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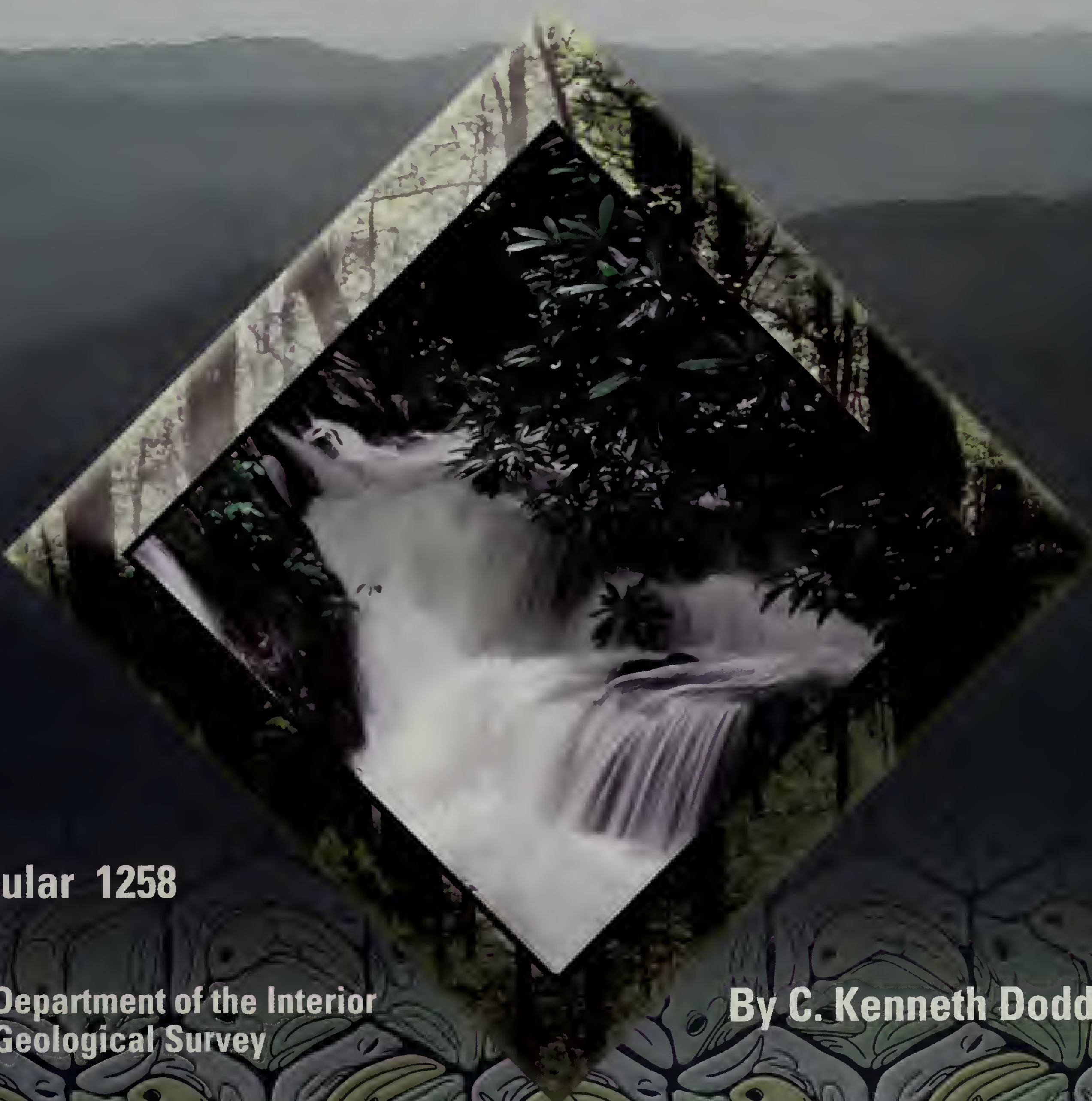
# Monitoring Amphibians in Great Smoky Mountains National Park



Circular 1258

U.S. Department of the Interior
U.S. Geological Survey

By C. Kenneth Dodd, Jr.

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U.S. DEPARTMENT OF THE INTERIOR
GALE A. NORTON, Secretary

U.S. GEOLOGICAL SURVEY
CHARLES G. GROAT, Director



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# *Monitoring Amphibians in Great Smoky Mountains National Park*

## *By* C. Kenneth Dodd, Jr.


### — Abstract —

Amphibian species have inexplicably declined or disappeared in many regions of the world, and in some instances, serious malformations have been observed. In the United States, amphibian declines frequently have occurred even in protected areas. Causes for the declines and malformations probably are varied and may not even be related. The seemingly sudden declines in widely separated areas, however, suggests a need to monitor amphibian populations as well as identify the causes when declines or malformations are discovered.

In 2000, the President of the United States and Congress directed Department of the Interior (DOI) agencies to develop a plan to monitor the trends in amphibian populations on DOI lands and to conduct research into possible causes of declines. The DOI has stewardship responsibilities over vast land holdings in the United States, much of it occupied by, or potential habitat for, amphibians. The U.S. Geological Survey (USGS) was given lead responsibility for planning and organizing this program, named the Amphibian Research and Monitoring Initiative (ARMI). Authorization carried the mandate to set up a national amphibian monitoring program on Federal lands, to develop the sampling techniques and biometrical analyses necessary to determine status and trends, and to identify possible causes of amphibian declines and malformations.

The biological importance of Great Smoky Mountains National Park has been recognized by its designation as an International Biosphere Reserve. As such, it is clearly the leading region of significance for amphibian research. Although no other region shares the wealth of amphibians as found in the Great Smokies (31 species of salamanders, and 13 of frogs), the entire southern and mid-section of the Appalachian Mountain chain is characterized by a high diversity of amphibians, and inventories and monitoring protocols developed in the Smokies likely will be applicable to other Appalachian National Park Service properties.

From 1998 to 2001, USGS biologists carried out a pilot inventory and monitoring research project in Great Smoky Mountains National Park. A variety of inventory, sampling, and monitoring techniques were employed and tested. These included wide-scale visual encounter surveys of amphibians at terrestrial and aquatic sites, intensive monitoring of selected

plots, randomly placed small-grid plot sampling, litterbag sampling in streams, monitoring nesting females of selected species, call surveys, and monitoring specialized habitats, such as caves. Coupled with information derived from amphibian surveys on Federal lands using various other techniques (automated frog call data loggers, PVC pipes, drift fences, terrestrial and aquatic traps), an amphibian monitoring program was designed to best meet the needs of biologists and natural resource managers after taking into consideration the logistics, terrain, and life histories of the species found within Great Smoky Mountains National Park.

This report provides an overview of the Park's amphibians, the factors affecting their distribution, a review of important areas of biodiversity, and a summary of amphibian life history in the Southern Appalachians. In addition, survey techniques are described as well as examples of how the techniques are set up, a critique of what the results tell the observer, and a discussion of the limitations of the techniques and the data. The report reviews considerations for site selection, outlines steps for biosecurity and for processing diseased or dying animals, and provides resource managers with a decision tree on how to monitor the Park's amphibians based on different levels of available resources. It concludes with an extensive list of references for inventorying and monitoring amphibians. USGS and Great Smoky Mountains National Park biologists need to establish cooperative efforts and training to ensure that congressionally mandated amphibian surveys are performed in a statistically rigorous and biologically meaningful manner, and that amphibian populations on Federal lands are monitored to ensure their long-term survival. The research detailed in this report will aid these cooperative efforts.

## Introduction

The Florida Caribbean Science Center (now Florida Integrated Science Center) received funding in 1997 from the U.S. Geological Survey (USGS) Inventory and Monitoring (I&M) Program to conduct a pilot project for amphibians in the southeastern United States. After considering several locations, Great Smoky Mountains National Park (fig. 1) was selected for the survey because of its amphibian diversity and the large number of potential threats to its varied ecosystems (Brown, 2000). During the course of the next 4 years, a field research team of enthusiastic young biologists was assembled to collect information on the species richness and distribution of the Park's amphibians. Researchers used a variety of sampling techniques, including 10 x 10-meter survey plots, "permanent" 30 x 40-meter plots, coverboards, litter-bag surveys, and a great number of time-constrained litter and stream searches. The team looked for previously reported rare species, sampled historic locations, investigated unique habitats (such as caves), and examined museum records and published literature. Survey activities and techniques were designed to optimize the use of available personnel within budget and logistic constraints. Survey teams sampled more than 500 sites (fig. 2) and recorded data on more than 10,000 amphibians. All parts of the Park were visited in all seasons and in all weather conditions.

The objectives of the Great Smoky Mountains National Park I&M program were to: (1) provide a geographically referenced inventory of the amphibian resources of the Great Smoky Mountains National Park; (2) provide indices of abundance of Park selected amphibian species, referenced to locations and habitat types; (3) develop and transfer to the Great Smoky Mountains National Park and National Park Service a series of protocols suitable for long-term monitoring of amphibian populations in the "Smokies" and other Appalachian parks; (4) evaluate current distributions and abundance of amphibian species as possible in the Park with literature reports of past investigations. This manual fulfills the third objective of the I&M program. Additional information on amphibian natural history, distribution, landscape ecology, trends analysis, and protocol development are published in Dodd and others, (2001), Waldron and others, (2003); Dodd, (2004), or is under development.

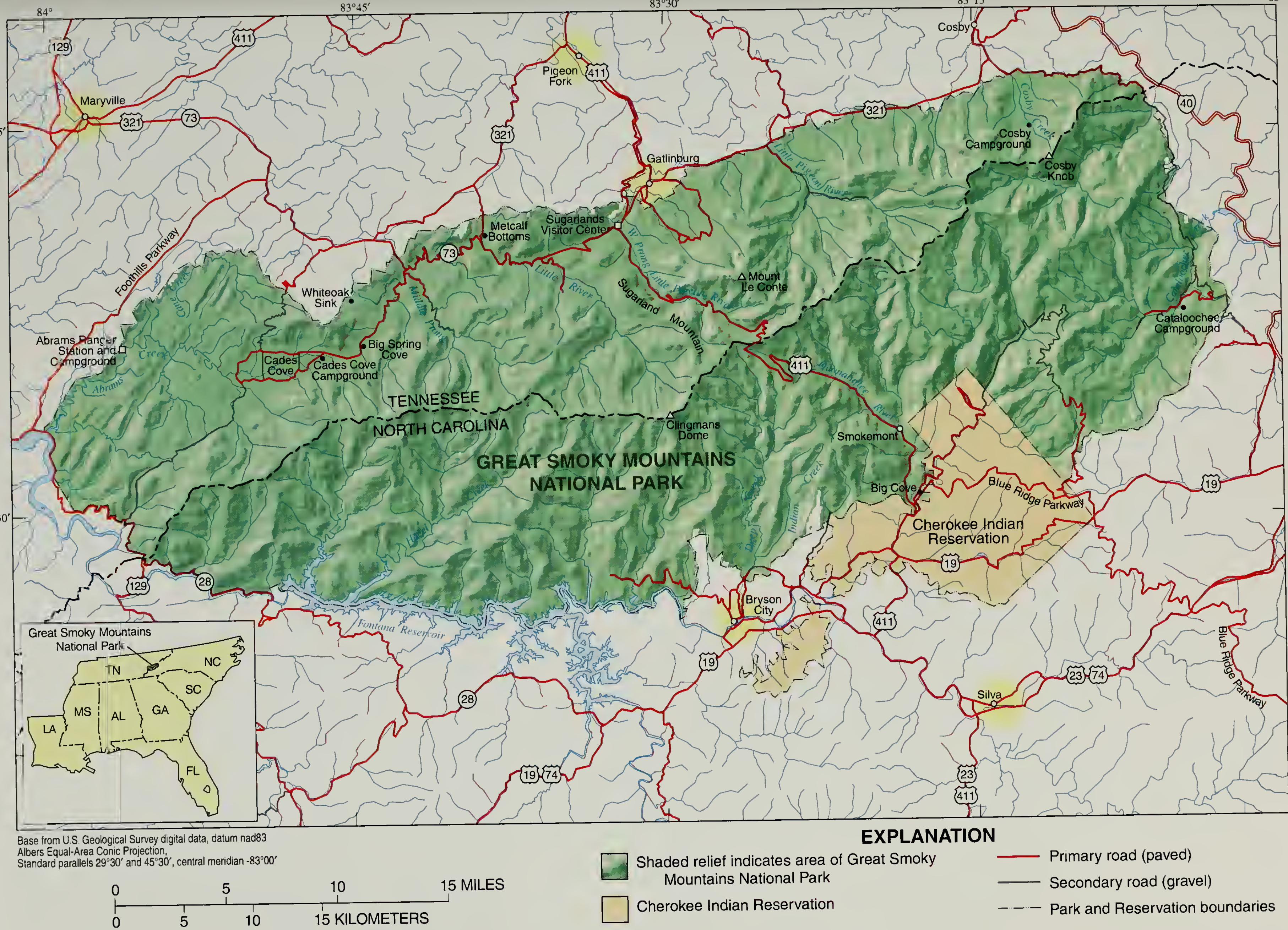


**Figure 1.** Great Smoky Mountains National Park, North Carolina and Tennessee.

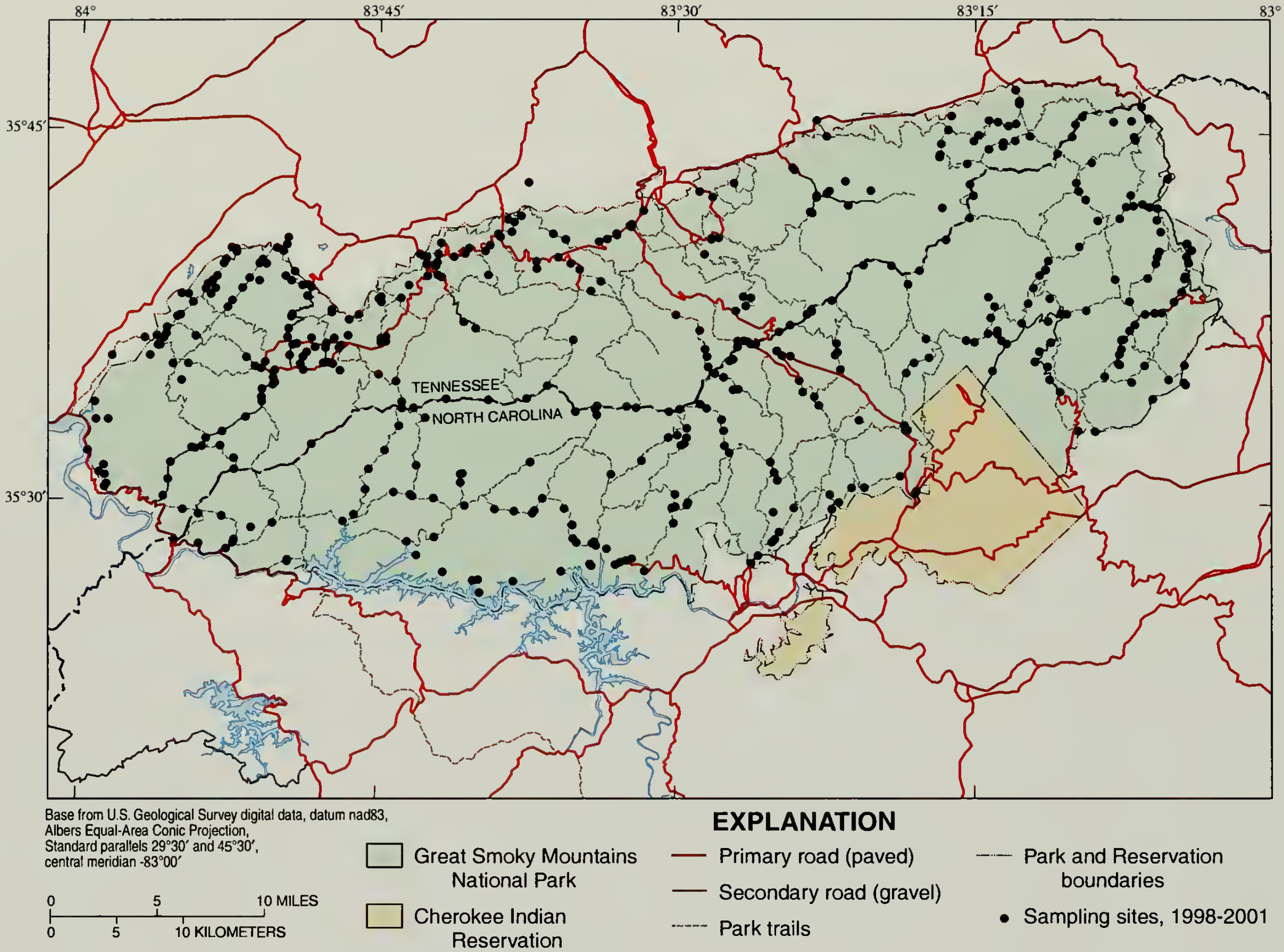


**Figure 2.** Location of U.S. Geological Survey sampling sites in Great Smoky Mountains National Park, 1998 to 2001.


## Acknowledgments

This manual is the result of collaborative survey efforts during 4 years of hard field work between 1998 and 2001 in the Great Smoky Mountains. The author would like to thank the following individuals for their support, assistance, dedication, and companionship:

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For reading the manuscript and making valuable comments: Jamie Barichivich, Marian Griffey, Russell Hall, Steve Johnson, Keith Langdon, and Jennifer Staiger.

*Monitoring Amphibians in Great Smoky Mountains National Park* is meant to help National Park Service natural resource biologists, university researchers, nongovernmental biologists, and the interested public understand and overcome some of the biological and nonbiological constraints to setting up a large-scale inventory and monitoring program for amphibians inhabiting the Great Smoky Mountains. Some of the information applies only to amphibians within the Great Smokies, whereas information on setting up inventory and monitoring programs may have more broad applications with regard to Appalachian amphibians. Many persons who use this guide will be familiar with basic amphibian biology, but others will require a refresher course or will be unfamiliar with amphibian life histories.

This guide serves as a companion volume to Dodd (2004) and, for that reason, information in that work has not been duplicated except when absolutely necessary. There is usually an exception to every generalization discussed below, and biologists should expect to encounter species outside of their "normal" habitat, that often do not fit identification information, or that have unusual behavioral patterns. Extensive information is not included on threats to amphibians (for example, habitat loss and alteration, disease, nonindigenous species, climate change, toxic chemicals, UVB, malformations) or the various reasons why amphibians are vulnerable to environmental problems (including their biphasic life cycle, skin permeability, and the complex morphological and biochemical transformations which accompany metamorphosis). These topics are dealt with in more detail elsewhere (Dodd, 1997, 2004; Alford and Richards, 1999; Corn, 2000; Houlahan and others, 2000).

All of the potential sampling protocols, techniques, and methods of data analysis that may accompany, or be required for, a large-scale amphibian inventory and monitoring program cannot be discussed within one short guide. For this reason, many specialized techniques are not discussed, instructions are not provided for making traps, and statistical programs are not considered in detail. However, references are provided at the end of this guide (see **References on Inventorying and Monitoring Amphibians**).

Future amphibian monitoring within Great Smoky Mountains National Park will be linked to the U.S. Department of Interior (DOI) Amphibian Research and Monitoring Initiative (ARMI). Standardized methods of data collection, entry, and analysis currently are being developed by ARMI researchers for all DOI lands. Pertinent information will be made available to Federal agencies and ARMI partners through ARMI's web site:

edc2.usgs.gov/armi/

**A cautionary note**: There is always the danger that site information will be misused by criminal elements to find amphibians in order to collect them. This is true in National Parks and on other Federal lands, as well as on private lands. None of the amphibians found in Great Smoky Mountains National Park are endangered or threatened under the Federal Endangered Species Act of 1973, as amended, although several species, such as Hellbenders, are protected by state law. Locations of many of the Park's amphibians, including its endemic salamanders, are well known via the published scientific literature and on records attached to museum specimens. Therefore, it seems unlikely that mentioning Park locations in this guide will increase the probability of collection, especially when these species are found readily, and often in greater abundance, outside the Park. For example, the Mole Salamander, Southern Zigzag Salamander, and Mud Salamander might be considered "rare" or "isolated" within Great Smoky Mountains National Park, yet very large and widespread populations of these species are found in the Tennessee Valley and elsewhere. Still, Park Service employees and research scientists working within the Park, including field survey teams, must be observant for illegal collectors and immediately report suspicious activities to law enforcement personnel.

# Amphibians of the Great Smoky Mountains

## *Species Richness*

A total of 31 salamanders and 13 frogs have been recorded from the Great Smoky Mountains National Park. Note that common names are capitalized, and that species names (consisting of a genus and specific epithet) are italicized. Species codes allow data to be entered in shorthand format. To minimize data entry errors, species codes should be either all capitalized or all in lower case letters. Capitals and lower-case letters should not be intermixed. Using accepted and standardized common and scientific names (Crother, 2000), the amphibians are:

| Common name | Scientific name | Suggested species code |
|---|---|---|
| **Salamanders** | | |
| Spotted Salamander | *Ambystoma maculatum* | AMA |
| Marbled Salamander | *Ambystoma opacum* | AOP |
| Mole Salamander | *Ambystoma talpoideum* | ATA |
| Green Salamander | *Aneides aeneus* | AAE |
| Hellbender | *Cryptobranchus alleganiensis* | CAL |
| Seepage Salamander | *Desmognathus aeneus* | DAE |
| Spotted Dusky Salamander | *Desmognathus conanti* | DCO |
| Imitator Salamander | *Desmognathus imitator* | DIM |
| Shovel-nosed Salamander | *Desmognathus marmoratus* | DMA |
| Seal Salamander | *Desmognathus monticola* | DMO |
| Ocoee Salamander | *Desmognathus ocoee* | DOC |
| Black-bellied Salamander | *Desmognathus quadramaculatus* | DQU |
| Santeetlah Salamander | *Desmognathus santeetlah* | DSA |
| Pigmy Salamander | *Desmognathus wrighti* | DWR |
| Three-lined Salamander | *Eurycea guttolineata* | EGU |
| Junaluska Salamander | *Eurycea junaluska* | EJU |
| Long-tailed Salamander | *Eurycea longicauda* | ELO |
| Cave Salamander | *Eurycea lucifuga* | ELU |
| Blue Ridge Two-lined Salamander | *Eurycea wilderae* | EWI |
| Spring Salamander | *Gyrinophilus porphyriticus* | GPO |
| Four-toed Salamander | *Hemidactylium scutatum* | HSC |
| Common Mudpuppy | *Necturus maculosus* | NMA |
| Eastern Red-spotted Newt | *Notophthalmus viridescens* | NVI |
| Northern Slimy Salamander | *Plethodon glutinosus* | PGL |
| Jordan's Salamander | *Plethodon jordani* | PJO |
| Southern Gray-cheeked Salamander | *Plethodon metcalfi* | PME |
| Southern Appalachian Salamander | *Plethodon oconaluftee* | POC |
| Southern Red-backed Salamander | *Plethodon serratus* | PSE |
| Southern Zigzag Salamander | *Plethodon ventralis* | PVE |
| Mud Salamander | *Pseudotriton montanus* | PMO |
| Black-chinned Red Salamander | *Pseudotriton ruber* | PRU |
| **Frogs** | | |
| Northern Cricket Frog | *Acris crepitans* | ACR |
| American Toad | *Bufo americanus* | BAM |
| Fowler's Toad | *Bufo fowleri* | BFO |
| Eastern Narrow-mouthed Toad | *Gastrophryne carolinensis* | GCA |
| Cope's Gray Treefrog | *Hyla chrysoscelis* | HCH |
| Spring Peeper | *Pseudacris crucifer* | PCR |
| Upland Chorus Frog | *Pseudacris feriarum* | PFE |
| American Bullfrog | *Rana catesbeiana* | RCA |
| Northern Green Frog | *Rana clamitans* | RCL |
| Pickerel Frog | *Rana palustris* | RPA |
| Northern Leopard Frog | *Rana pipiens* | RPI |
| Wood Frog | *Rana sylvatica* | RSL |
| Eastern Spadefoot | *Scaphiopus holbrooki* | SHO |

Amphibian taxonomy and systematics within the southern Appalachians are topics of intense debate among biologists. Rationale for using the listed names is provided by Dodd (2004).

### *Habitats and Distribution*

Five major forest communities are recognized within the Great Smoky Mountains National Park, although 80 percent of the Park falls within the Eastern Deciduous Forest Ecosystem (Houk, 1993). Some botanists have further subdivided the vegetation into as many as 67 florally distinct communities. No one species of amphibian is associated entirely with a single forest community, although some of the high-elevation salamanders (*Plethodon jordani, Desmognathus ocoee, D. wrighti*) are more often found in the spruce-fir community than in other community types. Habitat structure, particularly one that retains moisture and high humidity, is important in shaping salamander distribution. The high-elevation coniferous forest appears ideal in providing shade, cover (in the form of coarse woody debris), and abundant surfaces for moisture condensation.

Five major forest communities are recognized within the Great Smoky Mountains National Park. . . .

The ***spruce-fir forest*** (fig. 3) is dominated by Red Spruce (*Picea rubens*) and Fraser Fir *(Abies fraseri*), and is found generally above 1,676 m (5,500 ft), although the community descends to 1,372 m (4,500 ft) in some locations and individual Red Spruce are found at even lower elevations. This is the Canadian Zone boreal forest of high moisture, cool or cold temperatures, and high humidity (Houk, 1993). Ground surface is often dense with fallen tree branches and trunks, and carpeted by thick layers of tree needles. Wet, rotten, woody debris and dense needle mats provide ideal hiding places for terrestrial salamanders. Streams originate in this habitat, usually beginning as small seeps and springs. As streams trickle through

**Figure 3.** Spruce-fir forest at Indian Gap.

the dark-green forest, they gather momentum. Even at higher elevations, aquatic salamanders, particularly duskies (*Desmognathus*) and Blue Ridge Two-lined Salamanders (*Eurycea wilderae*), may be plentiful within the headwater streams.

At somewhat lower elevations (1,067-1,524 m; 3,500-5,000 ft), ***deciduous northern hardwoods*** (fig. 4) predominate, such as Sugar Maples (*Acer saccharum*), American Beech (*Fagus grandifolia*), and Yellow Birch (*Betula alleghaniensis*). Many terrestrial and aquatic salamanders reach their lower or upper distributional range within this community; frogs are scarce. ***Cove hardwoods***, the third community, comprise the most diverse forest community in the Smokies, one that is endemic to the southern Appalachian Mountains. It occurs generally below 1,372 m (4,500 ft) in sheltered valleys, and is dominated by Tulip Poplar (*Liriodendron tulipifera*), Dogwood (*Cornus florida*), Red Maple (*Acer rubrum*), Sweetgum (*Liquidamber styraciflua*), White Basswood (*Tilia americana* var. *heterophylla*), Yellow Buckeye (*Aesculus flava*), and Black Birch (*Betula lenta*). Both hardwood communities have complex understory vegetation, often with much coarse woody debris, which provides cover for terrestrial salamanders. The streams through these hardwood forests are rocky and fast paced, and salamanders are very common along streamsides and in the water.

Two somewhat specialized forest communities are found in the Smokies. The ***hemlock*** community (fig. 5) is dominated by Eastern Hemlocks (*Tsuga canadensis*), commonly

**Figure 4.** Deciduous forest at Lynn Hollow.

Hemlocks are massive with tall, straight trunks. When they fall, they provide excellent habitat for salamanders....

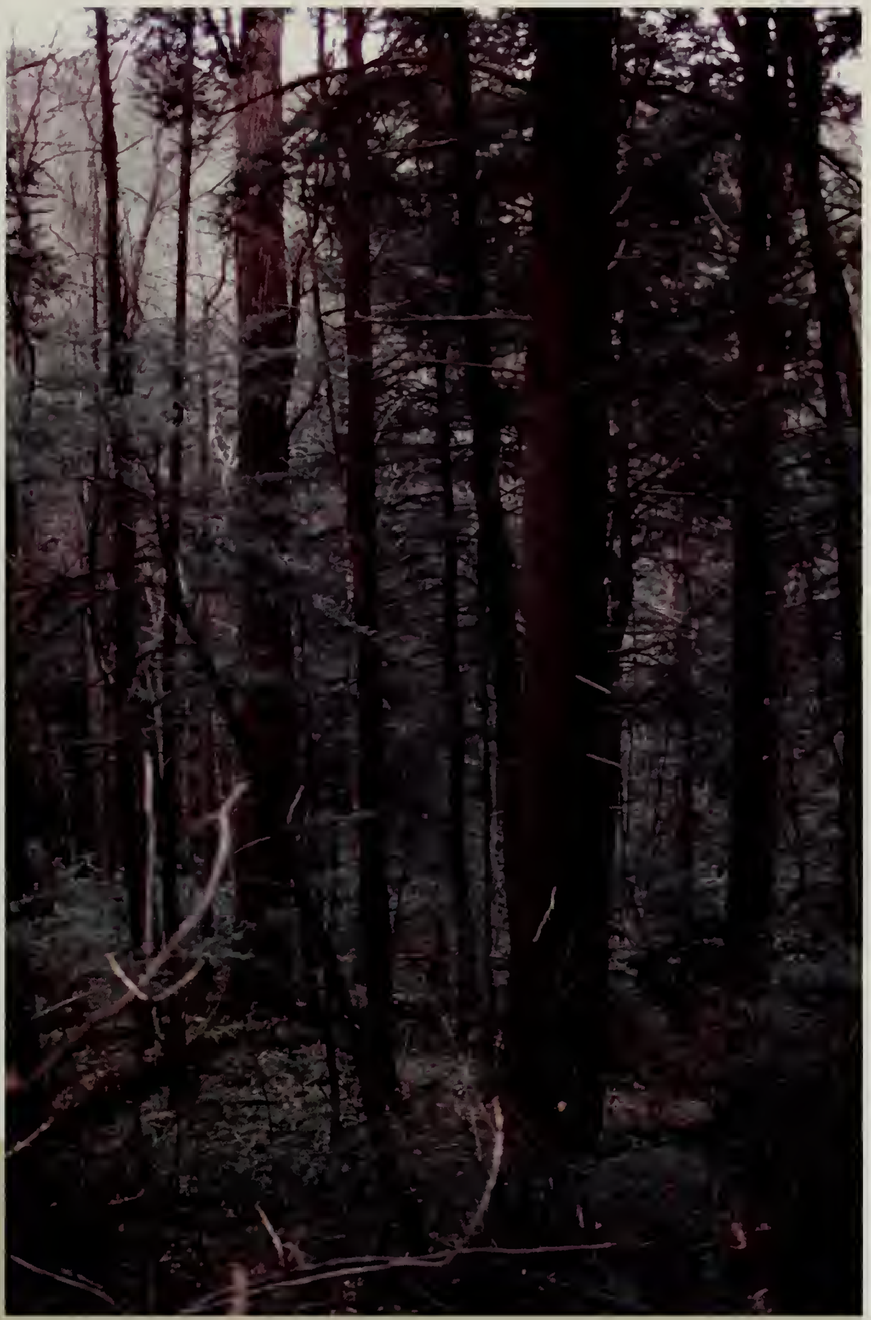


**Figure 5.** Hemlock forest at Chinquapin Knob.

called "spruce-pines" by natives of the southern mountains, and is common between 1,067-1,524 m (3,500-5,000 ft) in elevation. Hemlocks descend to much lower elevations along cold mountain stream valleys, and overlap considerably with both hardwood forests and the spruce-fir forest of the higher elevations. Hemlocks are massive with tall, straight trunks. When they fall, they provide excellent habitat for salamanders, both in the rotting wood and under exfoliating bark (fig. 6).

