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National Capital Region Network Amphibian Monitoring Protocol

Revision 2.0, 2024



Spotted salamander at Manassas National Battlefield Park
NPS / THOMAS PARADIS

National Capital Region Network amphibian monitoring protocol: Revision 2.0, 2024

Science Report NPS/SR—2025/275

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Abstract

This document describes the protocol and standard operating procedures for monitoring amphibians and amphibian habitat within the National Capital Region Network (NCRN). Amphibians were identified as a vital sign to be included in the network-wide monitoring program (National Park Service 2005). A pilot study and early work beginning in 2005 was conducted in a few of the network parks to test the sampling and data analysis methodology, and the monitoring program was subsequently expanded to all parks in the following years. This protocol and procedures within have been updated to align with advances in field methodology, data management, data analysis, and our overall knowledge gained from the network-wide amphibian community.

The second half of this document contains detailed information on the sampling framework, field and survey methodology, and the collection and management of both field and GIS data. Techniques are described to estimate amphibian occupancy and trends within the NCRN.

List of Acronyms

ANTI: Antietam National Battlefield

ARMI: U.S. Geological Survey's Amphibian Research and Monitoring Initiative

CATO: Catoctin Mountain Park

CHOH: Chesapeake and Ohio Canal National Historical Park

GWMP: George Washington Memorial Parkway

HAFE: Harpers Ferry National Historical Park

MANA: Manassas National Battlefield Park

MONO: Monocacy National Battlefield

NACE: National Capital Parks-East

NEARMI: Northeast Amphibian Research and Monitoring Initiative

NWI: National Wetlands Inventory

PAO: Proportion of Area Occupied

PRWI: Prince William Forest Park

ROCR: Rock Creek Park

SOP: Standard Operating Procedures

SSURGO: GIS Soil Survey Geographic Database

WOTR: Wolf Trap National Park for the Performing Arts

Background and Objectives

Rationale for Monitoring of Amphibian Populations

Amphibian malformations and diseases, changes in historical range sizes and locations, and declines of amphibians at the population level have been noted on a global basis, though the extent of these declines is not well understood (Green 1997; Alford and Richards 1999; Houlahan et al. 2000; Lannoo et al. 2003), and robust long-term data quantifying amphibian declines without bias are lacking (Schmidt 2003). The life histories, dispersal abilities and physiological tolerances of this clade of organisms make them potentially more susceptible to environmental change and the introduction of multiple, synergistic stressors at many life history stages (Corn 2000; Sparling et al. 2000; Semlitsch 2003). Because of these characteristics, amphibians may be good indicators of local and regional ecosystem change and perturbation, and many researchers have urged greater attention to this taxon (Semlitsch 2003 and chapters therein).

Responses of amphibians to anthropogenic landscape change are well documented, but in a rapidly urbanizing region, the potential to abate the loss of species or the change in occupancy is not well understood from a park management standpoint. An understanding of the spatial trends in anthropogenic ecosystem alterations and associated changes to amphibian occupancy must be monitored in order to identify potential mechanisms for ecosystem degradation, and appropriate time intervals within which to manage affected ecosystems.

Road networks have major negative effects on populations of organisms (Andrews 1990; Trombulak and Frissell 2000). The construction of new roads or alteration of existing roads results in physical alterations due to the loss of habitat, fragmentation of existing habitat patches, and introduction of patch edges (and associated edge effects). In addition, road networks result in direct mortality of animals trying to cross between patches of natural habitat, avoidance and disruption of natural dispersal corridors, and increase human-wildlife interactions. Road networks can serve as dispersal corridors for predators associated with human developments (i.e., subsidized predators), and ultimately reduce the quality of the remaining habitat fragments. Trail networks, while less well understood, have similar mechanisms to affect the quality of a forest patch, as the presence of a trail network facilitates human traffic into the interior of a habitat patch (Matlack 1993).

Human decision making is important in urban systems and can influence ecological patterns and processes (Grimm et al. 2000). Changes in land use surrounding a park unit, or changes within a park itself are guided by a set of dynamic human values, which can be in response to some environmental change (e.g., destruction of a trail or road due to flooding) or through changes in public perception about the value of natural resources or the use of natural systems in urbanized environments (e.g., the limitations on road traffic). Not incorporating an understanding of the dynamic nature of both the natural and social systems and interactions of these systems will lead to improper management decisions (Grimm et al. 2000). Changes in either the ecological integrity of the natural system, or in public perceptions or attitudes related to the value of ecological integrity can lead to important feedbacks in both the natural system and in management decisions regarding the biotic parameters of interest.

Amphibians are characterized by distinct biological and life history differences, which makes developing a coherent monitoring plan for multiple species of frogs and salamanders difficult. Amphibians occupy many discrete habitats, including intermittent and low-order perennial streams (e.g., northern red salamander [*Pseudotriton ruber*], long-tailed salamander [*Eurycea longicauda*], northern dusky salamander [*Desmognathus fuscus*]), temporary, semi-permanent and permanent wetlands (e.g., wood frog [*Lithobates sylvatica*], four-toed salamander [*Hemidactylium scutatum*], southeastern chorus frog [*Pseudacris feriarum*], southern leopard frog [*Lithobates sphenocephala*]), and moist woodlands (e.g., slimy salamander [*Plethodon glutinosus*]). Specific protocols must therefore be selected based on the habitat and species found in those habitats. Because species of amphibians may respond differently to changes in the environment (e.g., Lanoo et al. 1994), the selection of a subset of species cannot serve as a surrogate for all species which share the same habitats.

MacKenzie et al. (2003, 2004, 2005) have developed multi-season models for use with detection/non-detection data which can be applied to estimate the changes in species assemblages over a specified time at long-term monitoring sites. Multi-season occupancy data can be analyzed to assess changes in community assemblages at a single site, or among four or less species at more than one site.

