

National Capital Region Network

Amphibian Monitoring Protocol

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CONTENTS

Background and Objectives 1

 Issue Being Addressed and Rationale for Monitoring of Amphibian Populations 1

 Sampling Design 3

 Special Circumstances..... 10

 Field Methods..... 12

 Data Management..... 13

 Analysis and Reporting 13

Personnel Requirements and Training 14

Operational Requirements..... 14

 Procedure for Revising the Protocol and Storage of Archived Protocols 16

References..... 17

ACRONYMS

ARMI	U.S. Geological Survey's Amphibian Research and Monitoring Initiative
CATO	Catoctin Mountain Park
CHOH	Chesapeake and Ohio Canal National Historic Park
GWMP	George Washington Memorial Parkway
HAFE	Harpers Ferry National Historic Park
MANA	Manassas National Battlefield Park
NEARM	Northeast Amphibian Research and Monitoring Initiative
NWI	National Wetland Inventory
PAO	proportion of area occupied
ROCR	Rock Creek Park
SOP	Standard Operating Procedures
SSURGO	GIS Soil Survey Geographic database

National Capital Region Network Amphibian Monitoring Protocol

BACKGROUND AND OBJECTIVES

ISSUE BEING ADDRESSED AND 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 are 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 is 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, Tromulak 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 [*Rana sylvatica*], four-toed salamander [*Hemidactylium scutatum*], southeastern chorus frog [*Pseudacris feriarum*], southern leopard frog [*Rana sphenoccephala*]), 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 4 or less species at more than one site.

Data collected under this protocol will assist parks in achieving specific management goals:

1. Determine if the proportion of habitats occupied by amphibian species is:
 - a. increasing, decreasing or staying the same over time.
 - b. related to landscape variables including surrounding land use, road density or distance to nearest road, and proximity to or density of other potential breeding sites.
 - c. related to water quality variables, other biotic and abiotic variables, hydroperiod, or climatic conditions.
 - d. changing with respect to community composition over time.
2. Identify priority areas within individual Parks for amphibian habitat management and protection.

Our objective is to provide the National Capital Region parks with long-term amphibian monitoring protocols 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; and 4) to identify and evaluate management actions to reverse declining trends or to increase the occupancy of potential habitats. 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). In designing Standard Operating Procedures (SOPs) and the protocol narrative document, we have followed NPS guidelines (Oakley et al. 2003).

Historical Development of Amphibian Monitoring in National Capital Region Parks

The National Capital Region Network has 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 will have completed inventories of amphibian species (Shawn Carter, *pers. comm.*) (see Table 1), yet the status of amphibian populations was not evaluated, and currently, no statistically robust, long-term datasets have been collected with which to assess potential changes in amphibian populations in these parks. To assess the status of these populations and to document changes, we outline protocols for amphibian monitoring using the methods described below.

Measurable Objectives

The objectives of the protocols are to:

1. Establish a systematic sampling framework to evaluate changes to regional-level species diversity in amphibian communities.
2. Determine changes in species richness over time within the National Capital Region Network.
3. Provide monitoring information to park managers on long-term trends in distribution of amphibian species, and to aid in management by providing information on the potential impacts of urbanization, habitat loss/degradation, management practices or other identified stressors on amphibian populations.
4. Document the occurrence of amphibian malformations and disease outbreaks and establish SOPs for collection and vouchering of infected, dying or dead specimens.

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, and 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).

Recently, the proportion of area occupied (PAO) metric has been 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 will 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. We may account for violations of assumption 2 by analyzing single season datasets as part of a multiple season analysis within PRESENCE (MacKenzie et al. 2005), while violation of assumption 4 may be explored and corrected using goodness-of-fit test (MacKenzie and Bailey 2004).

Rationale for Selecting Urbanization as a Stratification Variable

There is increasing concern for the effects of rapid development in the region on natural systems (e.g., Palmer et al. 2002, Moore and Palmer 2005), a trend that is becoming increasingly typical of natural areas across the globe. In the Mid-Atlantic and Northeastern United States, historical land use change has resulted in conversion of forestland to agriculture, and subsequent urban and suburban development which induce changes to natural systems. These large-scale, persistent changes in the structure and spatial arrangement of habitat patches, combined with other interacting environmental factors, likely structure the distribution of populations on the landscape. Enigmatic

population declines of amphibians have occurred throughout the world, even in “natural islands” like National Park land (Pounds and Crump 1994, Drost and Fellers 1996).

We recommend that stratification be focused, where possible, on the impact of urbanization variables (e.g., trail and road networks) adjacent to (e.g., within 1km) the national park boundary, or within the park boundary. Future projections (within 10 years) about increases in traffic, locations of new trails or roads or increases in visitor use of existing trails or roads within or adjacent to the park boundary should be included in locating monitoring sampling sites.

Site Selection

Based on the distribution of potential amphibian habitat, all or a subset of the parks listed below will be included in the amphibian monitoring protocols:

Antietam National Battlefield
Catoclin Mountain Park
Chesapeake and Ohio Canal National Historic Park
George Washington Memorial Parkway
Harpers Ferry National Historic Park
Manassas National Battlefield Park
Monocacy National Battlefield
National Capitol Parks-East
Prince William Forest Park
Rock Creek Park
Wolf Trap National Park for the Performing Arts

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) will be implemented in the parks.

Lentic and lotic sampling sites can be identified through a number of methods described in SOP #03, 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. Lentic and terrestrial sites, to the extent feasible under the modeling framework, should be located at or in close proximity to existing stream sampling and vegetation monitoring stations, respectively.

Within the stratification framework (relating to urbanization variables), terrestrial sampling sites should be located in two strata: (1) near (<150 m) from a road, and (2) far (>150 m)

from a road. Lentic sampling sites should be stratified in a similar manner. Lotic samples should be located in relation to disturbance strata. Because many of the streams have headwaters located outside of the jurisdiction of the National Park Service managers, 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. Samples should be stratified by road proximity (or trail crossing, where an impacted section sample would be taken below the road or trail crossing). 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 principle 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 was collected at two parks (Chesapeake and Ohio Canal National Historic 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 each year 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 the following SOP #05-07. In addition, site selection is described in SOP #03, "Selection of monitoring sites by habitat type and species," in the final protocol document.

Amphibian Populations Targeted in this Sampling Design

Sampling will be limited to the breeding and activity season of amphibians in the Mid-Atlantic region (mid February through October), though inference can only be made for the active period of each species (i.e., February through June for *Ambystoma maculatum*, May-June and Sept-October for members of the genus *Plethodon*) (Appendix 1).

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 1). Problems of declines, malformations or diseases are not restricted to amphibians from one of these habitats alone. Sampling will be limited to the expected breeding or active seasons of the species found in these habitats, but will need to remain flexible due to uncertain climatic conditions. Specific information regarding sampling periods and survey techniques is addressed in the sections below for: lentic (SOP #05), lotic (SOP #06), and terrestrial (SOP #07) habitats. Not all habitats will be 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..

Lentic Surveys (SOP #05): All anurans and some caudates (see Appendix 1) 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 NAAMP protocol; see: <http://www.mp2-pwrc.usgs.gov/NAAMP/protocol/>; 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, 2 visual encounter surveys and 2 dip net surveys will be conducted at wetland survey sites to detect species presence (see SOP#05). 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 SOP#05).

Because of the difficulty in surveying for the spadefoot toad (*Scaphiopus holbrookii*) and the constraints on personnel, no special arrangements will be made to survey for this species. Detection of the spadefoot toad will be opportunistic, through calling and dipnet surveys. Inference for this species will therefore be limited. All anurans and some caudates (see Appendix 1) 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.

Lotic Surveys (SOP #6): 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 mi²; 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 sampling sites will be integrated with existing water quality sampling sites when possible. 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 (ψ) from this pilot survey, we recommend final survey methodology in SOP #6. In general, we recommend 2 sampling visits to 2 sets of paired transects on each of 20 streams (see SOP#6).

Terrestrial-Specific Surveys (SOP #7): 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 x 3m) and coverboard arrays (3 pine coverboard stations; each station consisting of 2 large boards (26x26 cm) and 3 small boards (13x26 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 SOP#7). 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., SOP #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 is always collected after amphibian sampling has been conducted, to avoid disturbing the amphibians.

SPECIAL CIRCUMSTANCES

While not a focus of the monitoring protocols, there is a specific SOP (#12, “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 web site: http://www.nwhc.usgs.gov/research/amph_dc/amph_sop.html; SOPs for “Biosecurity, Mailing, and Collection of Amphibians,” and “Restraint and Handling of Live 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 #5,6,7). 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 analysis in program PRESENCE.

Appropriate sampling frequency and intensity are recommended in each of the habitat specific SOPS (05-07). 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 SOP #5 for specific results). Based on these findings, reasonably precise estimates of occupancy (and presumably changes in occupancy) can be obtained with 4 well-timed visits (2 VES and 2 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 SOP#05). 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 SOP#05).

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 SOP #5 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 SOP#5 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 SOP #6 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 an SOP (#7) 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 SOP #7 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; presumably to provide precision on estimates of change in occupancy or to best differentiate among park locations experiencing different levels of urbanization..

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). 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 the appended datasheets should be copied, with at least 50% on weatherproof paper. Sampling equipment (including waders, dipnets and traps) should be clean, washed in 10% bleach solution, and rinsed well.

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, 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 should enter the day's field data directly into the database.

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

End-of-Season Procedures

Refer to SOP #10.

DATA MANAGEMENT

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). Data from the surveys described in this narrative and related SOPs will be entered and stored in the NEARMI local database, and all data will be checked for quality and accuracy. Data will be transferred between the NEARMI local database and the NPS Natural Resource Database once per year.

Data Entry, Verification, Editing

Data will be collected in the field on data sheets, and these data will be entered into the NEARMI local database by the primary field person(s) who collected the data at each park. Data verification (proofing) will be conducted within a month of data entry. During proofing, if any discrepancies or errors are found on the data sheets, 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 local database.

Metadata Procedures

Metadata for the amphibian monitoring project will be prepared in collaboration with Cheryl Solomon, metadata specialist for the USGS-BRD (Solomon@gcmd.nasa.gov).

Data Archival Procedures

Hard copy data and Access databases on CDs will be archived at the National Capital Region Network headquarters. Data will not be archived until the data is 5 years older or more.

ANALYSIS AND REPORTING

Reporting Schedule

Annual reports based on the current year's data will be prepared by 31 December.

Recommendations for Routine

Data Summaries and Statistical Analyses to Detect Change

Data summaries presenting the proportion of sites occupied by each species encountered and species detection probabilities will be prepared annually. Statistical analyses to detect change will be conducted after the first 5 years and annually thereafter.

Recommended Report Format with Examples of Summary Tables and Figures

Report format will conform to other NPS reports.

Recommended Methods for Long-term Trend Analysis (e.g., every 5 or 10 years)

After five years and annually thereafter.

PERSONNEL REQUIREMENTS AND TRAINING

Roles and Responsibilities

The PI and co-PI of the NE ARMI 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 data manager and database technician. In collaboration with the PI and co-PI, 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 a cassette tape or CD of calling anurans for review (Elliott 2004) and a guide covering all amphibian species and their life stages (including morphological, habitat associations and natural history) encountered in the National Capital Region parks. The tape/CD and guide will be provided as part of this project. We will also require all personnel participating in the surveys to score 70% or above on FroqQuiz (NEARMI: NCR quiz title), a new training tool which has been developed by the NAAMP program. 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. Table 2 shows a list of field equipment needed by one crew. We envision that two to three crews will be working simultaneously on any given day, requiring multiple sets of equipment.

**TABLE 2. LIST OF EQUIPMENT AND MATERIALS
TO BE TAKEN INTO THE FIELD FOR AMPHIBIAN SURVEYS.**

- | | |
|------------------------------|--|
| • Aquarium dip nets (2) | • Hip waders (2) |
| • Bleach spray bottle | • Li-ion Batteries for GPS, Camera, etc. |
| • Cell phone | • Mechanical Pencils with erasers |
| • Chest waders (2) | • Meter stick |
| • Clicker counters (2) | • Rinse spray bottle |
| • Clipboard (2) | • Datasheets on Rite-in-the-rain paper |
| • Compass (2) | • Rulers (in centimeters) |
| • Cooler and Ice packs | • Scrub brush |
| • Digital camera | • SOPs, CD and field guide(s) |
| • D-shaped dip nets (2) | • Specimen containers and labels |
| • Fiberglass tape (50 m) (2) | • Spherical densitometer (2) |
| • First aid kit | • Thermometer |
| • Flagging tape | • Wire flags |
| • GPS unit | • Water testing equipment |
| • Hand lens (10x) | • Zip-lock bags |
| • Headlamps (2) | • Aquarium dip nets (2–4 per site) |

Startup Costs and Budget Considerations

Habitat location and ground-truthing surveys was conducted by 6 NE ARMI personnel (2 staff, 4 technicians) at three sites (CHOH, ROCR, MANA) during the summer of 2004. Data management personnel expenses will cover a database technician's time to develop and maintain the NEARMI local database. Startup costs included the purchase of equipment for three crews (see Table 2) and purchase of coverboard materials.

Personnel expenses for field work are based on two crews of two people. We anticipate that NE ARMI personnel will be able to continue to survey lentic and lotic sites at the lower half of CHOH, lotic and lentic sites at ROCR, and perform pilot studies at 1–2 additional parks each year. If additional long-term monitoring is desired future allocation of effort among sites may need adjusting. National Capital Region personnel will be consulted to advise appropriate allocation of effort among parks in the region.

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. Such changes will be documented in a narrative detailing (1) the need for updating the protocol or any SOPs (including literature cited), and (2) how the protocol or SOPs are changed.

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Appendix 1. Amphibian species, occurrence (D = documented, P = possible occurrence, <-> = no occurrence or information) in the National Capital Parks Network (ANTI = Antietam National Battlefield, CATO = Catocin Mountain Park, CHOH = Chesapeake and Ohio Canal National Historic Park, HAFE = Harpers Ferry National Historic Park, MANA = Manassas National Battlefield Park, MONO = Monocacy National Battlefield, NACE = National Capital Parks-East, PRWI = Prince William Forest Park, WOTR = Wolf Trap National Park for the Performing Arts), primary habitat (lotic, lentic, terrestrial) and detection period (corresponds to breeding season*). Information derived from Petranka (1998), Thompson (2000), Mitchell (2000), Hulse et al. (2001), Pauley and Watson (2003).

Amphibian Species	Scientific Name	ANTI	CATO	CHOH	GWMP	HAFE	MANA	MONO	NACE	PRWI	ROCR	WOTR	Primary Habitat	Detection Period
ANURA														
Eastern Cricket Frog	<i>Acris c. crepitans</i>	P	P	D	D	P	D	P	D	D	P	D	Lentic	May - July*
Eastern American Toad	<i>Bufo americanus</i>	P	D	D	D	D	D	D	D	D	D	D	Lentic	March - May*
Fowler's Toad	<i>Bufo fowleri</i>	P	D	D	D	D	P	D	D	D	P	D	Lentic	May - June*
Cope's Gray Treefrog	<i>Hyla chrysoscelis</i>	-	-	D	P	-	P	-	D	D	D	P	Lentic	May - July*
Green Treefrog	<i>Hyla cinerea</i>	-	-	P	P	-	-	-	D	-	-	-	Lentic	April - July*
Common Gray Treefrog	<i>Hyla versicolor</i>	D	D	D	D	D	P	D	-	D	-	-	Lentic	May - July*
Northern Spring Peeper	<i>Pseudacris c. crucifer</i>	D	D	D	D	D	D	D	D	D	D	D	Lentic	February - May*
Upland Chorus Frog	<i>Pseudacris f. feriarum</i>	P	P	D	D	P	P	D	D	D	D	P	Lentic	February - April*
American Bullfrog	<i>Rana catesbeiana</i>	D	D	D	D	D	D	D	D	D	D	P	Lentic	May - July*
Northern Green Frog	<i>Rana clamitans melanota</i>	P	D	D	D	D	P	D	D	D	D	P	Lentic	May - July*
Pickereel Frog	<i>Rana palustris</i>	D	D	D	D	P	D	D	D	D	D	D	Lentic	March - April*
Northern Leopard Frog	<i>Rana pipiens</i>	-	P	P	-	-	-	-	-	P	-	-	Lentic	March - May*
Southern Leopard Frog	<i>Rana sphenoccephala</i>	-	-	D	D	-	D	-	D	D	P	D	Lentic	February - April*
Wood Frog	<i>Rana sylvatica</i>	D	D	D	D	D	D	D	D	D	D	D	Lentic	February - April*
Eastern Spadefoot	<i>Scaphiopus holbrookii</i>	-	-	P	-	-	-	-	D	-	D	-	Lentic	March - September*
CAUDATA														
Jefferson Salamander	<i>Ambystoma jeffersonianum</i>	P	P	D	-	D	-	-	-	-	-	-	Lentic	February - April*
Spotted Salamander	<i>Ambystoma maculatum</i>	D	D	D	D	D	D	D	D	D	D	D	Lentic	March - April*
Marbled Salamander	<i>Ambystoma opacum</i>	D	D	D	D	P	D	D	D	D	D	P	Lentic	September - November*
Northern Dusky Salamander	<i>Desmognathus fuscus</i>	P	D	D	D	D	P	D	D	D	D	P	Lotic	Year-round (peak spring-early summer)
Seal Salamander	<i>Desmognathus monticola</i>	-	-	P	-	D	-	-	-	-	-	-	Lotic	spring; late summer-fall
Northern Two-lined Salamander	<i>Eurycea bislineata</i>	D	D	D	D	D	P	D	D	D	D	D	Lotic	Year-round
Three-lined Salamander	<i>Eurycea guttolineata</i>	-	-	-	P	-	P	-	-	D	-	D	Lotic	April - October
Long-tailed Salamander	<i>Eurycea l. longicauda</i>	D	D	D	-	D	-	D	-	-	D	-	Lotic	April - October
Northern Spring Salamander	<i>Gyrinophilus p. porphyriticus</i>	D	D	D	-	D	-	D	-	-	-	-	Lotic	Year-round (peak spring-early summer)
Four-toed Salamander	<i>Hemidactylium scutatum</i>	D	D	D	P	P	P	D	D	D	D	P	Lentic	March - October
Red-spotted Newt	<i>Notophthalmus viridescens</i>	D	D	D	D	D	P	D	D	D	P	P	Lentic	March - April* (some fall*)

Appendix 1. (continued)

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

Preparations for the Field Season Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.2	01/05/2007	Grant		Update	1.3

1. Purpose

The purpose of this document is to help prepare field crews for amphibian monitoring including becoming familiar with relevant species and assembling the proper field gear.