The ***pine-oak forest*** (fig. 7) occupies the drier areas of the Park, particularly the area west of Cades Cove and at mid-elevations on the North Carolina side of the Park. This forest is dominated by Southern Red (*Quercus falcata*),

**Figure 6.** Coarse woody debris in Cove forest at Roaring Fork. Note the pink survey flags marking the position of transects.

Northern Red (*Q. rubra*), Scarlet (*Q. coccinea*), Black (*Q. velutina*), and Chestnut (*Q. prinus*) Oaks, and by Pitch (*Pinus rigida*), White (*P. strobus*), and Table Mountain (*P. pungens*) Pines. Soils are dry, as is the leaf litter. Prior to human intervention, this community burned frequently in the western regions of the Park, and a fire-adapted vegetation community resulted. Terrestrial salamanders are few, and usually found only during cool, wet times of the year. Aquatic-breeding salamanders and frogs are found along streamsides, where they likely remain close to water. The bottomlands along Cane Creek and Abrams Creek likely formed a corridor from the Tennessee Valley into Cades Cove. As a result, amphibian species richness is surprisingly high, particularly for frogs.

Amphibians are not uniformly distributed throughout the Great Smoky Mountains National Park. There are wide-ranging species, species restricted to specialized habitats, and species found in only one area of the Park.

Monitoring programs will need to take the distribution of species into account to optimize time and financial resources. A few generalizations can be made about amphibian distribution and habitats within the Park.

### SALAMANDERS

***Terrestrial salamanders*** (see **Life History**) include species that are: restricted in distribution in the Great Smokies; wide ranging but not common species; and wide ranging in higher or lower elevations, and generally common. Because they do not have larvae, they must be sampled where they carry out their entire life cycle, usually on the forest floor and under leaf litter and other debris.

Amphibians are not uniformly distributed throughout the Great Smoky Mountains National Park.


Photographer: Todd Campbell, University of Tampa

**Figure 7.** Oak-pine forest.

**Table 1.** Identification and life history of the nonpermanently aquatic salamanders of Great Smoky Mountains National Park

[mos, months; yr, year; mm, millimeter; TL, total length; SVL, snout-vent length; ~, approximately; <, less than; >, greater than]

| Species | Egg deposition | Hatching | Larval period | Hatching size | Size and time of Metamorphosis | Spots on dorsum | Dorsal pattern | Belly pattern | Tail attributes | Notes |
|---|---|---|---|---|---|---|---|---|---|---|
| *Ambystoma maculatum* | Jan. to late Mar. (mountains-late Feb. to early Mar.); 4-7 weeks incubation | April-May | 2-4 mos | 12-17 mm TL | 29-32 mm SVL; 43-60 mm TL (to 75 mm TL if overwinter); mid-June to August | | dull olive green, no conspicuous markings | white or light | tail fin lightly mottled or finely stippled; dark at tip | Breeding occurs in 2-3 bouts following rain; pond type larvae |
| *A. opacum* | Oct-Nov (in pond by Sept.); 9-15 days incubation, but must be flooded 1-2 days | winter | 5-7 mos | 10-14 mm TL | ~33 mm SVL; 49-58 mm TL; late March mid-June | | blackish, drab; older larvae have mottling on body | throat stippled; scattered melanophores on lateral sides | dorsal fin extends almost to front limbs | Pond type larvae; series of ventrolateral light spots forming a line below limb insertions |
| *A. talpoideum* | Sept. to Mar. (winter) | winter to early spring | 3-4 mos, but variable | ~10 mm SVL | 32 to 50 mm SVL; May to Sept. | | black and yellow blotches along midline of back | dark band on midline (poor in some specimens) | yellow and black on tail fin | Pond type larvae; variable life histories with regard to timing of events |
| *Desmognathus conanti* | early May to early July, perhaps to mid-August; 45-60 days incubation | July to early fall | <1 yr | 8-12 mm SVL; 12-20 mm TL | 9-12 mm SVL, to 20 mm SVL; July to early fall? | 5-8 pairs of even or alternating spots or blotches | sides with dorsolateral stripe; dorsum variable | | spot pattern continues on tail | In older larvae, spots or blotches may fuse |
| *D. imitator* | late spring to early summer? | | | | | | | | | |
| *D. marmoratus* | late spring to early summer?; 10-12 weeks incubation | mid-Aug to mid-Sept. | 3 yrs (10-20 mos) | 11 mm SVL | 26-38 mm SVL; May to Oct. | 2 rows light spots | dark, conspicuous light flecks on sides | | spatulate | more slender, with longer legs than DQ |
| *D. monticola* | mid-June to mid-August; 2 mos incubation | early summer to fall; Sept. | 10-11 mos | 11-12 mm SVL | June-July | 4-5 pairs light dorsal spots between limbs | | | | |
| *D. ocoee* | July to early Aug. to Sept.; 52-74 days incubation | Aug to late Sept. | 9-10 mos | 13-18 mm TL | 11-15 mm SVL; May to June | 4-6 pairs of alternating light spots on dorsum | | | | round snouts |
| *D. quadramaculatus* | May to June | July to Sept. | 3-4 yrs | 11-16 mm SVL | 35-42, to 54 mm SVL; mid-summer | 6-8 pairs light spots between limbs | light brown | | | much larger than all other Desmogs; lots of yolk 1-2 mos after hatching |
| *D. santeetlah* | early May to early July, perhaps to mid-August; 45-60 days incubation | July to early fall | <1 yr | 8-12 mm SVL; 12-20 mm TL | 9-12 mm SVL, to 20 mm SVL; July to early fall? | 4-5 pairs of even or alternating spots or blotches | | | | |

**Table 1.** Identification and life history of the nonpermanently aquatic salamanders of Great Smoky Mountains National Park (Continued)

[mos, months; yr, year; mm, millimeter; TL, total length; SVL, snout-vent length; ~, approximately; <, less than; >, greater than]

| Species | Egg deposition | Hatching | Larval period | Hatching size | Size and time of Metamorphosis | Spots on dorsum | Dorsal pattern | Belly pattern | Tail attributes | Notes |
|---|---|---|---|---|---|---|---|---|---|---|
| *Eurycea guttolineata* | winter | early to mid-Mar? | 3.5-5.5 mos (< 1 yr), but may over-winter | 11-12.5 mm SVL | 22-27 mm SVL, to 32 mm SVL; June to August | no paired light spots | cream; uniformly stippled; then dark broad dorsolateral stripe; narrow mid-dorsal stripe | immaculate | dorsal fin does not extend for-ward of rear legs | stream type |
| *E. junaluska* | at least by mid-May | early June? | 1-2 yr | 7-9 mm SVL; 11-13 mm TL | 34-42 mm SVL; mid-May to August | | deep olive green to brown | no iridophores | | dense, well-defined cheek patches; lower margin of dark pigmentation straight |
| *E. longicauda* | late autumn to early spring | Nov-March after 4-12 weeks | normally <1 yr (4-7 mos) | 18-21 mm SVL; 40 mm TL; | 23-28 mm SVL; > 50 mm TL if over-wintering; mid-June-July | | cream colored; then uniformly dark, simi-lar to adults; no paired spots | immaculate | | |
| *E. lucifuga* | Sept. to Feb. | | 6-18 mos; most 12-15 mos | 9-12 mm SVL; to 17.5 mm TL | 31-37 mm SVL; to 70 mm TL; spring | | sparse pigmentation with 3 longitudinal series of spots on the side | | | |
| *E. wilderae* | Feb. to May; 4-10 weeks incubation | May to August | 1-2 yr | 7-9 mm SVL; 11-14 mm TL | 18-19 mm SVL in 1 yr, to 32 mm SVL in 2 yr; April to July | 6-9 pairs light dor-solateral | dusky | light with iri-dophores | | stream type; tail fin stops near insertion of rear limbs; red-dish gills; square snouts |
| *Gyrinophilus porphyriticus* | summer | late summer to autumn | to 4 yr | 18-22 mm TL | 55-65 mm SVL, to 70 mm high eleva-tion; late June to August | | light yellow brown to gray with fine fleck-ing | | | long truncated snouts with small eyes |
| *Hemidactylium scutatum* | Feb to May | May-June? | 21 to 61 days | | 11-15 mm SVL; 17-25 mm TL; July? | | nondescript, yellow brown; dorsal fin extends to head | | | pond type larvae; joint nesting occurs; brooding |
| *Pseudotriton montanus* | autumn to early winter | winter | 15-17 mos to 29-30 mos | <13 mm SVL | 35-44 mm SVL; mid-May to Sept. | | light brown; older with widely scattered spots | immaculate | | stream type; over-wintering occurs; larvae can be very large |
| *P. ruber* | autumn to early win-ter; 3 mos incubation | mid-Dec to mid-Feb | 1.5 to 3.5 yr (27-31 mos) | 11-14 mm TL | 34-46 mm SVL; 62-86 mm TL; May to July | | light brown; weakly mottled or streaked | dull white | | stream type; no black chins or dor-sal spots |

Monitoring programs can target each type of distributional pattern or habitat listed below, depending upon the objectives of the researchers and the funds and personnel available. For example, whereas a few people can easily monitor the status of the Southern Zigzag Salamander, a much more elaborate protocol will be necessary to monitor populations of the Southern Red-backed Salamander. A number of these species are syntopic, making multispecies monitoring a realistic objective. As much as possible, single species sampling and monitoring should be avoided in favor of multispecies sampling and data recording. Some examples of typical distribution patterns follow:

**Species restricted in distribution**

Southern Zigzag Salamander (*Plethodon ventralis*).

**Wide ranging, but not common, species**

Southern Appalachian Salamander (*Plethodon oconaluftee*).

**Species that are common and wide ranging at higher elevations**

Pigmy Salamander (*Desmognathus wrighti*); Jordan's Salamander (*Plethodon jordani*); Southern Gray-cheeked Salamander (*Plethodon metcalfi*).

**Species that are common and wide ranging at lower elevations**

Northern Slimy Salamander (*Plethodon glutinosus*); Southern Red-backed Salamander (*Plethodon serratus*).

***River-dwelling salamanders*** inhabit only the largest of the Smokies' rivers (fig. 8), including Little River, Middle Prong, Oconaluftee River, Little Pigeon River, Abrams Creek, the lower reaches of Deep Creek and, perhaps, Hazel Creek. There are only two true river-dwelling salamanders in the Great Smokies, the Hellbender (*Cryptobranchus alleganiensis*), known presently only from Little River, Oconaluftee River, and Deep Creek

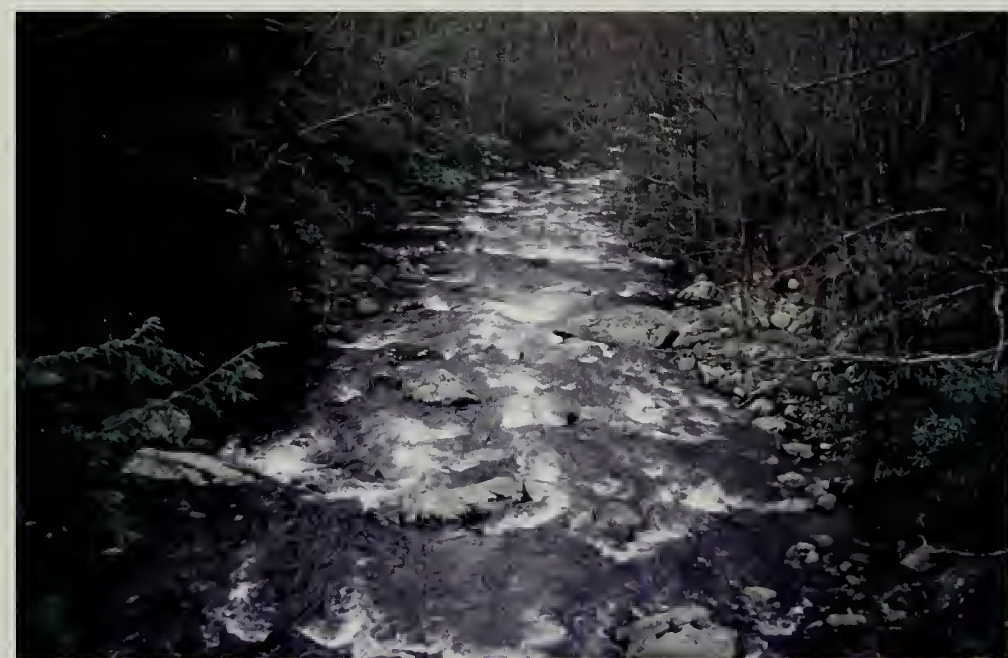


**Figure 8.** Middle Prong at Tremont.

**Figure 9.** Ideal habitat for Hellbenders in Lower Abrams Creek.

(Nickerson and others, 2002; Dodd, 2004) (fig. 9), and the Common Mudpuppy (*Necturus maculosus*), known only from Little River and Abrams Creek. One additional salamander, the Junaluska Salamander (*Eurycea junaluska*), tends to be associated with some of the Park's larger western and northwestern streams and rivers on the Tennessee side of the Smokies. Larvae are found near the shore, and the adults inhabit streambanks for at least part of the year. However, this species also inhabits some smaller streams, and it is by no means a "river-dwelling" species.

***Creek and stream salamanders*** have larvae that develop in the creeks and streams of the Park (figs. 10-12), whereas the adults may be

**Figure 10.** Small stream in unnamed tributary to Falls Branch.

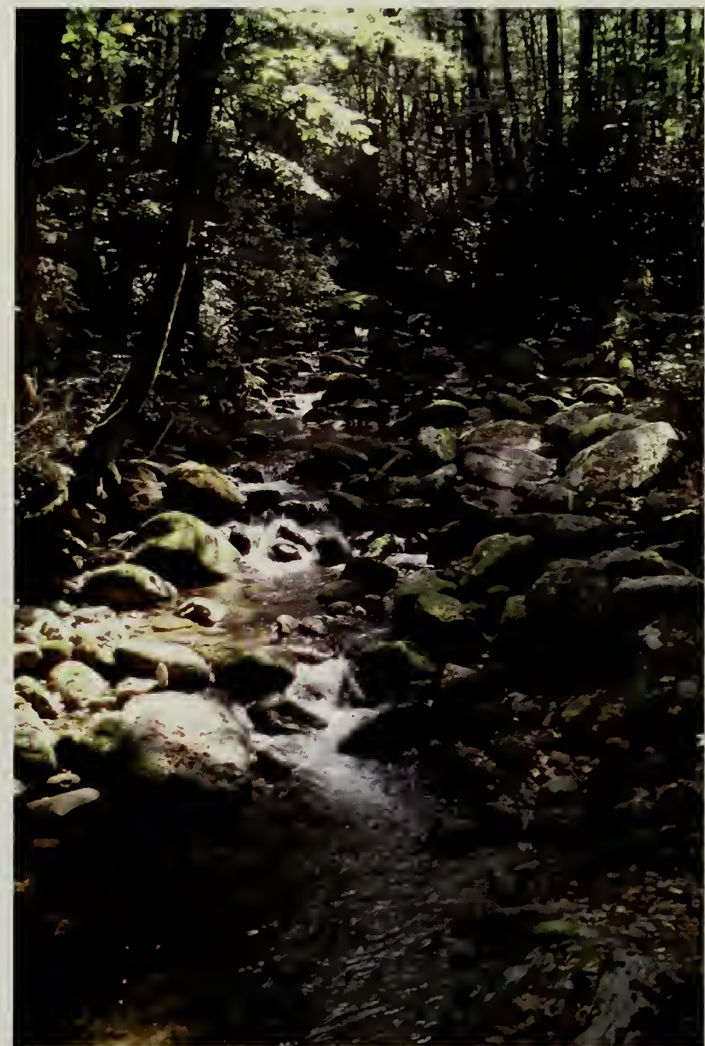


Figure 11. Medium-sized stream in normal flow at Roaring Fork.

aquatic, semi-aquatic, or even terrestrial to a greater or lesser degree. Many of these species are widespread in the Park because of the large number of creeks and streams available for colonization. A few species are found only at higher mountain elevations (for example, the Ocoee and Imitator Salamanders), whereas others are lowland species (Spotted Dusky, Three-lined, and Long-tailed Salamanders). Instead of a circumscribed area, their habitat is often linear, following the streams and streamsides. The dusky salamanders (*Desmognathus*) are prominent in this group, but there are many exceptions to each habitat categorization listed below. Even Black-bellied Salamanders have been found well above the forest floor in rock crevices among boulders at considerable distances from water. Monitoring adults and larvae of these species requires very different techniques, and may require sampling very different types of habitats.

**Nearly aquatic species**

Shovel-nosed Salamander (*Desmognathus marmoratus*).

**Predominantly aquatic and streamside species**

Spotted Dusky Salamander (*Desmognathus conanti*); Seal Salamander (*Desmognathus monticola*); Black-bellied Salamander (*Desmognathus quadramaculatus*); Santeetlah Salamander (*Desmognathus santeetlah*);

Many of these species are widespread in the Park because of the large number of creeks and streams available for colonization.

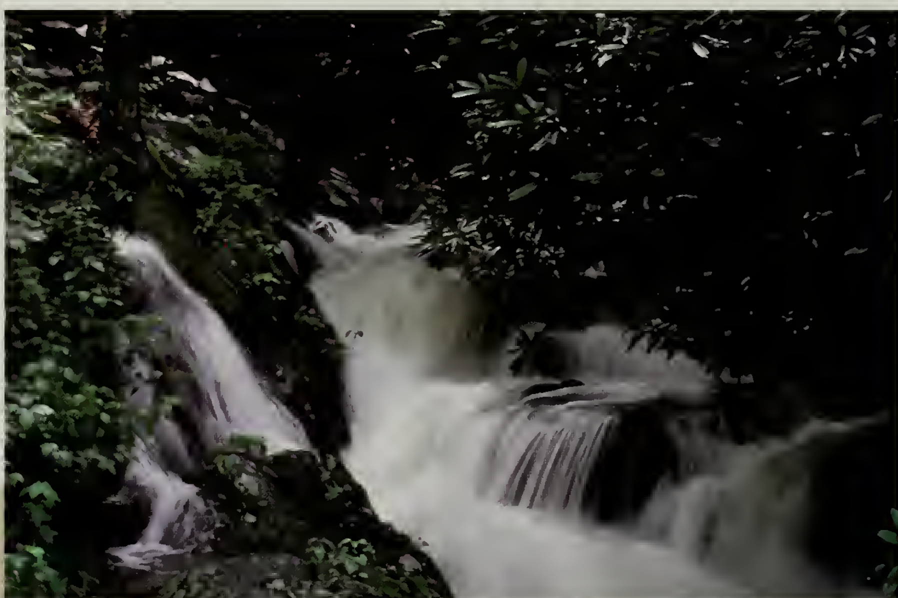


Figure 12. Medium-sized stream in high water at Whiteoak Flats Branch.

Junaluska Salamander (*Eurycea junaluska*). The extremely high elevation areas where some streams first appear may be devoid of salamanders if the water emanates from the Anakeesta rock formation (Dodd, 2004).

**Species with aquatic larvae but are largely terrestrial as adults**

Imitator Salamander (*Desmognathus imitator*); Ocoee Salamander (*Desmognathus ocoee*); Three-lined Salamander (*Eurycea guttolineata*); Long-tailed Salamander (*Eurycea longicauda*); Blue Ridge Two-lined Salamander (*Eurycea wilderae*).

A few ***salamanders*** require very ***specialized habitats*** in the Great Smokies, or at least are usually found in these habitats. Some of these species have larvae which are found in the same streams and creeks as the preceding species, although the adults prefer to leave the streams. Whereas the larvae may be relatively easy to survey, adults often can be quite difficult to find with any regularity. One species, the Seepage Salamander, does not have a larval stage, and the adults are only found in wet seeps.

**Cave inhabitants (fig. 13)**

Cave Salamander (*Eurycea lucifuga*).

Known only from Stupkas Cave, the Calf caves, and one record from Whiteoak Sink. Other salamanders in the Smokies may live in caves, especially around the entrances (Dodd and others, 2001). The larvae of some salamanders (for example, *E. longicauda* in Gregorys Cave) develop in pools well inside cave passages (fig. 14).

**Rock face inhabitants (fig. 15)**

Spotted Dusky Salamander (*Desmognathus conanti*); Seal Salamander (*Desmognathus monticola*).

Permanent to near permanent wet rock walls with hiding places, particularly along trails, road cuts, and in the vicinity of waterfalls, especially at lower elevations.

**Figure 13.** Entrance to Gregorys Cave.

Figure 14. Rimstone pools and cave pool at Gregorys Cave. Salamander larvae develop in the pools, although they are unlikely to complete metamorphosis.

A few salamanders require very specialized habitats in the Great Smokies, or at least are usually found in these habitats.

**Figure 15.** Rock face near Double Gap.

## Spring Inhabitants

Spring Salamander (*Gyrinophilus porphyriticus*); Black-chinned Red Salamander (*Pseudotriton ruber*).

## Inhabitants of swampy and mucky habitats (fig. 16)

Mud Salamander (*Pseudotriton montanus*).

Known only from a few scattered locations in the lowlands of the northern side of the Park.

## Inhabitants of wet seepages (fig. 17)

Seepage Salamander (*Desmognathus aeneus*).

Known only from drainages on the southwestern side of the Park.

Finally, there are ***salamanders that breed in ponds***, and it is virtually only at this time that these species can be censused. Five species fall into this category: the Spotted Salamander (*Ambystoma maculatum*); Marbled Salamander (*A. opacum*); the rare Mole Salamander (*A. talpoideum*); Four-toed Salamander (*Hemidactylium scutatum*); and Eastern Red-spotted Newt (*Notophthalmus viridescens*). Breeding ponds are limited within the Park, being concentrated in Cades Cove and nearby Big Spring Cove (the four Finley-Cane sinkhole ponds), the Cane Creek drainage, and at scattered localities between Sugarlands and Cades Cove along Little River (at the Sinks and ditches along the road to Tremont). These locations are on the Tennessee side of the Park. Although beaver ponds are found in Bone Valley and Big Cove in North Carolina, and small scattered ditches and wetlands occur in Cataloochee Valley, no pond salamanders are known to breed in them.

Figure 17. Seep at Big Spring Cove.

**Figure 16.** Former trout pond mucky habitat in Cataloochee.

Mud Salamanders are known only from a few scattered locations in the lowlands of the northern side of the Park.

## FROGS

Frogs in the Great Smoky Mountains National Park require water for breeding and for tadpole development. As such, the diversity and distribution of frogs are not as great in the mountains as in the adjacent lowlands of the Tennessee Valley and Atlantic Coastal Plain. In the Smokies, four major types of breeding sites are used by frogs and toads: ponds (natural, as well as of beaver or human origin); woodland pools; grassy ditches, pools, and rivulets; and larger streams and rivers.

### Ponds

Pond distribution is limited in the Smokies, being confined mostly to Cades Cove, Big Spring Cove, and two beaver ponds. The

**Figure 18.** Gum Swamp at Cades Cove in high water.

**Figure 19.** Gum Swamp at Cades Cove when dry.

most important frog-breeding ponds are Gum Swamp (figs. 18, 19), Gourley Pond (figs. 20, 21), Methodist Church Pond (fig. 22), and the sewage-treatment pond (all in Cades Cove); the four sinkhole ponds in Big Spring Cove (also known as the Finley-Cane ponds); and the beaver ponds in Bone Valley and Big Cove (fig. 23). Species that commonly use these ponds are the American Toad (*Bufo americanus*), Cope's Gray Treefrog (*Hyla chrysoscelis*), Northern Green Frog (*Rana clamitans*), Pickerel Frog (*R. palustris*), Wood Frog (*R. sylvatica*), and Eastern Spadefoot (*Scaphiopus holbrooki*), known only from Gum Swamp. American Bullfrogs (*R. catesbeiana*) also have been heard at the beaver pond in Big Cove. Some of these ponds dry completely as the summer progresses, particularly Gum Swamp (fig. 19), Gourley Pond (fig. 21), and the Finley-Cane sinkhole ponds.

**Figure 20.** Gourley Pond at Cades Cove in high water.

**Figure 21.** Gourley Pond at Cades Cove when dry.

**Figure 22.** Methodist Church Pond at Cades Cove.

**Figure 23.** Beaver pond at Big Cove.

### Woodland Pools

Woodland pools are scattered at various areas within the Park. They range from a few centimeters deep to about 0.5 m, and they usually dry as summer progresses. Woodland pools are located in level ground at Cosby, Sugarlands, Metcalf Bottoms, Big Spring Cove, Little Cataloochee Valley, throughout the Cane Creek drainage (fig. 24), Cades Cove (especially along Abrams Creek at the western edge of the cove), and doubtless in other areas of the Park. Amphibians that use these small pools for breeding include the Eastern Red-spotted Newt (*Notophthalmus viridescens*), American Toad (*Bufo americanus*), Cope's Gray Treefrog (*Hyla chrysoscelis*), Northern Green Frog (*Rana clamitans*), Pickerel Frog (*R. palustris*), and Wood Frog (*R. sylvatica*).

**Figure 24.** Woodland drainage pool at Cane Creek.

### Grassy Ditches, Pools, and Rivulets

Grassy ditches, pools, and rivulets are generally shallow, open-canopied habitats, with a grassy vegetation where concealment and breeding sites are available (fig. 25). Only two places in the Park contain much of this habitat: Cades Cove and Cataloochee Valley. Frogs found here include the American Toad (*B. americanus*); Eastern Narrow-mouthed Toad (*Gastrophryne carolinensis*), known only from grassy pools at the Abrams Creek Ranger Station and at Shields Pond in Cades Cove; Spring Peeper (*Pseudacris crucifer*); Upland Chorus Frog (*Pseudacris feriarum*); and the ubiquitous Wood Frog (*R. sylvatica*). These habitats normally dry rapidly with the warm weather, although the rivulets and some pools in Cades Cove may persist well into summer.

### Streams and Rivers

A few species of frogs breed in the shallows of rivers and larger streams. In the Great Smokies, the American Bullfrog's (*R. catesbeiana*) large tadpoles are conspicuous in Abrams Creek near the Abrams Creek Ranger Station. Additional species, such as Fowler's Toad (*B. fowleri*), breed in the backwaters formed from flooding along streams and rivers. Other frogs, such as Northern Green Frogs (*R. clamitans*), are found along streambanks during the nonbreeding seasons.