This long-term amphibian monitoring protocol is designed to: 1) determine the distribution of amphibian species and document the proportion of area occupied by amphibian species in the parks, 2) detect trends in the proportion of area occupied by amphibian species, 3) provide information to aid in the generation of hypotheses for determining causes of differential abundance or long-term changes in the proportion of area occupied among species, habitats, and areas; 4) to support identification of potential management actions to reverse declining trends or to increase the occupancy of potential habitats, and 5) to identify priority areas within individual parks for amphibian habitat management and protection. Results from concurrent monitoring efforts will be integrated with collection of amphibian occupancy data as is feasible, so that hypotheses may be tested with respect to covarying changes in other abiotic and biotic parameters (e.g., water quality, aquatic invertebrates).

Historical Development of Amphibian Monitoring in National Capital Region Parks

The National Capital Region Network identified amphibians as a priority taxonomic group for its Inventory and Monitoring program. The goals of this program are to document at least 90% of the species in its parks and to determine whether the integrity and status of plant and wildlife (e.g., amphibian) populations are changing over time. As of 2004, all of the parks had completed inventories of amphibian species, yet the status of amphibian populations was not evaluated, and no statistically robust, long-term data sets had been collected up to that point with which to assess potential changes in amphibian populations in these parks.

Amphibian monitoring began in CHOH and ROCR in 2005, whereas MANA was added in 2007 (note that early monitoring did not consistently implement methods as described in this protocol: see early protocol implementation methods described in Grant et al 2022). In order to expand the

monitoring effort and allow probabilistic sampling to be conducted Network-wide, lentic and lotic habitat mapping was initiated in NCR parks in 2013. Mapping and habitat identification were conducted following the methods established in SOP3: Establishing and Marking Sampling Plots (v. 2.0, 2024 update). In 2013, CATO and HAFE were mapped for lentic and lotic habitats; MANA and MONO in 2014; GWMP, NACE, and WOTR in 2015; and ANTI and PRWI in 2015. This mapping effort was largely conducted during the fall seasons. Further site visits were required over the subsequent years to determine if these locations held water and were capable of supporting amphibian breeding. Sites with no signs of water retention after multiple visits and across multiple years were removed from the list of potential survey sites. Sites erroneously mapped outside park boundaries were also removed from the potential survey site list.

In 2018, a dynamic, multi-region, community occupancy model was developed and used to analyze lentic sampling data in all parks (Grant et al. 2022). Previous analyses up to this point used the program PRESENCE to estimate amphibian occupancy at each individual park, whereas this new method shares data for species occupancy estimates across the entire NCRN network.

To assess the population status and to document changes, we established a systematic sampling framework and outlined protocols for amphibian monitoring (methods documented in this protocol).

Objectives

1. Estimate species richness and determine changes over time within the National Capital Region Network.
2. Provide monitoring information to park managers on long-term trends in distribution of amphibian species and changes in community composition over time, and to aid in management decision-making by providing information on the potential impacts of urbanization, habitat loss/degradation, management practices, or other identified stressors on amphibian populations.
3. Document the occurrence of amphibian malformations and disease outbreaks.

Specific Monitoring Questions

1. How do populations of amphibians respond to changes in landscape composition surrounding national parks in the National Capital Region?
2. Do changes in species distributions relate to changes in habitat quality resulting from increased urbanization in the region?
3. Can park management activities influence the distribution and occupancy of amphibians in the National Capital Region?

Sampling Design

Rationale for Selecting the Proportion of Area Occupied Sampling Design

Many sampling approaches have been developed to survey amphibians (e.g., Heyer et al. 1994). Multiple survey techniques, targeting species found in particular habitats, certain life stages or behaviors (e.g., emigration and immigration from breeding sites, calling), are often required to detect the suite of amphibian species found in a given area. Long-term monitoring surveys are typically designed to record changes in species assemblages or population size over time within a particular area. The identification of trends in populations is related to four factors: sampling variability, temporal heterogeneity, spatial heterogeneity, and the detectability of the organism of interest (Williams et al. 2002). Variability in sampling and seasonality can be addressed through a robust sampling design for the entire population of interest, accounting for any temporal differences which may affect detection of a species. Spatial heterogeneity must be addressed in the placement of survey locations, which can be stratified by habitat types or landscape features or located at random throughout the area of interest. However, a probabilistic sampling design cannot account for differences in habitat parameters, observer abilities, or species-specific differences in behavior, which can affect the detectability of a species (Grant et al. 2005). The true abundance (N) of an organism of interest is related to the count (C) recorded during a survey and the probability that an organism present will be included in the count (p), by the formula $E(C)=Np$, where E denotes statistical expectation. Thus, raw counts alone are not useful in monitoring programs unless 1) the relationship between the recorded count data (C) and the true population size (N) is linear and 2) the expected, or average, detection probability does not vary over time and space, which is unlikely for most species. Unadjusted counts will not provide robust estimates of change in population size, and the estimation of detection probability thus becomes critical to inferences about population size and change. Many options exist for calculating the detection probability p, including the addition of a second observer (Nichols et al. 2000), and mark-recapture or removal sampling approaches (Williams et al. 2002).

The proportion of area occupied (PAO) metric was developed to aid the U.S. Geological Survey's Amphibian Research and Monitoring Initiative (ARMI) in the collection of large-scale data on amphibians in the United States (MacKenzie et al. 2002, 2003, 2005; Bailey et al. 2004). This approach focuses on the proportion of habitat (or area) occupied by 1 or more target species, rather than focusing on true abundance or population size. Still, it is rare that target species are always detected at occupied sites, and 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, the 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 is to provide spatial and temporal estimates of change in species occupancy within the 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 multiple visits to sites (e.g., ponds, streams) 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, researchers must estimate the likelihood of a species being present even when it is not detected. The precision of an occupancy estimate can be increased by increasing the number of visits to a site. The approach of MacKenzie et al. (2002) 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 proportion of sites occupied.