2. Scope and Applicability

This document applies specifically to the NCRN Amphibian Monitoring Program and should be reviewed by any and all personnel involved with amphibian monitoring.

3. Reference Documents

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4. Procedures and General Requirements

Prior to the field season each year, preferably in January, all personnel should review the protocol narrative document and all included Standard Operating Procedures (SOPs) (#1–13). Data sheets and field notes from previous years should be reviewed to identify appropriate field conditions (e.g., air temperatures) and to aid scheduling of sampling. A calendar of tentative sampling dates for habitat-specific protocols (SOP #5, 6, and 7) at each study location should be prepared, especially if technicians will be expected to work on multiple projects at several locations.

Particular attention should be paid to studying and reviewing the identification of amphibian species at all life stages, including egg, tadpole/larvae, juvenile and adult life stages. Calling anurans should be studied and reviewed, and all personnel participating in field surveys must take the USGS frog quiz, an internet-based assessment tool designed for the North American Amphibian Monitoring Program (NAAMP), available at <http://www.pwrc.usgs.gov/frogquiz>. All personnel are expected to score 70% or higher on the “NEARMI: NCR” frog quiz prior to the field season.

4.1 General Preparation

- 4.1.1 Review field notes and data sheets from the previous year(s) to identify unique access issues and field conditions at sites.
- 4.1.2 Review amphibian species encountered in previous years.
- 4.1.3 GPS waypoints of sampling locations should be loaded in the handheld GPS units. These waypoints are combinations of X (easting) and Y (northing) coordinates in UTM, datum NAD 83. Sampling locations will be stored in the NCRAMP database.
- 4.1.4 Assemble, clean and disinfect, and repair all field equipment so that it is in excellent working condition as outlined in section III below.

Preparations for the Field Season Standard Operating Procedure

4.2 Scheduling Field Work

- 4.2.1 Field surveys should not begin prior to the middle of February, and should extend no later than the middle of October. Exact starting and ending dates will depend on local climatic conditions and thus, seasonal breeding phenology.
- 4.2.2 Conducting field work within the National Capital Region will require 2-4 person crews per park. One group of 2 personnel is expected to survey 8-10 sampling sites each field day. Hours during the field season may be extended (i.e., more than 8 hours per day), due to the short detection period for some amphibian species, and the need for multiple surveys throughout the day (e.g., visual encounter surveys during the day and calling surveys after sundown).
- 4.2.3 Whenever field work is to be conducted after sundown, field personnel should notify one other person who serves as the contact point for that park and who is not participating in the survey with the planned schedule and sampling locations. In some cases, the contact person may be law enforcement personnel at the park. At least one technician should have a cellular phone on hand in case of emergencies, and should designate a person to be “on call” during the sampling.

4.3 Field Equipment

- 4.3.1 All field equipment should be assembled, cleaned and checked for correct operation at least four weeks before the anticipated start of the field season, so that failing equipment can be repaired or replaced.
- 4.3.2 Field technicians should prepare a spray bottle of 10% bleach solution and a second large (1 gallon) spray bottle of rinse water which are used to clean boots and equipment (e.g., dip nets) after surveying each habitat. Bottles with bleach should be resistant to corrosive materials, and be clearly labeled with non-fading marks on at least three sides of the container. Bleach bottles and rinse water should not be interchanged; designated bottles should be devoted to either bleach or rinse water.
- 4.3.3 To properly clean field equipment, all mud, algae, or other debris must be completely cleaned from all surfaces. Items are washed with the bleach solution, then rinsed with clean or treated water, and allowed to dry separately from equipment used for other sites.
- 4.3.4 In general and where feasible, nets, boots and other sampling equipment should be dedicated for each individual site to minimize the spread of disease among sites. If equipment has been used at a site with suspected or known disease problems, that equipment should not be used for any other site.

Preparations for the Field Season

Standard Operating Procedure

- 4.3.5 Field personnel should bring a subset or as many of the following reference materials in the vehicle and/or field in case species identification is an issue. Reference materials for amphibians in the Mid-Atlantic Region include:
- Conant, R. and J. T. Collins. 1998. A Field Guide to Reptiles and Amphibians of Eastern and Central North America. Third edition, expanded. Houghton Mifflin Company, Boston.
 - Elliott, L. 2004. The calls of frogs and toads. Stackpole Books.
 - Meteyer, C.U. 2000. Field guide to malformations of frogs and toads with radiographic interpretations. Biological Science Report USGS/BRD/BSR-2000-0005.
 - Online tadpole identification key. <http://www.pwrc.usgs.gov/tadpole/>
 - Parmelee et al. (2002): A field guide to amphibian larvae and eggs of Minnesota, Wisconsin and Iowa.
 - Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC.
 - Pfingsten, R. A., and F. Downs, eds. 1989. Salamanders of Ohio. Ohio Biol. Surv. Bull. New Series Vol. 7, No. 2. 315 pp.
 - Wright, A.H., and A.A. Wright. 1949. Handbook of Frogs and Toads of the United States and Canada. Comstock Publ. Co., Ithaca, NY.

4.4 List of Field Equipment for Each 2-Person Survey Team

- | | |
|---|---|
| <ul style="list-style-type: none"> • Aquarium dip nets (2) • Bleach spray bottle • Cell phone • Chest waders (2) • Clicker counters (2) • Clipboard (2) • Compass (2) • Cooler and Ice packs • Digital camera • D-shaped dip nets (2) • Fiberglass tape (50 m) (2) • First aid kit • Flagging tape • GPS unit | <ul style="list-style-type: none"> • Hip waders (2) • Li-ion Batteries for GPS, Camera, etc. • Mechanical Pencils with erasers • Meter stick • Rinse spray bottle • Datasheets on Rite-in-the-rain paper • Rulers (in centimeters) • Scrub brush • SOPs, CD and field guide(s) • Specimen containers and labels • Spherical densitometer (2) • Thermometer • Wire flags • Water testing equipment |
|---|---|

Preparations for the Field Season Standard Operating Procedure

- Hand lens (10x)
- Headlamps (2)
- Zip-lock bags

Training Observers Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.2	01/05/2007	Grant		Update	1.3

1. Purpose

The most important data collected on each survey visit are the amphibians which are present at a site. Because these data are used to estimate the occupancy of available habitats, it is absolutely essential that observers are trained to correctly identify amphibians in all life stages. A false identification is a serious violation of the assumptions used in the analyses of data for occupancy estimation. The purpose of this document is to help ensure that field crews are properly trained.

2. Scope and Applicability

This document provides training guidelines for the NCRN Amphibian Monitoring Program.

3. Reference Documents

- Elliott, L. 2004. The calls of frogs and toads. Stackpole Books, PA.
- Parmelee, J.R., M.G. Knutson, and J.E. Lyon. 2002. A field guide to amphibian larvae and eggs of Minnesota, Wisconsin and Iowa. U.S. Geological Survey, Biological Resources Division, Information and Technology Report USGS/BRD/ITR-2002-0004, Washington, DC., iv + 38 pp.

4. Procedures and General Requirements

4.1 Identification of Amphibians by Sight

- 4.1.1 Observers should review reference materials (see SOP #1, section 3 and section 4.3.5), and be familiar with identifying characteristics of all species and their life stages found in National Capital Region Parks.
- 4.1.2 Eggs, tadpoles and larvae are the most difficult life stages for species identification. A useful guide to some species of amphibian larvae is Parmelee et al. (2002). An online identification key (<http://www.pwrc.usgs.gov/tadpole/>) for tadpoles can be useful for troublesome identification, though many characters may require an anesthetized specimen and a hand lens or dissecting microscope (i.e. labial tooth row formula).

Training Observers Standard Operating Procedure

- 4.1.3 If a visual species identification cannot be confirmed in the field, several digital photographs should be taken of dorsal, ventral and side views and any distinguishing features of the amphibian in natural positions (e.g., submerged in clear water if an aquatic life stage to show gills), and identification should be confirmed immediately upon returning from the field. However, digital photographs are never a suitable surrogate for identification in the field.

4.2 Identification of Adult Anurans by Call

- 4.2.1 Observers should practice identification of adult anurans by their calls using Elliott (2004).
- 4.2.2 Observers can use the frog call lookup section of the USGS frog quiz (see SOP #1) to determine the list of species expected in the region, and should be able to identify the calls of these species.
- 4.2.3 All observers, whether or not they will participate in after-sundown call surveys, must take the USGS frog quiz and score at least 70% or better on the quiz for the National Capital Region.
- 4.2.4 Observers should practice estimating chorus levels (e.g., 1 = individuals can be counted, calls do not overlap, 2 = calls overlap, but individuals are distinguishable, 3 = full chorus, calls continuous and overlapping, can not distinguish individuals.)
- 4.2.5 Observers may carry a tape recorder to record calls in the field and use them to compare to training materials (e.g., Elliot [2004] or USGS frog quiz) immediately after returning from the field, in order to assist in identification. Field identification of calls is critical, as recorded calls cannot offer the same sound quality and subtleties heard in the field.

4.3 Sampling Techniques: Amphibians and Habitat Protocols

- 4.3.1 Observers should review the SOPs for Lentic (SOP # 5), Lotic (SOP # 6), and Terrestrial (SOP # 7) habitat surveys and habitat data collection.
- 4.3.2 Field crew leaders should provide training to new observers in field sampling techniques at least one day prior to the field season for lentic, lotic and terrestrial sampling at a site which is not included in the monitoring program sampling frame.
- 4.3.3 Previously trained observers should always accompany new observers on the first day of sampling for each habitat type to ensure proper understanding of sampling techniques.

Establishing and Marking Sampling Plots Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.1 (08/05)	01/06	S. Mattfeldt	Updated on reflect completion of pilot study	Pilot data	1.2 (02/06)
1.2	12/2006	S. Mattfeldt	Updated based on second year of data collection	New Data	1.3
1.3		Grant		Update	1.4

1. Purpose

This document outlines the procedures to be used when establishing an amphibian monitoring plot.

2. Scope and Applicability

3. Reference Documents

- Bailey, L.L., T.R. Simons, and K.H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14: 692-702.
- Barbour, R.W., J.W. Hardin, J.P. Schafer, and M.J. Harvey. 1969. Home range, movements and activity of the dusky salamander, *Desmognathus fuscus*. *Copeia* 1969: 293-297.
- Forman, R.T.T. 2000. Estimate of the area affected ecologically by the road system in the United States. *Conservation Biology* 14: 31-35.
- Grant, B.W., A.D. Tucker, J.E. Lovich, A.M. Mills P.M. Dixon, and J.W. Gibbons. 1992. The use of coverboards in estimating patterns of reptile and amphibian biodiversity. Pp. 379-403 in: McCullough DR and Barrett RH, eds. *Wildlife 2001: Populations*. Elsevier Applied Science, New York, NY, USA.
- Grant, E.H.C., R.E. Jung, J.D. Nichols, and J.E. Hines. 2005. Double-observer approach to estimating egg mass abundance of vernal pool breeding amphibians. *Wetland Ecology and Management* 13: 305-320.
- Jung, R.E., J.A. Royle, J.R. Sauer, C. Addison, R.D. Rau, J.L. Shirk, and J.C. Whissel. 2005. Estimation of stream salamander (Plethodontidae, Desmognathinae and Plethodontinae) populations in Shenandoah National Park, Virginia, USA. *Alytes* 22: 72-84.

Establishing and Marking Sampling Plots

Standard Operating Procedure

- MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S. Droege, J.A. Royle, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83: 2248-2255.
- MacKenzie, D.I., J.D. Nichols, J.E. Hines, M.G. Knutson, and A.B. Franklin. 2003. Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology* 84: 2200-2207.
- MacKenzie D.I., L.A. Bailey, and J.D. Nichols. 2004. Investigating species co-occurrence patterns when species are detected imperfectly. *Journal of Animal Ecology* 73: 546-555.
- MacKenzie, D.I. and J.A. Royle. 2005. Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology* 42: 1105-1114.
- Mader, H.J. 1984. Animal habitat isolation by roads and agricultural fields. *Biological Conservation* 29: 81-96.
- Semlitsch, R.D. and J.R. Bodie. 2003. Biological Criteria for Buffer Zones around Wetlands and Riparian Habitats for Amphibians and Reptiles. *Conservation Biology* 17:1219-1228.
- Southerland, M.T., R.E. Jung, D.P. Baxter, I.C. Chellman, G Mercurio, and J.H. Vølstad. 2004. Stream salamanders as indicators of stream quality in Maryland, USA. *Applied Herpetology* 2: 23-46.
- Tiebout, H.M. 2003. An inventory of the herpetofauna of Valley Forge National Historical Park. National Park Service. Technical Report NPS/PHSO/NRTR-03/088.
- Weir L.A., J.A. Royle, P. Nanjappa, R.E. Jung, and W.G. Hildebrand. Submitted. Modeling anuran detection and site occupancy on North American Amphibian Monitoring Program (NAAMP) routes in Maryland. *Journal of Herpetology*.
- Willson, J.D. and M.E. Dorcas. 2003. Effects of habitat disturbance on stream salamanders: Implications for buffer zones and watershed management. *Conservation Biology* 17: 763-771.

4. Procedures and General Requirements

The location of sampling sites will vary based on habitat type and stratification with respect to urbanization-associated variables (e.g., categorical distance to roads and/or trails). A systematic grid of points spaced at 150 x 150 meters has been established within each park boundary. Points are then assigned to a particular strata based on their strata location, and an equal random sample of points is chosen within each strata. This design allows inference to be made across the strata with respect to park-specific occupancy parameters, while allowing tests of hypotheses related to the stratification variables. We suggest that parks stratify the terrestrial and lentic habitats based on the distance to a paved road, with sites less than 100 m considered within the “road effect zone” (Mader 1984, Forman 2000). We recommend that parks stratify the lotic habitats based on these same strata, but the location of a road crossing, the presence or absence of storm water

Establishing and Marking Sampling Plots Standard Operating Procedure

inputs into the stream, and/or the percent coverage of forest or impervious surface in the stream watershed should also be included as potentially important covariates (Southerland et al. 2004, Willson and Dorcas 2003).

From previous studies, we can expect that some amphibians will have consistently high detection probabilities. Detection probability is the probability that a member of N appears in C . The relationship between counts and the actual population size can be expressed as $E(C) = Np$, where N is the true or actual population size, C is the count collected during the survey, E denotes statistical expectation, and p is the detection probability. For example, the probability of detecting an individual egg mass is > 0.90 for *Ambystoma maculatum* and *Rana sylvatica* egg masses (Grant et al. 2005).

Using the PAO approach, where occupancy (not abundance) is the state variable of interest, detection probability refers to the probability of detecting at least 1 individual of the target species at occupied sites. Previous amphibian occupancy studies have suggested that detection probability varies among species and detection methods: for example, *Pseudacris crucifer* exhibits $p = 0.89$ in calling surveys (Weir et al. 2005), while other species have consistently low detection probabilities [e.g. *Desmognathus fuscus* $p = 0.40$ under artificial cover objects (Bailey et al. 2004), *Bufo americanus* $p = 0.19$ in calling surveys (Weir et al. 2005)]. The number and frequency of visits to survey sites will depend on the estimated or anticipated probability of occupancy and detection for each species (MacKenzie and Royle 2005), with fewer visits and sites required to estimate species with consistently high detection probabilities (e.g., *A. maculatum* occupancy via egg mass detections) and more visits at a greater number of sites for species with consistently low ones (e.g., *B. americanus* via calling surveys). Integration of several techniques (where possible) at a survey site will increase the likelihood of detecting a species. For each habitat-specific protocol (see SOPs #5, 6, and 7), several techniques are being implemented. For example, in SOP #5, we are using egg mass counts, calling surveys, visual encounter surveys and dip netting to determine amphibian species presence.

For all surveys, the guidelines below for establishing and marking sampling sites will be followed:

- 4.1 During the survey, observers will navigate to all terrestrial, lentic and lotic survey sites using a handheld GPS unit. At a later date, a Trimble or mapping grade GPS unit can be used to provide more precise (sub-meter) survey locations.
- 4.2 The location of lentic and lotic sites will depend on the number and distribution of available wetland (lentic) and stream (lotic) habitats. Prior to identifying survey locations for these habitats, a map of small ($< \frac{1}{4}$ ha) wetland sites and small-order (seeps, 1st and 2nd order) streams should be provided by park personnel. These habitats are often not mapped on 7.5 minute USGS topographic quads, yet are important habitats for amphibians. Special effort should be made to identify and GPS the locations of vernal pool wetlands, which are a unique type of wetland which typically dries completely by the end of the summer, excluding the establishment of predatory fish populations. GPS locations can be used to create a map which will make up the sampling frame for the park.

Establishing and Marking Sampling Plots Standard Operating Procedure

Once a map of the wetlands and streams has been assembled, these can be stratified on urbanization variables. Lentic sites should be stratified based on distance to a road or trail as the urbanization variable. These sites will be surveyed following SOP # 5 (Habitat-specific survey protocol: Lentic). Lotic sites should be stratified based on the location of a road or trail crossing (survey sites with a crossing above the sampling point should be considered impacted) and covariates such as the percent of forest or impervious surface in the stream watershed should be collected. These sites will be surveyed following SOP # 6 (Habitat-specific survey protocol: Lotic).

