

AMPHIBIAN



Monitoring Methods



Field Guide



Smithsonian
National Zoological Park

Sponsored by The National Zoological Park's
Conservation & Research Center

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INTRODUCTION TO AMPHIBIAN MONITORING

A. Goals and Objectives of this Program



The goal of this program is to give teachers the opportunity to bring textbooks-to-life by using real-world situations to teach scientific principles. Using the same techniques and methodology employed routinely by scientists at the Smithsonian Institution and other research organizations, students will be able to census and monitor amphibian populations in their own local community while learning about worldwide amphibian conservation issues.

This program focuses on: 1 basic amphibian ecology, 2 classifying and identifying individual species, 3 anatomy and physiology, 4 developing effective monitoring programs to assess population change over time and, 5 understanding current conservation concerns.

This manual is designed to assist educators with the development of inventory and monitoring programs that focus on amphibian populations in northern Virginia and the surrounding area. It provides information on techniques used to identify larval and adult stages of the species most likely encountered in this area, and it describes several methodologies that will enable schools to accumulate data that can be used to examine future population trends.

Teachers and others who engage in amphibian monitoring programs should use other reference material (see Appendix 1 and 2 for other resources and related literature) and web sites pertaining to amphibians, herpetology, and conservation. No one field guide can illustrate or describe the full range of variation in morphology, color, pattern, and behavior exhibited by frogs, toads, and salamanders. This, in part, is why the study of amphibians can be so exciting and rewarding.

B. The Roles of Scientists and How Students Can Contribute

The role of scientists is to measure a variety of parameters relating to ecosystems and to the diversity of species found in those ecosystems. By seeking to understand patterns found in comparisons of their data in conjunction with potential causes (and the implications of those comparisons), scientists hope to discover new ways of caring for life on our planet.

Scientists do not always know the best way to measure many of the traits they suspect are important in trying to understand life on earth. They do not always know the best way to sustain ecosystems and still acknowledge the needs of humans living in or near those ecosystems. But that is a part of what it means to be a scientist—to endlessly question; to explore all sorts of possibilities; to try new ways and to use new technologies to test ideas; to discover fresh ways to think about species and ecosystems.

This program seeks to turn school students into scientists; to bring them to the edge of what science now tells them and to ask them to explore what might lie beyond. If students are able to imagine what lies beyond current knowledge, and if they design methods to test their imagination, then they have become scientists.

Conducting an amphibian monitoring program can be a rewarding exercise for students. Not only do students learn how science is done using a real-world situation, but they are also able to contribute possible solutions to conservation issues. Their studies can lead to a host of other activities, including assisting in the education of other people in their community and evaluating laws and regulations that impact local and worldwide species and ecosystems. Hands-on studies allow students to become directly involved with the natural world and to discover their place in it. Above all, the study of amphibians can be fun.

C. About the Author and Sponsor

Joseph C. Mitchell, Ph.D. is a self-employed vertebrate ecologist who has conducted a wide variety of biodiversity inventories nationwide. His endangered species research and management studies include work for the National Park Service, National Forest Service, U.S. Fish and Wildlife Service, Virginia Department of Game & Inland Fisheries, Department of Defense, Virginia Division of Natural Heritage, Virginia Museum of Natural History, the Port Authority of Portland, Oregon, and several private environmental firms and corporations. Dr. Mitchell is an adjunct professor with the School of Continuing Studies at the University of Richmond. He has over 200 publications in peer review journals and wildlife magazines on the biology and management of amphibians and reptiles. Books include: The Reptiles of Virginia, 1994, Smithsonian Institution Press; Amphibians and Reptiles of Assateague and Chincoteague Islands, 1994, Virginia Museum of Natural History; Atlas of Amphibians and Reptiles of Virginia, VA Department of Game & Inland Fisheries (1999); and The Amphibians of Virginia, (in manuscript).

Recent and current research activities include: 1 inventory and management of amphibians and reptiles in a proposed reservoir site for the City of Newport News, VA, 2 establishment of standardized protocols for long-term amphibian monitoring sites on three military bases and in two national parks in Virginia, 3 evaluation of the effects of timber harvesting and development of a conservation plan for the endemic Peaks of Otter salamander in The Blue Ridge Mountains, 4 habitat assessment for amphibians, reptiles and small mammals on Fort A.P. Hill for the Virginia GAP Analysis Project, 5 development of a conservation and habitat management plan for the endangered bog turtle in Virginia, 6 evaluation of the conservation status of the western painted turtle in a wetlands complex in Portland, Oregon, and 7 evaluation of landscape use by amphibians and development of standardized long-term monitoring protocols on three military bases in North Carolina.

The National Zoological Park's Conservation & Research Center (CRC) is the focal site for the Smithsonian Institution's initiatives in conservation biology. Located on 3,200 acres in the foothills of the Blue Ridge Mountains just south of Front Royal, Virginia, CRC is a field station and laboratory that links regional and global conservation, basic and applied research, training and education.

CRC scientists realize that their programs have the potential to educate and inspire the public, especially children, about fundamental science, conservation and environmental issues. For this reason, CRC has initiated a educational program aimed specifically at training teachers in current conservation and research protocols, methods, and techniques.

CRC currently offers a selection of hands-on science outreach programs and teacher training opportunities designed to educate and inspire students about fundamental science, conservation and environmental issues. All programs are founded on current CRC research and endangered species breeding programs and allow students to "become scientists" by applying the same scientific methods and procedures used by our researchers locally and worldwide.

Information on other CRC research programs or training opportunities can be obtained by contacting the Education Office at the Conservation & Research Center by calling 540/635-6540 or by mail at 1500 Remount Road, Front Royal, VA 22630 or e-mail to jbuff@crc.si.edu.

D. Author's Acknowledgment*

This manual could not have been written without the help provided by numerous people who, over the years, have shaped my experience with amphibians in the mid-Atlantic region. They are far too many to acknowledge here by name but each one who has accompanied me in the field and helped me with data collection has my thanks. Some of my field research has been funded by The Legacy Resource Management Program (U.S. Department of Defense) and The Virginia Department of Game and Inland Fisheries.

For the development of this training manual, I especially thank Dr. Chris Wemmer of the Conservation & Research Center for having the vision to produce a manual designed to give teachers and other educators techniques to develop school programs to study amphibians in the field. Reviewers of the various drafts of the manuscript include Ronald Heyer, David McFadden, and Jamie Reaser, who deserve special thanks for helping to make this publication accurate and useful. I thank Jamie Reaser for providing the line drawings and Molly Griffin for the tadpole and salamander larvae. This project was made possible by funding from the Smithsonian Institution's Women's Committee with additional support from the Smithsonian Institution's Educational Outreach Fund.

INTRODUCTION TO AMPHIBIAN MONITORING

A. Amphibians and Scientists



Amphibians (frogs, toads, salamanders, tropical caecilians) have become recognized widely as sensitive indicators of environmental change. They have permeable skin, gills, and eggs that allow the uptake of chemicals. Most individuals have a complex life cycle in which they inhabit aquatic environments as larvae and terrestrial environments as adults. Many amphibians live and hibernate in soils and wetlands that expose them to toxic chemicals. They serve as both predator and prey in terrestrial and aquatic food webs (Dunson et al., 1982). Increasing numbers of amphibians are listed as endangered, threatened or are considered sensitive species under federal and state laws.

Unfortunately, several species have mysteriously gone extinct during the past two decades in some parts of the world. Other species, once widespread, are now restricted to only a few places (e.g., Heyer et al., 1988; Phillips, 1990,1994; Livermore, 1992; Drost and Fellers, 1996; Laurance et al., 1996; Lips, 1998). Reasons for the worldwide decline in amphibians are diverse and often local. These reasons range from habitat loss and/or alteration, thinning of the ozone layer and increasing the levels of ultraviolet radiation, chemical pollution of air and water, introduction of pathogens, fish and other animals into ecosystems either intentionally or unintentionally, and commercial exploitation (Freda and Dunson, 1986; Hayes and Jennings, 1986; Blaustein et al., 1994a,b,c, 1995; Berger et al., 1998; Tyler et al., 1998). Of these, the most pervasive and widespread cause of amphibian decline is loss of habitat.

Amphibians in the mid-Atlantic region have apparently not yet experienced the dramatic declines documented in other parts of the world. However, amphibians in this region have suffered population declines from historical and current habitat loss and alteration (Freda and Dunson, 1985, 1991; Tiner, 1987). Acid precipitation may play an important role in the near future. For example, the lowering of acidity in soil and water has reduced the amount of breeding habitat available for one terrestrial salamander and has shifted the genetic make-up of another pond-breeding salamander (Wyman, 1988; Wyman and Hawksley-Lescault, 1987; Bogart and Klemens, 1997). In Virginia, habitat loss and modification from urbanization (roads, home construction, shopping malls, office complexes, etc.) has caused the complete demise of many local amphibian populations (Mitchell, 1996), leaving the remaining populations in smaller and smaller fragments which are increasingly isolated from one another. Declines and losses of populations are local and the causes are usually obvious.

Fortunately, most of the amphibian species in the mid-Atlantic region of the United States are found in multiple ecosystems and are not as impacted by change or loss of one habitat. Many of these species can be considered generalists. Except for the terrestrial salamanders, Virginia amphibians require an aquatic habitat (e.g., pool, pond, or stream) in which eggs and larvae can survive to metamorphosis. They also need appropriate terrestrial habitat (e.g., intact hardwood forest) in which juveniles and adults can forage, grow, and be sheltered from harsh weather. Amphibians can be found in most places wherever these habitats co-occur.

The problem for scientists and others concerned about these animals is that we lack the long-term data sets necessary for realistic evaluations of population trends. Are populations in a given area declining, stable, or increasing? Are populations declining in some areas and not others? Answers to such questions require information from numerous places over many years where scientists are using the same methodologies. Standardization of methods used to obtain population information is critical to evaluations of how the amphibians are doing.

B. Amphibian Life Histories

Several closely related aspects of amphibian biology greatly influence how these animals interact with their environment. Their eggs have no protective casing (shell) and thus they must be deposited in water. Females do not retain their eggs in the oviducts for protection during development like reptiles, but deposit them at an early embryonic stage in water. Aquatic larvae need a swimming body form that includes gills for respiration.

Developing in water enables amphibians to exploit a rich food base in a relatively benign thermal environment. However, aquatic environments used by amphibians in the mid-Atlantic are seldom stable and safe, and frogs and salamanders use them at great risk. There are numerous vertebrate and invertebrate predators. The water can dry up, sometimes before the larvae are ready to metamorphose.

Metamorphosis requires dramatic changes in morphology and physiology when larvae change to lung-breathing, walking (or hopping) animals. The change is dramatic for frogs, which have to convert from a swimming form with a tail to a jumping form with no tail. Once out of the water, amphibians face a new set of problems, including a new set of predators, fluctuating temperatures and humidity, and changing habitats. Not all of these dynamic interrelationships have been described or are understood by the scientific community. Thus, these interactions offer a wealth of opportunity for study.

Frogs and toads:

The life histories of frogs and toads in the mid-Atlantic region can be grouped into two general categories based on the type of aquatic habitats they use for reproduction.

Species such as bullfrogs and green frogs require permanent bodies of water like ponds and lakes that may or may not have fish predators. Reproducing adults remain in and around the pond much of their adult lives, and tadpoles take as long as two years to complete metamorphosis. Juveniles, however, disperse widely and live temporarily in small, ephemeral (vernal) pools.

In contrast, other species, like wood frogs, gray treefrogs and American toads, reproduce in ephemeral pools that lack fish. Because the water dries up unpredictably, the larval period has to be short enough to enable metamorphosis to occur before the pools dry up and disappear. If metamorphosis does not happen quickly enough, entire cohorts of offspring may die during some years.

Populations of frogs in permanent water fluctuate less dramatically than those in ephemeral pools. Adults of all species of frogs require intact terrestrial habitats nearby for shelter outside of the breeding season. Adults and juveniles of many species have been documented hundreds of yards away from their breeding sites. Some have even been found over a mile away from their breeding sites (Dodd, 1996; Sexton et al., 1998; Pauley et al., 2000)!

Salamanders:

Salamanders in the mid-Atlantic region exhibit a greater diversity of life histories than frogs. Perhaps the most well known is the strategy of laying eggs in temporary pools in late winter (February-March) but living underground in the forest during the rest of the year. This approach typifies several salamander species, including the Jefferson, spotted, and marbled salamanders.

Larvae remain in the pools until May - July but are often at risk due to early pool drying. Red-spotted newts, on the other hand, live in both permanent water and temporary pools as adults and larvae. They disperse widely in the forest as brightly-colored juveniles (efts) until reproductive maturity is reached. Adults seldom leave permanent water. However, those living in temporary pools and ponds are able to withstand dry periods in moist microhabitats in the forest. Life in temporary pools is described and illustrated in Mitchell (1997a,b).

Seepage areas in riparian zones of streams and rivers and streams themselves support several species grouped into the assemblage called streamside salamanders. Two-lined salamanders, dusks, seals, and northern red salamanders, occupy this habitat and seldom leave except to disperse into the adjacent forest when it is wet. Reproduction takes place in the shallow water and below the substrate.

The most unique group of salamanders is comprised of species in the genus *Plethodon*, or terrestrial salamanders. These amphibians do not need water for reproduction. They are completely terrestrial and live in hardwood forests where they lay eggs in and under logs and rocks deep in moist soil. All of the larval development occurs within the egg capsule and the hatchling is a miniature adult form that is fully terrestrial.

Except for newts and the ambystomatid salamanders, few of the salamanders likely to be encountered in the mid-Atlantic region possess lungs.

The diversity of life histories and habitats used by amphibians in the Virginia and the mid-Atlantic region offer challenges to scientists and others who seek to monitor their populations.

C. How to Identify Amphibians

Identification of frogs, toads, and salamanders requires some basic knowledge of amphibian anatomy and understanding of key terms. This manual lacks dichotomous keys and assumes that a species in hand or one observed will be identified by comparing it with the illustrations and descriptions.

Adult amphibians of different species differ in body form, certain key anatomical features, color, and pattern, juveniles of the species included in this manual should not be difficult to identify but some individuals may exhibit challenging variations. The most difficult amphibians to identify are the larval forms: tadpoles and salamander larvae. The descriptions found in Appendix 6 of this manual will enable most individuals species to be identified correctly. However, the youngest larvae may be difficult because some features used in descriptions may not be fully developed.

A field guide to amphibians with accurate photographs and drawings is a must for anyone seeking to learn to identify frogs, toads and salamanders. A tape with recordings of frog and toad calls and vocalizations will also aid in identifying species.

Adult and juvenile frogs possess the same unique body plan: a robust body with no tail, short forelegs, and relatively long rear legs used for jumping. All of the phenotypic diversity in frogs is modifications of this same basic theme. In the mid-Atlantic region, variations are expressed as smooth or bumpy skin, presence or absence of a fold of skin along each side of the back (the dorsolateral ridge or fold), structure of the toes, color of the dorsum and venter, and size.

Adult and juvenile salamanders have elongated bodies, a long tail and, for most species, four limbs all about the same size used for walking. Much of the variation in this group occurs in body and tail length, snout length, number of toes on the forefeet, color, and pattern.

The larvae of these frogs/toads and salamanders differ dramatically.

Frog larvae (called tadpoles or pollywogs) have robust to globular bodies with a tail that has a distinct fin above and below. The mouth parts consist of several tooth rows and an upper and lower beak used for scraping algae and other microorganisms from surfaces. The gills are internal, covered by a flap of skin called an operculum. Most species possess a tubular opening called a spiracle on the left side of the body through which water exits the gill chambers. Tadpoles do not have legs at hatching but develop them as they mature. The rear legs emerge and develop first, followed by the forelimbs. During the process of metamorphosis to the juvenile stage tadpoles absorb their tails and switch from gills to lungs.

Salamander larvae are of two types: pond and stream. Pond type salamander larvae are elongated with all four legs present at hatching and have external, feathery gills. Their tails have high fins above and below the tail musculature and may extend onto the body. The fins of stream type salamander larvae are much less pronounced and occur only on the more elongated tail. Salamander tails are not absorbed during metamorphosis. The primary change is loss of the external gills. Some species develop lungs internally but others do not develop them at all. Instead, these species rely on oxygen-carbon dioxide exchange across the moist skin. Hatchling pond type salamander larvae possess a pair of small, elongated structures (one on each side of the head) called balancers. These are lost during development.

Identification of adults, juveniles and larvae of frogs, toads and salamanders requires close attention to the anatomical structures, color, and pattern described for each species. Once you have learned adults and juveniles, only a few of the larvae may remain difficult.

P. How to Conduct Field Studies

Before starting on a project with amphibians, you will need to answer the first question that should be asked of all field projects — What is the objective of the study?

If the objective is to conduct an inventory of an area or selected habitat, then one set of methods can be used. If the objective is to initiate a long-term study of a single species or a community of amphibians to evaluate trends, then a specific methodology or set of methodologies must be selected. If your objective is to determine the population size of a target species, then a mark-release-recapture method may be warranted.

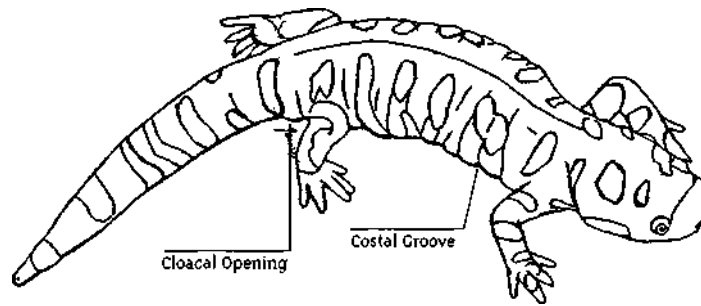
The objectives you choose for your study will greatly influence which methodology is right for the project you are conducting.

Most readers of this manual will have a specific area or habitat chosen for their study site. Temporary or permanent wetlands may be present or not. This is especially true for teachers who wish to conduct an amphibian study on school property. These logistical constraints will largely dictate what kind of project can be conducted. They will also influence the types of objectives for a study to be conducted at the site.

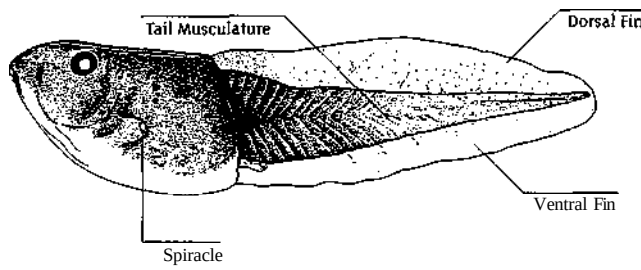
This manual describes how inventory and monitoring projects on amphibians can be conducted in three habitat types. Two focus on aquatic habitats and the other on terrestrial habitats. The methodologies described here are not meant to be the only way a project can be pursued. One can deviate from the approach presented, however, the teacher should describe in detail each and every step taken in written form so anyone (including other teachers or scientists) will know exactly how the project was conceived, organized, and executed. A detailed log in a permanent notebook or binder describing your methods is a good idea, whatever course is taken.

