Joint EMAN / Parks Canada National Monitoring Protocol for Plethodontid Salamanders

Version 1. July, 2004.

Paul Zorn[1], Valerie Blazeski[1] and Brian Craig[2]

1. Parks Canada, Ontario Service Centre, Ecosystem Conservation Section. 2630 Sheffield Rd., Ottawa ON K1A 0M5
2. Ecological Monitoring and Assessment Network Coordinating Office. Environment Canada. Canada Centre for Inland Waters. 867 Lakeshore Road. Burlington ON L7R 4A6

ACKNOWLEDGMENTS

The authors would like to thank the following people for their input into the development of this report: Tim Bellhouse, Tara Crewe, Sam Droege, Dan Lantz, Kim Mawhinny, Lisa Moore, Bruce Pauli, Dean Pheonix, Sarah Quinlan, Justin Quirouette, Christine Rikley, Alissa Sugar, Jim Wilson. In particular, the authors would like to thank Kristiina Ovaska for her detailed comments and revisions.

Table of Contents

List of Tables

List of Figures

List of Boxes

Joint EMAN / Parks Canada National Monitoring Protocol for Plethodontid Salamanders

See appendix 1 for a "methods summary" of this protocol.

1. Introduction

This document outlines a standard for monitoring plethodontid salamanders (family Plethodontidae) using artificial cover objects (ACO). This method consists of placing ACOs, such as wooden boards, on the forest floor in a specified pattern and inspecting them periodically for salamanders that seek refuge under them. These ACOs cause little disturbance to the natural habitat and are used as a basis for monitoring. This protocol contains information on:

- why plethodontids are useful indicator species,
- the types of monitoring questions that this protocol can be applied to,
- how to set up a sampling design for your monitoring program,
- methods for collecting data on plethodontid numbers,
- how to analyze the data this protocol generates,
- how to refine your monitoring program after baseline data has been collected, and
- when a plethodontid population trend should raise an early warning signal.

This protocol is based on, and is designed to be compatible with, existing terrestrial salamander monitoring programs in North America (e.g., Droege, et al. 1997, Sugar et al. 2001). This document builds upon these initiatives by presenting a protocol that is developed to be integrated with EMAN's suite of forest health indicators monitored through a 20 x 20 m or 1ha SI/MAB plot design (see Box 1).

BOX 1: EMAN's (Ecological Monitoring and Assessment Network) Suite of Forest Health Indicators

This monitoring protocol for plethodontid salamanders is part of a suite of forest health indicators that are integrated through a 20x20 m or 1ha plot design. Other available forest health protocols include:

- terrestrial vegetation monitoring
- worm species richness (Worm Watch)
- lichen abundance and diversity
- annual decay rates
- downed woody debris (Ontario Ministry of Natural Resources)
- exotic plant abundance
- tree health
- regeneration and sapling survey
- plant phenology (Plant Watch)
- soil temperature
- anuran abundance and phenology (Frog Watch)

For information on monitoring protocols for these forest health indicators visit the EMAN website at http://www.eman-rese.ca/.

Why did EMAN and Parks Canada collaborate on a national monitoring protocol for Plethodontids?

EMAN (Ecological Monitoring and Assessment Network) is a national network of long term monitoring and research sites that strive to provide a national perspective on how Canadian ecosystems are being affected at an ecozone scale. To this end, EMAN facilitates the development and implementation of monitoring initiatives that attempt to address federal, provincial, regional and local environmental needs.
(source: http://www.eman-rese.ca/eman/faq.html)

Parks Canada's top priority in the management of national parks is the maintenance or restoration of ecological integrity (Canada National Parks Act. 2000. c.32, 8(2)). To assess progress towards this goal national parks develop and implement long term ecological integrity monitoring programs.

Through collaborating in the development of this protocol Parks Canada gains a method for monitoring another forest-related indicator that can be integrated with the rest of a national park's ecological integrity monitoring program. EMAN benefits through the expansion of their national network, the increase in the number of sites implementing standardized monitoring methods, and the ability to use national parks as reference sites when comparing trends in human-settled landscapes.

1.1 Plethodontid Salamanders

What are plethodontid salamanders? The family Plethodontidae are lungless salamanders and represent the largest group of salamanders in the world. All salamanders in this family lack lungs and rely on their moist skin and roof of their mouth for respiration. There are seven genera and nine species of plethodontids native to Canada, they are (Cook 1984):

Dusky Salamander *(Desmognathus fuscus)*
Two-lined Salamander *(Eurycea bislineata)*
Spring Salamander *(Gyrinophilus porphyriticus)*
Four-toed Salamander *(Hemidactylium scutatum)*
Eastern Red-backed Salamander *(Plethodon cinereus)*
Western Red-backed Salamander *(Plethodon vehiculum)*
Coeur d'Alene Salamander (*Plethodon idahoensis*)
Ensatina *(Ensatina eschscholtzii)*
Wandering Salamander *(Aneides vagrans;* formerly the Clouded Salamander, *A. ferreus)*

This protocol focuses on the woodland forms of plethodontids, in Canada represented by the genera *Plethodon*, *Ensatina* and *Aneides*. These salamanders are entirely terrestrial and complete their entire life cycle on the forest floor. Typically, woodland plethodontids can be found under ACOs in sufficient numbers such that accurate indexes of population trends can be estimated. Terrestrial phases of other salamanders can also be found under ACOs but typically not in large enough numbers to estimate population trends; however, exceptions may exist at particular localities. These species include:

Rough-skinned Newt (*Taricha granulosa*)
Red-spotted Newt (*Notophthalmus viridescens*)
Blue-spotted Salamander (*Ambystoma laterale*)
Jefferson Salamander (*Ambystoma jeffersonianum*)
Yellow-spotted Salamander (*Ambystoma maculatum*)
Northwestern Salamander (*Ambystoma gracile*)
Smallmouth Salamander (*Ambystoma texanum*)
Long-toed Salamander (*Ambystoma macrodactylum*)
Tiger Salamander (*Ambystoma tigrinum*)
Coastal Giant Salamander (*Dicamptodon tenebrosus*)

Woodland plethodontids are the target of this protocol. They are useful indicator species for forested ecosystems due to their 1. life history traits, 2. sensitivities to anthropogenic stresses, and 3. population sampling properties (also see Box 2). The Canadian ranges for species of *Plethodon*, *Ensatina* and *Aneides* are shown in figure 1.

1.1.1 Life History Traits of Woodland Plethodontids

Woodland plethodontids have adapted to forest ecosystems. These salamanders do not have an aquatic larval stage, but eggs develop directly into miniature versions of adults in nest sites on land. Woodland plethodontids reply upon damp soils and forest litter to maintain their moist skin for respiration. During cool, wet, calm weather, usually during evenings in the spring and fall, they disperse across the forest floor to satisfy their requirements for feeding, courting and mating (Droege et al. 1997).

Plethodontids generally have long life spans (10+ years), high annual rates of survivorship, and low birth rates, which typically result in stable population sizes under normal conditions. Plethodontids display site fidelity and have small home ranges; some species are territorial (Droege et al. 1997). These characteristics facilitate long-term monitoring at

BOX 2: Why do woodland plethodontid salamanders make good indicator species?

The rationale for monitoring woodland plethodontid salamanders rests upon:

- their key role and high densities in many forests,
- the stability in their counts and populations,
- their vulnerability to air and water pollution,
- their sensitivity as a measure of change,
- the threatened and endangered status of several species across North America, and
- their inherent beauty and appeal as a creature to study and conserve.

(Droege et al., 1997)

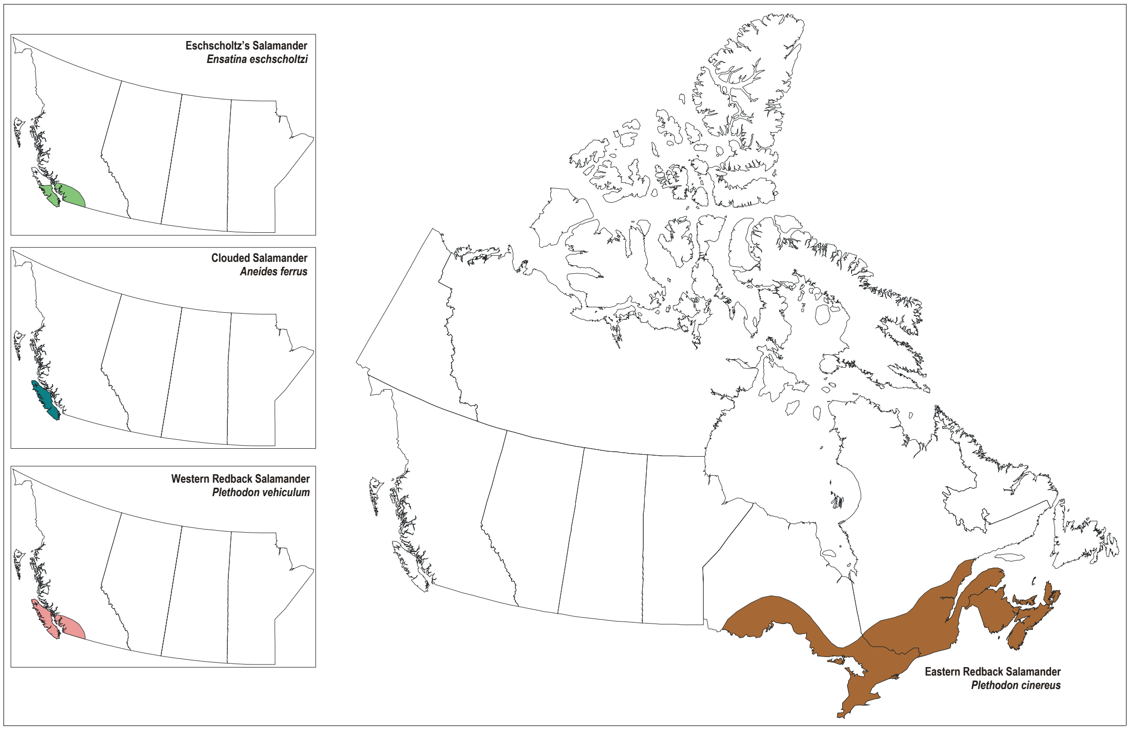


Figure 1 Canadian species range maps for woodland forms of plethodontids. Images recreated with permission from CARCNET (Canadian Amphibian and Reptile Conservation Network).

particular sites because they increase the likelihood that a change in population is an indication of some stress to a forest ecosystem rather than simply due to shifts in home range.

Plethodontids are also an important species in terms of their role in forest ecosystem processes. They are incredibly efficient at metabolizing their prey, insects and other soil invertebrates, and hence can, in suitable habitats, achieve such high densities that they equal or surpass the biomass of any other vertebrate group (Burton and Likens 1975). Plethodontids, therefore, represent an important food source in forest ecosystem food webs by transferring energy up trophic levels.

1.1.2 Woodland Plethodontids' Sensitivities to Ecological Stress

Woodland plethodontids are also useful indicator species of forest health because they are sensitive to a range of ecological stressors, particularly those that influence micro-climate and air and water quality. These include human activities such as logging, development, atmospheric pollution or climate change, and natural disturbances such as insect defoliation, storms or fire. Any event that directly or indirectly alters soil moisture, exposure to direct sunlight, or soil quality (i.e., pollutants from air or water) will likely have a negative impact on plethodontid populations. Their sensitivity to these stresses relates to the plethodontids' lack of lungs. Since respiration occurs through their skin, the transfer of oxygen is strongly affected by body moisture and the contact between their skin and contaminants (Droege et al. 1997).

1.1.3 Population Sampling Properties of Woodland Plethodontids

From a logistical perspective, plethodontid salamanders are also attractive for monitoring projects. First, plethodontids are relatively easy to identify in the field and require minimal training on behalf of observers. Since only 9 species of plethodontids occur in Canada, accurate species identification is very feasible with volunteers and changes in observers from year to year. Second, woodland plethodontids are attracted to artificial cover boards (ACOs) – which is the basis for the method described in this protocol. This allows for sampling that is non-destructive to their habitat and, through arrangement of ACOs around 20x20 m or 1ha forest health monitoring plots, can be integrated with the suite of other EMAN forest health indicators. Third, due to the relative stability of plethodontid populations in undisturbed forests relative to other amphibian species, population trends are easier to detect with smaller sample sizes than trends for those species with higher variability.

1.2 What May it Mean if a Decline in Plethodontid Populations is Detected?

Figure 2 represents a simplified conceptual model of the role of plethodontids in a forest ecosystem. This model places plethodontids in the context of various ecosystem biotic components (i.e., flora and fauna – yellow rectangular boxes), abiotic components (i.e., soil properties – green rounded-rectangular boxes), ecological processes and stresses (i.e., nutrient cycling – pink hexagons), and climate. Due to their sensitivities to changes in forest floor conditions and their importance to food web dynamics, a significant change in the numbers of plethodontids observed over time may be an early warning indicator that one of these components, processes or stressors have changed resulting in degraded forest ecosystem health.

How one determines if a significant change in plethodontid populations has occurred over time is based on the “monitoring target” or threshold and is discussed in section 11(Monitoring Threshold and Data Interpretation). If an early warning signal is raised due to population trends of plethodontids exceeding the monitoring threshold, then a decision point should be triggered to investigate the potential impact to one or many of the forest ecosystem elements contained within this conceptual model.

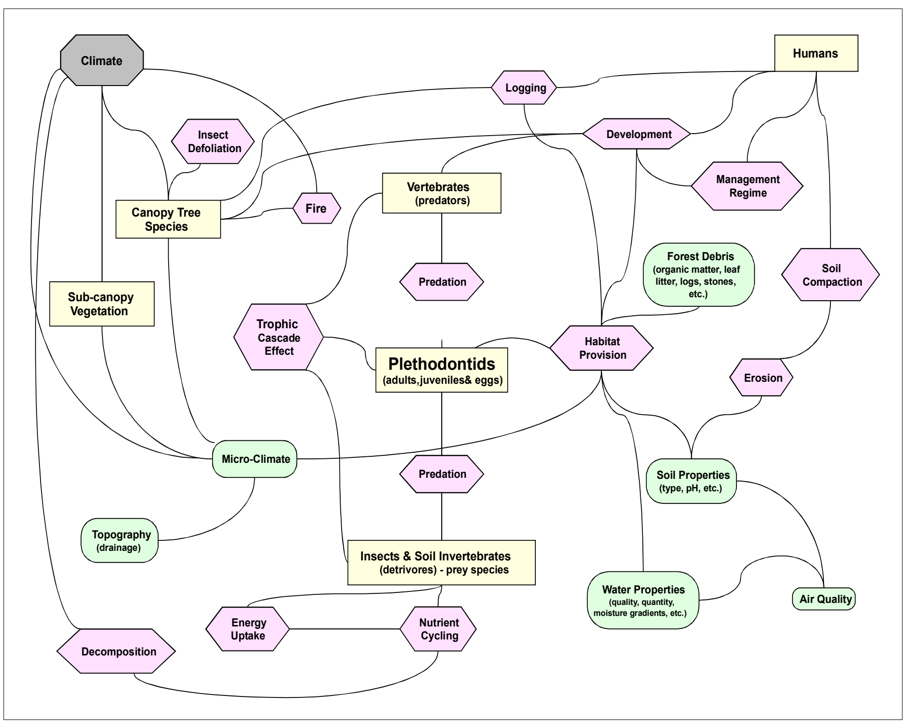


Figure 2 Conceptual model of the role of plethodontid salamanders in a forest ecosystem.