- 4.3 Lentic sites will be sampled randomly within each strata, with equal effort among the strata.
- 4.4 Lotic sites will be sampled from the headwaters and spaced 100 m apart along the length of a stream reach. Stream reaches will be chosen at random from the universe of known headwater streams, and the number of sample sites will be informed after analysis of the pilot data. Paired transects (15 m length), located on opposite banks, will survey 1 meter in the water and 2 meters on the bank. This sampling design will target both aquatic larvae and terrestrial adults associated with stream habitats.
- 4.5 Terrestrial survey points will be based on a grid, spaced at 150 m intervals across the park area. Points will be assigned to one of two stratification classes, determined by their proximity to roads or trails as the urbanization stratification variable. Independence is assumed between sampling points in one survey period based on terrestrial movements of individuals and published home range sizes within one activity season (for example, the maximum home range for *D. fuscus* is 114.5m²; Barbour et al. 1969). A subset of sampling points for actual sampling will then be randomly selected such that approximately equal numbers of each stratification class are represented.
- 4.6 Terrestrial survey sites will consist of three sets (stations) of coverboards, where each set consists of two 26x26 cm boards and three 13x26 cm boards, cut from 1.9 cm (¾ inch) pine sheathing. Artificial cover objects can increase the capture rate of terrestrial salamanders (Grant et al. 1992; Tiebout 2003). These artificial cover objects will be located along a transect, spaced 10 m apart, and usually oriented North unless features of the landscape preclude this placement scheme. The first station at each site is marked with duct tape on a tree branch at approximately eye level. The artificial cover object transect will parallel a 3x20 m natural cover transect, in which an observer turns all natural cover objects within the 3 x 20 m area to find amphibians.
- 4.7 The number of sampling sites has been determined based on pilot data. Advances in modeling and simulation allow estimation of the number of visits and number of sites needed to gain an acceptable level of precision for occupancy estimates (e.g. MacKenzie et al. 2002, 2003, 2004, MacKenzie and Royle 2005), using repeated survey data and knowledge of p (detection probability) and ψ (occupancy) for individual species. The pilot study allowed estimation of these parameters for use in simulation exercises allowing the estimation of number of sites and visits needed. See the Habitat-specific protocols for sampling designs based on the pilot study (SOP# 05-07).

Establishing and Marking Sampling Plots Standard Operating Procedure

- 4.8 The site selection outlined here sets out a framework for collection of data with respect to existing patterns of urbanization. We do not anticipate near future road or trail construction in the parks, though we expect increases in use of these infrastructural features.

Handling Live Amphibians Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

1. Purpose

While conducting Lentic (Standard Operating Procedure [SOP] #5), Lotic (SOP #6), and Terrestrial (SOP #7) habitat surveys, observers will often have to handle amphibians for identification and/or detailed examination. This SOP details the guidelines in place for the Amphibian Research and Monitoring Initiative (ARMI), as assembled by the USGS National Wildlife Health Center (NWHC).

2. Scope and Applicability

A summary of the procedures is provided below (but see specific measures including biosecurity practices for handling diseased or dead animals or eggs in SOP #12). Sites with known or suspected disease problems should be treated with caution, to protect the health of individual animals.

3. Reference Documents

- 3.1 ARMI SOP # 100: "Restraint & Handling of Live Amphibians," USGS NWHC (http://www.nwhc.usgs.gov/research/amph_dc/amph_sop.html) revised 16 February 2001.SOP – Water Site Selection

4. Procedures and General Requirements

- 4.1 Anurans: Adult frogs and toads will attempt to escape capture, primarily by kicking and attempting to jump or hop away. This type of kicking can cause dislocations of leg or hip joints and could break or fracture the back. To prevent these types of injuries, hold adult frogs and toads by grasping them firmly around the waist with their legs fully extended.
- 4.2 Caudates: Adult salamanders can drop (autotomize) their tails, or could injure their backs trying to twist free of capture. To prevent this, salamanders should never be grasped by their tails but should be grasped mid-body between fore- and hindlimbs.
- 4.3 Larvae: Tadpoles and salamander larvae have very thin skin that is easily damaged. In particular, salamander larvae have delicate external gills that are extremely fragile. To avoid tearing, puncturing, or damaging skin or external delicate tissues, never directly handle tadpoles and larvae and do not keep tadpoles or larvae out of water. Instead, use a

Handling Live Amphibians Standard Operating Procedure

small net and/or coax larvae into a clear plastic bag or container with enough water to cover the entire larva (including gills in salamander larvae). In transferring tadpoles and larvae from a dipnet or small net to a container or plastic bag with water, do not pick them up with your hands but invert the dip net and dip the larva into the water so that it can swim into the water freely. Examine larvae and tadpoles through the bag or container, and release gently.

Habitat-specific Protocol: Lentic Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.1	Winter 2006	S. Mattfeldt	Updated based on pilot data	Pilot data	1.2
1.2	12/2006	S. Mattfeldt	Updated based on second year of data collection	New data and updated analysis	1.3

1. Purpose

Lentic habitats are characterized by still water. All anurans and some caudates (Appendix 1) rely on a variety of lentic sites for breeding as well as foraging or hibernation. Lentic sites include vernal pools, permanent and semi-permanent ponds, marshes, forested swamps, bogs, tidal-influenced wetlands, wet meadows, and other areas which have a significant amount of surface water for at least a portion of the year.

See SOP #3: “Establishing and marking sample plots” for survey site selection protocols.

2. Scope and Applicability

This SOP provides instructions for sampling lentic habitats associated with the amphibian monitoring program in the National Capital Region parks. See descriptions of data fields (below) for lentic sampling. Habitat data and sampling procedures are described below the amphibian sampling protocols.

3. Reference Documents

- 3.1 Altig, R. 1970. A key to the tadpoles of the continental United States and Canada. *Herpetologica* 26:180-207.
- 3.2 Arntzen, J.W. 2002. Testing for equal catchability of *Titurus* newts by dip netting. *Journal of Herpetology* 36:272-276.
- 3.3 Cowardin, L.M., V. Carter, F.C. Golet, and E.T. LaRoe. 1979. Classification of wetlands and deepwater habitats of the United States. U.S. Department of the Interior, Fish and Wildlife Service, Washington, D.C. 131 pp.
- 3.4 Crisafulli, C.M. 1997. A habitat-based method for monitoring pond-breeding amphibians. Chapter 6, pp. 83-111. In Olson, D.H., W.P. Leonard, and R.B. Bury (eds.), *Sampling Amphibians in Lentic Habitats, Northwest Fauna 4*. Society for Northwestern Vertebrate Biology, Olympia, Washington.

Habitat-specific Protocol: Lentic Standard Operating Procedure

- 3.5 Crow, G.E. and C.B. Hellquist. 2000. Aquatic and wetland plants of northeastern North America: a revised and enlarged edition of Norman C. Fassett's A manual of aquatic plants. Volume 1: Pteridophytes, gymnosperms, and angiosperms: dicotyledons. The University of Wisconsin Press, Madison, WI.
- 3.6 Gleason, H.A. and A. Cronquist. 1991. Manual of the vascular plants of the United States and adjacent Canada. Ed. 2. Bronx.
- 3.7 Heyer, R.W., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster (eds). 1994. Measuring and Monitoring Biological Diversity: Standard methods for amphibians. Smithsonian Institution Press, Washington DC.
- 3.8 Holmgren, N.H. 1998. Illustrated Companion to Gleason and Cronquist's Manual: Illustrations of the Vascular Plants of Northeastern United States and Adjacent Canada. New York.
- 3.9 Knutson, M.G., J.E. Lyon, and J.R. Parmalee. 2002. Resources for monitoring pond-breeding amphibians in the northcentral USA. *In* Farm ponds as critical habitats for native amphibians: Final Report. Submitted to the Legislative Commission on Minnesota Resources by U.S. Geological Survey Upper Midwest Environmental Sciences Center, La Crosse, Wisconsin, USA, June 2002. 27 pp.
(http://www.umesc.usgs.gov/terrestrial/amphibians/mknutson_5003869.html)
- 3.10 MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S. Droege, J.A. Royle, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83: 2248-2255.
- 3.11 MacKenzie, D.I., J.D. Nichols, J.E. Hines, M.G. Knutson, and A.B. Franklin. 2003. Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology* 84: 2200-2207.
- 3.12 MacKenzie, D.I., L.A. Bailey, and J.D. Nichols. 2004. Investigating species co-occurrence patterns when species are detected imperfectly. *Journal of Animal Ecology* 73: 546-555.
- 3.13 MacKenzie, D.I. and J.A. Royle. 2005. Designing occupancy studies: general advice and allocating survey effort. *Journal of Applied Ecology* 42: 1105-1114.
- 3.14 Parmelee, J.R., M.G. Knutson, and J.E. Lyon. 2002. A field guide to amphibian larvae and eggs of Minnesota, Wisconsin and Iowa. U.S. Geological Survey, Biological Resources Division, Information and Technology Report USGS/BRD/ITR-2002-0004, Washington, D.C., iv + 38 pp.
- 3.15 Stranko, S., L. Erb, J. Kilian, L. Phu. 2005. A provisional key to the reptiles and amphibians of Maryland. Maryland Department of Natural Resources, Annapolis, MD.

Habitat-specific Protocol: Lentic Standard Operating Procedure

- 3.16 Thoms, C., C.C. Corkran, and D.H. Olson. 1997. Basic amphibian survey for inventory and monitoring in lentic habitats. Chapter 3, pp. 35-46. *In* Olson, D.H., W.P. Leonard, and R.B. Bury, Sampling Amphibians in Lentic Habitats, Northwest Fauna 4. Society for Northwestern Vertebrate Biology, Olympia, Washington
- 3.17 Wilson, J.J. and T.J. Maret. 2002. A comparison of two methods for estimating the abundance of amphibians in aquatic habitats. *Herpetological Review* 33: 108-110.

4. Procedures and General Requirements

4.1 Pilot Study

A pilot study was conducted in 2005 (March 07–June 16) at the Chesapeake and Ohio Canal National Historic Park, employing three sampling techniques: visual encounter surveys (VES), dipnet surveys, and calling surveys. The purpose of the pilot study was to determine the number of sampling sites and visits needed to optimize long-term amphibian monitoring in these National Park areas. Occupancy modeling of the pilot study data gives initial estimates of p (detection probability) and ψ (occupancy) for each individual species; using this information with simulation results allow approximation of the optimal number of visits and number of sites needed to gain an acceptable level of precision for occupancy estimates (MacKenzie and Royle 2005).

We sampled 35 wetland sites in the Chesapeake and Ohio Canal NHP. Each site was visited four times by two observers, conducting visual encounter surveys during the first three visits and a dip-net survey during the last visit. Fourteen call survey points were visited a minimum of 22 times by two observers. During the visual encounter and dipnet surveys we observed 12 amphibian species: *Ambystoma maculatum*, *A. opacum*, *Bufo americanus*, *B. fowleri*, *Hemidactylium scutatum*, *Notophthalmus viridescens*, *Pseudacris crucifer*, *Rana catesbeiana*, *R. clamitans*, *R. palustris*, *R. sphenoccephala*, *R. sylvatica*. During the call surveys we heard 9 species: *B. americanus*, *B. fowleri*, *Hyla chrysoscelis*, *P. crucifer*, *R. catesbeiana*, *R. clamitans*, *R. palustris*, *R. sphenoccephala*, *R. sylvatica*

R. sylvatica, *A. maculatum* and *P. crucifer* were the first species to breed in the spring allowing for detection of adults and egg masses early in the season. *R. palustris*, *R. sphenoccephala*, and *H. scutatum* were the next species observed breeding, followed by *B. americanus* and *B. fowleri*, and then *R. clamitans* and *R. catesbeiana*. Over-wintering *R. catesbeiana* tadpoles, *R. clamitans* tadpoles and *A. opacum* larvae were detected as soon as it was warm. In this study, adult *N. viridescens*, *R. clamitans*, *R. catesbeiana*, *R. palustris*, and *H. scutatum* were detected earlier than *B. americanus* and *B. fowleri*. The seasonal phenology of lentic amphibians is useful in planning sample designs that target species and increase efficiency (Figure 5.1).

To determine the number of surveys and sites that would provide precision in estimates of occupancy we analyzed species by age class (i.e., *R. sylvatica* tadpoles, *R. sylvatica* adults) using the program Presence. We included age class since different age classes are available for detection at different times of the year, may have different detection probabilities, and may not have the same occupancy probability.

Habitat-specific Protocol: Lentic Standard Operating Procedure

H. scutatum and *R. sphenoccephala* were rarely observed and did not have sufficient detections for formal occupancy analysis. *B. americanus* and *B. fowleri* tadpoles are very similar in appearance and were treated as the *Bufo* spp. tadpole complex. Furthermore, some age classes were rarely observed for some species. The species by age combinations that were analyzed for probability of detection and site occupancy are *A. maculatum* larvae and egg masses, *A. opacum* larvae; *Bufo* spp. tadpole complex, *N. viridescens* adults, *P. crucifer* tadpole, *R. catesbeiana* adults and tadpoles, *R. clamitans* adults and tadpoles, *R. palustris* adults and *R. sylvatica* tadpoles and egg masses.

Among the species and age combinations analyzed for probability of detection and site occupancy, the probability of detection varied among species ranging from 0.1847 to 0.9333; the proportion of sites occupied also varied among species ranging from 0.0871 to 0.5661 (Table 5.1). We looked at several urbanization covariates that may contribute to variation in site occupancy. These covariates were the proximity of the wetland to a road, pH and conductivity. The proximity of the wetland to a road did not seem important in the estimation of occupancy for any of the species, while pH and conductivity were important for several of the species (Table 5.1).

The species observed during the call surveys generally had a low probability of detection and high site occupancy (Table 5.2). Proximity of the call survey site to a road did not seem important in the estimation of occupancy for any of the species.

Based on the pilot study, to achieve optimal precision and efficiency, we recommend 4 visits by two observers to 35 wetland sites (Table 5.3). Visual encounter surveys should be conducted during the first two visits and dipnet surveys should be conducted during the second two visits. In addition, we recommend 6 visits by two observers to the call surveys sites (Table 5.3). Based on the seasonal phenology of lentic amphibians the first VES should be conducted mid-March-April, the second VES should be conducted from mid-April through late-April, the first dipnet survey should be conducted from mid-May through early June, and the final dipnet survey should be conducted in June (Figure 5.1). The second dipnet sample is beyond the season of the pilot study, and is for the purpose of increasing detection of *Hyla* spp. Call surveys should target *B. americanus* and *B. fowleri*, which are not distinguishable as tadpoles during the dipnet surveys. The call surveys should therefore occur between mid-April and June.

4.2 Multiple Season Study

Pilot Data

In 2006 we conducted the second year of lentic amphibian surveys at 33 wetlands in the Chesapeake and Ohio Canal NHP. We eliminated 2 of the 35 sites visited in 2005 because it was determined that these wetlands were truly not isolated, nor were they likely to ever be isolated. Weather patterns were different among the 2 years: while overall rainfall was similar over the entire survey season (March-June rainfall: 41 cm and 49 cm in 2005 and 2006, respectively), the timing of the rainfall was quite different. In 2006, no rain was reported early in the season, followed by little precipitation in April and May (8 cm and 6 cm, respectively), followed by a single, large storm in June (35 cm). In contrast, in 2005 there was 11 cm rainfall in March, followed by more evenly distributed rainfall readings of 11, 12, 7 cm in April, May and June,

Habitat-specific Protocol: Lentic Standard Operating Procedure

respectively. The rainfall totals during the breeding season for these 2 years were greater than the 10 year average for rain for the period of the survey season (March-June 1997-2006, average = 36 cm). The lack of rain in March 2006 is noteworthy, as March is vital part of the breeding season for several lentic amphibians (*A. maculatum*, *R. sylvatica*, *P. crucifer*, *R. palustris* and *R. sphenoccephala*; Figure 5.1). Also in 2006 a greater number of wetlands dried before the end of the survey season (2006 n=14 vs. 2005 n=10). Finally, the substantial rain in June 2006 caused the river to flood two of the study wetlands possibly washing out amphibian eggs and larvae and introducing fish and other aquatic predators.

During 2006 two visual encounter surveys and two dipnet surveys were performed: the same 13 amphibian species were observed as in 2005: *A. maculatum*, *A. opacum*, *B. americanus*, *B. fowleri*, *Hemidactylium scutatum*, *Hyla spp.*, *N. viridescens*, *P. crucifer*, *R. catesbeiana*, *R. clamitans*, *R. palustris*, *R. sphenoccephala*, and *R. sylvatica*. *A. opacum*, *Hemidactylium scutatum*, *Hyla spp.*, and *R. sphenoccephala* were rarely observed and did not have sufficient detections for formal occupancy and vital rate analysis. Amphibians were not observed at 9 of the 33 lentic sites in either year; most of these sites dried (n=8) during at least once survey season. All wetlands that dried at least once (n=14) had low species richness, and several species were never observed at these sites (*N. viridescens*, *P. crucifer*, and *R. palustris*).

Methods and Hypothesis

We used multi-season models to estimate year-specific occupancy probabilities for each species, as well as colonization and extinction probabilities between years (MacKenzie et al. 2006). In this analysis we utilized all available information, including detections of all life-history stages. We acknowledge that detection probability may not be the same for a species among its life history phases, and thus maintained detection covariates (discussed above) that we expected to co-vary with the amphibian's life history stage(s) (e.g. water temperature). *B. americanus* and *B. fowleri* tadpoles are very similar in appearance and were treated as the *Bufo spp.* complex. Building on results from the analysis of the 2005 pilot data, we included urbanization variables (minimum pH, maximum conductivity, distance to the towpath) as well as 5 other habitat variables (maximum canopy cover, flood potential, maximum percent aquatic vegetation, wetland drying, minimum wetland surface area) when modeling initial site occupancy. These urbanization variables influenced initial (2005) occupancy estimates for several species. (see Table 5.5 for details). Moreover, we tested whether urbanization variables (pH and conductivity), as well as 3 other habitat variables (flood potential, minimum wetland surface area, wetland drying) were important in estimates of local extinction and/or colonization. *A priori* we predicted that:

1. Wetlands with lower pH levels might have a higher extinction probability because these wetlands may not consistently recruit juvenile amphibians or may recruit fewer/ smaller juvenile amphibians due to the lethal and sub-lethal effects of low pH on amphibian embryos and larvae (Freda et al. 1991, Sadinski and Dunson 1992).
2. Wetlands with higher conductivity might have a higher extinction probability as several studies (Turtle 2000; Glooschenko et al. 1992) have shown a negative relationship between conductivity and amphibian presence or embryonic survivorship.

