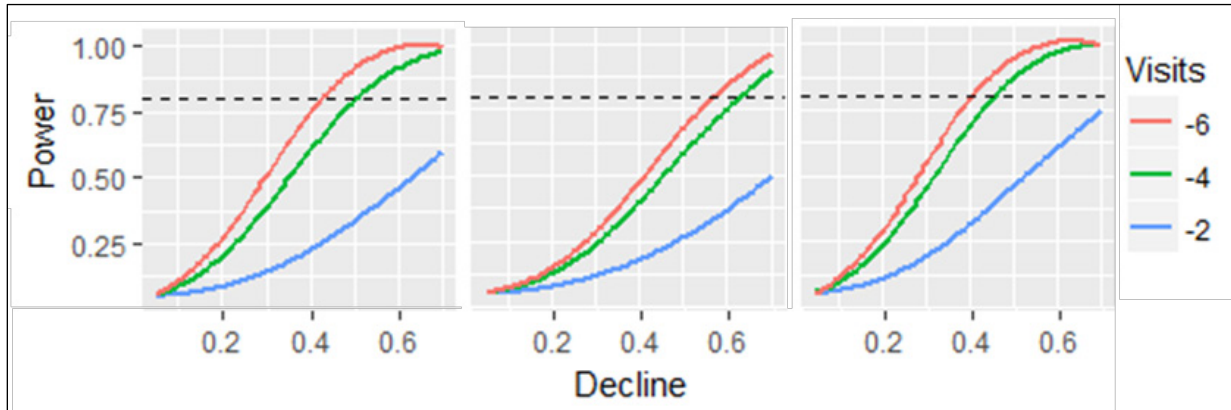


Figure 4. Power to detect declines (using the two-point method of Guillera-Aroita and Lahoz-Monfort 2012) under different levels of annual effort, assuming constant rates and initial occupancies of a) the historic range (70% initial occupancy for 53 sites), b) the range edge (30% initial occupancy for 100 sites) and c) overall (40% initial occupancy for 153 sites).

Again, it is important to note that the sampling design articulated here is virtually the maximum sampling allowable given inherent restrictions on potential sampling areas and the maximum sampling frequencies allowable. With these inherent constraints, we view the power noted at six revisits (Figure 4) as the most that is reasonably attainable for this species and sufficient for monitoring objectives.

Field Methods

Field season preparations and equipment setup

Prior to the field season, all personnel will review field protocols, study identification guides for the species (SOP #1 — Preparations for the Field Season, and SOP #2 — Training Observers) and review best practices for handling amphibians (SOP #2 — Training Observers). Because correct identification of species is a major assumption of occupancy sampling design, the competency of observers is a central component of the sampling protocol, and substantial time should be invested in training and review of species identification.

All equipment should be assembled, and the appended datasheets should be copied, with at least 50% on weatherproof paper.

Permitting and compliance consultation with NPS, the U.S. Fish and Wildlife Service (FWS) and VDGIF should occur in conjunction with annual review and/or permit report from each field season. This will allow for appropriate permits to be issued and renewed as needed in a timely manner prior to the start of field work.

Details of field sampling (i.e., taking measurements)

All field crews should consist of at least two observers; it is preferable for multiple teams of two observers to share sampling duties during the season. If more than two people are available, the survey sites should be rotated among the observers at each revisit, to reduce the potential for observer-induced detection bias.

Terrestrial sites will consist of a pair of 32 m x 2 m perpendicular natural cover object transects that share a common origin point. After navigating to the sampling site, observers will establish transects at the appropriate location and bearing using a compass and a 50 m tape. During the first site visit, a number of site covariates will be collected including slope, aspect, elevation. Sampling covariates (temperature, weather, time) are collected on each visit. Following covariate collection, sampling for salamanders will begin. Observers simultaneously start the survey at the distant end of the 32 m x 2 m transect and systematically search under all cover objects (rocks and logs that can be moved) greater than 6 cm in diameter (or maximum length if ellipsoid) and within 1 m of the transect tape until the entire area is searched. All amphibians found on the surface or underneath cover objects will be captured by hand, identified, measured, and recorded. The number of cover objects is also recorded. Following salamander sampling, the transect % cover type (soil, cobble, boulder, moss, large woody debris, leaf litter, other) is visually estimated. Prior to leaving a site, observers should review all datasheets for omissions or errors. Prior to handling salamanders in another site, observers will clean their hands with ethyl alcohol to minimize disease transmission. Example field forms and occupancy survey sampling details can be found in SOP #3 — Occupancy Site Surveys.