2. Monitoring Objectives

This protocol is intended to address monitoring objectives from an EMAN and Parks Canada perspective. It is also intended to address potential objectives that may be posed by an environmental or community group interested in forest health for conservation or education purposes.

2.1 EMAN's Objective for Plethodontid Monitoring

EMAN's mandate includes tracking environmental changes at a broad, ecozone scale over time. EMAN's objective for this protocol, and other protocols associated with their suite of indicators for the early detection of ecological change, is to catalyze the creation of monitoring programs by a range of groups creating a network of monitoring sites across Canada. Through this network EMAN attempts to address another objective – to track change in forested ecosystems across the country. By considering trends in forest health at EMAN sites (20x20 m and 1ha plots) distributed throughout different ecozones, EMAN will provide an early warning system for identifying future environmental issues.

2.2 Parks Canada's Objective for Plethodontid Monitoring

Parks Canada's mandate includes maintaining or restoring the ecological integrity of national parks. To assess a national park's progress towards this goal each national park selects a suite of ecological integrity indicators as the basis for their long-term monitoring program (for more information see www.parkscanada.gc.ca). The Parks Canada Agency objective for this protocol, therefore, is to provide a standardized method for consistently monitoring plethodontids where they have been identified by a park as an ecological integrity indicator. The park-level monitoring objective for this protocol, however, will vary depending on the specific park management question asked. For example, a national park may be interested in issues across multiple plots that represent a range of forest conditions, stress gradients or management regimes, thereby allowing managers to address goals within their park management plan.

BOX 3: Potential Applications for this Monitoring Protocol on Plethodontid Salamanders

Consistent implementation of this protocol over time can provide a group with a statistically rigorous estimate of population trends of plethodontid salamanders at a plot level. Given the role that plethodontids can play in forest ecosystems these trends may provide an early warning indication of other, potentially more serious, issues of environmental change.

Through the integration with other forest monitoring indicators and the careful placement of plots, with replication, this protocol can be useful for addressing a range of questions that may be posed by a variety of groups.

2.3 Applications for this Monitoring Protocol

The objective for monitoring salamander populations will vary depending on the needs to the group implementing it (*the objective of the protocol - to provide standard methodology - will not vary; the monitoring objectives of different groups may vary*). Depending on a group's monitoring question this protocol could have many applications (see Box 3). Generally speaking, groups interested in this monitoring protocol will have one or many of the following issues in mind:

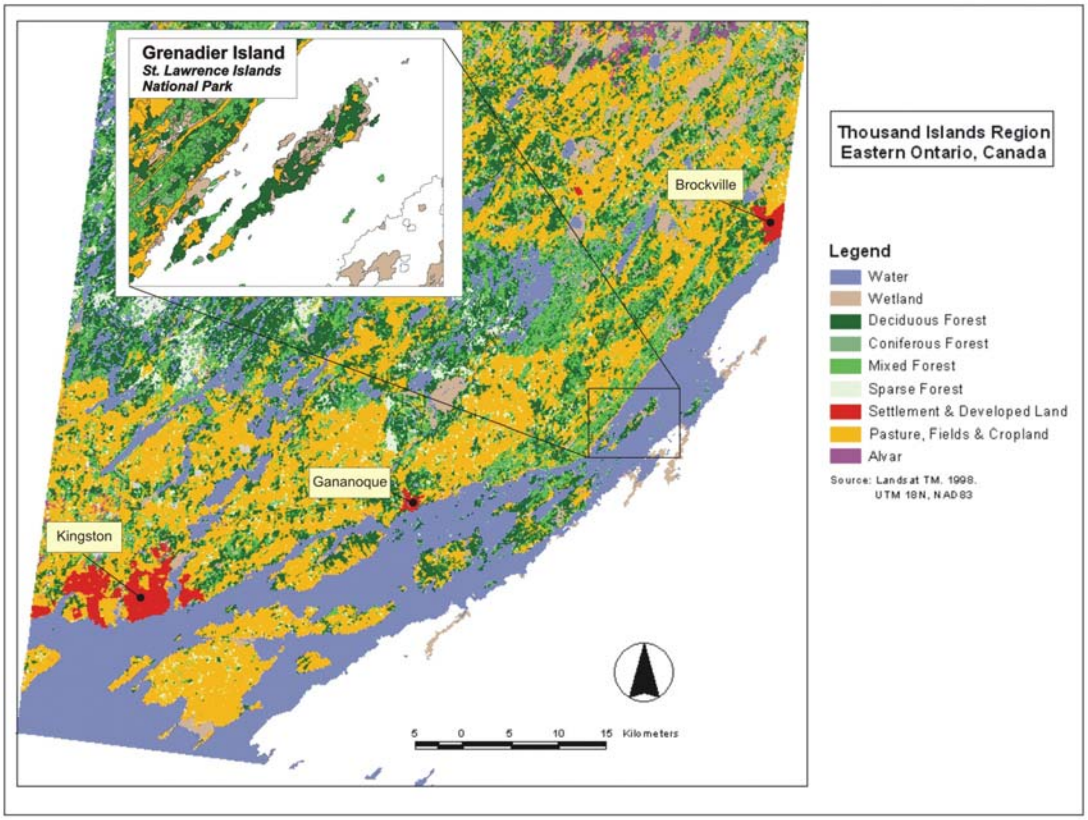
- salamander monitoring as a tool for environmental education,
- collection of baseline information for a protected area,
- plethodontid trends as an indicator of changing forest ecosystem conditions for an area using a number of replicate plots,
- plethodontid trends as an indicator of the impact of management regimes on forest ecosystems (i.e., impacts of clearcut logging versus no clearcut logging, impacts of visitor use in a national park),
- plethodontid trends as an indicator of the impact of anthropogenic stressors on forest ecosystems (i.e., climate change, acid rain).

The objective of the group implementing this protocol will influence the number and location of monitoring plots required. Given the wide range of possible objectives, this protocol cannot be exhaustive in terms of providing complete information on study design or data analysis for all situations. To assist groups interested in monitoring plethodontids this protocol will focus on steps required for establishing 1 plot. An example based on a more complex question requiring replicate plots is also provided (see Box 4). If more guidance is needed, please contact a volunteer monitoring advisor listed in appendix 2.

BOX 4: Example of a Plethodontid Salamander Monitoring Program – *Grenadier Island, St. Lawrence Islands National Park, Ontario.*

Throughout this document Grenadier Island will be used as a hypothetical example to demonstrate the application of this protocol related to study design and data analysis based on a monitoring question posed by St. Lawrence Islands National Park.

Note the land cover types on Grenadier Island from the map legend.



3. Step One: Confirming Presence of Plethodontids

If a group is interested in implementing this protocol, the first step should be to undertake an initial assessment to confirm that plethodontids are present in your study area (if it is not already known). This small pilot project should consist of the following:

What:	This initial assessment should consist of up to three 20 minute searches under natural cover (i.e., rocks, decaying logs, leaf litter).
Where:	The intended study area for your monitoring program.
When:	3 sampling periods should be conducted over three weeks (one site visit per week) during cool, moist, calm weather in the spring or fall.
How:	Begin at a point that is away from forest edge and thoroughly search natural cover objects in a pattern for 20 minutes, remaining within the same habitat type. Once the presence of plethodontids has been confirmed further searches are not required. If no plethodontids are observed during the 3 sampling periods then that study area is likely not suitable because plethodontids are either not present or occur at such low densities that too few individuals will be observed to measure meaningful trends.

4. Step Two: Monitoring Question

After the presence of plethodontids has been confirmed, the next step to designing your monitoring program is to define your monitoring question. This seemingly simple step is not as easy as it appears and will influence all aspects of your program. A properly posed and precise monitoring question can help you to...

- define program goals and objectives,
- adopt appropriate protocols,
- select an effective sampling design,
- choose methods for analysis,
- communicate to partners the intent and results of the monitoring program, and
- better integrate monitoring information into decision making.

All useful monitoring questions should have some common elements. Specifically, a monitoring question should contain:

1. the specific variable (with units) to be monitored,
2. a spatial delineation of the target population,
3. the magnitude and direction of change of interest, and
4. the temporal scale over which trends will be assessed.

The difference in these four elements is evident when comparing the following 2 monitoring questions: “Is the abundance of the Eastern Red-backed Salamander *(Plethodon cinereus)* changing through time?” versus “Is an index of population size (counts) of the Eastern Red-backed Salamander in the greater Fundy National Park ecosystem changing more than ±15% over a 5 year period?”. The first question is too vague and doesn’t suggest a monitoring strategy. The latter question is clear on what specifically is being monitored (an index of population size (counts) of eastern redback salamanders), where it is being monitored (the greater Fundy National Park ecosystem), the time horizon over which trends will be assessed (5 year period), and at what magnitude and direction of change an early warning signal is raised (±15%).

BOX 5: How do you know if your monitoring question is precise enough?

A good rule of thumb when trying to define your monitoring question is to write it such that the criteria for answering it is in the question itself. The only answer required should be “yes” or “no”.

Consider the question: “Is an index of population size (counts) of eastern redback salamanders (Plethodon cinereus) in the greater Fundy National Park ecosystem changing more than ±15% over a 5 year period?”. The answer to this monitoring question is either “yes - counts in eastern redback salamanders changed greater than ±15% over a 5 year period in the greater Fundy National Park ecosystem” or “no - counts in eastern redback salamanders did not change greater than ±15% over a 5 year period in the greater Fundy National Park ecosystem”. If the answer is yes then an early warning signal is raised and more investigation is required. If the answer is no then status quo. The decision point is clear.

Other sections within this protocol help define some of the 4 common elements to good monitoring questions. The specific variable (with units) to be monitored by this protocol is described in section 9 (Data Collection). The magnitude and direction of change of interest, as well as the temporal scale over which trends should be assessed, is discussed in section 11 (Monitoring Threshold and Data Interpretation). The spatial delineation of the target population is determined by a group’s area of interest.

When a monitoring program is beginning for the first time, often not enough information exists to accurately define an appropriate monitoring target or threshold that should be included into your monitoring question. In these cases the first 5 years of implementation should be considered as a *pilot phase* during which baseline data are collected that will then be used to refine the program.

Specific monitoring questions will be influenced by the objectives of the group implementing this protocol. It is not possible, therefore, to provide a list of questions that will satisfy all needs. However, examples of increasingly complex monitoring questions below may be useful in guiding you to pose your own question(s) (also see Box 6).

Example of monitoring question if only 1 plot is established.
Is an index of population size (counts) of plethodontids changing more than ±15% over a 5 year period at Long Point Biosphere Reserve?

- *1 plot representing one type of environment*

Example of monitoring question concerned with trends over time in a range of forest conditions (in this case, mixed forest sites with soils over limestone versus mixed forest sites with soils not over limestone) .
Is an index of population size (counts) of plethodontids changing more than ±20% over a 10 year period in mixed forest sites with soils over limestone compared to mixed forest sites with soils not over limestone within the greater Bruce Peninsula National Park ecosystem?

- *requires replicate plots representing 2 strata*

Example of monitoring question concerned with trends over time in a range of forest conditions and human impact gradients (in this case, mixed forest sites with soils over limestone and mixed forest sites with soils not over limestone that have been clearcut versus those that have not been clearcut).
Is an index of population size (counts) of plethodontids changing more than ±50% over a 3 year period in the Niagara Escarpment Biosphere Reserve in the following sites: 1. clearcut areas of mixed forest with soils over limestone, 2. non-clearcut areas of mixed forest with soils over limestone, 3. clearcut areas of mixed forest with soils not over limestone, and 4. non-clearcut areas of mixed forest with soils not over limestone?

- *requires replicate plots representing 4 strata*

NOTE: These monitoring questions, and the type of trend they describe, are merely examples. The magnitude, direction and temporal scale of the trend of interest will vary and must be carefully considered by each group implementing this protocol.

BOX 6: Example - Monitoring Question.
Grenadier Island, St. Lawrence Islands National Park, Ontario.

St. Lawrence Islands National Park would like to monitor plethodontids as an indicator of future visitor impacts on the forests of Grenadier Island. When planning for their monitoring program they consider the following:

- The park has few staff to allocate to monitoring projects but those staff who are dedicated will be able to monitor on an ongoing basis.
- The park decides to use zoning to represent spatial patterns of visitor use. Zone 1 areas are designated "Special Preservation" and are areas where visitor use is controlled. The park decides to compare trends between Zone 1 areas and other areas where visitor use is more prevalent.
- Given their few staff the park decided to focus monitoring in dense deciduous forest types only as this is the dominant forest type on Grenadier Island. Monitoring across a greater range of forest conditions would require setting up more monitoring plots than the park can handle.
- Early in the planning process the park undertakes a pilot project to survey Grenadier Island for eastern redback salamanders (the most abundant plethodontid in the region). They discover that the relative abundance is 12% (with an average variation of ±5%) higher in the Zone 1 deciduous forest areas compared to non-Zone 1 areas of the same forest type. Based on this baseline information the park tentatively decides that a change beyond 17% (12% + 5%) between Zone 1 and non-Zone 1 areas would be beyond what they would reasonably expect given current visitor use patterns and would indicate an early warning signal.
- The park decides to analyze trends over a 5 year period to coincide with their park management plan review.

Given the above...

The specific variable (with units) to be monitored = index of plethodontid population size (counts)
Spatial delineation of target population = deciduous forest in Zone 1 compared to deciduous forest not in Zone 1 on Grenadier Island
Magnitude and direction of change of interest = ±17% change in Zone 1 areas compared to non-Zone 1 areas
Temporal scale over which trends will be assessed = 5 years

Therefore, they define their monitoring question as:

"Is an index of population size (counts) in eastern redback salamanders changing more than ±17% over a 5 year period in deciduous forest - Zone 1 areas compared to deciduous forest - non-Zone 1 areas on Grenadier Island?".

Their monitoring data will give them a "yes" or" no" answer.

As you can see, a lot of work can go into defining an appropriate monitoring question. The effort is worth it as careful planning can save a great deal of time and money if instead you invest in a monitoring program whose data does not meet your management needs.

5. Step Three: Identifying Methods for Data Analysis

To some the identification of methods for data analysis may not seem as the logical next step. Often times this important step is not considered until after data have been collected. After all, why worry about data analysis if data have not been collected yet? There are two main reasons why methods for data analysis should be considered here. First, if no plan for analyzing monitoring data is developed ahead of time, then often no analysis takes place. In the end a great deal of effort may be spent in collecting field data, but no actual monitoring will have occurred. After all, if data aren't properly analyzed and interpreted, then your monitoring question can't be answered! Second, the method for data analysis must be identified before you can define your sampling frame (step 4). Sampling frame is critical to any monitoring program as it specifies how large of a sample size is required to detect what magnitude of change with what level of confidence. This information is vital to defining appropriate sampling effort, assigning tasks for staff, managing resources, etc.

Appropriate methods for data analysis are determined by the question being asked and the data type(s) used in the analysis. There are four data types:

BOX 7: Excel Spreadsheet for Automated Analysis of Plethodontid Monitoring Data.

A spreadsheet accompanies this protocol that provides a template for data entry and provides some basic descriptive statistics and trend analysis of your monitoring data. This spreadsheet will be useful for basic types of analyses that address basic types of monitoring questions.

A copy of this spreadsheet can be found at the EMAN website.

1. Nominal: Data are categories without order (i.e., yes / no, present / absent, male / female).

2. Ordinal: Data are categories with order (i.e., high, medium, low).

3. Interval: Data are categories with order and with meaningful differences between the categories. Counts are interval data. They have order (you count 1 salamander before you count 2 salamanders) and there are meaningful distances between the categories (2 salamanders are double 1 salamander and half of 4 salamanders).