Habitat-specific Protocol: Lentic Standard Operating Procedure

3. Wetlands that are more likely to be flooded by the Potomac River might have a higher extinction probability because flooding may wash out amphibian eggs and larvae, and potentially introduce aquatic predators (Dorcas et al. 2006). We define ‘flood potential’ as a continuous covariate, calculated as the product of distance (m) $\times$ slope ($^{\circ}$) from river.
4. Larger wetlands might have a higher colonization probability and lower extinction probability because they are larger targets for dispersing amphibians and they may be more stable habitats (Seale 1982, Armstrong 2005).
5. Wetlands that dried during the survey season (denoted as ‘wetland drying’ and treated as a categorical variable: 0= dried, 1= non-drying) may have higher extinction probabilities because they do not consistently recruit juvenile amphibians each year, and thus may be considered less suitable amphibian breeding habitat (Shoop 1974, Skelly 1999, Babbitt et al. 2003).

Results

Estimates of occupancy did not vary greatly between 2005 and 2006 (Figure 5.2). While the estimates of the occupancy were relatively constant, the occupancy state did change among sites. In other words, some sites were colonized and others failed to support breeding and/or foraging activity (i.e. went locally “extinct”), resulting in non-zero estimates of extinction and colonization probabilities (Table 5.4). None of the *a priori* factors were important in estimates of local colonization for any species. The estimated likelihood of flooding by the Potomac River was important in estimates of extinction for several species (Table 5.5). pH was important in estimates of extinction for *R. catesbeiana*, suggesting that sites with lower pH levels were more likely to go locally extinct. Wetland drying was important in estimates of extinction for *Bufo* spp.; wetlands that were prone to drying had a higher extinction probability.

Habitat-specific Protocol: Lentic Standard Operating Procedure

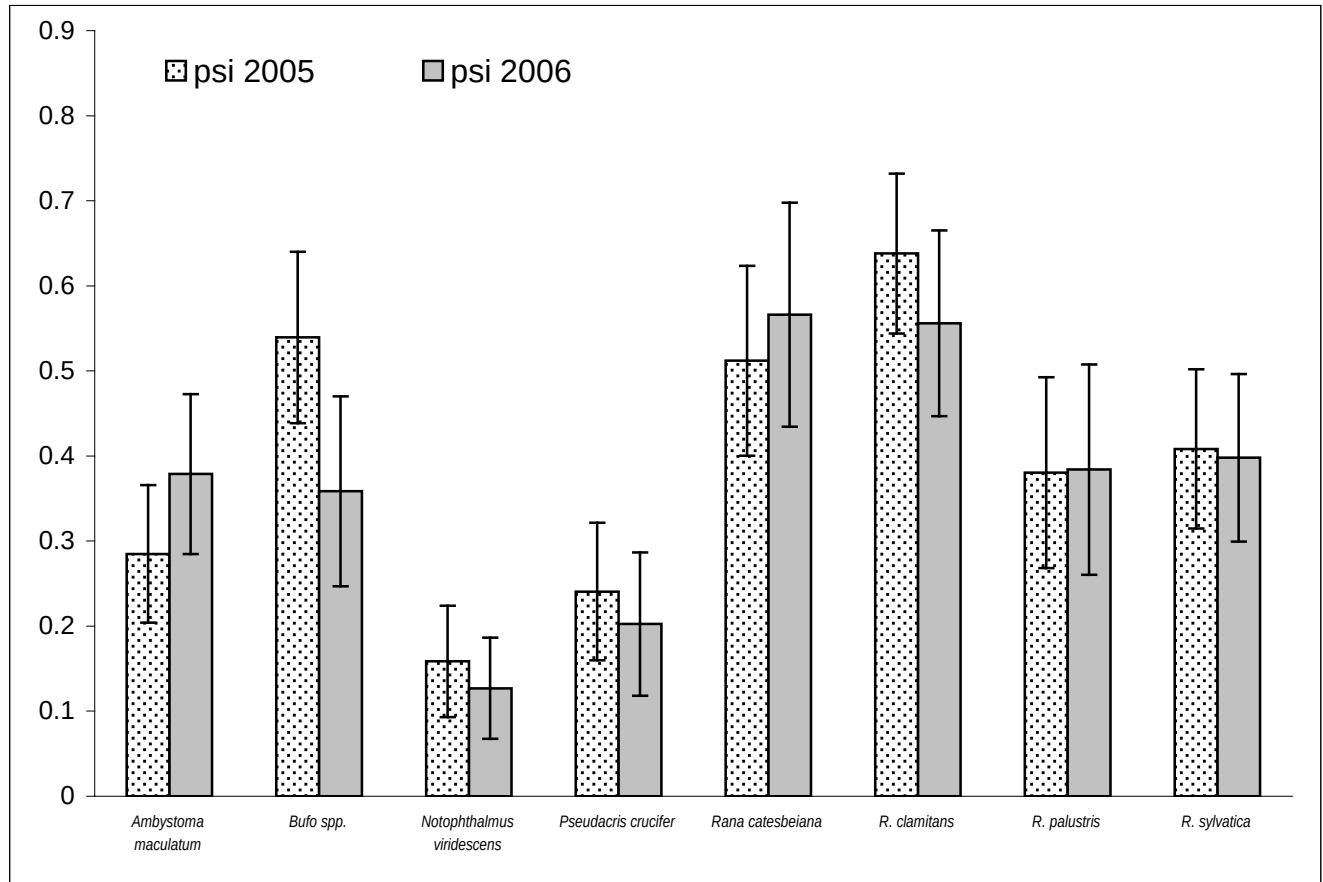


Figure 5.2. Change in occupancy (psi) of lentic sites based on data collected during visual encounter and dipnet surveys at the Chesapeake and Ohio NHP, pilot study 2005-2006.

Habitat-specific Protocol: Lentic Standard Operating Procedure

Table 5.4. Parameter estimates based on data collected during visual encounter and dipnet surveys at the Chesapeake and Ohio NHP, pilot study 2005- 2006. For each species there are estimates of probabilities of occupancy in 2005 (Ψ_{2005}) and 2006 (Ψ_{2006}), detection (p), colonization (γ), and extinction (ϵ) based on a model that assumed constant rate and detection parameters. Numbers in parentheses are the associated standard errors.

Species	Ψ_{2005} (.)	Ψ_{2006} (.)	p (.)	γ (.)	ϵ (.)
<i>Ambystoma maculatum</i>	0.28 (0.08)	0.38 (0.09)	0.27 (0.17)	0.13 (0.15)	0
<i>Bufo spp.</i>	0.54 (0.10)	0.36 (0.11)	0.46 (0.05)	0.08 (0.10)	0.41 (0.17)
<i>Notophthalmus viridescens</i>	0.16 (0.07)	0.13 (0.06)	0.54 (0.06)	0	0.20 (0.18)
<i>Pseudacris crucifer</i>	0.24 (0.08)	0.20 (0.08)	0.37 (0.05)	0.13 (0.09)	0.56 (0.19)
<i>Rana catesbeiana</i>	0.51 (0.11)	0.57 (0.13)	0.25 (0.04)	0.41 (0.20)	0.28 (0.17)
<i>R. clamitans</i>	0.64 (0.09)	0.56 (0.11)	0.49 (0.03)	0.21 (0.20)	0.25 (0.10)
<i>R. palustris</i>	0.38 (0.11)	0.38 (0.12)	0.21 (0.04)	0.14 (0.14)	0.22 (0.20)
<i>R. sylvatica</i>	0.41 (0.09)	0.40 (0.10)	0.40 (0.04)	0.08 (0.09)	0.14 (0.11)

Long-term Sampling Design Options

One objective of long-term monitoring programs is to detect changes or trends in occupancy. For example, a common monitoring ‘goal’ is often stated as detecting a level of decline in a set time frame (i.e., to detect a 50% decline in site occupancy over five years). We used the program GENPRES, which allows investigators to explore the trade-offs between different survey designs, under a specified monitoring objective. Using data from the two years of lentic surveys at Chesapeake and Ohio Canal NHP, we explored the ability of several different survey designs (varying the number of sites and frequency of visits) to detect change in amphibian populations within this biological system (see scenarios below). Four different survey designs were explored, each having different levels of survey effort: 35 sites visited every year; 70 sites visited every year; 35 sites visited every other year; 70 sites visited every other year. Program GENPRES is currently rather limited in the total number of sampling occasions in an analysis, thus we have to condense the number of sampling occasion within each season to 2. We compensate for this shortcoming, and maintain loyal to the current level of sampling by redefining a detection probability, p^* , as the probability of detecting the species at least once in 4 person-surveys (2 observers x 2 visits). Thus we can represent our current 8 surveys per year effort adequately despite the limitations currently imposed by program GENPRES.

One of the measurable objectives of this project is to provide management information on the potential impacts of urbanization and how urbanization may influence site occupancy and vital rates. Thus we chose to explore the ability of different survey designs to detect a change in two species that are likely to be sensitive to urbanization: *A. maculatum* and *N. viridescens* (Rubbo and Kiesecker 2005). While other lentic amphibian species may also be sensitive to urbanization (*A. opacum*, *R. palustris*, and *R. sylvatica*), *A. maculatum* and *N. viridescens* have different initial occupancy levels in this system (0.33 and 0.15, respectively), contrasting adult habitat preferences (forest vs. pond) and different breeding habitat requirements (intermediate vs. long hydroperiod) (Paton and Crouch 2002; Rubbo and Kiesecker 2005). Thus we chose these

Habitat-specific Protocol: Lentic Standard Operating Procedure

species because they are likely to exhibit a negative relationship to urbanization and may be at different stages of possible ‘decline’.

To determine whether sampling designs affected the ability to detect a change in amphibian populations we used likelihood ratio test to approximate power (Burnham et al. 1987 p.214-7). In each case the “true” generating model was considered the alternative hypothesis (i.e. time specific occupancy estimates), and the null hypothesis was represented by models representing no change in occupancy or its related vital rates (e.g. extinction probabilities). We approximated test power (assuming $\alpha=0.05$ and $\alpha=0.10$) by using the resulting chi-square statistic as the noncentrality parameter, λ , and calculating power from a non-central chi-squared distribution (Burnham et al. 1987). It is important to note that this methodology is only an approximation of power that we utilize to qualify power as poor, medium or good, and rank different sampling design options.

Scenario 1: 50% decline in occupancy assuming time-constant rate parameters (γ_t, ϵ_t)

Using estimates from the pilot data, we set *N. viridescens* parameter values at: initial occupancy $\Psi_1=0.15$, colonization $\gamma=0.05$, $p=0.96$. Extinction probability had to be high, $\epsilon=0.60$, to result in a 50% decline in occupancy after 5 years. Applying these parameter values, occupancy in year five would be $\Psi_5=0.078$. The power to detect time-specific occupancy estimates was low (Table 5.6), but this was expected since the number of initially occupied sites was small (expected $n=5$) and a 50% decline in occupied sites (expected $n=2-3$) would be difficult to detect. If more sites are surveyed, assuming the same occupancy probabilities apply, then more sites are truly occupied and available for detection, thus a ‘50% decline’ can be more easily detected (Table 5.6). The power to detect the change in occupancy decreased slightly when sites were visited in alternate years (Table 5.6). The decrease in power is small because the colonization and extinction rates were assumed to be constant and initial occupancy probabilities are low. In this type of scenario the power approximation would suggest that the ability to detect change is poor with realistic sample sizes.

Habitat-specific Protocol: Lentic Standard Operating Procedure

Table 5.6. Likelihood ratio test for the null hypothesis (H_0 : no change in occupancy) vs. the alternative hypothesis (H_a) of time-specific occupancy estimates for *Notophthalmus viridescens*. Power is approximated using the resulting chi-square statistic as the noncentrality parameter in a noncentral chi-square distribution.

Sampling Frequency	Number of Sites	χ^2	df	power (α 0.05)	power (α 0.1)
Every year	35	1.28	3	14%	22%
Alternating year	35	1.18	3	13%	21%
Every year	70	2.56	3	24%	35%
Alternating year	70	2.35	3	22%	33%

For *A. maculatum*, the pilot data show an influence of wetland drying on occupancy probability, with higher occupancy in sites that did not dry during the survey season (March- June) (Table 5.5), as a result we modeled two groups: Dried (D) and Non-Drying (ND) with 15 and 20 sites respectively. Based on pilot data we set the parameter values for non-drying sites at: initial occupancy $\Psi_{ND}=0.55$, colonization $\gamma_{ND}=0.10$. Extinction probability was set at $\epsilon_{ND}=0.35$ to result in a 50% decline in occupancy after 5 years. We set the initial occupancy for sites that dried as $\Psi_D=0.05$; since these sites are likely to be less reliable as habitat we set the colonization probability low ($\gamma_{ND}=0.05$) and the extinction probability high ($\epsilon_{ND}=0.9$). The overall initial occupancy probability was 0.34, and applying these parameter values occupancy in five years would be 0.17 ($\Psi_{ND}=0.25$; $\Psi_D=0.05$). Pilot data showed that water temperature was important in the probability of detection, so we calculated a detection value for early and late in the season: $p_{Early} = 0.98$ and $p_{Late} = 0.87$. The power to detect the change in occupancy probability was higher when more sites were surveyed and decreased slightly when sites were visited in alternate years (Table 5.7). Again, the relatively slight difference in the power approximation among annual and alternating year scenarios is in part because the colonization and extinction rates were assumed to be constant in this scenario (see scenario 2). When vital rates vary over time, the power difference among these sample designs is more pronounced (see next scenario).

Habitat-specific Protocol: Lentic Standard Operating Procedure

Table 5.7. Likelihood ratio test for the null hypothesis (H_0 : no change in occupancy) vs. the alternative hypothesis (H_a) of time-specific occupancy estimates for *Ambystoma maculatum*. Power is approximated using the resulting chi-square statistic as the noncentrality parameter in a noncentral chi-square distribution.

Sampling Frequency	Number of Sites	χ^2	df	power (α 0.05)	power (α 0.1)
Every year	35	5.08	6	34%	47%
Alternating year	35	4.72	6	32%	44%
Every year	70	10.02	6	64%	76%
Alternating year	70	9.52	6	62%	73%

Scenario 2: 50% decline in occupancy assuming variable rate parameters (γ_t , ϵ_t)

The previous scenario assumed constant vital rates. With only 2 years of data, we do not know if vital rates are relatively constant or vary widely across time, though presumably they will vary as a result of changing weather and hydrological conditions. Thus, using the same initial occupancy probabilities ($\Psi_{ND}=0.55$ and $\Psi_D=0.05$; initial overall occupancy $\Psi=0.34$) we modeled a scenario of one “good” year (higher colonization, lower extinction) followed by two bad years (lower colonization, higher extinction), then repeated the cycle. In “good” years we set the parameter values for non-drying (ND) sites at: colonization $\gamma_{ND}=0.15$ and extinction was set at $\epsilon_{ND}=0.2$; for sites that dried (D) we set our parameters as $\gamma_D=0.05$ and $\epsilon_D=0.7$. In “bad” years we set the parameter values for non-drying sites at: colonization $\gamma_{ND}=0.05$, and extinction was set at $\epsilon_{ND}=0.55$; for sites that dried we set our parameters as $\gamma_D=0.03$ and $\epsilon_D=0.95$. Applying these parameter values the overall initial occupancy of 0.34 would decline by 50% in five years to 0.17 ($\Psi_{ND}=0.25$; $\Psi_D=0.05$). We used values for the probability of detection based on water temperature $p_{Early} = 0.98$ and $p_{Late} = 0.87$. The power to detect a change in occupancy probability and extinction probability was higher when more sites were surveyed and lower when sites were visited in alternate years (Table 5.8).

Habitat-specific Protocol: Lentic Standard Operating Procedure

Table 5.8. Likelihood ratio test for the null hypothesis (H_0 : no change in occupancy and no change in extinction) vs. the alternative hypothesis (H_a) of time-specific occupancy and extinction probability estimates for *Ambystoma maculatum*. Power is approximated using the resulting chi-square statistic as the noncentrality parameter in a noncentral chi-square distribution.

Sampling Frequency	Number of Sites	χ^2	df	power (α 0.05)	power (α 0.1)
Every year	35	11.53	6	72%	81%
Alternating year	35	6.23	6	42%	55%
Every year	70	23.05	6	97%	99%
Alternating year	70	12.46	6	76%	84%

Scenario 3: 50% increase in occupancy (assuming constant rate parameters (γ , ϵ))

Estimates of occupancy by *A. maculatum* in the Chesapeake and Ohio Canal NHP (Table 5.4, Figure 5.2) is lower than naïve levels observed by other studies in the northeastern United States (New Hampshire: 0.56; Maine: 0.70; Rhode Island: 0.70 (Herrmann et al. 2005; Calhoun et al. 2004; Egan and Paton 2004)). It is possible that the distribution of *A. maculatum* may expand in the area resulting in an increase in occupancy probability. Thus, we tested the power to detect a 50% increase in site occupancy. We set the parameter values for non-drying sites at: initial occupancy $\Psi_{ND}=0.55$, colonization $\gamma_{ND}=0.4$; extinction was set at $\epsilon_{ND}=0.1$. For sites that dried we set the parameters as $\Psi_D=0.05$, $\gamma_D=0.1$ and $\epsilon_D=0.7$. The overall initial occupancy was 0.34 and applying these parameter values the overall occupancy in five years would be 0.50 ($\Psi_{ND}=0.78$; $\Psi_D=0.12$). We used values for the probability of detection based on water temperature $p_{Early} = 0.98$ and $p_{Late} = 0.87$. The power to detect a change in the occupancy probability was higher when more sites were surveyed (Table 5.9).

Habitat-specific Protocol: Lentic Standard Operating Procedure

Table 5.9. Likelihood ratio test for the null hypothesis (H_0 : no change in occupancy) vs. the alternative hypothesis (H_a) of time-specific occupancy estimates for *Ambystoma maculatum*. Power is approximated using the resulting chi-square statistic as the noncentrality parameter in a noncentral chi-square distribution.