Collecting data for PAO analysis typically does not require sophisticated field techniques for many species, and protocols are more easily implemented by collaborators. Detection/non-detection data are more reliably and easily collected than counts or estimates of population size, and do not require labor-intensive methods such as marking or removing animals. 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 data sets on amphibian populations, cannot be matched with count data alone (Schmidt 2003).

The state variable of interest in this monitoring program is the proportion of area occupied (PAO) by a species, and data may be analyzed using the program PRESENCE (MacKenzie et al. 2002), which has been developed to deal specifically with this data structure. The program PRESENCE uses standard maximum likelihood techniques to estimate occupancy and detection probability parameters in the model, which can be modeled as functions of collected covariates (MacKenzie et al. 2005).

Data collected using the PAO approach 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., the species is likely to be present at a site if the site is suitable to be occupied and the occupancy state of that site does not change over the survey period), 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.

Site Selection

Based on the distribution of potential amphibian habitat, the parks listed below are included in the amphibian monitoring protocols (Table 1).

Table 1. List of parks where amphibians are monitored.

Park	Abbreviation
Antietam National Battlefield	ANTI
Catoctin Mountain Park	CATO
Chesapeake and Ohio Canal National Historical Park	CHOH

Table 1 (continued). List of parks where amphibians are monitored.

Park	Abbreviation
George Washington Memorial Parkway	GWMP
Harpers Ferry National Historical Park	HAFE
Manassas National Battlefield Park	MANA
Monocacy National Battlefield	MONO
National Capitol Parks-East	NACE
Prince William Forest Park	PRWI
Rock Creek Park	ROCR

Potential amphibian habitats must be identified at the individual park level. Once habitat data have been assembled, survey techniques specific to detection of amphibians in discrete habitat types (e.g., lentic, lotic or terrestrial; method details described below) are implemented in the parks.

Lentic and lotic sampling sites can be identified through a number of methods described in SOP3, including information from park personnel, GIS Soil Survey Geographic database (SSURGO) attributes (e.g., drainage [classes P, SE, SP, VP], grtgroup, hydric, order, wlwet), National Wetland Inventory (NWI) attributes (i.e., PUB, POW, PFOE, PEM, PSS, R3/4, RUB, RSS, REM) and small-order stream spatial data in the regional hydrology coverage. All sites must be field-verified in the first year to determine the sampling frame from which to define the set of long-term amphibian monitoring sites. Terrestrial sites should be located within mature forest cover, though lentic and lotic sites can be included in the sample even if they are not located under a forest canopy. Because many of the streams have headwaters located outside of the jurisdiction of the National Park Service, samples will include threats that are both extrinsic and intrinsic to the individual park. Where possible, sampling sites should be located along headwater, first and second order streams, and at any seeps that occur within the NPS boundary. In addition, indication of stormwater input into a stream would result in classification of the section of stream downstream from the point source as impacted.

The precision of the occupancy estimate increases with greater sampling effort, both in terms of number of sampling sites or visits, and will 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 principal step in choosing the number of sampling sites is to estimate the detection probabilities for the species of interest. In the first year (2005), pilot data were collected at two parks (Chesapeake and Ohio Canal National Historical Park, Rock Creek Park) to estimate the parameters needed to gain an acceptable level of precision in the estimates of site occupancy and changes in occupancy ($p_{species}$: detection probability of each species, t: number of sampling occasions, n: number of sites). Following this, the protocol document was updated and will be updated as needed as more information on these parks and others become available. The recommended number of sites per park unit, divided among the specific habitats, (i.e., lentic, lotic and terrestrial), along with a complete description of the number of sampling visits per site, are reported in SOP5: Habitat-specific Protocol:

Lentic, SOP6: Habitat-specific Protocol: Lotic, and SOP7: Habitat-specific Protocol: Terrestrial. In addition, site selection is described in SOP3.

Amphibian Populations Targeted in this Sampling Design

Sampling is limited to the breeding and activity season of amphibians in the mid-Atlantic region (mid-February through October), though inferences can only be made for the active period of each species (e.g., February through June for *Ambystoma maculatum*, May–June and September–October for members of the genus Plethodon).

Different species of amphibians have unique life history characteristics specifically adapted to a set of habitats. It is therefore impossible to identify a single sampling methodology to effectively sample for all species at all habitat types. Amphibians in the region of interest are known to inhabit ponds, vernal pools and other temporary wetland habitats, streams, rivers, and terrestrial habitats including meadows and forests. Most species rely on the proximity of water for some stage of development, with the notable exception being species of the genus *Plethodon*, which inhabit cool, damp deciduous forests, and lay eggs in moist areas under rocks or logs or in underground chambers which undergo direct development.

To assess the full suite of amphibian species present in the National Capital Region parks, it is essential to survey lentic, lotic, and terrestrial habitats, which represent the primary habitats utilized by amphibians primarily in the breeding season (Appendix A). Problems such as declines, malformations or diseases are not restricted to amphibians from one of these habitats alone. Sampling is limited to the expected breeding or active seasons of the species found in these habitats, but needs to remain flexible due to uncertain climatic conditions. Specific information regarding sampling periods and survey techniques is addressed in the sections below for: lentic (SOP5), lotic (SOP6), and terrestrial (SOP7) habitats. Not all habitats are sampled in all parks in the region. Therefore, the targeted population is amphibians which breed in a variety of lentic sites or are associated with lotic or terrestrial habitats for all or part of their life cycle within the National Capital Region parks of interest, and which are present during the survey periods. See Appendix A for a list of the species monitored.