The protocols and techniques in this manual are based on the methods used by scientists working in the region and worldwide.

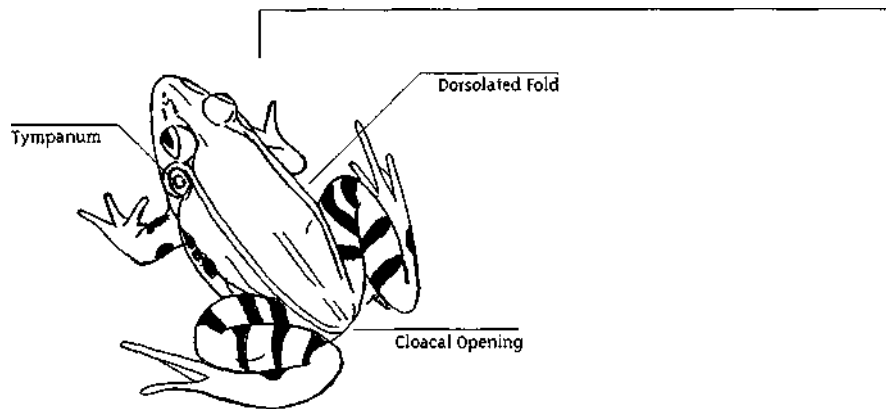
Salamander



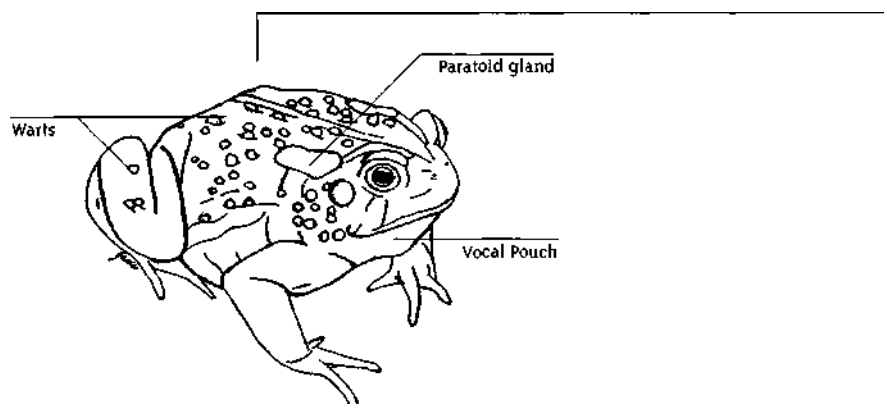
Bullfrog Tadpole



Ranid frog



American Toad



DESIGNING SCHOOL MONITORING PROJECTS

A. Level of Detail



When developing an amphibian survey or monitoring program, it is important to determine clear objectives. These objectives can range from identifying species presence/absence in a habitat, determination of relative abundance, or measuring population growth/decline over a period of time. Objectives determine the level of detail needed in developing protocols for your project. The level of detail needed in a project dictates the amount of effort and organization that will have to be expended. For example, a project focusing on species presence/absence in a habitat requires the least amount of effort, and only basic data (detail) are required in a census. Projects which focus on population size over time require the most effort, but

provide a great deal of information (data) on how a population performs in a habitat and ecosystem.

Many methods used for amphibian census and monitoring provide information on species presence/absence and indices of relative abundance for the species encountered. The protocols outlined in this document stress these two approaches.

NOTE: Long-term studies utilizing mark-recapture protocols require significant skill and expertise and are not addressed in this manual.

B. Site Selection

The number of sites selected for study depends on the objective(s) of the project and what areas are available for school use. If your objective is to conduct a simple census of amphibians found in a specified location, then the sites chosen for study should represent the range of wetlands and terrestrial habitats containing amphibians found in the area. If the objective is to monitor amphibians in a specific habitat type, then the sites selected for the project should fit the criteria for the specific habitat type of interest (e.g., vernal pool, oak-hickory forest, etc.).

Two general criteria should also be considered when selecting sites for study. These are:

- 1 Ease of access:** You should not have to hike extended distances to reach the study site, whether it is a wetland, stream or woodland.
- 2 Accessibility at night:** Several of the methodologies in this manual include night censusing. Care should be taken to select a site that can be located easily in the dark, even under adverse weather conditions.

C. Sampling Frequency

Amphibians are highly seasonal animals. It is difficult, and for some species nearly impossible, to find the adults outside of the breeding period. Project objectives will strongly influence the number of times sites will need to be visited. In general, the census techniques used for a presence/absent study will require fewer site visits than monitoring techniques yielding relative abundance data.

NOTE: Studies usually requiring the most site visits usually involve mark-recapture techniques, as the number of recapture incidences effects on the population estimate calculations.

P. Data Collection

The means by which data are collected and the level of accuracy required are important decisions that should be addressed before initiating any amphibian inventory or monitoring project. There is a trade-off between how much information is collected at a site and how much time it takes to collect that information at each site being studied (Fellers, 1997).

Not all data collected during the course of a study are considered equal. Some information must be collected accurately and precisely using the same methods each time it is collected. Other information is less essential and may be collected periodically or using different methods. For example, it is imperative that species and location be identified accurately, while some environmental information, such as water turbidity, is difficult to obtain the same way each time. Data sheets should be developed that reflect the accuracy needed for all information (data) to be collected.

The types of data that must be collected on each and every field trip are outlined below. Types of data [collected in a study should include] information on the locality, species identification and measurements, habitat type and environmental factors such as water quality, temperature, etc.

Following are items from Fellers and Freel (1995) listing information frequently collected during amphibian census or monitoring projects:

- site location: state, county
- plot/quadrat name or number
- date
- begin time of survey
- total time of survey
- observers
- topographic map name
- exact locality (corresponding to map)
- elevation
- « weather
- wind speed/direction
- air temperature
- water temperature
- site description (habitat type, size)
- drainage (from topographic map; alterations)
- ® substrate type
- © vegetation (description, canopy cover)
- ® species found (adults, juveniles, eggs, larvae)
- 9 other species present (reptiles, fish, birds, mammals)
- ® individual data (sex, body size, tail length, weight)
- ® for aquatic habitat: (1) maximum and average depth, (2) water flow, (3) turbidity, (4) mid-day shade, (5) emergent and floating vegetation, (6) fish present?, (7) fishing tackle present?
- photographs taken (roll and frame numbers)

Sample data sheets frequently used for frog call surveys, amphibian larval surveys, and inventories of amphibians in small ponds are included in Appendix 4; however, teachers should make up their own data sheets to suit their own specific criteria or situation. Other samples of data sheets can be found in Heyer et al. (1994), Fellers and Freel (1995), and Olson et al. (1997).

E. Species Identification

It is absolutely critical to the success of any amphibian inventory and monitoring project to identify correctly every individual encountered. Unfortunately, this is not an easy task, as amphibians are notoriously variable. Sometimes identification must be made using the single note of a frog call caught on tape or a fleeting glimpse of an escaping salamander. Pattern, color, and even body shape of a single species can vary greatly between habitats and geographic regions.

Accuracy in identification of species and recognition of life history stages (egg, larvae, adult) is extremely important in a census or monitoring project. Teachers and students should know the range of variation for all species in the area under study. Training and practice are essential.

It is most important to learn to identify each species found at your site before starting any amphibian census or monitoring project. Practice frequently. This includes being able to recognize all life history stages, preferred habitat and the behavior of each species.

If you have the opportunity, train with a qualified instructor. However, a good field guide to amphibians is essential and will aid in learning the colors, patterns, anatomy, sexual dimorphism, life histories, and ecology of each species. There are many good field guides and other publications that provide a wide variety of information (see Appendix 1).

Access to a voucher collection (specimen collection) at a local museum or university can also aid in acquiring identification skills (see Section 4).

F. Measurement Accuracy__

Being able to collect accurate measurements is another essential part of conducting an amphibian census or monitoring project.

Animals captured should be measured at least to the nearest millimeter and weighed to the nearest gram. Calipers work best for measuring length, width and depth; hand-held or digital scales should be used to weigh the animal to the nearest gram. Standardized measurements should be determined prior to the census or monitoring project. If the animals cannot be captured, then assign a size class (small, medium, large, etc.) beforehand. Weight should be taken on as many specimens as possible, as long as it does not compromise the time needed to collect other data. Once measurement collection methods have been initiated, they should become standard and routine.

Following are guidelines for measuring amphibians. All measurements should be taken to the nearest millimeter.

Frogs and Toads:

The standard size measurement for post-metamorphic (juvenile and adult) frogs is body length, without the legs. This is called "snout to vent length". The measurement is taken from the tip of the snout to the cloacal opening (vent). Make sure that the frog's body is flattened, not hunched.

Salamanders:

Snout to vent length, tail length and total length of salamanders are measured in a straight line using relaxed animals (not always an easy thing to accomplish). Snout to vent length is measured from the tip of the snout to the posterior margin of the cloacal opening (vent). The tail length is measured from the posterior cloacal margin to the tip of the tail.

Larvae:

Tadpoles and salamander larvae are measured in a straight line for body length (tadpoles), snout-to-vent length (salamanders) and total length.

Notes on Tail Measurements:

Not all tadpoles and salamander larvae and adults will have complete, original tails. Tails are frequently incomplete due to predation attempts or breakage. Adult salamanders regenerate their tails, although the color and pattern of the regenerated portion usually differs from the original. One should always note whether the tail is broken or regenerated on all individuals measured.

If an adult salamander has a regenerated tail, two measurements should be taken: (1) the length of the original portion from the posterior margin of the vent to the point of breakage and (2) from that point to the end of the regenerated portion.

These data provide insights into the frequency of tail breakage in a population and where on the tail the breakage most frequently occurs.

Other Abnormalities: Descriptions of abnormalities (e.g., missing or additional limbs, misshapen body parts), injuries, parasites (leeches, fly larvae, etc.) ,and odd colors and patterns should always be written down on the data sheets or in the field notebook.

MONITORING TECHNIQUES BY HABITAT TYPE



Most teachers who use the protocols and techniques found in this manual will probably be constrained by the availability of habitat types on or near their school grounds. This section outlines techniques for conducting a species inventory or census (development of a species list for a habitat or ecosystem) and for developing monitoring programs over several years (develops information on population size and changes over time). The techniques are grouped by habitat type in order to help users find the appropriate methods for their own unique situation.

Suppliers and equipment for each protocol and technique are noted in the Appendices.

A. Wetland Habitats

The selection of study sites depends on the range of wetland habitats available. Wetland types include human-made ponds and lakes, beaver ponds, ephemeral (vernal) pools, and freshwater marshes. If the number of wetlands in one's target area is small, then all of them can be monitored. If the area and number of wetland types available is large and all the wetlands of an area cannot be monitored effectively, then a similar number of each type should be selected. In this case an example or two of each wetland type should be included in the study plan. All sites should be indicated on a map of the area, assigned a code or place name, and coordinates determined.

Techniques used in wetlands habitat include the following four surveys:

Technique 1: Direct Visual Observations

Technique 2: Frog Vocalization Surveys

Technique 3: Egg Mass Surveys

Technique 4: Larval Surveys

Technique 1: Direct Visual Observations

One relatively easy way to study amphibians is to simply observe them in their natural habitat by walking around the edge of a wetland or watching from a vantage point. Unfortunately, amphibians are not as easily observed as birds because they are often hiding beneath vegetation or in water, are well camouflaged, and often remain still for long periods of time. Many species are active only at night and are thus even harder to observe directly. Daytime observations are still useful in many instances and can supplement other methods, especially when the goal is to conduct a simple inventory of amphibians present in a selected habitat.

Binoculars can be used to scan the shoreline of the wetland one is studying. Bullfrogs, green frogs, and others species may be observed sitting on the bank or on vegetation out in the water and their microhabitats can be cataloged and behavior studied without disturbing them.

Amphibians may also be encountered walking to a wetland study site or while hiking through the woods. This is especially true of toads. Records of these individuals provide insights into how a habitat is used.

Some species of frogs call periodically during the daytime and more frequently at night, especially during the height of the breeding season and sometimes for weeks afterwards. Hearing a frog or toad calling is another form of direct observation; the sense of hearing is used rather than the sense of seeing. Records of frogs species based on calls may be the only way one can determine their presence during daytime searches. (Techniques for conducting a census with vocalizations are addressed in greater detail below.)

On the initial approach to a vernal pool or pond, teacher and students should first record the species of calling frogs on their data forms as disturbance may cause them to stop vocalizing. Next, scan the shoreline and nearby riparian areas for frogs sitting on the ground or perched on low vegetation. Before disturbing the water by wading or dipping, air and water temperatures and other environmental variables should be taken. Count each individual adult frog or salamander observed and catch a many as possible for measurement and identification (see Section 2 for measurement data collection).

NOTE: Water depth and the amount of vegetation along the wetland edge may limit one's movement around the pond.

Technique 2: Frog Calls

Frog calls and vocalizations are an integral part of the reproductive and social biology of these animals. Only males advertise their presence by vocalizing in order to attract females and to warn off other males in the vicinity. Females do not call. Each species has its unique call (or vocalization). This enables scientists to: (1) identify individual species, (2) conduct an inventory or estimate of the number of individuals of a species, (3) study their activities, and (4) document the dynamics of the population.

In the mid-Atlantic region, frog species are reproductively active at different times of the year. Generally, some species breed in late-winter and early spring and others in late-spring and summer, and there is little or no overlap in calling times (Mitchell, 1986).

Frogs also respond to variations in weather. Dates frogs begin calling are different each year, the length of the male calling period varies within each season, and the times which females breed are all weather-dependent. In addition, rainfall events strongly influence when frog activities occur.

Wetland hydrology is a major player in frog biology. Frogs in permanent wetlands exhibit different phenologies (timing of biological activities during a season) than frogs in vernal pools and puddles.

Knowledge of such variation is important if one wishes to understand the dynamics of frog populations and how to interpret how they are affected by changes in their habitats and changes in weather patterns. Because so much variation exists from year to year and season to season, spot-checking for frogs by recording vocalizations only once or twice during a season will not provide information about population dynamics. This technique requires that it be repeated frequently throughout the breeding season.

Frequent frog call censusing also enables the researcher to accumulate data on reproductive biology and habitat use. Several methods can be employed in conjunction with frog call censusing (e.g., direct visual observations during the day or night) to obtain information on the dynamics of local frog populations.

Several skills and techniques must be learned and implemented to conduct a successful frog call census. These skills include:

- 1 Learning frog calls and, other types of vocalizations (eg., release calls);
- 2 How to set up a site visit schedule;
- 3 Recording frog calls, and
- 4 Keeping accurate records.

1 Learning frog calls: It is possible to become familiar with frog calls by listening repeatedly to commercial tapes that include all the species in your area. However, calls may also be learned by combining training sessions with tapes in addition to field trips to locations to hear them in context. Calls will sound differently when coming out of a speaker or headset than they will when you hear them in the pond or other type of wetland. A good knowledge of frog calls and other vocalizations they make is necessary to conduct an accurate census using these methods. Practice, practice, practice.

2 How to set up a site visit schedule: How many times during a year or season a site is visited depends on the objective of the study determined ahead of time. If information on the phenology of the species in the study area is a primary goal, then this methodology should be carried out at least once per week from early March, when frogs first become active, through the end of June. Because several species in some areas call until the end of summer (including green frogs, cricket frogs), the weekly methodology could extend from March until mid- to late-September.

Conducting a frog call census twice a month will provide information on species presence/absence, relative abundance, and habitat use. More site visits during a month will give more accurate information on species phenology. If a census is conducted only once a month, species will be missed and important events ignored.

3 Recording frog calls: An inexpensive but durable cassette tape recorder will enable calls of male frogs to be recorded during a census. Because some frogs will stop calling when someone approaches (also called the 'observer effect'), taping should be conducted before other censusing activities take place. Taping should be conducted by one person who points a hand-held directional microphone toward calling males (or the portion of the pond from which the calls are being produced). Avoid making any sounds during the taping session. Recording time should be at least five minutes (at a minimum) to ten minutes in length in order to increase the chance of recording all of the species present.

A FrogLogger is an automated animal vocalization recorder (Peterson and Dorcas, 1994) which will do most of the work for you. FrogLoggers record animal vocalizations during time periods established by the researcher by using timers attached to a recorder. For example, a researcher may set the FrogLogger to record 12 seconds during each hour for seven days (on one side of a cassette tape).

A FrogLogger is set outside in secure areas and chained to an immovable object. Microphones are placed within a plastic soda bottle cut in half to form a sound funnel, securely attached to a branch of a tree or shrub and pointed to the area to be recorded. The teacher or students must turn over or change the cassette tape on a regular basis, determined by the recording time set on the timer and the length of the cassette tape. In addition, battery life may be affected by local conditions and the teacher or students will need to replace them as needed. If FrogLoggers are to be used for extended periods of time, solar panels may be established to bypass the use of batteries (M. Dorcas, personal communication).

The primary advantage of using a FrogLogger, rather than a conventional tape recorder, is the regular, systematic recording of animal vocalizations over an extended period of time without having to be there. FrogLoggers also provide general information on size of the frog chorus and will record shy, secretive, and sensitive species which are easily disturbed by the presence of humans. One quickly obtains information on the day and night patterns of amphibian behavior. In addition, FrogLoggers will record birds, insects, and some mammals, too.

NOTE: FrogLoggers are not available commercially. You can make them following instructions found in Peterson and Dorcas (1994). Creative people may be able to devise their own automated animal vocalization recorders by adapting these instructions to meet their needs.

It is important to remember that cassette tapes from FrogLoggers accumulate rapidly. The researcher should identify the calls on the tape and transfer the information to data sheets as soon as possible. It can take more than two hours for a person well versed in frog call identification to record the data from a single 90 minute tape. Some people have created computer programs that are able to translate frog calls and enter the information directly into spreadsheets programs.

The following information should be recorded on the cassette tape before a census takes place:

- date
- location
- time
- © pond name and/or number
- © name(s) of project participants

See Heyer et al. (1994) for more information on recording accurate and useful frog calls.

4 Keeping accurate records: In addition to recording calls on cassette tape (or by using a FrogLogger), all frog vocalizations must be identified and the species recorded on a data sheet. The tape continues to serve as a back-up system. It should not be viewed as the primary source of identification and data records.

Technique 3: Egg Mass Surveys

Several species of amphibians in the mid-Atlantic region breed routinely in small ponds and vernal (ephemeral) pools. These include all species of salamanders in the genus *Ambystoma*, newts, as well as several frog species. Wood frogs (*Rana sylvatica*) breed exclusively in vernal pools which are devoid of fish predators. These small ponds and vernal pools are often relatively easily to access and study.

Amphibians which use these aquatic habitats for breeding leave conspicuous egg masses which can be counted to obtain quantitative measures of relative abundance or indices indicative of population size. Unfortunately, there is no recent reference or guide to field identification of amphibian egg masses for Virginia or the mid-Atlantic region. (See Appendix 6 for detailed descriptions of egg masses. Other sources for egg mass descriptions include: Bishop (1941), Wright and Wright (1949), Pfingsten and Downs (1989), and Petranka (1998).)