Sampling Frequency	Number of Sites	χ^2	df	power (α 0.05)	power (α 0.1)
Every year	35	4.08	6	28%	40%
Every year	70	8.17	6	54%	66%

Scenario 4: Detecting change in distribution (i.e. variable rate parameters (γ_t , ϵ_t)) but no trend in occupancy probability over 5 years

Colonization and extinction rates may not be constant. We modeled the scenario of one “good” (higher colonization, lower extinction) year followed by two bad years (lower colonization, higher extinction) and then a “good” year; with no trend in occupancy after 5 years. In “good” years we set the parameter values for non-drying sites at: colonization $\gamma_{ND}=0.25$, and extinction $\epsilon_{ND}=0.10$; for sites that dried we set our parameters as $\gamma_D=0.10$ and $\epsilon_D=0.5$. In “bad” years we set the parameter values for non-drying sites at: $\gamma_{ND}=0.05$, and $\epsilon_{ND}=0.3$; for sites that dried we set our parameters as $\gamma_D=0.05$ and $\epsilon_D=0.85$. After five years the overall occupancy probability would be the same as the initial occupancy, but the distribution of *A. maculatum* among dried and non-dried sites would have changed (initial distribution: $\Psi_{ND}=0.55$, $\Psi_D=0.05$ vs. distribution in year 5: $\Psi_{ND}=0.47$, $\Psi_D=0.12$). We used values for the probability of detection based on water temperature $p_{Early} = 0.98$ and $p_{Late} = 0.87$. The power to detect the change in distribution was higher when more sites were surveyed and lower when sites were visited in alternate years (Table 5.10).

Habitat-specific Protocol: Lentic Standard Operating Procedure

Table 5.10. Likelihood ratio test for the null hypothesis (H_0 : no change in occupancy and no change in extinction) vs. the alternative hypothesis (H_a) of time-specific occupancy and extinction probability estimates for *Ambystoma maculatum*. Power is approximated using the resulting chi-square statistic as the noncentrality parameter in a noncentral chi-square distribution.

Sampling Frequency	Number of Sites	χ^2	df	power (α 0.05)	power (α 0.1)
Every year	35	7.75	6	52%	64%
Alternating year	35	1.36	6	11%	19%
Every year	70	15.51	6	86%	92%
Alternating year	70	2.73	6	19%	29%

Conclusions

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. Our models indicate that wetland drying influences the probability of occupancy for most of the amphibians found in Chesapeake and Ohio Canal NHP. Wetland drying may vary with climatic conditions, causing the availability of lentic habitats for amphibians to be year/survey specific. In addition, the probability of a wetland drying may vary due to changes outside of the park (watershed modifications, water diversion, or global climate change) or changes within the park (i.e. changes in the canal water level). The urbanization variables (minimum pH, maximum conductivity) influenced the initial occupancy probability of several species (Table 5.1 and 5.5), but the urbanization variables did not seem to influence the turnover in site occupancy. However, several habitat variables (estimated likelihood of flooding by the Potomac River, and wetland drying) were important in the estimates of extinction. These results are based on only a single estimate of the vital rate parameters, and future data may reveal more information on the factors influencing turnover in site occupancy. Analyses of this data are crucial because they give estimates of the vital rates, which are difficult to come by in the literature, and they can be used to explore sampling design tradeoffs.

The scenarios we examined to explore sampling design tradeoffs are realistic to the Chesapeake and Ohio Canal NHP. Results varied among species, for example, in the first scenario, given the same level of survey effort and the same survey design (number of sites; years), the power to detect a 50% decrease in occupancy probability after 5 years was higher for *A. maculatum* than for *N. viridescens*. A difference in the power to detect a change in occupancy may vary by species due to either the detection probability or the initial occupancy. The detection probability of both species was high (>0.9) however, the initial occupancy was higher for *A. maculatum* than *N. viridescens* (0.34 and 0.15, respectively). This means that if there were 35 sites in the populations and all are surveyed, a 50% decline would represent a decrease to 6 occupied sites for

Habitat-specific Protocol: Lentic Standard Operating Procedure

A. maculatum, and only 2-3 sites for *N. viridescens*. For rare species with a low probability of occupancy, such as *N. viridescens*, survey designs should maximize the number of sites with the purpose of increasing the power to detect a change in site occupancy. This is in agreement with results found by Mackenzie and Royle (2005) which suggest sampling more sites is most efficient for occupancy studies of rare species.

If management agencies are unsatisfied with the current study's ability to detect change in amphibian occupancy and associated vital rates, we recommend surveying more sites (Tables 5.6-5.10). There are many lentic sites available to be surveyed since we are currently surveying 20% of the lentic sites within the Potomac Gorge area of the Chesapeake and Ohio Canal NHP. If an increase in the number of sites is not possible, due to logistic or financial constraints, then the management agencies may need to survey for more years before changes in occupancy can be detected (MacKenzie et al. 2006, p.219-221, Bailey et al. *in press*).

We recommend continuing to survey each year. In this pilot study (2005-2006), the estimates of occupancy were relatively constant, however the occupancy state did change among sites. In other words, some sites were colonized and others failed to support breeding and/or foraging activity (i.e. went locally extinct), resulting in non-zero estimates of extinction and colonization probabilities (Table 5.4). An alternate year survey design cannot be used to estimate annual rates of change in any parameter of interest and the ability to detect temporal trends in occupancy and other vital rates is sufficiently reduced in most scenarios (Table 5.8 and 5.10). At this point we do not know if extinction and colonization rates are constant or if they vary widely across time due to either environmental conditions (i.e. rainfall, pH), biotic conditions (presence of prey, predators, competitors) or changes in these conditions due to urbanization, and thus caution against an alternate year survey design.

Wetland drying indirectly represents habitat availability. It may be beneficial in the future to simultaneously model both habitat availability, then conditional on available habitat, the probability that amphibians occupy it. Developing this type of joint model is a current area of research by USGS scientists and collaborators (J. Nichols and D. MacKenzie, *personal communication*). We anticipate being able to apply these types of models to data collected at C&O in the near future.

Habitat-specific Protocol: Lentic Standard Operating Procedure

4.3 Procedures

- 4.3.1 Prior to the field day, prepare a list of sites to be sampled and the order in which they will be sampled. Prepare data sheets, filling in plot numbers and latitude and longitude coordinates prior to going in the field, and be sure that points are uploaded into the GPS unit.
- 4.3.2 Determine what type of sampling will be conducted [e.g., VES, calling (initiated at least ½ hour after sundown), or dipnet survey]. See appropriate protocols below.

4.4 Visual Encounter Survey

- 4.4.1 Visual encounter surveys (VES) should be conducted during daylight hours, and may continue until light conditions prevent viewing objects further than 20 ft away (usually until just after sundown).
- 4.4.2 Do not survey under severe weather conditions (i.e. when wind and/or sky codes > 6).
- 4.4.3 Two observers should conduct the VES, and observers should carry notepaper to record data while conducting the survey (this may be automated with use of PDA technology). Observers begin at opposite sides of a wetland site, and walk along the perimeter of the wetland in the same direction, taking extreme care to approach the wetland perpendicular to the perimeter without disturbing amphibians which are basking at the edge. Both observers will circumnavigate the wetland. No communication between observers should be made during the survey. Adult, juvenile, or larval individuals which are caught in dip nets and all other observations (calling, visual encounters without handling) should be recorded discretely.
- 4.4.4 Observers should slowly walk along the perimeter (observing 1 m on the bank and 1 m into the water) of a defined lentic habitat, recording the species and tallying the number of individuals of each species found. Species that cannot be identified with utmost confidence should not be included in the data, but all diagnostic features should be recorded on the data sheets, and several photographs of dorsal, lateral and ventral views of the animal should be taken, to clarify identification once observers have returned to the office. An incorrect identification is a serious violation of the protocol, and all attempts should be made to avoid this error.
- 4.4.5 The wetland perimeter should be searched for egg masses, including a careful search of vegetation at the margin of the lentic site, as many amphibians lay their eggs attached or hidden within vegetation at the lentic site margin. Record

Habitat-specific Protocol: Lentic Standard Operating Procedure

species name, number of egg masses (or individual eggs if that is appropriate for a given species), and elapsed survey time.

- 4.4.6 All terrestrial cover (rocks, logs and coarse woody debris; >6 cm) on the shoreline within 2 m of a lentic site's edge should be turned and searched for amphibians. Record the number of cover objects searched and the elapsed time for the search, the species, numbers, and age classes of all animals encountered (if possible). All cover which is too large or cumbersome to turn and search should not be searched or included in the counts. Leaf litter should not be searched unless it is under a cover object.
- 4.4.7 If malformed or diseased (unnaturally lethargic, swollen, or sickly) amphibians are encountered, refer to SOP #12 (Malformed or Diseased Amphibians).

4.5 Calling Survey

- 4.5.1 Calling surveys should be conducted at predefined survey locations, and should always be conducted from the same location in each successive survey. Observers should record all species heard calling from nearby habitats, estimating the direction (and approximate distance if possible) from the survey site.
- 4.5.2 Following the NAAMP protocol (<http://www.pwrc.usgs.gov/NAAMP/protocol/index.html>), calling surveys should begin at least 30 minutes after sundown and should conclude before 0100 hours. Surveys should be conducted under appropriate local weather conditions (sky code ≤ 5 , wind ≤ 3 , see code definitions below). The best time to survey is after a rain when temperatures are greater than 5.6° C, or when relative humidity is high.
- 4.5.3 Two observers should participate in each calling survey, and observers should not communicate during the survey. Each observer will record calling survey data on their own data sheet. Having two observers simultaneously participate in a calling survey is not only a consideration for the safety of the observers, but will allow us to estimate the detection probability for each species and observer bias.
- 4.5.4 At each stop (if a road survey) or at each wetland, observers should wait 1 minute to allow anurans to begin calling again. During this waiting period, observers should take care to remain still and quiet. Temperature, wind and sky codes, and other physical variables can be recorded at this time.
- 4.5.5 After the initial waiting period, observers listen for five (5) minutes and record a calling code (1-3) for calling intensity of each species detected based on the NAAMP protocol. For calling codes 1 and 2, the approximate number of individuals of each species detected should be recorded.

Habitat-specific Protocol: Lentic Standard Operating Procedure

4.6 Dipnet Survey

Protocols for dipnet surveys were compiled from published recommendations and data, including Heyer et al. (1994), Crisafulli (1997), Thoms et al. (1997), Arntzen (2002), Knutson et al. (2002), and Wilson and Maret (2002).

- 4.6.1 Dipnet surveys are used primarily to identify species or life stages not detected during VES or calling surveys. Whereas the VES and calling surveys aid in detection of adult or juvenile individuals (and also egg masses in the VES), dipnet surveys aid primarily in detection of larval amphibians.
- 4.6.2 During dipnet surveys, VES of adults, juveniles, or egg masses should still take place (see below).
- 4.6.3 Larval amphibian keys and guides by Parmelee et al. (2002) and Altig (1970) can help if there are problems with species identification. Proper identification of species is essential to inferences about occupancy and cannot be overemphasized.
- 4.6.4 Two observers should survey a single lentic site during one visit. Observers begin at opposite sides of a wetland site, and walk along the perimeter of the lentic site in the same direction, and should carry notepaper in order to record counts for species observed. Observers should mark their starting point with a wire flag. During the first part of the survey, both observers are first observers for the half of the pond they are surveying. Observers should note the point at which they reach the starting point of the other observer, as at that point they have become the second observer for the remaining half of the pond.
- 4.6.5 The number of dipnet samples should be based on the area of the pond; ponds that are greater than 1000 m² should have dipnet samples every 10-15 m, those with an area greater than 300 m² should have samples every 7-10 m, and those with an area smaller than 300 m² should have samples every 5-7 m. The location of the dipnet samples should also be guided by knowledge of the biology and behavior of the animals. For example, at exactly 5 m, there may be no vegetative cover, or tadpoles may not be visible in the water column. However, tadpoles may be visible or favorable habitat may be present within 1 m of the sample point. In such cases, observers can use informed judgment to adjust the sample location. Samples should remain at minimum 3 m apart to preserve the independence of samples.
- 4.6.6 Record start time for each observer.
- 4.6.7 Prior to taking each sample, observers should take care to visually search the shoreline near and a few meters ahead of the sample location for basking adults or egg masses; such **encounters** should be recorded. In the case of egg masses, care should be taken to avoid disturbing these during the dipnet sample. Adjust the sample location as necessary if egg masses are present.

Habitat-specific Protocol: Lentic Standard Operating Procedure

- 4.6.8 Carry a container in which to place animals during each dipnet sample.
- 4.6.9 Each dipnet sample will consist of three sweeps; each sweep should include 2-3 cm of bottom substrate, should proceed from deeper water to shallow water (perpendicular to shore), and the swath length should be 1 m. With each sweep, place animals into the bucket, with enough water for them to easily swim. Record the number of samples you made, species captured and their counts when you were the primary observer separately from when you were the secondary observer. Carefully release animals back into pond slightly behind the sample location. Care should be taken to avoid stirring up substrate when releasing animals.
- 4.6.10 Record final end time for each observer.

4.7 Lentic Habitat Data and Sampling Procedures

Sampling Covariates (covariates that change with each sampling occasion). Survey methods for which the sampling covariates are used are noted after the name of the covariate: VES (VES), Calling (C), and Dipnet (D). Modeling of pilot survey data supports the continued collection of these sampling covariates (Table 5.3; Table 5.5). Most of these covariates influence the detection probability of one or more species. For the VES and dipnet surveys, sky condition, wind speed, and rainfall did not appear important in the probability of detection of any lentic species. Conductivity and pH contributed to estimates of site occupancy.

- 4.7.1 Sky code (VES, C, D): Do not conduct surveys if sky codes are 6 or above.

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

- 4.7.2 Wind code (VES, C, D): Do not conduct surveys if wind codes are 6 or above (for VES and D), or 4 or above (C).

Code	mph	Indicators of Wind Speed
0	0	< 1 calm, smoke rises vertically
1	2–3	light air movement, smoke drifts

Habitat-specific Protocol: Lentic Standard Operating Procedure

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

4.7.3 Air Temperature (VES, C, D)

Record to nearest 10th of a degree in centigrade (e.g., 20.2°C)

4.7.4 Water Temperature (VES, C, D)

Record to nearest 10th of a degree in centigrade (e.g., 20.2°C)

4.7.5 Moon Code (C)

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

4.7.6 Turbidity at Lentic Site (VES, D): Record degree of visibility in the water column

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

4.7.7 Canopy Closure (VES, C, D)

Measure the extent of canopy closure over the center of the lentic site using a spherical densiometer.

4.7.8 Aquatic Vegetation (VES, C, D)

Measure the % cover of various types of aquatic vegetation (submerged aquatic vegetation, emergent, shrub, tree, and floating vegetation) at the lentic site.

4.7.9 Water Depths (VES, C, D)

Habitat-specific Protocol: Lentic Standard Operating Procedure

Measure maximum water depth and depths 4 times along maximum length and width at 1.0 m intervals. Record both the depth of the water and the point along the maximum length at which the depth is taken. If a pond is less than 8 m wide or long, then measure 4 equally spaced depths along the maximum length.

4.7.10 Maximum Length (VES, C, D)

Measure the maximum length of lentic site in meters.

4.7.11 Maximum Width (VES, C, D)

Measure the maximum width of lentic site in meters.

4.7.12 Water pH (VES, C, D)

Record using pH meter at each visit during the field season. The pH is to be measured with a voltmeter attached electrically to a glass electrode. Calibration is accomplished using two buffer solutions (pH = 4 and 7) and should be performed at the beginning of each survey day. The accuracy of the pH meter should be 0.1 pH units.

4.7.13 Water Conductivity/Specific Conductance (VES, D)

Record using conductivity meter at each visit over the field season. Specific conductance is to be measured in units of micro Siemens per centimeter ($\mu\text{S}/\text{cm}$) using a probe that measures the electric current that passes through the water sample. Calibration is accomplished by setting the zero point in air and setting the upper range slope using a solution of known value.

4.7.14 Number of Cars (C)

If individual cars can be distinguished tally with a clicker counter the number of cars that pass by the lentic site during the 5 minute calling survey.

4.7.15 Rainfall (VES, D, C)

Record the presence/absence of rainfall within 24 hours prior to the survey. Data sources can include Park weather stations, or data from nearest weather station to lentic site from AnythingWeather.com, which synthesizes data from the National Weather Service, National Oceanic and Atmospheric Administration, and the National Climatic Data Center. If possible, record approximate duration/amount of precipitation within a 24 hour time period.

4.7.16 Survey Date (VES, C, D)

Record the Day, Month and Year of survey as DD Month YYYY (e.g., 25 June 2005).

Habitat-specific Protocol: Lentic Standard Operating Procedure

4.7.17 Visit Number (VES, C, D)

Record whether this is the first, second, third, etc. visit to the lentic site. If multiple survey methods (VES, C, D) are employed on the same survey date, the visit number should still be recorded as the same for each (e.g., if VES and D are conducted at a particular lentic site on the same day - 30 March 2004 – that day would constitute visit number 1)

4.7.18 Observer Name (VES, C, D)

Record the name of each observer.

4.7.19 Survey Times (VES, C, D)

Record the begin and end search times for each survey method.

4.7.20 Number of Dipnet Samples (D)

Record number of dipnet samples at a lentic site. Each sample consists of 3 dipnet sweeps at the same location.

4.7.21 Number of Natural Cover objects (VES)

Record number of natural cover objects (wood and rock) overturned during each half of a VES at a lentic site.

Site Covariates (covariates that do not change over the course of sampling occasions). Only record these covariates once during the field season; note that all of these are applicable to VES, C, and D surveys. Modeling of pilot survey data support the continued collection of these site covariates (Table 5.1, Table 5.5) Most of these covariates influence the estimates of occupancy of one or more species. For the VES and dipnet surveys, the distance to the road did not seem important in the estimation of occupancy for any of the species. GIS variables were not available during the pilot study, but will be included in future analysis, as soon as the information becomes available.

4.7.22 Distance to Nearest Road

Record the distance from the lentic site to the nearest road in meters.

4.7.23 GIS Variables

From appropriate GIS databases/data layers, record the following variables per lentic site:

- Percent land cover/land use (urban, agricultural, forest, impervious surface, etc. within 1 km²)
- Road density (density of roads within 1 km² area)

Habitat-specific Protocol: Lentic Standard Operating Procedure

- Nearest wetland (distance in meters to nearest wetland)
- Wetland density (number of wetlands within 1 km²).
- Flood potential (distance to the river (m) × slope from the river (degrees)).