4. Ratio: Data are a special type of interval data that have an absolute zero value (i.e., height, weight).

This protocol calls for monitoring the trends in plethodontid counts at a plot level over time. Both plethodontid counts and time (as measured by a typical calendar) are interval data and so an appropriate method for analysis may be *simple linear regression*. However, one may also be interested in these trends between plots in a protected area versus outside a protected area. This additional variable is nominal (2 categories – inside protected area and outside protected area) and so an appropriate method for analysis to compare trends between these 2 groups

BOX 8: Example - Methods for Data Analysis
Grenadier Island, St. Lawrence Islands National Park, Ontario.

St. Lawrence Islands National Park is interested in 1. tracking trends at a plot level over time and, 2. trends across plots located in deciduous forests in Zone 1 areas versus plots in deciduous forests in non-Zone 1 areas.

1. Tracking trends at a plot level over time...

dependent variable = annual plethodontid counts from plot (interval)
independent variable = time, years (interval)
method of analysis = simple linear regression

2. Tracking trends across plots over time...

dependent variable = annual plethodontid counts from plots (interval)
independent variable = Zone 1 areas / Non-Zone 1areas (nominal)
time, years (interval)
method of analysis = general linear model

may be an *independent samples t-test*. If this additional variable had 3 or more categories, such as categories combining areas of different soil type in and out of a protected area, then the appropriate analytical method may be *ANOVA (analysis of variance)*.

Another analysis method that should be of particular interest for monitoring is general linear models (GLM). GLMs are very flexible and allow one to consider relationships between variables of varying data types, are tolerant to unbalanced data, and can incorporate the functions of simple linear regression with t-tests or ANOVA. Through GLMs it is possible to simultaneously consider trends over time (through repeated measures tests) and across space (by using variables to create spatial units for comparison – i.e., Zone 1 areas versus non-Zone 1 areas) while also testing for the influence of bias variables such as observer changes or weather patterns (see section 9.1 for more information on bias).

This protocol cannot be too prescriptive in outlining methods for analysis as they will change depending on the monitoring question of a particular group. It is recommended when designing your monitoring program that you consult with someone familiar with statistics.

As described in Box 7, a Microsoft Excel spreadsheet is available for this protocol to assist with many analysis needs. This spreadsheet provides a standard template for data entry and provides some automated analysis functions. Please see this spreadsheet for more information on methods of data analysis for this protocol.

6. Step Four: Sampling Frame

6.1 An Introduction to Sampling Frame

Once the method for data analysis has been identified, the sampling frame for your monitoring program should be considered. Sampling frame refers to the relationship between the sample size, the minimum magnitude of change that your monitoring program can detect, and the probability that the trend you observe in your sample over time reflects the true trend (minus the probability of making an error). This relationship is important to consider because during the initial development of a monitoring program the following questions are typically asked:

"How large of a sample size do I need to collect to detect a certain minimum detectable trend?" (the required level of precision for the monitoring program is known ahead of time but the needed sample size to achieve this precision is not), or

"How small of a change can I reliability detect with a given sample size?" (the capacity of the group implementing the monitoring program, and therefore the sample size, is fixed and known ahead of time but the level of precision this sampling effort provides is not).

A monitoring program's sampling frame is defined through the identification of 3 primary elements: probability of error, minimum detectable trend (also referred to as "effect size"), and sample size.

Probability of Error

Probability of error refers to the chance that the trend you detect in your sample does not reflect the true trend. There are 2 types of errors that can be made. First, you may conclude, based on your sample, that a significant change has occurred when in fact it hasn't. This false alarm is referred to as a Type 1 error. The probability of not making a Type 1 error is called the confidence level. Second, you may conclude, based on your sample, that a significant change has not occurred when in fact it has. This failure to detect a trend is referred to as a Type 2 error. The probability of not making a Type 2 error is called sample power.

Traditionally, for scientific publications, the standard for confidence level is set at 95% and sample power is 80%. For conservation purposes, however, these values are not ideal. A confidence level of 95% may not be proactive enough, since by the time you monitor to the point that you are 95% confident that a trend has actually occurred and it is not a false alarm, it may be too late for management action. A sample power of 80% may also not be appropriate. From a conservation perspective, Type 2 errors (failing to detect a significant change) are more important than Type 1 errors as they result in a failure in our monitoring program to detect the type of trend it was designed to detect. Based on a precautionary principle, therefore, a conservation agency may choose to increase their standard for sample power.

In the end it is up to each group to determine their standards for probability of error for themselves. Detecting a negative trend with a confidence level of 70% may be enough to motivate one group into action where it may not be for another group depending on the risks involved. The important idea is that each group undertaking monitoring should be aware of the probabilities of errors associated with their program and how they may influence their decisions.

For government conservation agencies, such as EMAN and Parks Canada, it is recommended that a *confidence level of 80%* and *sample power of 90%* be used when determining sample frame and required sample sizes. These values are consistent with the Terrestrial Salamander Monitoring Program (Droege et al., 1997) developed in the United States and with other monitoring programs in Canadian national parks (Zorn, 2000).

<u>Minimum Detectable Trend</u>

Larger trends are more obvious than smaller trends and so are easier to detect. Larger trends can be detected with smaller sample sizes with higher levels of confidence and sample power. Trends that naturally have low variability are also easier to detect than trends that have higher variability. The minimum trend that a monitoring program can detect given its sampling frame should be known as precisely as possible. This will allow managers to determine if their sampling effort can detect minimum trends that are ecologically meaningful.

As a *minimum standard*, EMAN and Parks Canada recommends a sampling effort associated with a minimum detectable trend of *at least a 50% decline over 20 years*. Again, this recommendation is consistent with the Terrestrial Salamander Monitoring Program (Droege et al., 1997). It is expected that many groups implementing this protocol, such as national parks, will exceed this minimum and be able to detect smaller trends.

<u>Sample Size</u>

Depending on the confidence level, sample power, amount of variability in plethodontid counts, and the minimum detectable trend, a required sample size can be estimated. The process of determining an appropriate sample size from these sampling frame parameters is called <u>power analysis</u>. From this required sample size the number of ACOs to be placed at each 20x20 m or 1 ha forest health monitoring plot can be determined.

6.2 Power Analysis and Determining the Number of ACOs Needed

Methods of power analysis for identifying appropriate sample sizes vary according to the method of data analysis (which is why "Identifying Methods for Data Analysis" is step 3). Given the possible range of monitoring questions and associated analytical methods, therefore, this protocol can not be too prescriptive on how to conduct a power analysis for your monitoring program. As with data analysis it is recommended that you consult with someone in your area familiar with statistics or contact a support person listed in appendix 2. Assuming some common questions, however, some advice can be given:

Power Analysis for Estimating Trends at the Plot Level

For monitoring questions concerned with tracking trends in plethodontid populations at a plot level (and analyzing data using linear regression with salamander counts as the dependent variable and time as the independent variable), a useful – and free – tool for conducting power analysis is software called MONITOR (available at http://www.mbr-pwrc.usgs.gov/software/monitor.html). A wide range of other on-line and commercial power analysis software exists that can address a host of data analysis methods. See Thomas & Krebs (1997. A Review of Statistical Power Analysis Software. Bulletin of the Ecological Society of America. 78(2): 126-139.) for a discussion of these software packages (available for download at http://www.zoology.ubc.ca/~krebs/power.html).

Determining the Initial Minimum Number of ACOs Needed for each Plot

Conducting a power analysis at the beginning of a monitoring program poses some problems as a number of factors are usually unknown, such as:

- the average number of plethodontids found at a plot is unknown,
- the variability in the number of plethodontids found over successive surveys is also unknown,
- the density of plethodontids in the study area is unknown, which makes it difficult to estimate the number of ACOs per plot needed to reach an appropriate sample size for your sampling frame.

Given this lack of information, some "rules of thumb" are provided in table 1 that suggest an initial <u>minimum</u> number of ACOs per plot. These "rules of thumb" are based on experiences from existing terrestrial salamander monitoring programs across the country.

Table 1. "Rules of thumb" for initial minimum number of ACOs at a 20x20m or 1ha forest health monitoring plot.

Initial ACOs for British Columbia = 20
Initial ACOs for Eastern Canada = 40

Conducting a Review to Refine Number of ACOs Needed

Every monitoring program in its initial stages should be viewed as a pilot project. The first few years of data collection are essential for determining the characteristics of your samples and the parameters needed to undertake a detailed power analysis. Through power analysis, managers can make sure that an adequate monitoring effort is applied and they can be confident that the data the program generates will be robust enough to answer their monitoring questions.

After installing the number of ACOs at a plot according to the "rule of thumb" for your area, and after implementing the data collection portions of this protocol for 3 years, a review of your program should be undertaken to refine the number of ACOs (and, therefore, the sampling frame) for your plot. These 3 years of data will provide the information required to conduct a power analysis specific to your monitoring program. From this information, the required sample size and number of ACOs needed can be adjusted to ensure that your monitoring question(s) are adequately answered with a known probability of error. Support for conducting power analyses can be found by contacting EMAN or Parks Canada (see appendix 2).

BOX 9: Review and Power Analysis of Baseline Monitoring Data After 3 Years.

After the first 3 years of implementation a formal review of your plethodontid monitoring program should take place. These 3 years will provide enough baseline information to conduct a power analysis and refine your sampling effort – including ensure enough ACOs are in place to attract an adequate sample of plethodontids.

Data from the first year of implementation will not be representative as the wooden cover boards require one full year of weathering before they are likely to effective in attracting salamanders.

Data from the following two years can be used as inputs into a power analysis. This review period is essential but often lacking in many monitoring programs. This review and power analysis will ensure that sample sizes are large enough given your situation to answer your monitoring question.

If no support for power analyses is available in your area, contact EMAN or Parks Canada from the list of volunteers in appendix 2.

6.3 Autocorrelation

An important consideration in a monitoring program's sampling frame and data analysis is autocorrelation. Autocorrelation occurs when samples are not independent and observations do not represent a full degree of freedom in statistical tests. The lack of independence violates data assumptions associated with many types of analysis. The result is that statistical tests will overestimate the true degrees of freedom and may conclude that trends are statistically significant when, in fact, they are not (a type 1 error). Common sources of autocorrelation in monitoring programs are spatial autocorrelation (often due to samples that are spatially clustered) and temporal autocorrelation (when samples taken close together in time are not independent, they are temporally clustered.). Since monitoring is the sequential recording of observations (in this case, number of terrestrial salamanders) through time, temporal autocorrelation is particularly common. The potential influence of autocorrelation on a monitoring program is so important that it has recently motivated the removal of the MONITOR 7.0 power analysis software for download (as of summer 2004), however, other power analysis programs are available.

A full discussion on autocorrelation is beyond the scope of this protocol but it is recommended that readers become familiar with this term and its relevance for monitoring. It is also recommended that groups consult with someone familiar with statistics and autocorrelation for assistance in the development of your monitoring program.

It is assumed that most groups using this protocol will not have in-house expertise related to the issue of autocorrelation. However, we do not recommend that autocorrelation become a barrier to motivated groups becoming active in monitoring. Therefore, it is our advice that groups should be especially cautious in the development of their sampling frame. If sampling effort is based on a cautiously high confidence level and power (80% and 90% respectively or greater) then it is more likely that a statistically significant trend will be of management relevance, even if degrees of freedom are deflated because of autocorrelation.

It is also important to note that statistical significance does not necessarily infer ecological significance or management relevance. Monitoring data may provide valuable information even in the presence of autocorrelation.

7. Step Five: Acquiring ACOs

From step 4 (Sampling Frame) you have an initial number of ACOs required for monitoring and a plan for refining this number after a 3 year review. The next step is to acquire the ACOs.

ACOs are typically wooden boards placed on bare soil or the litter on the forest floor. A number of plethodontid studies have been conducted that use wooden boards of varying tree type, size and design. Table 2 describes the variety of wooden ACOs used. Figure 4 depicts examples of cover board designs that have been used in monitoring studies. The consensus from this experience is that consistency in ACOs over time at a given plot is more important than the specific type of wood used, board design or size (given that the board is a minimum of 8 inches wide). The exception to this is ACOs for monitoring the Wandering Salamander *(Aneides vagrans*). For this species, layered cover boards are recommended, as the spaces between boards mimic its natural microhabitats within decaying logs (Davis 1997).

Table 2. Summary of the board dimensions and type of wood used by researchers during terrestrial salamander surveys in the field.

Author	Board Dimensions	Type of Wood
Droege et al. (1997)	1 foot x 1 foot x 1 inch	rough cut oak
Sugar et al. (2001)	LAYERED Bottom: 30 x 8 x 1 inch Top: 2 bds of 4 x 1 x 30 inch	rough cut spruce, pine, fir
Sugar et al. (2001)	30 x 8 x 1 inch	rough cut spruce, pine, fir
Davis (1997)	LAYERED Bottom: 180 x 30.5 x 5 cm; top: two boards, 180 x 15 x 2.5 cm each	untreated, rough cut, full dimensional lumber of Douglas Fir
Enright (1999)	100 x 20 cm	hemlock
Hackett et al. (1999)	76 x 20x 2.5 cm	
Szuba (1999)	76 x 20 x 2.5 cm	rough cut pine
Brooks (1999)	100 x 25 x 4 cm	rough cut hemlock (pine in talk)
Morneault (1999)	76 x 20 x 2.5 cm	seasoned red pine
DeGraaf & Yamasaki (1992)	100 x 20 2.5 cm	pine
Kolozsvary & Swihart (1999)	125 x 30 x 15 cm	pine
Pauley (1999)	26 x 18 x 2 cm	
Ovaska and Sopuck (2001)	LAYERED Bottom: 90 x 30.5 x 5 cm; top: two boards, 90 x 15 x 2.5 cm each	untreated, rough cut, full dimensional lumber; Douglas Fir
Jaeger et al. (2001)	20 x 24 x 2 cm	untreated pine
Craig (unpublished)	LAYERED (angled) Bottom: 61 x 25 x 5 cm Top:61 x 25 x 25 cm Middle: strip 61 x 3.5 x 1.2 cm	rough cut, untreated white pine
Craig (unpublished)	LAYERED (V-shaped) Bottom: 61 x 25 x 5 cm Top: 2 bds of 61 x 12 x 5 cm	rough cut, untreated white pine
Craig (unpublished)	30.5 x 25 x 5 cm	rough cut, untreated white pine
Craig (unpublished)	30.5 x 25 x 2.5 cm	rough cut, untreated white pine
Monti et al. (2000)	10 x 25 cm	thin cedar shingles

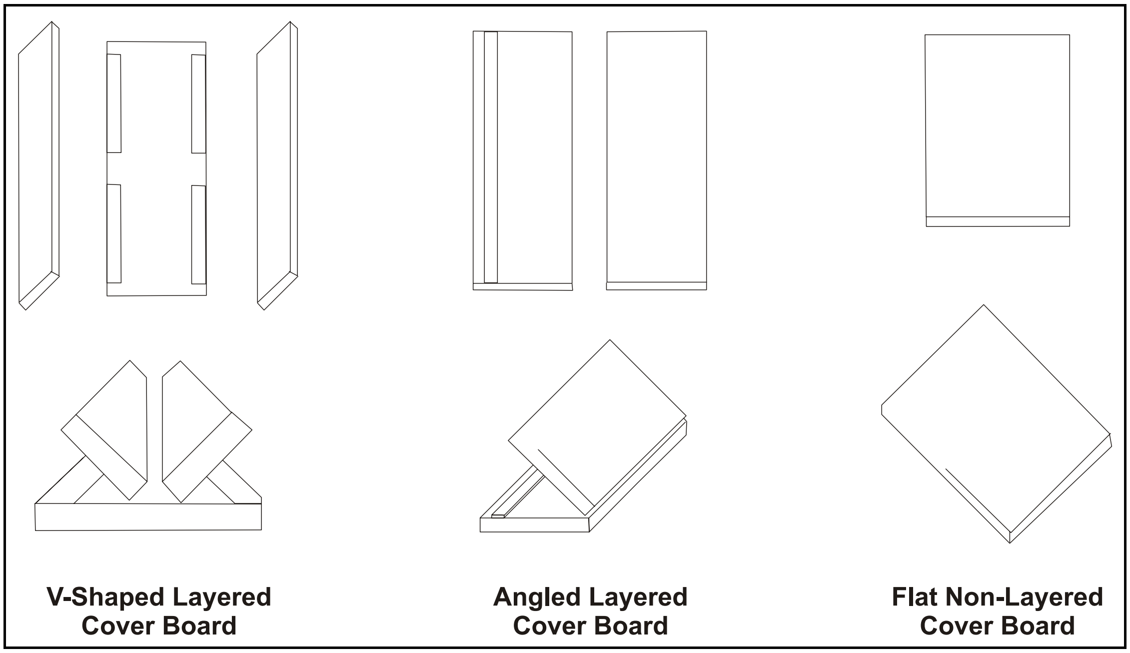


Figure 3. Common designs of wooden artificial cover boards used for plethodontid monitoring.