Egg masses can often be identified in the field. If a positive identification is not possible, then a full description may enable a later identification. Photographs are very useful as well. Use terms like "globular," "round," "firm jelly," "loose jelly," "string," "surface film," "attached to branch or grass," "approximate depth," "clumped" and "separated," when describing egg masses. Approximate counts of eggs in the mass and the measurement of jelly mass length and width will also help with identification.

Each egg mass found in a wetland area under study should be identified and counted. In some locations this might be difficult, perhaps due to deep water. If an innovative way to census the entire area is not found, use a standard distance around the margin of the wetland and count all the egg masses found within this band. If the pool is shallow enough to allow wading, then a quadrat grid or a transect marked at meter intervals can be laid out and the eggs counted within each grid cell or along the transect. If transects are used, then they should cover the entire pool to provide complete coverage. Counts for each species should be kept separate. For each egg mass, record the distance from the pool margin and water depth at which the mass is found. If possible, count the number of eggs in the egg mass, and determine how many are viable or are dead (dead eggs are milky white in color). Repeat this procedure at each pond or pool in a study area. This method can be conducted during the day or at night; however, nighttime is best because glare and other visual effects are more of a problem during daylight hours.

NOTE: These techniques may work best with spotted salamanders, wood frogs, gray treefrogs, and green frogs.

Technique 4: Larval Surveys

Assessing presence and relative abundance of amphibian larvae (including tadpoles and aquatic salamander larvae) can be an effective way to study and monitor amphibian populations. This type of census provides useful information on an often neglected aspect of frog biology. This is especially important because studying tadpoles through metamorphosis provides information on reproductive success and phenology of local populations.

The major advantage of doing a larval survey (besides being relatively inexpensive) is that they can be conducted during the day or night in a wide variety of wetland types. Larval surveys obtain information on quantitative estimates of relative abundance. The primary disadvantage of a larval survey is that tadpoles and salamander larvae are often hard to identify.

Researchers must be well-trained in larval identification and must be able to easily recognize a wide variety of variation within each species (as well as between species). Tadpole and salamander color, shape, and pattern can be influenced by the local environment. Larvae of a species found in muddy road rut pools will be the color of the mud, whereas the same species found in woodland pools with dark organic substrate will be dark in color.

Several techniques may be used to obtain information on tadpole populations and each is effective in different habitats. These include:

- 1 Seine nets
- 2 Dip nets
- 3 Minnow traps

1 Seine nets: Seines can be used to assess presence or absence of tadpole species in bodies of water lacking thick vegetation and snags and where one does not sink into the muck. Standard techniques should be applied: two people drag the seine rapidly across the substrate over a specified distance. In small wetlands, the entire body of water can be sampled. The number of samples taken will depend on the size of the wetland; larger ones require more samples. Relative abundance can be obtained by counting the number of individuals of each species in each sample. In small bodies of water that can be seined completely, one may be able to obtain complete population sizes. Seine mesh size needs to be taken into consideration, as early stage amphibian larvae can be quite small and fragile. Samples should be taken several times a season, preferably 2-4 times per month to account for changes in larval size, habitat utilization, and metamorphosis.

2 Dip nets: Sturdy dip nets can be used in a wide variety of wetlands, especially those that are small and are surrounded by heavy vegetation. Nets with a D-shaped ring are better than those that are round because the flat portion of the dipnet can be dragged across the substrate much like a small-scale seine. The length of a sweep should be standardized (e.g., 1 meter of drag in the water). Sweeps in all but the smallest wetlands (small pools, road ruts) should be at least 5 meters apart to approximate independent sampling for statistical treatment (Shaffer et al., 1994).

The required number of sweeps of the dipnet depends on the size of the pond. Small ponds and wetlands (15 m in diameter) may be sampled with as few as ten sweeps. Larger ponds and wetlands should be sampled with 20 to 30 sweeps. Once the number of sweeps has been determined for each pond, the same number of sweeps should be taken each sampling session to facilitate season-to-season and year-to-year comparisons. All of the parts of the pond system should be sampled to increase the chances of encountering larvae.

The numbers of each identified species found in each sweep are recorded on a data sheet, along with all pertinent environmental data (including water/air temperature, weather, etc.). Salamander larvae and tadpoles can be measured and their life stage identified using developmental schemes found in Gosner (1960) to provide important information on population structure. An assessment of relative abundance (based on the total sample) can be calculated and, at least for some sites, a statistical analysis can be performed (using numbers of each species per sweep sample).

The following steps should be used when using a dipnet:

- 1 Extend the dipnet approximately 1 meter or more away from the shoreline.
- 2 Dig all the way to the bottom and then in a vigorous and rapid motion, drag the dipnet toward the shore with all the debris and leaves. (The rapid movement of the sweep reduces the chances that the larvae will escape and it helps the net pass through vegetation.)
- 3 Pull all the material which is caught up in the dipnet onto shore.
- 4 Check the dipnet before emptying it for any adult or juvenile amphibians that may escape once the net is emptied.
- 5 Once you have emptied the contents on the ground, a plastic sheet or into a shallow pan, poke carefully through the debris and leaves for salamander larvae and tadpoles. A spoon is a very useful tool for this part and it also helps to scoop up larvae. These animals may be small, so it is advisable to take your time. Look at every leaf.
- 6 All animals caught for observation can be placed into one or two plastic bags or a shallow pan containing a quantity of water. Do not combine small larvae with large larvae because the larger ones may eat the smaller ones.
- 7 Record on a data sheet the number of every species found in each sweep of the dipnet, along with all pertinent environmental data (including water/air temperature, weather, etc.). Remember that the species of larvae and the numbers of individuals of each species should be recorded for each sweep of the dipnet.
- 8 Return all larvae to the wetland after recording the data.

3 Minnow Traps: Trapping larval stages of amphibians using small-mesh traps (such as minnow traps) enables a wide variety of wetland types to be censused, some of which may be impossible to census using seines and dipnets (e.g., deep ponds with rocky substrates). Traps can be set completely in deep water or partially exposed in shallow water. If a trap is set in deep water, they must be checked frequently (at least several times per day) so that drowning of non-target animals (e.g., newts, snakes, turtles) and predation by other animals, including predatory salamander larvae, can be avoided. Minnow traps work best if left overnight.

There is no bait known to work well for trapping larval stages of amphibians, so it is not necessary to bait a trap. However, it might be a nice class experiment to bait several traps with different types of bait to test food preference.

Trapping is an effective supplement to dipnetting. Combining these two techniques increases the chances of obtaining data on most, if not all, species in the wetland.

The numbers of each species of tadpole caught should be counted in each trap and the data expressed as number per trap day. If traps are placed at least five meters apart, then the results can be treated as independent samples and analyzed statistically (Heyer et al., 1994).

B. Stream Habitats____

Several species of salamanders in the mid-Atlantic region occur primarily in streams, especially in higher elevation streams. Stream habitats pose an entirely different set of challenges and limitations than do wetland habitats such as vernal pools, ponds, and freshwater marshes. Streams can be studied from April through October because the salamanders are active near the surface throughout this period. Most salamanders can be found under rocks along the edge of the stream. During wet seasons, some individuals may also be encountered under logs and rocks in terrestrial habitat in the adjacent forest.

Three techniques are often used to obtain data on streamside salamanders:

Technique 1: Time-Constrained Census

Technique 2: Quadrat Sampling

Technique 3: Leaf Litter Bag Census

Technique 1: Time-Constrained Census

A time-constrained census of salamanders along a stream habitat is performed by recording the starting and ending times of each survey. Rocks and other objects along the stream's edge are overturned systematically and swiftly in search of salamanders at any life stage (larvae, juvenile and

C. Terrestrial Habitats

Techniques for monitoring terrestrial salamander populations are fundamentally different from techniques dealing with aquatic amphibians. These animals (including salamanders in the genus *Plethodon*, red efts, four-toed salamanders and some streamside salamanders) are active on the surface of the ground in forested habitat only for short periods of time. They may be found foraging on the surface and low vegetation during warm, moist nights (rain or fog) and they may be found under objects on the surface at certain other times. These amphibians aestivate or hibernate underground when the surface is dry or frozen.

In general, there are two periods of time to monitor terrestrial salamanders in the mid-Atlantic region, spring (April-June) and late-summer to early fall (September-October).

The primary techniques used for monitoring include:

Technique 1: Transect Sampling

Technique 2: Quadrat and Plot Sampling

Technique 3: Coverboards

Technique 1: Transect Sampling

Scientists create transects (measured linear strips) along the forest floor to measure populations of terrestrial amphibians. Transects can be various sizes, but 1 x 50 meter or 2 x 50 meter transects are most often used (Heyer et al., 1994; Mitchell et al., 1996).

If the transect is temporary (less than one year), the centerline can be marked by flagging tied to trees or other vegetation at 10 meter intervals. If the transect is to be used long-term, the end points can be marked with Rebar® stakes or other permanent markers. Five to six transects need to be established within an area for statistical analysis. Each transect should be marked on a topographic map.

Once established, the transect can be censused at night during warm rains when the salamanders are active on the surface. Field personnel walk bent over or crawl on the ground and look carefully for adult and juvenile salamanders on the surface of the ground, on tree trunks, leaves, fallen limbs, and herbaceous vegetation.

Each salamander is identified, measured, and sexed and its position is recorded on a map. Measurements include snout-vent length, tail length (measure both original portion and regenerated portion for partial or re-grown tails), and weight (see Section 2 for measuring techniques).

If possible, all transects in an area are censused on the same night. If this is not possible, transects should be censused during the same rain event. At the very least, all transects should at least be censused during the same month to account for seasonal differences.

If night field trips are not possible, then students can conduct a census during the day. Day trips should be scheduled to occur within two to three days of a rain event while the ground cover is still moist. Ground cover (leaves, rocks, logs, etc.) should be overturned and replaced carefully during the census.

NOTE: Cover objects may be unattractive to salamanders for several days or weeks following a severe disturbance. Thus, it may be best to limit the daytime studies to once per spring or fall seasons.

Technique 2: Quadrat and Plot Sampling

Quadrat Sampling: One meter square frames (called quadrats) can be placed within a habitat and searched thoroughly for terrestrial salamanders (Jaeger and Inger, 1994).

Quadrats can be placed randomly throughout an area or can be placed along a linear transect that is itself established randomly. Resulting data yield species presence, relative abundance, and population densities. The number of quadrats needed in order to conduct an adequate census depends on the habitat size and the individual encounter rate. A general rule of thumb is that the more quadrats censused the better.

Quadrat frames can be made of 0.5 - 0.75 inch PVC pipe using elbows for connections. The frame can be taken apart for storage and transport. (An alternative method is to measure the quadrat on the ground and stake the four corners, however, this adds considerable time to the census.)

Quadrats can be sampled at any time, however, since terrestrial salamanders are most active during and following rains, it is more effective to conduct a census immediately after rain events. All surface objects are carefully overturned and replaced and ground cover and leaf litter thoroughly searched. Record all individuals of all sizes and sexes of each species encountered in each numbered quadrat.

Plot Sampling: Plots are larger than quadrats (up to 10-15 meters square) and are usually marked with string. Fewer plots are needed to conduct an accurate census but searching time is longer. Plot placement should be random within a habitat.

All cover objects are carefully overturned and leaf litter is raked in search of salamanders. All cover types and microhabitats should be mapped and measured.

NOTE: Due to the amount of disturbance in both quadrats and plots, a plot site should not be censused again for a year or more.

Technique 3: Coverboards

The use of coverboards for amphibian monitoring is a logical extension of a favorite activity of many field herpetologists — looking under logs and rocks, and takes advantage of the fact that amphibians like to hide underneath things.

Only a small portion of the total terrestrial salamander population forages on the surface at any specific time. Salamanders use surface objects for cover in their territories; man-made coverboards may also be used in place of natural cover.

A wide variety of materials can be used to make a coverboard but sheets of untreated rough cut plywood (measuring 12 x 12 inches) are recommended (Fellers and Drost, 1994). They should not be too large, as dry patches can develop and limit their effectiveness (T. Pauley, personal communication).

Coverboards can be arranged in grids of various sizes and configurations determined by the researcher to work best for the habitat and species to be studied. Grid arrangement may vary from rectangular in shape to a domino configuration (an X-shape with a board at each corner and one in the center). All coverboards are numbered individually.

Once deployed, coverboards can be checked by lifting them and capturing the amphibians (and reptiles) underneath. This technique requires at least two people for the best results: one person lifts the board and the second quickly captures any animal found underneath.

Each animal is identified, sexed, measured, weighed and marked. Data on each animal caught should also include the board number and data on environment and microhabitat.

After all data are collected on an amphibian, the coverboard board is replaced as close to the original position as possible and the animal is released at its edge. Coverboard grids should be checked several times during the animal's active season.

A coverboard may be occupied by a single individual for most of an entire season which may prevent other individuals from using it. Mark-recapture-release techniques must be used to account for the sighting or counting of an individual more than once. This requires considerable expertise and the capture and handling of each individual. Mark-recapture techniques are not addressed in this manual. Without a mark-recapture protocol, a study is limited to a population census only, with little information on the status of the population or trends in population size.

The effectiveness of the coverboard census technique varies dramatically due to weather, season, soil moisture, habitat type, and species behavior. Coverboards are less effective in dry habitats. In addition, coverboard size and grid pattern can potentially affect capture and monitoring success. This is a relatively new census technique and there have not been enough formal field tests to know their effectiveness. However, grids of 0.66 x 1.33 m plywood coverboards have been used extensively in South Carolina forests (Grant et al., 1992) and arrays of 20 x 10 x 1.3-2.5 cm and 175 x 23 x 19 cm rough-cut boards have been used in West Virginia (T. Pauley, personal communication).

USE OF AMPHIBIANS IN CLASSROOM STUDIES

A. Tips on Handling Amphibians_____



Amphibians have sensitive skin and whatever is on your hand may be easily absorbed by the animal in your hand. Frogs and salamanders are also sensitive to the heat of the sun and other drying conditions.

The following is a list of precautions that will minimize the stress and potential death of an individual you have captured:

- 1 Never use bug spray or other chemicals on your hand while working with amphibians.
- 2 Keep the frog or salamander out of the hot sun and do not let it overheat.
- 3 Keep the animal moist.
- 4 Do not keep tadpoles or salamander larvae out of

water for more than a minute or so. Return them to the water as soon as possible.

- 5 Do not squeeze frogs any tighter than is necessary to restrain them. Holding them firmly around the waist is the best method.
- 6 Do not squeeze tadpoles. They are turgid with water and may burst open.

B. Amphibian Health Issues

An important recent development in the scientific evaluation of the worldwide amphibian decline crisis is the realization that things scientists do to census and monitor amphibians in the field may actually harm the animals that are being studied.

The Declining Amphibian Populations Task Force (DAPTF) is a network of concerned scientists based in the United Kingdom. In 1998, DAPTF released a Fieldwork Code of Practice 1998 to address this issue.

Seven points to the code are included here to make teachers and students aware of the risks to amphibian populations during field census and monitoring projects. These codes serve as guidelines and their use is left up to the discretion of the individual researcher.

- 1 Remove mud, snails, algae, and other debris from nets, traps, boots, vehicle tires and all other surfaces. Rinse cleaned items with sterilized (e.g., boiled or treated) water before leaving each study site.
- 2 Boots, nets, traps, etc. should then be scrubbed with 70% ethanol solution and rinsed clean with sterilized water between study sites. Avoid cleaning equipment in the immediate vicinity of a pond or wetland.
- 3 In remote locations, clean all equipment as described above (or with a bleach solution) upon return to the lab or "base camp". Elsewhere, when washing machine facilities are available, remove nets from poles and wash with bleach on a "delicate" cycle, contained in a protective mesh laundry bag.
- 4 When working at sites with known or suspected disease problems, or when sampling populations of rare or isolated species, wear disposable gloves and change them between handling each animal. Dedicate sets of nets, boots, traps, and other equipment to each site being visited. Clean and store them separately at the end of each field day.
- 5 When amphibians are collected, ensure the separation of animals from different sites and take great care to avoid indirect contact between them (e.g., via handling, reuse of containers) or with other captive animals. Isolation from unsterilized plants or soils which have been taken from other sites is also essential. Always use disinfected/disposable equipment.

- 6 Examine collected amphibians for the presence of diseases or parasites soon after capture. Prior to their release or the release of any progeny, amphibians should be quarantined for a period and thoroughly screened for the presence of any potential disease agents.
- 7 Used cleaning materials (liquids, etc.) should be disposed of safely and if necessary taken back to the lab for proper disposal. Used disposable gloves should be retained for safe disposal in sealed bags.

C. Voucher Collections and Photographs

Voucher Collections: The decision to collect, properly preserve, and store a representative set of voucher specimens is an important one, although sometimes controversial. In some circumstances, the removal of even a small number of individuals from a declining population may cause additional harm. However, voucher specimens often provide the only means of scientific verification (evidence) for species identification. Voucher collections also provide a great deal of information about the biology of a species, as well as the ecology of the habitat; sort of like a book on a shelf.

If a voucher specimen or series of specimens is collected, it must be: (1) euthanized humanely, (2) preserved promptly (to avoid loss of reproductive and diet information), (3) preserved properly and (4) stored correctly.

Adult specimens are preserved in 10% formalin for hardening of tissues and then transferred to an airtight container containing 70% ethanol. Larvae must be stored in 10% buffered formalin and never transferred to alcohol, else they deteriorate.

Voucher specimen are worthless without the accompanying collection data (date of collection, exact location, etc.).

Some researchers take tissue samples from these specimens for DNA analysis prior to preservation, involving yet another layer of procedures.

Proper preparation of voucher specimens is a time-consuming task and the techniques must be learned and practiced before the inventory and monitoring program is initiated. Simmons (1987) and McDiarmid (1994) provide instructions on proper preservation techniques.

Photography: Photographic images of specimens, instead of preserved specimens, are preferred by a growing number of people. They have the advantage of leaving the animal alive in its habitat.

One must ensure, however, that the photographic image is taken properly and that it illustrates all the necessary features that will allow proper identification at a later date. This is difficult for larval forms. One cannot retake a picture of an animal once it has been released.

Images should be labeled properly as soon as they are processed with all collection information and stored location with proper storage facilities. Improper storage can lead to loss and disarray.

Color slides are preferred over prints. Slides using K-14 processing (Kodachrome) last about 25 years when stored in dark, whereas slides using E-6 processing (Fujichrome, Ektachrome) will last longer (Fellers, 1997).

The use of slides for vouchers may be a reasonable approach for well-known, common species that are taxonomically stable (e.g., bullfrogs). However, many species are not stable taxonomically and the systematic relationships have not been settled. Slides of such individuals will probably not enable one to see newly-recognized features, and they will certainly not allow measurements or internal examination. If the identification of a specimen becomes dependent on these measurements, then a photographic image will not suffice and the animal's identification may come into question. Thus, preserved vouchers are preferred over slides when this option can be taken.