4.7.24 Wetland Classification Type

Record wetland type based on Cowardin et al. (1979) to Subclass level. Utilize resources including Crow et al. (2000 a, b), Gleason and Cronquist (1991), Holmgren (1998) and the Northeast Wetland Flora Field Office Guide to Plant Species (USDA Natural Resources Conservation Service, Northeast National Technical Center, Chester, PA) to identify wetland plants.

4.7.25 Adjacent Land Cover Types/Riparian Buffer Zone

Write Y/N (Yes/No) for the following adjacent land cover types within 50 meters of the transect:

- Mature Forest (> 12 inches dbh of most trees)
- Immature Forest ($\leq$ 12 inches dbh of most trees)
- Clearcut/Logged Area
- Old Field/Pasture/Orchard/Cropland
- Lawn
- Road
- Trail
- Residential
- Parking Lot/Industrial/Commercial
- Other: Describe

Habitat-specific Protocol: Lotic Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.1	Winter 2006	E.H. Campbell Grant	Updated based on pilot data	Pilot data	1.2
1.2	Jan 2007	E.H. Campbell Grant	Updated based on pilot data	Pilot data	1.3

1. Purpose

Lotic sites are characterized by moving water and include seeps and first-, second-, and higher order streams. Some lotic sites have defined inlets and outlets, but lotic sites are typically linear in form and have continuous movement of water through the habitat. Lotic sites are important habitat for some anurans that use streams for foraging and breeding in slow-moving or stagnant pools along the streams, but primarily lotic sites support certain salamander species who rely on these habitats for all life history stages (Appendix 1).

See Standard Operating Procedure (SOP) #3: “Establishing and marking sample plots” for survey site selection protocols.

This SOP provides instructions for sampling lotic habitats associated with the amphibian monitoring program in the National Capital Region parks. See descriptions of data fields (below) for lotic sampling. Habitat data and sampling procedures are described below the amphibian sampling protocols.

2. Scope and Applicability

This SOP applies to conducting amphibian monitoring in lotic habitats in the National Capital Region of the National Park Service.

3. Reference Documents

- 3.1 Kazyak, P.F. 2001. Maryland Biological Stream Survey Sampling Manual. Maryland Department of Natural Resources, Monitoring and Non-Tidal Assessment Division, Annapolis, MD (available at http://www.dnr.state.md.us/streams/pubs/2001mbss_man.pdf).
- 3.2 Strahler, A.N. 1957. Quantitative analysis of watershed geomorphology. American Geophysical Union Transactions. 38: 913-920.
- 3.3 Waldron, J.L., C.K. Dodd, Jr. and J.D. Corser. 2003. Leaf litterbags: factors affecting capture of stream-dwelling salamanders. Applied Herpetology. 1:23-36.

Habitat-specific Protocol: Lotic Standard Operating Procedure

4. Procedures and General Requirements

4.1 Pilot Study: 2005 data

A pilot study was conducted in 2005 (23 June–29 July) at the Chesapeake and Ohio Canal National Historic Park and Rock Creek National Park, employing two sampling techniques: visual-encounter transect surveys, and leaf litterbags. The purpose of the pilot study was to determine the number of sampling sites (paired transects) and visits needed to optimize long-term amphibian monitoring in the National Capital Region of National Parks. Occupancy modeling of the pilot study data yielded estimates of p (detection probability) and ψ (occupancy) for individual species; combining these initial estimates with simulation results allow approximation of the optimal number of visits and number of sites needed to gain an acceptable level of precision for occupancy estimates (MacKenzie and Royle 2005).

We sampled 10 streams with 25 paired transect sites in the parks (5 streams in CHOH and 5 streams in ROCR). Each site was visited twice by two observers, conducting both transect surveys and leaf litterbag searches on each visit. We observed four species of stream salamanders: *Desmognathus fuscus*, *Eurycea bislineata*, *E. longicauda*, and *Pseudotriton ruber*.

We did not encounter *E. longicauda* with enough frequency to include in the formal analysis, and due to the time period of sampling, we were not able to detect all age classes of all species. To determine the number of sites and surveys that would provide precision in estimates of occupancy of stream salamanders, we pooled all captures by species, regardless of age class.

The probability of detection ranged from 0.42 to 0.79, and the proportion of sites occupied also varied from 0.14 to 0.71. We included several urbanization-related variables in the analysis which we expected would influence variation in occupancy (proximity to a road (i.e., <100 m) and pH). Proximity to a road was important to estimation of occupancy only for *D. fuscus*, but pH was not important for the combined age class data. We will investigate other measures of urbanization at the watershed level which may apply specifically to stream systems (e.g., watershed impervious surface, watershed road density and proximity).

To achieve optimal precision and efficiency (based on data from the pilot study), if the same 11 streams from the pilot study are surveyed, we recommend 2 visits to each stream, surveying 2 sets of paired 15 m transects (separated by at least 100 m) at each visit. To expand the lotic sampling to other parks in the National Capital Region, we could survey 20 streams with 2 sets of paired 15 m transects (and 2 visits to each set of paired transects), which would result in a standard error of $\psi < 0.09$. These surveys should occur during June-July and repeat visits should be spaced by at least two weeks.

We do not recommend leaf litterbags as a survey method. Litterbags are expensive to construct and time consuming to maintain in the field. Though leaf litterbags were better at detecting salamanders, the relative difference in detection probability was quite small (e.g., litterbag $p = 0.1897$ vs. transect $p = 0.1397$).

4.2 Update to the Protocol: Incorporating 2006 data

Habitat-specific Protocol: Lotic Standard Operating Procedure

In 2006, we expanded the stream surveys to Prince William Forest Park (PRWI), Triangle, VA, USA. We visited 53 streams which were identified in GIS coverages provided by the park, finding that only 19 had sufficient surface water flow to accommodate two sets of transects spaced by 100m (note: 2006 was a dry year, with only one significant rain event occurring in mid-June, after the first surveys had been conducted). These 19 streams were surveyed according to the protocol outlined in this document. During the second visit, 6 stream channels had water above the first transect pairs. On these streams, an additional pair of transects was sampled during the second visit, in place of the third transect pair. An independent experiment suggests that stream salamanders are likely to be available for detection in dry streambeds once they begin flowing (EHC Grant, in preparation).

Across the 3 parks, we observed five species of salamanders: *Desmognathus fuscus*, *Eurycea bislineata*, *E. longicauda*, *E. guttolineata*, and *Pseudotriton ruber*. We did not encounter *E. longicauda* or *E. guttolineata* with enough frequency to include in the formal analysis, and due to the time period of sampling, we were not able to detect all age classes of all species. *E. longicauda* and *E. guttolineata* were not found in the same parks, with *longicauda* found in CHOH and *E. guttolineata* in PRWI.

We found that multiple survey passes greatly increased the detection probability of all species (especially *P. ruber*), and therefore we summed the first two survey passes for each detection occasion for each species. All age classes (larval, juvenile and adult *D. fuscus*, larval and adult *P. ruber*, and larval and adult *E. bislineata*) were combined into a single detection/nondetection matrix for analysis, since the majority of individuals detected were in only one age class (larval *P. ruber* and *E. bislineata*, and adult *D. fuscus*).

Multiple season pilot data

We conducted a multiple season analysis on data from the 11 streams at CHOH and ROCR, to test whether these stream sites had measurable colonization or extinction between 2005 and 2006. Our estimates for extinction and colonization for *D. fuscus*, *P. ruber* and *E. bislineata* were not different than zero (i.e., sites that were occupied in 2005 were likely to be occupied in 2006 and sites that were not occupied in 2005 were not likely to be occupied in 2006). We therefore combined data from 2005 and 2006 for the following analysis.

Analysis of 2005-2006 data

We visited a total of 32 stream reaches (with 62 transect pairs) in 2005 and 2006 (8 at CHOH, 5 at ROCR and 19 at PRWI). We related occupancy of each species (all age classes combined) to several site covariates [Table 1; stream network configuration (NETWORK; having two confluent first order branches vs. unbranched before entering a higher order stream), minimum pH (MIN_pH), located within 100 m of the park boundary (NEAR_BOUND)] and sampling covariates (season, number of cover objects). We included the variable NEAR_BOUND as a proxy for watershed impervious surface, as we were unable to create suitable watersheds for our analysis scale, and all streams that began inside a park had a visibly larger proportion of forest cover than those that began near the park boundary. We did not include number of cover objects in models for *P. ruber*, because larval *P. ruber* are more likely to be found in leaf litter packs

Habitat-specific Protocol: Lotic Standard Operating Procedure

(Bruce 2005, Mattfeldt and Grant 2007). We also estimated a park-specific parameter (PARK) and tested whether transect location (TRANSECT; survey conducted at the stream headwaters versus 150 m from stream origin) influenced occupancy. We expected *D. fuscus* and *P. ruber* to be associated with headwater stream locations, while *E. bislineata* was expected to be widespread and associated with transects lower on the stream reach. For each species, we used Akaike's information criterion (AIC; Burnham and Anderson 2002) to compare models representing covariates we *a priori* believed would be related to stream occupancy. A global model for each species was included to test the fit of the data to the most parameterized model. The global model fit well for *D. fuscus* and *P. ruber* (Chat = 1.1 and 0.9, respectively), but the *E. bislineata* global model did not support inclusion of the PARK covariate. The global model had some suggestion of lack of fit (Chat = 2.7), but was not indicative of serious problems with model structure (Burnham and Anderson 2002). We used an adjusted QAIC value to rank our candidate models (Table 1).

Table 1. Candidate models considered for all species (*Eurycea bislineata*, *Desmognathus fuscus*, *Pseudotriton ruber*) detected at stream survey sites in CHOH, ROCR and PRWI streams during 2005-06 survey events. Variable definitions as follows: park name (park), whether the stream began within 100 m of the park boundary (near_bound), dummy variable representing the stream spatial layout (Network = 1 if branched, 0 if unbranched), the lowest pH recorded during the survey period (min_pH), and the location of the stream survey transect pair (1 if at the headwaters, 0 if located 150 m from the headwaters).

MODEL	Model Name	Psi covs				
		Park	Near_bound	Network	Min_pH	Transect location
Global	PSI(park, Near_bound, Network, min_ph, transect)	x	x	x	x	x
1	PSI(park, Near_bound, Network)	x	x	x		
2	PSI(park, Near_bound)	x	x			
3	PSI(park, Near_bound, transect)	x	x			x
4	PSI(park, Network, transect)	x		x		x
5	PSI(park, Network)	x		x		
6	PSI(park)	x				
7	PSI(park, transect)	x				x
8	PSI(park, min_ph)	x			x	
9	PSI(park)	x				
10	PSI(Near_bound, Network, transect)		x	x		x

Habitat-specific Protocol: Lotic Standard Operating Procedure

11	PSI(Near_bound, transect)		x			x
12	PSI(Near_bound)		x			
13	PSI(Network, transect)			x		x
14	PSI(Network)			x		
15	PSI(Network,min_ph)			x	x	
16	PSI(min_ph)				x	

Species-specific results

Observations from the field, and *a posteriori* investigation of the land use history of the study areas, suggested that the stream habitats in PRWI were potentially very different than those in ROCR and CHOH. To this end, we modeled an interaction between the study location (PRWI versus ROCR and CHOH combined) and stream network configuration. A model with the interaction was supported only by the *P. ruber* data. Because only two sites had detections of *D. fuscus* at PRWI, we discuss results (below) from all sites combined, with an additional analysis for the ROCR and CHOH data separately. Land use history appears to have a large effect on the occupancy of streams in the National Capital Region. It will be of great interest to monitor changes in occupancy as unoccupied habitats become recolonized in the future, especially for species with suspected lower vagility (e.g., *D. fuscus*, *P. ruber*).

Model selection for *P. ruber* did not support the inclusion of any covariates in models of occupancy (i.e., $\Delta AIC > 2.0$ for all covariate models). However, by specifying *a priori* models, we can investigate the direction and magnitude of the parameter estimates on our covariates of interest (Table 1). As expected, minimum pH was negatively related to site occupancy across all models that included this covariate. The spatial layout of the stream network also appeared important, but the direction of the effect depends on the National Park. Based on a model that included separate estimates for the influence of stream spatial layout (i.e., branched vs. unbranched streams) across the parks, we found that branched streams in PRWI had a lower probability of occupancy, while branched streams in CHOH and ROCR had a higher probability of occupancy. This is interesting, and may be related to the land use history of the study areas. Streams near the park boundaries (at all parks) had a lower probability of occupancy than those interior to the park, suggesting that *P. ruber* may be affected by land use change outside the park boundary. Finally, sites that were located at the headwaters were more likely to be occupied than sites further downstream (i.e., a positive sign on the covariate describing location along the stream reach).

For *D. fuscus*, three models can be interpreted as having good support (i.e., $\Delta AIC < 2.0$). These three models all include the variable PARK, and all have a higher probability of occupancy in CHOH and ROCR streams. This is well supported by the detection data, as we only detected *D. fuscus* in two unbranched streams (of 19 surveyed) at PRWI. This may be related to historical land use differences among the parks, and is an area of monitoring interest as PRWI forests mature, and the opportunity for dispersal among unoccupied streams potentially increases. One

Habitat-specific Protocol: Lotic Standard Operating Procedure

of the three models also indicated that the stream spatial layout (branched vs. unbranched) was related to site occupancy, with unbranched streams having higher occupancy than branched streams, though removing the PRWI sites changes the sign of the relationship. This indicates that branched streams are more likely to be occupied by *D. fuscus*, unless they are in PRWI. In contrast to the results for *P. ruber*, for *D. fuscus* and *E. bislineata*, we found a positive association with location near the park boundary. However, in models without the PRWI sites, we could not fit models including this variable. Finally, one of the three top models included the longitudinal location of the transect, indicating that sites at the stream headwaters are less likely to be occupied by *D. fuscus* (and *E. bislineata*) than transects located further downstream. This result for *D. fuscus* was surprising, as we expected this species to prefer headwater stream sites.

There was substantial model uncertainty for *E. bislineata*, and all models that we were able to fit to the data had some support (i.e., $\Delta AIC < 2.0$). We were not able to fit models including PARK, and this species was detected at all but two of the streams. Occupancy of this species was positively associated with both branched streams and streams located near the park boundary. We were surprised by the positive relationship with branched streams, as we expected no relationship between stream spatial layout and occupancy for this species, though the difference in point estimates was small (0.92 for branched streams, 0.82 for unbranched streams). The interpretation of the positive relationship with the park boundary is also interesting, and may represent several competing hypotheses, including absence of conspecifics (*D. fuscus* and *P. ruber*). However, point estimates for the variable NEAR_BOUND are also extremely close (0.92 vs. 0.84), and the high uncertainty in model selection may represent a lack of data to support the estimated relationship.

Investigating sampling intensity

To examine the tradeoff between efficient survey design and logistical constraints associated with surveying a large number of sites (with both temporal and spatial replication), we evaluated the response of the occupancy state variable to decreasing the number of transects which are surveyed on each stream. We removed observations (individual transect data) in one of two ways: 1) using only headwater transect data (i.e., survey data from within the first 45 m of the stream origin) and 2) using only data from one transect at the headwaters and one located 150 m from the headwaters. We compared occupancy estimates from these reduced datasets with an analysis using all transect data (4 transects per stream when available). We found a small negative bias for *D. fuscus* and a small positive bias for *E. bislineata* and *P. ruber* in case (2), and a larger positive bias in all species when using only data in case (1). Based on this analysis, and to balance the total number of streams surveyed with replicate surveys at each stream, we will always visit one transect at the headwaters and one 150 m from the headwaters, and will survey the additional transects (one additional at the headwaters and one additional 150 m from the headwaters) when time allows. Surveying more transects per stream (stream reach-level replication) can provide more precise estimates for occupancy and associated rate parameters, but this may come at the cost of greater spatial distribution of sampling locations. The survey design, as outlined here, provides a compromise between bias and spatial breadth of survey locations.

Habitat-specific Protocol: Lotic Standard Operating Procedure

Conclusions and recommendations

The most interesting result from the multiple season analysis indicates that the spatial configuration of streams may be important to occupancy of stream salamanders (Grant et al. *in preparation*). Continued monitoring, and incorporation of study sites from projects outside the National Capital Region will allow further investigation of this pattern. If branched streams do indeed have higher occupancy, then one management implication would be that natural recolonization of confluent stream reaches may have a higher likelihood than in unbranched streams, which may require greater effort (e.g., translocation and reintroduction programs) to restore local extinctions. Further, our results suggest that stream salamanders differentially respond to streams located near the boundaries of these protected areas, which is of interest as landscapes surrounding parks in the region are expected to continue to urbanize. All species are apparently affected by low pH, and greater consideration should therefore be made to activities that may lower stream pH. Finally, differences in park-specific occupancy is interesting, suggesting that Prince William may still be recovering from historically intensive agriculture prior to its establishment as a National Park. We suggest continued monitoring of the stream sites included in this study, with potential expansion to an additional park in 2007, depending on whether low-order streams and seeps are mapped in other parks in the monitoring network.

Because stream salamanders are expected to have small rates of turnover due in part to the relative stability of stream habitats (e.g., Davic and Welsh 2004), we suggest that the same 32 stream segments be sampled for an additional 3 years, and an assessment of site-specific colonization and extinction probabilities is suggested after 5 years of data have been collected. At this future date, an assessment of the frequency and number of sample sites for long-term monitoring can be made.

4.3 Procedures

- 4.3.1 Prior to the field day, prepare a list of sites to be sampled and the order in which they will be sampled. Prepare data sheets, filling in lotic site numbers and latitude and longitude coordinates prior to going in the field, and be sure that points are uploaded into the GPS unit.

4.4 Transect Survey

- 4.4.1 Stream reaches will be chosen at random from the universe of known headwater, 1st streams and seeps, and the number of long term monitoring sites will be informed after analyses of the pilot data. Transect pairs will be conducted during the day (between two hours after sunrise and one half hour before sunset). The first transect pair will occur at the headwaters of the stream, if located within the park, or from the edge of the park boundary, and subsequent transects will be spaced 100 - 200 m apart along the length of a stream reach. Paired transects (15 m length), located on opposite banks, will consist of surveys of 1 meter in the water and 2 meters on the bank. Transects within each pair should be separated by 15 m. If the wetted channel of the

Habitat-specific Protocol: Lotic Standard Operating Procedure

stream section is < 1 meters in width, the entire wetted width of the channel will be surveyed. This sampling design will target both aquatic larvae and terrestrial adults associated with stream habitats.