**Table 2.** Identification and Life History of the Frogs of Great Smoky Mountains National Park

[<, less than; >, greater than; mm, millimeter; cm, centimeter; m, meter; m$^2$, square meters]

| Species | Eggs | Tadpole description | Breeding times | Larval period | Metamorph size |
|---|---|---|---|---|---|
| *Acris crepitans* | eggs deposited singly; 1 gelatinous envelope, >2.3 mm in diameter; deposited in shallow water among stems of grass or on bottom; 250 eggs per complement | a medium-sized light to medium-gray tadpole; throat light; tail musculature mottled or reticulated; usually a very distinctive "black flag" on the tail tip; tail long and narrow; anus dextral (to the right); oral disk emarginate; most 30-36 mm total length, rarely to 46 mm | April to June, possibly into July | 35-70 days, based on *Acris crepitans blanchardi* | 10-15 mm |
| *Bufo americanus* | eggs in strings with gelatinous casings; 2 envelopes present; strings long, to 60 m; 15-17 eggs per 25 mm; 4,000-12,000 eggs on bottom of quiet pools | body round or oval in dorsal view; eyes dorsal (looks cross-eyed); nostrils large; color dark brown to black; dorsal portion of the body unicolored; venter with aggregate silvery or copper spots; snout sloping in lateral view; tail musculature distinctly bicolored; anus medial (in the center); spiracle is distinctly on left side of body | spring (March-April) | 50-65 days | 7-12 mm |
| *B. fowleri* | eggs in strings with gelatinous casings; 1 envelope present and <5 mm in diameter; strings 2.4-3 m with 17-25 eggs per 25 mm; 5,000-10,000 eggs; in tangled mass around vegetation | body round or oval in dorsal view; eyes dorsal (looks cross-eyed); nostrils large; color dark; dorsal portion of body slightly mottled; snout rounded in lateral view; tail musculature often not distinctly bicolored; anus medial (in the center); spiracle is distinctly on left side of body | April to July | 40-60 days | 7.5-11.5 mm |
| *Gastrophryne carolinensis* | eggs in small surface film that has a mosaic structure; envelope a truncated sphere; mass round or square; 10-150 eggs per mass; in any depression with water, but not deep pools | a small jet-black tadpole with lateral white to pink stripes on posterior portion of body extending to the tail musculature. Viewed from the side, the head comes to a point; body round in dorsal view; eyes wide set and lateral; anus median; jaws do not have keratinized sheaths, and the oral disc and labial teeth are absent | mid-May to mid-August | 20-70 days | 8.5-12 mm |
| *Hyla chrysoscelis* | eggs in small surface film, but envelope not in truncated sphere; no mosaic structure; 5-40 eggs per mass; in shallow ponds attached loosely to vegetation, or free. Air bubbles present. | small to medium-sized grayish tadpole with a high dorsal tail fin; dorsal tail fin height equal to or greater than musculature height; tail long, with black blotches; background color of mature tail orange to scarlet; throat rarely pigmented; dorsal fin never extends anterior to midway between the spiracle and eye; anus dextral (to the right); oral disk not emarginate | April to June, but calls occasionally heard at other times of the year | 45-65 days | 13-20 mm |
| *Pseudacris crucifer* | eggs deposited singly in shallow water near bottom among vegetation; one gelatinous envelope. | a small-sized deep-bodied tadpole with a medium-sized tail; tail musculature mottled; fins clear or with blotches; no dots on body; snout square when viewed dorsally; anus dextral (to the right); oral disk not emarginate | late winter to early spring (February to April); calls occasionally heard at other times of the year | 90-100 days | 9-14 mm |
| *P. feriarum* | egg mass in lump, but loose irregular cluster; 1 envelope, 3.6-4.0 mm; deposited in marshy areas and pools in matted vegetation | small olive to black tadpole with a bronze belly; tail medium; anus dextral (to the right); oral disk not emarginate; tadpoles develop rapidly | February to April. | 50-60 days | 8-12 mm |

**Table 2.** Identification and Life History of the Frogs of Great Smoky Mountains National Park (Continued)

[<, less than; >, greater than; mm, millimeter; cm, centimeter; m, meter; $m^2$, square meters]

| Species | Eggs | Tadpole description | Breeding times | Larval period | Metamorph size |
|---|---|---|---|---|---|
| *Rana catesbeiana* | eggs in large surface film in form of a disc; 10,000-12,000 eggs per disc; deposited among water plants or brush; 1 gelatinous envelope | large olive to grayish green tadpole with small widely spaced small spots (dots) covering the body and tail; venter straw; eyes bronze; body oval and round in dorsal view; eyes dorsal or dorsolateral; nostrils small compared with eyes; lower jaw wide; anus dextral (to the right); oral disk emarginate | late spring and throughout the summer. Calls may be heard at other times of the year | 1-2 years | 31-59 mm |
| *R. clamitans* | eggs in surface film; mass <0.09 $m^2$; 1,000-5,000 per mass; attached to vegetation or free; 2 gelatinous envelopes | large (but not deep bodied) olive green tadpole with large dark spots, generally with a white throat; belly deep cream without iridescence; body oval and round in dorsal view; eyes dorsal or dorsolateral; nostrils small compared with eyes; tail green mottled with brown; lower jaw wide; anus dextral (to the right); oral disk emarginate | late April to late July or even early August. Calls may be heard at other times of the year | to 1 year | 23-38 mm |
| *R. palustris* | eggs in firm regular cluster; brown above and yellow below; mass a sphere 38-100 mm in diameter; 2 envelopes present; 2,000-4,000 eggs; mass deposited 75-100 mm to 91 cm under water; attached to debris and vegetation | large, full, deep-bodied tadpole; olive green shading through yellow on sides; venter cream, back marked with fine black and yellow spots; belly with blotches of white; venter iridescent, viscera visible; tail very dark, black blotches can aggregate to purple-black; body oval and round in dorsal view; eyes dorsal or dorsolateral; nostrils small compared with eyes; lower jaw narrow; anus dextral (to the right); oral disk emarginate | late winter to spring (mid-March-April) | 70-80 days | 19-27 mm |
| *R. pipiens* | mass a firm regular cluster; 3,500-6,500 eggs close together in mass; 2 envelopes present; outer envelope 5 mm; eggs black above and white below; deposited near surface, usually attached to grasses and vegetation, sometimes free | large, deep-bodied tadpole; dorsally dark brown, covered with small gold spots; belly deep cream, with bronze iridescence; viscera visible; throat translucent and more extensive than Pickerel Frog; similar in appearance to Green Frog, but darker; body oval and round in dorsal view; eyes dorsal or dorsolateral; nostrils small compared with eyes; lower jaw narrow; anus dextral (to the right); oral disk emarginate | probably early March to early May | 60-80 days | 18-31 mm |
| *R. sylvatica* | eggs in firm regular cluster; black above and white below; mass a sphere 38-100 mm in diameter; 2 envelopes present; 2,000-4,000 eggs; mass deposited 75-100 mm to 91 cm under water; attached to debris and vegetation | medium-sized tadpole with usually very dark to gray coloration, and with a faint light stripe of cream, white or gold along the upper jaw (like a mustache); venter cream with belly slightly pigmented at sides; body oval and round in dorsal view; eyes dorsal or dorsolateral; nostrils small compared with eyes; anus dextral (to the right); oral disk emarginate; tail quite long; dorsal crest high extending on to body | winter and early spring (mid-December to March) | 45-85 days | 16-18 mm |
| *Scaphiopus holbrooki* | eggs in loose irregular cylinder or band; mass 25-75 mm wide and 25-305 mm long; deposited on stems of plants/grass; 1 gelatinous envelope; 200 per packet | a small dark tadpole, bronze to brown with close-set tiny orange spots; body round or oval in dorsal view; eyes close-set and dorsal, iris black; head wide relative to body width; tail short, with tip blunt and rounded; anus medial (in the center); spiracle is ventrolateral. Often found in "schools" of hundreds of tadpoles | only heard calling once (July 12, 1999). Probably any time from March to October | 14-60 days | 8.5-12 mm |

**Figure 25.** Grassy pool at Cades Cove.

### Other Breeding Sites

Four minor types of wetlands and aquatic sites are used occasionally by frogs for breeding in the Great Smokies. American toads (*B. americanus*) breed in the backwaters along the north shore of Fontana Reservoir, although **reservoirs** (fig. 26) are generally depauperate of amphibians. Small, usually closed-canopied, **swampy and mucky wetlands** (for example, those found along Indian Creek, at Smokemont, and at the old trout pond in Cataloochee; see fig. 16) are used by Wood Frogs (*R. sylvatica*). Wood Frogs are quite variable in their choice of breeding sites, even to depositing eggs in human-enlarged **spring pools** and **roadside ditches**. Indeed, virtually any pool in late winter to early spring is likely to be colonized by breeding Wood Frogs.

## *Life History*

*Terrestrial Salamanders (*Plethodontidae*).* The life cycle of terrestrial plethodontids takes place in a multidimensional space. Naturalists tend to think of salamanders as surface-dwelling, but surface activity is only a small part of the life cycle of a terrestrial salamander. Most terrestrial species probably do not have a very large home range on the ground surface, including beneath debris and litter. They spend a considerable part of their lives underground, and biologists really know very little about their life history, especially their time spent underground and the depth and range of underground lateral movement. In addition, terrestrial species occasionally become arboreal during the night or under rainy conditions; salamanders often take refuge under loose bark. Salamanders at different life stages may remain nearly

**Figure 26.** Chilhowee Lake at mouth of Abrams Creek.

entirely underground (tiny juveniles perhaps; adults during egg deposition and mating) or on the surface (adult feeding and territoriality, environmental conditions permitting). It is by no means clear that space is used similarly by different life stages. Thus, detection probabilities may change with life stage within a habitat. The eggs of some terrestrial species have never been seen, and nests have been located only with extreme infrequency. Some plethodontids may be long-lived (5-10 years).

*Semi-Aquatic Salamanders (*Ambystomatidae, Plethodontidae, Salamandridae*).* All attributes that apply to terrestrial salamanders apply to semi-aquatic salamanders in terms of surface and underground habitat use. Semi-aquatic salamanders, however, require water for reproduction. For mole salamanders (*Ambystoma*) and newts (*Notophthalmus*), breeding sites are usually standing water (ponds, ditches) free of fishes. For semi-aquatic plethodontids, breeding sites include seeps and streams from little trickle trails to sizeable streams or rivers. Adults (mole salamanders and newts) may migrate synchronously to breeding sites in a quite orderly fashion, although temporally constrained to one or a few nights during the breeding season. Breeding adults and egg masses can be censused, but herpetologists know little about what proportion of a population breeds annually, and from what area they are drawn. Males and females may not stay for equal amounts of time during the breeding season, even when the breeding season is extended.

Stream-breeding species may live permanently in the streams (*Desmognathus marmoratus*), streamsides (many other *Desmognathus*), or at various distances from the stream (*D. imitator, Gyrinophilus, Pseudotriton*). Distances may range from a few meters to hundreds of meters away, and breeding migrations are not synchronized. Little is known about spatial distribution during terrestrial nonbreeding times. For some species (for example, *Hemidactylium scutatum*) virtually nothing is known about their lives away from woodland pools and streams/ ditches outside of the breeding season. For certain species (*D. quadramaculatus*) adults can be censused streamside, whereas adults of other species (*D. imitator*) can be readily found in terrestrial habitats; some species (*Pseudotriton*) can be found terrestrially as adults usually only by luck, and the adults of a few species (egg-brooding adult female *Hemidactylium*) are observed only during the breeding season.

All eggs of semi-aquatic salamanders are deposited in water, and the egg masses of some species (*Ambystoma*) can be censused easily. All semi-aquatic species have larvae which remain in a larval stage from a few months to as long as 2-3 years. Paedomorphosis (the ability to breed while maintaining a larval appearance) occurs in a few species (*Ambystoma talpoideum*) under favorable conditions, but no salamanders from the Park are known to be paedomorphic. Larvae metamorphose and presumably take up adult habits, but nothing is known concerning dispersion for most species. Maturation can range from one to many years, depending on species. Individuals of some species (*Ambystoma, Notophthalmus*, large *Desmognathus*) may live 10-15 or more years.

*Aquatic salamanders (*Cryptobranchidae, Proteidae*).* Little is known about the life history of most of these species, except for *Cryptobranchus*. Species within these families are entirely aquatic. The spatial use of habitat is largely unstudied except for Hellbenders, which are known to have home ranges and to guard nesting sites. Fully aquatic species (*Cryptobranchus, Necturus*) inhabit medium to large streams and rivers in the southern Appalachians. Hellbenders may live 25 or more years. Nothing, however, is known about longevity of the Common Mudpuppy (Proteidae: *Necturus*), because the larvae are little known and, for the most part, rarely seen.

*Frogs*. All of the frogs in the southern Appalachians have a "typical" amphibian life cycle. Adults move to a breeding site, deposit eggs that hatch into larvae (tadpoles), metamorphose to juveniles, disperse, and grow until they are ready to repeat the cycle. For most species, however, many questions about the life cycle remain unanswered (what percentage is breeding in any one year, where do juveniles go, how far do adults disperse). Larval periods may be extremely brief (days in *Scaphiopus*) to extremely long (years in some *Rana*). Breeding may be synchronous (spadefoots, many ranids) or extended (*Rana catesbeiana*). Even when synchronous and explosive (*Rana sylvatica*), the actual breeding date may extend over a period of months (December to March) as adults wait for the right combination of environmental conditions. Adults (and perhaps juveniles) of many frog species spend most of their lives away from the breeding sites. Individuals have been found hundreds (or even thousands) of meters from the nearest breeding sites. Frogs are often exceptionally hard to locate outside the breeding season, much less to sample them. However, the terrestrial sites are extremely important to survival since individuals spend most of their lives as terrestrial predators.

Although most species of frogs call during the breeding season, some species do not or they have only weak voices that do not carry far. Calling times are variable among species; some call during the day, some call at dusk and during the early evening, and some call only between midnight and early dawn. Some species call only during rains, whereas others will call most evenings of the breeding season. Some frogs breed in winter (even in the mountains of the South), others breed in the spring or summer, whereas others call during an extended breeding season. Calling times and seasons also vary latitudinally and perhaps with elevation.

## *Areas of Particular Amphibian Species Richness*

Three areas within Great Smoky Mountains National Park are particularly rich in amphibians. Two (Cades Cove, Cane Creek drainage) are lowland sites, whereas the third is the high-elevation spruce-fir forest. The lowland sites are similar in amphibian species composition; they are rich in species because they are the only two sizeable lowland areas within the Park with a large variety of wetlands. As such, they contain most of the frogs and pond-breeding salamanders. Both areas share species affinities with the herpetofauna of the Tennessee Valley, from whence lowland amphibians colonized Cane Creek and Cades Cove (via Abrams Creek). On the other hand, the high-elevation amphibians are composed entirely of salamanders, and two species (*Plethodon jordani, Desmognathus imitator*) are virtually endemic to the Park (*D. imitator* is found also in the Plott Balsams). Other high-elevation species in the spruce-fir forest (for example, *D. ocoee, D. wrighti, P. metcalfi*) are found in other restricted regions of the Southern Appalachians. These three areas should be the special focus of amphibian monitoring activities.

## *Identification*

Most biologists working at Great Smoky Mountains National Park should be able to identify the majority of the amphibians that they observe by using a combination of the color photographs, species descriptions, and identification/life history tables found in this manual and in Dodd (2004). Some individual animals may be impossible to identify with certainty. Larvae, especially small salamander larvae and tadpoles, often cannot be distinguished without microscopic examination. Adult salamanders, especially the duskies (*Desmognathus*), are notoriously variable with overlapping phenotypic and genotypic characters. Field biologists have found it increasingly difficult to place some individual animals into a species category because of the range of genetic and color variation observed in natural populations. As a result, sometimes an animal must be recorded to genus, species complex, or as "unknown" in field notes.

One of the best ways to identify salamander and frog larvae, in addition to color and morphology, is to examine their habitats and the times of year they are found. This can most easily be done through a comparative table. Morphological and life history characteristics are listed in tables 1 and 2 to help field biologists identify the species that are being examined. These data can be used in conjunction with the information in Dodd (2004).

### SALAMANDERS

All salamanders in the Great Smoky Mountains have four limbs with four (*Necturus, Hemidactylium*) or five (all others) toes on each hind foot. They all have tails, lack dry scales covering the body (lizards have dry scales), and have skins that are moist or wet to the touch. The skins of a few species, such as Jordan's Salamander (*Plethodon jordani*), are sticky because of glandular secretions, but only the Hellbender and Common Mudpuppy are truly slimy.

Biologists take two standard measurements with regard to length. The total length (TL) is the length of an animal from the tip of the snout to the tip of the tail. Because some salamanders lose their tails (or parts thereof) to predation, another common measurement recorded is the snout-vent length (SVL). SVL is measured from the tip of the snout to the posterior portion of the vent (the opening of the cloaca, the common receptacle for the digestive, excretory, and reproductive tracts). All scientific measurements are recorded in metric units, usually millimeters.

Salamander larvae sometimes are divided into two general groups, depending on morphology and the type of wetland in which they develop. The pond form (fig. 27A) is stout bodied, with long filamentous gills and a wide dorsal fin which extends well onto the body. Mole salamanders (*Ambystoma*), for example, have this type of larva. Pond larvae develop in still water, and use the extra surface area of the body and fin as aids in swimming. Stream larvae (*Eurycea*, *Pseudotriton*) are slimmer than pond larvae, with more streamlined bodies, shorter gills, and a narrower tail fin that does not extend onto the body (fig. 27B). These larvae usually live in swift flowing water, where extra surface area on the body would be a distinct disadvantage.

A number of useful characters are available which can be used to identify salamanders to genus or family. A few illustrative examples are provided, but more detailed comparisons are found in Dodd (2004) under the heading "Similar Species."

*Desmognathus*: All dusky salamanders have a light line which extends from the back of the eye to the angle of the jaw. The duskies also have well-developed muscles on the sides of their heads. They need these muscles to raise the upper jaw in order to open their mouths, since the lower jaw is fused to the skull.

*Gyrinophilus* versus *Pseudotriton*: Although these colorful salamanders are superficially similar in appearance, Spring Salamanders (*Gyrinophilus*) have a canthus rostralis, a large white line bordered by black lines, that runs from in front of each eye to the nostrils. Salamanders of the genus *Pseudotriton* do not have this line. Spring Salamanders use the canthus rostralis as a "gunsight" to zero in on prey.

Plethodontidae versus all other salamander families: All lungless salamanders have a nasolabial groove that extends from each nostril to the upper jaw. The nasolabial groove transmits chemicals to the salamander from the substrate; no other salamander family has this groove.

## FROGS

Like most salamanders, frogs have four legs with four toes on the front limbs and five toes on the rear limbs. The hind limbs are much larger than the front limbs, and are used to propel the body when walking, hopping, or jumping. Frogs are measured in TL, that is, from the tip of the snout to the end of the body between the hind limbs (that is, at the end of the

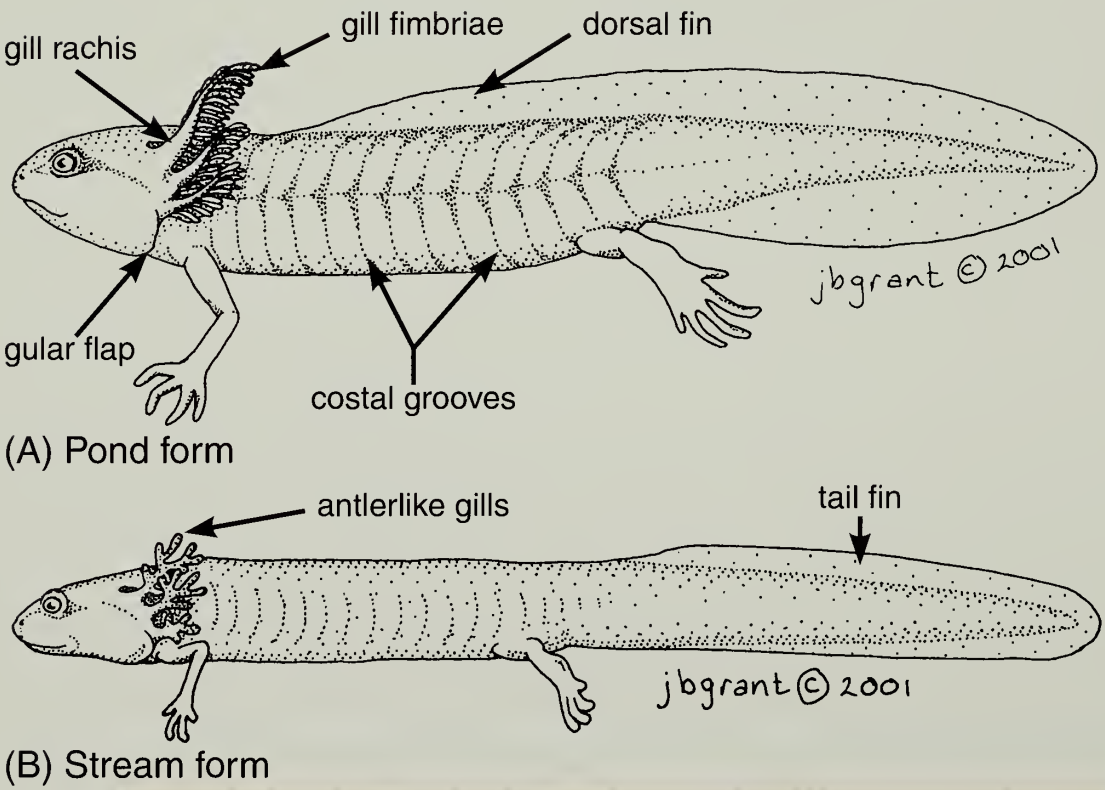



**Figure 27.** Body morphology of a salamander larva: (A) pond form; (B) stream form.

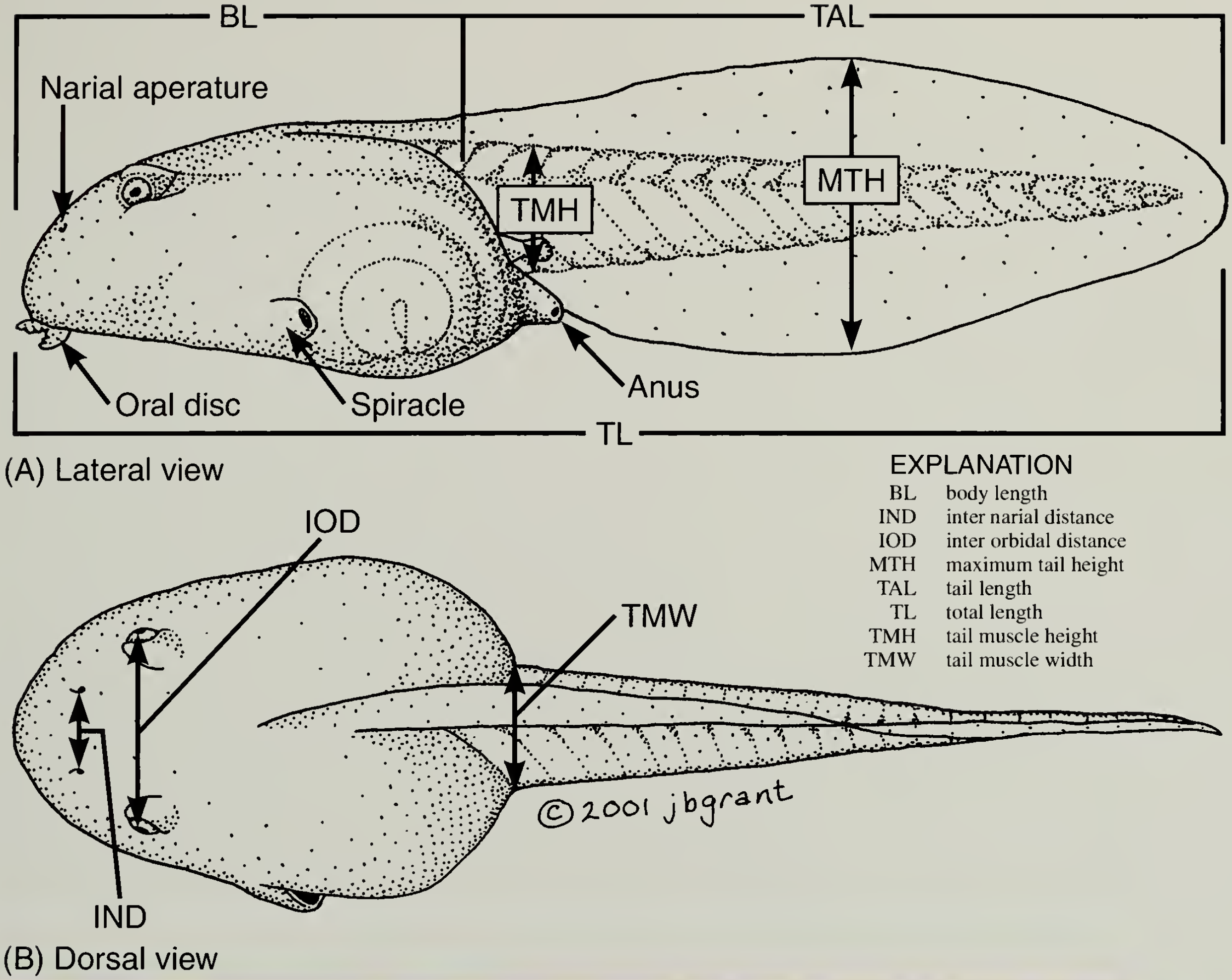
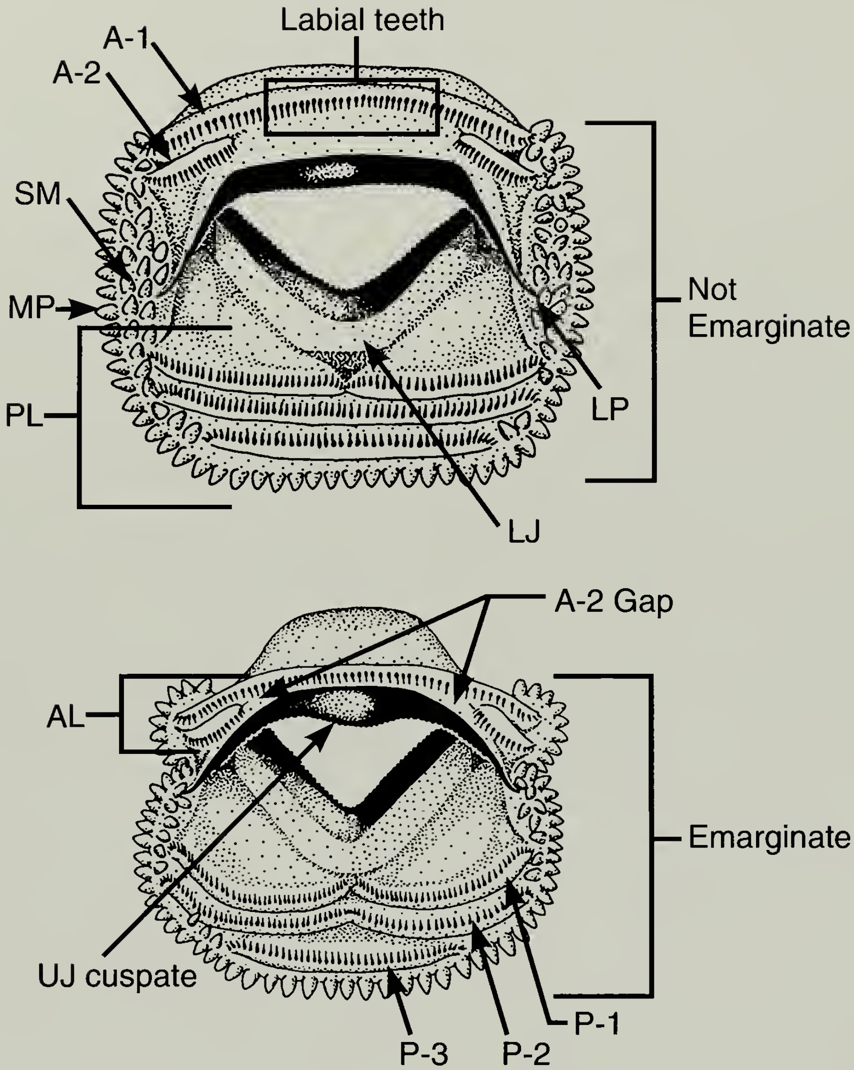



**Figure 28.** Body morphology of a tadpole.

urostyle). Of course, there are many other measurements which could be made, such as the length of the various sections of the hind limb, but these data generally are not important in amphibian monitoring-programs except in studies of fluctuating asymmetry (Alford and others, 1999, but see McCoy and Harris, 2003).

Tadpoles are morphologically complex. As with salamander larvae, there are two general tadpole body types, the pond type and the stream type. Pond-type tadpoles have deeper bodies and higher tail fins than do stream-type tadpoles. Structures important in the identification of tadpoles are labeled on figure 28. The oral disk consists of the mouth parts; the narial aperture is the opening to the nostrils; the spiracle is the opening from the gills (water is taken in through the mouth, passes over the gills, and is expelled via the spiracle); the anus is the opening from the digestive tract. The total length (TL) consists of the body length (BL) and tail length (TAL). Sometimes additional morphological measurements are taken, such as the maximum width of the tail musculature (TMH) or the maximum tail depth (MTH). The location and size of these characters, or their ratios in relation to one another, are useful in identifying what otherwise appears to be just another drab, olive-green, or black tadpole.




EXPLANATION

| | |
|---|---|
| AL | anterior (upper) labium |
| A-1 and A-2 | first and second upper tooth rows |
| A-2 GAP | medial gap in second anterior tooth row |
| LJ | lower jaw sheath |
| LP | lateral process of upper jaw sheath |
| M | mouth |
| MP | marginal papilla |
| OD | oral disk |
| PL | posterior (lower) labium |
| P-1, P-2, P-3 | first, second, and third posterior (lower) tooth rows |
| SM | submarginal papilla |
| UJ | upper jaw sheath |

**Figure 29.** Oral disc (mouthparts) of a tadpole.

The tadpoles of different species of frogs often appear identical to one another, but the structure of their mouthparts readily separate them. Biologists may need to examine mouthparts to determine which species is in hand. For this reason, a diagram has been included of tadpole mouthparts is provided in figure 29. The nomenclature follows Altig and McDiarmid (1999). The location, number, and degree of separation among labial teeth and papillae are important characters for identifying tadpoles. Examining tadpole oral disks (sometimes incorrectly termed "teeth") also gives researchers an opportunity to check the health of the tadpole. For example, the horny jaw sheaths drop out when the tadpole is exposed to certain toxic compounds and to the dangerous disease, chytridiomycosis. However, tadpoles should not be held too long before examination or preservation, since some tadpoles may shed denticles in the laboratory.

As with salamanders, there are certain useful defining characteristics that help to identify certain superficially similar animals. Some of these are listed below (also see "Similar Species" in Dodd, 2004).

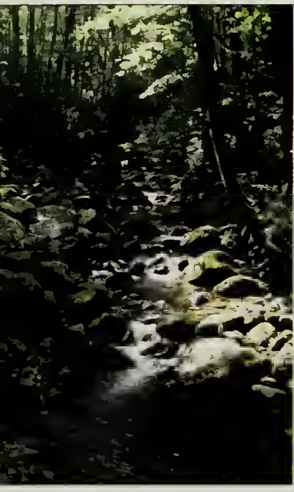
Bufonidae versus Pelobatidae: Frogs in both of these families are terrestrial. However, the true toads (*Bufo*) are dry-skinned and "warty," and have prominent cranial crests and parotoid glands. The spadefoot toads (*Scaphiopus*) are smooth-skinned, lack cranial crests and parotoids, and have a sharp digging spade on their hind feet.

Hylidae versus other frog families: all hylid frogs (*Acris, Pseudacris, Hyla*) in the Great Smokies have slightly to completely expanded toepads, but only in the treefrogs (*Hyla*) are these greatly expanded for climbing; the other hylids are mostly ground-dwelling (however, note that Spring Peepers, *Pseudacris crucifer*, often call from the trees from late fall to early spring before descending to breeding ponds).

*Rana palustris* versus *R. pipiens*: these very similar frogs are both green and spotted. In *R. palustris*, the spots are squarish, paired and of nearly equal size, whereas in *R. pipiens* they are smaller, rounded, and more randomly scattered on the frog's back.

## Additional Information

Information on the etymology, identification of adults, larvae, and eggs, similar species and how to differentiate them, taxonomic problems, distribution both within the Park and elsewhere in North America, life history, abundance and status, and remarks on interesting aspects of the biology of the species are found in Dodd (2004). Data on 44 amphibians are presented, including information on species no longer thought present (for example, *Aneides aeneus*) or which were reported historically from the Park, but whose actual occurrence may be doubtful (*Acris crepitans*). Distribution maps, color photographs of amphibians from the Park, and original color illustrations accompany each account.



## *Why Monitor Amphibians?*

The problem of declining amphibian populations has been recognized worldwide, with credible reports of diminishment or disappearance of amphibians from many regions and habitat types. No single cause for declines has been demonstrated, although acid precipitation, environmental contaminants, introduction of exotic predators, disease agents, parasites, and effects of ultraviolet radiation have been suggested as factors in declining numbers. Indeed, no one cause may be implicated, and several factors may interact in such a manner as to threaten populations (Carey and Bryant, 1995). A major factor in the loss of amphibian populations has been and continues to be the loss of habitat. The severity and apparent complexity of the problem led the National Park Service in 1997 to list amphibian declines as among its highest priority research and information needs.

> The problem of declining amphibian populations has been recognized worldwide....

In terms of its significance to amphibians, the Great Smoky Mountains National Park is more important than almost anywhere else in North America. Thirty-one species of salamanders have been recorded in the Park, and that number could conceivably increase as molecular genetic techniques are used to unravel the complex relations among populations. Of particular note are the salamanders of the family Plethodontidae, a largely North American group that has a center of evolution and distribution in the southern Appalachians (Dodd, 2004). Jordan's Salamander (*Plethodon jordani*) is known to occur only in the Park, and the salamander fauna is believed to represent several evolutionary series progressing from the more aquatic species to those which are almost totally terrestrial. Thirteen species of frogs and toads are historically reported to inhabit the Park. The biological importance of the Park has been recognized in its designation as an International Biosphere Reserve. Although no other region and no other National Park shares the wealth of amphibians found in the Great Smokies, the entire southern and midsection of the Appalachian chain is characterized by a high diversity of amphibians, and inventories and monitoring protocols developed in the Great Smokies may be applicable to National Park Service, U.S. Forest Service, Nature Conservancy, or other properties in the Appalachians.