Since consistency is more important than the characteristics of ACOs, the following is recommended:

- Local saw mills should be contacted on availability and cost of wooden cover boards (look in your local yellow pages).
- ACO size should the consistent with no edge shorter than 8 inches.
- ACOs can be of any type of wood provided that it is consistent over time. The type of wood used should be predicated on what is readily available and affordable from local saw mills.
- ACOs should be simple, non-layered cover boards unless *Aneides* is the target species for monitoring (in which case V-shaped cover boards are recommended).

See appendix 3 for details on the design of wooden cover boards.

8. Step Six: Study Design

> "The most critical stage of implementation and completing a monitoring study is not data collection, presentation or interpretation, but rather design. Careful design will increase effectiveness, reduce costs and lead to improved interpretation." (Jones, K.B. 1986. The Inventory and Monitoring Process. pp. 1-10 in A.Y. Cooperrider, R.J. Boyd and H.R. Stuart (eds.). Inventory and Monitoring of Wildlife Habitat.)

Careful planning for monitoring programs is essential. By following this protocol so far you have...

- established why plethodontids are valuable indicator species for forest health (section 1, Introduction),
- confirmed that plethodontids occur in your study area (section 3, Confirming Presence of Plethodontids),
- defined your specific monitoring question (section 4, Monitoring Question),
- created a plan for analyzing your monitoring data (section 5, Identifying Methods for Data Analysis), and
- identified how many ACOs you will initial install at your 20x20m or 1ha plot with a plan for refining this number after 3 years.

The next step in the planning process is to determine where to locate your 20x20m or 1ha plot(s) – if they are not already established -- and how to arrange the ACOs around the plot(s).

8.1 How to Choose a Monitoring Location

Site selection for monitoring is one of the most important decisions to make when developing a program. It is vital that your monitoring sites adequately represent the types of conditions identified in your monitoring question. Through randomly placing monitoring sites, with replication, in areas that represent specific conditions data can be applied to your monitoring questions and be used for decision making more readily.

Not surprisingly, an appropriate strategy for study design is influenced by the monitoring question. More complicated questions require more complicated study designs. Once again, due to the possible range of questions groups who are interested in this protocol may ask, it is not possible to describe an exhaustive strategy for study design. If a group is wanting to create a monitoring program that will involve multiple forest health plots and would like some advice, please contact one of the advisors listed in appendix 2.

BOX 10: The 3 R's of Study Design – Representation, Randomization and Replication

Proper representation of the condition(s) of interest is a common feature of well designed monitoring programs. Representation of a range of conditions is often accomplished through dividing the study area into strata. Strata are homogeneous units that represent a unique condition. For example, to represent a range of conditions in a forest ecosystem one may delineate strata that represents each soil type and dominant canopy cover type present in a study area.

Once strata are delineated that represents the range of conditions of interest, well designed monitoring programs strive to randomly locate sites within these strata. Randomization of monitoring locations is essential to infer trends in your study area from plot-based trends. Inferences from randomly located monitoring sites are possible because every possible location has an equal probability of being chosen. If this probability is not equal, then it is possible that the monitoring location is biased and does not truly represent the condition of interest. An assumption of probabilistic sampling is violated.

Well designed programs also strive to replicate monitoring sites. This replication takes place over space (multiple plots within strata) and over time (multiple sampling points over time). Replication ensures that the variation in environmental conditions is better represented. Replication also strengthens a program's sampling frame as it increases sample size and allows smaller trends to be detected at higher levels of confidence and power.

Study Design Advice for Establishing 1 Forest Health Plot

A group may be interested in establishing only one monitoring plot, perhaps for educational purposes. If a group is planning on establishing only one plot, its location does not have to be random. In these instances plot location should be determined on more logistical criteria (i.e., access to property, difficulty in reaching plot). Before the plot is established, make sure Step One of this protocol is completed and the presence of plethodontids is confirmed.

Study Design Advice for Establishing Multiple Forest Health Plots

Achieving replication by setting up multiple monitoring plots is strongly recommended. If multiple monitoring plots are planned, then a concerted effort to develop an appropriate study design is warranted. Each study design will be unique; however, the following are some generic guidelines that may be helpful:

Before Going into the Field...

- Collect spatial information on the range of forest conditions in your study area. If possible, this information should include not only dominant land cover (i.e., from satellite imagery or aerial photographs) but also sub-canopy vegetation, soil type and geology. Below canopy level information is important to represent the range of forest conditions. Ideally, geographic information system (GIS) technology will be available. Collect other spatial information as required to address the specific monitoring question.
- From this spatial information delineate strata such that the forest conditions within each strata is homogeneous.
- Create random point(s) within each strata. Ensure that points are no closer than 50 m from the edge of each strata to control for possible edge effects (Droege 1999). Also,

ensure that points are well dispersed (not spatially clustered) within the strata. These randomly generated points represent plot locations. Generate more random points than required in case some are found to be not appropriate after a site visit.

In the Field...

- Find random plot locations on the ground using map, compass and GPS.
- Evaluate whether the random point adequately represents the desired forest condition(s). Frequently, random points do not adequately represent the forest condition of interest due to coarse, incomplete or inaccurate information used to delineate the strata.
- If the random point is appropriate, then establish monitoring plot.
- If the random point is deemed to be not appropriate then a new site must be found. With a compass and tape measure (or GPS) move 50 m north from the random point and assess whether this new location is adequately representative. If yes, then establish plot. If no, then return to the original random point and move 50 m east and repeat. Continue clockwise (south and west) until an adequate location is found. If no appropriate site can be found, then abandon the location and move to a new random point generated for that strata. Continue process until an appropriate location is found. (While potentially labour-intensive, ensuring proper representivity is vital to a good study design.)

8.2 How to Arrange ACOs at Forest Health Monitoring Plots

Once the location of monitoring sites have been found, the ACOs need to be placed. Figure 4 displays the arrangement of ACOs about the 20x20 m plots. Figure 5 shows the arrangement for a 1ha SI/MAB plot. ACOs are to be arranged around the perimeter of the plot at 10 m from the plot edge. A systematic design is recommended with the ACOs placed at equal distance from each other. Since home ranges are typically small and many plethodontids display territorial behaviour, no ACO is to be closer than 5 m to another. Depending on the number of ACOs to be placed at a plot (the number of ACOs may be adjusted from the initial recommended number – section 6.2) more than one perimeter row of ACOs may need to be installed adjacent to a plot edge.

When installing each ACO, place them completely flat on the ground to maximize the amount of contact with the forest floor (they can be placed on forest litter and need not be in direct contact with bare soil). If the ground is uneven such that placing an ACO flat is not possible then move the location further up the perimeter until a flat area is found (remember to keep subsequent ACOs 5m away). If the duff layer on the forest floor is too deep so that reaching bare soil is not possible then simply remove the top leaf litter until moist debris is found and install the ACO. <u>Cover ACO by replacing debris and individually mark their location with a pin so they can be more easily found once they are buried. Assign each ACO a unique identification number, and clearly mark them (e.g. with numbered aluminum tags stapled to the upper right corner).</u>

After installing the ACOs around the plot, the ACOs will need to weather for one full winter.

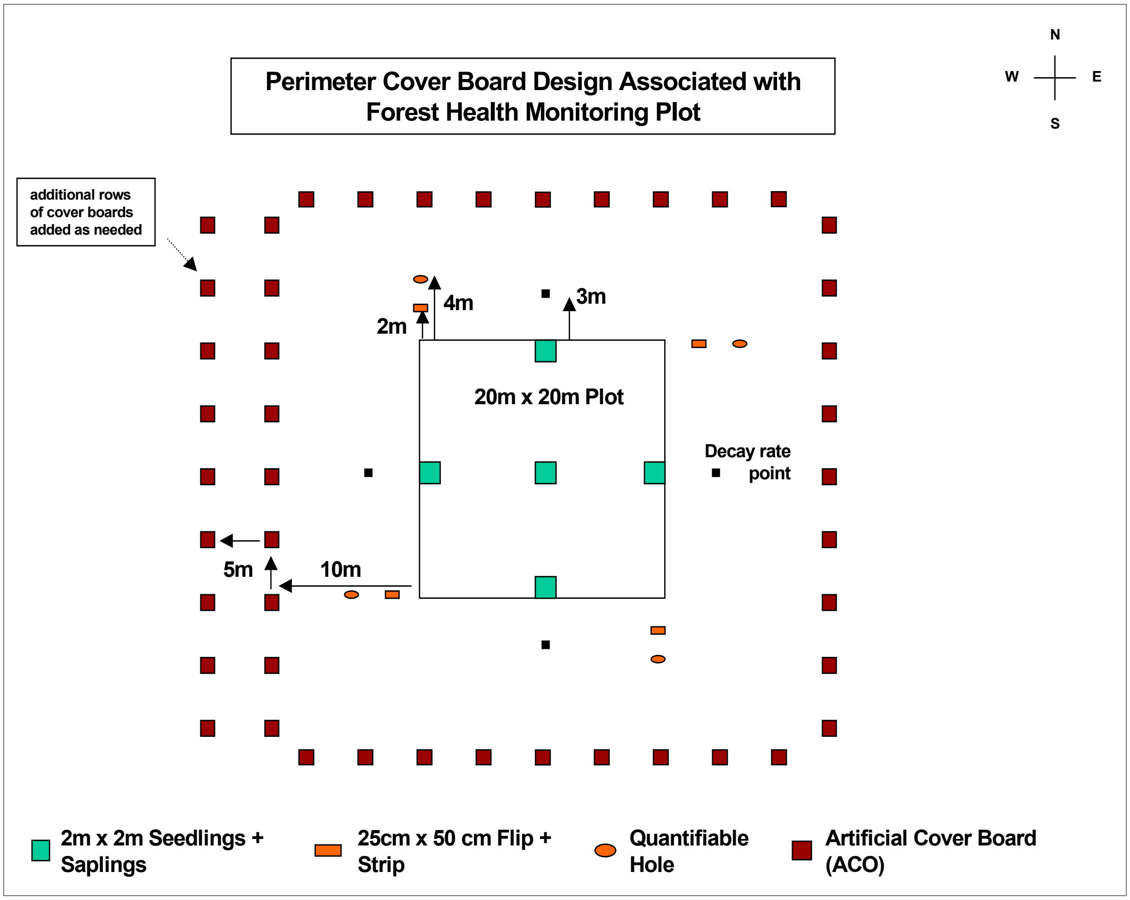


Figure 4. Arrangement of ACOs at a 20x20m EMAN forest health monitoring plot. Other sampling points (i.e., decay rate point, 2x2m seedlings & saplings) are associated with other EMAN forest health indicators.

Experience with other plethodontid monitoring programs across North America agree that new cover boards are less effective in attracting salamanders than are weathered boards. After the ACOs have been installed and weathered, then data collection can begin.