D. Permit Requirements

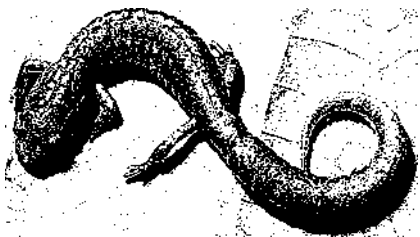
The State of Virginia requires that anyone possessing more than five individuals of any species that is not endangered or threatened must have a valid Scientific Collection Permit. Permits to conduct research on protected species are rarely provided.

If collections of voucher specimens are planned or if more than five individuals are to be housed in the classroom, then teachers should contact the Virginia Department of Game & Inland Fisheries and ask for application forms for educational use permits. Such permits will require an annual report.

Obtaining proper classroom permits is not as daunting a task as it may seem at first and there are many resources available to teachers who wish to set up living or preserved classroom voucher collections (including amphibians, mammals, reptiles, etc.). The Wildlife Center of Virginia has a helpful informational brochure entitled "Wildlife Laws: What Virginia Educators Need to Know." This brochure can be obtained by writing P.O. Box 1557, Waynesboro, VA 22980 or by calling 540/942-9453.

Additional information on educational use permits may be obtained directly from the Virginia Department of Game & Inland Fisheries, Wildlife Education Office, P.O. Box 11104, Richmond, VA 23230 or by calling 804/367-0188.

COLLECTING ENVIRONMENTAL DATA



Taking accurate measurements of environmental variables may be difficult. Naturally-induced variation makes it difficult to be consistent in collection methods. It is important to collect each measurement the same way every time.

For example, in a wetland, water temperature can vary due to shading, depth and presence of vegetation, and one must decide how many temperatures need to be collected and averaged to be most accurate. Another example is that wetlands may not have clear, discrete edges due to the nature of the slope and vegetation along the margin. Should

the wetland size be measured to the nearest 0.1 meter or nearest 1 meter?

Decisions on standardization of your environmental measurements must be made in the planning stages of the project.

Physical parameters that could be sampled at a study site include:

- 1 temperature (in Celsius) of the air and water
- 2 an estimate of turbidity (cloudiness)
- 3 pH
- 4 dissolved oxygen (DO) (a DO meter calibrated for elevation is necessary for this measurement)
- 5 alkalinity
- 6 salts and heavy metals
- 7 other chemicals such as herbicides and pesticides

A. Temperature and Water Measurements

Recording air and water temperature is essential. Air temperature should be taken about 1 meter above the ground in shade while you are standing near the edge of the pond. Make sure that the thermometer is held away from you to avoid body temperature influence. Water temperature should be taken in about the same place in the pond where the water sample was obtained. (See below) Take several readings from several locations. Both of these measurements should be taken before entering the pond to sample larvae.

B. Water Samples for Chemical Analysis

A number of chemical factors influence the presence and survival of amphibians. These include pH, alkalinity (or acid neutralizing capacity), dissolved oxygen, salts, and heavy metals. Most of these measurements are obtained easily, either with field meters or by having them analyzed in a chemistry lab. Other chemicals, like pesticides and herbicides, can be evaluated but are often expensive. It may be possible to obtain data on many of the basic water chemical parameters by engaging the chemistry department in your school. This interdisciplinary approach has many advantages, including collaboration with colleagues and other students.

Water sample bottles require special preparation because the acid-base chemistry required for many chemical analysis can be altered by contaminated bottles. The usual procedure is to wash bottles in hydrochloric acid, rinse thoroughly, and store with de-ionized water. Enough bottles

should be prepared for all census locations ~~BEFORE~~ the field season starts to avoid having to prepare or obtain them on short notice.

Take the water sample after recording observations on species present and other environmental data like temperatures but before you disturb the area to sample or census amphibians. This will minimize disturbance of the sediment on the bottom and lessen the chances of getting undesirable material in the bottle.

The deionized water stored in the bottle should be dumped out immediately before obtaining a water sample from the pond. The bottle must be rinsed three times with wetland water before taking the final sample. The final sample should be taken from an untouched portion of the wetland, avoiding plant material and bottom debris. Take the sample in a portion of the wetland that has at least 20 cm of water. Fill the bottle completely.

Hands should never come in contact with the column of water being sampled or the uncovered mouth of the bottle. Plastic gloves can be used to prevent contamination, though this is not necessary.

Once the sample has been taken and the bottle capped, the bottle should be wiped dry, placed into a separate plastic bag and labeled (either inside the bag or with permanent marker on the bag itself) with the date, pond name and/or number and location sample was taken from. Store the water samples in a cooler with ice packs.

If you work with a professional lab, check with them for water collection protocols, as they may differ from the ones listed here.

TIPS FOR TEACHERS



The following several checklists are for teachers to use when planning a school amphibian census or monitoring program. These lists are not a traditional syllabus or lesson plan but should serve as an organizational tool and be modified to fit each teacher's particular situation and project requirements. Discussions with students about methodologies, the scientific method, data analysis, and scientific writing can be interjected anywhere each teacher deems appropriate.

These lists include points to conduct a full species inventory and portions not applicable to the specific methodology you are using should be omitted.

A. Wetlands Habitat Monitoring Checklist

Classroom preparation:

- 1 Familiarize students with all procedures that will be used, including student and amphibian safety points, species identification techniques and data collection protocol.
- 2 Prepare and organize supplies and equipment to be used during the census.
- 3 Assign specific tasks to each student and review protocol.
- 4 On the day of the census, briefly review all procedures and protocols and conduct an equipment check.

In the field:

- 1 Conduct a direct visual and an auditory census upon approaching the wetland. Record all frogs calls heard and amphibians seen on a data sheet.
- 2 If night field trip, tape record frogs.
- 3 Collect water samples and record air and water temperatures.
- 4 Record environmental and habitat information on data sheet.
- 5 Photograph sites and document census techniques as needed.
- 6 Obtain dipnet samples & record results for each sweep; preserve examples of larvae if necessary.
- 7 Review tapes to make sure recording procedures worked.
- 8 Review data sheets for completeness.
- 9 Re-pack equipment and supplies in a bucket or other waterproof container, and organize all samples and/or specimens to be returned to school.
- 10 Check equipment prior to leaving study site to ensure against loss.
- 11 Repeat steps 1-10 for each site to be censused.

Classroom processing post field trip:

- 1 Check labels of all samples.
- 2 Place water samples in refrigerator.
- 3 Put specimen jars in safe storage location.
- 4 Copy all data sheets.
- 5 Clean equipment and supplies; re-pack for next field trip.
- 6 Place all data sheets and tape recordings into files.

B. Stream and Terrestrial Habitat Monitoring Checklist

Classroom preparation:

- 1 Familiarize students with all procedures that will be used, including student and amphibian safety points, species identification techniques and data collection protocol.
- 2 Prepare and organize supplies and equipment to be used during the census.
- 3 Assign specific tasks to each student and review protocol.
- 4 On the day of the census, briefly review all procedures and protocols and conduct an equipment check.

In the field:

- 1 Establish transects, quadrats or plots in the terrestrial habitat to be censused. Establish transects and sections for time constrained searching along streamside habitats to be censused.
- 2 Photograph sites and procedures as needed.
- 3 Record habitat information as noted on data sheet.
- 4 Take water sample and environmental data as needed.
- 5 Execute transect, quadrat or plot search and record all observations, including individual salamanders censused, on data sheets.
- 6 Review data sheets for completeness.
- 7 Re-pack equipment and supplies and organize all samples and/or specimens to be returned to school.
- 8 Check equipment prior to leaving study site to ensure against loss.
- 9 Repeat steps 1-8 for each site to be censused.

Classroom processing post field trip:

- 1 Check labels of all samples.
- 2 Place water samples in refrigerator.
- 3 Put specimen jars in safe storage location.
- 4 Copy all data sheets.
- 5 Clean equipment and supplies; re-pack for next field trip.
- 6 Place all data sheets and tape recordings into files.

C. Processing and Analyzing Data

- 1 Write the original objective or hypotheses on a handout or on a blackboard. (Objectives and hypotheses should be determined before the start of the census or monitoring project.)
- 2 Enter data collected in the field into computer spreadsheets following a simple format that will allow you to obtain statistics and graphs (individual students or student teams can divide the work load). This spreadsheet should include data on species identification and measurements, site information and environmental data.
- 3 Perform basic statistical calculations (e.g., means, standard deviations, and minimum-maximum for number of observations per quadrant, transect, or dip net sweep) as needed.
- 4 Develop graphs and charts that illustrate and summarize the results.
- 5 Organize non-numerical results and observations by species and habitat type.
- 6 Assign writing tasks to individual students or student teams. Student reports should be written using standard formats (see Section 7).

P. Equipment Checklist

The list of supplies and equipment listed for each of the major types of methods described above is not exhaustive. You may find other items that suit your needs.

Abbreviations:
FC = frog call surveys
EM = egg mass surveys
LV = larval surveys
ST = streamside salamander surveys
TR = terrestrial habitat survey

Equipment List	FC	EM	LV	ST	TR
Aquarium dipnets			X		
Binoculars		X	X		
Blank cassettes (60 min. non-metallic)	X				
Cassette recorder, with extra batteries	X				
Collecting permit	X	X	X	X	X
Data forms with clipboard and pencil/pen	X	X	X	X	X
Dipnets		X	X		
DO meter	X	X	X		
FrogLoggers	X				
Flagging tape		X		X	X
Hammer for pounding stakes				X	X
Handlens (10X)			X	X	X
Headlamp with extra batteries	X	X			
Metric rulers (plastic preferred)	X	X	X	X	X
Metric tape measure (50 meter)	X	X	X	X	X
Microphone with switch	X				
Minnow traps			X		
Pesola® scales	X	X	X	X	X
pH meter	X	X	X	X	
Plastic bags	X	X	X	X	X
Plastic netting (leaf bags)				X	
Rainsuit	X	X	X	X	X
Sling psychrometer	X	X	X	X	X
Spoons			X		
Stakes (Rebar®)				X	X
Thermometer (Celsius)	X	X	X	X	X
Tools for rocks and boards •				X	X
Waders	X	X	X	X	
Watch with second hand or stopwatch	X			X	

E. Student Safety

Teachers and students should always practice appropriate safety measures in the field. Common sense safety in the field should be emphasized routinely throughout the course of the study. Hands should be rinsed thoroughly after handling any amphibian.

Some amphibians, like gray treefrogs and pickerel frogs, produce mucus that is irritating to mucous membranes. Poisonous plants and venomous insects to which students may be allergic should be avoided. Venomous snakes are rarely encountered, but everyone in the field should know where their feet and hands are at all times, and everyone should be able to identify local venomous snakes from non-venomous snakes on sight.

ANALYZING DATA AND MAKING RESULTS USEFUL

A. Forming a Hypothesis and Analyzing Data



How one analyzes data depends on the hypotheses established at the beginning of the study. Data analysis can be relatively simple, like comparing the lists of species discovered in different habitats or at different times in the same habitat. It can also be complicated if too many questions are asked.

Differences might be found by looking at various habitats, weather conditions, or field techniques. Comparisons using numbers (such as number of salamanders found along several transects or the number of egg masses in two or more pools) can be made in several ways. One can compare total numbers or one can perform elementary statistics on the samples

if there are replicates. Comparisons of differences and similarities can be illustrated with pie charts, histograms, or other appropriate graphs.

It pays off at the end of a project to have asked very specific questions at the beginning. Here are two examples:

- 1 Do spotted salamanders deposit more egg masses in large vernal pools than in small vernal pools in my area?

Obtaining counts of all the egg masses in each pool and comparing the totals would provide a direct answer. Then one can ask if this relationship is maintained over a period of several years or at other study sites. You can also ask if there are more egg masses in pools located near hardwood forests than in pools in the open or in pine forests.

- 2 Are there more terrestrial salamanders in a patch of hardwood forest than a patch of pine woods?

One would establish an equal number of transects of equal length in each habitat type, execute the protocol and compare total numbers observed. If there were three or more transects in each habitat, then a statistical test may be used to provide a more rigorous evaluation.

One can then ask questions about similarities or differences in recent weather or number of cover objects. The list of potential questions one can ask is almost endless. The more precise your question up front, the easier it will be to determine how you need to analyze your data at the end.

B. Writing Reports and Papers

There are many ways of writing a scientific paper. Many people believe that science writing is rigid and dry, but in fact, there can be a great deal of creativity involved.

The primary difference between writing scientific papers and essays for an English class is the structure.

The basic structure and order of a scientific paper follows:

- 1 Title. The title of a paper should be informative and not too long (see some examples in the "literature cited" section of this manual).
- 2 Name(s) of Author(s).

- 3 Abstract. A clear and concise abstract of the project should be written that is no more than a half of a page in length and which includes brief information on background, hypothesis, and results.
- 4 Introduction . The introduction should begin with a paragraph setting the stage for the project. For example, an introduction can include a paragraph about current conservation issues on amphibian decline worldwide, followed by a paragraph describing why you are doing this project. An introduction will also include a clear statement of the objectives or hypothesis for the study.
- 5 Materials and Methods. The materials and methods section includes a description of your study site, some basic natural history of the study animals, descriptions of methods used, how animals were measured and handled, and how the data were treated statistically (including the computer software used).
- 6 Results. The results section includes summaries of your observations and the results of your analysis, including references to tables and graphs that support your statements about differences and similarities between samples or sites or species.
- 7 Discussion. The discussion section should evaluate the results of a study in light of the questions asked or hypotheses established before the project began. It is often the hardest part to write because it must evaluate the results of the study in context with what has already been published. In the case of a classroom paper, coverage of previously published scientific literature may be limited; however, students can still compare their results with information derived from a few key papers provided by the teacher or from studies found on the Internet.
- 8 Acknowledgments. The acknowledgments section is where the authors thank key people or organizations instrumental in the implementation and completion of the study, including major funders and other supporters.
- 9 Literature Cited. A scientific paper must cite all other literature used in the text and should follow a standard format (See Appendix 2 for examples on citing literature).
- 10 Tables and Figures. Tables and figures in an unpublished manuscript are placed at the end of the paper after the literature cited section. Publishers will then take these tables and figures and imbed them within the body of the text when they print a scientific paper in a journal. Teachers may allow students to imbed them in the body of the pages.

Writing a scientific paper as an individual or group project can be fun. It is only when the paper is written that one can see what you have done, how you did it, and how your results provide insights into the natural world. Teachers may wish to compile all student reports into one protocol manual or binder and store in a safe place. These protocol binders then become a resource to students planning census or monitoring projects in future years.

C. Archiving Data

If all the animals are identified correctly and the counts and measurements were taken under a standard protocol, then the resulting data set becomes valuable from both scientific and historical perspectives. The data accumulated are likely to have value beyond the classroom exercise. Such data aid in a greater understanding of the natural history of each species and provide a background against which future data sets may be compared.

Make several copies of all data (both data sheets and electronic files) and keep them in more than one safe place. Keep two copies for yourself and send another (both hard and electronic copy) to the Virginia Department of Game & Inland Fisheries or other state agency charged with maintaining information on amphibians in their states. This should include any maps, field notes, data sheets, and electronic copies of spreadsheets.

APPENDIX I

ADDITIONAL RESOURCES

Bibliography

There are numerous sources of information about amphibians on the Internet. Many journals provide lists of their contents. General searches on herpetology or amphibians will reveal a host of web sites and much information. The list below includes some general references on amphibians for the mid-Atlantic area. The first one includes a web site for an on-line journal that deals with amphibians (and reptiles).

- Altig, R., R.W. McDiarmid, and K.A. Nichols. 1998. "A key to the Anuran Larvae of the United States and Canada." Contemporary Herpetology 1 (on-line: <http://vmsweb.selu.edu/pcsd4805/>)
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- Wright, A.H., and A.A. Wright. 1949. Handbook of the Frogs and Toads of the United States and Canada. Cornell University Press, Ithaca, NY. 640 pp.

Frog Call Identification Tapes

- The Calls of Frogs and Toads. NorthWord Press, Inc., P.O. Box 1360, Minocqua, WI 54548 (800-336-5666)
- Voices of the Night. Library of Natural Sounds. Cornell Laboratory of Ornithology, 159 Sapsucker Woods Rd., Ithaca, NY 14850)
- Sounds of North American Frogs. C.M. Bogart. Smithsonian Institution, Folkways Cassette Series 06166. (Center for Folklife Programs and Cultural Studies, 955 L'Enfant Plaza, Washington, D.C. 20560)

Sources of Equipment and Supplies

- Forestry Suppliers, Inc., P.O. Box 8397, Jackson, MS 39284-8397 (800-647-5386) (clipboard with cover; thermometers (Celsius); Justrite headlamp - FS 02155; extra bulbs for headlamp; Pesola scales; tape measures; Rite-in-the-Rain copy paper; pH and DO meters)
- Ben Meadows Co., 3589 Broad St., Atlanta, GA 30341 (800-241-6401) (clipboard with cover; thermometers (Celsius); Justrite headlamp -BM 130594; extra bulbs for headlamp; Pesola scales; tape measures; Rite-in-the-Rain copy paper)
- Carolina Biological Supply, 2700 York Rd., Burlington, NC 27215 (800-334-5551) (ethanol, chloretone, field supplies)
- Wards Natural Science Establishment, Inc., 5100 West Henrietta Rd., P.O. Box 92192, Rochester, NY 14692-9012 (800-962-2660) (dipnet, D-frame - 10W0620; extra dipnet bags - 10W0625)
- Diagger and Company, Inc., 199 Carpenter Ave., Wheeling, IL 60090 (800-621-7193; FAX 800-320-7200). (Nalgene® water sample bottles)

Sources of Equipment and Supplies

University Products, Inc., PO Box 101, 517 Main St., Holyoke, MA 01041 (800-628-1912) (100% rag, 28 lb, 8.5x11 inch paper, 100 sheets)

Fisher, Inc. (formaldehyde, 37% stock solution)

Bass Pro Shops, 2500 East Kearney, Springfield, MO 65898-0123. (800-227-7776) (minnow traps, waders)

Local sources (plastic bags, quart size; spoons; pens/pencils; cassette recorder with external microphone on cord; cassette tapes; batteries for cassette player; batteries for headlamp; magic markers (black); Styrofoam boxes for shipping water bottles; shipping tape; box for specimen jars)

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APPENDIX 3

SAMPLE DATA SHEETS

Appended here are several data sheets that may be used as examples for various amphibian monitoring projects. Data sheets do not have to be elaborate or fancy. Their primary use is to organize the collection of data in the field, remind field personnel the categories of data to take consistently, and provide a permanent hard-copy record of all the data obtained. Data sheets should be designed after you have settled on your specific questions or hypotheses and have designed the methodology. They should include all the data fields that are necessary to accomplish the task.