Lentic Surveys (SOP5)

All anurans and some caudates rely on a variety of lentic sites for breeding. Lentic sites (vernal pools, permanent and semi-permanent ponds, marshes, swamps, tidal-influenced wetlands) will be selected in one of two ways within a given park. For parks with few known (≤ 10) lentic sites (e.g., Rock Creek), all lentic sites within the park will be included in the study. For other parks, a random sample of appropriate habitats will be selected, with the number of sample sites determined after initial data on the distribution of habitats and detection of the species of interest in each habitat are collected.

We will employ various survey techniques at these sites to determine species presence. At all lentic sites we will employ visual encounter surveys and calling surveys (following North American Amphibian Monitoring Program (NAAMP) protocol; see: North American Amphibian Monitoring

Program; Weir and Mossman 2005) in the early spring through summer to estimate species occurrence. The number and timing of calling surveys is based on detectability estimates from the 2005 pilot study at Chesapeake and Ohio Canal NHP. Based on these results, two visual encounter surveys and two dip net surveys will be conducted at wetland survey sites to detect species presence (see SOP5). All larvae captured in the dipnet surveys will be returned to the site. Calling surveys, if conducted, require 6 calling visits to estimate occupancy with a similar level of precision (see SOP5).

Because of the difficulty in surveying for the spadefoot toad (*Scaphiopus holbrookii*), no special arrangements will be made to survey for this species. Detection of the spadefoot toad will be opportunistic, e.g., through calling and dipnet surveys. Inference for this species will therefore be limited.

Lotic Surveys (SOP6)

Stream salamanders (in the genera *Desmognathus, Eurycea, Gyrinophilus, Pseudotriton*) inhabiting low-order (headwater and 1st order streams, i.e., stream segments with a watershed area less than 1 mi^2; Ohio EPA 2002) will be a focus of these surveys (Rocco and Brooks 2000; Welsh and Ollivier 1998). Lotic sites will be selected in one of two ways within a given park. For parks with few known streams (≤ 5), all stream segments will be surveyed. For parks with > 5 streams, a subset of streams will be randomly selected for the survey. Stream surveys should be conducted on paired transects (i.e., two 15 m transects spaced 15 m apart) using cover object visual encounter surveys on both the stream bank and within the stream channel. Each transect is surveyed with multiple passes (2–3), and 2 sets of paired transects should be surveyed along a stream segment (with one pair at the headwaters and another 100–200 m downstream). Surveys should be conducted twice in the summer (June, July).

We collected data during a 2005 pilot study, with 2 visits to 25 paired transects located on 11 streams (6 streams at CHOH and 5 streams at ROCR). Based on the detection probabilities (p) and estimates of occupancy (psi, ψ) from this pilot survey, we recommend final survey methodology in SOP6. In general, we recommend 2 sampling visits to 2 sets of paired transects on each of 20 streams (see SOP6).

Terrestrial-Specific Surveys (SOP7)

Terrestrial surveys for *Plethodon* species were conducted using coverboard and natural cover object surveys in Chesapeake and Ohio Canal NHP and Rock Creek Park. Sites were selected on a park- and habitat type-basis. Coverboards are useful as artificial refugia to increase the capture rate of terrestrial salamanders (Grant et al. 1992; Tiebout 2003). Natural cover transects (20 × 3m) and coverboard arrays (3 pine coverboard stations; each station consisting of 2 large boards (26 × 26 cm) and 3 small boards (13 × 26 cm)) were employed in habitats appropriate for terrestrial salamanders. Spatial and temporal sampling of terrestrial habitats was integrated with the lentic and lotic surveys to increase the efficiency of the monitoring program. Pilot surveys were conducted at least 3 times each in the spring at 38 and 49 terrestrial survey sites in Chesapeake and Ohio Canal NHP and Rock Creek Park, respectively. Survey methodology based on the detection probabilities from these pilot surveys suggest two visits with two transects per visit (see SOP7). However, due to the large investment of effort, and the dominance of *P. cinereus* in the pilot data (*P. cinereus* usually tolerates

a range of forest management and moisture regimes, Pough et al. 1987, Brooks 1999, Grover 2000, Bailey et al. 2004), we do not recommend sampling terrestrial sites for this monitoring program.

Collecting Habitat Data

Specific details about collecting habitat data are included in the habitat-specific survey protocols (i.e., SOPs 5–7, lentic, lotic and terrestrial), as several variables are collected within one habitat type and not in another. All habitat data are measured within 50 m of a sampling site location. Some variables are collected once per year (e.g., distance to road, etc.), while others are collected on each sampling occasion (e.g., temperature). Habitat data are always collected after amphibian sampling has been conducted to avoid disturbing the amphibians. Details for recording sampling covariates are listed in Appendix B: sky condition codes, wind speed codes, moon phase codes, and turbidity condition codes.

Special Circumstances

While not a focus of the monitoring protocols, there is a specific SOP (SOP11: Malformed or Diseased Amphibians) for incidental observations of amphibian malformations, diseased individuals, and die-off events. This SOP follows the protocols in place for the ARMI program and includes protocols for submitting specimens and documenting the occurrence of malformed, diseased, sick, or dead amphibians encountered during the normal course of surveys (see USGS National Wildlife Health Center website; SOP for "Collection, preservation, packaging and shipping of Amphibians").

Sampling Frequency and Replication

All sites selected for monitoring must be surveyed at least twice each year [more survey visits are recommended for most species; see specific SOPs (SOPs 5–7)]. Because of the phenology of amphibian breeding, the survey period may vary slightly each year, as influenced by climatic events, in order to satisfy the site closure assumption for occupancy analysis.

Appropriate sampling frequency and intensity are recommended in each of the habitat specific SOPs (SOPs 5–7). Surveys will be conducted at the same sites every year. Because of the high potential variation in populations of amphibians (Gibbs et al. 1998), it is important to survey sites each year, rather than in a rotating sampling frame.