- 4.4.2 Each transect is searched by a single observer, though two observers can simultaneously conduct separate transects along the same stream segment. In future surveys, observer assignment to each transect should be random, to prevent potential observer bias.
- 4.4.3 The observer starts at the downstream end of the transect in the wetted channel, and records a begin time for the survey. The observer proceeds upstream, turning over and counting all surface layer cover objects > 6 cm in diameter (or maximum length if ellipsoid). The observer moves back and forth across the 1 meter width of the in-stream part of the transect, turning and recording all cover within the stream channel and capturing all salamanders encountered. Every 1 m, the leaf litter should be sampled by dipnetting under points where leaf litter has accumulated in the stream (Mattfeldt and Grant 2007).
- 4.4.4 The observer uses a net to facilitate the capture of amphibians encountered. Any salamander found is removed from the transect and placed in a moistened plastic zipper-lock bag for measurement and identification and to ensure that no individual is counted twice in one survey event. Observers will follow SOP # 4 (Handling Live Amphibians) for capturing and retaining amphibians. All amphibians are returned to their original location after the survey is complete.
- 4.4.5 Once the observer reaches the top of the transect, the observer works his or her way back down along the bank, turning and recording all cover objects in a 2 meter band along the bank adjacent to the wetted channel of the transect and capturing all amphibians encountered. All cover objects should be replaced as originally found at the end of each survey.
- 4.4.6 For the pair of transects located at the headwaters of the stream multiple passes will be conducted to allow for detection of the salamander *Pseudotriton ruber*, whose detection probability was related to the number of transect passes (from analysis of pilot data). A minimum of three passes will be completed.
- 4.4.7 If malformed or diseased (unnaturally lethargic, swollen, or sickly) amphibians are encountered, refer to SOP #12 (Malformed or Diseased Amphibians).

4.5 Lotic Habitat Data and Sampling Procedures

Sampling Covariates (covariates that change with each sampling occasion).

- 4.5.1 Sky Code: Do not conduct surveys if sky codes are 6 or above

Code	Sky Condition
0	Clear or few clouds (< 20% of sky)

Habitat-specific Protocol: Lotic Standard Operating Procedure

- 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

4.5.2 Wind Code: Do not conduct surveys if wind codes are 6 or above

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

4.5.3 Air Temperature

Record to nearest 10th of a degree in centigrade (e.g., 20.2°C)

4.5.4 Water Temperature

Record to nearest 10th of a degree in centigrade (e.g., 20.2°C)

4.5.5 Water pH

Record using pH meter at each survey over the course of the field season. pH is to be measured with a voltmeter attached electrically to a glass electrode. Calibration is accomplished using two buffer solutions (pH = 4 and 7) and should be performed at the beginning of each survey day. The accuracy of the pH meter should be 0.1 pH units.

Habitat-specific Protocol: Lotic Standard Operating Procedure

4.5.6 Water Specific Conductance

Record using conductivity meter at each survey over the course of the field season. Specific conductance is to be measured in units of micro siemens per centimeter ($\mu\text{S}/\text{cm}$) using a probe that measures the electric current that passes through the water sample. Calibration is accomplished by setting the zero point in air and setting the upper range slope using a solution of known value.

4.5.7 Maximum Stream Width

Record stream width (width of wetted stream channel perpendicular to direction of stream flow) in centimeters at 0 m, 7.5 m and 15 m along the transect.

4.5.8 Maximum Stream Depth

Record maximum stream depth in centimeters at 0 m, 7.5 m and 15 m along the transect.

4.5.9 Embeddedness

Measure the percentage that the surface area of gravel, cobble, and boulders are surrounded by fine sediments or flocculent material on the stream bottom (Kazyak 2001). Observations of embeddedness should be taken in the upstream and central portions of riffles and cobble substrate areas.

4.5.10 Substrate Types

In the wetted channel use a 20 x 20 cm transect grid with 25 intersections to record the number of intersection with each of the following substrate types. Should sum to 25 for each location.

- Silt/Clay (< 2 mm, greasy, clay and fine organic)
- Sand (< 2 mm, gritty texture)
- Gravel (2 – 32 mm)
- Pebble (33-64 mm)
- Cobble (65-256 mm)
- Boulder (> 256 mm)
- Bedrock
- Artificial (cement, pipe, etc.)

Habitat-specific Protocol: Lotic Standard Operating Procedure

4.5.11 Riffle/Run/Pool Length

Record the length of riffle, run or pool habitat within each 15 m transect where riffle is defined as areas where shallow water passes over coarse substrate and produces riffles, run is defined as an area of smooth moving water, and pool is defined as an area of still or slow moving water. Should total 15 meters.

4.5.12 Rainfall

Record the presence/absence of rainfall within 24 hours prior to the survey. Data sources can include Park weather stations, or data from nearest weather station to lotic site from AnythingWeather.com, which synthesizes data from the National Weather Service, National Oceanic and Atmospheric Administration, and the National Climatic Data Center. If possible, record approximate duration/amount of precipitation within a 24 hour time period.

4.5.13 Survey Date

Record Day, Month and Year of survey as DD Month YYYY (e.g., 15 April 2005).

4.5.14 Visit Number

Record visits as number for visits to the lotic site within the June- July sample window.

4.5.15 Survey Times

Record begin and end search times for each survey method.

4.6 Site Covariates (covariates that do not change over the course of sampling occasions). Only need to record these covariates once during the field season.

4.6.1 Distance to Nearest Road

Record distance from the lotic site transect to the nearest road in meters. Specify if road is paved, gravel or dirt.

4.6.2 Distance to Nearest Trail

Record distance from the lotic site transect to the nearest trail in meters. Specify if trail is paved, gravel or dirt.

Habitat-specific Protocol: Lotic Standard Operating Procedure

4.6.3 Adjacent Land Cover Types/Riparian Buffer Zone

Write Y/N (Yes/No) for the following adjacent land cover types within 50 meters of the transect:

- Mature Forest (>12 inches dbh of most trees)
- Immature Forest ($\leq$ 12 inches dbh of most trees)
- Clearcut/Logged Area
- Old Field/Pasture/Orchard/Cropland
- Lawn
- Road
- Trail
- Residential
- Parking Lot/Industrial/Commercial
- Other: Describe

4.6.4 Channelization/Culvert

Write Y/N (Yes/No) if there evidence of a culvert, channel straightening or dredging within 50 meters of the transect. If Yes, check which type(s) are present:

- Culvert (pipe, metal)
- Concrete
- Rip-Rap
- Other: Describe

4.6.5 GIS Variables

From appropriate GIS databases/data layers, record the following variables per lotic site:

- Percent land cover/land use (urban, agricultural, forest, impervious surface, etc. within 1 km²)
- Road density (density of roads in watershed)
- Nearest stream (distance in meters to nearest stream)
- Percent impervious surface (in watershed)

4.6.6 Stream Order Code

Habitat-specific Protocol: Lotic Standard Operating Procedure

Hierarchical ordering of streams based on the degree of branching. A first-order stream is an unforked or unbranched stream. Two first-order streams flow together to form a second-order stream, two second-order streams combine to make a third-order stream, etc. (Strahler 1957). This ordering method starts at zero at each terminal segment, and proceeds towards the root stream. Each time a bifurcation node is encountered, if both daughter branches have the same order, then the order is increased by one, otherwise the largest order is used.

Name	Code	Description
Seep	0	area where water is oozing or seeping out of ground
First Order	1	unforked or unbranched stream
Second Order	2	stream segment fed by two or more first order streams
Third Order	3	stream segment fed by two or more second order streams

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
1.1	Winter 2006	S. Mattfeldt	Updated based on pilot data	Pilot data	1.2

1. Purpose

Terrestrial habitats, in the context of amphibian surveys in the National Capitol Region Parks, consist of deciduous, coniferous and mixed forests. Forests are primarily occupied by members in the genus *Plethodon*, which occur underground or under rocks and logs during the day and on the surface foraging in the spring and fall on rainy or humid nights (Appendix 1; Petranka 1998). Anurans and lotic salamanders forage, hibernate, or traverse across terrestrial habitats and may also be found during terrestrial surveys.

See Standard Operating Procedure (SOP) #3 (Establishing and Marking Sample Plots) for survey site selection protocols, if applicable.

2. Scope and Applicability

This SOP provides instructions for sampling terrestrial habitats associated with the amphibian monitoring program in the National Capital Region parks. See descriptions of data fields (below) for terrestrial sampling. Habitat data and sampling procedures are described below the amphibian sampling protocols.

Terrestrial sites will consist of an artificial cover object transect and a paired 20 × 3 m natural cover transect. A pilot study was conducted in 2005 (March 15–May 30) at the Chesapeake and Ohio Canal National Historic Park and Rock Creek Park. We sampled 38 terrestrial sites in the Chesapeake and Ohio Canal NHP and 49 terrestrial sites in Rock Creek Park. Each site was visited three times with one observation of the natural cover transect and one observation of an artificial cover object transect during each visit. During the terrestrial surveys at Chesapeake and Ohio Canal NHP we observed 3 amphibian species: *Plethodon cinereus*, *Hyla spp.*, and *P. glutinosus*.

For the Chesapeake and Ohio Canal National Historic Park we determined the number of surveys and sites that would provide precision in estimates of occupancy by analyzing species by age class (i.e. *Plethodon cinereus* adults, *Plethodon cinereus* juveniles) using the program PRESENCE. We included age class since different age classes may have different detection probabilities and may not have the same occupancy probability. *Hyla spp.* and *P. glutinosus* were rarely observed and did not have sufficient detections for formal occupancy analysis.

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

For *P. cinereus* adults and juveniles the probability of detection was 0.4052 (SE = 0.0432) and 0.3628 (SE = 0.0548), respectively, while the proportion of sites occupied was 0.7556 (SE = 0.0862) and 0.4682 (SE = 0.0915), respectively (Table 1). We looked at several urbanization covariates that may contribute to variation in site occupancy. These covariates were the proximity to a road and the proximity to a trail. The proximity to a road did not seem important in the estimation of occupancy for *P. cinereus* adults but did seem important for *P. cinereus* juveniles (Table 1).

Table 1. Relative difference in AIC (Δ AIC), β coefficients, Model weight, detection probabilities (p), overall estimate of the fraction of sites occupied by each species (Ψ), and associated standard error (SE). Numbers in *italics* are naïve estimates of site occupancy. Variables are defined as: survey method (0=coverboard transect, 1=natural transect), soil (average soil temperature), days since rain (the number of days since last rainfall), and nearRoad (0= more than 100 meters from road, 1= within 100 meters of a road). Soil temperature was standardized.

Model, by species	Δ AIC	Ψ β coefficients (SE) ($\beta_0, \beta_1, \beta_n$)	p β coefficients (SE) ($\beta_0, \beta_1, \beta_n$)	Model weight	P (range)	Ψ (SE) <range>
<i>Plethodon cinereus</i> adult						<i>0.6842</i>
$\Psi(.)p(\text{survey method})$	0.00		-0.2858 (0.1819), 0.0023 (0.0010)	0.3228	0.4290 to 0.4295	0.7721 (0.0865)
$\Psi(.)p(\text{soil})$	0.05		-0.2852 (0.1819), 0.0023 (0.0010)	0.3148	0.4280 to 0.4303	0.7721 (0.0866)
$\Psi(.)p(\text{days since rain})$	0.10		-0.2929 (0.1817), 0.0023 (0.0010)	0.3071	0.4278 to 0.4318	0.7725 (0.0866)
$\Psi(.)p(.)$	6.17			0.0048	0.4052	0.7556 (0.0862)
<i>Plethodon cinereus</i> juvenile						<i>0.4211</i>
$\Psi(\text{nearRoad})p(\text{soil, days since rain})$	0.00	0.7731 (0.5450), -2.1162 (0.8487)	-1.3102 (0.2412), - 0.2499 (0.0016), 0.2497 (0.0016)	0.7200	0.1736 to 0.76624	0.4707 (0.0822) <0.2070 to 0.6841>
$\Psi(\text{nearRoad})p(.)$	3.15	0.7444 (0.5451), -2.1463 (0.8433)		0.1491	0.3651	0.4630 (0.0814) <0.1975 to 0.6779>
$\Psi(.)p(\text{soil, days since rain})$	5.22		-1.3708 (0.2445), - 0.2603 (0.0016), 0.2600 (0.0016)	0.0529	0.1636 to 0.7737	0.4853 (0.0946)
$\Psi(.)p(.)$	8.83				0.3628	0.4682 (0.0915)

P. cinereus was the only species found at Rock Creek Park, where the naïve occupancy was 0.347 and 0.286 for *P. cinereus* adults and juveniles, respectively. Modeled site occupancy is likely close to that of Chesapeake and Ohio Canal NHP because detection probability was lower at Rock Creek NP. Unlike Chesapeake and Ohio Canal NHP most of the individuals were found in the artificial cover transects. The number of natural cover objects available was slightly lower in Rock Creek Park (mean = 9.0) than at Chesapeake and Ohio Canal NHP (mean = 11.8). When

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

detection is lower more surveys are likely necessary to achieve the same level of precision for occupancy estimates. Since the artificial cover transects had higher detection probabilities sample designs for parks with less natural cover should include the use of artificial cover.

Pilot study from Chesapeake and Ohio Canal National Historic Park and Rock Creek National Park suggests discontinuing or reprioritizing terrestrial sampling. The only species detected at adequate frequencies to monitor was *P. cinereus*, a species that is often found in a wide variety of habitat perturbations and moisture regimes (Pough et al. 1987, Brooks 1999, Grover 2000, Bailey et al. 2004, but see Harpole and Hass 1999). However, if further or future terrestrial sampling is desired, the following procedures are recommended based on the pilot study data at Chesapeake and Ohio Canal NHP: to achieve optimal precision and efficiency, we recommend visiting 38 terrestrial sites 2 times in the spring with one observation of the natural cover transect and one observation of an artificial cover object transect during each visit (Table 2).

Table 2. Terrestrial habitat-specific sampling design based on pilot study data collected at the Chesapeake and Ohio Canal National Historic Park in 2005. Each visit has two transect surveys (artificial cover and natural cover) and thus there are two occasions per visit.

Species, age class	Pilot study data				Sampling Design: 1 sampling period with 2 visit at 38 sites	
	Number of sites	Number of Visits (Occasions)	p (SE)	Ψ (SE)	Number of Visits (Occasions)	Predicted SE of Ψ
<i>Plethodon cinereus</i>, adult						
	38	3 (6)	0.4052 (0.0432)	0.7556 (0.0862)	2 (4)	0.0975
<i>Plethodon cinereus</i>, juvenile						
	38	3 (6)	0.3628 (0.0548)	0.4682 (0.0915)	2 (4)	0.1047

3. Reference Documents

- 3.1 Bailey, L.L., T.R. Simons, and K.H. Pollock. 2004. Estimating site occupancy and species detection probability parameters for terrestrial salamanders. *Ecological Applications* 14: 692-702.
- 3.2 Brooks, R.T. 1999. Residual effects of thinning and high white-tailed deer densities on northern redback salamanders in southern New England oak forests. *Journal of Wildlife Management* 63:1172-1180.
- 3.3 Grover, M.C. 2000. Determinants of salamander distributions along moisture gradients. *Copeia* 2000: 156-168.
- 3.4 Harpole, D.N. and C.A. Haas. 1999. Effects of seven silvicultural treatments on terrestrial salamanders. *Forest Ecology and Management* 114: 349-356.
- 3.5 Pough, F.H., E.M. Smith, D.H. Rhodes, and A. Collazo. 1987. The abundance of salamanders in forest stands with different histories of disturbance. *Forest Ecology and Management* 20: 1-9.

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

- 3.6 Petranka, J.W. 1998. Salamanders of the United States and Canada. Smithsonian Institution, Washington, DC.

4. Procedures and General Requirements

- 4.1 Coverboard arrays at the terrestrial survey sites should be set in place at least four months prior to the start of the field season. This allows the coverboards time to develop a suitable microhabitat underneath which salamanders can use (“seasoned”).
- 4.2 Prior to the field day, prepare a list of sites to be sampled and the order in which they will be sampled. Prepare data sheets by filling in terrestrial site numbers and latitude and longitude coordinates prior to going in the field, and be sure that points are properly uploaded into the GPS unit.
- 4.3 Observers navigate to the point which begins a linear transect of three coverboard stations spaced 10 m apart. In most cases, the transect will run North of the start point, except in instances when features of the landscape do not allow this placement. Notes from coverboard placement should clarify the direction of the transect.
- 4.4 Adjacent to (5 m) the coverboard transect is the natural cover transect (20 × 3 m). The position of the natural cover transect (i.e., either west or east of the coverboard transect) is chosen randomly at the first visit. One observer conducts the coverboard survey and one observer conducts the natural cover transect. Observers alternate responsibilities at each sample point.
- 4.5 Coverboard Surveys
- 4.5.1 During the day (at least two hours after sunrise and one hour before sunset), observers turn over all coverboards at a point (5 coverboards per station, 3 stations per transect), recording data for all amphibians found underneath (see detailed data collection below). Each coverboard station consists of two large (26 × 26 cm) boards and three small (13 × 26 cm) boards; all boards are ¾ inch pine sheathing.
- 4.6 Natural Cover Transect
- 4.6.1 Observers begin at one end of the 20 × 3 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) until the entire area is searched. Observers should always begin at the same start point (0 m point) each time. All amphibians found underneath natural cover objects will be measured, sexed and recorded.
- 4.7 Terrestrial Habitat Data: Sampling vs. Site Covariates
- 4.7.1 Sampling covariates are recorded at each visit, using the “Terrestrial habitat survey datasheet.” One datasheet can be used for both coverboard and natural

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

transects. The site covariates are only recorded on the first visit using the “Terrestrial site covariates datasheet.”

- 4.7.2 Sampling Covariates (covariates that change with each sampling occasion). Survey methods for which the sampling covariates are used are noted after the name of the covariate: Coverboard (C), Natural Cover (N). Record sampling covariates at each sampling occasion.

4.8 Survey Date (C, N)

Record Day, Month and Year of survey as DD Month YYYY (e.g., 15 April 2005).

4.9 Observer (C, N)

Record the full name of the observer.