Frog Call Survey Field Data Form

Location:

Date:

Site:

Observers:

Begin time: _____ End time: _____ Air temp: _____ Water temp: _____ pH: _____

Cloud cover: _____ (clear = 1; partial clouds = 2; overcast = 3; fog = 4; drizzle = 5, showers = 6)

Wind speed: _____ (0= smoke rises vertically; 1= wind direction shown by smoke drift; 2= wind felt on face; 3= leaves in constant motion; 4= small branches moved; 5= high wind gusts)

Water turbidity: clear to fully turbid 1 2 3 4 5 (circle one)

Species	Frog call	Egg mass/larva	Notes
American bullfrog			
American toad			
Eastern spadefoot			
Fowler's toad			
Gray treefrog (H. chry.)			
Gray treefrog (H. vers.)			
Green frog			
Narrow-mouthed toad			
Northern cricket frog			
Pickerel frog			
Southern leopard frog			
Spring peeper			
Upland chorus frog			
Wood frog			
Other			
Other			
Other			

Chorus:

1 = single individual calling; 2 = individuals can be counted (space between calls);
3 = individual calls can be distinguished (intermediate between 2 and 4);
4 = full chorus (continuous and overlapping calls)

²Microhabitat:

1 = in open water; 2 = in vegetation in water; 3 = on vegetation above open water;
4 = in open on bank; 5 = in or under low vegetation on bank;
6 = in vegetation above bank; 7 = in adjacent woods

³Life history:

* egg clusters; * amplexant pairs; * observed but not calling; etc.

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Pond:

Date:

Observers:

District: _____ Air temp: _____ Water temp: _____ Canopy: ☐ Full ☐ Partial ☐ None

Pool length: _____ Width: _____ Depth: _____ Substrate: ☐ Clay ☐ Organic ☐ Other

Pool vegetation:

Turbidity: ☐ clear ☐ turbid ☐ stained Water sample: ☐ Yes ☐ No Disturbed: ☐ Yes ☐ No

pH: _____ mv: _____ temp: _____ DO: _____ %: _____ mg/l temp: _____

Weather (check all that apply): ☐ day ☐ night ☐ dusk ☐ clear ☐ cloudy ☐ pcloudy ☐ fog
☐ rain ☐ other _____

Species	Pool 1	Pool 2	Pool 3	Pool 4	Notes

NOTES:

Amphibian Monitoring in Ponds

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Pond:

Date:

Observers:

District: _____ Air temp: _____ Water temp: _____ Canopy: ☐ Full ☐ Partial ☐ None

Pool length: _____ Width: _____ Depth: _____ Substrate: ☐ Clay ☐ Organic ☐ Other

Pool vegetation:

Turbidity: ☐ clear ☐ turbid ☐ stained Water sample: ☐ Yes ☐ No Disturbed: ☐ Yes ☐ No

pH: _____ mv: _____ temp: _____ DO: _____ %: _____ mg/l temp: _____

Weather (check all that apply): ☐ day ☐ night ☐ dusk ☐ sunny ☐ cloudy ☐ pcloudy ☐ fog

☐ rain ☐ other _____

Species Observed	Adults y n #	Calls y n #	Egg masses y n #	Notes

Sweep #	Species	Number	Notes

NOTES:

APPENDIX 4

GLOSSARY

Definitions of the terms in this glossary are from Peters (1964), Heyer et al. (1994), and a standard Webster's collegiate dictionary.

Aggregate: a group of tadpoles that remain together after hatching from eggs.

Ambystomatids: the group of salamanders in the genus *Ambystoma*, including Jefferson, spotted, and marbled salamanders.

Amplexus: the grasping of the female's body by the male's forelimbs during egg laying in frogs.

Balancers: a slender, elongated projection from the mouths of salamander larvae in the early stages of development.

Chromosomes: the DNA molecules in the nucleus of living cells; the numbers of chromosomes vary among species.

Cloaca: the common chamber at the base of the tail of salamanders and at the posterior body of frogs into which intestinal, urinary, and reproductive tracts discharge their contents.

Cloacal lip: the area around the cloacal opening that is usually swollen in males during the breeding season.

Coverboard: an artificial object such as a board of plywood, piece of metal (tin), or a section of a log that is placed on the forest floor to serve as a hiding place for amphibians.

Dorsal: that portion of the upper surface of the body; the back.

Dorsolateral ridge: the enlarged ridges running from behind the eye to the posterior body along the sides of the dorsum of some frogs.

Dorsum: pertaining to the dorsal or back side of an animal.

Ephemeral: pertaining to the length of the hydrological period in wetlands in which the water dries out completely during the course of a single year.

Gelatinous: the jelly-like covering of amphibian eggs.

Hydrology: the study of how water moves in the landscape and in wetlands.

Immaculate: pertaining to a portion of the body of a frog or salamander that lacks an obvious pattern.

Keratin: a tough, fibrous non-soluble protein, like fingernails, that form hardened structures on amphibians, such as spades.

Mental gland: a round, gland that enlarges during the breeding stage of some male salamanders used for courtship.

Metamorphosis: the process of changing from an aquatic larval stage to a terrestrial stage in frogs and salamanders that usually involves substantial changes in morphology and physiology.

Microhabitat: a subset of the habitat in an area usually defined by the presence of an amphibian, such as leaf litter adjacent to a stream.

Natal: pertaining to the hatching stage of a frog or salamander.

Operculum: the portion of the skin that develops over the gills in frog tadpoles leaving only the spiracle for an opening.

Parotoid: the large, swollen glandular area behind each eye on the shoulder region in toads of the genus *Bufo*.

Phenology: the study of the timing of biological and life history events during the seasons of the year.

Pollex: the first toe on the forefoot in frogs that bears a swollen area during the breeding period that is used to aid in amplexus with females; the thumb.

Quadrat: a square frame or area, usually one meter square, laid down on a substrate used to delineate an area to be sampled for amphibians.

Riparian: pertaining to the floodplain of a river or stream that is only occasionally inundated by water and has habitats distinct from the surrounding uplands.

Spade: the keratinized projection on the rear feet of spadefoot toads and true toads used for digging into the substrate.

Spermatophores: a gelatinous mass consisting of a jelly-like stalk and a packet of sperm on top produced by male salamanders during courtship.

Spiracle: the opening on the left side of tadpoles through which water passes from the gill chamber.

Stippling: patterns of dark pigment formed by multiple combinations of black dots.

Subterranean: pertaining to the microhabitat beneath the floor of a forest.

Symbiotic: living together; pertaining to an association of two or more species living in the same habitat, such as a pond.

Transect: a defined linear path along which amphibians are counted or on which quadrats are placed at predetermined points.

Tympanum: the visible external eardrum of frogs.

Venter: pertaining to the lower surface of an individual amphibian; the belly.

Vernal: spring; in reference to wetlands that hold water for only a period of time each year, usually from late winter to summer.

Vocal pouch: a thin-walled pouch on the chin or jaws of male frogs used to aid in producing sound.

Voucher: one or more individuals that serves to document the occurrence of a species at a particular locality or site. These individuals are collected, properly preserved, and deposited with all collection information in a museum and is available for study.

Warts: raised skin glands on amphibians, particularly on toads.

APPENDIX 5

IDENTIFICATION GUIDE TO AMPHIBIANS IN THE MID-ATLANTIC REGION

The following species accounts provide a means to identify the 17 frogs and toads and 18 salamanders one may encounter during inventory and monitoring projects in northern Virginia and nearby portions of the mid-Atlantic region. These descriptions may not work on every individual encountered because of the wide range of variation exhibited by some species. Additional sources should be consulted when you are unsure of your identification. These include Martof et al. (1980), Pfingston and Downs (1989), Conant and Collins (1998), Altig and McDiarmid (1998), and Petranks (1998).

Listing of amphibians in this guide:

Frogs and Toads: any anuran amphibians of various families with long, powerful hind legs, short forelegs and webbed feet, (frogs - smooth skin; toads - a rough, warty skin)

American Bullfrog

(*Rana catesbeiana*)

American Toad

(*Bufo americanus americanus*)

Cope's Cray Treefrog

(*Hyla chrysoscelis*)

Eastern Spadefoot

(*Scaphiopus holbrookii*)

Fowler's Toad

(*Bufo fowleri*)

Cray Treefrog

(*Hyla versicolor*)

Green Treefrog

(*Hyla cinerea*)

Northern Cricket Frog

(*Acris crepitans crepitans*)

Northern Green Frog

(*Rana clamitans melanota*)

Northern Spring Peeper

(*Pseudacris crucifer crucifer*)

Pickerel Frog

(*Rana palustris*)

Southern Leopard Frog

(*Rana sphenoccephala*)

Upland Chorus Frog

(*Pseudacris feriarum*)

Wood frog

(*Rana sylvatica*)

Salamanders: any amphibians of the Caudata order with limbs, a tail, and soft moist skin

Eastern Mud Salamander

(*Pseudotriton montanus montanus*)

Four-toed Salamander

(*Hemidactylium scutatum*)

Jefferson Salamander

(*Ambystoma jeffersonianum*)

Long-tailed Salamander

(*Eurycea longicauda longicauda*)

Marbled Salamander

(*Ambystoma opacum*)

Northern Dusky Salamander

(*Desmognathus fuscus fuscus*)

Northern Red Salamander

(*Pseudotriton ruber ruber*)

Northern Spring Salamander

(*Gyrinophilus porphyriticus porphyriticus*)

Northern Two-lined Salamander

(*Eurycea bislineata*)

Red-backed Salamander

(*Plethodon cinereus*)

Red-spotted Newt

(*Notophthalmus viridescens viridescens*)

Seal Salamander

(*Desmognathus monticola*)

Spotted Salamander

(*Ambystoma maculatum*)

Three-lined Salamander

(*Eurycea guttolineata*)

White-Spotted Slimy Salamander

(*Plethodon cylindraceus*)

Other species in the Mid-Atlantic Region

Barking Treefrog

(*Hyla gratiosa*)

Greater Siren

(*Siren lacertina*)

Narrow-mouthed Toad

(*Castrophryne carolinensis*)

Northern Slimy Salamander

(*Plethodon glutinosus*)

Squirrel Treefrog

(*Hyla squirella*)

Valley and Ridge Salamander

(*Plethodon hoffmani*)

FROGS AND TOADS



Adults: This large, stout frog (to about 6 inches [152 mm] body length) has large eyes, long limbs with extensive webbing between the toes, and no dorsolateral ridge (raised line) along each side of the back. Body color varies from green to brown and may or may not have a netlike pattern of gray to dark brown, or a series of large obscure blotches. The venter is white with a highly variable mottling of gray. The snout and sides of the head are green. The diameter of the tympanum in males is larger than the diameter of the eye; they are about the same size in females. During the breeding season males have a yellow chin and throat and a dark, swollen patch on their thumbs (the pollex).

Juveniles: Similar to adults in color but there may be numerous small black dots on the back.

Advertisement calls: Males are well known for their deep, bass jug-o'-rum calls. Juveniles emit a loud, high-pitched squeenik when startled as they jump from their resting sites along the bank of a pond.

Eggs: Females lay 10,000 to nearly 30,000 eggs in a large surface film among vegetation in ponds and lakes.

Larvae: The larvae of this species are the typical pollywog type of tadpole and can be large (up to 150 mm total length). Dorsal body color is uniformly greenish brown to olive with a clear yellowish venter. Distinct, small, black dots occur on the body, musculature of the tail, and at least the dorsal fin. The dorsal fin is not as wide as the width of the tail musculature.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Bullfrogs begin calling and establishing territories in late-April to early May in the northern Virginia and mid-Atlantic region. Males call from that time throughout the summer. Egg laying occurs late-May to July. Tadpoles can be found in every month of the year, even under the ice, because they often take up to two years or more to complete metamorphosis.

Habitat: Adults inhabit permanent water, such as beaver ponds and human-made lakes, that may or may not have fish. Streams are also used by bullfrogs for shelter and breeding. Juveniles disperse widely from breeding sites and may be found in ephemeral pools in roads and woods, roadside ditches, and other shallow wetlands long distances from permanent water. Tadpoles can be found in ponds, lakes, and streams.

Identification notes: This frog is distinguished from green frogs (*Rana clamitans*) by the lack of the raised dorsolateral ridge on each side of the back. Green frogs have similar coloration but are smaller and possess dorsolateral ridges.

American Toad

(*Bufo americanus americanus*)



Adults: American toads (to 4 inches [100 mm] body length) have numerous large warts (glands) on the back, including a large one (the parotoid) behind each eye, and a broad, squat body. American toads usually have one wart per dark spot or blotch on the back. The warts are brown to yellowish in color on the dark brown to black spots. Warts on the thighs are usually capped with a spine. A light line usually occurs down the center of the back. Body color is brown to nearly brick red. The venter usually has black spots on or near the chin and anterior abdomen. Males are usually smaller than females and the skin on the chin is dark brown to almost black in color during the breeding season.

Juveniles: Smaller versions of adults; the raised warts on the thighs and the single wart per dark blotch on the back will usually distinguish these juveniles from those of Fowler's toads.

Advertisement calls: A long, musical trill, lasting 15-30 seconds. Usually heard at night but males may call occasionally during the day.

Eggs: The 2,500-8,000 black eggs are laid in two long, gelatinous strings in shallow water usually unattached to vegetation.

Larvae: Tadpoles are dark brown to black at all developmental stages, with fine gold-colored dots in some individuals. The body is ovoid in shape, broader posteriorly than anteriorly. The eyes are located more on top of the head than in tadpoles of other frogs. The anal opening lies along the midline, not curved to the right as in frogs. The upper half or more of the muscular portion of the tail is dark and lower half is light. The fins are about the same size above and below the muscular portion and somewhat opaque. Toad tadpoles often aggregate and occur in large numbers.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: American toads call during warm spells in late-winter and early spring. They are usually heard March to April, depending on the weather. Eggs are laid during this period. Sporadic calls may be heard in February and May. The larvae are present for up to two months but may metamorphose earlier if the pool dries.

Habitat: Adults are terrestrial and usually underground outside of the breeding season. They are found in shallow water (road ruts, shallow depressions in fields, marshes, pond margins) in the open when calling and laying eggs. They may be found in back yards, gardens, and school grounds in suburban areas, agricultural fields, hardwood forests, and mixed hardwood/pine forest patches.

Identification notes: The combination of the time of year (late-winter and early-spring) for calling males, eggs, and young tadpoles will help to distinguish this toad from the Fowler's toad. Rely on the raised warts on the thighs for a positive identification for all post-metamorphic stages.

Cope's Cray Treefrog (*Hyla chrysoscelis*)



Adults: These are moderate-sized treefrogs (to 2 inches [61 mm] body length) with expanded toe tips used for climbing. The skin is not smooth but variously bumpy. Body color is usually gray but may be greenish during the breeding season. Temperature plays an important role and individuals may be dark when they are cold. Irregular dark brown to nearly black blotches, sometimes outlined in black, occur on the dorsum. A small, white patch occurs beneath each eye. The inside of the thighs and lower abdomen is bright yellow with dark mottling.

Juveniles: Colored and patterned as adults.

Advertisement calls: A fast, rasping trill that lasts about 3 to 10 seconds, depending on temperature.

Eggs: The dark-colored eggs are laid in multiple, small gelatinous egg masses on top of shallow water in ephemeral pools. Number per mass is about 20-200. Total number of eggs produced by individual females range from 1,000 to 2,500.

Larvae: Cope's gray treefrog tadpoles are dark gray to yellowish-brown to olive in color with high tail fins. The venter is white to light cream and may be iridescent. The dorsal fin is larger than the ventral fin and its anterior point on the dorsum of the head is at midbody. Both fins are variously mottled with patches of gray pigment. The fins of many gray treefrog tadpoles are red along the margins with black pigment along the edge.

Distribution: Eastern margin of northern Virginia and vicinity, including eastern Fairfax, Prince William, and Stafford counties, eastern Maryland, and Delmarva.

Seasonality: Cope's gray treefrogs begin calling in mid- to late-April, depending on the weather, and continue well into August when conditions are moist or wet. Most egg laying occurs in May and June but also occasionally in July-August. Males may call from trees all summer, especially when it rains. Tadpoles can be found in pools throughout the summer but are most prominent May-July. Metamorphosis occurs June through August.

Habitat: Adults and juveniles use a wide variety of terrestrial habitats that contain shrub vegetation to hardwood trees. Many of these areas occur around wetland breeding sites. Riparian areas along streams are used extensively by juveniles. Outside of the breeding season, adults may be found on various human constructions, like swimming pools, bath houses, and walls of one's home, as well as in forested habitat. The primary breeding habitat is shallow, ephemeral pools and in small bodies of water in open places near woody vegetation. Such pools usually lack fish.

Identification notes: This species is identical in morphology, color, and pattern as the eastern gray treefrog (*Hyla versicolor*). However, the calls are distinctly different. See that species account for additional notes.

Eastern Spadefoot (*Scaphiopus holbrookii*)



Adults: Spadefoot toads (to 2 inches [57 mm] body size) are brown to blackish brown with two, wide, irregular yellowish lines extending down the back to form a lyre-like pattern. Each line originates at the eye. An additional light yellowish line occurs on each side of the body. Body color depends on temperature; cooler spadefoots are darker. The pupil of the eye is vertical, not round, and the eyes protrude well above the head. There are no large glands on the back. The venter is white. A characteristic feature is the single black spade located on the inner border of each foot.

Juveniles: Colored and patterned as adults.

Advertisement calls: Male vocalizations are moderately short, explosive grunts described as a low-pitched wank repeated about every two or more seconds, depending on the temperature.

Eggs: Several thousand eggs are laid in irregular bands along grass stems or in the open in shallow water.

Larvae: The body color of this tadpole is very dark brown to black with a yellowish stripe along the body and the tail musculature. The body is somewhat flattened. The dorsal and ventral fins are not wider than the width of the tail musculature, and the dorsal fin barely extends onto the body. The fins are usually clear but may have some scattered dark pigment.

Distribution: Most known populations in northern Virginia and Maryland occur east of the Blue Ridge Mountains. They also occur on the Delmarva peninsula and in New Jersey. Scattered populations occur in the Shenandoah Valley and south-central Pennsylvania.

Seasonality: These frogs appear sporadically on the surface and are seldom seen except during or immediately after heavy rain storms. The few records known from the northern Virginia area occur within the period of April - September. Tadpoles have a very short developmental time; they reach metamorphosis in about three weeks following hatching.

Habitat: Most places known to harbor this species are sandy or of a soil type that enables spadefoots to burrow underground. Agricultural fields, grasslands, mixed hardwood and pine forest patches, and abandoned open areas may have these frogs. Breeding locations are shallow, ephemeral pools that form during rain storms or are depressions that hold water for varying lengths of time. Most of these places are in the open or in sparse vegetation, and they all lack fish.

Identification notes: The "pop eyes", vertical pupil, single spade, and yellowish lyre pattern will distinguish this frog from all others in the area. Toads have raised warts on the skin, black blotches on the back, a large gland behind each eye, and two spades on each foot.

Fowler's Toad (*Bufo fowleri*)



Adults: Fowler's toads (to 3 inches [76 mm] body length) usually have more than one wart, usually 2-4, per dark spot or blotch on the back. The warts are yellowish in color on the brown to nearly black spots. Warts on the thighs are not capped with a spine; they are small and flattened. A light line occurs down the center of the back. Body color is brown to grayish. A large parotid gland occurs behind each eye. The venter usually lacks spots or other distinct dark markings. Males are usually smaller than females and the skin under their chins is dark in color during the breeding season.