Level of Change That Can be Detected for the Amount / Type of Sampling

Statistical power to detect a trend varies by the taxa of interest. The number of sites, frequency of visits, and the desired trend level will influence the structure of the monitoring program. The optimal number of sampling occasions depends on occupancy and detection probabilities estimated through a pilot study.

In 2005, the estimated occupancy probabilities for the 12 species of amphibians detected in lentic habitat during visual encounter surveys and dipnet surveys ranged from 0.0871 to 0.5661. Likewise, detection probabilities varied among these species ranging from 0.1847 to 0.9333 (see SOP5 for specific results). Based on these findings, reasonably precise estimates of occupancy (and presumably changes in occupancy) can be obtained with four well-timed visits (two visual encounter surveys and two dipnet) using double independent observers. We recommend that 35 sites be

sampled within Chesapeake and Ohio Canal NHP for 2005; our analysis revealed no reason why the sites initially chosen for the pilot study cannot be used for long-term occupancy monitoring.

In the first two years (2005–2006) of lentic amphibian surveys, there was only a slight change in the probability of occupancy, but for most species there was turnover, indicating that the same sites are not occupied each year (see SOP5). We recommend continuing annual sampling and increasing the number of sites if management agencies are unsatisfied with the current study's ability to detect change in amphibian occupancy and associated vital rates (see SOP5).

Estimated occupancy probabilities for the 9 species detected in the call survey ranged from 0.5715 to 0.8571 and detection probabilities varied among these species from: 0.2201 to 0.4520 (see SOP5 for specific results). Based on these findings, reasonably precise estimates of occupancy can be obtained with 6 visits using double independent observers, if only 10–14 sites are surveyed (see SOP5 for details).

For lotic surveys, the estimated occupancy probabilities for the three species ranged from 0.137 to 0.710 and detection probabilities varied among the species from 0.42 to 0.79 (see SOP6 for specific results). Reasonably precise estimates of occupancy can be quantified by surveying 20 streams, using 2 sets of transect pairs on each stream, separated by at least 100 m (for a total of 40 sets of paired transects). Each set of paired transects should be sampled on two separate visits (between June and August). Our analysis does not suggest that streams surveyed in the pilot study are unsuitable for long-term monitoring for occupancy.

In the first two years (2005–2006) of lotic amphibian surveys, estimates of colonization and extinction for 32 stream sites were not different than zero (i.e., parameter estimates had wide estimated confidence intervals could not exclude the possibility that there was no colonization or extinction of the survey sites). Because stream salamanders are suggested to have lower rates of turnover due to their association with more reliable habitats (Davic and Welsh 2004), we recommend continuing annual sampling and assessing turnover after at least 5 years of data have been collected. At this time, we will be able to determine the number of sites that should be included in the long-term monitoring program in the National Capital Region Network.

For terrestrial surveys, *Plethodon cinereus* was the only species with sufficient detections for formal occupancy analysis. We do not recommend continuing terrestrial salamander monitoring, but have provided SOP7, which describes the pilot study methods and results. Estimated occupancy probabilities differed between juveniles and adults (0.4682 and 0.7556, respectively). The detection probability of both age classes was around 0.39. Based on these findings, reasonably precise estimates of occupancy (and presumably changes in occupancy) can be obtained with 2 visits with two transect surveys per visit (see SOP7 for specific results). If a park is interested in terrestrial salamander surveys, we would recommend that at least 40 sites be sampled.

The program GENPRES (Bailey et al. 2006) can be used to estimate the number of sites and the frequency of visits necessary to detect a trend in population change. Evaluation of data collected in the pilot year provided further guidance on the selection of number of sites and frequency of visits to

obtain the desired level of precision in estimates of change in habitat occupancy. Critical information that is still lacking includes (1) multiple years of data to estimate changes in occupancy and explore factors that are hypothesized to affect change (e.g., urbanization variables), and (2) occupancy estimates from other National Capital Region parks that will quantify spatial variation and help determine how sampling effort should be allocated among the parks in the region (i.e., how many habitat-specific sites to sample at each park). Lotic pilot data suggested a difference in stream salamander occupancy among Chesapeake and Ohio Canal NHP and Rock Creek NP. We would expect park-specific differences as pilot studies are conducted at more parks. With this information, and input from the National Capital Region Park personnel, optimal spatial allocation of sampling location among parks can be explored (e.g., Brand et al. 2017; Wright et al. 2022) to provide precision on estimates of change in occupancy, or to best differentiate among park locations experiencing different levels of threats.

Field Methods

Field Season Preparations and Equipment

The benefit of the PAO approach is that it requires no special sampling equipment, unlike the mark-recapture theory, which underlies this method. However, sampling equipment and methodologies vary by habitat, species, and season. Because the phenology and habitats of amphibians are related to climatic events (e.g., timing of spring melt, precipitation events, temperature), some flexibility in the scheduling of field sampling is necessary.

Prior to the field season, all personnel will review field protocols, study anuran calls and identification guides for amphibians in the mid-Atlantic region (SOP 1–2). Because correct identification of species at all life stages is a major assumption of the proportion of area occupied 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 lentic and lotic data sheets should be copied on weatherproof paper. Sampling equipment (including waders, dipnets and traps) should be cleaned, washed in 10% bleach solution, and rinsed well. For blank copies of lentic and lotic data sheets, see SOP5 and SOP6 in NPS DataStore.

Sequences of Events During Field Season

All field crews should consist of at least 2 observers; it is possible for two teams of 2 observers to share sampling duties during the season. If 4 people are available, the survey sites should be rotated among the observers, to reduce the potential for observer detection bias.