Several known stressors potentially affect amphibians in the 2,071.2 km$^2$ (521,000 acre) Park (reviewed by Dodd, 2004). Air pollution, particularly long-distance pollution from cities in the nation's mid-region, is a nationally recognized problem. Reduced visibility, damage to plants, and fish kills are documented to be associated with sulfurous and nitrogenous compounds and atmospheric ozone. Low pH is known to have affected survivorship in at least one aquatic salamander species in the Park. Exotic pathogens and parasites have seriously affected some forest communities, with unknown effects on ecosystems. Finally, the pressure of ten million visitors per year--more than any other National Park--seems relatively benign, but could potentially have subtle effects on sensitive amphibian populations. The existence of these and other unknown stressors suggest that an inventory and a monitoring program are needed to ensure the protection of amphibian populations.

**Amphibian Research and Monitoring Initiative (ARMI) —** In 2000, the President of the United States and Congress directed Department of the Interior (DOI) agencies to develop a plan to monitor the trends in amphibian populations on DOI lands and to conduct research into possible causes of declines. The DOI has stewardship responsibilities over vast land holdings in the United States, much of which is occupied by or is potential habitat for amphibians. The U.S. Geological Survey (USGS) was given lead responsibility for planning and organizing this program, named the Amphibian Research and Monitoring Initiative (ARMI), in cooperation with the National Park Service, U.S. Fish and Wildlife Service, and Bureau of Land Management. Results of the monitoring program will be available to cooperators, land managers, the scientific community, and the general public. ARMI's Internet site is:

http://edc2.usgs.gov/armi/

**National Park Service (NPS)** — Recent legislation (National Parks Omnibus Management Act of 1998) and policies of the National Park Service require that park managers know the condition of natural resources and that they monitor long-term trends in those resources. To comply with legal and policy requirements, the NPS Inventory and Monitoring Program focuses on attaining the following major long-term goals: (1) establish natural resource inventory and monitoring as a standard practice throughout the NPS that transcends traditional programs and activities; (2) inventory the natural resources and park ecosystems under NPS stewardship to determine their nature and status; (3) monitor park ecosystems to better understand their dynamic nature and condition and to provide reference points for comparisons with other, altered environments; (4) integrate natural resource inventory and monitoring information into NPS planning, management, and decision making; and, (5) share NPS accomplishments and information with other natural resource organizations and form partnerships for attaining common goals and objectives. Information on the National Park Service Inventory and Monitoring Program can be found at: http://www1.nature.nps.gov/im/monitor/index.htm #Legislation, and in publications by Silsbee and Peterson (1991) and Peterson and others (1995).

**All Taxa Biodiversity Inventory (ATBI)** — A research effort designed to compile a comprehensive inventory of all life forms in Great Smoky Mountains National Park, ATBI is sponsored by Discover Life in America, a private nongovernmental organization working in partnership with the NPS. The initiative has a goal of completing the inventory in as few as 10 years and is, therefore, an intensive undertaking. Before the project is completed, it will employ the expertise of taxonomists, data specialists, zoologists, botanists, and ecologists, among others. Once completed, the ATBI will provide baseline data from which to measure species change through time. ATBI's objectives are to: (1) complete a comprehensive "checklist" of life forms in the Park; (2) gather data to create range maps for each Park species; (3) compile natural history information on each species, including its relative abundance, its response to various climatic conditions, photographs of each of its life stages, its role in the greater ecosystem, its relationship with other species, and digital recordings of its calls or sounds; and, (4) organize the information gathered and make it available to scientists, educators, land managers, students, and all other interested parties via the Internet and other media. More information can be found at:

http://www.discoverlifeinamerica.org

## *Things to Consider During Planning*

There are at least 10 major items which need to be addressed before starting an inventory or monitoring program for amphibians, especially when under financial or personnel constraints. These are discussed briefly below and, in some cases, more extensively elsewhere within the guide.

1. <u>There are many amphibians in the southern Appalachians and the southeast</u>. A total of 31 species of salamanders and 13 species of frogs have been recorded historically as occurring in the Park. Extending the area of interest to the greater southern Appalachians, the figure increases substantially, by 21 salamanders and 1 frog, because of the high levels of endemism of many salamander species. Extending the area of interest even more, there are approximately 85 species of salamanders and 58 species of frogs within the southeastern United States (or 49.6 percent of the species in the entire United States). This figure does not include different subspecies, nor does it include the many genetic variants that have been described.

2. <u>The systematic status of many species of southeastern amphibians is in a flux.</u> It is likely that there are a number of new and unrecognized species of amphibians in the

southern Appalachians, particularly among the salamanders. In addition, there is considerable debate among salamander taxonomists over what constitutes a species in terms of genetic uniqueness, phylogeny, and reproductive compatibility. Particularly in the genera *Plethodon* and *Desmognathus*, many new "genetic" species have been described in recent years, especially in the southern mountains. Unfortunately, morphology and coloration may be only of limited assistance in identification; many individuals are impossible to distinguish phenotypically in the field. There also are areas where considerable introgression or hybridization occurs, especially in the Great Smoky Mountains. This has led to the recognition of species complexes (for example, the slimy salamanders of the *Plethodon glutinosus* complex), or even of size-based guilds among the dusky salamanders (*Desmognathus*). As systematists closely examine other genera (*Eurycea, Pseudotriton*), the situation will probably become more complicated. Systematic certainty may be no better in the frog world, especially in the genera *Pseudacris* and *Rana*, although the taxonomy of frogs within the southern Appalachians will probably remain stable.

3. <u>Species and life stages are sometimes difficult to distinguish</u>. Even experienced herpetologists sometimes have difficulty identifying adult amphibians, and eggs and larvae pose special identification problems. Color and morphology vary considerably among individual amphibians. The ability to distinguish species based on egg mass and tadpole morphology is exceptionally difficult and is an ability that is rapidly being lost, as such identification is rarely taught, and the pool of naturalists who are knowledgeable concerning identification is diminishing. There are very few current color guides to amphibian eggs and larvae, even on a local basis.

4. <u>Amphibians have complex life cycles</u>. Because of the extremely varied life histories of many amphibians (see **Life History**), inventory and monitoring programs must consider such variation when planning when and where amphibians will be monitored, and what biases may be associated with interpreting sampling results.

   For example, egg mass counts might tell a researcher about the number of egg masses deposited and, therefore, the number of females that reproduced that year. Egg mass counts cannot be used to determine population size (often used as a measure of status), however, unless the operational sex ratio (that is, the sex ratio of adults that actually bred successfully) is known for that year. This ratio is usually assumed to be 1:1, but if it is not, estimates of population size could be in error by several orders of magnitude. Also, not all individuals breed every year and, thus, population size at a breeding pond may not be indicative of overall population size. Even with such data available, population sizes still cannot be estimated inasmuch as the ratio of juveniles to adults is not known for most species. In addition, counting egg masses says nothing about whether reproduction was successful, since a variety of factors (disease, desiccation, predation) can interact to prevent hatching and metamorphosis. Consequently, it might be possible to count large numbers of egg masses, yet have none of them actually result in juvenile recruitment to the population. Status and long-range impacts to the population could be easily misinterpreted.

   When inventorying and monitoring amphibians with complex life histories, multiple sampling techniques may be required, and status interpretation must be restricted to the sector of the population actually sampled. This rather obvious approach is often ignored, as authors often make general statements as to status and trends when only a portion of the animal's life cycle was sampled.

5. <u>In the field, detectability of amphibians is likely influenced by the following variables, to a greater or lesser extent, depending on species</u>. Some of these variables include:

   ***Annual cycles of reproduction***–The reproductive season may be prolonged, or extend for only a few days or weeks. Some amphibians may be effectively sampled only during the breeding season (*Ambystoma* sp., *Hemidactylium*, many frogs), whereas breeding females of other species may disappear underground to brood eggs (*Plethodon*) and thus be undetectable.

***Seasonal events (cold, drought, heat, storms) that are usually unpredictable–*** Cold, heat, and drought generally make amphibians more difficult to find, whereas tropical depressions and hurricanes, with their heavy rains, may actually bring amphibians to the surface in incredible numbers.

***Diurnal versus nocturnal activity–*** Many amphibians are more conspicuous at night, when they leave hiding places to forage, than they are in the day. This is true for both terrestrial and aquatic species.

***Air, water, and substrate temperature–*** Amphibians often have rather narrow tolerances or preferences for particular air, water, or substrate temperatures. Some species prefer rather cool temperatures (for example, salamanders living at high elevations, and the winter-breeding frogs), whereas others prefer the warm temperatures of summer. Since temperature changes with elevation (Dodd, 2004), activity patterns of broadly distributed species tend to change seasonally with an increase in elevation.

***Soil moisture and rainfall–***Terrestrial amphibians are active when soils are moist and during rainfall, much more so than when soils are dry. Breeding movements may be triggered by a combination of seasonal gonadal development, favorable temperature, and rainfall.

***Relative humidity–*** High humidity favors amphibian activity; low humidity depresses activity.

***Barometric pressure–*** Barometric pressure is indicative of changing weather conditions; a falling barometer is associated with weather fronts and rain, and a rising barometer is associated with clearing or fair weather. Therefore, a change in barometric pressure may influence amphibian activity patterns and, thus, detectability.

***Cloud cover/moon brightness–*** Amphibians tend to be more active on cloudy nights when humidity levels are higher than they are on clear nights. A bright moon tends to inhibit activity, since predators may be more effective at detecting prey on bright nights.

***Prey availability–*** Amphibians are likely to be more abundant in areas with a high diversity of prey items than in areas depauperate of prey. A few amphibians (Hellbenders) have specialized diet preferences. When prey are absent or scarce, specialist feeders will also be scarce despite the otherwise seemingly appropriateness of habitats.

Note that many of the variables discussed above change daily, seasonally, or annually (for example, during El Niño versus La Niña years).

6. Species and populations occur in a landscape. Some amphibian species are extremely localized geographically (*Ambystoma opacum* in the Great Smokies), whereas others are very widespread (*Desmognathus quadramaculatus*). Populations may be geographically isolated to an extreme degree (cave species or the crevice-dwelling *Aneides aeneus*), occur very patchily in a larger landscape, occur in a metapopulation structure (*Bufo*) with considerable (or little) interchange between or among metapopulations, or occur over hundreds of square kilometers of deciduous forest where it is difficult to define the limits of a population (many *Plethodon*). Individuals may be naturally rare or exceptionally abundant. Because a species is unusual or difficult to sample, is not a reason to bypass its study. Some of the most specialized amphibian species are those biologists know have declined or are imperiled in the southeastern states.

   Although some populations may be huge (some terrestrial woodland salamanders, *Plethodon*, for example), others seem small, isolated, and vulnerable (crevice-dwelling, cave, or ravine species).

Little is known about how and when these species disperse or about what mechanisms allow for the long-term persistence of small populations. Perhaps individuals move more than is recognized; even rare immigration is sufficient to ensure genetic exchange and prevent stochastic extinction. The demography and "spatial biology" of most amphibians is still poorly understood. Even if known for a few species, the diversity of life histories suggests that generalizations about persistence will not be easily forthcoming.

7. Populations may be stable or fluctuate widely. Much of what is known concerning amphibian populations has been derived from studies of frogs and salamanders breeding in temporary ponds. The number of breeding adults and their reproductive output (larvae, metamorphs) varies to extreme proportions from one year to the next, perhaps in response to environmental and ecological conditions (weather, hydroperiod, prey availability). Some species may live in an area for years, disappear for years, then reappear. For example, populations of European *Rana* seem to fluctuate cyclically on an 8-year cycle. On the other hand, terrestrial plethodontid populations appear rather stable from one year to the next. Detectability may be influenced by weather (drought) even if populations are stable. Not much is known concerning the stability or fluctuation of semi-aquatic and most aquatic species and populations, especially in the southern Appalachians.

...biologists have enough data to advance hypotheses about the persistence and stability of amphibian populations...

Still, biologists have enough data to advance hypotheses about the persistence and stability of amphibian populations, while keeping in mind the caveat concerning exceptions. Species that live in stable environments tend to have stable populations from one year to the next, whereas species that live or breed in unstable or fluctuating environments tend to have populations that fluctuate to a much greater degree. Perhaps population stability can even be viewed on a gradient with environmental stability. If this is true, declines or disappearances of species living in stable environments might be more cause for concern than declines in species living or breeding in fluctuating environments, unless the fluctuating environments are highly isolated. In this case, isolation may prevent recolonization from source populations and, thus, lead to declines throughout the landscape.

8. Virtually nothing is known concerning emigration, immigration, and natural extinction. It seems quite reasonable that during the course of ecological and evolutionary history, extinction and recolonization naturally occur, especially in small populations, isolated populations, or populations structured in metapopulations (as sources and sinks). Yet herpetologists understand little of these processes in southern Appalachian amphibians. The Europeans seem to have more data in attempts to understand landscape-level population changes, but their environment has been influenced by people for so long that it is difficult to separate anthropogenic from "natural" causes of extinction. In any case, colonization and other forms of interpopulation movements may not move in a straight line overland. Animals might follow sinuous topography, watersheds, streams and rivers, or even subsurface passages.

Populations of amphibians certainly experience natural turnover (recruitment, mortality), but little is known about this process or how long it takes for any southern Appalachian species. Just because some individuals have the potential for considerable longevity does not mean that populations turn over slowly. Biologists need information on the generational times for various species.

9. Amphibian sampling techniques. There are as many ways to sample amphibians as there are amphibians (see **Sampling Techniques**).

Each technique has its own underlying assumptions, biases, and limitations. Until relatively recently, these biases were unrecognized, not discussed, or simply ignored. Currently, sampling protocols have been receiving a great deal of experimental examination. It is unlikely that a single sampling technique can be used to sample an entire community. Some of the techniques listed below are not mutually exclusive.

***Active sampling*** (easy to use)

- Time constrained--
  number of observers x time sampled; catch; visual encounter
- Area constrained--
  using plots, transects [visual encounter surveys], habitat defined
- Sweep samples--for larvae
- Call surveys--
  breeding or territorial adult frogs
- Egg mass counts

***Easy passive sampling***
(observer need not be present; no harm to animals)

- Coverboards--
  various sizes, shapes, configurations, materials
- PVC pipes--in ground or on trees
- Larval litterbags
- Automatic audio data loggers--
  for recording calling frogs

***Intensive passive sampling***
(labor, time, and financially expensive).

- Traps and fences must be checked regularly, generally daily, for accurate results and to prevent mortality.
- Traps (aquatic or terrestrial): funnels, bottles, minnow, wire basket
- Drift fences, with pitfalls and/or funnel traps, sometimes in conjunction with PVC pipes or coverboards

10. The human-based constraints on sampling, inventorying, and monitoring amphibian populations on Federal lands must to be considered at the outset. These include:

***Money*** *(equipment, personnel, emergencies, meetings, data analysis, publication)* – The single biggest limitation

Highly qualified researchers and field technicians are absolutely essential for conducting inventory and monitoring programs.

affecting inventory and monitoring projects is the amount of money available to conduct the programs, which ultimately will determine the number of researchers hired, the type of techniques used, the number of species monitored, and the number of locations visited. Inventory and monitoring programs should be designed to make the best use of the available funding to ensure scientific rigor, rather than try to be "all things to all people."

***People*** *(principal investigator, experienced field crews, biometricians, GIS, administrative support, field support)* – Highly qualified researchers and field technicians are absolutely essential for conducting inventory and monitoring programs. The identification of amphibians in the Great Smoky Mountains and elsewhere in the southern Appalachians is often difficult, and there is no substitute for experienced judgement. Resource managers should not assume that field assistants can be trained easily and quickly, or that volunteers can take the place of experienced biologists. Just as few persons would expect ecologists to conduct genetic analyses, current field research is a collaborative effort needing a variety of experts. When planning an inventory or monitoring program, agreements or arrangements need to be in place to ensure that field researchers have the needed biometric, landscape, and other types of support necessary for data analysis and interpretation.

***Time*** – Inventory and monitoring programs take time to carry out. For amphibian monitoring programs, a minimum of 10 years of data collection is not unreasonable to begin to understand population status and to measure the extent of variation associated with sampling data. Sampling time is dependent upon the life history characteristics of the species in question.

For example, a monitoring program might provide reliable trend-analysis data for a short-lived species if sampling was conducted every year for 10 years at locations throughout the species' range within the Park; for a long-lived species, the duration of sampling might have to extend for 20 to 30 years before researchers could be confident in recognizing trends. In addition, trends resulting from human perturbation sometimes are difficult to separate from natural, often stochastic, population changes, except during catastrophic population collapse. It might be difficult to separate human-caused change from natural population variation without a long-term data set. Unfortunately, conflicts may arise when answers are needed by resource managers (for example, "We need to know the status of the Park's amphibians for the annual report"). However, resource managers must recognize that short-term projects are ineffective and may give misleading results. Inventory and monitoring programs need time and patience.

***Safety*** – The minimum number of persons necessary to conduct amphibian field research involves two-person field crews. This is to ensure safety in case of injury, accident, or other medical emergency. Assume that emergencies *will occur.* Field crews should carry radios or cell phones and emergency first aid kits. Both heat stress and hypothermia are possible when sampling amphibians over long time periods in the southern Appalachians. Yellowjackets, venomous snakes, and bears are other park denizens requiring occasional attention.

***Logistics*** – Can researchers get to locations with the people and equipment in a reasonable amount of time and effort? Given logistical constraints, how many sites can be sampled and over what area? The failure to consider logistical constraints is one of the most common errors when setting up inventory and monitoring programs.

***Regulations*** *(permits, access, restrictions on research techniques, collecting)* – Regulations can impede research results and limit the types of data collected. Researchers need to clearly understand the limitations imposed upon them by regulations, whether local, state, or national. Likewise, administrators need to recognize that some regulations can impede scientific progress. In some cases, it may be impossible to obtain scientific data given impositions upon research access or techniques.

***Collaborations*** *(intra-agency, Federal, state, other researchers, land managers)* – Biologists working on amphibian inventory and monitoring programs should be knowledgeable about previous research and keep other researchers informed of their progress. When possible, ongoing research should be incorporated into the inventory or monitoring program to facilitate data sharing and partitioning of resources. Agency personnel need to facilitate research, especially for congressional or departmentally mandated programs.

***Administrative Policy*** *(hiring restrictions, equipment-ordering procedures, contracts)* – Administrative delays need to be anticipated and alternative plans or policy established to allow science crews to be in the field conducting research when the animals are likely present.

## Species and Locations to Monitor

Of the 44 amphibian species historically reported from the Park, two species (Green Salamander, Northern Cricket Frog) probably no longer occur within the Park; one species (Northern Leopard Frog) may not occur, and four species (Mole Salamander, Common Mudpuppy [perhaps], Mud Salamander, Eastern Spadefoot) are so rare that designing a meaningful species-based monitoring program for them is impossible. However, two of these species (the Mole Salamander and the Eastern Spadefoot) are known only from the same locality (Gum Swamp), which is also a major amphibian breeding site within the Park. Monitoring the amphibians at this site may result in occasional observations of these two restricted and rare species. Likewise, a monitoring program developed for the Hellbender might result in additional captures of the Common Mudpuppy, thus making it feasible to sample both species simultaneously.

The following suggestions are made to facilitate monitoring the amphibians of Great Smoky Mountains National Park. It is unlikely that all species within the Park can be monitored every sampling year, although careful planning may help to increase the number of species monitored through time.

1. ***Concentrate on certain species***, especially those that may be in biological decline elsewhere within their range or are limited in distribution within the Park. Some of these species are:
   - Large stream and river-dwelling species: Hellbender.
   - Pond-breeding species: Spotted Salamander, Marbled Salamander, Eastern Red-spotted Newt, Four-toed Salamander, Northern Green Frog, Wood Frog.
   - Stream-associated species (especially with conspicuous larvae): Black-bellied Salamander, Blue Ridge Two-lined Salamander, Black-chinned Red Salamander, Spring Salamander.
   - (Primarily) Terrestrial salamanders: Jordan's Salamander, Southern Gray-cheeked Salamander, Northern Slimy Salamander, Southern Red-backed Salamander, Southern Zigzag Salamander, Imitator Salamander, Pigmy Salamander.
2. ***Concentrate on areas of special species richness***, such as the Cane Creek drainage, Cades Cove (especially Gum Swamp (fig. 18), Gourley Pond (fig. 20), Methodist Church Pond (fig. 22), Stupkas Sinkhole Pond (fig. 30), Big Spring Cove (the Finley-Cane sinkhole ponds), and the high-elevation spruce-fir forest (fig. 3).
3. ***Concentrate on problem areas***. The only currently recognized problem area for

**Figure 30.** Biologist sampling with sweep net in Stupkas Sinkhole Pond in Cades Cove.

amphibians in Great Smoky Mountains National Park is Gourley Pond in Cades Cove. Amphibians breeding at this site have contracted iridovirus infections, and large numbers of larvae have died. Because of the disease threat (Chinchar, 2002), this location should be monitored every year throughout the breeding and metamorphic season, about mid-March to late July, depending on water levels.

4. ***Periodically check areas of known occurrence for certain species.*** There are a few areas within the Park where certain salamanders and frogs are known to occur with regularity; these locations can be visited periodically to determine continued presence and, possibly, relative abundance. The following are examples: Long-tailed Salamanders in Gregorys Cave and at other cave entrances; Cave Salamanders in Stupkas Cave; Southern Zigzag Salamanders in Whiteoak Sink and in the uvala surrounding Bull Cave; Seepage Salamanders along the road bordering Hazel Creek; American Bullfrog tadpoles in Abrams Creek at the Abrams Creek Ranger Station; Eastern Narrow-mouthed Toads at Shields Pond (fig. 31). If sampled during appropriate seasonal and weather conditions, these species should be found at the locations mentioned; if not, it could be an indication of concern. Unfortunately, it may be difficult to interpret such present/not observed data without information on the same species outside the Park.

5. ***If particularly cost-effective monitoring techniques are available for certain species, use them.*** For example, all breeding male frogs in the Park emit loud calls to attract females. Species that are extremely difficult to find at most times of the year, such as the Upland Chorus Frog (*Pseudacris feriarum*), can be readily detected calling on a wet spring night throughout

**Figure 31.** Biologist looking for tadpoles at Shields Pond in Cades Cove.

Cades Cove. The presence and relative abundance of other breeding frogs that are spatially limited within the Park (such as the Eastern Narrow-mouthed Toad, *Gastrophryne carolinensis*, at Shields Pond in Cades Cove; fig. 31) can be detected by using automated call-monitoring devices without the continued presence of observers. As another example, the presence of certain salamander larvae can be detected passively using inconspicuously placed leaf litterbags. Larval Spring and Black-chinned Red Salamanders are detected in higher numbers using these bags compared to other search methods.

## Choosing Sampling Sites

***Pond-woodland pool breeding amphibians* –** If researchers decide to monitor the pond-woodland pool breeding amphibians within Great Smoky Mountains National Park, no great difficulty is encountered. This is because there are so few known locations that visiting each site two or more times per year can be planned very easily. One visit should be planned in the early spring (late March to mid-April), with a second visit in early summer (late May to mid-June). Coupled with at least one or two call surveys in Cades Cove and periodic call surveys at other locations, biologists should be able to determine whether most species are present, obtain counts of egg masses, and categorize the abundance of calling males. Because of the existing disease threat, Gourley Pond should be visited at least once every 3 to 4 weeks from February/March to July/August.

***Large stream and river-dwelling amphibians*** – The Hellbender is the sole large stream- or river-dwelling species to be monitored in Great Smoky Mountains National Park. The largest population inhabits Little River from the Park entrance at Townsend for several kilometers within the Park, although the maximum distance upstream has not been determined. Smaller populations are found in lower Deep Creek and in the Oconaluftee River. The Little River population would, therefore, be the most important population to monitor annually. Periodic sampling should be conducted at the

...there are at least 25 watersheds within Great Smoky Mountains National Park, totaling > 3,400 km of streambed.

other locations and in potential habitat elsewhere within the Park (see Nickerson and others, 2002).

***Streams and creek-dwelling amphibians*** – Depending on how precisely watersheds are defined, there are at least 25 watersheds within Great Smoky Mountains National Park, totaling > 3,400 km of streambed. Nearly each meter of every stream likely contains salamanders. Sampling the amphibian fauna of these streams depends largely on: (1) objective (certain species or areas of interest); (2) money and personnel (how many field crews are available and can be hired); and, (3) time available to conduct the surveys. Obviously, it is necessary to define these limitations prior to undertaking a stream monitoring program. When deciding where to conduct a stream/creek amphibian monitoring program, researchers should decide first what they hope to accomplish. For example, if using "percentage of area occupied" (PAO) analyses (see **Data Handling**), many more sites can be sampled than by using intensive sampling or mark-recapture techniques. The objective will fit the analysis; this will be discussed in more detail in **Data Handling**.

Given the caveats of people and time constraints, it will be necessary to narrow the choice of stream locations to be sampled. Some ideas are listed in the following section. However, a biologist needs to remember that, as a rule, the more sites that are sampled, the greater confidence are the results. The goal of sampling is to determine reliable estimates of variance associated with capture or sighting probabilities, or with estimates of population size; variance estimates will be more reliable with a greater number of sites surveyed than with a small number of sites.

## SAMPLING WATERSHEDS

Limit sampling to a subset of watersheds: randomly pick watersheds to sample from throughout the Park. Each watershed is assigned a number and a computer program can then be used to select a random subset of the watersheds for survey. Streams to be sampled within the watershed are randomly selected in the same manner. The location of the exact part of the stream to be sampled can be specified randomly (very impractical in difficult-to-access mountainous country) or stratified by stream order, elevation, vegetation type, access, or some other selective criterion. For example, biologists may limit their survey to second order streams between 900 and 1,400 m within 1 mile by trail from a road access. A GIS can be used to generate the extent of such habitat with these criteria, locate potential sampling sites, and randomly select those to be sampled.

## SAMPLING STREAMS

Limit sampling to a subset of streams: randomly select streams for sampling from throughout the Park. Each stream is assigned a number, and a computer program can be used to select a random subset of the streams for sampling. The location of the exact part of the stream can be specified randomly or stratified by stream order, elevation, vegetation type, access, or some other selective criterion, as in the example above. A GIS can be used to generate the extent of such habitat with these criteria, locate potential sampling sites, and randomly select those for sampling.

## SAMPLING LOCATIONS

Specific locations can be selected for sampling, such as all streams draining into Tennessee, all streams draining into Cades Cove or Cataloochee Valley, or, all streams located on the western side of the Park. The same general procedure for site selection and stratification is followed. However, the more limited the area sampled, the more restricted generalizations about status must become. Researchers could not sample all the streams draining Mt. LeConte and then extrapolate their results concerning stream-dwelling salamander status to the entire Park, the eastern side of the Park, or even to nearby Mt. Guyot.

***Terrestrial amphibians*** – Choosing terrestrial sites to sample for terrestrial salamanders is very similar to choosing stream sites, but without the streams. There is no well-defined physiographic feature, such as a watershed or stream course, with which to initially stratify the area to be sampled. Biologists are left with the questions: which species or amphibian community should be sampled, what habitats should be targeted, what areas should sampling be concentrated, and what degree of access is possible? Because the Park covers a large area (2,071.2 $km^2$), much of it in difficult terrain and without easy trail access, stratification of the terrestrial area to be sampled is absolutely necessary. How many sites can be sampled will depend on personnel, time available for sampling, and logistics. As with stream sampling, active sampling rather than passive sampling techniques will allow for more sites to be sampled, but the types of information that may be obtained will be correspondingly limited.

***Unusual terrestrial amphibians*** – There are only a few salamanders that may qualify in this category, such as the Southern Zigzag Salamander currently known from only two areas within the Park (Whiteoak Sink; entrance to Bull Cave), and the cave entrance-inhabiting salamanders of Gregorys Cave, the Calf Caves, and Stupkas Cave (especially Long-tailed and Cave Salamanders). As with sampling pond-breeding amphibians, these sites could be checked annually to verify the presence of these species. Detailed studies, using mark-recapture techniques, would be necessary to establish population size and trends through time.

In the section that follows, brief examples are listed of how certain techniques have been used to sample amphibians. As stated in **Things to Consider During Planning**, there may be vastly different amounts of time associated with using the different techniques, different reasons for choosing them, and different biases when interpreting the results. In every instance, researchers should quantify the amount of search time or sampling effort involved in the survey.

## *Active Sampling*

***Time constrained*** **–** In this technique, a predetermined amount of time is set for sampling the area or habitat. The presence of different species and the number of individuals (or even sex and life stage—males, females, juveniles) observed are recorded. Visual encounter protocols are followed; that is, animals are counted as they walk over the forest floor or stream bottom, hide in crevices or cling to cave walls, found by turning over surface debris (figs. 32, 33), heard calling, or captured in random dip (fig. 34) or sweep nets (fig. 30). The number of observers x total amount of time sampled is recorded. In terrestrial and aquatic situations, times may be set for 15 or 30 minutes, occasionally longer, depending on the number of observers and the amount or quality of habitat to be surveyed.

*Example.* A sampling protocol is set whereby three researchers hike along Noland Divide Trail for 30 minutes, conduct a 30 minute time-constrained survey, hike another 30 minutes followed by another 30 minute sample, and so on throughout the day. Four to six sites per day can be sampled with this method, depending on trail conditions and terrain. The sampling effort would be 3 x 30 = 90 person-minutes at each site sampled. Sample data might be 3 adult *D. imitator*, 5 *P. jordani* (2 males, 3 juveniles), and 1 subadult *E. wilderae* at site 1, with similar data recorded at every sampling location.

**Figure 32.** Turning logs in time constrained survey at Beech Flats.

Figure 33. Terrestrial time-constrained survey in thickly vegetated habitat at Balsam Mountain.

**Figure 34.** Dip netting for salamander larvae in Abrams Creek.

**What this tells the observer.** Time-constrained surveys provide information on: (1) species presence (but not absence) at the time of sampling; (2) life history information, such as when eggs are deposited, larval presence, and activity patterns; and (3) habitat information. Sampling effort is easily quantified.

**Limitations.** Detectability is influenced by all the factors listed in **Things to Consider During Planning**. Even if every attempt is made to standardize sampling (for example, by sampling at the same time of day and during the same time of year), environmental factors likely will be different and thus influence whether a species will be observed. Because environmental variables influence the number of animals observed, differences in counts over time may be more reflective of differences in environmental conditions during the sampling periods among years than changes in status. It is very difficult to determine any kind of trend based on periodic counts because it is unknown what the relationship is between the counts and actual abundance. In addition, there may be considerable variation in the ability of the field observers to locate and count animals; some observers may find animals easily, whereas others might have great difficulty finding amphibians. Observer bias, thus, could skew count data in a manner which has nothing to do with the actual abundance of the animals counted.