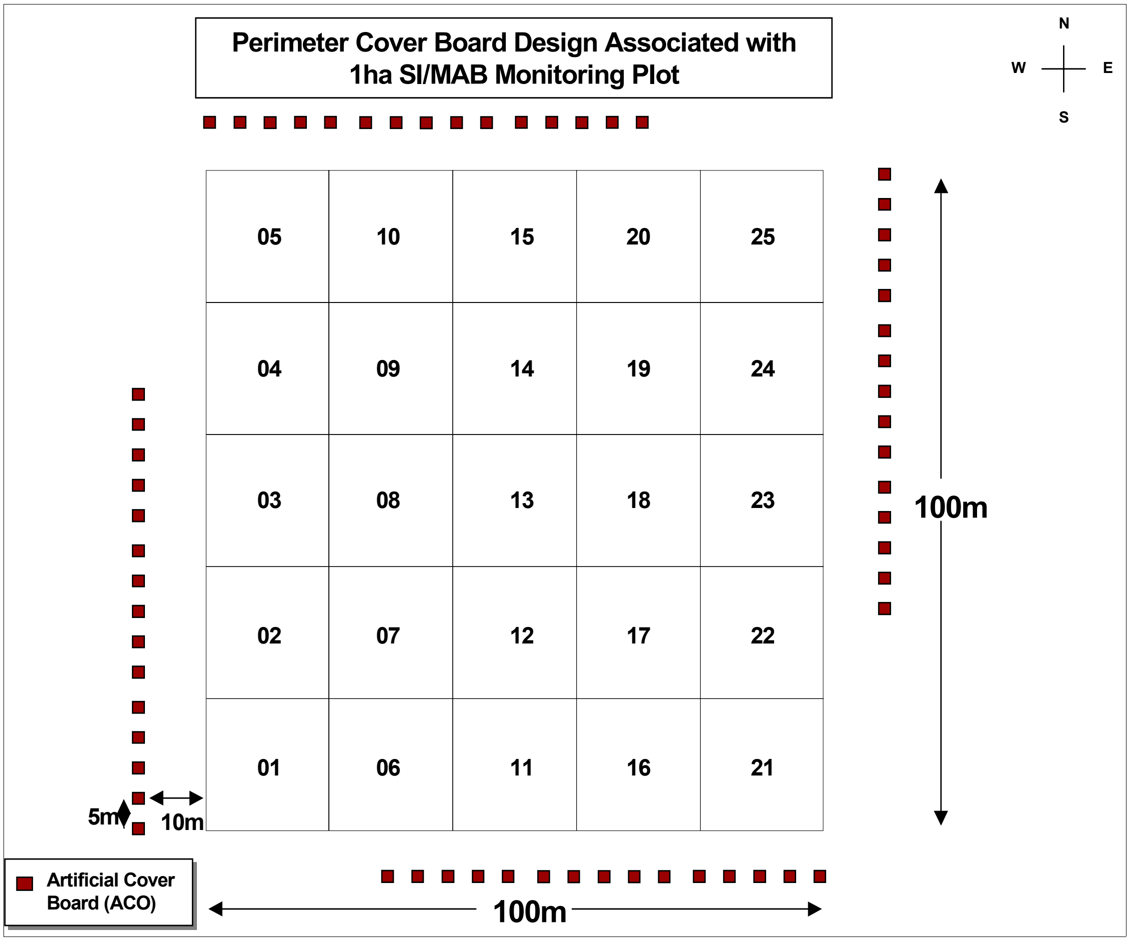
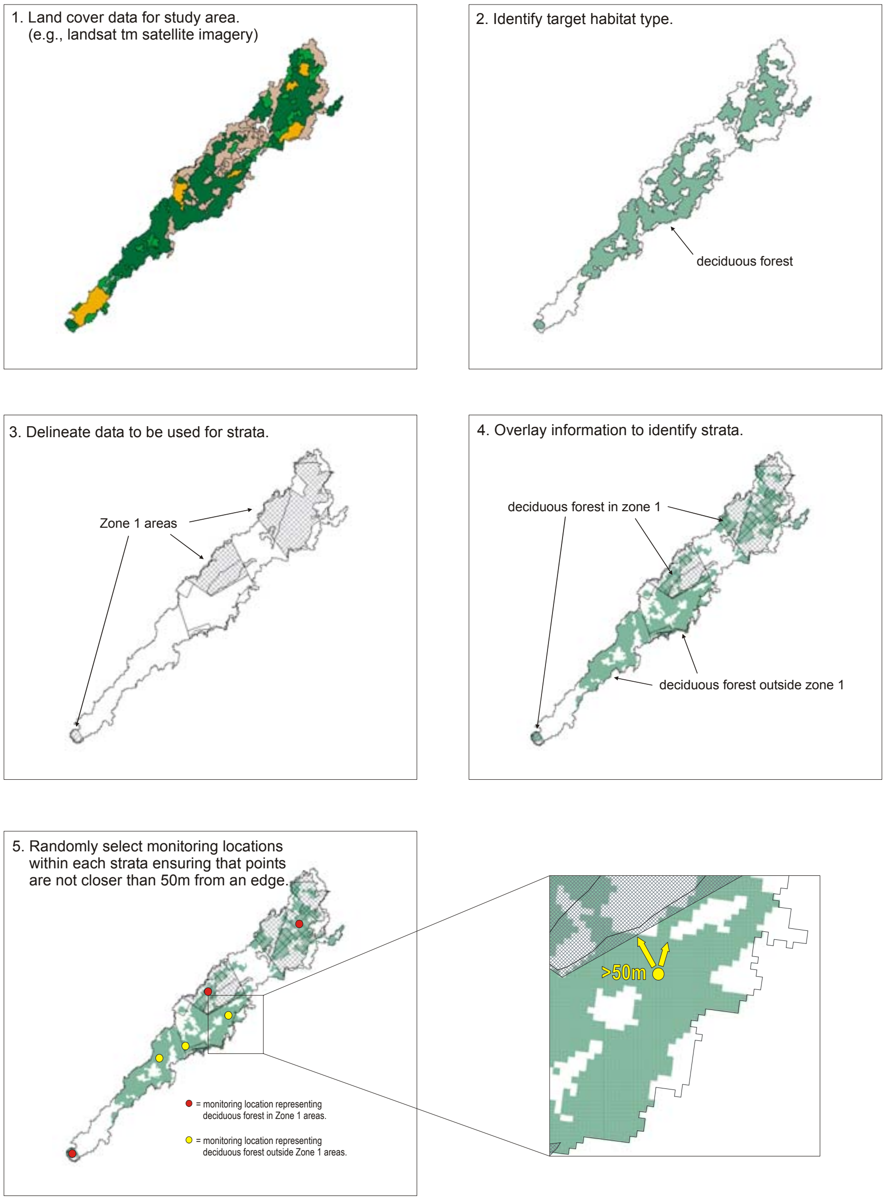


Figure 5 ACO placement around a 1ha SI/MAB monitoring plot. The total number of ACO's are divided by 4 and evenly distributed around each plot side.

BOX 11: Example - Study Design. Grenadier Island, St. Lawrence Islands National Park, Ontario.

St. Lawrence Islands National Park is interested in monitoring trends in forest health in Zone 1 areas versus non-Zone 1 areas on Grenadier Island. Using GIS, the park undertakes a 5 step process for identifying potential monitoring sites for 20x20m forest health plots. The park focuses on deciduous forest since it is the dominant forest type on the island. From natural resource inventory data the park confirms ahead of time that the island is relatively homogeneous in terms of soils and geology.



9. Step Seven: Data Collection

<u>Variables to be Collected</u>

There are two lists of variables that data are to be collected for – a mandatory list and a preferred list. *All groups implementing this protocol are to fully complete data collection for the mandatory list.* If a group has the capacity and interest, then supplementary "preferred" variables can be added (these additional variables are useful if a group is conducting a more intensive study).

> **BOX 12: Data Comparability Among Different Plethodontid Monitoring Programs**
>
> A common concern when developing a monitoring program is the comparability of data with other programs that may have different protocols. Given the wide range of monitoring objectives from various government and non-government organizations it is not surprising that more than one protocol may be available for a particular indicator – including plethodontids.
>
> The comparability of data from different plethodontid monitoring programs depends on the intended "comparability measure". It is not appropriate to pool raw data from monitoring programs that employ different methods as these differences represent a form of bias. However, it is appropriate to compare programs if the "comparability measure" is the direction and magnitude of trend. It is reasonable to expect that if multiple monitoring programs are reporting a consistent negative trend over a large spatial scale that this result should be of concern, even though these multiple programs may have different protocols.

<u>Mandatory Variables</u>: plot location, name of organization, number of salamanders observed per species per plot, ground temperature (air temperature at ground level), air temperature (at chest height), precipitation during last 24 hours (yes or no), Beaufort wind and sky codes (see appendix 4), time of day, date, observer names, ACO identification number, number of days since last ACO check, age of ACOs, disturbance to ACO (if any), additional comments (if any).

<u>Additional Preferred Variables</u>: wind speed (km/h) , soil moisture (kPa - soil / water trension), soil pH, snout-vent length per salamander (mm), vent-tail length per salamander (mm), weight (grams), age class per salamander (adult, juvenile, larvae, egg mass), sex of salamander.

<u>Equipment Needed</u>

For mandatory variables...

(for locating plots)

map of site, compass and GPS unit, 50 m measuring tape

(for data collection)

Field Sheet A from appendix 5, clip board, pencil or waterproof pen, thermometer

For preferred variables...
Field Sheet B from appendix 5, wind speed meter, relative humidity meter, soil pH meter, soil moisture meter, calipers, plastic bag with moist towel or spray bottle (to keep hands moist when handling salamanders), clear plastic container

How to Monitor Plethodontids Using ACOs

- 2 people at a minimum (preferably 3) are needed for data collection. One person will be the data recorder using Field Sheet A provided in appendix 5. The other 1 or 2 people will be the observers.

Mandatory Variables:

1. Record information about the monitoring location and the names of the field crew. Included here should be date and time of data collection, time since last check, air temperature, ground temperature, precipitation in last 24 hours and Beaufort wind and sky codes. For air and ground temperature collect 2 readings along each perimeter row of ACOs (8 readings total) and record the average value.
1. Record information about the ACO – its unique number, age (number of years), and disturbance (i.e., moved by animal, infested with ants).
2. Observers lift each ACO, identify and count the numbers of each species of salamander seen under the cover board. Observations are called out to the recorder and the recorder calls back the data entry to ensure correct data input (this minimizes entry errors).
3. Replace the ACO as quickly as possible ensuring no rocks or pieces of wood have fallen into the space keeping the boards from lying directly in contact with the ground (if ACOs are not replaced quickly or a space is left, the soil will dry out making the habitat unsuitable (Fellers and Drost, 1994). ***If the salamander(s) have moved replacing the board my crush the salamander. In this case capture the salamander(s) gently in your hand, replace the board, and release the salamander near the board edge so that the salamander can again crawl under the board. Ensure that your hands are not contaminated with insect repellent or any other compound that would affect the health of the***

Figure 6. Snout-vent length being recorded on two eastern redback salamanders using moist, clean hands, a plastic container and a millimeter ruler. (photo - Long Point Biosphere Reserve, Ontario)

salamander. Remember, plethodontid salamanders will absorb chemicals through there skin which could seriously impair their health, or even kill.

4. Record any additional comments or observations that may be of interest (e.g. disturbed ACOs from animals, ant colonies under boards, vandalism, etc.).

Additional Preferred Variables:

1. From each ACO, collect salamanders from under the boards (Ensure that your hands are free of bug spray, sun screen or any other chemical so that it is not absorbed by the plethodontid's skin). Moisten hands ahead of time using wet towel or spray bottle.
2. Place salamanders into moist plastic container. For each individual identify the species and measure snout-vent length, vent-tail length and weight. Identify the specimen's age and sex if possible (see appendix 6).
3. For environmental variables (wind speed, soil moisture, soil pH) use appropriate meters (see Equipment Needed) and take 2 measurements along each perimeter of the plot (8 records total) and record the plot average.
4. ***Release the salamanders next to the ACO and allow them to seek refuge on their own (Fellers and Drost, 1994). The likelihood of crushing an animal is therefore eliminated.***

When to Monitor Plethodontids Using ACOs

Seasonality of Sampling

Plethodontids are more active on the forest floor in cool, wet weather, and so monitoring for them is best done in the spring or fall. Since their behaviour is weather dependent, the specific week to begin monitoring from year to year may change. Based on previous North American experience in monitoring plethodontids the following is recommended:

- Monitoring can take place in the spring and/or fall but, if monitoring in both seasons, data must be kept separate (data from spring and fall should not be pooled).
- In spring, monitoring should take place when temperatures are above 5 C and the ground is wet. In eastern Canada, the conditions are typically suitable for surveys in the first 5 weeks following winter thaw. The surveys should be avoided on days following frosty nights.
- In fall, monitoring should take place after rains have started and the ground is wet. In eastern Canada conditions are suitable during the final 5 weeks before winter freeze.
- Experience suggests that spring surveys may be more effective than fall surveys in terms of seasonality. If a group has limited capacity such that this protocol can be implemented in 1 season only, then spring is preferred. Results from fall only, however, will also be valuable.
- Once a monitoring program has begun sampling should be consistently undertaken in the same season from year to year (eliminates seasonal bias).

Frequency and Timing of Sampling

- Plethodontid monitoring should take place every year during the chosen season(s).
- As a minimum standard ACOs at a given plot should be checked 4 times during a given season (up to 8 checks per season would be ideal). (The greater the number of checks per year the larger your sample size. Greater sample size will allow for the detection of smaller changes and/or will reduce the probability of error. To determine the appropriate number of checks per year see step 4 - Sampling Frame.)
- ACO checks should take place at least 1 week apart (sampling every 2 weeks is preferred). More frequent checks will cause disturbance and trampling to the plot.
- Timing of ACO checks during the day is not critical but morning is preferred (potentially cooler and wetter than afternoons). Night searches are not necessary for sampling using ACOs and might be less effective, as salamanders are likely to be away from covers on the forest floor.
- As with seasonality the consistency of sampling frequency and timing over time is important.

9.1 Bias and Supplementary Data

Biases are factors that confound or mask trends recorded through monitoring. When comparing monitoring data between sites or when comparing data from one site across years, it is important to assess changes in potential bias. This is because observed trends may not be due to ecosystem changes but simply a product of the influence from bias (i.e., changing weather patterns). Being able to distinguish between true trends, natural variation, and bias is why long time spans of monitoring data are often required.

Below is a list of potential sources of bias that may confound observed trends in annual counts of plethodontids. Some types of bias can be controlled for through sampling design. For example, the influence of “Time of Year” bias can be controlled by consistently monitoring during the same season each year. Other sources of bias are more difficult or impossible to control for (e.g., territoriality). If potential sources of bias cannot be controlled for, it is important that those biases at least be consistent over time. For this reason it is recommended that bias variables be measured when conducting monitoring as supplementary data. Groups can amend the field data sheets provided with this protocol to include bias variables that are of concern. For advice on addressing bias in your monitoring program contact an advisor listed in appendix 2.

Habitat

Certain salamanders prefer a particular forest type or a specific niche within the forest. It is therefore imperative to know what species live in which habitats and select monitoring sites accordingly. Otherwise, not detecting a species of salamander could simply be a function of improper sampling and not true absence (Davis, 1997).

Time of Year

The time of year the boards are inspected will influence their use by salamanders (Resources Inventory Committee, 1998). Monitoring efforts should therefore coincide with the peak activity period of the species in the study region (Steinhilber et al., 2002). Remember the optimal time of year will vary regionally and along latitudinal gradients (Fellers and Drost, 1994). Consistency with respect to the seasonality of monitoring will ensure the comparability of the data. Maintain searches within the same season on an annual basis. Spring and fall have been proposed as the optimal times to inspect boards for terrestrial salamanders (Cook, 1984). Summer months are dry and unfavourable for salamanders, so monitoring at this time should be avoided (Cook, 1984, Davis, 1997).

Time of Day

The activity level, and probability of detection, can vary according to time of day (i.e., day versus night). To minimize the effects of this type of bias check ACO's during a similar time of day.

Weather/Moisture(Precipitation)/Temperature

Weather not only influences the activity of some species, it affects the ability of observers to find what they are looking for (Resources Inventory Committee, 1998). Weather conditions, particularly litter moisture, has a strong influence on surface activity of salamanders. Rainy nights draw salamanders out from beneath the ground and out from under cover objects into the leaf litter in search of food (Jaeger, 1980). Otherwise, outside of the rainy season, it is best to check cover boards when the soil is moist (Fellers and Drost, 1994).

Effort

The greater the sampling effort, the greater the number of captures (Resources Inventory Committee, 1998). To minimize effort bias, the numbers of times a site is visited should be standardized and observers should not exceed the predetermined number of visits. See the Sampling Frame section.

Territoriality

Territories are defended by many species of terrestrial salamanders (Jaeger and Forester, 1993). The defence of territories is often centred around a cover object (Jaeger et al., 1982). Two behaviours could therefore influence the numbers of salamanders seen under the cover boards. The same individuals could be captured under the same board week after week (Brooks, 1996 as cited by Monti et al., 2000) or individuals could be absent from boards because of competitive exclusion (Brooks, 1996 as cited by Monti et al., 2000). Monti et al. (2000) investigated the above using the Eastern Red-backed Salamander, *Plethodon cinereus*, and found that although there were some recaptures under the same boards after repeated inspections during one season, it was rare. Additionally, they found that salamanders do remain within the same general area (Monti et al., 2000) - individuals did not move from one monitoring plot along the transect to another. In contrast, Davis and Ovaska (2001 and unpublished data) repeatedly found individuals of the Western Red-backed Salamander (*P. vehiculum*) under the same artificial cover-objects during weekly inspections. It is therefore unclear whether territoriality and site-fidelity plays a role in use of artificial cover objects. Laying down paired boards is suggested as a means of minimizing territorial exclusion (Droege et al., 1997).

<u>Species/Sex/Age Class</u>

Some individuals of a given species are easier to detect because of their sex or age class. Adults and juveniles often display markedly different behaviours and occupy different niches in the same environment. This phenomenon has been proposed for The Eastern Red-backed Salamander. Adult males have been found to use natural cover objects and to defend those covers over time. Juveniles on the other hand have been found to occur most frequently in the duff layer of the forest floor foraging for food, particularly on damp, cool nights. It has been proposed that the addition of artificial cover object may provide additional cover that would attract individuals that would otherwise be excluded from natural cover objects making the census unrepresentative of the true population structure, i.e. there may be more available cover for juveniles to move to from within the leaf litter. A study by Monti et al. (2000) found that individuals of the Eastern Red-backed Salamander, found under artificial cover objects were similar in body size to individuals occupying natural cover objects, i.e. all were adults. They suggested that the source population for salamanders found under artificial cover objects is from the leaf litter on the forest floor. Their hypothesis differs from original studies that found that juveniles primarily occupied a niche within the leaf litter. The discrepancy implies that further research needs to be conducted to evaluate source populations of salamanders moving to artificial cover objects. Recording size class data (relative sizes or actual measurements) will provide additional information to assess the comparability to a natural population of terrestrial salamanders and their use of cover objects.