Sampling methods are specific to each habitat strata (e.g., lentic, lotic or terrestrial habitats), though adjacent sampling locations can be surveyed on the same days. A minimum of 24 hours should separate sampling periods at a single location, and observers should follow the sampling windows, weather providing.

Details of Taking Measurements

Included in SOPs 5–7.

Processing of Samples Taken

Upon returning from the field each day, observers will prepare any biological samples collected for shipment. Samples will be stored in a lab freezer until they are shipped for analysis.

Completed data sheets will be stored in the office and data from them will be entered into the online database within a few weeks' time post survey.

All field equipment should be washed in a solution of 10% chlorine bleach by volume, between visits to sites.

End-of-Season Procedures

Refer to SOP9: After the Field Season.

Data Management

Overview

The success of any monitoring program depends on high quality data that is properly maintained. Therefore, data management procedures are an essential part of any monitoring protocol in order to provide detailed guidance on how the data are to be collected, checked, and maintained. The following is a general overview of the data management procedures associated with this monitoring protocol. Please refer to the data management SOP (SOP12: Data Management) for more details.

Database Design

A relational database in Microsoft SQL server has been established on the USGS National Web Server System (NatWeb) and is maintained by personnel in the Amphibian Research and Monitoring Initiative (ARMI), located in San Diego, CA. Data from the surveys described in this narrative and related SOPs will be entered and stored in the NEARMI online database. This system provides a secure location for long-term data storage and data are included in regular backup routines to protect against catastrophic hardware failure and database corruption.

Data Entry, Verification, Editing

Data will be collected in the field on paper data sheets or electronic tablets. All field data will be entered into the NEARMI online database by the primary field person(s) who collected the data at each park. Data verification (QA/QC) will be conducted at the end of the field season after survey data entry is completed. During QA/QC, if any discrepancies or errors are found on the data sheets, staff will consult with the personnel who collected the data and, if necessary, make corrections both on the data sheet itself (noting the date and person making the correction) and in the NEARMI online database.

Data Archival Procedures

Data entered by the NEARMI Program are stored on USGS file servers at EESC, in the NEARMI online database, and in shared Microsoft OneDrive folders. Onsite data are backed up and transferred offsite once per month and the online database is backed up nightly.

Data Dissemination

After all QC for a field season has been completed, an updated cumulative dataset will be published to the NPS DataStore, along with relevant metadata.

Analysis and Reporting

The USGS NEARMI program is required to submit annual reports to the NCRN I&M program detailing activities and findings from the previous field season. Report formatting and schedules will vary and will be detailed in the annual workplans and financial agreements. Additional details about the report standards can be found in SOP13: Reporting.

Reporting Schedule and Recommendations for Routine Analysis

Reporting milestones will be set annually. Typically, routine data summaries will be produced by late Spring of the year following field sampling.

These summaries present the proportion of sites occupied by each species encountered and species detection probabilities. See recent annual data summary products here: DataStore - Series: Amphibian Monitoring in the National Capital Region (nps.gov)

Trend and synthetic analysis and reporting will be produced regularly, but less frequently, following IMD reporting guidance (DataStore - Data analysis and reporting requirements (nps.gov)) and information needs

Recent reporting includes:

Grant EHC and Others. 2022. Summary and synthesis of 15 years of the amphibian vital sign monitoring in the National Capital Area Inventory and Monitoring Network. Natural Resource Report. NPS/NCRN/NRR—2022/2356. National Park Service. Fort Collins, Colorado. https://doi.org/10.36967/nrr-2291380

Wright, A.D., Grant, E.H.C., Zipkin, E.F., 2022. A comparison of monitoring designs to assess wildlife community parameters across spatial scales. Ecological Applications, e2621.

Wright AD, Campbell Grant EH, Zipkin EF. 2020. A hierarchical analysis of habitat area, connectivity, and quality on amphibian diversity across spatial scales. Landscape Ecology. 35:529–544

Miller DA and Grant EH. 2015. Estimating occupancy dynamics for large-scale monitoring networks: amphibian breeding occupancy across protected areas in the northeast United States. Ecology and Evolution. 5(21)

All completed reports and articles will be publicly available from multiple sources including the NCRN website (https://www.nps.gov/im/ncrn/publications.htm) and the NPS DataStore (NPS DataStore Home).

Personnel Requirements and Training

Roles and Responsibilities

The PI and staff of the NEARMI program have served as 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 data manager and database technician. In collaboration with the PI and staff, the data manager and technician will develop data entry forms and other database features (e.g., quality assurance, report generation). The data manager and technician are responsible for data archiving, data security, dissemination, and database design.

Qualifications and Training

Prior to each field season, personnel who will be participating in the surveys, including the lead biologist and the research technicians, should review the protocol and all associated SOPs. As part of the SOP, we will also require that all personnel participating in the surveys receive training and a guide covering all amphibian species and their life stages (including morphological, habitat associations and natural history) which may be encountered in the National Capital Region parks. Training will also include demonstrations and participation in field survey methods at sites which are not included in the monitoring program. Because the PAO approach assumes that a species is never falsely detected (i.e., misidentified), review and competence in species identification at all life history stages which 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.

Operational Requirements

Annual Workload and Field Schedule

Amphibian surveys will begin no sooner than mid-February and extend no later than mid-October, a period that covers the breeding and active seasons of all amphibians in the National Capital Parks region. Survey work is not continuous during this period, and inclement and variable weather conditions preclude scheduling specific sampling events. The SOPs will specify weather and seasonal conditions under which surveys should be conducted (e.g., calling surveys are conducted in specific sampling windows under certain weather conditions). All monitoring efforts will require a minimum of a two-person crew. Five to ten lentic surveys and three to five lotic 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, type of sampling, and number of sampling sites.