Juveniles: Recently metamorphosed Fowler's toads resemble adults with the 2-4 warts per blotch on the back and the flattened warts without spines on the thighs. A hand lens may be necessary to examine these features.

Advertisement calls: A short (less than 5-10 seconds), nasal call that sounds something like a bleating of a sheep - something like w-a-a-a-h (as described in Conant and Collins, 1998).

Eggs: The round eggs are black, numbering 2,000-8,000; laid in long, gelatinous strings in shallow water usually unattached to vegetation.

Larvae: Fowler's toad tadpoles are black at all developmental stages and are similar in shape and eye position to American toads. The anal opening lies along the ventral midline. Most of the muscular portion of the tail is dark and only the extreme lower side is light in color, not distinctly bi-colored as in American toads.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Males call from late-April through August and egg strings may be found at any time

during this period. Male Fowler's toads usually start calling 1-2 weeks later than American toads, although in some years they may overlap in mid-April. Length of larval period is about one month.

Habitat: When they are not at shallow breeding pools, Fowler's toads can be found in many different terrestrial habitat types. These include backyards, gardens, and school grounds in suburban areas, agricultural fields, hardwood and mixed hardwood/pine forest patches, and old fields. Breeding locations are usually shallow pools in dirt roads, wet depressions in grassy fields, ditches along roadsides, and the margins of ponds.

Identification notes: Adult Fowler's and American toads are usually not difficult to distinguish in the mid-Atlantic region because they are not known to hybridize in this region, although they do elsewhere. Recently metamorphosed juveniles and tadpoles may be more difficult, but paying attention to the type of warts on the thighs of juveniles and when early stage tadpoles are present will help.

Cray Tree frog (*Hyla versicolor*)



Adults: These are moderate-sized treefrogs with expanded toe tips used for climbing. Body size is up to 2 inches (57 mm). They are identical to Cope's gray treefrog in color, pattern, and morphology.

Juveniles: Colored and patterned as adults.

Advertisement calls: A slow, melodic trill that lasts about 5 to 20 seconds, depending on temperature. The call has been described as a resonant flute-like trill and a sound like that of the red-bellied woodpecker (Conant and Collins, 1998).

Eggs: Eggs are laid in multiple, small gelatinous egg masses on top of shallow water in ephemeral pools. Total number produced by females ranges from 1,000 to 2,500.

Larvae: Tadpoles of this species are identical to those of the Cope's gray treefrog. The two larval drawings illustrate variations in gray treefrog patterns.

Distribution: Western portions of northern Virginia and the mid-Atlantic region. Populations west of Fairfax and Prince William counties in Virginia and central and western Maryland are likely to be this species.

Seasonality: Eastern gray treefrogs begin calling in mid- to late-April, depending on the weather, and continue well into August. Most egg laying occurs in late-May through July but also occasionally in August. Males call from trees periodically all summer when it is wet.

Habitat: Adults and juveniles utilize a wide variety of terrestrial habitats like those described for Cope's gray treefrogs. The primary breeding habitats are shallow, ephemeral pools and in small bodies of water in open places near woody vegetation that usually lack fish.

Identification notes: The only ways to tell this species apart from Cope's gray treefrog (*Hyla chrysoscelis*) is by the male's call (slow trill for *H. versicolor* versus fast trill for *H. chrysoscelis*) or by counting the individual's chromosomes. *Hyla versicolor* has four sets of 24 chromosomes and *H. chrysoscelis* has two sets. Learning the male call is the best way to distinguish these two species. Geographic location will assist as well, especially with tadpoles and non-calling individuals. Populations in the contact zone of these two species will need to be evaluated carefully for accurate identification.

Green Treefrog (*Hyla cinerea*)



Adults: This is a bright green treefrog with toe pads. Body size is up to 2 _ inches (57 mm). A prominent, narrow white stripe may or may not be present along each side of the body. If present, the stripe may extend completely from the snout to the rear legs or may terminate on the body. The venter is white. Body color will vary with temperature, from bright green to nearly yellowish to grayish brown. Small golden flecks may occur on the back. During the breeding season males possess wrinkled pink-colored skin under the chin (location of the vocal sac).

juveniles: Similar to adults in color and pattern.

Advertisement calls: A loud, nasal queenk-queenk repeated rapidly for several seconds.

Eggs: Females lay about 400-1,500 eggs in small egg masses on or near the surface attached to vegetation.

Larvae: The body of the tadpole is yellowish green above and pale cream to yellowish below. The tail musculature is similar in color but may have irregular dark patches. The heights of both fins are approximately equal and are clear with patches of dark pigment. The dorsal fin inserts about midway on the body.

Distribution: Green treefrogs occur along the eastern portion of northern Virginia and Maryland and on the Delmarva Peninsula.

Seasonality: This is the late-spring and summer frog of the coastal mid-Atlantic region. Males call from May to August. Larvae occur from June to August.

Habitat: Green treefrogs are most often found in vegetation along the margins of lakes, ponds, and marshes in coastal areas and wetlands associated with streams and rivers near the Chesapeake Bay. Adults often utilize human shelters, such as bath houses, when they are not breeding and can be seen in water-saving postures (legs under the body) in corners, above doors, and in shower stalls.

Identification notes: This treefrog is more slender and has smooth skin compared to gray treefrogs that may occur in the same area.

Northern Cricket Frog (*Acris crepitans crepitans*)



Adults: These small frogs (to 1 3/8 inches [35 mm] body length) have a blunt snout, a dark, ragged-edged stripe along the inside of the thigh, and legs that are not much longer than the body; the heel does not extend beyond the snout when the leg is extended along the side of the body. A dark stripe usually occurs along the side of the body and a dark, triangular patch occurs between the eyes on the dorsum of the head. Several raised, slightly elongated, bumps resembling warts occur on the body. Body color is highly variable within populations: gray, green, and brown. Various combinations of red, yellow, orange, brown, green, and gray occur on the dorsum as a distinct stripe or sometimes as patches. The venter is white. Males have orange-brown skin

under the chin (the vocal pouch) during the breeding season, whereas females have white chins.

Juveniles: Colored and patterned as adults.

Advertisement calls: Male vocalizations sound like pebbles being clicked together - gick-gick-gick-gick, etc. The call starts slowly and picks up speed during the 15-30 second call period. Males will call day and night.

Eggs: About 150-300 small, cream-colored eggs are laid singly in vegetation in shallow water.

Larvae: The most distinguishing feature of cricket frog tadpoles is the black tail tip. On some tadpoles it looks as if the tip of the tail had been dipped in India ink. Body color is olive to yellowish brown with varying intensities of darker pigment. The venter is pinkish. The dorsal fin on the tail is higher than the ventral fin and its most anterior point is about mid-body. Both upper and lower fins have black pigment.

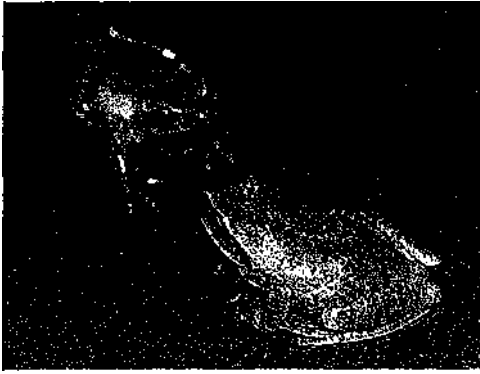
Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Males start calling in mid- to late-April, depending on the weather, and can be heard well into September. Tadpoles may be found in wetlands throughout the summer. Metamorphosis occurs in August and September.

Habitat: These frogs are most often found in grassy areas along the margins of ponds and lakes where they breed in shallow water. They also occur in shallow pools in dirt roads, depressions in grasslands, and riparian corridors along creeks and streams. Cricket frogs are seldom encountered far away from water.

Identification notes: The triangular head patch, dark stripe on the thigh, and the various colors will distinguish this small frog from others of its size.

Northern Spring Peeper (*Pseudacris crucifer crucifer*)



Adults: This small frog (to 1 inches [37 mm] body length) has a light brown to tan to grayish body with a distinct dark cross on its back in the form of an imperfect "X." A dark bar occurs between the eyes on the top of the head, but it may be obscured in dark frogs. The venter is white to cream and may have small dark spots. The darker throat of males distinguish them from females during the breeding season.

Juveniles: Colored and patterned as adults.

Advertisement calls: This is the well-known harbinger of spring with its clear, high-pitched "peep-peep-peep-peep" repeated several times at an interval of a second or less. Males call during the day and at night when choruses are most intense.

Eggs: Up to about 1,600 cream-colored eggs are laid singly by females among vegetation and on dead leaves on the bottom of shallow water wetlands.

Larvae: Spring peeper tadpoles are gray, brown, or nearly black depending on the type of substrate with patches of black pigment on the tail musculature and in the tail fins. Older tadpoles have more pigment than younger tadpoles. The venter is iridescent gold. Dorsal and ventral fins are nearly equal in size and the dorsal fin inserts on the posterior portion of the body.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Males begin calling with the first warm days of late winter and continue, depending on the weather, well into April. Air temperatures in the 50s and 60s F (10-15°C) in January and February will stimulate these frogs to call. Choruses are most intense in this area in March and April. Females lay eggs during this time. Males can be heard calling from trees periodically in Autumn (September-December) on cool days. Tadpoles can be found in shallow water breeding sites March through June.

Habitat: Spring peepers occur in forested habitats outside of the breeding season where they seek shelter in trees, shrubs, and beneath leaf litter. During the breeding season they occur in vegetation surrounding, and sometimes well away from, a wide variety of wetland types, including vernal pools in woodlands, road ruts, and grassland depressions, roadside ditches, freshwater marshes, and alongside small ponds that lack fish. Tadpoles can be abundant in these wetlands.

Identification notes: The call of this species is easily learned and distinguishes it from all others. Other frogs in this size range lack the "X" on the back.

Pickerel Frog (*Rana palustris*)



Adults: These moderate sized frogs (to 3 inches [76 mm] body length) are brownish with two rows of dark, squarish spots between the two prominent and light-colored dorsolateral ridges. The spots on the back may be separate or joined together in some individuals to form wide, partial stripes. Squarish spots usually occur on the sides. The venter is white but the undersides of the legs are bright yellow. Males are similar to females in color and pattern but have paired vocal pouches and darkened, swollen thumbs during the breeding season.

Juveniles: Patterned as adults but darker, often dark olive green.

Advertisement calls: Males emit a low-pitched snore lasting 1-3 seconds. These calls are seldom audible away from the breeding sites.

Eggs: Females lay up to 3,000 eggs in a single, globular egg mass attached to a twig or branch in shallow water.

Larvae: These tadpoles are dark olive green to dark brown with blotches of white to yellowish pigment on the iridescent venter. There is a variable number of fine yellowish spots on the dorsum. The dark markings on the tail musculature and dorsal and ventral fins increase in density toward the tail tip, sometimes making the tail look nearly purplish black. Dorsal and ventral fins about equal in size and are not as wide as the tail musculature.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Males call in late winter and early spring, approximately February through early April in the northern Virginia area. Females usually lay eggs in March and early-April. Active, non-reproductive individuals may be seen at all times of the year except winter. Tadpoles can be found throughout the summer with metamorphosing occurring in late-May to July.

Habitat: Pickerel frogs occur in permanent ponds with abundant vegetation around the margin, beaver ponds, freshwater marshes and swamps in riparian zones, and along streams. Juveniles are found occasionally in vernal pools. Tadpoles also occur in these habitats.

Identification notes: The irregular, squarish spots, light-colored dorsolateral ridge, and yellow on the inside of the rear leg distinguish this frog from others in the area. Southern leopard frogs have distinctly round spots and lack the yellow legs.

Northern Green Frog (*Rana clamitans melanota*)



Adults: Green frogs (to 3 inches [89 mm] body length) are similar in shape to bullfrogs but are proportionally smaller. A dorsolateral ridge is present on each side of the back. Body color varies from greenish brown to nearly all brown, sometimes with small dark spots on the back, especially on young frogs. A green patch is present below the eye on the upper lip. The venter is white but may have dark markings on the upper chest and legs. The tympanum is larger than the diameter of the eye in males but similar in size in females. During the breeding season males have yellow throats and the dark pollex is enlarged.

Juveniles: Body color is usually brownish with numerous small dark spots on the back. Dorsolateral ridges are present.

Advertisement calls: Males emit several types of vocalizations, the most common of which is the one often described as a plucked and rather explosive loose banjo string. The call may be made once or several times with the notes becoming progressively less audible. This species emits a loud, high-pitched squeenk when startled as they jump from their resting sites along the bank of a pool or pond.

Eggs: Between 1,000 and 4,000 dark eggs are laid by a female in a single, clear surface egg mass attached to vegetation in shallow water.

Larvae: Tadpoles are similar to bullfrog tadpoles but are olive green with a cream-colored, non iridescent venter. These tadpoles may be nearly all brown in color, resembling the clay of road rut pools or very dark with an abundance of dark blotches in pools filled with blackened leaves. The dark markings on the body, tail musculature, and tail fins are irregular in shape, often in the form of diffuse black spots. The dorsal fin is not as wide as the width of the tail musculature.

Distribution: Throughout northern Virginia and the mid-Atlantic.

Seasonality: Green frogs begin calling as early as late-April and will call throughout the summer into September. Eggs are laid May through September. Tadpoles may be found in every month of the year because they take a year or more to complete metamorphosis.

Habitat: Green frogs occupy a wide variety of freshwater wetland habitats, including margins of permanent beaver and human-made ponds and lakes, vernal pools in woodlands, road ruts, and grassland depressions, roadside ditches, streams, and rivers. Juveniles disperse long distances from breeding sites through terrestrial habitats to ephemeral pools and other shallow bodies of water. Tadpoles are found in all of these wetlands.

Identification notes: The dorsolateral ridge and green patch on the upper lip below the eye will distinguish this species from other frogs in the genus *Rana* in the area.

Southern Leopard Frog (*Rana sphenocephaly*)



Adults: Body coloration in this species varies from green to brown with a highly variable series of distinctly dark, round spots in the back between the light-colored dorsolateral ridges. There are few spots on the sides. Maximum body size reaches about 2 inches (64 mm). The snout is distinctly pointed, there is a white spot in the center of the tympanum, and the venter is white. Males are similar to females but have paired vocal pouches and a pollex during the breeding season.

Juveniles: Similar to adults in color and pattern.

Advertisement calls: Males emit a series of short, chuckle-like calls that sound similar to that produced by rubbing an inflated balloon.

Eggs: A single, clear, globular egg mass of up to 3,500 eggs is laid in shallow water usually where live or dead grasses are present.

Larvae: The body of leopard frog tadpoles is dark olive green with large, diffuse black spots on the tail musculature and both fins. The coiled intestines are clearly visible through the body wall. The venter is pinkish. The dorsal tail fin is about equal in height as the tail musculature and inserts on the head at about mid-body. Populations of larvae in wetlands with high densities of predators, like dragonfly nymphs, exhibit a black tail tip.

Distribution: Scattered populations of this frog occur in eastern portions of northern Virginia, eastern Maryland, and throughout the Delmarva peninsula.

Seasonality: Southern leopard frogs usually call in March and April in northern Virginia and vicinity. Juveniles disperse widely in Summer and Fall following metamorphosis.

Habitat: These frogs may be found in lowland riparian corridors along stream courses, freshwater marshes, beaver ponds, and in shallow pools in grassy fields. Adults may be found along the margins of wetlands during spring and summer, juveniles disperse widely and may be found well away from water as well as in isolated ephemeral pools, small ponds, and riparian areas. Tadpoles occur in pond margins, marshes, and ephemeral pools.

Identification notes: Southern leopard frogs have distinctly round spots and lack the yellow legs seen in pickerel frogs.

Upland Chorus Frog (*Pseudacris feriarum*)



Adults: This small frog (to 1 3/8 inches [35 mm] body length) has small toe pads and little to no webbing between the toes. Body color is brown to tan to slate gray usually with three narrow, dark stripes on its back. The stripes may or may not be complete and in some individuals may be broken into a series of elongated spots or lacking altogether. The upper lip is white. A dark line runs from the snout passing through the eye to groin. A dark triangular patch usually occurs between the eyes on the top of the head. The venter is white often with a variable amount of dark stippling in the upper abdomen.

juveniles: Colored and patterned as adults.

Advertisement calls: Calls of males sound much like a fingernail running over the teeth of a stiff comb,

like a loud creek or preep repeated regularly to intermittently depending on the temperature.

Eggs: About 30-150 eggs are laid in each of several clear egg masses attached to grass stems near the surface in shallow water. Total clutch size is up to about 700 eggs.

Larvae: Chorus frog tadpoles are dark brown to nearly black dorsally and bronze ventrally. Fine dots of greenish yellow occur on the upper body and tail musculature. The dorsal and ventral fins are about equal in height and the anterior insertion of the dorsal fin is on the posterior portion of the body. The dorsal fin has elongated dark markings.

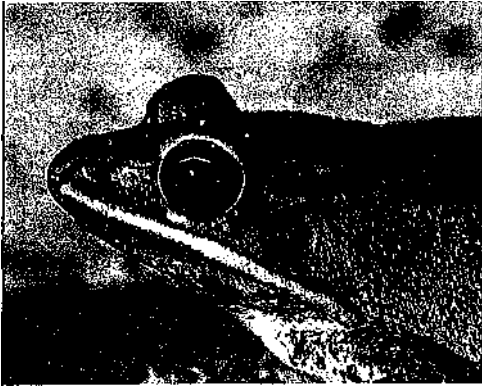
Distribution: Throughout northern Virginia and vicinity.

Seasonality: Males call early in the year, usually beginning when air temperatures are above freezing (40-50 F) for three or more days, sometimes when snow is still on the ground. Breeding occurs over a short, several day period in February or March but males will call from then into early-April. Tadpoles are seldom found after April to mid-May.

Habitat: Males call from grassy areas associated with shallow water in wetlands without fish. These include tire ruts, pools in dirt roads, roadside ditches, and pools in fields in the open. Outside of the breeding season adults and juveniles remain hidden in the leaf litter of hardwood forests and among shrubby and grassy vegetation not far from the vicinity of breeding sites. Riparian areas and saturated forested wetlands are utilized extensively for shelter.

Identification notes: This species is distinguished from other small frogs found in winter and early spring by the presence of the three stripes down the back, the white upper lip, and the dark stripe on the side of the body. The distinctive call will enable one to identify calling males.

Wood Frog (*Rana sylvatica*)



Adults: Wood frogs exhibit a wide range of body colors, from pink to reddish to brown to nearly black. There is almost no pattern on the back but there are two dark spots on each side of the chest. The one feature that will identify this frog is the dark, somewhat triangular patch that lies behind the eye on each side of the head. The patch or "mask" extends to behind the tympanum and down to the insertion of the forelimb. Dorsolateral ridges are present. Maximum body size reaches about 3 inches (76 mm). During the mating season, males are dark and the females are light in color, and males possess a pollex on each thumb.

juveniles: Similar to adults in color and pattern but usually darker. They also show the dark "mask."