10. Step Eight: Data Management and Analysis

Following data collection attention must be paid to proper data management and analysis. Far little attention is often paid to this step, and many readers may know of personal examples where field data sits in paper data sheets without being electronically inputted into a database for effective storage, data retrieval and analysis. A great deal of time and money has been wasted due to inadequate data management.

To assist in data management this protocol is accompanied by a MS Excel spreadsheet that is linked to Field Sheets A and B. This spreadsheet can be downloaded at the EMAN web site. This spreadsheet provides a consistently formatted data entry worksheet. This will ensure that each group's data will be formatted the same, which will facilitate compiling data from multiple plots. In order to create archived backups of plethodontid monitoring data and to assist in the sharing of information each group should email their completed Excel spreadsheet after each year's field season to **eman@ec.gc.ca**.

Once the data has been entered the Excel file will automatically generate some initial analyses. Fortunately, in step 3, a strategy for data analysis has already been created to specifically address your monitoring question(s) given your data. This strategy can be used to supplement the initial analyses provided by the downloadable spreadsheet. Once again, if support is needed to further analyze your plethodontid data please contact an advisor from appendix 2.

11. Step Nine: Monitoring Threshold and Data Interpretation

"What is the answer to my monitoring question?". "Is the trend I observed beyond what one would reasonably expect?". "Is any further action required?". After your data have been electronically entered and analyzed the observed trends need to be interpreted within the context of your monitoring question and compared to some preset monitoring threshold.

As with many elements of a monitoring program, setting an appropriate threshold (the point at which an early warning signal should be raised) will depend on your question. Given the possible range of questions a group may pose, a threshold(s) cannot be recommended here for all circumstances. This protocol, therefore, will focus on setting a monitoring threshold for trends at a plot level.

A valuable attribute of plethodontids as an indicator species is their low natural variability at a population level and their sensitivity to disturbance. Compared to many other species, a smaller change in plethodontid populations over time should trigger alarm bells. With this in mind a monitoring threshold should be set at *"a statistically significant change in plethodontid counts at a plot level over 5 or more years"*. Based on the experience of the reviewers for this protocol, if a change in plethodontid numbers is statistically significant (confidence level ≥ 80%, power ≥ 90%) then there is sufficient cause for concern. This generic threshold can be applied to a variety of different monitoring questions.

In terms of magnitude of trend (effect size) at the plot level, a recommended monitoring threshold is: *50% change over 20 years (for long term trends) and 20% change over 5 years (for shorter term trends)*. Again, these thresholds assume a confidence level ≥ 80% and sample power ≥ 90%.

Including confidence levels and sample power as part of the threshold ensures that monitoring programs will likely have adequate sample sizes and that observed trends are likely to reflect true changes.

It is important to note that these are suggested generic thresholds. Depending on the goals and objectives of a particular group more specific criteria for threshold(s) may need to be established.

BOX 13: What does it mean if a monitoring threshold is surpassed?

A surpassed monitoring threshold means that an early warning signal should be raised and more intensive investigation is called for to determine if a management problem exists and to identify possible actions.

The conceptual model of the role of plethodontid salamanders in a forest ecosystem (figure 2) presents a guide as to stressors or processes which may have resulted in the observed trend in plethodontids at a monitoring plot(s).

12. Step Ten: Program Review

An annual program review, especially during the first 5 years of implementation, should be a part of every monitoring program. During the first 5 years of implementation baseline data is collected that will help managers to refine their programs ensuring that an appropriate, consistent sampling effort is applied to meet the defined sampling frame. Without ensuring that a program's sampling frame is met then there is the possibility that insufficient data was collected to detect the monitoring threshold and a group's monitoring question may go unanswered.

During the first 5 years a formal program review should assess each step in this protocol (bringing you back to step 1) and, if necessary, changes should be made to ensure that the monitoring program is robust enough to meet goals and objectives. As more information is made available, a group can refine their monitoring questions and associated methods of analysis, sampling frame and study design. This 5 year review period is essential for long term monitoring programs to ensure that considerable effort and expense is not spent needlessly on data collection that, in the end, is not as useful as it could have been. This strategy of continual program assessment and evaluation is the foundation of an adaptive management approach to monitoring.

BOX 14: Example - Monitoring Program Review
Grenadier Island, St. Lawrence Islands National Park, Ontario.

After 3 years of data collection St. Lawrence Islands National Park conducts a formal review of their plethodontid monitoring program as recommended in section 6.2. At 6 monitoring plots the park installed 40 ACOs per plot. Following the protocol the park surveyed these plots through 5 weekly visits during the spring after winter thaw.

From their 3 years of data the average number of eastern redback salamanders found at each plot per survey occasion was 8 with a standard deviation of 4. Occurrences of other terrestrial salamander species were made but there were too few to assess population trends (occurrences of these species were recorded and used to update the park's species list). Using the software program Monitor 7.0 the park discovers that this sample size only allows them to detect a change of 50% over 20 years with a sample power of 82.8% (with a confidence level of 80%). Since the recommended sampling frame for this protocol suggests a sample power of 90% the park determines that it needs to slightly increase its sample size.

Using Monitor 7.0 the park determines that an average count of 10 eastern redback salamanders per plot per survey occasion (an increase of 25%) would increase their sample power from 82.8% to 91%. In order to achieve this increase of 25% the park decides to increase the number of ACOs per plot by 25% from 40 to 50. The park refines their monitoring program by installing an additional 10 ACOs in each of their 6 plots and keeps the same number of 5 weekly visits during the spring. The increase in time required to check 50 ACOs instead of 40 is small. This sampling effort become standardized and the park plans on consistently monitoring at this level over the long term.

13. References

Journals and Reports

Ash, A. 1997. Disappearance and return of Plethodontid salamanders to clearcut plots in the southern Blue Ridge Mountains. Conservation Biology 11(4):983-989.

Bonin, J. and Y. Bachand. 1997. The use of artificial covers to survey terrestrial salamanders in Quebec. In Amphibians in decline: Canadian studies of a global problem. edited by D. M. Green. Herpetological Conservation 1:175-179.

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(4):1172-1180.

Burton, T. M. and G. E. Likens. 1975. Salamander populations and biomass in the Hubbard Brook Experimental Forest, New Hampshire. Copeia 1975(3):541-546.

Cohn, J. P. 1994. Salamanders slip-sliding away or too surreptitious to count? BioScience 44(4):219-223.

Davis, T. M. 1997. Non-distruptive monitoring of terrestrial salamanders with artificial cover objects on southern Vancouver Island, British Columbia. In Amphibians in decline: Canadian studies of a global problem. edited by D. M. Green. Herpetological Conservation 1:161-174.

Davis, T. M., and Ovaska, K. 2001. Individual recognition in amphibians: assessment of toe-clipping and fluorescent-marking with *Plethodon vehiculum* (Caudata: Plethodontidae). J. Herpetol. 35:217–225.

DeGraaf, R. M. and M. Yamasaki. 1992. A nondestructive technique to monitor the relative abundance of terrestrial salamanders. Wildlife Society Bulletin 20:260-264.

deMaynadier, P.G. and M. L. Hunter Jr. 1998. Effects of silvicultural edges on the distribution and abundance of amphibians in Maine. Conservation Biology 12(2):340-352.

Droege, S., D. Lantz, and L. Monti. 1997. The terrestrial salamander monitoring program. USGS Unpublished Report.

Dupuis, L. A. 1997. Effects of logging on terrrestrial amphibians of coastal British Columbia. In Amphibians in decline: Canadian studies of a global problem. edited by D. M. Green. Herpetological Conservation 1:185-190.

Dupuis, L. A., J. N. M. Smith, and F. Bunnell. 1995. Relation of terrestrial-breeding amphibian abundance to tree-stand age. Conservation Biology 9(3):645-653.

Feder, M. E. 1983. Integrating the ecology and physiology of plethodontid salamanders. Herpetologica 39(3):291-310.

Fellers, G. M. and C. A. Drost. 1994. Sampling with artificial cover. In W. R. Heyer, M. A. Donnelly, R. W. McDiarmid, L. C. Hayek, and M. S. Foster. Editors. Measuring and Monitoring Biological Diversity: Standard Methods for Amphibians. Washington, Smithsonian Institute Press. p. 146-150.

Jaeger, R. G., M. G. Peterson, G. Gollmann, B. Gollmann, and V. R. Townsend, Jr. 2001. Salamander social strategies: living together in female-male pairs. Journal of Herpetology 35(2):335-338.

Jaeger, R. G., and D. C. Forester. 1993. Social behavior of Plethodontid salamanders. Herpetologica 49(2):163-175.

Jaeger, R. G., D. Kalvarsky, and N. Shimizu. 1982. Territorial behaviour of the red-backed salamander: expulsion of intruders. Animal Behaviour 30:490-496.

Jaeger, R. G. 1980. Microhabitats of a terrestrial forest salamander. Copeia 1980(2):265-268.

Jung, R. E., S. Droege, J. R. Sauer, and R. B. Landy. Evaluation of terrestrial and streamside salamander monitoring techniques at Shenandoah National Park. Environmental Monitoring and Assessment 63:65-79.

Herbeck, L. A., and D. R. Larsen. 1999. Plethodontid salamander response to silvicultural practices in Missouri Ozark forests. Conservation Biology 13(3):623-632.

Kolozsvary, M. B. and R. K. Swihart. 1999. Habitat fragmentation and the distribution of amphibians: patch and landscape correlates in farmland. Canadian Journal of Zoology 77:1288-1299.

Monti, L., M. Hunter, Jr., and J. Witham. 2000. An evaluation of the artificial cover object (ACO) method form monitoring populations of the redback salamander *Plethodon cinereus*. Journal of Herpetology 34(4):624-629.

Moore, A. L., C. E. Williams, T. H. Martin, and W. J. Moriarity. 2001. Influence of season, geomorphic surface and cover item on capture, size and weight of *Desmognathus ochrophaeus* and *Plethodon cinereus* in Allegheny Plateau riparian forests. American Midland Naturalist 145(1):39-45.

Ovaska, K., and Gregory, P.T. 1989. Population structure, growth and reproduction in a Vancouver Island population of the salamander *Plethodon vehiculum.* Herpetologica 45:133–143.

Ovaska, K., and Sopuck, L.G. 2001. Potential of terrestrial gastropods and salamanders as indicators for monitoring ecological effects of variable-retention logging practices. Unpubl. report prepared by Biolinx Environmental Research Ltd. for Weyerhaeuser Company Ltd., Nanaimo Office, B.C.

Peacock , R. L., and Nussbaum, R. A.. 1973. Reproductive biology and population structure of the Western red-backed salamander, *Plethodon vehiculum* (Cooper). Journal of Herpetology 7:215-224.

Pough, F. H., E. M. Smith, D. H. Rhodes, and D. Collazo. 1987. The abundance of salamanders in forest stands with different histories of disturbance. Forest Ecology and Management 20:1-9.

Quinn, V. S. and B. M. Graves. 1999. A technique for sexing red-backed salamanders (Plethodon cinereus). Herpetological Review 30(1):32.

Sattler, P. and N. Reichenbach. 1998. The effects of timbering on Plethodon hubrichti: short-term effects. Journal of Herpetology 32(3):399-404.

Smith, C. K., and J. W. Petranka. 2000. Monitoring terrestrial salamanders: repeatability and validity of area-constrained cover object searches. Journal of Herpetology 34(4):547-557.

Steinhilber, M., A. Russell, L. Powell, B. Eaton, A. Didiuk. 2002. Monitoring amphibian and reptile populations in Canada: a manual of techniques for estimating relative abundance. Unpublished.

Sugar, A., T. Bellhouse, D. Phoenix, N. Dawson, G. Holborn. 2001. A sampling protocol for red-backed salamander (Plethodon cinereus) populations in Ontario: the 2nd pilot study. Wildlife Assessment Program, Ontario Ministry of Natural Resources WAP-2001-01.

Waldick, R. C., B. Freedman, R. J. Wassersug. 1999. The consequences for amphibians of the conversion of natural, mixed-species forests to conifer plantations in southern New Brunswick. The Canadian Field Naturalist 113(3):408-418.

Waldick, R. 1997. Effects of forestry practices on amphibian populations in eastern North America. In Amphibians in decline: Canadian studies of a global problem. edited by D. M. Green. Herpetological Conservation 1:191-205.

Welsh, H. H., and S. Droege. 2001. A case for using Plethodontid salamanders for monitoring biodiversity and ecosystem integrity of North American forests. Conservation Biology 15(3):558-569.

Wise, S. E. and B. W. Buchanan. 1992. An efficient method for measuring salamanders. Herpetological Review 23(2):56-57.

Zorn, P. 2000. Bullfrog Population Monitoring. Initial Analysis and Review. St. Lawrence Islands National Park. Parks Canada. Mallorytown Landing, ON.

<u>Books</u>

Ballinger, R. E., and J. D. Lynch. 1983. How to Know the Amphibians and Reptiles. Wm. C. Brown Publishers, U.S.A.

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, U.S.A.

Cook, F. R. 1984. Introduction to Canadian Amphibians and Reptiles. National Museums of Canada, Ottawa, Ontario, Canada.

Green, D. M., and R. W. Campbell. 1984. The Amphibians of British Columbia. British Columbia Provincial Museum, Victoria, B.C.

Johnson, B. Familiar Amphibians and Reptiles of Ontario. Natural Heritage/Natural History Inc. Toronto, Ontario, Canada.

Consultation of Workshop Proceedings

Brooks, R. T. 1999a. Use of artificial cover objects (boards) to assess the effects of timber harvests on terrestrial salamanders. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Droege, S. 1999. The North American terrestrial salamander monitoring program: sampling with coverboards. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Enright, L. 1999. An evaluation of techniques for determining the relative abundance of salamanders in hardwood forest ecosystems in Algonquin Park, Ontario. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Hackett, D., M. Twiss, T. Bellhouse, K. Cowcill. 1999. A comparison among four techniques for monitoring forest salamanders near North Bay. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Harpole, D. 1999. Effects of silvicultural alternatives to clearcutting on terrestrial salamander abundance in southern Appalachian hardwood forests. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Morneault, A. 1999. Using cover boards to assess the effect of shelterwood harvest and site preparation on the relative abundance of redback salamanders in white pine stands. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Pauley, T. 1999. Terrestrial salamander monitoring in the higher elevations of West Virginia: a 12-year study of a sensitive species. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Szuba, K. 1999. Cover boards as indicators of the population density of red-backed salamanders. Workshop proceedings from the Monitoring Salamanders Workshop, October 26-29, North Bay, Ontario.