Facility and Equipment Needs

The nature of amphibian survey work does not require special facilities beyond normal office space, equipment storage needs and use of a vehicle to travel between survey sites. A list of field equipment needed by one crew (SOP1: Preparations for the Field Season) is shown below. We envision that two to three crews will be working simultaneously on any given day, requiring multiple sets of equipment.

List of Field Equipment for Each 2-Person Survey Team

- Aquarium dip nets (2)
- Batteries for equipment
- Bleach spray bottle
- Cell phone
- Chest waders (2)
- Clicker counters (2)
- Clipboard (2)
- Compass (2)
- Cooler and Ice packs
- Data sheets on Rite-in-the-rain paper
- Digital camera
- D-shaped dip nets (2)
- Fiberglass tape (50 m) (2)
- First aid kit
- Flagging tape

- GPS unit
- Hand lens (10x)
- Headlamps (2)
- Hip waders (2)
- Mechanical Pencils with erasers
- Meter stick
- pH & conductivity meter
- NPS & state permits
- Rinse spray bottle
- Rulers (in centimeters)
- Scrub brush
- SOPs, field ID notes, site photos and maps
- Specimen containers and labels
- Spherical densitometer
- Thermometer
- Wire flags
- Ziplock bags

Procedure for Revising the Protocol and Storage of Archived Protocols

On occasion, the protocol document will be updated as new information on species occurrence, detection, or improvements in survey techniques or modeling approach are developed and documented in the peer-reviewed literature.

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Appendix A: List of Species Monitored

Table 2 shows the occurrence of anurans and caudates in NCR parks.

Table 2. Occurrence of amphibian species in National Capital Region parks [A]. D = documented, P = possible occurrence, and "–" = no occurrence or information. The table also includes the primary habitat (lotic, lentic, terrestrial) and detection period for each species. Information derived from Petranka (1998), Thompson (2000), Mitchell (2000), Hulse et al. (2001), Pauley and Watson (2003). Updated in 2023.

Taxon	Amphibian Species	Scientific Name	ANTI	CATO	CHOH	GWMP	HAFE	MANA	MONO	NACE	PRWI	ROCR	WOTR	Primary Habitat	Detection Period
Anura	Eastern Cricket Frog	*Acris c. crepitans*	P	P	D	D	D	D	P	D	D	P	D	Lentic	May–July [B]
	Eastern American Toad	*Anaxyrus americanus*	D	D	D	D	D	D	D	D	D	D	D	Lentic	March–May [B]
	Fowler's Toad	*Anaxyrus fowleri*	D	D	D	D	D	D	D	D	D	D	D	Lentic	May–June [B]
	Cope's Gray Treefrog	*Hyla chrysoscelis*	–	–	D	D	–	D	–	D	D	D	P	Lentic	May–July [B]
	Green Treefrog	*Hyla cinerea*	–	–	P	P	–	–	–	D	–	–	–	Lentic	April–July [B]
	Common Gray Treefrog	*Hyla versicolor*	D	D	D	D	D	D	D	D	D	D	–	Lentic	May–July [B]
	Northern Spring Peeper	*Pseudacris c. crucifer*	D	D	D	D	D	D	D	D	D	D	D	Lentic	February–May [B]
	Upland Chorus Frog	*Pseudacris f. feriarum*	P	D	D	D	D	D	D	D	D	D	P	Lentic	February–April [B]
	American Bullfrog	*Lithobates catesbeiana*	D	D	D	D	D	D	D	D	D	D	P	Lentic	May–July [B]
	Northern Green Frog	*Lithobates clamitans melanota*	D	D	D	D	D	D	D	D	D	D	P	Lentic	May–July [B]
	Pickerel Frog	*Lithobates palustris*	D	D	D	D	D	D	D	D	D	D	D	Lentic	March–April [B]
	Northern Leopard Frog	*Lithobates pipiens*	–	P	D	–	–	–	–	–	P	–	–	Lentic	March–May [B]
	Southern Leopard Frog	*Lithobates sphenocephala*	–	D	D	D	D	D	D	D	D	D	D	Lentic	February–April [B]

[A] ANTI = Antietam National Battlefield, CATO = Catoctin Mountain Park, CHOH = Chesapeake and Ohio Canal National Historical Park, GWMP = George Washington Memorial Parkway, HAFE = Harpers Ferry National Historical Park, MANA = Manassas National Battlefield Park, MONO = Monocacy National Battlefield, NACE = National Capital Parks-East, PRWI = Prince William Forest Park, ROCR = Rock Creek Park, WOTR = Wolf Trap National Park for the Performing Arts.

[B] Corresponds to breeding season.

Table 2 (continued). Occurrence of amphibian species in National Capital Region parks [A]. D = documented, P = possible occurrence, and "–" = no occurrence or information. The table also includes the primary habitat (lotic, lentic, terrestrial) and detection period for each species. Information derived from Petranka (1998), Thompson (2000), Mitchell (2000), Hulse et al. (2001), Pauley and Watson (2003). Updated in 2023.