***Area constrained*** **–** In this technique, a defined amount of habitat is selected for sampling. For example, researchers might choose to sample large, randomly selected plots (such as 30 x 40 m plots; fig. 35); they might survey smaller plots (for example, 10 x 10 m plots) during a hiking survey; or they might survey a pond, wetland, or cave entrance, regardless of how much time is required. Plots may be singular or in groups (fig. 36). As above, the presence of different species and the number of individuals (or even sex and life stage–males, females, juveniles) observed are recorded. Visual encounter protocols also are followed; that is, animals are counted as they walk over the forest floor or stream bottom, hide in crevices or cling to cave walls, found by turning over surface debris, heard calling, or captured in random dip or sweep nets. The number of observers x total amount of time sampled is recorded.

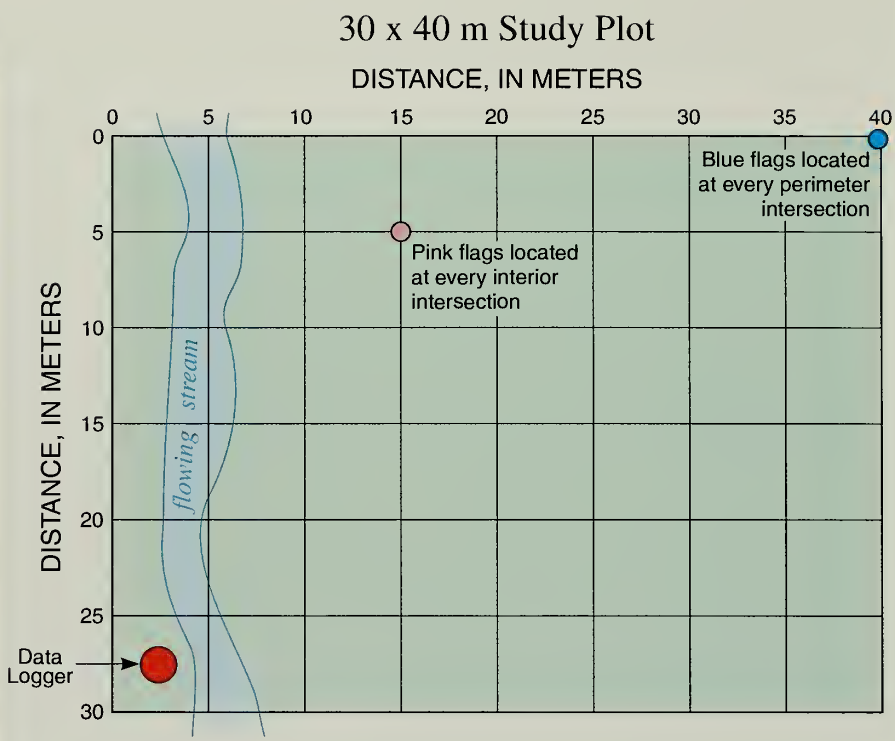
*Example.* Two persons search Gourley Pond for 67 minutes. The sampling effort is 2 x 67 = 134 person-minutes. Sample data might be: larval *A. opacum* (> 50 observed), 14 egg masses of *A. maculatum*, larval *R. sylvatica* (hundreds of tadpoles), 4 *P. crucifer* heard calling.

**What this tells the observer.** Area-constrained surveys provide information on: (1) species presence (but not absence) at the time of sampling; (2) life history information, such as when eggs are deposited, larval presence, and activity patterns; (3) habitat information; and (4) in some cases, a very crude estimate of density (the amount of area ÷ number of animals). Sampling effort is easily quantified.

**Limitations.** Detectability is influenced by all the factors listed in **Things to Consider During Planning**. Even if every attempt again is made to standardize sampling, environmental factors likely will be different and thus influence whether a species is observed. Since environmental variables influence the number of animals observed, differences in counts over time may be more reflective of differences in environmental conditions during the sampling periods among years rather than changes in amphibian population status. As with time-constrained sampling, it is very difficult to determine any kind of trend based on periodic counts because the relationship between counts and actual abundance is unknown.

***Transects*** **–** Transect sampling can be conducted using simple visual encounter survey techniques, such as by walking a preselected line transect at night and counting all the salamanders seen, or it can be used in conjunction with passive sampling techniques, such as the placement of coverboards along a preselected survey line. When using transects, sampling locations are determined through a stratified random process. A survey line of a prescribed length is selected, and observers use the line as a base from which to make observations.

Figure 35. Schematic of a 30 x 40-meter sampling plot. The grid is marked off in 5-meter intervals. The outside of the grid is marked with blue survey flags, whereas the rows are marked with pink survey flags. A stream is included on the left margin of the plot, so that both stream and terrestrial salamanders may be surveyed. Automated data loggers (red dot, DL) can be installed to record air and water temperature and relative humidity. Researchers walk up the survey lines turning coarse woody debris, rocks, and leaf litter. In addition to information on the species, size, and age class of salamanders observed or captured, the distance from water also can be recorded. This gives an idea of the spatial distribution of species across the plot.



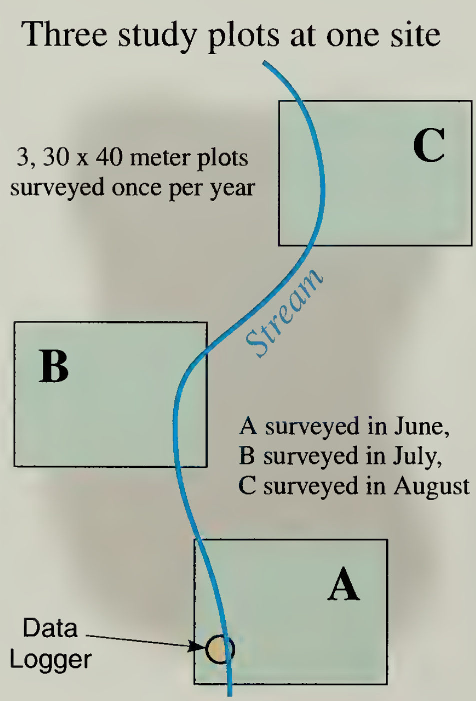




Figure 36. Diagram of the relationship of three 30 x 40-meter fixed sampling plots at a location. Plots need not be isolated. In this schematic, three plots are located along the course of a stream. Each plot is surveyed once per year during the summer, all in the same order (A in June; B in July; C in August), for the length of the study. A single data logger station is located at one of the plots.

*Example 1.* Researchers select 50 locations in the fir-spruce forest where transects of 100 m length will be established. During the day, a starting point for the transect is selected. The direction of the transect is then determined from a set of random numbers from 1 to 360 (based on the number of degrees in a circle). Using a compass and a 100-m survey tape, fluorescent tape is used to designate the survey line. After dark, two researchers walk along the transect line, 5 minutes apart, and count all the salamanders, categorized by species, observed in their flashlight beams. The distance from the starting point where the salamanders were observed also is recorded. Using two researchers allows for a measure of potential observer bias.

*Example 2.* A three-party survey crew samples the Little River for Hellbenders. The total amount of the river to be sampled is marked off in 100-m sections on a map, and ten 100-m sections are selected for sampling based on a random numbers chart. At the river, a starting point and an end point are marked using red survey flagging. Wearing wet suits, two observers snorkel along parallel transects about 4 m from the shore and look for Hellbenders under rocks, ledges, and other underwater hiding places. Observations are relayed to the third researcher walking parallel to the shore.

*Example 3.* Researchers select 50 stream locations on the northern side of the Park for sampling; the locations are selected based on elevation and accessibility. At each location, the stream is marked off in 5-m transects for a total of 100 m of stream length. Using a random numbers chart, seven transects are selected for sampling. A two-person team turns over all the rocks and searches hiding places, beginning downstream and working upstream, capturing and measuring salamanders (fig. 37). They call out the data (species, sex, length, age-class) to a third researcher walking parallel to the stream who records the information (fig. 38).

**Figure 37.** Stream sampling at Balsam Mountain.

**Figure 38.** Checking identification and recording data during stream sampling at Balsam Mountain.

*Example 4.* Researchers select 50 locations in the fir-spruce forest where transects of 100 m in length will be established. A starting point for the transect is selected. The direction of the transect is then determined from a set of random numbers (from 1 to 360, based on the number of degrees in a circle). Using a compass and a 100-m survey tape, fluorescent tape is used to mark the survey line. At every 10-m increment, a series of eight coverboards are laid out in a grid parallel to the transect line (fig. 39). The coverboards are then monitored periodically for salamander presence (see ***Coverboards***).

□ **What this tells the observer.** Area-constrained surveys provide information on: (1) species presence (but not absence) at the time of sampling; (2) life history information, such as when eggs are deposited, larval presence, size-class structure, and activity patterns; (3) habitat information; and (4) in some cases, a very crude estimate of density (for example, a minimum number of salamanders inhabiting the selected length of the stream surveyed). Sampling effort is easily quantified.

**Limitations.** Detectability is influenced by all the factors listed in **Things to Consider During Planning**. Even if every attempt is made to standardize sampling (for example, by sampling at the same time of day and during the same time of year), environmental factors likely will be different and thus influence whether a species is observed. Because environmental variables influence the number of animals observed, differences in counts over time may be more reflective of differences in environmental conditions during the sampling periods among years than changes in amphibian population status. It is very difficult to determine any kind of trend based on periodic counts, because it is unknown what the relationship is between the counts and actual abundance. On the other hand, the life-history information obtained using transect surveys may be valuable for understanding the basic biology and demography of the species sampled.

*Sweep samples* – Sweeping a large, small-mesh dip net through the water column or in submerged leaf litter in ponds or larger wetlands allows observers to capture amphibian larvae and sometimes breeding adults. Sample locations may be completely randomized or some measure of design can be incorporated into sampling, such as by sampling areas along pond margins every 10 or 15 m, depending on the circumference of the area to be sampled. Species richness, the number of larvae in each sweep, and the total number of sweeps are recorded.

*Example.* Two persons search the entire circumference around Gourley Pond by sweeping a dip net five times every 15 m. If the pond margin is 600 m, then 40 locations could be sampled and 200 sweeps could be made. The sampling effort is 200 sweeps. Sample data might be: 240 larval *A. opacum*; 6 egg masses of *A. maculatum*; and, 1,246 larval *R. sylvatica*. The amount of area sampled in relation to available habitat could be estimated visually.

■ **What this tells the observer.** Sweep surveys provide information on: (1) larval species presence at the time of sampling; (2) life history information, such as when eggs are deposited and tadpole developmental stage; (3) habitat information, such as microhabitat preferences and distribution of various larvae; and, (4) in some cases, an estimate of density (number of animals ÷ the amount of area sampled in reference to available habitat). Sampling effort is easily quantified.

> ....differences in counts over time may be only reflective of differences in environmental conditions during sampling periods.

**Limitations.** Detectability may be influenced by many of the factors listed in **Things to Consider During Planning**. Even if every attempt is made to standardize sampling, environmental factors (for example, water availability and depth; water temperature) likely will be different among sampling occasions and thus influence whether a species is observed. Since environmental variables influence the number of animals observed, differences in counts over time may be only reflective of differences in environmental conditions during sampling periods. As with time-constrained sampling, it is very difficult to determine any kind of trend based on periodic counts, because the relationship between counts and actual abundance is unknown. Also, the number of larvae observed may not reflect the number of breeding adults, or tell anything about future reproductive success and the rate of successful metamorphosis.

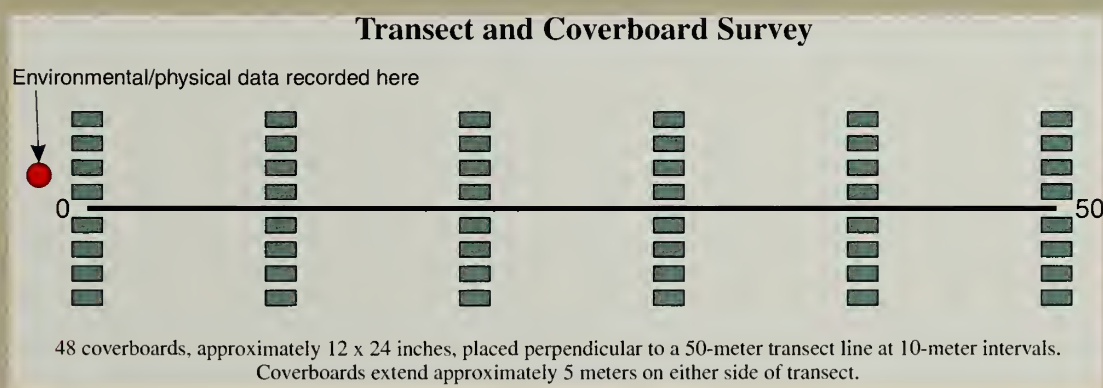



**Figure 39.** Schematic of a combined transect/coverboard survey design. A series of eight coverboards are located at the origin and thereafter at 10-meter intervals along a 50-meter transect. In this design, the boards are placed perpendicular to the transect. This survey design could be combined with a night survey, whereby a team of observers walks along the transect, spotlighting and counting salamanders. The boards would not be disturbed during such survey. Using dual observers at close intervals helps quantify observer bias. See text for layout details.

For example, the wetland could dry 10 days after a sampling visit, and all larvae could perish.

***Call surveys –*** All species of male frogs in Great Smoky Mountains National Park call to establish breeding territories and attract females. Species that may be quite difficult to find throughout most of the year can be readily heard at this time, their breeding sites identified, and relative abundances of adult calling males estimated. Call surveys are easy to conduct. A biologist simply periodically visits wetlands or drives park roads at night during the breeding season and records the locations of species heard calling. In very large choruses, it may be necessary to record abundance in terms of categories: 1 = 0 frogs calling; 2 = 1 individual calling; 3 = < 5 individuals calling; 4 = > 5 to 10 individuals calling; 5 = > 10 individuals calling.

Call surveys must be conducted at multiple occasions during the potential breeding season.

Areas appropriate for call surveys within the Great Smokies include the Cades Cove Loop Road and associated roads in Cades Cove, the road through Cataloochee Valley, Laurel Creek Road, Little River Road, lowland areas of Newfound Gap Road at Sugarlands and Smokemont, Big Cove Road, and the entry roads to Greenbrier, Cosby, and Deep Creek. Two methods may be used: (1) drive slowly and listen for frog choruses, or (2) conduct systematic searches using periodic stops with defined amounts of time for listening.

*Example.* Starting at the entry gate to Cades Cove Loop Road, drive slowly and stop every 0.5 miles. At each stop, turn off the engine, and listen for 5 minutes. Record the species heard and the compass direction from which the call is heard; possible breeding sites can be identified during daylight hours as time permits.

□ **What this tells the observer.** Call surveys provide information on: (1) adult male presence at the time of sampling; (2) the dates and environmental conditions when males call; (3) the location of breeding sites; and (4) an estimate of breeding male relative abundance can be attained through the use of the abundance categories. Sampling effort is easily quantified.

**Limitations.** Detectability may be influenced by many of the factors listed in **Things to Consider During Planning**. Even if every attempt is made to standardize sampling, environmental factors (for example, weather, temperature, rainfall patterns) likely will be different among sampling occasions and thus influence whether a species is heard. Since environmental variables influence the number of animals calling, differences among abundance categories over time may be only reflective of differences in environmental conditions during sampling periods. Thus, call surveys must be conducted at multiple occasions during the potential breeding season. Further, call surveys tell nothing about the presence and number of females and nonbreeding males, or whether reproduction was successful. Call surveys are best implemented where researchers have access by road; isolated breeding sites could be overlooked, or ignored when access is difficult (such as along lower Hazel and Eagle Creeks). Since frogs often call diurnally or during different intervals of the night (several hours after dusk or before dawn), species could be missed or relative abundances underestimated. One way to circumvent this problem is to use automated data loggers to periodically sample frog calls throughout the day and night.

***Egg mass or nest counts –*** A number of amphibians (Spotted Salamander, Wood Frog) deposit globular egg masses that are readily identified and can be counted. Other species (Marbled Salamander, Four-toed Salamander) deposit eggs in terrestrial habitat on dry pond bottoms or in the vegetation bordering ponds. As the pond fills, the eggs are inundated and hatching occurs (Marbled Salamander) or the eggs hatch and larvae wiggle through the vegetation to reach the pond (Four-toed Salamander). Counting egg masses or nests should give an indication of reproduction during the sampling period. This method has been used in the Great Smokies by James Petranka and Charles Smith; Crouch and Paton (2000) have suggested that the method is an effective way to gage trends in Wood Frog population size and reproduction.

*Example 1.* Researchers visit Gum Swamp shortly after Wood Frogs have bred. Each separate egg mass can be identified and a flag placed next to it. Flags mark the distribution of the egg masses, are easily counted, can be left in place to follow reproductive parameters (for example, whether successful hatching takes place), and help to reduce observer bias (single observers can miss 10 percent or more of the egg masses (Crouch and Paton, 2000)). Because each female deposits one mass, the number of breeding females at a pond can be monitored through time.

*Example 2.* The dry pond basin at Gum Swamp can be searched in October when female *A. opacum* have deposited their eggs and are sitting over them until the autumn rains arrive. By carefully turning logs, researchers can locate nests, place flags in the ground adjacent to them, and obtain an idea of the number of nests and their spatial distribution. Numbers of females and males can be counted (see Dodd, 2004, for sex determination criteria).

□ **What this tells the observer.** Egg mass or nest surveys provide information on: (1) the number of females breeding successfully in a year; (2) the dates and environmental conditions when eggs are deposited; and (3) egg masses that can be followed through time to obtain an idea of the extent of successful reproduction. Crude estimates of the number of metamorphs produced can be obtained (number of egg masses x the percentage of masses with successful hatching x the mean number of eggs per mass). In the case of nests, the reproductive potential (number of nests x the mean number of eggs per nest) can be determined. Sampling effort is easily quantified as the amount of time spent searching an area.

**Limitations.** Counting egg masses assumes that there is one female per egg mass. This assumption seems to hold true for those species depositing large, globular, jelly masses. However, this assumption will not be valid for all species depositing eggs in nests (for example, the Four-toed Salamander) because nests may include the eggs of more than one female. Be sure to check information on life history (Dodd, 2004). Counting egg masses generally does not give an indication of the number of males or nonbreeding females (but see Crouch and Paton, 2000). Unless the hatching success of egg masses is recorded, counting egg masses will not provide an estimate of the number of metamorphs produced during the breeding season. Care must be taken not to disturb brooding females because nest abandonment virtually ensures reproductive failure. Although some species are more tolerant of disturbance than others, a nest should not be disturbed repeatedly.

**Figure 40.** Coverboards.

## *Easy Passive Sampling*

***Coverboards –*** Herpetologists have a long history of turning over surface cover objects to look for terrestrial salamanders and reptiles. Coverboards are simply an extension of this search technique, albeit with a more formalized sampling design. Coverboards may be made of many types of materials (for example, wood, tarpaper shingles, plastic sheets), but the most common material is nonchemically treated plywood. The boards are cut into small sizes (for example, 20 x 25 cm; 35 x 35 cm; fig. 40) and placed in a grid of various design. Boards should not be too large, because the leaf litter underneath them becomes dry in the center and discourages salamander residency. Pressure-treated boards should never be used.

In the Great Smoky Mountains, National Park Service personnel have used four boards placed within a few centimeters of one another at each sampling site along a long transect. Sampling sites might be located at 10-m intervals along the transect, such that a 50-m transect would have 24 coverboards placed along it (stations 0-5 x 4 boards/station). Coverboards *must* be placed in location for at least a month prior to beginning a survey to ensure they age properly and provide secure hiding places. Ideally, coverboards *should* be set out in the autumn of the preceding year prior to sampling. Some researchers scrape the ground underneath coverboards to ensure that the area underneath is not too large to discourage residence or will not increase air flow. Coverboards should be checked once every week or two; too much disturbance will inhibit salamander occupancy.

*Example.* In a study of sampling techniques on the north side of Mt. LeConte, Hyde and Simons (2001) used two sizes of coverboards (three 13 x 26 cm; two 26 x 26 cm) placed at 10-m intervals along a 50-m transect (5 boards x 5 sampling stations = 25 boards/transect). Using a stratified sampling design to locate transect sites, they sampled 101 locations and captured 1,224 salamanders over a 2-year period. Coverboards were only checked three times the first year, and four times the second year.

□ **What this tells the observer.** Coverboard surveys provide information on: (1) species presence at the time of sampling; (2) life history information, such as data on size-class structure, reproduction, and activity patterns; and (3) habitat information. If used in conjunction with mark-recapture techniques, they also might be used to examine site fidelity, movement, and population size. Sampling effort is easily quantified (number of coverboards x number of days sampled).

**Limitations.** Capture probability is influenced by all the factors listed in **Things to Consider During Planning**. Even if every attempt is made to standardize sampling, environmental factors likely will be different and thus influence whether a species is observed. Because environmental variables influence the number of animals observed, differences in counts over time may be more reflective of differences in environmental conditions during the sampling periods among years than changes in status. It is very difficult to determine any kind of trend based on periodic counts, because it is unknown what the relationship is between the counts and actual abundance. Hyde and Simons (2001) found that counts of terrestrial salamanders in the Great Smokies were highly variable and that sampling variability and detectability were not constant among species or even habitat type. Recapture rates of marked salamanders also are notoriously low, making estimates of population size unreliable. Finally, coverboards may provide artificially favorable cover, although preliminary evidence suggests this capture bias may not be as serious as previously believed. Some size classes of terrestrial salamanders are more likely to use coverboards than other sizes (for example, data from Virginia suggest that hatchlings and juveniles are found less often under coverboards than they are under natural cover objects). Coverboards are labor intensive to cut and haul to a sampling site. They are subject to vandalism, and bears and pigs will readily turn them over or move them around.

***PVC pipes –*** A method that has proved successful in the southeastern United States for monitoring treefrog (*Hyla*) populations is to place polyvinyl chloride (PVC) pipes in the ground or to mount them on trees (Boughton and others, 2000; http://www.fcsc.usgs.gov/posters/Herpetology/Artificial_ Refugia/artificial_refugia.html). The pipes are readily colonized by treefrogs, even during the non-breeding season when the treefrogs are dispersed away from ponds. The placement of the pipes and their characteristics (diameter, structure, possibly color) are important. Frogs are captured most often in pipes of 3.8 to 5.0 cm (1.75-2 inch) in diameter located 2- to 4-m high, on a large trunked, deciduous, hardwood tree; they are captured much less frequently in pipes on tree trunks near the ground, in pipes of larger diameter, or in pipes located on pine trees

**Figure 41.** PVC pipes on trees in Okefenokee National Wildlife Refuge.

(fig. 41). Pipes capped on the bottom to allow some standing water within the shaft and presumably to increase humidity also capture more frogs than pipes that are open on both ends. Free-standing pipes (91.4 cm; 36 inches) sunk directly in the ground near breeding ponds also are used by treefrogs.

*Example.* A series of PVC pipes are to be placed around Gourley Pond to monitor the population of Cope's Gray Treefrog (*Hyla chrysoscelis*). Twenty transects are established evenly spaced around the pond perimeter at its edge (fig. 42). Each transect consists of five pairs of pipes (N = 10/transect; total N = 200 pipes) spaced 10 m apart, and radiates outward perpendicular to the pond's edge, similar to the spokes of a wheel. The first two pairs are in-ground pipes, whereas the last three pairs are nailed to hardwood trees (if possible) at a 2-m height. Each pair of pipes consists of one 3.8- and one 5.0-cm pipe. The pipes on trees are fitted with bottom caps, with a hole drilled 9 cm above the base to allow drainage. Pipes are painted camouflage green on the outside for concealment, and each pipe is marked with a distinct number. Pipes are checked once a week from March through September. The number of frogs observed is recorded. Frogs could be marked via individual or cohort toe clips, or digitally photographed for identification. Recording the data separately for unmarked animals and recaptures is important, because results from other studies show that frogs take up residency within pipes.

□ **What this tells the observer.** PVC pipe surveys provide information on: (1) species presence at the time of sampling; (2) life history information, such as when animals arrive at breeding ponds, how long they stay, sex ratios, size-class structure; (3) movement patterns while at the ponds; and (4) information on the direction and distance of dispersal. Sampling effort is easily quantified (number of pipes x the number of 24-hour periods sampled).

**Limitations.** The only species that can be monitored in the Park using PVC pipes is Cope's Gray Treefrog. Even then, sampling results for this species have revealed mixed results at other locations where pipes have been used. In some areas, Cope's Gray Treefrogs will use pipes as retreats, whereas in other areas they seem to avoid PVC pipes. Whether they will use PVC pipes in the Great Smokies is unknown. If simple presence data are needed, call surveys would be more appropriate, although PVC

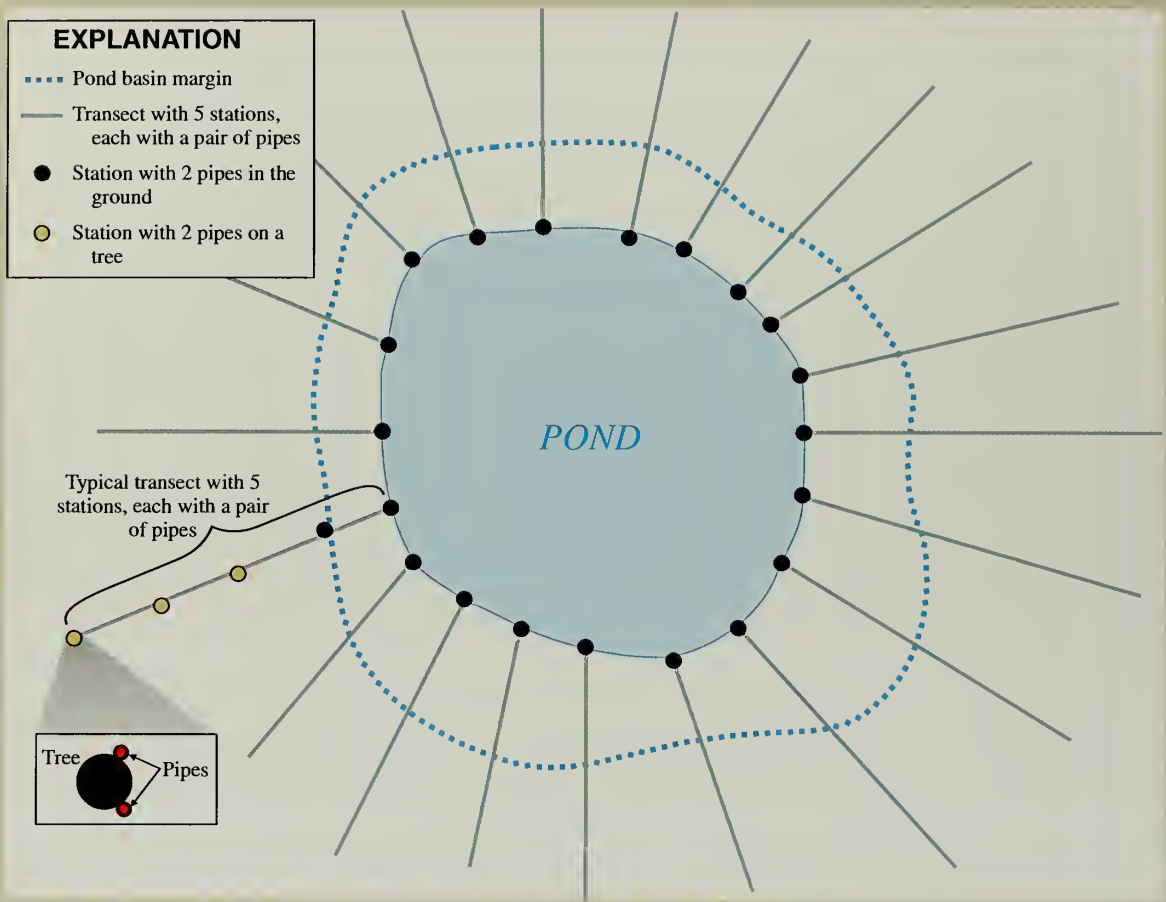



**Figure 42.** Schematic of a survey design using paired PVC pipes located at 10-meter intervals around a pond's perimeter. The first set of pipes is located at the pond's margin, and thereafter at 5- or 10-meter intervals perpendicular to the pond. The second set of pipes is located at the margin of the pond basin (dashed line). The first two sets of pipes are ground pipes (black dots), whereas the last three (gold dots) are located (preferably) on large-diameter deciduous hardwoods. Pipes are placed at a height of 2 meters on opposite sides of the trunk (red dots).

sampling might prove valuable if more detailed life-history information is required. PVC pipes are likely to be stolen or vandalized. Bears, in particular, seem to be attracted to PVC and will often bite it or carry pieces around.

***Larval litterbags*** – One relatively new method for inventorying and sampling most stream-dwelling salamanders, especially larvae, involves the use of artificial refugia (leaf litter-bags) placed in shallow streams (fig. 43). In 2000, Waldron and others (2003) tested the utility of using litterbags to sample salamanders in Great Smoky Mountains National Park. Three transects of six litterbags each (two large, two medium, and two small) were placed in five small, medium, and large streams. A total of 690 larval, juvenile, and adult stream-dwelling salamanders from 11 species were captured from June to November in the 90 litterbags. Sampling salamanders in small streams was most productive using large and medium-sized litterbags, although all bag sizes worked equally well in medium and large streams. The number of salamanders captured varied seasonally, with most captures occurring in June and July. The depth of bag submergence significantly influenced litterbag use by adult and larval salamanders, but had no effect on use by juvenile salamanders. The ease of deployment and non-destructive sampling methodology suggest that

Figure 43. Leaf litterbag in Little River.

litterbags could be useful in determining salamander presence during large-scale inventory programs, especially when the time available for sampling a large number of individual sites is limited and when sampling for secretive or uncommon larvae, such as *Pseudotriton* or *Gyrinophilus*.

*Example.* Litterbags of two sizes (70 x 70 and 90 x 90 cm) are constructed as outlined in Waldron and others (2003). In the field, three or four small rocks are placed in the netting to give the bag weight, then covered with leaves. Once filled with leaf litter, the corners of the netting are pulled together and tied with plastic cable ties to form a bag. Blue flagging is tied to the top of each bag so that researchers can easily locate bags in the field. Precautions are taken to prevent the loss of bags from fast-flowing water and flooding by placing one or two large rocks against or just downstream from each bag, and by tethering each bag to the nearest root, log, or large rock using monofilament fishing line.

Streams are selected using a stratified sampling protocol for size, location, and ease of access (see *Sampling Streams*). All streams are < 50 cm in depth at the sampling site. Sampling sites are spaced so that a watershed can be sampled in 1 day, allowing all of the sites to be completely sampled in 1 week. One 50-m transect is set up in each stream study area. Eight bags, four of each size class, are placed 10 m apart along transects. The order of presentation of medium and large bags from 0 to 50-m is randomized along the transect. Litterbags are sampled biweekly from April through September. Prior to sampling each litterbag, the percentage of litterbag submergence under water is recorded. Bags are removed quickly from the stream and gently shaken over a white dishpan for approximately 15 seconds to remove salamanders (fig. 44). Adult, juvenile, and larval salamanders that fall into the dishpan are identified to species, measured for total length (TL, tip of snout to end of the tail) and snout-to-vent-length (SVL, tip of snout to the posterior end of the cloacal opening), and released. If field identification is not possible, individuals

Figure 44. Checking leaf litterbag at Little River.

are taken to the laboratory for identification, and later released into their respective streams.