Advertisement calls: Males have a low powered

call described as a "hoarse crackling sound that suggests the quack of a duck " (Conant and Collins, 1998) and a rasping "craw-aw-auk" (Martof et al., 1980).

Eggs: Females lay up to 1,000 dark eggs in a loose globular mass in shallow water. They often aggregate to lay their eggs in the same portion of the pool, resulting in a large mat of numerous egg masses. Once the jelly around the eggs has been in the water for several days the mass takes on the appearance of a bunch of grapes.

Larvae: Tadpoles are uniform light to dark gray in color on the body and tail musculature with fine gold-colored spots. The venter is uniform cream in color. A cream-colored line extends along the snout above the mouth parts. The tail fins are clear and are larger than the width of the tail musculature. The dorsal fin inserts on the dorsum of the body less than halfway between the base of the tail and the eye.

Distribution: Populations of this frog occur throughout northern Virginia and the mid-Atlantic region where appropriate habitat exists.

Seasonality: Wood frogs call earlier than most other species, usually in February or March in the mid-Atlantic. Adults remain at breeding sites only for two to three weeks at most. Juveniles disperse widely into the forest in summer and fall following metamorphosis in May and June.

Habitat: These frogs occur in hardwood forest habitats that contain or are near shallow, ephemeral pools in which they breed. Pools and ponds with fish are avoided (Hopey and Petranks, 1994). Juveniles disperse widely and may be found well away from water as well as in isolated ephemeral pools, small ponds, and riparian areas.

Identification notes: Southern leopard frogs and pickerel frogs have spots on the back and lack the dark mark on the side of the snout. Green frogs have a green snout and bullfrogs lack the dorsolateral ridges.

Eastern Mud Salamander (*Pseudotriton montanus montanus*)



Adults: These are somewhat stocky salamanders with short tails and a very short snout that reach a maximum total length of about 7 inches (190 mm). Body color can be pinkish, bright red, orange red, or light yellowish orange. The variable number of black spots on the back diffuse into the body color; they lack sharp perimeters. Color darkens with age as older adults tend to be reddish brown with obscure spots. The venter is pale and has brownish flecks or mottling. The eyes are brown in living specimens.

Juveniles: Colored and patterned as young adults but brighter.

Eggs: About 75-190 eggs are laid by females among plant roots and debris in seepages and springs.

Larvae: The larval stage of this salamander is light brown dorsally and cream in color ventrally without pattern. There may be some dark brown streaking along the sides. The external gills are small.

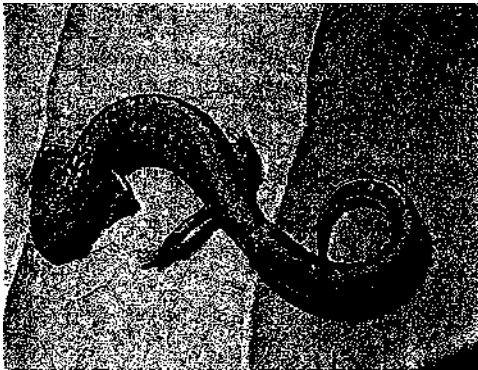
Distribution: Mud salamanders occur in the eastern portion of northern Virginia and Maryland, and also on the Delmarva peninsula into New Jersey.

Seasonality: Adults and larvae may be found in all but the coldest months. Adults can be found well away from water in moist habitats well away from water in Spring and Summer. Eggs are laid in late fall and early Winter. Metamorphosis occurs 1.5-2.5 years after hatching.

Habitat: Mud salamanders occupy seepages from springs and muddy seepages in riparian areas associated with streams in areas with hardwood forests. Adults and larvae can be found in the mud and under leaf litter.

Identification notes: The short snout and the brown eyes will distinguish this species from red salamanders when the body spots are difficult to discern.

Four-toed Salamander (*Hemidactylium scutatum*)



Adults: Four-toed salamanders are small bodied (reaching up to 3 inches [89 mm] total length) with only four toes on each rear foot (most salamanders have five) and a distinct constriction at the base of the tail. The constriction is associated with the tendency for the tail to break easily. Body color is brown with scattered small black dots on the back. The white venter is uniquely patterned individually with small black spots.

juveniles: Colored and patterned as adults.

Eggs: Females lay about 29-80 eggs in sphagnum moss, decomposing moist stumps and logs, and leaf litter and usually remain with them until hatching.

Larvae: Gilled larvae resemble Ambystoma larvae in body form. They are yellowish-brown in color with

an upper tail fin that extends to the head and a lower tail fin that extends to the base of the tail. The heads of larvae can be colored yellow to orange with small dark spots that make a Y-shaped pattern on the back of the head.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Adults migrate from overwintering sites in late-Winter and early-Spring and can be found until Summer in association with wet areas and shallow wetlands. They may be found at other times of the year, except for the coldest months, under logs and other material in moist terrestrial habitats. Eggs are laid from late-March to mid-May and larvae metamorphose about a month later.

Habitat: This salamander is usually associated with seepage areas with sphagnum moss but may be found around small ponds without fish in intact hardwood forests and other moist areas. Hardwood forests and seepages seem to be required, although they will disperse into other habitats outside of the breeding season.

Identification notes: The four toes on the rear feet, the constriction around the base of the tail, and the black and white pattern on the venter will distinguish this salamander from all others.

Jefferson Salamander (*Ambystoma jeffersonianum*)



Adults: This large salamander (to about 8 inches [203 mm] total length) is nearly uniformly gray to brownish in color with bluish flecks on the sides and limbs. Old individuals may completely lack the blue flecks. The venter is pale and the area surrounding the cloacal opening is gray. The toes are longer and the body more slender than other members of the genus Ambystoma and the long tail is compressed laterally. In males, the cloacal region is swollen during the breeding season.

juveniles: Similar to adults but with conspicuous bluish or yellowish flecking on the sides and limbs.

Eggs: Two to 70 eggs are laid in clear gelatinous masses usually attached to vegetation in ponds and pools. Females may lay up to 200 eggs in several egg masses. A symbiotic algae turns many of the egg masses green but does no harm.

Larvae: Larval Jefferson salamanders are gray in color with a heavily mottled, broad tail fin, and a light stripe along the side of the body. The white venter is immaculate.

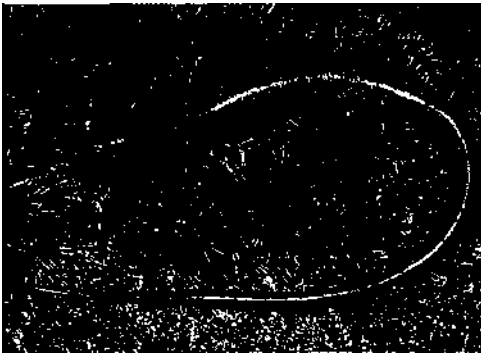
Distribution: Higher elevations in the mountainous portion of the mid-Atlantic region.

Seasonality: Jefferson salamanders breed in February and March in high elevation ponds and pools. Adults are seldom observed outside of the breeding season when they live underground in hardwood forests. Larvae metamorphose in July and August and disperse into the forest often great distances from their natal ponds.

Habitat: In this region, Jefferson salamanders are subterranean (underground) inhabitants of hardwood forests at high elevations. They may be found occasionally under logs and rocks on the wet forest floor but most of their time outside of the breeding season is spent underground. Breeding locations are permanent and temporary ponds and pools.

Identification notes: Adults may be confused with white-spotted slimy salamanders but this species has a distinctly round tail and irregular white spots on a black body. Spotted salamanders have distinct, large round spots on a nearly black and robust body.

Long-tailed Salamander (*Eurycea longicauda longicauda*)



Adults: These are long, slender salamanders reaching a maximum total length of about 8 inches (203 mm). Body color is yellow orange to orange red with many small, irregular black spots on each side and along the sides of the tail. Small black dots occur on the back. The pattern on the tail is distinctive: alternating vertical orange to yellow and black bars forming a slanting pattern in many individuals. The venter lacks a pattern. The tail of adults may be as much as 2/3 of total body length.

Juveniles: Patterned as adults but yellowish and with a relatively shorter tail.

Eggs: About 60-100 eggs are laid underground, beneath rocks in water or in caves.

Larvae: Long-tailed salamander larvae are elongated and streamlined with short gills and a dorsal tail fin that does not extend beyond the rear legs. The dorsum is brownish to yellowish with dark sides and a faint mid-dorsal stripe. The venter is cream in color, sometimes with tiny black dots on the throat. The dark pigment on the tail musculature forms a slanting pattern with vertical dark bars.

Distribution: West of the Blue Ridge Mountains in northern Virginia and Maryland.

Seasonality: Long-tailed salamanders can be found throughout all the warm months of the year in streams and riparian areas but year-round in caves. Adults disappear underground in the fall where mating and egg-laying take place and reappear in early spring when they move to summer foraging habitats in moist areas near streams.

Habitat: This species occurs in rocky streams and margins of rivers, especially those with limestone and shale substrates where they take shelter under rocks. They occur in and under moist logs and rocks in riparian areas over 30 meters from water and are frequent inhabitants of caves.

Identification notes: Only the two-lined salamander may be confused with this species in its range, but it is shorter, distinctly yellow, and lacks the dark slanting pattern and the long tail.

Marbled Salamander (*Ambystoma opacum*)



Adults: These are short (to 4 inches [108 mm] total length), stocky salamanders with several gray to white crossbars on a black to bluish black body. The crossbars may be broken in some individuals or run together to form parallel white stripes. Crossbars occur on head, body, and tail. The venter is black. Males have white crossbars and swollen cloacal lips during the breeding season. Females have gray crossbars and a flattened cloacal area.

Juveniles: Recently metamorphosed juveniles may lack the crossbars, instead having scattered light flecks to an irregular light mottling pattern on a brownish to blue black body.

Eggs: Eggs numbering from about 50 to 200 are laid singly by females under logs and rocks in moist shallow depressions where vernal pools will form with winter rains. The eggs are not encased in a gelatinous mass.

Larvae: Larvae are dark brown to grayish to tan in color without distinct markings on the dorsum. Venter is cream with tiny black peppering under the chin and around the fold of skin between the chin and abdomen. The peppering may be indistinct and best observed with a hand lens. A line of distinct white dots is present along each side in older larvae. The broad tail fin may be heavily mottled with dark pigment.

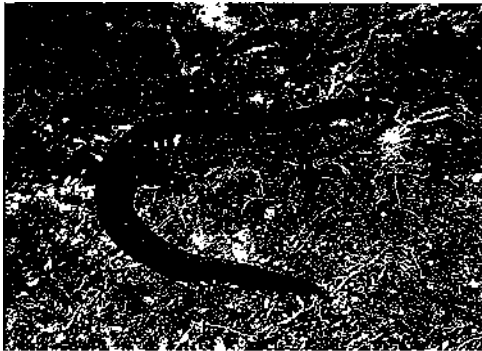
Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Males and females mate on the forest floor in September and October when forest conditions are wet. Females then deposit eggs under moist logs in and along the margins of woodland pools and other ephemeral pools and remain with them until winter rains fill the depressions. Embryos hatch upon contact with water and the female then leaves the pool for her underground retreat in the forest. Larvae over winter in the pool and metamorphose May-June the following year. Adults and juveniles live underground in the forest outside of the breeding period but may be found occasionally under logs when the forest floor is wet.

Habitat: Adults prefer the floor of hardwood forests in the mid-Atlantic area where they live in rodent burrows, decayed root channels, and other pockets beneath the surface. They breed during September and October rains on the edge of and in depressions in the forest and other low areas that will accumulate water. Agricultural areas and some suburban areas with patches of forest and wetland pools remaining may support this species.

Identification notes: The adults are unique with their stocky bodies and light-colored crossbars. Larvae are more difficult to identify but the combination of the light stippling under the chin and their much larger size during the time when spotted and Jefferson salamander larvae are small (e.g., March - May) should allow correct identification in the field.

Northern Dusky Salamander (*Desmognathus fuscus fuscus*)



Adults: This gray to brown salamander (maximum total length 4 3/4 inches [120 mm]) has a highly variable dorsal pattern that ranges from a broad reddish-brown stripe edged in black scalloping to a broad, indistinct field of tiny black spots. The dorsal pattern of reddish brown edged by black is usually present on the dorsum of the tail in younger adults in this area. The tail is flattened laterally and has a distinct keel (knife-edged above). A light-colored line that runs diagonally from the eye to the rear corner of the mouth is present on all but the oldest individuals. The venter has black and cream colored stippling across most of the entire venter (a salt and pepper effect).

Juveniles: Body color is gray to brown with a more distinct reddish dorsal stripe than found in adults. The dorsal stripe extends onto the tail where it is distinctly reddish. Young juveniles possess a series of 5-8 yellowish spots along the back connected by a dark wavy band.

Eggs: The approximately 10-35 eggs are laid under logs, rocks, and moss cover adjacent to streams.

Larvae: Larvae are colored and patterned as the youngest juveniles described above but have short gills best seen with a hand lens.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Dusky salamanders may be found in every month of the year if one digs for them in winter. Most activity takes place from about April through October. Females lay eggs in June to mid-August. The larval period is approximately 8-10 months in duration.

Habitat: Mountain and Piedmont streams, especially those with margins consisting of small rocks. This salamander is especially abundant in patches of seepage habitat and headwater portions of mountain streams.

Identification notes: The light eye-jaw stripe will identify the genus *Desmognathus* from all other salamanders in the area. Seal salamanders have irregular black spots on the back and the venter lacks the salt and pepper pattern, at least along the chin and middle of the venter. Juvenile seal salamanders lack the reddish stripe on the upper part of the tail base.

Northern Red Salamander (*Pseudotriton ruber ruber*)



Adults: Adults are brightly colored red to reddish orange and reach a maximum total length of about 7 inches (178 mm). Numerous distinct black spots occur on the back but the pattern on the sides is black flecking. The venter is pale salmon pink in color with dark spots. Old adults may be purplish with obscure spotting on the back. Eye color is yellow. The margin of the chin may be lined with black flecks.

Juveniles: Colored and patterned as adults but brighter.

Eggs: Females lay about 30-130 eggs following courtship and mating in summer. Eggs are laid deep underground attached to rocks in seepage areas and springs.

Larvae: The larvae are robust but are elongated and have a paddle-like tail. They are dark brown to reddish brown in color with indistinct black spotting on the dorsum. The tail fins have a variable amount of mottling and the venter is pale white without a pattern.

Distribution: Throughout northern Virginia and the mid-Atlantic region, except for the southern portion of the Delmarva peninsula.

Seasonality: Red salamanders spend winters deep underground in seepages and springs. They emerge about March or April and move to the forest floor. Females move back to springs and seepages in late Summer and lay eggs in the Fall months. The larval stage lasts for 2-3 years, and metamorphosis occurs in Summer.

Habitat: Adults may be found in early Spring and Fall in seepages, springs, and margins of mountain streams. In late Spring, Summer, and early Fall adults may be found under logs and rocks in moist soil in hardwood forests. The combination of springs or seepages and hardwood forest is essential habitat for this species.

Identification notes: The longer snout, numerous distinct black spots on the back, and the yellow eyes will distinguish this species from mud salamanders. Spring salamanders have longer and broader snouts with a pale line bordered by black between the eye and nostril.

____ Northern Spring Salamander (*Gyrinophilus porphyriticus porphyriticus*)



Adults: This large streamside salamander reaches a total length of about 8.5 inches (216 mm). It has a stout body, a broad, flattened snout, and a flattened knife-like tail. Body color is flesh-colored to salmon pink to orange-red. A highly variable number of black flecks occurs on the body and tail. The venter lacks markings. There is a distinct yellowish line usually border by black between the eye and nostril.

Juveniles: Juveniles are large and resemble adults in color and pattern.

Eggs: From about 15 to 100 eggs are deposited by females on the undersides of rocks in seepages and under water in flowing streams in summer months.

Larvae: The larvae of this species are larger than any other salamander in the region. Individuals up to 4 inches with gills are not uncommon. They have broad, elongated snouts and a body color of light yellowish brown to light purple. The pattern is a relatively uniform pattern of fine reticulations on the dorsum and paddle-like tail. The venter is cream to pinkish and lacks pattern.

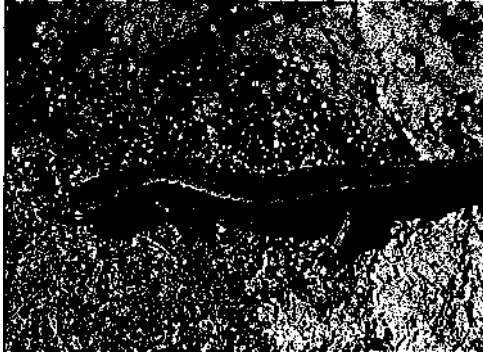
Distribution: Mountainous regions and uplands of northern Virginia and the mid-Atlantic region.

Seasonality: These salamanders can be found 12 months of the year, although in winter months they are below the ice deep in flowing water. They may be found under rocks along stream margins from late-March through November. Metamorphosis occurs in Summer, about three to four years after hatching from eggs.

Habitat: Spring salamanders are most commonly encountered under rocks in and along the margins of cool mountain streams. Adults can sometimes be found under logs in moist forest habitats near streams and they inhabit caves.

Identification notes: This species may be confused with adults and larvae of the northern red salamander. This species has a much shorter snout and distinct black spots on a red body. Larval red salamanders have shorter snouts and large eyes. Larval spring salamanders have a broad snout and small eyes.

Northern Two-lined Salamander (*Eurycea bislineata*)



Adults: This a slender yellow to greenish-yellow salamander (maximum total length to 4 inches [100 mm]) with two dark brown stripes that border a broad yellowish to brownish stripe on the back. The broad light stripe along the back is usually peppered with black dots of varying densities. In some individuals, there may be a dark line down the center of the back and in others the back is dark. The venter is plain yellow to cream in color and translucent.

Juveniles: Similar to adult in color and pattern.

Eggs: Fewer than 100 eggs are deposited by females under rocks, among root masses and vegetation, and under leaves and other objects under water in flowing streams.

Larvae: Two-lined salamander larvae are elongated and streamlined. The anterior margin of the tail fin occurs behind the rear legs. Body color is gray to light brown with a variable mottling pattern, the venter is cream in color, and the tail fin is variously mottled.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Although this salamander can be found in most months of the year, it is most commonly encountered in Spring and Fall. Females lay their eggs in April and May. Hatching occurs one to two months later and the larvae metamorphose in one to two years.

Habitat: The margins of rocky streams are utilized extensively by this salamander. Adults may venture far into the adjacent forest during wet periods where they can be found under logs and rocks. Two-lined salamanders appear to be more abundant in mountain streams than in Piedmont streams.

Identification notes: The yellow coloration distinguishes this species from all other streamside salamanders in this region. The larvae differ from other streamside species by the lack of a definable pattern on the body and tail. The gills on the larvae are longer than those in dusky and seal salamander larvae.

Red-backed Salamander (*Plethodon cinereus*)



Adults: These small terrestrial salamanders reach a maximum total length of about 4 inches (100 mm). Background body color is dark gray to black with often indistinct tiny brass flecks. Some individuals also have a broad red, reddish orange, or yellowish stripe along the back that extends at least half way down the tail. This is the red-back phase (morph). Individuals without the stripe are grouped in the lead-back phase (morph). All individuals have a salt-and-pepper venter, about an equal mixture of dark and cream to brass-colored flecking.