Taxon	Amphibian Species	Scientific Name	ANTI	CATO	CHOH	GWMP	HAFE	MANA	MONO	NACE	PRWI	ROCR	WOTR	Primary Habitat	Detection Period
Anura (cont.)	Wood Frog	*Lithobates sylvatica*	D	D	D	D	D	D	D	D	D	D	D	Lentic	February–April [B]
	Eastern Spadefoot	*Scaphiopus holbrookii*	–	–	D	–	–	–	D	D	–	D	–	Lentic	March–September [B]
Caudata	Jefferson Salamander	*Ambystoma jeffersonianum*	P	P	D	–	D	–	–	–	–	–	–	Lentic	February–April [B]
	Spotted Salamander	*Ambystoma maculatum*	D	D	D	D	D	D	D	D	D	D	D	Lentic	March–April [A]
	Marbled Salamander	*Ambystoma opacum*	D	D	D	D	P	D	D	D	D	D	P	Lentic	September–November [B]
	Northern Dusky Salamander	*Desmognathus fuscus*	D	D	D	D	D	D	D	D	D	D	P	Lotic	Year-round (peak spring-early summer)
	Seal Salamander	*Desmognathus monticola*	–	–	P	–	D	–	–	–	–	–	–	Lotic	spring; late summer-fall
	Northern Two-lined Salamander	*Eurycea bislineata*	D	D	D	D	D	D	D	D	D	D	D	Lotic	Year-round
	Three-lined Salamander	*Eurycea guttolineata*	–	–	–	P	–	P	–	–	D	–	D	Lotic	April–October
	Long-tailed Salamander	*Eurycea l. longicauda*	D	D	D	–	D	–	D	–	–	D	–	Lotic	April–October
	Northern Spring Salamander	*Gyrinophilus p. porphyriticus*	D	D	D	–	D	–	D	–	–	–	–	Lotic	Year-round (peak spring-early summer)
	Four-toed Salamander	*Hemidactylium scutatum*	D	D	D	D	P	D	D	D	D	D	P	Lentic	March–October

[A] ANTI = Antietam National Battlefield, CATO = Catoctin Mountain Park, CHOH = Chesapeake and Ohio Canal National Historical Park, GWMP = George Washington Memorial Parkway, HAFE = Harpers Ferry National Historical Park, MANA = Manassas National Battlefield Park, MONO = Monocacy National Battlefield, NACE = National Capital Parks-East, PRWI = Prince William Forest Park, ROCR = Rock Creek Park, WOTR = Wolf Trap National Park for the Performing Arts.

[B] Corresponds to breeding season.

Table 2 (continued). Occurrence of amphibian species in National Capital Region parks [A]. D = documented, P = possible occurrence, and "–" = no occurrence or information. The table also includes the primary habitat (lotic, lentic, terrestrial) and detection period for each species. Information derived from Petranka (1998), Thompson (2000), Mitchell (2000), Hulse et al. (2001), Pauley and Watson (2003). Updated in 2023.

Taxon	Amphibian Species	Scientific Name	ANTI	CATO	CHOH	GWMP	HAFE	MANA	MONO	NACE	PRWI	ROCR	WOTR	Primary Habitat	Detection Period
Caudata (cont.)	Red-spotted Newt	*Notophthalmus viridescens*	D	D	D	D	D	D	D	D	D	P	P	Lentic	March–April [B] (some fall)
	Eastern Red-backed Salamander	*Plethodon cinereus*	D	D	D	D	D	D	D	D	D	D	D	Terrestrial	Year-round (peak spring and fall)
	White-spotted Slimy Salamander	*Plethodon cylindraceus*	–	–	–	P	–	P	–	–	D	–	D	Terrestrial	March–November
	Northern Slimy Salamander	*Plethodon glutinosus*	D	D	D	–	P	–	D	–	–	D	–	Terrestrial	March–November
	Valley and Ridge Salamander	*Plethodon hoffmani*	–	D	D	–	–	–	–	–	–	–	–	Terrestrial	spring; fall
	Eastern Mud Salamander	*Pseudotriton m. montanus*	–	–	D	P	–	–	–	P	D	P	–	Lotic	Unknown (may be similar to *Pseudotriton ruber*)
	Northern Red Salamander	*Pseudotriton r. ruber*	D	D	D	D	D	P	D	D	D	D	D	Lotic	Year-round (peak April–May; August)
	Greater Siren	*Siren lacertina*	–	–	–	–	–	–	–	P	–	–	–	Lentic	Unknown in this area

[A] ANTI = Antietam National Battlefield, CATO = Catoctin Mountain Park, CHOH = Chesapeake and Ohio Canal National Historical Park, GWMP = George Washington Memorial Parkway, HAFE = Harpers Ferry National Historical Park, MANA = Manassas National Battlefield Park, MONO = Monocacy National Battlefield, NACE = National Capital Parks-East, PRWI = Prince William Forest Park, ROCR = Rock Creek Park, WOTR = Wolf Trap National Park for the Performing Arts.

[B] Corresponds to breeding season.

Appendix B: Sampling Covariates

The following tables define codes for sky condition (Table 3), wind speed (Table 4), moon phase (Table 5), and turbidity condition (Table 6).

Table 3. Codes for sky condition.

Code	Sky Condition
0	Clear or few clouds (< 20% of sky)
1	Partly cloudy or variable (20–50% of sky)
2	Cloudy or overcast (> 50% of sky)
3	Fog
4	Mist
5	Showers or light rain
6	Heavy rain
7	Sleet/Hail
8	Snow

Table 4. Codes for wind speed.

Code	mph	Indicators of Wind Speed
0	0	< 1 calm, smoke rises vertically
1	2–3	light air movement, smoke drifts
2	4–7	light breeze, wind felt on face, leaves rustle
3	8–12	gentle breeze, leaves/twigs in constant motion, raises dust
4	13–18	moderate breeze, small branches move
5	19–24	fresh breeze, small trees begin to sway
6	25–31	strong breeze, large branches move
7+	> 31	strong winds

Table 5. Codes for moon phase.

Code	Moon Condition
0	Moon not visible (e.g., not risen, hidden by clouds)
1	New moon or Waxing/Waning Crescent moon
2	First and Last Quarter moon
3	Full moon or Waxing/Waning Gibbous moon

Table 6. Codes for turbidity condition.

Code	Turbidity Condition
Clear	Can see the bottom of the wetland
Slightly turbid	Visibility slightly impaired by suspended particulate matter,
Turbid	Visibility substantially impaired by suspended particulate matter
Opaque	Unable to see into water column; visibility greatly impaired

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