Websites

http://www.mp2-pwrc.usgs.gov/amphibs
http://www.npwrc.usgs.gov/narcam/idguide.htm
http://www.aquatic.uoguelph.ca/amphibians/amphib/accounts.htm
http://collections.ic.gc.ca/amphibians/taxa.html
http://www.npwrc.usgs.gov/narcam/idguide.htm
http://www.aquatic.uoguelph.ca/amphibians/amphib/accounts.htm
http://collections.ic.gc.ca/amphibians/taxa.html
http://www.livingbasin.com/endangered/Amphibians-Reptiles.htm
http://www3.gov.ab.ca/srd/fw/amphib/lts.html
http://www.watertoninfo.ab.ca/r/salamander.html

Personal Communication

Francis R. Cook
Sam Droege
Dan Lantz
Kristiina Ovaska

Appendix 1. Methods Summary

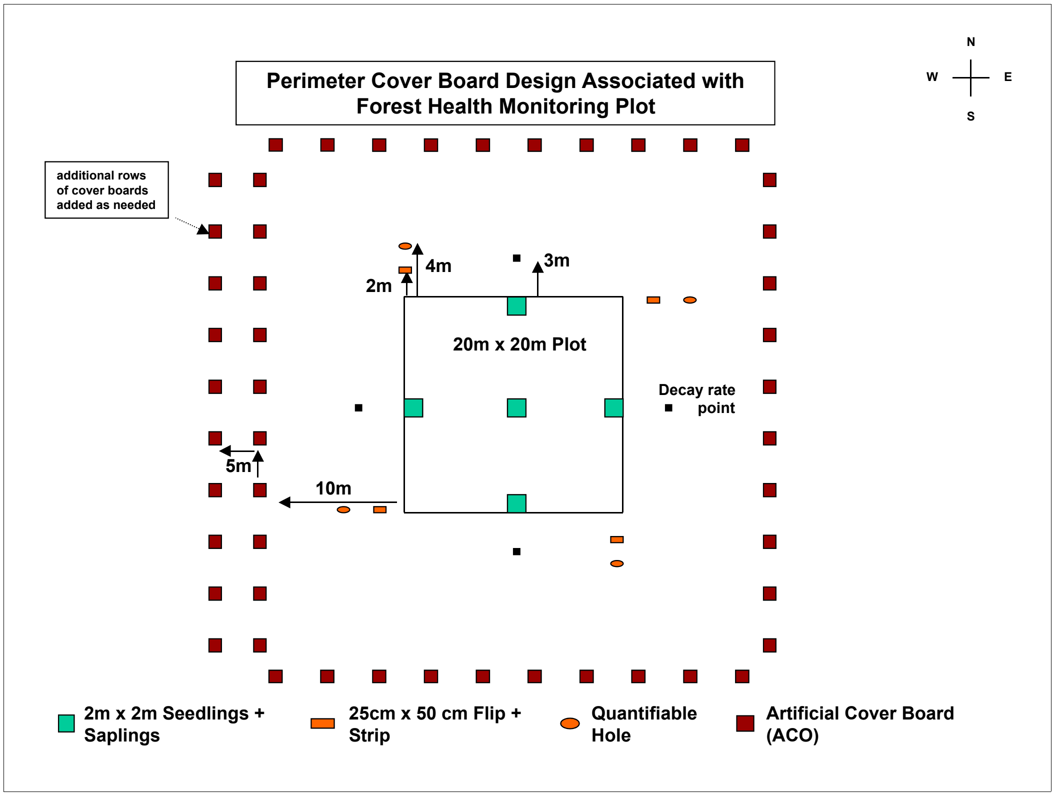


Figure 10. Arrangement of ACOs at a 20x20m EMAN forest health monitoring plot. Other sampling points (i.e., decay rate point, 2x2m seedlings & saplings) are associated with other EMAN forest health indicators.

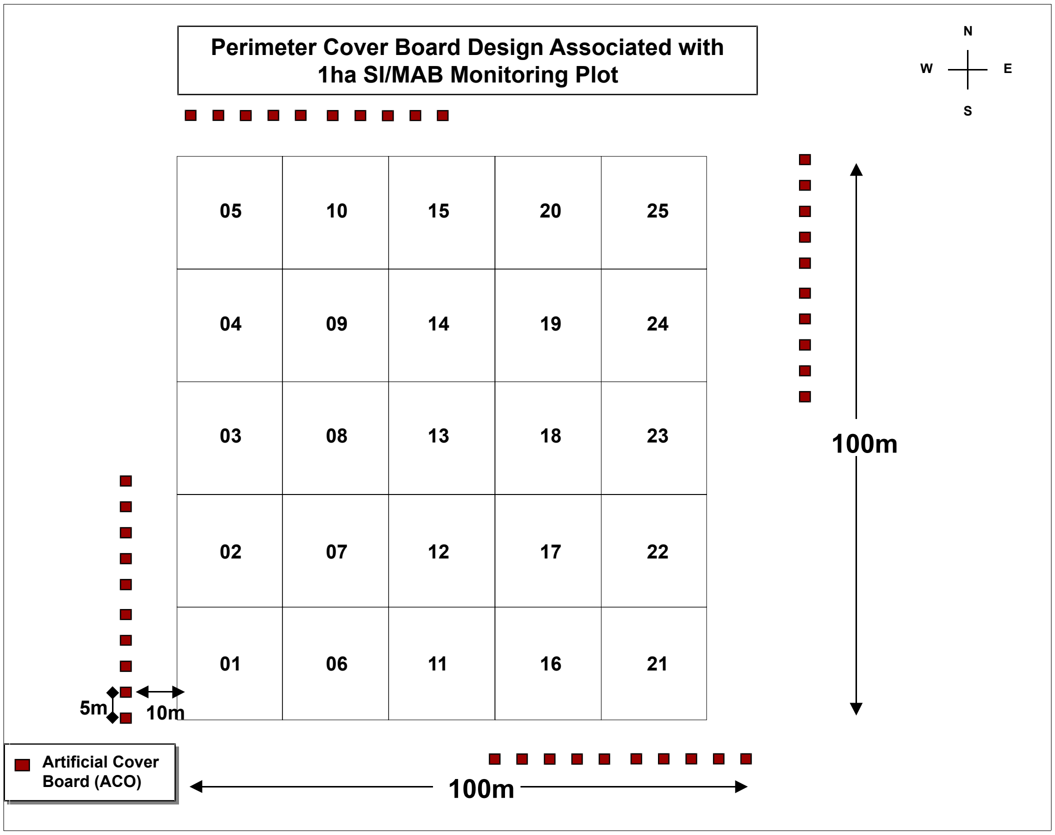


Figure 11 Arrangement of ACO's at a 1ha SI/MAB plot.

How to Arrange ACOs at your EMAN 20x20m or 1ha Forest Health Monitoring Plot

- ACOs are to placed around the perimeter of 20x20m or 1ha forest health monitoring plot.

- First perimeter is to be 10 m from plot edge to minimize disturbance to ACOs while conducting other forest health monitoring protocols.

- To minimize territoriality bias due to plethodontid behaviour ACOs are not to be closer than 5 m from each other.

- Add additional rows of ACOs as required ensuring a minimum distance of 5 m from other ACOs.

Minimum Number of ACOs to Install When First Implementing this Protocol

"Rules of thumb" for initial minimum number of ACOs at a 20x20m or 1ha forest health monitoring plot.

Initial ACOs for British Columbia = 20
Initial ACOs for Eastern Canada = 40

This initial number of ACOs is to be refined after 3 years of data collection to ensure a sampling effort that will likely meet the program's sampling frame.

Number of People Needed to Implement this Protocol

- At a minimum, 2 people are required to implement this protocol (3 are preferred).
- Sampling is to follow a "call back" procedure. One person is to be the recorder. One or two people are to be the observers. During data collection observers are to call out observations to the recorder who will then call back the observation back to the observer while it is being entered into the field sheet. This "call back" procedure is effective in reducing data input errors.

When to Conduct Plethodontid Monitoring

- Monitoring can take place in the spring and/or fall but, if monitoring in both seasons, data must be kept separate (data from spring and fall should not be pooled).
- In spring, monitoring should take place in the first 5 weeks following winter thaw.
- In fall, monitoring should take place during the final 5 weeks before winter freeze.

- Experience suggests that spring may be slightly more effective than fall in terms of seasonality. If a group has limited capacity such that this protocol can be implemented in 1 season only then spring is preferred. Results from fall only, however, will also be valuable.
- Once a monitoring program has begun sampling should be consistently undertaken in the same season from year to year (eliminates seasonal bias).

How Often to Conduct Plethodontid Monitoring

- Plethodontid monitoring should take place every year during the chosen season(s).
- As a minimum standard ACOs at a given plot should be checked 4 times during a given season (up to 8 checks per season would be ideal).
- ACO checks should take place at least 1 week apart (sampling every 2 weeks is preferred). More frequent checks will cause disturbance and trampling to the plot.
- Timing of ACO checks during the day is not critical but morning is preferred (potentially cooler and wetter than afternoons). Night searches are not necessary for sampling using ACOs.
- As with seasonality the consistency of sampling frequency and timing over time is important.

Variables to be Collected with this Protocol

Mandatory Variables (Field Sheet A)

(all groups implementing this protocol should collect data for these variables)

plot location
name of organization
number of plethodontids observed per species
ground temperature
air temperature
precipitation during last 24 hours (yes or no)
Beaufort wind and sky codes
time of day
date
observer names
ACO identification number
number of days since last ACO check
age of ACOs
disturbance to ACO (if any)
additional comments (if any)

Additional Preferred Variables (Field Sheet B)

wind speed
wind speed (km/h)
soil moisture (kPa - soil / water trension)
soil pH
snout-vent length per salamander (mm)
vent-tail length per salamander (mm)
weight (grams)
age class per salamander (adult, juvenile, larvae, egg mass)
sex of salamander

Equipment Needed

For Mandatory Variables...

(for locating plots)
map of site, compass and GPS unit, 50m measuring tape

(for data collection)
Field Sheet A, clip board, pencil or waterproof pen, thermometer

For Additional Preferred Variables...

Field Sheet B
wind speed meter
relative humidity meter
soil pH meter
soil moisture meter
calipers
plastic bag with moist towel or spray bottle
clear plastic container

How to Monitor Plethodontids Using ACOs

Mandatory Variables:

1. Record information about the monitoring location and the names of the field crew. Included here should be date and time of data collection, time since last check, air temperature, ground temperature, precipitation in last 24 hours and Beaufort wind and sky codes. For air and ground temperature collect 2 readings along each perimeter row of ACOs (8 readings total) and record the average value.
1. Record information about the ACO – its unique number, age (number of years), and disturbance (i.e., moved by animal, infested with ants).
2. Observers lift each ACO, identify and count the numbers of each species of salamander seen under the cover board. Observations are called out to the recorder and the recorder calls back the data entry to ensure correct data input (this minimizes entry errors).
3. Replace the ACO as quickly as possible ensuring no rocks or pieces of wood have fallen into the space keeping the boards from lying directly in contact with the ground (if ACOs are not replaced quickly or a space is left, the soil will dry out making the habitat unsuitable, Fellers and Drost, 1994).
4. Record any additional comments or observations that may be of interest (e.g. disturbed ACOs from animals, ant colonies under boards, presence of bugs/worms under boards, vandalism, etc.).

Additional Preferred Variables:

1. From each ACO, collect salamanders from under the boards (Ensure that your hands are free of bug spray, sun screen or any other chemical so that it is not absorbed by the plethodontid's skin). Moisten hands ahead of time using wet towel or spray bottle.
2. Place salamanders into moist plastic container. For each individual identify the species and measure snout-vent length, vent-tail length and weight. Identify the specimen's age and sex if possible (see appendix 6).

Figure 12. Snout-vent length being recorded on two eastern redback salamanders using moist, clean hands, a plastic container and a millimeter ruler. (photo - Long Point Biosphere Reserve, Ontario)

3. For environmental variables (wind speed, soil moisture, soil

pH) use appropriate meters (see Equipment Needed) and take 2 measurements along each perimeter of the plot (8 records total) and record the plot average.

4. Release the salamanders next to the ACO and allow them to seek refuge on their own (Fellers and Drost, 1994). The likelihood of crushing an animal is therefore eliminated.

Appendix 2. Plethodontid Monitoring Support

For primary support for this joint EMAN / Parks Canada monitoring protocol contact...

Brian Craig
Network Science Advisor
Ecological Monitoring and Assessment Network Coordinating Office, Environment Canada.
Canada Centre for Inland Waters.
867 Lakeshore Road
Burlington, ON L7R 4A6
tel: (905) 336-4431
email: brian.craig@ec.gc.ca

Paul Zorn
Ecological Integrity Monitoring Specialist
Parks Canada, Ontario Service Centre
Ecosystem Conservation Section
2630 Sheffield Road, Ottawa, ON
K1A 0M5
tel: (613) 998-7248
email: paul.zorn@pc.gc.ca

Appendix 3. Artificial Cover Board (ACO) Design

This appendix contains recommended dimensions for ACOs to be used for plethodontid monitoring. As per section 7 (Step 5: Acquiring ACOs) of the protocol, it is more important that the type of ACO used at a plot is consistent over time than it is to follow these dimensions exactly (given that an ACO is at least 8 inches wide). The dimensions of wood planks available to make ACOs and their cost will, therefore, greatly influence the size and dimensions of ACO that will be used. Except for *Anedies* simple, non-layered perimeter cover boards are recommended (for *Anedies* use V-shaped or angled layered cover boards).

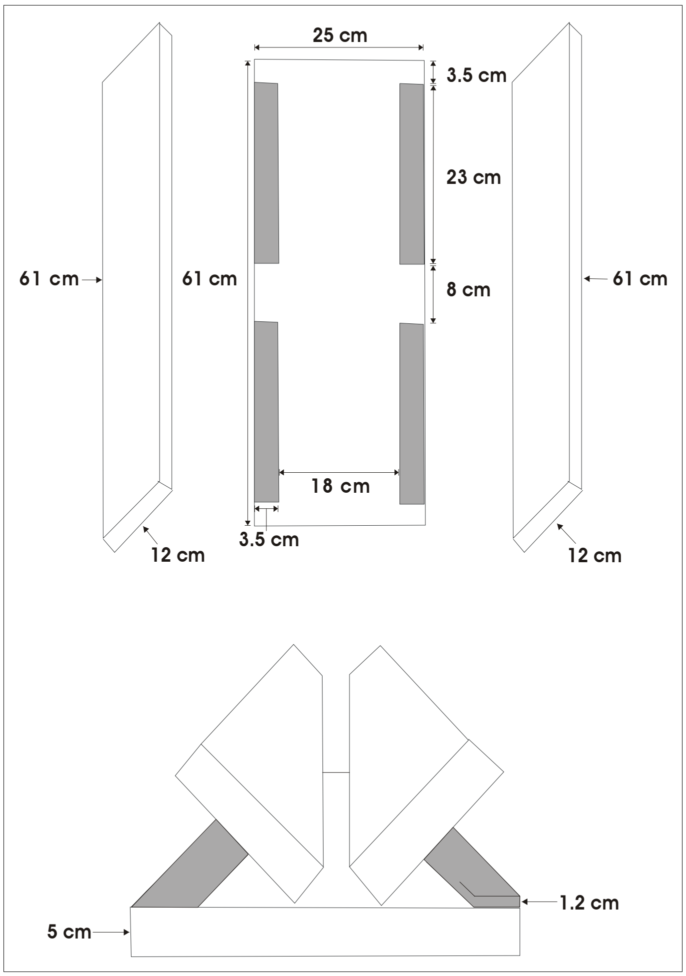


Figure 1. Recommended dimensions for V-shaped wooden cover board.