□ **What this tells the observer.** Leaf-litter-bag surveys provide information on: (1) species presence (but not absence) at the time of sampling; (2) life-history information, such as larval size and activity patterns; and (3) habitat information. Sampling effort is easily quantified.

**Limitations.** Although the technique may be effective for determining the presence of many stream-dwelling salamander larvae in Great Smoky Mountains National Park, the variation in the numbers of individuals captured and the inability to relate captures to overall abundance make trends impossible to monitor without considerable additional effort, such as by employing mark-recapture techniques on, often, very small larvae. Capture may be influenced by the factors listed in **Things to Consider During Planning**. Even if every attempt is made to standardize sampling (for example, by sampling at the same streams during the same time of year), environmental factors, as well as natural variation in reproductive output, likely will be different among years and locations and thus influence whether a species is captured. Since environmental and other variables influence the number of animals captured, differences in counts over time may not reflect changes in status. Additionally, it is difficult to determine whether the bags are selected by adult and large larval salamanders as places of retreat or for foraging, and to determine the amount of area actually being sampled using the method.

***Automated frog call data loggers* –** Automated data loggers have been used successfully to determine the presence of calling frogs at breeding sites (fig. 45). They can be set to record at variable time intervals for various amounts of time throughout the entire day, or they can be programed to record only at certain times of a 24-hour period, such as from dusk to dawn. Frog calls are easily discerned by listening to the tapes, and it is sometimes

Figure 45. Recording data in field as storm approaches at Cataloochee Divide.

possible to gain an index of calling intensity, provided large choruses are not involved.

*Example.* At a pond the size of Gum Swamp, three data loggers could be installed to monitor chorusing frogs: one on the east shore, one on the west shore, and one on either the north or south shore midway between the other two. The program could be set to record for 5 minutes every hour throughout the day, or for 5 minutes only from dusk to dawn (the starting and ending times would vary with season to account for day length). Both sides of the tape can be used, thus extending the amount of time between tape changes. Data loggers measuring water and air temperature, and barometric pressure, could be placed near the call logger to account for environmental influences on calling activity.

❑ **What this tells the observer.** Automated frog call data loggers provide information on: (1) species presence at the time of sampling (species likely to be overlooked during time-constraint sampling can be recorded with greater reliability); (2) life history and phenology information, such as when frogs call (especially if different species call at different times of the day), what environmental influences affect calling; and (3) a relative index of the number of males calling.

**Limitations.** Although species can be easily identified, categorizing abundance may be very difficult in even moderately sized choruses because of call-overlapping interference. It is also often not possible to separate individual callers, allowing the possibility that a single calling male could be counted multiple times. Since environmental variables influence the number of animals calling, differences among abundance categories over time may be only reflective of differences in environmental conditions during sampling periods. Thus, call surveys using automated data loggers must be conducted at multiple occasions during the potential breeding season. Further, call surveys tell nothing about the presence and number of females and nonbreeding males, or whether reproduction was successful. Frog call surveys using automated data loggers are best implemented where researchers have limited access by road (such as along lower Hazel and Eagle Creeks) or when rare species are suspected.

Whereas automated frog call data loggers are relatively easy to assemble (appendix IV), they are somewhat expensive (about $350 in 2002). Unfortunately, there are no computer programs currently available that can identify calls and categorize abundance by reading the tapes. Thus, researchers must listen to tapes and manually record the results, a time-consuming, tedious exercise. At the Florida Integrated Science Center, two observers independently listen to the tapes as a measure to reduce and quantify observer bias. Automated data loggers must be well hidden to reduce theft and vandalism, and this can limit their effectiveness. Curious bears have been known to investigate and attempt to dismember the data loggers.

Various types of aquatic traps have been used to sample amphibian larvae....

### *Intensive Passive Sampling*

***Traps (aquatic or terrestrial): funnels, bottles, minnow, wire basket*** – Various types of aquatic traps have been used to sample amphibian larvae; on occasion, some of these traps have been used to capture adults, such as the Common Mudpuppy, in fine wire-mesh basket traps. They are all based on the premise that an animal entering the trap will be unable to escape because it would be difficult to exit through the inward-directed funnel opening. However, few studies have examined this assumption, and unhindered movement into or out of a trap (termed trespass) undoubtedly occurs with varying degrees of frequency. Minnow traps come in wire-mesh, collapsible soft, and plastic variations. Wire-mesh minnow traps seem to capture the most larvae, whereas plastic-mesh traps seem to have the least capture success. A drawback to wire-mesh traps is that they cause injury to tadpoles, even when checked every day, because the animals tend to beat themselves against the metal mesh attempting to escape. Wire-basket traps are usually larger with larger mesh, and are more often used to sample fishes and turtles than amphibians. In Florida, a modified crayfish trap with a fine mesh plastic insert is used to capture aquatic salamanders (Amphiumas, Sirens) (http://www.fcsc.usgs.gov/posters/Herpetology/Sirens_and_Amphiuma/sirens_and_amphiuma.html). The trap has not been tested specifically to capture amphibians in more temperate habitats. Wire-mesh screen funnel traps have been used for both aquatic and terrestrial sampling. These traps are placed flush with a downed log, rock, or drift fence. As the animal enters the trap, it falls to the center and, presumably, cannot find its way back out of the trap. None of these traps are baited, although larvae may attract invertebrate and vertebrate (turtles, snakes) predators.

*Example.* Researchers place 15 wire-mesh minnow traps around the perimeter and in the center of Big Cove Beaver Pond. Traps are spaced at about 5 m apart, secured to a branch to prevent loss, and placed in such a manner that trapped air-breathing animals have access to surface air. Traps are checked daily, perhaps even once in the morning and once at night. The number of animals caught are recorded by species, size, and developmental stage, then released. Sampling should only require a few days at each location, although a location may be trapped more than once per season to capture both early and late breeders. Sampling effort is easily quantified (number of traps x number of days = number of trap days).

◘ **What this tells the observer.** Funnel traps are used to detect a species' presence, and perhaps to obtain a crude abundance estimate (that is, very large numbers of larvae versus very few larvae). Counts have little meaning except in this context. Funnel trapping is often used during mark-recapture studies, especially if there are no known capture biases (that is, trap avoidance or trap happiness). Traps might be useful in sampling for rare species.

**Limitations**. Some types of traps require assembly, whereas others can be purchased ready-to-use directly from a supplier. They are subject to vandalism by both wildlife (bears, pigs) and people; minnow traps, in particular, may be stolen. Trapped animals are vulnerable to drowning, predation, and injury, making daily checking, preferably in the early morning, absolutely essential to minimize mortality. Traps capture nontarget organisms, such as invertebrates and fish. Even if every attempt is made to standardize sampling (for example, by sampling at the same exact location and during the same time of year), environmental factors likely will be different and thus influence whether a species is captured. It is very difficult to determine any kind of population trend based on periodic counts since it is unknown what the relationship is between the counts and actual abundance. Captures also may be biased by trap avoidance or trap happiness (that is, returning to a trap again and again because of the availability of food or shelter). It may be necessary to conduct a pilot study prior to employing trapping methods to determine sampling biases.

***Drift fences –*** Drift fences are the most labor intensive method for sampling amphibians. In brief, the idea is to intercept an animal during its daily wanderings, direct it along a fence constructed of metal (galvanized or aluminum) or cloth (highway department silt cloth; plastic sheeting) to where it either falls into a pitfall trap (a bucket or can sunk flush with the ground surface) or funnel trap (wire-mesh screening with inward-directed funnels; once the animal gets inside the funnel, it should be difficult for it to escape). Sometimes buckets and funnels are used simultaneously. There are a number of different array configurations, but they usually take some form of a Y or X shape; each arm is 7.5-10 m long. Drift fences also can be used to completely encircle breeding ponds. Each sampling unit may consist of three or four arrays randomly placed in an area. In a region the size of the Great Smokies, dozens of arrays would be necessary to sample the terrestrial amphibian communities. Arrays should be opened at least four times per year for a minimum of 2 weeks per sampling period; at high elevations, the winter sampling period could be skipped. There are several excellent descriptions of the technique and various configurations, and the reader is referred to chapters in Vogt and Hine (1982) and in Heyer and others (1994) for more information.

*Example.* Researchers decide to use a Y-shaped drift fence configuration to sample lowland, terrestrial amphibians in the Cades Cove region. Twenty sampling locations are randomly selected, and three arrays are placed at each location approximately 50 m from one another. The fence must be trenched so that animals cannot walk underneath the fence, and so that erosion does not create areas for under-fence trespass. Pitfalls may not be feasible because of the rocky soils, so two funnel traps are placed on each side of a fence arm (that is, 12 per array). Funnel traps may need to be shaded to prevent desiccation of trapped animals and are placed flush with the base of the fence. Traps must be checked daily to avoid animal desiccation and minimize predation. The number of captured individuals of each species for each funnel trap is recorded. Animals are released at least a few meters away in appropriate cover to minimize chances of recapture. Funnel traps are opened and checked four times per year for a period of 2 weeks per sampling occasion to ensure that different amphibian faunas are sampled (that is, those species which are active during the cool versus the warm times of the year).

◘ **What this tells the observer.** Drift fence surveys provide information on: (1) species presence (but not absence) at the time of sampling; (2) life history information, such as population size-class structure, reproduction, and activity patterns; and (3) when used with mark-recapture techniques (toe-clipping, elastomer marking, photographic identification), to obtain a measure of abundance. A drift fence-pitfall-funnel trapping regimen might be useful in capturing rare species or, when completely encircling a breeding site, in measuring reproductive effort and success. Sampling effort is easily quantified (number of buckets or funnels x the number of nights over which the sampling was conducted = number of bucket- or trap-nights).

**Limitations.** Drift fences take a great deal of work to install and maintain, even without digging holes for pitfalls and carrying heavy metal flashing to a study site. They are subject to vandalism by both wildlife (bears, pigs) and people; drift fence materials may also be stolen. Animals are very vulnerable in pitfalls and traps, making daily checking, preferably in the early morning, absolutely essential to minimize animal desiccation and predation from reptiles and small and large mammals. Pitfalls also capture large numbers of shrews which either eat the other animals present or die from stress.

...differences in captures over time may be more reflective of differences in environmental conditions...

As previously mentioned, the probability of catching an animal is influenced by all the factors listed in **Things to Consider During Planning**. Even if every attempt is made to standardize sampling (for example, by sampling during the same time of year), environmental factors likely will be different and thus influence whether a species is captured. Since environmental variables influence the number of animals that are active, differences in captures over time may be more reflective of differences in environmental conditions among the yearly sampling periods than changes in status. It is very difficult to determine any kind of trend based on periodic counts since it is unknown what the relationship is between the counts and actual abundance, unless mark-recapture techniques are employed.

Even with mark-recapture techniques, only a very small portion of the population may be sampled (for example, terrestrial plethodontids may be territorial and thus unlikely to move about very much), so it may be difficult to extrapolate estimates of abundance in a wide area where animals are patchily distributed. Recapture rates are notoriously low in most mark-recapture studies of terrestrial salamanders, making estimates of variance quite high and unacceptable. Many amphibians may not walk along a fence (treefrogs might just climb it, hop over, or just pass it by), enter a funnel, or fall into a pitfall; some amphibians may be readily able to crawl out of a pitfall. Little is known about capture biases, but data from other studies indicate that the color (Crawford and Kurta, 2000) and size of the bucket may influence capture; that some individuals learn to avoid buckets; and, that other individuals may come to recognize buckets as a source of shelter or food. Therefore, capture probabilities are likely to vary considerably among species, even if the species is locally abundant.

## Field Data

Field data should be recorded immediately when taken (fig. 45). Data may be recorded on data sheets, preferably in pencil using waterproof paper, or by using preprogrammed palm pilots. ARMI is currently developing a web based data entry program using palm pilots. Park researchers may desire to link their data collection with the DOI-sponsored national amphibian monitoring program. Palm pilot programs with project-specific formats also can be developed. In any case, the following data should be recorded at all sampling sites (note that all measurements should be recorded in metric units):

Date: month/day/year.

Site No.: a unique identifying site number. Example: BB-1 could indicate site 1 on the Bunches Bald Quadrangle. There are many ways this can be done, but site location codes should be consistent.

Personnel: initials or names of those persons conducting the survey.

Weather: at the time of the survey.

Altitude: in meters.

Wind: categorical judgement of wind speed 1 m above sampling area.

General location: a geographic description of the site location. Example: Garretts Gap on the Hemphill Bald Trail on Cataloochee Divide.

Specific location: using GPS or Topo® software.

Quadrangle: USGS 7.5' quadrangle map.

Start time and End time: in military time (that is, 0800 or 1600 hrs).

Standing water: at aquatic sites, record whether water is present.

Water level: deepest water level at sampling site. Can be estimated (example: > 0.5 m).

Air temperature (AT): recorded at 1 m above substrate in °C.

Water temperature (WT): recorded at 30 cm under water in °C.

Substrate temperature (ST): recorded at 30 cm under leaf litter in °C.

Relative humidity: recorded at 1 m above substrate in °C.

pH: when appropriate, recorded in soil/water with a calibrated meter.

Conductivity: when appropriate, recorded in water with a calibrated meter.

Habitat type: a general appraisal of the habitat type (circle one, see appendix II).

Vegetation: a general appraisal of the vegetation types (circle as many as appropriate, see appendix II).

Canopy: a categorical assessment of canopy cover (especially important at wetland sites).

Slope aspect: a compass direction of slope aspect.

Drainage direction: in which compass direction does a stream flow at the sampling location.

Amphibians. The species (using the three letter species code), sex (if discernible), life stage (adult, juvenile), number of individuals, and other notes (for example, reproductive condition, missing limbs) should be recorded. In some cases, the snout-vent length (for salamanders), total length (for frogs), mass, or other individual measurements may be required by a study's objectives. Measurements should always be in metric units.

Method of capture: specialized capture techniques may require a data form to reflect the types of data taken, in addition to the information listed above. For example, the identifying number of the trap, PVC pipe, or coverboard should always be recorded to discern possible capture biases. The distance an animal is captured or observed from a transect's origin and baseline helps indicate spatial distribution.

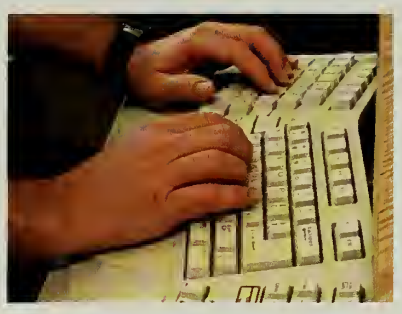
Invertebrates: the type (genus, order, class) and relative abundance of invertebrates may be very important in studies of amphibians, especially amphibians breeding in ponds and woodland pools.

Active sampling effort: the number of observers (exclusive of the person recording data, unless that person is also sampling animals) x the amount of time sampling occurs.

If data sheets are used, additional information concerning the site can be included on the back of the form, such as drawings of ponds or pools, sketches and notes of unusual color patterns or morphology, notes on the physical description of the sampling site, records of photographs taken, and the presence of unusual plants and animals. A sample data sheet is included as appendix II.

## *Spreadsheets and Databases*

Most U.S. Government agencies are now using Microsoft Excel® and Access® to generate spreadsheets and databases. Data from field data sheets should be transferred into one of these programs as soon as possible following a survey, or entered directly while in the field using palm pilots, using the same conventions as on the data sheets.



Both programs are compatible with a variety of statistical programs, such as SAS® (Statistical Applications Systems). Data accuracy should be checked to ensure quality control and prevent inaccuracy; the field data sheets serve as a backup from which to double check data records. Backup copies of data should be made weekly, at a minimum, and copies should be safely stored at different physical locations or in a fireproof data safe.

## *Analysis and Software*

The objective of monitoring the amphibians of Great Smoky Mountains National Park is to detect population trends so that actions can be taken, if possible, to reverse declines should they be detected. Inasmuch as many species' populations fluctuate from one year to the next, especially in unstable habitats such as temporary ponds, and that populations probably go extinct naturally (and vacant habitats are recolonized), trend analysis is not an easy task to apply to amphibian populations. Much ongoing research is focused on amphibian populations; new biometric methods are being developed to analyze trends in light of the complexities of amphibian biology.

Traditionally, population trends have been measured via changes in numbers or abundance of the animal in question. If the population size can be measured through time, then changes could indicate increasing or decreasing trends and, therefore, reflect changes in conservation status. To determine the size of a population, it is necessary to relate the numbers recorded during periodic counts to the overall population size. The most commonly used method to do this is to individually mark animals and to record the numbers recaptured during a period of extended sampling. Thus, each animal is accorded a capture history. If enough animals are captured and recaptured during a survey, it is possible to relate the counts mathematically to an estimate of actual population size within a certain degree of confidence. Although it is beyond the scope of this manual to discuss the nuances, theory, and assumptions of mark-recapture analysis, there is substantial literature available on this subject (Pollock and others, 1990; Nichols, 1992; Thompson and others, 1998).

Unfortunately, it is not easy to use mark-recapture techniques when studying populations of amphibians for two reasons:

1. Amphibians are not easy to mark "permanently." Various methods, such as toe clipping, elastomer implants, and photographic identification (ID), have been used, although each technique has limitations. Amphibians lose toes naturally and regrow clipped toes; elastomers are time consuming to apply and are difficult to read under field conditions, and photographic ID is not practical when hundreds or thousands of animals are involved or when animals are uniformly patterned or unpatterned. Observer error is an ever-present bias.
2. In most instances, very few recaptures are recorded in relation to the number of amphibians marked. In such cases, the variance of the population estimate can become quite large, thus negating the reliability of the estimate.

In the Great Smokies, there is only one species, the Hellbender, that is probably amenable to reliable mark-recapture population estimation. These large salamanders are territorial and relatively confined to a circumscribed habitat (only large rivers and streams) in a few areas of the Park. They can be permanently identified through implantation of an injectable passive integrated transponder (PIT) tag. As such, resurveys should be possible to track populations within certain sections of streams. Nickerson and others (2002) have marked Hellbenders in Little River using PIT tags, and National Park Service biologists should be able to track the status and size of this population annually using a transect-based snorkeling protocol.

Another technique that is gaining favor is to conduct repeated sampling at locations throughout a designated area, such as a Park or refuge, or in a particular subset of a habitat type within such an area. Through time, researchers can record a capture history for each species at each location. Thus, a data set is developed that in practice looks very much like the capture history of individuals in a typical mark-recapture study. By recording changes in these species' capture histories through time, biometricians can determine detection probabilities for each species. Trends can be determined by changes in the "percent of area occupied" (PAO) by a species and by changes in detection probabilities. More information on applying PAO analyses to monitoring amphibians is contained in MacKenzie and others (2002), and at:

http://www.mbr-pwrc.usgs.gov/software.html#presence

*SOFTWARE*

***Program MONITOR*** – Power analysis basically tells the researcher how reliable his or her data are considering a number of variables, such as sample size and the length of time that a program is conducted. Important caveats for interpreting the results of a monitoring program are contained in "Power Analysis of Wildlife Monitoring Programs: Exploring the Trade-Offs Between Survey Design Variables and Sample Size Requirements" by Paige C. Eagle, James P. Gibbs, and Sam Droege (http://www.pwrc.usgs. gov/resshow/droege3rs/salpower.htm). The USGS has developed a free software program, MONITOR, which uses

Power analysis basically tells the researcher how reliable his or her data are...

linear regression to estimate the statistical power of population monitoring programs relative to: the number of plots monitored, the magnitude of counts per plot, count variation, plot weighting schemes, the duration of monitoring, the interval of monitoring, the strength and nature of ongoing population trends, and the significance level associated with trend determination. MONITOR is available at:

http://www.mbr-pwrc.usgs.gov/software.html
(then click on POWER)

***Program MARK*** – Program MARK provides population parameter estimates (for example, survivorship and population rate changes) based on mark-recapture data. Re-encounters (captures or observations) can be recorded from animals found dead, live recaptures (for example, the animal is retrapped or resighted), radio tracking of an animal's movements, or from some combination of these sources. The time intervals between re-encounters do not have to be equal, but are assumed to be one time unit if not specified (for example, every week or month). Data can be subsetted, such as by sex or life history stage, so that population parameters can be estimated for the designated group. The basic input to program MARK is the encounter history for each animal (for example, the entry **1001101001** could result for an animal caught 5 times during 10 sampling periods where 1 = captured, 0 = not captured). MARK also can be used to provide estimates of population size for closed populations. Capture and recapture probabilities for closed models can be modeled by attribute groups and as a function of time, but not as a function of individual-specific covariates. Program MARK is available free from Colorado State University at:

http://www.cnr.colostate.edu/~gwhite/mark/mark.htm

***Program PRESENCE*** – The number and diversity of amphibians in the Great Smokies and elsewhere in the southeast makes monitoring all species difficult, if not impossible. Nonetheless, high species richness of amphibians is a hallmark of ecosystems in southeastern North America. Changes in ecosystems through disturbance, human activities, disease, environmental contaminants, or other factors could negatively impact the composition and richness of amphibian communities. Estimating variation in species richness through time and among different locations is one means of tracking the status of amphibians as a group. This type of analysis, termed percent of area occupied (PAO), may be more effective than focusing on abundance measures of individual species, which have been shown in most studies to lack statistical power because, in part, of the low recapture probabilities in mark-recapture studies of amphibians.

In the past, the main hindrance to making reliable inferences about variation in species richness has been the inability to count all species present in an area during a survey. Weather conditions, the behavior of different species, cryptic coloration, and observer skill are just some factors affecting detection (also see **Things to Consider During Planning**). Invariably, some species will be missed, thus biasing the estimates (Boulinier and others, 1998a,b). However, methods are now available which account for variation in detection probabilities, and which estimate species richness, standard error, and 95 percent confidence intervals (Nichols and Conroy, 1996). These methods have been extended to estimate several important vital rates in animal communities, which would be useful to assessing status, for example, rates of local species extinction, turnover, and colonization (Nichols and others, 1998a). They also have been used to test hypotheses concerning factors affecting temporal (Boulinier and others, 1998a,b) and spatial variation (Nichols and others, 1998b) in species richness.

The application of PAO methods to amphibian survey data is promising, not only because these methods can address important questions, but also because they may easily be applied to inventory surveys, intensive monitoring at preselected sites, and in extensive surveys (MacKenzie and others, 2002). Furthermore, detection of a change in species richness can alert biologists and managers to potential problems that may require more focused study. To facilitate PAO analyses in amphibian monitoring studies, USGS researchers have developed Program PRESENCE. This program is available free at: http://www.mbr-pwrc.usgs.gov/software.html#presence. This program is still being tested and developed; undoubtedly improvements will be forthcoming to enhance its performance and ease of use.

## EQUIPMENT AND TRAINING

Field researchers require adequate equipment and training before undertaking amphibian inventory and monitoring activities in Great Smoky Mountains National Park. Volunteers can be trained to conduct supervised activities, such as call surveys, but quality assurance and control must be maintained by a supervising biologist. Identifying the amphibians of Great Smoky Mountains National Park is often complex and difficult (Dodd, 2004). Even experienced herpetologists are sometimes unable to verify identification to species, especially among salamanders of the genus *Desmognathus* and for many salamander and frog larvae (notably very small animals). Experienced judgement is critical to a successful monitoring program.

Before going into the field, survey crews must be instructed in the proper use of survey techniques and map reading, and each crew member should be instructed in the use and care of each piece of equipment. Prior to beginning surveys, field trips should be conducted to examine the major amphibian communities, and to gain hands-on experience with identification, specifically with regard to key characters. Field crews should be taught why certain techniques are being used, the limitations of those techniques, and what the results will tell the researcher. Communication is important to minimize observer bias, a major cause of error in field studies. Individuals should be made to feel part of the team, and they should be credited for hard work under sometimes difficult conditions, as well as for the discoveries made.

To assist planning, a checklist is provided in appendix III for equipment needed at field sites during amphibian surveys and data collection. All crews should be briefed on the dangers of hypothermia, heat stress, lightning, and dangerous animals (yellowjackets and wasps, venomous snakes, pigs, bears, humans). Each vehicle should have appropriate first aid, safety, and communications supplies. Crews should be properly dressed for cold or heat and inclement weather, especially with regard to footwear. Never conduct surveys, even in streams, in bare feet or sandals because of the dangers of sharp rocks or glass. Crews should always provide a destination and estimated time of return to supervisors before setting out on surveys.

## BIOSECURITY AND DISEASE

Concern about disease and toxic contamination as causes of amphibian declines has increased considerably in recent years (Carey and Bryant, 1995; Daszak and others, 1999). A corollary of this concern is the need for field workers to avoid becoming vectors for transmitting disease organisms or toxic chemicals to and among study sites. The Declining Amphibian Populations Task Force (DAPTF) has developed a standard protocol for use by anyone conducting fieldwork at amphibian breeding sites or in other aquatic habitats. These procedures should be used for all routine surveys, but more stringent measures are necessary in areas with known diseases.

### *Biosecurity Protocol*

| Protective Wear & Equipment | Disinfecting & Sanitizing Methods |
|---|---|
| nonpermeable boots or waders | rinse in bleach solution immediately after leaving each study site[3] (fig. 46) |
| vinyl gloves[1] | dispose of gloves after each handling incident |
| nets | rinse in bleach solution immediately after leaving each study site |
| plastic bags (for holding specimens)[2] | properly dispose after each use |
| needles & syringes (for blood extraction) | properly dispose after each use |
| scalpel blades, PIT tag cannula, forceps, etc. | immerse in sterilizing solution |

[1]Only vinyl gloves should be used when handling amphibians. Some people are allergic to latex gloves, and latex gloves are toxic to amphibians (Gutleb and others, 2001).

[2]Use one bag per specimen.

[3]Premixed bleach solutions can be carried in containers large enough to step into and immerse boots, nets, and equipment. If this is not possible, bleach solutions can be carried in a spray backpack firefighting pump.

### *Solution Formulas*

| | |
|---|---|
| bleach | one (1) capful per gallon water |
| sanitizing solution (for instruments) | 70% methanol for 30 minutes, then flamed; or, 1% glutaraldehyde for 15 minutes; or, boiling water for 10 minutes |

**Figure 46.** Biosecurity. Washing boots and stump ripper in bleach solution.

## *Additional Precautions*

- Avoid contact between used and unused protective wear and equipment.
- Separately house specimens.
- Avoid contact between gloved hands and face, especially the area of the nose.
- Do not urinate in or near ponds and streams.
- Wash hands thoroughly with soap and water, or use a sanitary wipe, after urinating.
- Wash hands thoroughly with soap and water, or use a sanitary wipe, after handling specimens known or suspected of being diseased or contaminated.
- Wash hands thoroughly with soap and water, or use a sanitary wipe, after leaving each site.
- Do not use insect repellent on hands when handling amphibians.

## *Disease Protocols*

The following information is taken from the U.S. Geological Survey's **STANDARD OPERATING PROCEDURE** (Kathryn Converse and D. Earl Green; ARMI SOP No. 105; revised March 2, 2001) entitled "Collection, Preservation & Mailing of Amphibians for Diagnostic Examinations." It was developed by the National Wildlife Health Center, Madison, Wisconsin (http://www.nwhc.usgs.gov/research/amph_dc/sop_mailing.html).

The best diagnostic specimen is the live, sick amphibian. Live amphibians are necessary to obtain meaningful bacterial cultures and most types of fungus cultures. In addition, blood for various "blood tests" can be obtained only from live amphibians. Dead amphibians have limited usefulness because aquatic animals decompose much more rapidly than terrestrial animals which means amphibian carcasses nearly always will have large numbers of decompositional bacteria and fungi throughout their bodies. This rapid decomposition (autolysis) makes it very difficult to obtain meaningful or useful bacterial and fungal cultures, but dead amphibians may still have usefulness for virus cultures, histology and toxicological tests, if promptly and properly preserved.

If the amphibians will be captured and euthanized as part of other studies, then first observe and record their behavior. Blood should be collected and saved prior to euthanasia. If the euthanized amphibians will be preserved in a fixative, then collect swabs for bacterial, viral and fungus cultures from the mouth, vent, skin, and any skin abnormalities (lesions) prior to emersion of the animal in the fixative.

At a casualty site, the priority specimens for diagnostic examinations are live, sick amphibians. Divide dead amphibians into two groups: promptly preserve about half the carcasses (preferably the most recently dead amphibians) in 10 percent formalin (or 70-75 percent ethanol); promptly freeze the other dead amphibians (for virus cultures and possible poison tests). In cases involving less well known species, submission of live healthy amphibians as "control" or "baseline" specimens will be necessary to assist in the interpretation of findings in the sick or dead animals.

More than one lethal disease may affect a population simultaneously, so submission of multiple animals is always encouraged. Collect specimens that represent the species that are affected and the geographic areas. Do not place live and dead animals in the same container, and do not put multiple species in the same container (except, it is acceptable to put dead animals of multiple species in one container of formalin or ethanol).

If possible, submission of invading (alien or introduced) amphibians from the casualty site is desirable, even if they appear healthy or unaffected, because invasive species can be the vectors of infectious diseases. If any other endemic amphibians, fish, or reptiles are present at the casualty site, these animals also may need to be examined as part of a wider epizootiologic investigation into the cause of the casualties.

Many amphibian die-offs are fleeting. This means the casualties must be collected the hour and day they are found. Returning to the casualty site the next day to collect sick amphibians and carcasses invariably fails because of the highly efficient activity of scavengers during the night and rapid autolysis of carcasses.

# METHODS

## LIVE AND SICK AMPHIBIANS

**Eggs** – Place eggs in heavy mil plastic bag or plastic container. Equal volumes of air and water should be present in the bag or container to assure adequate oxygen exchange. Do NOT fill bags or containers completely with water. If bottled oxygen is available, it may be placed into the air cell in the bag or container, but this is optional. If possible, place plastic bags in a solid container for support and to avoid crushing specimens or puncture of the bag.

**Tadpoles, Larvae, and Neotenes** – Same as for eggs. For small amphibians (<2 grams each), multiple live animals may be placed in one container, but avoid mixing species. For larger aquatic larvae and neotenes, one animal per bag or container is recommended. Enough air must be present in each container; containers that have a large surface area of water to air are preferred; hence, flat food storage-type plastic boxes with lids (available at nearly any grocery store) are preferred to tall narrow plastic bottles. If bottled oxygen is available, oxygen may be placed into the air cell in the bag or container, but this is optional.