Juveniles: Colored and patterned as adults.

Eggs: Up to about 14 eggs are laid under rocks and in and under logs, sometimes deep in the ground. Female guard their eggs until hatching.

Larvae: There are no aquatic larvae as all development occurs within the egg.

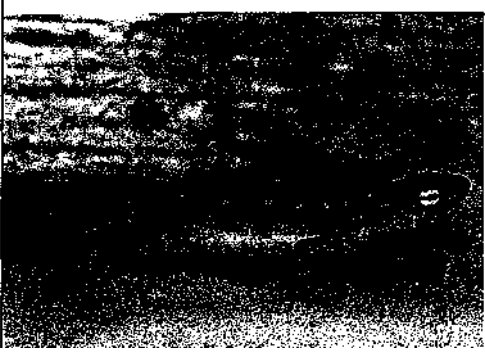
Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Adults and juveniles may be found under objects on the surface of the forest floor in Spring and Fall. Winter is usually too cold for surface activity and Summer (July and August) is often too hot and dry. They forage for prey on wet nights on the ground surface and low vegetation. Eggs are laid in May and June and hatchlings are found in the Fall.

Habitat: This salamander prefers mature hardwood forests with an intact leaf litter-humus forest floor and some downed woody debris for cover objects. It may be found in other habitats, including mixed pine-hardwood forest stands, forested riparian corridors, and old fields adjacent to hardwood forests and moist substrates as long as the ground is moist. They may occur in gardens and urban landscaped areas as long as there are forest remnants nearby.

Identification notes: The red stripe down the back and tail of this small, terrestrial salamander and the absence of white spots should enable positive identification.

Red-Spotted Newt (*Notophthalmus viridescens viridescens*)



Adults: Mature adult newts are yellowish brown, olive green, or dark olive brown above with an individualistic, dorsal pattern of small red dots bordered by black. The venter is yellow with black flecks. Maximum total length is 5.5 inches (140 mm). A narrow dark line passes from the snout through the eye to the neck on each side of the head. The skin is rough, not smooth as in other salamanders. In males, the tail fin is substantially higher (wider) than that of females, and possess black toe tips and a line of raised black patches on the inside of the thighs. Females lack the black toe tips and black line inside the thighs. These sexually dimorphic features are more pronounced in the breeding season.

Juveniles: The immature stage of this salamander (eft) is fully terrestrial and brightly colored. Body color ranges from red to orange to yellowish. The spots are red to orange encircled or nearly so by black. The bright color signals a warning to potential predators that the glands in the skin produce a toxic mucous. Efts metamorphose into adults in three to seven years.

Eggs: Females lay about 200-300 eggs and wraps each one of them in a leaf or blade of aquatic vegetation.

Larvae: The larvae of this salamander are light brown to yellowish-green in color with a dark line from the snout through the eye to the neck on each side. The dorsal tail fin extends onto the back. The legs are longer than any of the other larval salamanders in the area. The larvae metamorphose into the eft stage.

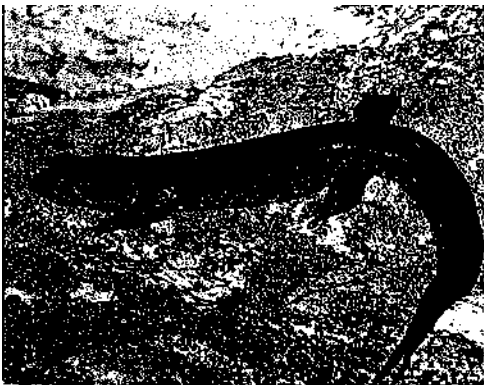
Distribution: Throughout northern Virginia and the mid-Atlantic region.

Seasonality: Adults remain in the aquatic habitat year round unless the pond dries up. If that happens, adult newts will seek shelter under debris in the dried pond or in the nearby forest. The extended breeding season lasts from about March through June. Larvae are present June through November.

Habitat: Many types of freshwater wetlands are used by adults and larvae of this salamander, including beaver ponds, farm ponds, road rut puddles, woodland pools, water-filled depressions in old fields, and pools in riparian areas. Fish may or may not be present. Efts wander widely in the forest from their home pool or pond, and may disperse as far as a half mile. Mature hardwood forest is the optimal habitat for efts.

Identification notes: No other salamander in this region has the rough skin, green body color, and red spots on the back. Pay attention to the dark stripe on the side of the head of larvae that passes horizontally through the eye. Head stripes on other salamander larvae do not pass prominently through the eye.

Seal Salamander (*Desmognathus monticola*)



Adults: These are light to dark brown salamanders (maximum total length to 6 inches [150 mm]) with a highly variable number of diffuse black spots on the back. The black spots join to form a netlike pattern in some individuals. Old seal salamanders and those that are cold are very dark and the pattern may be difficult to see (placing the salamander in a plastic bag with a little water may help). The light eye-jaw stripe is nearly always present. The tail is compressed laterally with a knife-edge shape near the tip. The venter is unpatterned cream, at least along the center, but a salt and pepper stippling pattern may occur along the sides.

Juveniles: Young juveniles have a series of yellowish spots along the back that may be connected

in a netlike pattern. This pattern breaks up into the pattern of black spots as the individual ages. The venter is usually patternless white.

Eggs: Females deposit clutches of 15-40 eggs beneath rocks, logs, and moss along the edge of streams and on the bank above the stream in moist areas. The eggs may stick to each other but are not encased in a gelatinous mass.

Larvae: Seal salamander larvae possess a series of 4-5 pairs of yellowish spots along the dorsum that may be connected to each other. Small external gills are present.

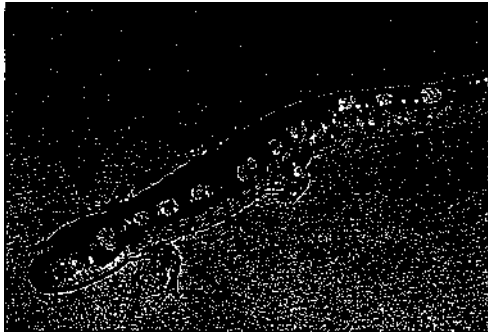
Distribution: Upland and mountainous regions of the mid-Atlantic region.

Seasonality: Adults and juveniles may be found in every month of the year beneath rocks along stream margins. They occur deeply below the surface in the colder months. Females lay eggs from mid-June to early August and eggs hatch in about September. The larval period is apparently long, 10-11 months. Transformation is in June and July the summer following hatching.

Habitat: This salamander is most often encountered along the margins of rocky mountain streams with forest canopy cover. Individuals may occasionally be found in leaf litter and under logs not far from the banks of the stream during wet weather in spring and summer.

Identification notes: The diffuse black spots on the dorsum and clear center portion of the venter will distinguish adults of this species from the northern dusky salamander in the northern Virginia area. Rely on the few dorsal spots and the absence of a reddish stripe on the dorsum of the tail to distinguish larvae and small juveniles from dusky salamanders.

Spotted Salamander (*Ambystoma maculatum*)



Adults: The distinctly round cream, yellow, or orange spots on a black to bluish black, robust body characterize this species from other large salamanders in the area. Maximum total length is about 7 inches (200 mm). Most individuals have at least a few spots on the head, body, or tail. The venter is slate-gray without spots. Males possess enlarged cloacal lips during the breeding season.

Juveniles: Generally patterned as adults with distinct spots. Very recent metamorphs may lack distinct spots.

Eggs: Up to 250 eggs are laid in one to several clear or opaque to white gelatinous masses attached to woody twigs or grass stems in shallow water. A symbiotic algae invades many egg masses soon after they are laid. Numbers of clear and white egg masses vary among ponds.

Larvae: Larval spotted salamanders are generally grayish above and cream below without a distinct pattern. Larvae in muddy pools are light tan. The broad tail fin has varying amounts of dark mottling. Older larvae may have a row of small white spots along each side of the body. The venter completely lacks pigmentation, especially on the throat.

Seasonality: Males in the mid-Atlantic region emerge from forest cover in February and March, rarely January, to lay spermatophores (short gelatinous stalks with a sperm packet on top) on the bottom of temporary pools and ponds. Females follow shortly thereafter and, after being courted by the males to pick up a spermatophore with the cloaca, lay one to several egg masses. Larvae hatch in 3-4 weeks and remain in the pool or pond until metamorphosis. Length of the larval period depends on pool temperature and elevation. Most larvae metamorphose in July-September but only rarely will they over winter and emerge the following spring.

Distribution: Throughout northern Virginia and the mid-Atlantic region.

Habitat: Adults live underground outside of the breeding season in hardwood forests. Individuals may be found under logs and rocks when the substrate is wet. They may migrate over a kilometer to breeding pools and ponds. Breeding sites include ephemeral pools in road ruts, grassland and woodland depressions without fish and in the shallows margins of ponds as long as there is abundant vegetation to allow shelter from fish predators.

Identification notes: The distinct round spots on the dark body will distinguish adults and juveniles of this species from others in the region. Marbled salamander larvae, with which this species is often found, have tiny black dots in the throat.

Three-lined Salamander (*Eurycea guttolineata*)



Adults: Three dark brown stripes on a yellowish brown to tan body (maximum total length to 6 inches [150 mm]) characterize this species. The stripe along the center of the back is usually black and is bordered by the lighter stripes. The black stripe ends on the tail base, whereas the yellowish stripes join to form the light stripe on the dorsum of the tail. The sides of the body and tail are dark brown with a pattern of vertical yellowish markings. The venter is tan with dark brown mottling.

Juveniles: Similar to adults in color and pattern.

Eggs: Little is known about egg deposition sites, but they are likely to be under water beneath rocks or other objects in streams. Egg number is not well known but clutches of between 8 and 15 have been found.

Larvae: The larvae of this species are elongated and streamlined with the tail fins starting behind the rear legs. Body color is brown to yellowish with a broad dark band along each side that extends onto the tail musculature. The venter is cream, and the mottling in the tail fin is variable. The pattern on the tail musculature of older larvae is like parallel slanting lines - pigment spots forming vertical, bar-like shapes.

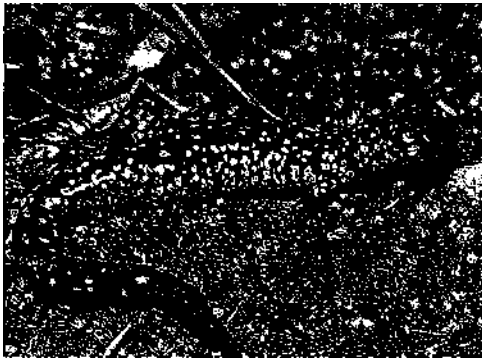
Distribution: East of the Blue Ridge Mountains in northern Virginia and vicinity.

Seasonality: This salamander is seldom found except during spring and fall months. It may be active on the adjacent forest floor during wet periods. Females apparently lay their eggs in early winter. Larvae have been found in Spring, Summer, and Fall, indicating a larval period of less than a year.

Habitat: Adults occupy Piedmont and Coastal Plain streams and seepage areas that usually run through forested habitat. Juveniles and adults can also be found under logs in terrestrial habitat near wetlands, especially forested riparian corridors during late-Spring and Summer.

Identification notes: The three dark lines on the dorsum of adults and juveniles will distinguish this species from others in the area. The pattern on the sides of the tail in juveniles and larvae should allow correct identification. Some populations east of the Blue Ridge Mountains, especially in some streams in Fairfax County, Virginia, have individuals that resemble long-tailed salamanders. The dark band on the sides of larvae will identify this species from larvae of the two-lined salamander.

White-Spotted Slimy Salamander (*Plethodon cylindraceus*)



Adults: Slimy salamanders are large (maximum total length 8 inches [203 mm]) black salamanders with white spots on the back and sides. The white spotting is more concentrated on the sides than the back; some individuals have nearly all white sides with little black pigment exposed. Adult males possess an enlarged, round raised area under the chin during the breeding season (the mental gland). The glands in the skin and particularly those on the tail emit a sticky mucus when handled that quickly dries on one's hand; it is hard to remove but will wear off.

Juveniles: Identical to adults in color and pattern.

Eggs: Females lay 10-30 eggs deep underground beneath rocks in moist soil in hardwood forests.

Larvae: There are no aquatic larvae, as all development occurs within the egg.

Distribution: Throughout northern Virginia (also see below).

Seasonality: Slimy salamanders spend much of the year underground. They are active on the surface and under cover objects from May to the end of October, although they escape dry periods in the Summer by going deep underground. Foraging for invertebrate prey occurs at night on the forest floor during wet periods. Egg-laying occurs in late summer and hatchlings appear in late Fall and the following Spring.

Habitat: Hardwood forests with pockets of moist soil, rocks, and logs are the preferred habitat of this species. Adults can be found under rocks, logs, and stumps on the forest floor when conditions are moist. This salamander is more limited in local distribution than red-backed salamanders because of its more restrictive moisture requirements. Mixed hardwood-pine sites are sometimes occupied as well as other less quality sites.

Identification notes: Red-backed salamanders lack the white pigment and have a salt and pepper pattern on the venter.

OTHER SPECIES

IN THE MID-ATLANTIC REGION

Six other species of amphibians occur around the primary area (northern Virginia) targeted by this guide. With two exceptions, the terrestrial salamanders *Plethodon hoffmani* (Valley and Ridge salamander) and *P. glutinosus* (northern slimy salamander), all are peripheral in distribution and not likely to be encountered by most teachers and other users of this guide.

Barking Treefrog (*Hyla gratiosa*)

This robust treefrog occurs only in a few scattered locations in Delaware and the Eastern Shore of Maryland (Conant and Collins, 1998). It is listed as Endangered in both states. This frog resembles green treefrogs but is larger, more robust, and has dark round spots on a bright to dark green body. Its call is a wonk-wonk-wonk and likened to a small dog barking in the distance.

Narrow-mouthed Toad (*Gastrophryne carolinensis*)

This small frog lives underground most of its life, coming to the surface to breed in shallow puddles formed by summer rains. It occurs in extreme southern Maryland (where it is protected), on lower the Delmarva peninsula, and south of Fredericksburg in Virginia. It has a narrow head and mouth, a broad dark dorsum with broad reddish-brown flanks, and a darkly mottled venter. Its call is like the bleating of a sheep.

Squirrel Treefrog (*Hyla squirella*)

This small treefrog has been found in only one location in eastern Fairfax County, Virginia near the Potomac River (Gotte and Ernst, 1987), well to the north of the rest of its known range. Its body color is highly variable from brown to green and may or may not have spots or a light stripe along the sides of its body. The call is likened to that of a duck but more nasal and raspy, sort of like a squirrel (Conant and Collins 1998).

Greater Siren (*Siren lacertina*)

This is a large, fully aquatic salamander known to occur in southern Maryland and southeast of Fredericksburg, Virginia, in the mid-Atlantic region. It is uniformly gray in color with large external gills even as adults and only one pair of legs, the forelegs. The rear legs are lacking completely. It inhabits streams and associated ponds that are choked with vegetation.

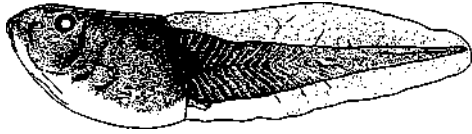
Northern Slimy Salamander (*Plethodon glutinosus*)

Slimy salamanders to the north and west of the northern Virginia area (e.g., Maryland, Pennsylvania) are recognized by some taxonomists as the northern slimy salamander (*P. glutinosus*). It differs from the white-spotted slimy by having a reduced number of brassy-colored spots on the back that are about 1 mm in diameter. The ecology of this species is nearly identical to that of the white-spotted slimy salamander.

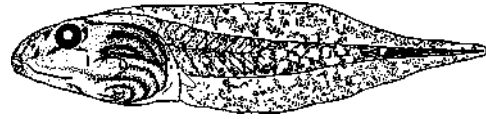
Valley and Ridge Salamander (*Plethodon hoffmani*)

This small, terrestrial salamander occurs in the mountains of western Virginia and Maryland and parts of the Appalachian region of Pennsylvania. It is nearly black with a variable amount of silvery flecking on the dorsum, a white chin, and a plain black venter that is mottled with white near the sides. Its habitat and ecology is similar to the red-backed salamander.

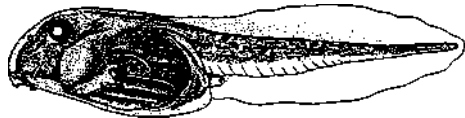
TADPOLES



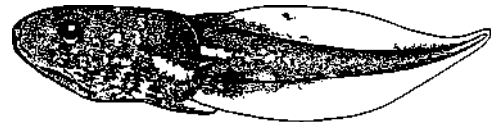
American Bullfrog Tadpole



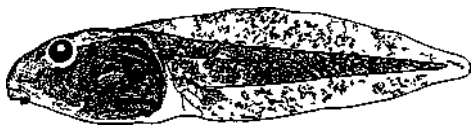
Green Tree Frog Tadpole



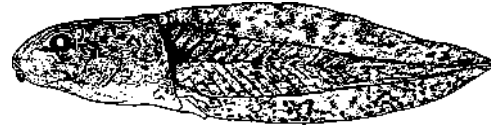
American Toad Tadpole



Narrow-mouth Toad Tadpole



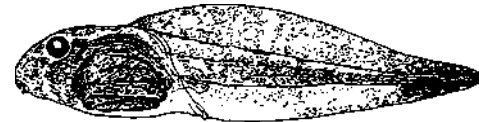
Cope's Cray Treefrog Tadpole



Northern Green Frog Tadpole



Eastern Cray Treefrog Tadpole



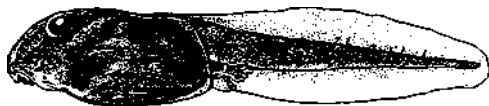
Northern Cricket Frog Tadpole



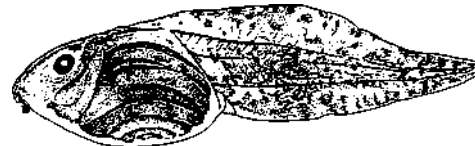
Eastern Spadefoot Toad Tadpole



Pickerel Frog Tadpole



Fowler's Toad Tadpole



Southern Leopard Frog Tadpole



Wood Frog Tadpole

Eastern Mud Salamander Larva



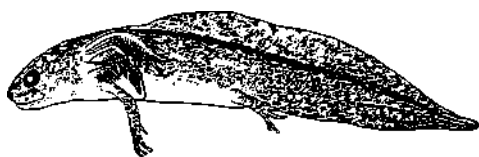
Greater Siren Larva



Jefferson Salamander Larva



Long-Tailed Salamander Larva



Marbled Salamander Larva



Northern Dusky Salamander Larva



Northern Red Salamander Larva



Northern Spring Salamander Larva



Northern Two-Lined Salamander Larva



Red-Spotted Newt Larva



Spotted Salamander Larva



Three-Lined Salamander Larva