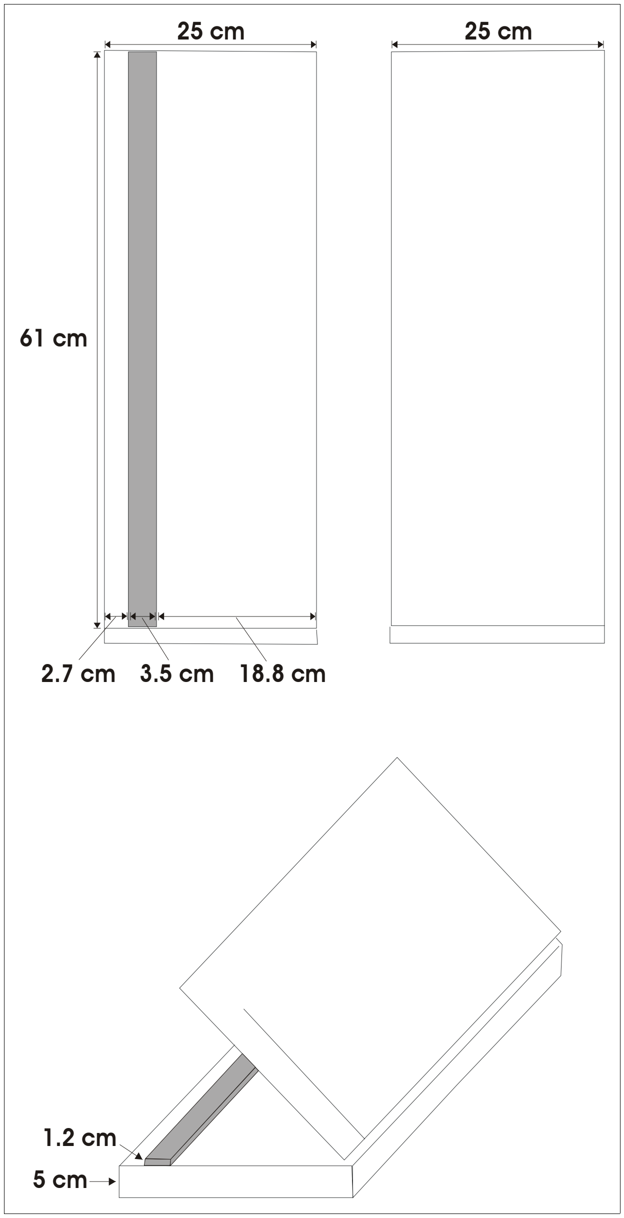


Figure 2. Recommended dimensions for angled cover board.

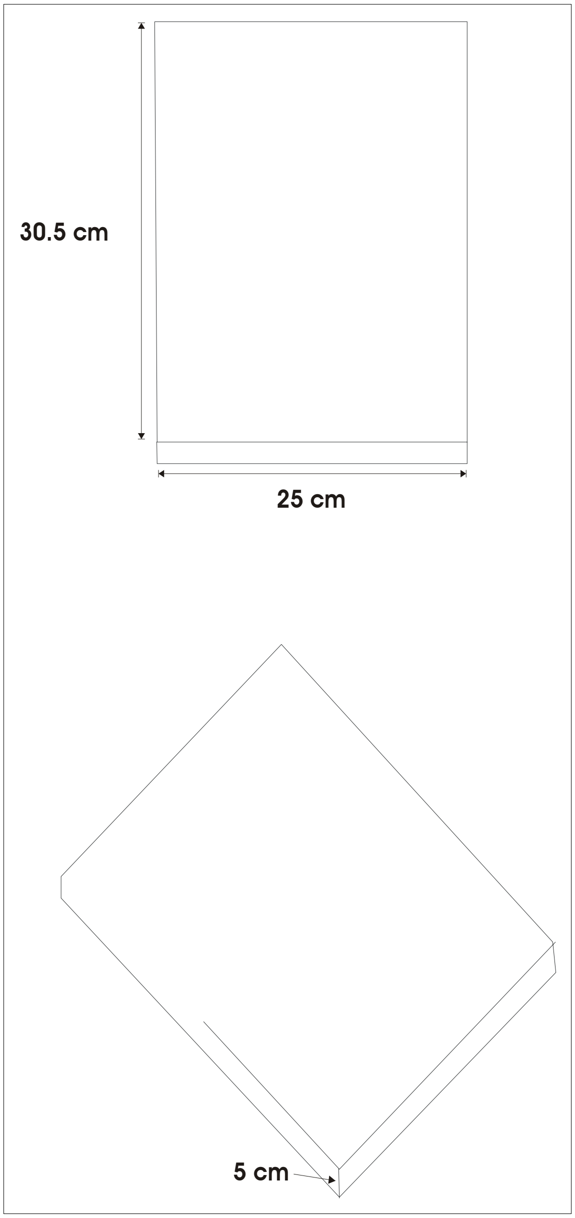


Figure 3. Recommended dimensions for simple, non-layered cover board.

Appendix 4. Beaufort Sky and Wind Codes

Beaufort Wind Codes

Beaufort Scale	Wind Speed (mph)	Wind Speed (km/h)	Description	Visual Cues
0	1	1.6	Calm	Smoke rises vertically
1	2	3.2	Light	Smoke drifts
2	5	8	Light Breeze	Leaves rustle
3	10	16	Gentle Breeze	Lighter branches sway
4	15	24	Moderate Breeze	Dust rises. Branches move
5	21	33.6	Fresh Breeze	Small trees sway
6	28	44.8	Strong Breeze	Larger branches move
7	35	56	Moderate gale	Trees move
8	42	67.2	Fresh gale	Twigs break
9	50	80	Strong gale	Branches break
10	59	94.4	Whole gale	Trees fall
11	69	110.4	Storm	Violent blasts
12	75	120	Hurricane	Structures shake

Beaufort Sky Codes

Sky Code	Description
0	Clear (no cloud at any level)
1	Partly cloudy (scattered or broken)
2	Continuous layer(s) of blowing snow
3	Sandstorm, dust storm, or blowing snow
4	Fog, thick dust or haze
5	Drizzle
6	Rain
7	Snow, or snow and rain mixed
8	Shower(s)
9	Thunderstorm(s)

Appendix 5. Field Data Sheets A & B

Adopted from A Sampling Protocol for Red-Backed Salamander (Plethodon cinereus) Populations in Ontario: The 2nd Pilot Study, Ontario Ministry of Natural Resources, 2001.

Field Sheet A

PLOT NAME: Name given to describe plot location.
GROUP NAME: Name of the organization conducting the sampling.
UTM EASTING: UTM Easting coordinate of plot centre. (m)
UTM NORTHING: UTM Northing coordinate of plot centre (m)
OBSERVER NAMES: Initials of people conducting the sampling.
DATE: Date the ACOs are checked. (dd-mm-yyyy)
TIME: Time of day sampling is conducted. (24 hour clock)
PRECIP IN LAST 24 HRS: Amount of precipitation during the 24 hour period prior to sampling. (mm)
AIR TEMP: Average air temperature from 8 checks recorded during sampling. (Celsius)
SOIL TEMP: Average soil temperature from checks under 8 random ACOs recorded during sampling. (Celsius)
BEAUFORT SKY CODE: 0 = few clouds, 1 = partly cloudy or variable sky, 2 = cloudy or overcast, 4 = fog or smoke, 5 = drizzle, 7 = snow, 8 = showers.
BEAUFORT WIND CODE: 0 = calm (smoke rises vertically), 1 = light air (rising smoke drifts), 2 = light breeze (leaves rustle, calm wind on face), 3 = gentle breeze (leaves and trees in constant motion), 4 = moderate breeze (moves some branches), 5 = fresh breeze (small trees begin to sway).
ACO NUMBER: Unique number used to identify specific artificial cover object.
SPECIES: Species of salamander found under ACO. (see species codes)
COUNT: Number of individual salamanders per species found under ACO. (number)
ACO TYPE: Type of ACO used at the plot. (S = simple, L = layered)
ACO AGE: Number of years ACO has been at the plot. (number of years)
ACO DISTURB: Presence of ACO disturbance from things such as vandalism, ants, etc. (yes or no)
COMMENTS: Record unusual observation associated with sampling including type of ACO disturbance if present.

Additional Variables from Field Sheet B

SOIL MOISTURE: Reading from soil moisture meter. (kPa)
SOIL pH: Reading from soil pH meter. (pH)
S-V LENGTH: Length of individual salamander from snout to vent. (mm)
V-T LENGTH: Length of individual salamander from vent to tail. (mm)
WEIGHT: Recorded weight of individual salamander. (g)
SEX: Sexof individual salamander. (m = male, f = female, u = unknown)
AGE CLASS: Estimated age class of individual salamander. (a = adult, j = juvenile)

Common Species Found Under Cover Boards

ERSA - Eastern Redback Salamander (*Plethodon cinereus*)
WRSA - Western Redback Salamander (*Plethodon vehiculum*)
ENSA - Ensatina Salamander (*Ensatina eschscholtzii*)
WASA - Wandering Salamander (*Aneides vagrans*)
CASA - Coeur d'Alene Salamander (*Plethodon idahoensis*)

Other Species that Could be Found Under a Cover Board

BLSA - Blue Spotted Salamander (*Ambystoma laterale*)
JFSA - Jefferson Salamander (*Ambystoma jeffersonianum*)
YESA - Yellow Spotted Salamander (*Ambystoma maculatum*)
SMSA - Smallmouth Salamander (*Ambystoma texanum*)
LTSA - Long-toed Salamander (*Ambystoma macrodactylum*)
TISA - Tiger Salamander (*Ambystoma tigrinum*)
NWSA - Northwestern Salamander (*Ambystoma gracile*)
PGSA - Pacific Giant Salamander (*Dicamptodon ensatus*)
DUSA - Dusky Salamander (*Desmognathus fuscus*)
TLSA - Two-Lined Salamander (*Eurycea bislineata*)
FOSA - Four-toed Salamander (*Hemidactylium scutatum*)
SPSA - Spring Salamander (*Gyrinophilus porphyriticus*)
RENE - Red-spotted Newt (*Notophthalmus viridescens*)
RSNE - Rough-skinned Newt (*Taricha granulosa*)

Field Sheet A

Plot Name:	**Group Name**:
UTM Easting:	**UTM Northing**:

Observer Names:	**Date**:	**Time**:
Precip in Last 24 hrs.:	**Air Temp.**:	**Soil Temp.**:
Beaufort Sky Code:	**Beaufort Wind Code**:	

ACO Number	Species	Count	ACO Type	ACO Age	ACO Disturb.

Comments:

Field Sheet B

Plot Name:	**Group Name**:	
UTM Easting:	**UTM Northing**:	
Observer Names:	**Date**:	**Time**:
Soil Moisture:	**Soil pH**:	

ACO No.	Species	s-v Length	v-t Length	weight	sex	age class

Comments:

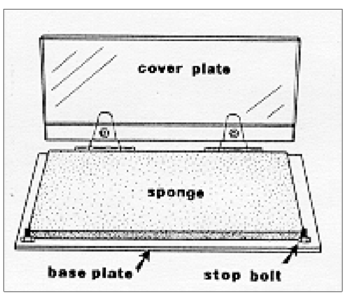
Appendix 6. Methods for Collecting Data on Preferred

Variables

Measuring Length

There are several methods that can be used to assist when measuring salamanders. To minimize stress to the individual, it is recommended that salamanders be measured in a transparent box (with a lid) at least 25 cm long (available where ever fishing tackle is sold) with a damp sponge lining the bottom. Make sure there is some space between the sponge and the lid so the salamander is not crushed upon closing the box.

Dampen sponge and the transparent lid and place the salamander belly side showing through the lid. Tilt the box upwards and the salamander will try to climb up the lid (if it does not do so on its own, gently prod it at the tail and it will try to walk up the slant). The body will straighten. Close the base plate (sponge side) over the salamander trapping it in the moistened sponge (Wise and Buchanan,1992).



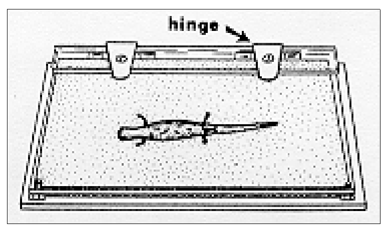


Figure 1. An example of the box design that can be used to measure salamanders (Wise and Buchanan, 1992).

Use calipers or a ruler and collect the information below:

1) Measure the snout-vent length (SVL). Place one end of the caliper at the tip of the salamanders snout and measure to the anterior angle of its vent (Davis, 1997, deMaynadier and Hunter, 1998).

2) Measure the vent-tail length (VTL). Measure from the anterior angle of the salamander's vent to the tip of its tail. A full length tail may not always be present. Record the length of what is there and make a not in the comments column.

Measuring Weight

Adult salamanders are not very large and thus do not weigh very much. More than 80% of salamander species weigh less than 1 gram (Pough, 1980). A 10 g spring scale is suitable; a small, portable digital scale can also be used. Weight measurements for salamanders should be recorded to the nearest 0.1g (Bury, 1983, Davis, 1997).

Place a salamander in a small, plastic bag (we don't moisten them - you don't want the salamander dripping wet, as the measurement will be inaccurate). Using a scale, measure the weight of the bag. Place the salamander in the bag and reweigh the bag with the salamander in it. Subtract the weight of the bag from the weight of the bag plus the salamander and the result is the weight of the salamander. (See equation below).

Weight of Salamander and moistened bag (g)	-	Weight of Moistened bag (g)	=	Weight of Salamander (g)

Identify the Age of the Salamander

Using the numbers attained above, determine the age of the salamander using the following guidelines:

Eastern Redback Salamander *(Plethodon cinereus)*
Juveniles: 25mm or less (first year young)
Adults: 35mm or greater

Western Red-backed Salamander (*Plethodon vehiculum*)
Young of the Year: < 30 mm SVL
Older juveniles: 30 - 40 mm SVL
Adults: > 40 mm SVL

The above values are based on information in Peacock and Nussbaum (1973) and Ovaska and Gregory (1989). Note that there is much variation in growth rates of individuals, resulting in overlap in body size among different age classes.

Identifying Sex of Adult Salamanders

Eastern Redback Salamanders *(Plethodon cinereus)*
While the salamander is in the bag, hold it up to bright light and look for the presence or absence of testes through the abdominal wall (Jaeger et al., 2001). Absence of testes identifies a female.

OR

Using calipers, measure the distance between the nostrils of the salamander. This distance differs between males and females (Quinn and Graves, 1999).

Males:	0.22 - 0.33 mm
Females:	0.13 - 0.23 mm

OR

Look for the following characteristics:

Males: Presence of a mental gland, lighter pigmentation (*under the throat*), and greater folding of the cloacal wall.
Females: Absence of the above (Kleeberger and Werner, 1982).

Western Red-backed Salamander (*Plethodon vehiculum*)
While the salamander is in the bag, hold it up to bright light and look for the presence or absence of testes through the abdominal wall (Jaeger et al., 2001). Absence of testes identifies a female.

OR

Adult males have enlarged premaxillary teeth that protrude through the upper lip. You can feel the rasping of these teeth by running your moistened thumb along the underside of the snout of the salamander. Females lack enlarged teeth.

OR

Vent lobes are present around the cloaca of adult males but are absent from females. These lobes are visible as two folds, one of each side of the vent, in males, whereas the vent of females appears somewhat concave and smooth. It takes some practice to see this difference.

Other species
The upper lip of adult males of the Ensatina (*Ensatina eschscholtzii*) is enlarged and appears swollen. The sex of the Wandering Salamander (*Aneides vagrans*) cannot be determined reliably from external features. Using method 1 for *Plethodon cinereus* (looking for the presence of testes through the abdominal wall) might be feasible with this and other species of salamanders but requires more investigation. Gravid females of plethodontids can often be identified from eggs that are visible through the translucent abdominal wall. However, because plethodontids from northern localities typically do not breed every year, individuals without visible eggs may be of either sex.