**Adult Amphibians (Terrestrial Amphibians)** – Plastic boxes or bottles with wide lids may be used for mailing. Sick amphibians should be mailed in separate containers. Two or more live adult amphibians of the same species may be placed in one container, but avoid crowding. Note: if an infectious disease is the cause of the casualties, the disease may be transmitted between amphibians in the container if more than one animal is placed in each container. Wet unbleached (brown) paper towels or wet local vegetation should be added to the container to prevent dehydration of the animal; do not use sponges, because many contain chemicals that are toxic to amphibians. Three or more small holes should be made in the lid of each container. Plastic bags are not recommended for terrestrial amphibians.

## DEAD AMPHIBIANS

About half the dead amphibians should be immediately placed into 10 percent buffered neutral formalin or 75 percent ethanol for histologic examinations. When possible, the freshest carcasses (those with the least amount of decomposition) should be selected for fixation. Prior to immersing the carcass in the fixative, slit open the body cavity along the ventral midline to assure rapid fixation of internal organs. For the first 3-4 days of fixation, the volume of fixative to volume of carcasses should be 10:1. After 3-4 days of fixation, the carcasses may be transferred to a minimal amount of fresh fixative that prevents drying of the specimen.

**Freezing** – About half the carcasses should be promptly frozen. Preferred freezing temperature is -40 degrees, but any freezing temperature is preferable to a chilled carcass. Do NOT freeze amphibians in water. Frozen carcasses can be used for virus cultures, toxicological examinations, and molecular (DNA) tests. Frozen and preserved carcasses are not suitable for bacterial and fungus cultures; generally, bacterial and fungus cultures will be attempted only on amphibians that are submitted live.

**Decomposed Carcasses** – Clearly decomposed carcasses may have some diagnostic usefulness for molecular testing and toxicological analyses. Very decomposed carcasses with fluffy growths of fungus on the skin; maggots in the mouth, vent, and body cavity; or carcasses of only skin and bones should be frozen and saved if fresher carcasses are not available.

## LABELS

Each container must be labeled. Paper labels written in pencil are preferred, especially if there is ethanol in any containers. Most ink will dissolve in ethanol or become streaked during freezing and thawing. Each label should have the following information:

- ➪ species
- ➪ date collected
- ➪ location (state/county/town)
- ➪ found dead or euthanized
- ➪ collector (name/address/phone)
- ➪ additional history on back of tag

## MAILING

**Shipping Container** – Use a picnic cooler or styrofoam-lined cardboard box.

**Ice** – Ice packs (blue ice) is preferred to wet ice to avoid leaking during shipment. Most amphibians from temperate climatic zones should be mailed with ice packs. Ice packs should be wrapped with about 5 layers of newspaper before being placed at the side of containers of amphibians. For live amphibians, position ice packs on the side of the shipping container, not under the specimens, as this allows live amphibians to move away from cold zones.

**Frozen Specimens** – Frozen samples should be mailed with dry ice. Ice packs are an alternative, especially if the ice packs were frozen in an ultra-low freezer (-40 or lower). Avoid mailing frozen specimens in the same shipping container as live animals or specimens in formalin. If frozen samples and live amphibians (or specimens in formalin) must be mailed in the same shipping container, never put dry ice in the shipping container. If frozen samples and live amphibians (or specimens in formalin) must be mailed in the same shipping container, separate the shipping container into two compartments with styrofoam panels and place the ice packs at one end of the container next to the frozen samples.

**Preserved Specimens** – Once specimens have fixed in a large volume of formalin or ethanol for 3-4 days, the preserved samples may be mailed in a minimal amount of preservative that prevents drying. It is not necessary to mail large volumes of liquid fixative. Preserved carcasses may be wrapped in gauze or a paper towel that is moistened with the fixative. If preserved specimens are transferred to plastic bags, always double-bag the specimen and pack it into the shipping box to avoid crushing the sample during transport.

**Packing the Shipping Container** – Plastic boxes and bags containing live amphibians may be stacked, but keep air holes clear; some plastic boxes will stack tightly on each other and may seal air holes of lower containers. Do not place live amphibians directly on top of ice packs, because this may cause water in the animal's container to freeze. After placing ice packs and specimen containers in the shipping box, add crumpled newspaper, plastic peanuts, or other filler around the containers to minimize shifting of contents during mailing and crushing the plastic-bag samples. If a styrofoam-lined cardboard box is being used for mailing, then line the box with a heavy mil plastic bag and place all ice packs and specimens into the bag to minimize leaks and moisture condensation into the cardboard box.

**Double Bagging** – Frozen samples and specimens in formalin (or ethanol) should be double bagged. This is especially important to avoid fixative leakage. If glass vials or jars must be mailed, these too should be placed into a plastic bag.

**Taping** – Tape should be wrapped completely across the lid, sides, and bottom of each plastic cooler in at least two places to prevent accidental opening of the container during mailing. Nylon-reinforced tape is recommended, but 2-inch-wide clear tape also may be used.

**Overnight Couriers** should be used for sick, live, and frozen amphibians.

**Dates for Mailing** – Only mail boxes of specimens by overnight couriers on Mondays, Tuesdays, and Wednesdays. Most diagnostic laboratories are not open on weekends, so specimens mailed on Fridays may be held in hot or freezing delivery vans over the weekend. A significant percentage of packages mailed by overnight courier on Thursdays, do not arrive in 24 hrs, and these can suffer the same fate.

**Mailing** – Overnight courier service should be used. Securely tape the cooler or box and mail to: National Wildlife Health Center, 6006 Schroeder Road, Madison, WI 53711. Note: in addition to the NWHC address, add DIAGNOSTIC SPECIMENS--WILDLIFE to the outside of the box. This label will direct coolers with specimens to our necropsy entrance. Do not label the container with statements like, "Live Animals," as this could interrupt or prohibit shipment because of courier policy. Contact NWHC (608-270-2400) (FAX 608-270-2415) prior to shipping animals by 1 day (overnight) service and after shipment to confirm the estimated time of arrival.

### QUARANTINE OF AMPHIBIANS

Amphibians (dead or alive) from a casualty site should be considered contagious specimens. Live, sick animals and carcasses should never be released or discarded at other sites and should not be taken into laboratory settings with other live amphibians, fish, or reptiles. Release of sick amphibians or discarding carcasses at other sites may result in the spread of infectious diseases.

## MALFORMATIONS

In certain parts of North America, particularly in the Midwest and northern New England, large numbers of malformed amphibians have been observed. Malformations involve missing or supernumerary digits, arms, or legs, missing eyes, and deformed jaws (Meteyer, 2000). Several hypotheses have been tested as causes, including parasite-induction during development (Morrell, 1999; Johnson and others, 2002), the effects of toxic chemicals (pesticides), and high levels of UV light; all have induced malformations under laboratory and field conditions. As with other environmental influences, however, it is possible that the malformations observed result from interactive causes. Much research is being directed toward understanding amphibian malformations.

Fortunately, no malformations of amphibians have been found in Great Smoky Mountains National Park. The U.S. Geological Survey has developed a standardized protocol for reporting and handling malformed amphibians (http://www.npwrc.usgs.gov/narcam/index.htm); should such individuals be found within the Park, these protocols should be followed.

# CONCLUSIONS

Concepts, problems, considerations, and approaches were outlined for establishing a monitoring program for the amphibians of Great Smoky Mountains National Park. The monitoring approach that is selected (which species will be monitored, where they will be monitored, how many sites will be monitored, and which techniques will be used) will be determined by the funding (and personnel) available and the specific objectives of Park managers. In this regard, a three-pronged approach to amphibian monitoring within the Park is presented in figure 47. The decision path is based on minimum, medium, and maximum levels of funding, although exact amounts are deliberately not specified.

**Decision Path for Monitoring Amphibians at Great Smoky Mountains National Park**

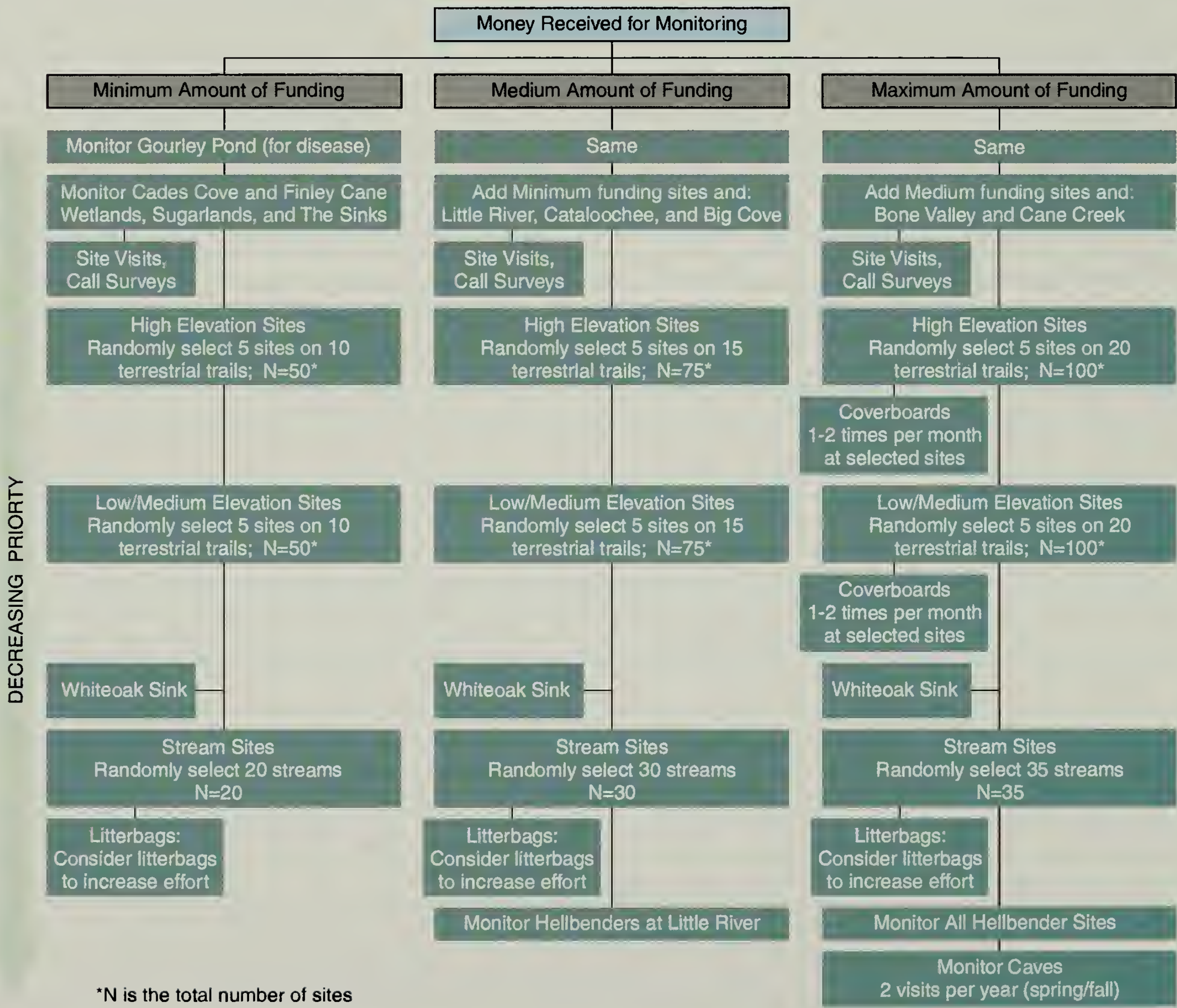



**Figure 47.** Decision path for helping design an amphibian monitoring program at Great Smoky Mountains National Park based on three levels of funding (see **Conclusion**).

***Minimum Funding***

1. In this and all tiers, Gourley Pond must be visited several times a year to monitor the effects of disease.
2. A minimum of two to three visits per year is specified for the Park's most critical wetlands. Three of the wetland sites (the Finley-Cane ponds, Sugarlands, the Sinks) are readily accessible by road; all of the sites in Cades Cove (Gum Swamp, Methodist Church Pond, Shields Pond, Stupkas Sinkhole Pond, Abrams Creek pools) could be visited easily in a single day. Nighttime call surveys would greatly increase the efficiency of wetland surveys in Cades Cove and elsewhere.
3. Time-constrained techniques could be used at the terrestrial and stream sites. If five sites could be visited per day, sampling these sites would take a two or three-person crew about 3 weeks to complete the data collection. Whiteoak Sink is singled out for sampling because of the presence of the Southern Zigzag Salamander *(Plethodon ventralis)* and because of all the readily accessible cave openings. Litterbags set early in the year could be checked easily throughout the season and thus record species that may be not encountered during stream time constraint sampling.

***Medium Funding***

1. In addition to the work considered above, the number of terrestrial and stream sampling sites could be increased.
2. Hellbenders should be monitored annually in the Little River.

***Maximum Funding***

1. In addition to the work considered above, the number of terrestrial and stream sampling sites could be increased further.
2. Coverboards could be used to increase long-term sampling effort at selected sites; they should be checked once or twice monthly.
3. Hellbenders should be monitored annually at all known locations in the Park.
4. Selected caves (Gregorys, Stupkas, the two Calf caves) should be surveyed thoroughly two or three times a year; other caves should be visited, especially in Whiteoak Sink, and the openings around the entrance and twilight zones searched for salamanders and frogs.

To increase sample size, the same terrestrial and stream sites need not be searched annually. For example, 50 terrestrial sites could be searched one year; a second 50 searched the second year; and a third 50 searched the third year, after which the cycle could be repeated. Unfortunately, however, there is a tradeoff with this approach. If a rotation is used, sample size is increased (a good thing), but the amount of time it takes to complete a cycle is greatly extended (12 years to get four samples per location). Amphibian populations may change dramatically in this amount of time, and trends could be missed or misinterpreted.

A rotating schedule could also be used to vary survey species or areas. For example, researchers might decide to alternate Hellbender and cave surveys every other year if money became limited. Or, Hellbenders could be monitored for 2 years in Little River, and at the other locations every third year. Planning is absolutely essential, and figure 47 is meant as a guide to approaches that might be considered rather than an absolute schedule.

## SUMMARY

In many regions of the world, amphibian species have inexplicably declined or disappeared, and serious malformations have been observed, particularly in the upper Midwest region of North America. Causes for the declines and malformations probably are varied and may not even be related. The seemingly sudden declines in many amphibians, however, suggests that a vigilant approach is necessary to monitor populations and to identify causes when declines or malformations are discovered.

In the United States, amphibian declines frequently have occurred in protected areas which should provide an ideal habitat against the most common causes of decline, habitat loss and changes in land use. In particular, declines in western National Parks have concerned biologists, resource managers, and legislators to the extent that Congress authorized the U.S. Geological Survey to set up a national amphibian monitoring program on Federal lands to develop the sampling techniques and biometrical analyses necessary to determine status and trends, as well as identify possible causes of amphibian declines and malformations when they are discovered.

Great Smoky Mountains National Park is the most visited park in the National Park Service system. It is also a center of salamander diversity in North America (with 31 species recorded historically) and contains a moderate number of frog species (13 species recorded historically). Because of this diversity, the Park was selected as a prototype amphibian monitoring location, and USGS biologists conducted intensive sampling throughout all regions and habitats from 1998 to 2001. This report presents the results of this intensive sampling, beginning with an overview of the Park's amphibians, the factors affecting their distribution, a review of important areas of biodiversity (particularly Cades Cove and the Cane Creek drainage), and a summary of amphibian life history in the southern Appalachians; it concludes with an extensive list of references for inventorying and monitoring amphibians.

As part of the project, a variety of inventory, sampling, and monitoring techniques were employed and tested. These included wide-scale visual encounter surveys of amphibians at terrestrial and aquatic sites, intensive monitoring of selected plots, randomly placed small-grid plot sampling, leaf-litterbag sampling in streams, monitoring nesting females of selected species, call surveys, and monitoring specialized habitats, such as caves. Coupled with information derived from amphibian surveys on Federal lands using various other techniques (automated frog call data loggers, PVC pipes, drift fences, terrestrial and aquatic traps), an amphibian monitoring program was designed to best meet the needs of biologists and natural resource managers within the Park after taking into consideration the logistics, terrain, and life histories of the species found within the 2,071 km$^2$ area of the Park. Each monitoring technique was described, including an example of how the technique was set up, what the results tell the observer, and limitations of the technique and the data derived from it.

Survey and monitoring projects are both time and labor intensive, and resource managers must make the best use of the resources available. For this reason, labor-intensive techniques, such as the use of drift fences with or without pitfall traps, and various types of trapping techniques which require continuous checking, are not recommended. Because only one species of frog (Cope's Gray Treefrog, *Hyla chrysoscelis*) is likely to be attracted to PVC pipe (as a hideaway), PVC is not recommended, particularly when the species of frog can more easily be detected by listening for calls or by employing automated frog call data loggers (AFCDL). AFCDL are effective at detecting frogs within Great Smoky Mountains National Park, but are best employed in areas with extensive wetlands, such as ponds within Cades Cove. An extensive guide is included as an appendix to this manual with instructions on the construction and deployment of AFCDL. Coverboards are not recommended because of

potential biases (in which species and age classes are observed) associated with sampling.

Extensive use of both small (10 x 10 m) and large (30 x 40 m) plots, either randomly sampled or "permanently" established, suggested that plot surveys are inefficient when compared with visual encounter (or time constraint) surveys. In addition, it is difficult to extrapolate counts obtained during plot surveys to actual amphibian abundance, despite efforts to standardize survey techniques, locations, and timing. Inasmuch as capture-recapture protocols are labor and time intensive, and that recapture rates are usually very low, capture-recapture surveys also are not recommended to park personnel.

The most consistent and effective survey technique to monitor amphibians within the Park, especially considering temporal, personnel, and logistic constraints, is to use visual encounter surveys based on repeated site visits. The use of leaf litterbags is also an effective nondestructive technique for determining the presence of secretive salamander larvae in streams. Data on presence (present/not detected), rather than abundance, is used to record a capture history for each species at each location. Thus, a data set is developed that, in practice, looks very much like the capture history of individuals in a typical capture-recapture study. By recording changes in these species' capture histories through time, biologists can determine detection probabilities for each species. Trends can be determined by changes in the percentage of area occupied by a species and by changes in detection probabilities. URLs for free, downloadable software are included in this report.

Amphibians in the Park should be monitored in a three-tiered approach, which will depend on the amount of funding available. With minimum funding, biologists should:

- Monitor Gourley Pond, where disease has been reported in the past,
- Monitor other Cades Cove wetlands and a few other wetlands with easy access,
- Use call surveys to record presence,
- Monitor 50 high-elevation sites (5 sites per each of 10 trails),
- Monitor 50 medium- to low-elevation sites (5 sites per each of 10 trails),
- Monitor amphibians in Whiteoak Sink, and,
- Monitor sites at 20 randomly selected streams (employ litterbags to increase sampling effort).

As funding levels increase, the number of sites monitored could be increased and species with specific habitat requirements (Hellbenders, *Cryptobranchus alleganiensis*; cave species) can be included. In all cases, visual encounter (or time constraint) survey techniques are recommended.

Because disease agents were found within the Park (iridovirus and fungus in several species at Cades Cove), biosecurity protocols must be employed after sampling each wetland within this region. All nets, boots, and equipment must be cleansed using a 10 percent bleach solution, and researchers should carry materials into the field which will allow them to process dead, dying, or live amphibians. Disease protocols and instructions for handling amphibians suspected of harboring disease were developed by the USGS National Wildlife Health Center, and are reprinted in this report.

Sampling a diverse amphibian assemblage in an area as large as Great Smoky Mountains National Park, and with limited physical access, is not an easy task. Randomization of sampling sites is not strictly possible, so some form of a stratified sampling paradigm must be employed. Depending on the amphibian species or community sampled, biologists must use trails, watersheds, hydrological units, elevation, or other parameters to narrow sampling focus. Ultimately, however, rarer species or those with specialized habits could be overlooked. Species identification also is challenging, and the use of experienced survey personnel is critical for obtaining factual data. In this regard, USGS and Park biologists must establish cooperative efforts and training to ensure that the congressionally mandated amphibian surveys are performed in a statistically rigorous and biologically meaningful manner, and that amphibian populations on Federal lands are monitored to ensure their long-term survival.

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# Appendixes I, II, and III

**Appendix I.** Location of selected wetland sampling sites

[Locations shown in latitude and longitude]

| | | |
|---|---|---|
| **Ponds** | | |
| Big Cove Beaver Pond | 35 30 27N | 83 18 02W |
| Bone Valley Beaver Pond | 35 31 07N | 83 40 51W |
| Finley-Cane Sinkhole Ponds (4) | 35 36 37N | 83 44 38W |
| Gourley Pond | 35 35 36N | 83 47 19W |
| Gum Swamp | 35 35 21N | 83 50 17W |
| Methodist Church Pond | 35 36 24N | 83 49 01W |
| Sewage Treatment Pond | 35 36 14N | 83 46 57W |
| Shields Pond | 35 35 33N | 83 48 54W |
| Stupkas Sinkhole Pond | 35 35 23N | 83 50 52W |
| **Swampy and mucky wetlands** | | |
| Cataloochee | 35 37 44N | 83 06 00W |
| Cataloochee Trout | 35 39 12N | 83 04 28W |
| Indian Creek | 35 28 51N | 83 24 51W |
| Little Cataloochee | 35 39 43N | 83 05 55W |
| Smokemont | 35 33 06N | 83 18 35W |
| The Sinks | 35 40 10N | 83 39 38W |
| **Woodland pools** | | |
| Abrams Creek | 35 35 40N | 83 50 41W |
| Big Cove Pool | 35 30 29N | 83 18 02W |
| Cane Creek | 35 39 01N | 83 53 15W |
| | 35 39 42N | 83 52 41W |
| | 35 39 07N | 83 53 05W |
| Gourley Sinkhole | 35 35 34N | 83 47 14W |
| Sugarlands | 35 41 11N | 83 32 17W |
| Tremont Roadside Ditches | 35 39 15N | 83 42 08W |
| | 35 39 07N | 83 41 47W |
| **Grassy pools (Cades Cove)** | | |
| | 35 36 19N | 83 47 44W |
| | 35 36 20N | 83 48 31W |
| | 35 36 03N | 83 48 33W |
| | 35 36 16N | 83 48 10W |

**Appendix II.** Example of a field data sheet prepared for amphibian surveys in Great Smoky Mountains National Park.

## GREAT SMOKY MOUNTAINS NATIONAL PARK
## AMPHIBIAN SURVEY FORM

**Date:**________________ **Site No**. __________ **Personnel** ____________________________

**Weather:** clear / partly cloudy / cloudy / rain / fog / other:_ ________________________________

**Altitude:**____________**ft/m** **Wind:** calm / slight breeze / moderate / windy

**General Location:** _ __________________________________________________________

**Specific Location (UTM):** E ______________; N ______________; Quad: _ __________________

**Start Time:** ___________ **Standing Water: Y / N** **Water Level:** ______________ **m**

**End Time:** _________ **AT:** _____; **WT:** ______; **ST:** ______; RH: ______; pH_____; **Cond** _ _____

**Habitat Type (circle one):** terrestrial / large stream / med. stream/ small stream / seep / pond / woodland pool / mucky area / open grassy pools / cave / rock face / other: ____ ____________________________

**Vegetation:** spruce-fir / deciduous / cove hardwood / oak / pine / hemlock / open field / other: _______

**Canopy:** open / sparsely covered / closed **Slope Aspect:**___________ **Drainage Dir:** _ ___________

| Amphibian Species | No. of Individuals | Life Stage | Method of Capture | Notes |
|---|---|---|---|---|
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |
| | | | | |

**Method of capture: tc= time constraint; ac= area constraint; em= egg mass count; nets= dip or sweep nets; PVC= pvc pipe; cb= cover board; ft= funnel trap; FL=frog logger; mt= minnow trap; pf= pitfall; tr= transect; V= visual; C= calling. Life Stage: A= adult, SA= subadult; L= larvae, E= eggs.**
**Invertebrates present: Y / N Species:**_____________________________________________
**Active Sampling Effort:** ____________________________________________________

**Further notes should be written on back of sheet**

**Appendix III.** Sampling equipment. Trademark names are mentioned for information purposes only, not as an endorsement of the U.S. Geological Survey.

## On the Trail

Weatherproof flat map case containing:

- USGS 7.5' topographic maps
- Great Smoky Mountains National Park trail guide
- Field Data Sheets (on Rite in the Rain® paper)
- Notebook
- Pencils/sharpener
- Copy of permits

Equipment (in a sealable rainproof bag):

- GPS
- Compass
- Clinometer
- Palm pilot (where appropriate for data entry)
- Digital temperature gauge
- Extra temperature gauge probe
- Digital relative humidity meter
- Camera, preferably digital
- Binoculars
- Pesola® spring scales (10 g, 50 g, 100 g)
- Ziploc® bags for carrying and weighing animals (various sizes)
- Clear plastic metric ruler
- Small mesh hand dip net (for larvae)
- Hand lens
- Stump ripper (Fuhrman® Diversified)
- Extra batteries
- Small pen light (for searching crevices)
- Leatherman® tool
- Fluorescent flagging

Biosafety:

- Ziploc® bags for dead or diseased animals (various sizes)
- Sanitary Handwipes
- Vinyl gloves (several pair)

Other:

- First-aid supplies
- Sting-eze® or other sting remedy, especially if anyone is allergic to yellowjackets
- Extra water
- Lightweight cell phone (may not work in valleys or remote areas)

## At Ponds (in addition to the above)

- Oxygen, pH, Conductivity meters
- Dip nets (both large and small of appropriate mesh size to capture larvae)
- Waders
- Shrimper (Lacrosse® Majesty 12") boots (excellent in small marshes and creeks)
- Tape measure (50 or 100 m) or laser range finder
- Meter ruler

Biosafety:

- Pre-mixed bleach solution in appropriately-sized tub or in a spray backpack firefighting pump

# Appendix IV

## Guidelines for building and operating remote field recorders (automated frog call data loggers)

*by*

William J. Barichivich

# Guidelines for building and operating remote field recorders (automated frog call data loggers)

*By* William J. Barichivich[1]

Automated frog call data loggers have been used successfully to provide information on: (1) species presence at the time of sampling (that is, species likely to be overlooked during time-constraint sampling can be recorded with greater reliability); (2) life history and phenology information, such as when frogs call (especially if different species call at different times of the day), what environmental influences affect calling; and (3) a relative index of the number of males calling. Although species can be easily identified, categorizing abundance may be very difficult in even moderately sized choruses because of call-overlapping interference. It is also often not possible to separate individual callers, allowing the possibility that a single calling male could be counted multiple times. Because environmental variables influence the number of animals calling, differences among abundance categories over time may be only reflective of differences in environmental conditions during sampling periods. Thus, call surveys using automated frog call data loggers must be conducted at multiple occasions during the potential breeding season. Further, call surveys tell nothing about the presence and number of females and non-breeding males, or whether reproduction was successful. Frog call surveys using automated data loggers are best implemented where researchers have limited access by road or when rare species are suspected.

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## What is an automated frog call data logger?

Automated frog call data loggers are recorders that can be programmed to operate for a specified duration at specified intervals (for example, one minute every hour) and over a specified period (for example, 18:00 until 06:00). They can operate remotely without maintenance for extended periods under most environmental conditions, including extreme heat, cold, rain, and snow. The automated frog call data logger described in this section is a conglomeration of stand-alone components, whereas the original design (Peterson and Dorcas 1992, 1994) required building several components on a printed circuit board or using an expensive commercial data logger to control the tape recorder. The literature regarding previous designs is helpful and should be reviewed not just for construction details but also for study design (see related literature).

## Why build an automated frog call data logger?

Automated frog call data loggers produce an archivable record that can be analyzed or confirmed at a later date. Unlike standard aural surveys, no observers are present, so the behavior of calling anurans is likely unaffected. Since automated frog call data loggers can be deployed prior to monitoring, they can synchronously monitor any number of sites 24-hrs/day.

## How do you build an automated frog call data logger?

The basic automated frog call data logger consists of an analog tape recorder, timer(s), power source (battery) and voltage step-down, container, and microphone (figs. 1-2). The battery powers the timer(s) that regulate power, also from the battery, to the tape recorder. The tape recorder, timer(s), battery and voltage step-down are housed in a weatherproof container and an external microphone picks up nearby sounds (for example, frog calls) and relays the signals to the tape recorder in the container (fig. 1).

### Construction

1. *Parts*-gathering materials can be time consuming. Vendors are often out of stock and no single source carries all the necessary components to build an automated frog call data logger (table 1).
   a. A wide range of analog **tape recorders** have been utilized and can range in price from tens to hundreds of dollars. Since the recorder is the heart of the system, consider the highest quality recorder within reason. The following features are highly desirable:
      (1.) **Stereo recording** provides left and right channel recording.
      (2.) **Extended record time** slows the speed of the tape so less tape is used to record a given interval.

**Continuous auto-reverse** changes the tape head direction after one side of the tape has been used. This avoids the need for a researcher

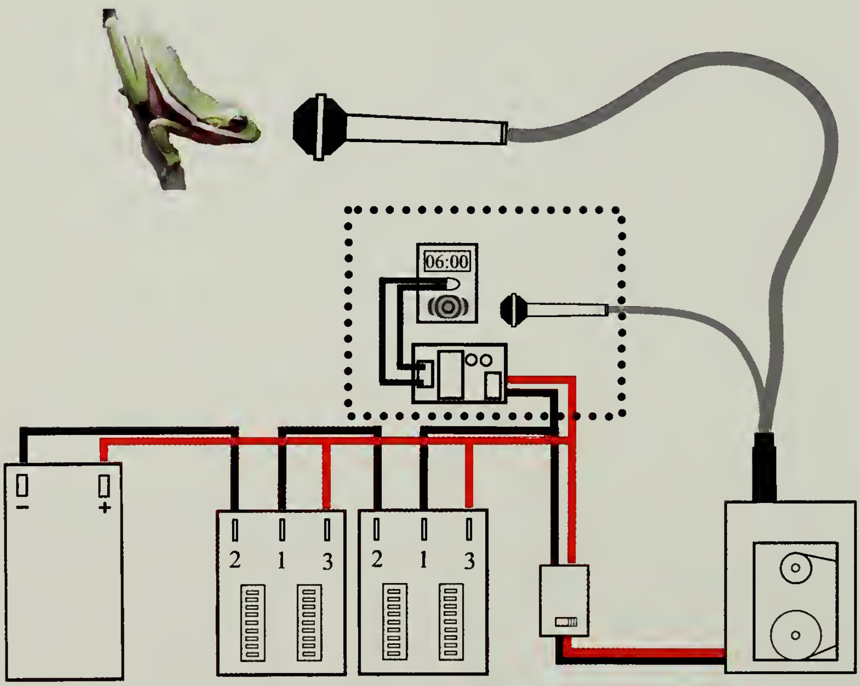


**Figure 1.** Wiring schematic for an automated frog call data logger. The components within the dotted box are required only for the voice time stamp.