Upon returning from the field each day, observers should enter the day's field data directly into the database.

End-of-season procedures

End-of-season procedures include cleaning and repairing all field gear (SOP #7 — After the Field Season). Field references, manuals, and unused data sheets are filed appropriately. A final review of all data sheets is completed.

Data Handling, Analysis, and Reporting

Metadata procedures

Metadata, including details of any updates to spatial information and relevant details for interpretation of survey data for the monitoring project will be prepared and updated annually for transfer to NPS.

Overview of database design

A relational database in Microsoft SQL server has been established at the Patuxent Wildlife Research Center and is maintained by personnel in the Northeast Amphibian Research and Monitoring Initiative (NEARMI).

The database is composed of three core tables including information on site location variables (e.g., site ID, latitude, longitude, etc.), habitat variables (e.g., bearing, elevation, % cover, etc.) and site visit data (e.g., sample date, observer, weather, salamander information, etc.). A data schema is provided in SOP #4 — Data Management and Archiving.

Data entry, verification, and editing

Data are collected in the field on data sheets and are later entered into the NEARMI local database by the primary field person(s) who collected the data. Data verification (proofing by comparing datasheets with entered values and assessing data for aberrant or unusual values) will be conducted within a month of data entry. During proofing, if any discrepancies or errors are found on the data sheets, personnel who collected the data will be consulted and, if necessary, corrections made on both the data sheet itself (noting the date and person making the correction) and in the NEARMI local database.

Data certification and archiving

Hard copy data will be archived at Patuxent Wildlife Research Center in Laurel, MD. Databases are backed up and maintained by USGS Patuxent Wildlife Research Center personnel daily. Data will be provided to the NPS on an annual basis. During all data management processes, the USGS will follow the best management practices as outlined on the USGS Data Management webpage ([Data Management \(usgs.gov\)](http://DataManagement.usgs.gov)).

Recommendations for routine data summaries and statistical analyses to detect change

Routine data summaries present the proportion of sites occupied by each species encountered and species detection probabilities. These analyses will be completed via the program PRESENCE (MacKenzie and Kendall 2002, MacKenzie et al. 2003). The program PRESENCE uses maximum likelihood techniques to estimate the probability of detection (p) and the probability of occupancy (ψ) at a set of habitats within a defined area of inference (e.g., historic habitat). This program allows the inclusion of site and sampling covariates to test the association between abiotic and biotic variables on detection and occupancy. At a minimum, the simplest model in PRESENCE [i.e., $p(\cdot)\psi(\cdot)$] will be assessed. This model assumes that site occupancy and detection probabilities are constant across time and sites. It is recommended that alternative models including variation across time or sites should

also be evaluated, including models that test whether site occupancy and detection probabilities are influenced by site, habitat, and sampling covariates. See SOP #5 — Data Analysis for a complete description.

The endangered status of the focal species suggests that trend analysis should occur after three years of collection under this monitoring design and on an annual basis thereafter. Given that 24 of the 130 sites already have more than five years of data, trend analysis may be initiated in some form as soon as the protocol is implemented. Generalized linear mixed models may be used to estimate mean trend in occupancy. Models may be fit using the lme4 package in the R programming language. To estimate mean trends over years, year is treated as a continuous covariate where year is standardized to have a mean of 0. Models are run using a log-link function to estimate relative rates of change in occupancy over years. The annual rate of change is $e^{\beta}-1$ where β is the instantaneous rate of change from the log-linear models. A likelihood-ratio test (LRT) can be used to evaluate the null hypothesis of no difference in trend.

Recommended report format with examples of summary tables and figures

Report format, outlined in SOP #6 — Reporting, will conform to standards of the NPS Natural Resource Data Series (see <https://www.nps.gov/im/publication-series.htm>). At a minimum, these reports will include an abstract, introduction, methods, results, discussion, and a literature cited section.

It is also recommended that a short 1–2 page resource brief including the abstract or other summary and the most relevant figures and results, be developed for each report.