4.10 3. Point Number (C, N)

Record the unique number assigned to the terrestrial point

4.11 4. Visit Number (C, N)

Record whether this is the first, second, third, etc. visit to the terrestrial site.

4.12 Sky Code (C, N): Do not conduct surveys if sky codes are 6 or above

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

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

- 4.13 Wind Code (C, N): Do not conduct surveys if wind codes are 6 or above

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

- 4.14 Canopy Closure (N)

- 4.14.1 Express the extent of canopy closure at the mid-point (10 m) of the natural cover transect as a percentage, using a spherical densiometer.

- 4.14.1.1 Take four densiometer readings from the center of each of the natural cover transects while facing north, south, east and west. Use the table on the back of the data sheet to calculate the average of these four readings.
- 4.14.1.2 Keep the instrument leveled (indicated by the round level in the lower right-hand corner). Hold the densiometer far enough away from your body so that your head is just outside the grid (12–18 inches away). Maintain the densiometer approximately 3 feet above the ground.
- 4.14.1.3 There are a total of 24, 1/8 inch × 1/8 inch squares in the grid. Each square represents an area of canopy opening (unfilled squares [image of sky]) or canopy cover (filled squares [image of vegetation]). Count the number of filled squares. If there are squares that are only partially filled, these can be added to make a complete square.
- 4.14.1.4 The covered area is determined by multiplying the number of filled squares by 4.17 to determine canopy cover in % (e.g., 10 filled squares × 4.17 = 41.7% canopy cover).

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

- 4.15 Previous day precipitation (C, N)
 - 4.15.1 Record the presence/absence (Y/N) of rainfall within 24 hours prior to the survey. Data sources can include Park weather stations, or data from nearest weather station to lentic site from AnythingWeather.com, which synthesizes data from the National Weather Service, National Oceanic and Atmospheric Administration, and the National Climatic Data Center. Record approximate *amount* and *duration* of precipitation within a 24 hour time period. Also record the amount (cm) and duration (hrs), if such information can be obtained.
- 4.16 Survey Times (C, N)
 - 4.16.1 Record begin and end search times separately for the natural cover and artificial cover transects.
- 4.17 Air Temperature (C,N)
 - 4.17.1 Record air temperature to the nearest 10th of a degree in centigrade (e.g., 20.2°C) at 1 inch (2.5 cm) height above ground in shade at the beginning (0 m), mid-point (10 m), and end (20 m) of the transects. Use a waterproof digital thermometer with a probe and an accuracy of $\pm 1^{\circ}\text{C}$ and a resolution of 0.1°C.
- 4.18 Soil Temperature (C,N)
 - 4.18.1 Record soil temperature to the nearest 10th of a degree in centigrade (e.g., 14.8°C) at 2 inch (5 cm) depth in soil in shade at the beginning (0 m), mid-point (10 m), and end (20 m) of the transects. Use a waterproof digital thermometer with a probe and an accuracy of $\pm 1^{\circ}\text{C}$ and a resolution of 0.1°C.
- 4.19 Leaf Litter Depth (N)
 - 4.19.1 Record the depth of the leaf litter every 2.0 m along the natural cover transect in millimeters using a ruler.
- 4.20 Species Observed:
 - 4.20.1 Station # (C only): Record at which station (1, 2, or 3) the species was observed
 - 4.20.2 Species: Record the four-letter code for species observed (see codes on datasheet) using the first letter of the genus name + the first three letters of the species name (e.g., *Plethodon cinereus* = PCIN).
 - 4.20.3 Age Class: Record whether the species is an Adult (A), Juvenile (J), or Undetermined (U)
 - 4.20.4 Sex: Record whether the species is Male (M), Female (F), or Undetermined (U)
 - 4.20.5 SVL: Record snout-vent length in mm

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

- 4.20.6 TL: Record total length in mm
- 4.20.7 Color: For *Plethodon cinereus* only – record R (red-backed) or L (lead-backed)
- 4.20.8 Cover Type: Record whether the species was found under natural cover: R = rock, W = wood, OR under a coverboard (C)
- 4.20.9 Length: Record the maximum length of the **natural** cover object in mm, if applicable.
- 4.20.10 Width: Record the maximum width of the **natural** cover object in mm, if applicable.
- 4.20.11 Depth: Record the maximum depth (height) of the **natural** cover object in mm, if applicable.
- 4.20.12 Coverboard (small/large): If the species was found under a coverboard, record whether it was a small (15 × 15 cm) or large (26 × 26 cm) coverboard.

NOTES: record any pertinent notes about the species (e.g., tail regenerating, malformed foot, etc.)

- 4.21 Number of Rocks (N)
 - 4.21.1 Record the total number of rocks greater than 6 cm in diameter (or maximum length if ellipsoid) overturned in the natural cover transect.
- 4.22 Number of Wood (N)
 - 4.22.1 Record the total number of woody objects (logs, sticks) greater than 6 cm in diameter (or maximum length if ellipsoid) overturned in the natural cover transect.
- 4.23 Site Covariates (covariates that do not change over the course of sampling occasions). These covariates are recorded once during the field season for the **natural** cover transect.
 - 4.23.1 Slope
 - 4.23.1.1 Record slope of natural cover transect using a clinometer from the beginning of the transect (0 m) looking toward the end of the transect (30 m).
- 4.24 Aspect
 - 4.24.1 Record aspect of natural cover transect using compass from the beginning of the transect (0 m).

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

4.25 Distance to Nearest Road

- 4.25.1 Record distance from the nearest part of the coverboard or natural cover transect to the nearest road in meters. Circle whether road is paved, gravel or dirt.

4.26 Distance to Nearest Trail

- 4.26.1 Record distance from the nearest part of the coverboard or natural cover transect to the nearest trail in meters. Circle whether trail is paved, gravel or dirt.

4.27 Adjacent Land Cover Types

- 4.27.1 Write Y/N (Yes/No) and provide an approximate percent for the following adjacent land cover types within 50 meters of the transect (percents should sum to 100):

- Mature Forest (>12 inches dbh of most trees)
- Immature Forest ($\leq$ 12 inches dbh of most trees)
- Clearcut/Logged Area
- Old Field/Pasture/Orchard/Cropland
- Lawn
- Road
- Trail
- Residential
- Parking Lot/Industrial/Commercial
- Other: Describe

4.28 GIS Variables (TO BE COMPLETED IN OFFICE)

- 4.28.1 From appropriate GIS databases/data layers, record the following variables for the natural cover transect:

- 4.28.1.1 Percent land cover/land use of each type (urban, agricultural, forest, impervious surface, etc. within 1 km²)
- 4.28.1.2 Road density (total linear length of roads within 1 km² area)

Habitat-specific Protocol: Terrestrial Standard Operating Procedure

- 4.28.1.3 Wetland density (number of wetlands, all classes and sizes, within 1 km²)
- 4.28.1.4 Stream density (number of streams, all classes and sizes, within 1 km²)
- 4.28.1.5 Distance to nearest wetland (in meters)
- 4.28.1.6 Distance to nearest stream (in meters)
- 4.28.1.7 Elevation in meters

Data Analysis Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

1. Purpose

This Standard Operating Procedure (SOP) explains methods for data analysis of amphibian monitoring data contained in the National Capital Region Amphibian Monitoring Program database.

2. Scope and Applicability

3. Reference Documents

- 3.1 Burnham, K.P. and D.R. Anderson. 2002. Model selection and multi-model inference: A practical information-theoretic approach. New York, Springer-Verlag.
- 3.2 MacKenzie, D.I., J.D. Nichols, G.B. Lachman, S. Droege, and C.A. Langtimm. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* 83:2248-2255.
- 3.3 MacKenzie, D.I., J.D. Nichols, J.E. Hines, M.G. Knutson, and A.B. Franklin. 2003. Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. *Ecology* 84: 2200-2207.
- 3.4 MacKenzie, D.I., L.L. Bailey, and J.D. Nichols. 2004. Investigating species co-occurrence patterns when species are detected imperfectly. *J. Animal Ecology* 73: 546-555.
- 3.5 MacKenzie, D.I. and L.L. Bailey. 2004. Assessing the fit of site occupancy models. *J Agric. Biol. Envir. S.* 9: 300-18.
- 3.6 MacKenzie, D.I., J.D. Nichols, J.A. Royle, K.H. Pollock, L.L. Bailey, and, J.E. Hines. 2005. Occupancy estimation and modeling: inferring patterns and dynamics of species occurrence. Academic Press, New York, USA.

4. Procedures and General Requirements

- 4.1 Make sure all data is entered correctly into the SQL database.

Data Analysis Standard Operating Procedure

- 4.2 Carefully read and review Burnham and Anderson (2002). This type of analysis is new and complex, and care should be taken to understand the principles behind it prior to applying it. Preferably, a quantitative ecologist or biometrician should perform these analyses. There are many ways in which the multiple visit data collected can be combined for analyses, and these alternatives should be discussed with someone familiar with options within the analysis program.
- 4.3 Carefully read and review Mackenzie et al. (2005). This text provides the foundation for understanding the application and interpretation of the PRESENCE model for detection/non detection data.
- 4.4 Use program PRESENCE (downloadable from <http://www.mbr-pwrc.usgs.gov/software.html> or <http://www.proteus.co.nz/home.html>; MacKenzie et al. 2002, MacKenzie et al. 2003) to conduct routine (annual) and long-term (>5 yr) analyses. 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., the National Capital Region). This program allows the inclusion of site and sampling covariates to test the association between abiotic and biotic variables on detection and occupancy.
- 4.5 Site-specific covariates do not change across sampling occasions within years (e.g., many habitat variables) and may influence either occupancy or detection probabilities. Sampling covariates (e.g., water temperature, weather) often change for each sampling occasion and may primarily influence detection probability. Covariates are included in a set of candidate models, and these competing models are tested using Akaike's Information Criterion (AIC, QAIC, AICc) to determine the model (and therefore, covariates) which best explain variation in the observed data. Because of the potential for a very large number of candidate models with the inclusion of an increasing number of covariates hypothesized to influence detection or occupancy of a species, much attention should be devoted to choosing relevant covariates prior to constructing models. Ideally the choice of covariates should be informed by *a priori* hypotheses and determined, by definition, before the initiation of sampling.
- 4.6 Refer to SOPs #5, 6, and 7 to determine data field definitions and which data fields are applicable for analyses. Covariates used in constructing candidate models should reflect the *a priori* hypotheses outlined prior to collection of field data (i.e., found in the protocol narrative).
- 4.7 The final data file should consist of detection (1) and nondetection (0) data at each visit to each site for species detected during lentic, lotic and terrestrial surveys. This species presence/absence (1/0) data should be assembled or downloaded from the database in the following matrix format for each of the species observed using the Habitat-Specific Protocols (Lentic, Lotic, Terrestrial) and the following data fields: Species (e.g., wood frog), Presence (e.g., 0 for absence, 1 for presence), Site Identifier (e.g., name of wetland – site 1, site 2, etc.), and Visit Number (e.g., 1, 2, 3, etc.):

Data Analysis Standard Operating Procedure

4.7.1 Wood Frog Data from Lentic Surveys

Site	Occasion 1	Occasion 2	Occasion 3	Occasion 4	Occasion 5
Site 1	0	1	1	1	1
Site 2	1	0	0	0	0
Site 3	0	0	1	1	1
Site 4	1	1	1	0	0
Etc.	...	...	...	...	...

- 4.8 For each species matrix table, prepare corresponding Sampling Covariate Tables to match the species presence/absence matrix data. For sampling covariates (covariates that change with each sampling occasion), which are used for analyses in relation to species detection probabilities and site occupancy rates, use the following format (water temperature in °C is used as the example here):

4.8.1 Lentic Surveys

Site	Occasion 1	Occasion 2	Occasion 3	Occasion 4	Occasion 5
Site 1	14.5	15.6	17.1	18.2	20.2
Site 2	16.0	14.2	17.8	19.1	20.9
Site 3	13.2	13.9	15.4	17.0	18.3
Site 4	12.2	12.9	14.1	15.6	17.8
Etc.	...	...	...	...	...

- 4.9 For each species matrix table, prepare corresponding Site Covariate Tables to match the species presence/absence matrix data. For site covariates (covariates that do not change with each sampling occasion), which are used for analyses in relation to site occupancy rates, use the following format (distance to road in meters is used as the example here):

Data Analysis Standard Operating Procedure

4.9.1 Lentic Surveys

Site	Site Covariate
Site 1	55
Site 2	19
Site 3	233
Site 4	5
etc.	...

- 4.10 Once all the tables are proofed, copy these tables into Program PRESENCE and conduct analyses. A variety of models will be tested. The simplest model ($p(.)\psi(.)$) assumes that site occupancy and detection probabilities are constant across time and sites. Alternative models include variation across time or sites. More complicated models will be used to test whether site occupancy and detection probabilities are influenced by a variety of site and sampling covariates (see habitat covariates in SOPs #5, 6, and 7), which have been specified in a set of *a priori* hypotheses. A goodness-of-fit test is available in program PRESENCE to assess how well the global or most parameterized model represents the data, and whether the data are overdispersed, in which case a variance inflation factor can be estimated and used to adjust standard errors and model selection procedures (Burnham and Anderson 2002; MacKenzie and Bailey 2004). Further descriptions of occupancy estimation methods are available elsewhere (MacKenzie et al. 2002, MacKenzie et al. 2003, MacKenzie et al. 2004; MacKenzie and Bailey 2004, MacKenzie et al. 2005). Analyses are best performed by someone familiar with model selection techniques and with program PRESENCE specifically.

After the Field Season Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

1. Purpose

This Standard Operating Procedure (SOP) explains procedures that all field observers using the Amphibian Monitoring Protocol for the National Capitol Region Parks should be familiar with and follow after the field season is completed.

2. Scope and Applicability

This Standard Operating Procedure (SOP) applies to all those involved with the NCRN Amphibian Monitoring program especially the field crews and those managing the data sheets and database.

3. Reference Documents

3.1 None.

4. Procedures and General Requirements

- 4.1 Clean and repair all equipment prior to returning them to their proper storage areas. All field equipment should be cleaned of mud, algae, and other debris, washed in 10% bleach solution, rinsed, and allowed to air dry before storage. Clean the insides and outsides of all vehicles used in the field. Any field equipment that is damaged or not functioning should either be discarded or repaired. All references and manuals should be re-shelved at the appropriate library and appropriate bookshelf. Other reference materials and extra (blank) data sheets should be filed in their appropriate file folders within designated filing cabinet(s).
- 4.2 Organize field data sheets and check that they have been filled out completely. As specified in SOPs #5, 6, and 7, all data sheets should to be reviewed for completeness at the end of each field day. However, some deficiencies in data recording may not be identified until all data sheets have been organized and reviewed as a group at the end of the field season (e.g., when habitat work has inadvertently been missed for a terrestrial, lotic or lentic study site). If this is the case and the pertinent missing data can still be collected, personnel responsible must return to the field site to obtain the missing data.

After the Field Season Standard Operating Procedure

- 4.3 Identify, obtain, and specify ancillary landscape and climate data. It is of critical importance that these data be incorporated into the amphibian monitoring efforts and entered appropriately onto datasheets or palm pilots and into the database.
 - 4.3.1 Landscape data as identified on the SOPs need to be compiled for that field season's data using National Park Service or other GIS data layers, whichever is most current.
 - 4.3.2 Climate can also influence amphibian numbers, both directly and indirectly. Excess precipitation can disrupt lotic surveys (e.g., visibility, search areas), while drought conditions may impact lentic and terrestrial surveys (e.g., ponds and soils are dry, creating habitat conditions unsuitable for pond breeders or foraging salamanders). Daily climate data will be obtained from the park weather station (if available) or from a region wide climatic database on the internet.
 - 4.3.3 Detailed metadata will be included in the database.
- 4.4 Logs of each field trip should be kept, including personnel, individual responsibilities within each survey, and unique field notes. These notes should be entered into the database, and will allow more detailed understanding of field conditions, and help clear data discrepancies once the data are being analyzed.
- 4.5 Prepare data for analysis as described in SOP #8.

Revising the Protocol Standard Operating Procedure

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #

1. Purpose

This Standard Operating Procedure (SOP) explains how to make changes to the Amphibian Monitoring Protocol Narrative for the National Capitol Region Parks and accompanying SOPs, and tracking these changes. Observers asked to edit the Protocol Narrative or any one of the SOPs must follow this outlined procedure in order to eliminate confusion in how data is collected and analyzed. All observers should be familiar with this SOP in order to identify and use the most current methodologies.

2. Scope and Applicability

This Standard Operating Procedure (SOP) applies to the versioning of the NCRN Amphibian Monitoring protocol and associated SOPs.

3. Reference Documents

3.1 None.

4. Procedures and General Requirements

- 4.1 The Amphibian Monitoring Protocol Narrative for the National Capitol Region Parks and accompanying SOPs were assembled with the goal of incorporating the sound methodologies for collecting and analyzing amphibian data. However, any and all protocols may require editing as new and different information becomes available.
- 4.2 Required edits should be made in a timely manner and should receive appropriate peer-review. All edits should be reviewed for clarity and technical reliability. Small changes or additions to existing methods should be reviewed in-house by NPS National Capital Region I&M staff. However, if a complete change in methods is sought, an outside review is required. Regional and National staff within the National Park Service possessing knowledge in amphibian research and data analysis will be utilized as reviewers as will experts in amphibian research and statistical methodologies outside of the National Park Service.
- 4.3 All edits and protocol versioning must be approved by the Project Manager. Once approved, all changes must be documented in the Revision History Log (see above) that

Revising the Protocol Standard Operating Procedure

accompanies the Protocol Narrative and each SOP. Log changes in the Protocol Narrative or SOP being edited only. Version numbers increase incrementally by tenths (e.g. version 1.1, version 1.2, ...etc) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0 ...). Record the previous version number, date of revision, author of the revision, identify paragraphs and pages where changes are made, and the reason for making the changes along with the new version number.

- 4.4 Inform the Data Manager about changes to the Protocol Narrative or SOP so the new version number can be incorporated in the Metadata of the project database. The database may have to be edited by the Data Manager to accompany changes in the Protocol Narrative and SOPs.
- 4.5 Post new versions on the internet and forward copies to all individuals with a previous version of the changed Protocol Narrative or SOP.