Personnel Requirements and Training

Roles and responsibilities

The principle investigator and personnel of the NEARMI program are the lead biologists and coordinators for implementing this monitoring protocol. They are responsible for collecting field data following all SOPs and for assigning, training, and overseeing other qualified personnel (NPS, USGS, contractors) to conduct the surveys following all SOPs. They are responsible for data verification and validation as well as data summary, analysis, and reporting. Data management will be the responsibility of the NEARMI personnel, who will develop data entry forms and other database features (e.g., quality assurance, report generation, etc.). NEARMI personnel are responsible for data archiving, data security, dissemination, and database design.

Qualifications and training

Training will include demonstrations and participation in field survey methods at sites which are not included in the monitoring protocol. Because the modeling approach assumes that a species is never falsely detected (i.e., misidentified), review and competence in species identification at all life history stages that will be encountered during the course of the survey work is paramount to obtaining precise estimates of occupancy. Only qualified and trained personnel will participate in surveys outlined in this document and associated SOPs. Each employee performing field work has a job hazard analysis (JHA) that is tailored to the specific position. This JHA will be reviewed prior to the start of field work (SOP #1 — Preparations for the Field Season).

Operational Requirements

Annual workload and field schedule

Spring surveys begin no sooner than mid-April and extend no later than mid-June. Fall surveys begin no earlier than early-September and extend no later than mid-October. Survey work is not continuous during these periods, and inclement and variable weather conditions preclude scheduling specific sampling events. The SOPs specify weather and seasonal conditions under which surveys should be conducted. All monitoring efforts require a minimum of a two-person crew. Seven to fifteen occupancy surveys should be scheduled for completion each field day during appropriate times according to SOPs, though actual sampling time will vary based on travel time and characteristics of individual sample sites. In addition, as noted above, care should be taken not to sample an individual site without an appropriate seven day “rest period” between sampling events.

Facility and equipment needs

The nature of terrestrial salamander survey work does not require special facilities beyond normal office space, equipment storage needs, and use of a vehicle to travel between survey sites. We envision that two to three crews will be working simultaneously on any given day, requiring multiple sets of equipment.

Startup costs and budget considerations

This protocol includes a number of implementation details that would make implementation by Shenandoah National Park a difficult endeavor. Logistically, these include bi-annual sampling periods with several months of potential field “down-time” between the seasons when field staff are needed to implement the protocol. Sampling periods are also relatively short (1–2 months), much shorter than the typical period of employment for seasonal staff. Data analysis for the protocol also requires a high level of understanding and modeling expertise with occupancy data. Though perhaps available at the national level in the NPS, this scale of knowledge is unlikely to be present or to be developed in SHEN. Lastly, implementation of the protocol would be a new effort, thus detracting from current staff obligations at SHEN.

As such, it is recommended that USGS NEARMI personnel implement this protocol. The NEARMI program has over a decade of experience working with *P. shenandoah* including pilot surveys and *P. shenandoah* research conducted by NEARMI personnel from 2007–2017. The NEARMI personnel have extensive experience with planning, implementation, and modification of monitoring plans to accommodate the needs of the funding agency, as well as expertise with analyzing and developing new statistical methods as value-added contributions to meeting the monitoring objectives outlined in this protocol. In addition, the NEARMI supports field crews that are highly experienced, available for the bi-annual sampling events, and flexible to adapt to variable weather conditions that affect sampling scheduling.

Protocol costs include personnel time, travel, and equipment. Program manager’s time includes administrative time for hiring and training personnel, oversight of field personnel and planning sampling, QA/QC to maintain the field-collected data and the NEARMI local database, as well as

preparation of reports or analyses associated with the monitoring program. Startup costs include the purchase of equipment for three crews (Table 4).

Table 4. Annual cost of protocol implementation by Northeast Amphibian Research and Monitoring Initiative (NEARMI).

Item	NPS Cost ¹	In kind contribution ¹
USGS Salary (Grant 5% of time)	\$5,000	\$5,000
USGS Salary (Brand 20% of time)	\$5,000	\$5,000
Seasonal field technicians (4*12wks, GS-04)	\$42,240	—
Fieldwork (travel, vehicle use, equipment)	\$3,800	\$1,000
USGS Overhead (44.33%) ²	\$24,843	\$11,969
Total estimated annual cost	\$80,883	\$15,876

¹2018 estimate

²Includes facilities costs (Patuxent Wildlife Research Center — USGS) at 2018 levels

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