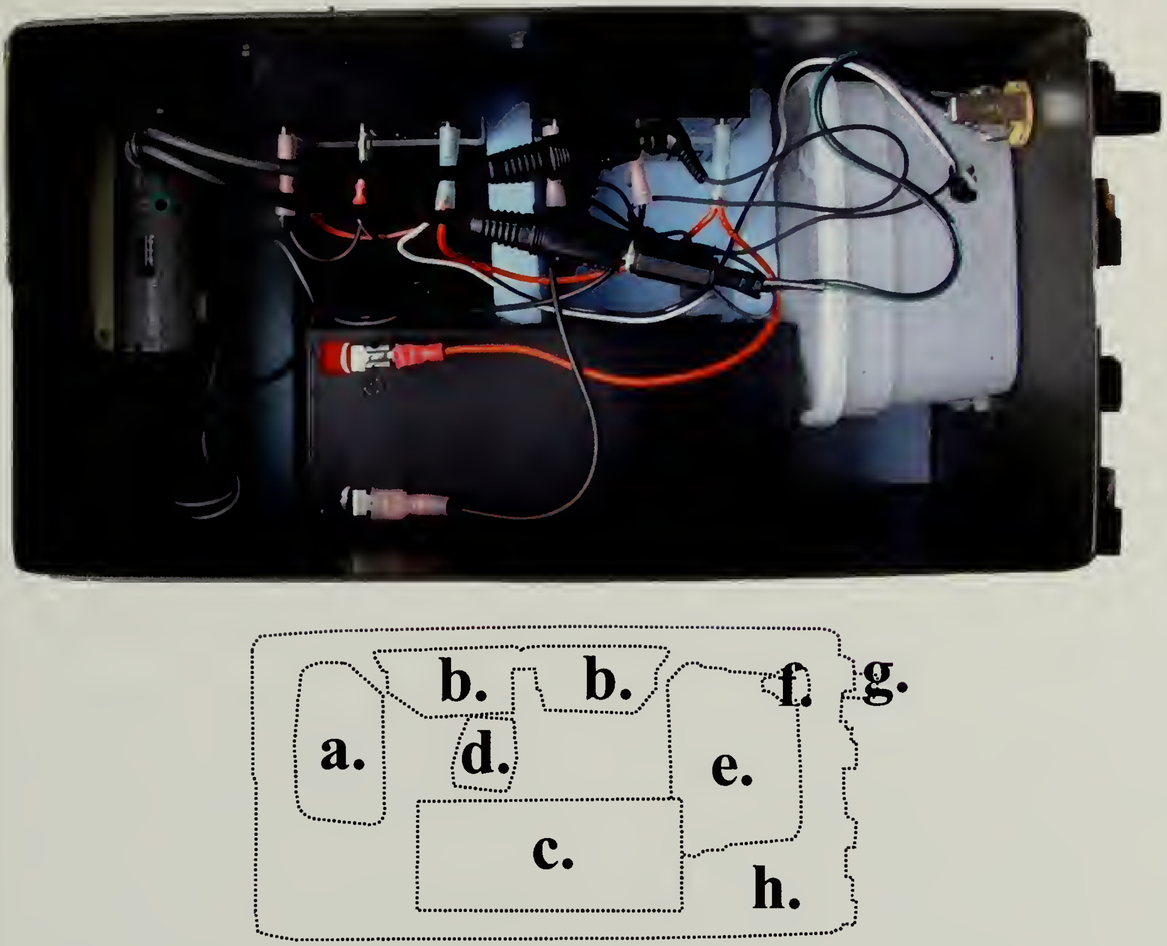


**Figure 2.** Example of the interior layout of an automated frog call data logger; a.) analog tape recorder, b.) two solid state recycle timers, c.) 12v, 7amp sealed lead acid battery, d.) voltage regulator, e.) voice stamp assembly, f.) ¼" microphone female jack, g.) ¼" male microphone jack on microphone cable, h.) 50-caliber ammunition can.

**Table 1.** Primary components used in the sample automated frog call data logger

| Items needed for each unit | Model | Approximate Price |
|---|---|---|
| Tape recorder | SONY TCS-60DV Pressman | 120 |
| Microphone | Shure Omnidirectional Dynamic | 50 |
| Recycle timer (Hour/minute) | SSAC RS1A34 | 55 |
| Recycle timer (Minute/second) | SSAC RS1A12 | 55 |
| 12-volt battery | 7Amp SLA | 15 |
| DC power converter | Cigarette lighter adapter | 10 |
| Container | 50-caliber ammunition can | 5 |
| Voice-time stamp | Keychain voice clock | 10 |
| | Timer/relay | 20 |
| | Microphone[1] | 0 |
| **Total** | | $340 |

[1]The secondary microphone used for the voice-time stamp was included with the tape recorder.

to flip tapes over before the first side is spent. This feature varies from standard auto-reverse in that continuous auto-reverse functions while the tape recorder is in record mode and standard operates only in play mode.

b. One or two 12-volt **timer**(s) are needed to run each automated frog call data logger. If the automated frog call data logger is intended to sample continuously (24-hr/day), then a single (minute/second) timer is necessary. If a specific period within a day is desired a second (hour/minute) timer is required. Solid-state encapsulated recycle timers have been widely used in automated frog call data loggers. These timers are programmed by adjusting two series of binary switches, one series for "ON" time and the other series for "OFF" time. The programmer must make absolutely sure the combined "ON" and "OFF" times equal 1 hour for the minute/second timer and 24 hours for the hour/minute timer. Greater detail regarding timer programming and technical data are available at the supplier's website (www.ssac.com). Other types of timers (555, BioQuip 12v DC timer) are available and have been used with success but require advanced knowledge of electronics, are less flexible to program, and can be less reliable.

c. Any single or combination of **batteries** totaling 12 volts will suffice. The greater the amperage the longer the automated frog call data logger can operate without changing or replacing the batteries. Rechargeable batteries are recommended including the 12-volt, 7-amp sealed lead acid battery (SLA) illustrated in the sample automated frog call data logger (fig. 2). If multiple batteries are used, a battery holder is recommended. No batteries are used in the tape recorder as the main battery powers the entire unit.

d. Although the recycle timers run on 12v, tape recorders typically require 3 to 6v DC power. Rather than build a **voltage step-down**, this design uses an automotive cigarette lighter adapter capable of converting from 12v to 9, 7.5, 6, 4.5, and 3v. The DC power input of the cigarette lighter adapter can be modified by cutting off the cylinder and contacts and splicing the timer outputs directly to the adapter input leads. The power adapter output will be connected to the external power jack or directly to the battery connections of the tape recorder. Attaching the power directly to the tape recorder battery terminals provides a more reliable unit than using the external power jack due to the small surface area of the external jack. This connection can easily be made by building insulated dummy batteries with the power connections at the ends (fig. 3).

e. The main purpose of a **container** is to protect the electronics from the elements in any easy-to-transport package. Figure 3 shows a very economical (< $5) surplus 50-caliber ammunition can. Alternatives include plastic pails, toolboxes, tackle boxes, and Otter® or Pelican® cases.

f. **Microphones** should be omni-directional and should not require an additional power source. In most cases monaural models are the only choices given these criteria, but they work adequately. Superior recordings are possible with DC powered stereo microphones, although the relatively short battery life can increase the maintenance schedule of the data loggers. The microphone cable can be passed through a port placed in the container or a microphone jack can be installed in the side of the container. Microphones can be shielded from the elements by placing them inside a cut-off plastic soda bottle. Additionally, foam can be placed between the bottle and the microphone head to reduce wind noise but this can introduce moisture wicking.

g. Most tape recorder manufacturers recommend **tapes** no longer than 90-minutes, because tapes of greater length are too thin and stretch under the tension of

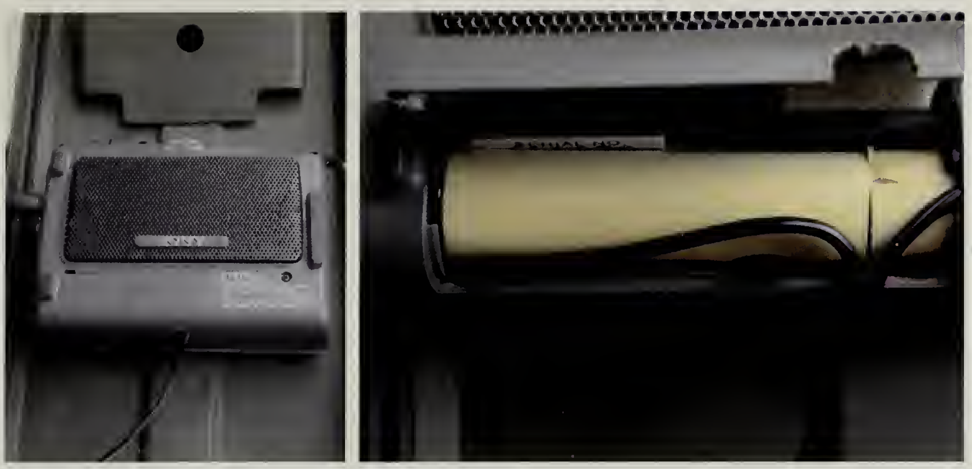

**Figure 3.** Example of a dummy battery, an external power jack alternative. The battery door on this tape recorder has been notched to allow clearance for the wires. Wooden dowels (3/8", 0.9525cm) were used as insulators between the battery terminals.

recording, thus reducing recording quality and reliability.

h. An optional **voice-time stamp** is highly recommended especially if calling phenology is of interest. This feature allows the researcher to know the time of each recorded interval with reasonable precision and makes reviewing the tapes considerably more easy. A voice-time stamp is made by triggering a talking clock at the same time the tape recorder is activated. This is accomplished by splitting the timer output to both the tape recorder and a relay that triggers the voice clock. To prevent the voice clock from continuously announcing the time during a recording, the relay must be supplied power only briefly at the beginning of the recording period. This can be accomplished by building a binary logic circuit or by using a timer. A second microphone picks up the announcement of the voice clock while the tape recorder records signals from both the primary external microphone and this smaller secondary internal microphone. The internal microphone should be placed away from the tape recorder and near the voice clock. In the example data logger, the voice clock, secondary microphone, and relay are housed in their own container and are at the opposite end of the can from the tape recorder (figs. 2-4).

2. *Tools*
   a. **Wire cutters** for cutting rolls of wire into shorter lengths.
   b. **Wire strippers** for removing the insulation from the ends of the wires.
   c. **Wire crimps** are necessary for making wireless connections.
   d. A **drill** or **Dremel®** can be useful for making modifications to the container.
   e. A **soldering iron** is necessary for making solder connections and should be used to prepare multistrand wire for solderless connections.
   f. A **multimeter** can be very helpful in troubleshooting connections as well as checking and maintaining batteries.
   g. A **12v automotive battery charger** can be used to charge a single battery or to run a bank-charging system (fig. 5). If a bank-charger is used, each battery should be individually fused and the fuse rating should be less than the maximum amperage of the battery and greater than the charge amperage. This will allow the batteries to be charged without blowing fuses. If a battery does short, however, it will blow only its fuse.

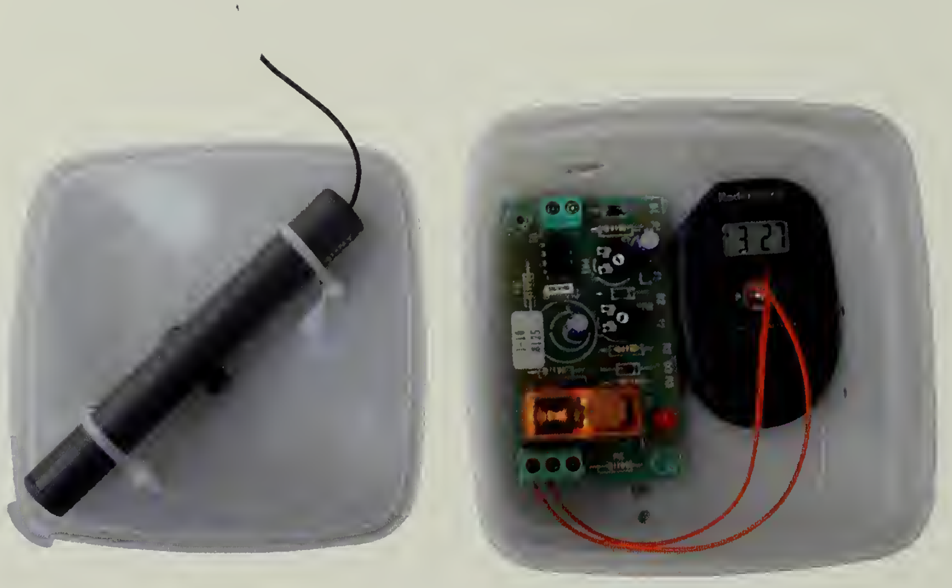

**Figure 4.** Example of a voice stamp assembly. The voice clock (far right) and timer/ relay are secured in a plastic food container and the microphone is mounted to the lid. Enclosing the assembly in its own container helps isolate the microphone from the sound of the tape recorder running.

3. Consumable materials
   a. Stranded hook-up **wire** (18AWG). Multiple colors can be helpful to prevent confusion in polarity.
   b. Female **terminal connectors** (1/4")
   c. Light-duty 60/40 rosin core **solder** (0.050" diameter).
   d. **Heat-shrink tubing** or **liquid tape**.
4. Assembly
   a. Charge all the batteries if using rechargeable batteries.
   b. Make any modifications to the container that may be necessary (for example, drilling holes for ports or jacks) and install the appropriate hardware.
   c. Program all timers to the desired schedule.
   d. Dry fit all the individual components in the container to determine the best placement and layout.
   e. Build the wiring loom to accommodate the location of the components.
   f. Outside the container, attach the wiring loom to all the components except the battery.
   g. Connect the battery to test the unit and make necessary corrections until the unit operates.
   h. Disconnect the battery and transfer the partially assembled unit into the container.
   i. The automated frog call data logger is now ready for use.

## Setup

1. **In the lab or office**
   The timers begin cycling when power is applied; therefore, make the power connections at the time the automated frog call data loggers are intended to begin recording. The voice clock should announce the correct time and the LED on the voltage step-down should remain on for the duration of the

**Figure 5.** Example of a six battery bank charger. Each battery is individually fused to prevent catastrophic failure should an accidental short occur.

programmed recording interval. In the example automated frog call data logger the batteries would be turned on at 06:00. At this point the timers would be cycling but the unit will not record until the record button is depressed in the field. This step should be performed the day before field deployment. Make sure the tape recorder is turned off. Label and insert a cassette tape into the recorder. Make sure the tape is rewound, on side "A," and the tape recorder, if it has auto-reverse, is set in the correct direction. If there is any interruption of power to the timers they will reset to the time the power was reapplied.

2. **In the field**
   Depress the record button on the tape recorder, connect the microphone, and close the case. The unit will not begin to record until the time the timers were started the preceding day. The microphone should be secured to woody vegetation or to a microphone stand if there is no structure available. It is important to place the microphone near the breeding site but the main unit can be placed anywhere the microphone cord can reach, which should be a secure site, away from possible flooding or vandalism. The data loggers can be locked closed and secured a tree or other sturdy object. Notify managers as to the location and appearance of the data loggers, because they could be easily mistaken as an explosive device (fig. 6). Tapes can be changed in the field, but it is not practical to change batteries without retrieving all the units.

### 3. Listening to tapes

Tapes can be reviewed at any time, and depending on the number of units deployed, it is easy to accumulate a backlog of tapes. Tape review should be conducted in a quiet area with as few distractions as possible. Listening requires about twice the recording time. All observations should be recorded on a data sheet (see fig. 7 for a sample data sheet). Important data fields should include the site, recording dates, time of each interval, and species calling. Additional data could include NAAMP call rank (fig. 7). Observations of sounds other than frog calls (for example, rain falling on the microphone, aircraft) can be useful in the interpretation of the tapes and should be noted.

**Figure 6.** Automated frog call data logger setup near a small pond.

### Tips

- Twist and tin (apply small amount of solder) all stranded wires before crimping if using solderless connectors, and use heat-shrink tubing to cover and protect all solder and crimp joints. This greatly improves the reliability, durability and longevity of the data logger.
- Start by building a single prototype unit and after all the "bugs" have been worked out, use an assembly line technique to speed the process of building the others.
- Consider the research questions/objectives before programming and deploying your data loggers. Listening to the tapes can be very time consuming, so recording time should be minimized while still meeting research goals.
- Use high quality headphones that completely cover the listeners' ears for reviewing the recorded material. While doing so, try not to multitask, because it can be easy to overlook a call if the listener is distracted.
- If recordings were made using an extended record time feature, then playback must be performed on a like unit. This could require purchasing additional tape recorders to review the tapes.

| Okefenokee NWR Frog Logger Data Sheet | | | | |
|---|---|---|---|---|
| Site Name/Number: ____________ | | | Dates: ____________ | |
| Logger Number: ____________ | | | Listener 1: ____________<br>Listener 2: ____________ | |
| Int/Hour | Night 1 | Night 2 | Night 3 | Night 4 |
| 1/ | | | | |
| 2/ | | | | |
| 3/ | | | | |
| 4/ | | | | |
| 5/ | | | | |
| 6/ | | | | |
| 7/ | | | | |
| 8/ | | | | |
| 9/ | | | | |
| 10/ | | | | |
| 11/ | | | | |
| 12/ | | | | |
| 13/ | | | | |
| 14/ | | | | |
| ** include species heard and NAAMP calling codes at each interval in parentheses<br>0= no frogs can be heard calling; 1= individual calls not overlapping; 2= calls are overlapping; but individuals are still distinguishable; 3= numerous frogs can be heard; chorus is constant and overlapping | | | | |
| Notes: | | | | |

**Figure 7.** Sample data sheet used to review automated frog call data logger tapes from Okefenokee National Wildlife Refuge.

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Mazanti, L.E.,1999, The effects of atrazine, metolachlor and chlorpyrifos on the growth and survival of larval frogs under laboratory and field conditions: Unpublished Ph.D. dissertation, University of Maryland.

Peterson, C.R. and Dorcas, M.E., 1992, The use of automated data-acquisition techniques in monitoring amphibian and reptile populations, *in* McCullough, D.R., and Barrett, V., eds., Wildlife 2001: Populations: London, Elsevier, Applied Science, p. 369-378.

Peterson, C.R., and Dorcas, M.E., 1994, Automated data acquisition, *in* Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C., and Foster, M.S., eds., Measuring and monitoring biological diversity: Standard methods for amphibians: Washington, DC, Smithsonian Institution Press, p. 47-57.

Rand, A.S., and Drewry, G.E., 1994, Acoustic monitoring at fixed sites, *in* Heyer, W.R., Donnelly, M.A., McDiarmid, R.W., Hayek, L.C., and Foster, M.S., eds., Measuring and monitoring biological diversity: Standard methods for amphibians, Washington, DC, Smithsonian Institution Press, p. 151-152.


## RELATED WORLD WIDE WEB SOURCES

http://www.uga.edu/srelherp/ecoview/Eco19.htm
http://www.parcplace.org/education/techniques/froglogger.htm
http://www.bedfordtechnical.com/index.htm
http://www.bio.davidson.edu/people/midorcas/research/StResearch/Cocklinetal00/Cocklinetal.htm
http://www.bio.davidson.edu/people/midorcas/research/StResearch/Briggs99/scottprop.html
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(315) 638-0333 (fax)
http://www.ssac.com

# PLATES

# *Amphibians*

A — Marbled Salamander (*Ambystoma opacum*). Stupka's Sinkhole Pond, Cades Cove.

B — Spring Salamander (*Gyrinophilus porphyriticus*). Andrews Bald.

**PLATE 1**


Shovel-nosed Salamander (*Desmognathus marmoratus*). Mill Creek, Cades Cove.


Jordan's Salamander (*Plethodon jordani*). Clingmans Dome.

**PLATE 2**

A

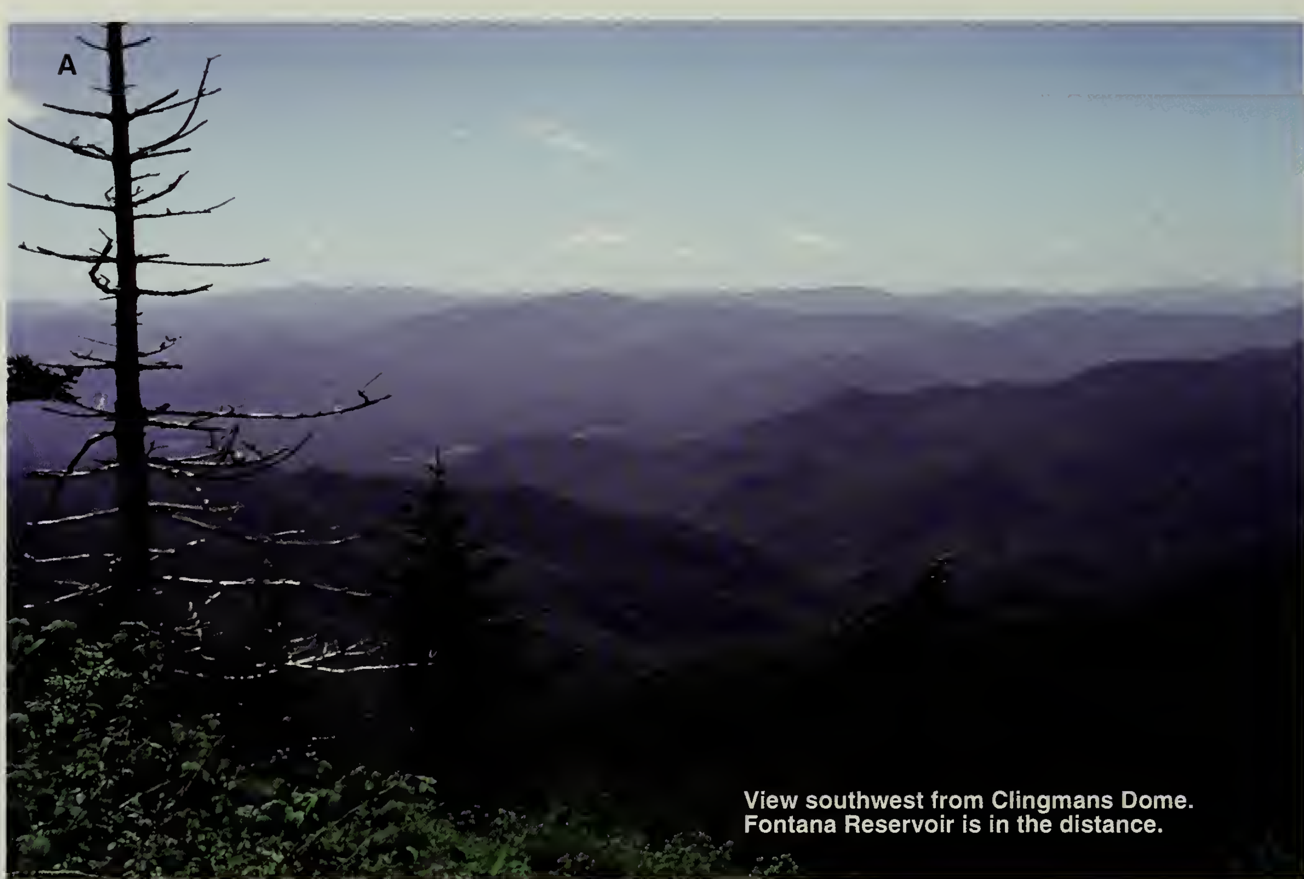
American Toad (*Bufo americanus*). Cades Cove.

B

Wood Frog (*Rana sylvatica*). Gregorys Cave, Cades Cove.

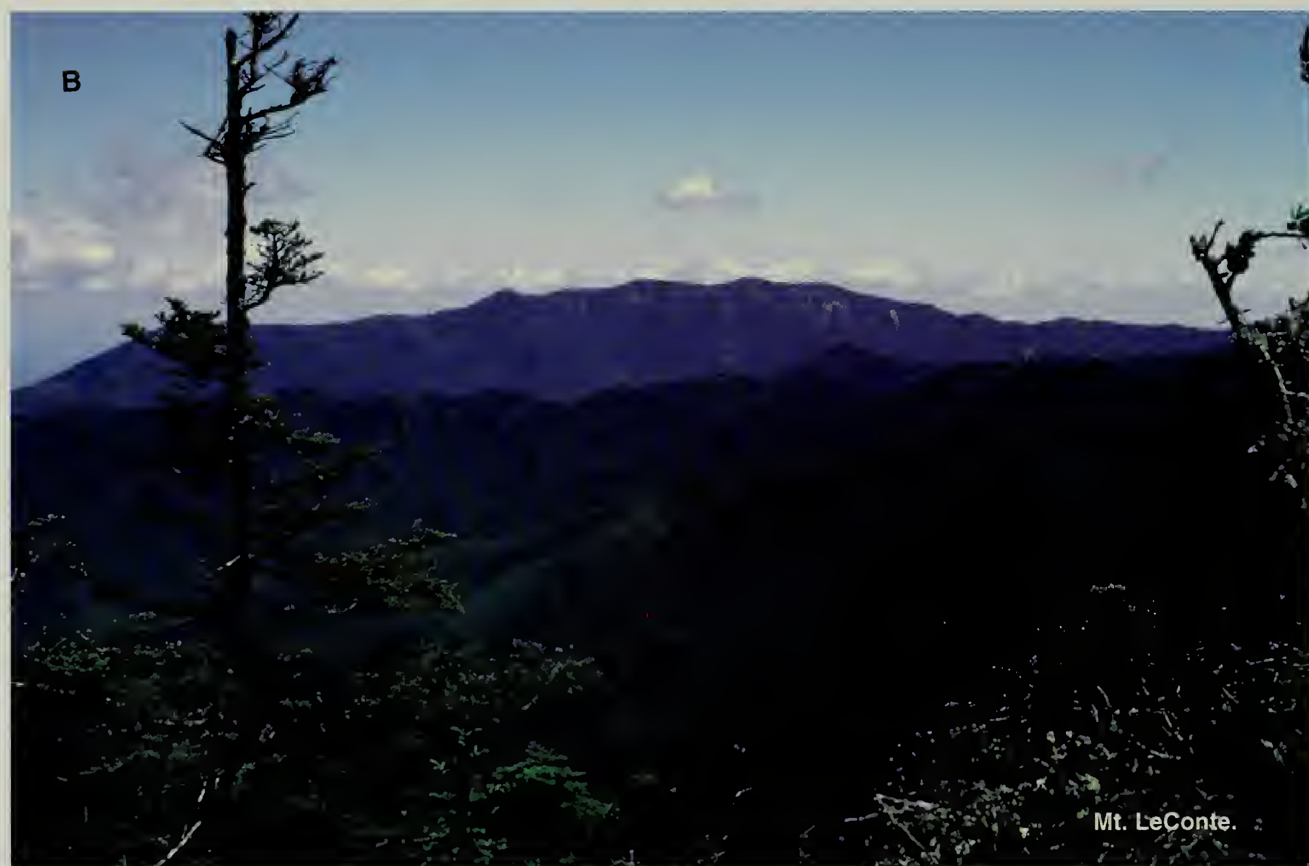
**PLATE 3**

# *Habitats*



A

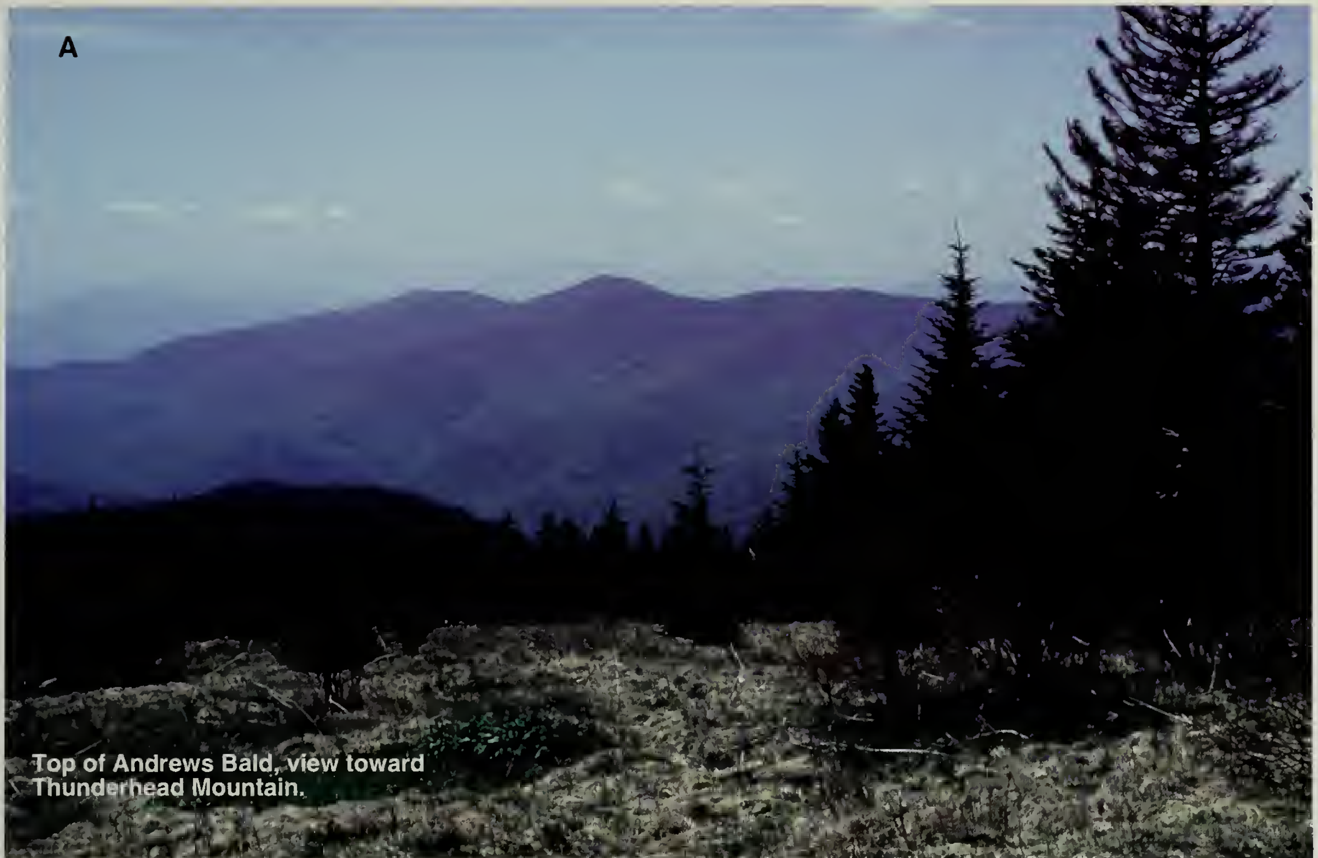
View southwest from Clingmans Dome. Fontana Reservoir is in the distance.



B

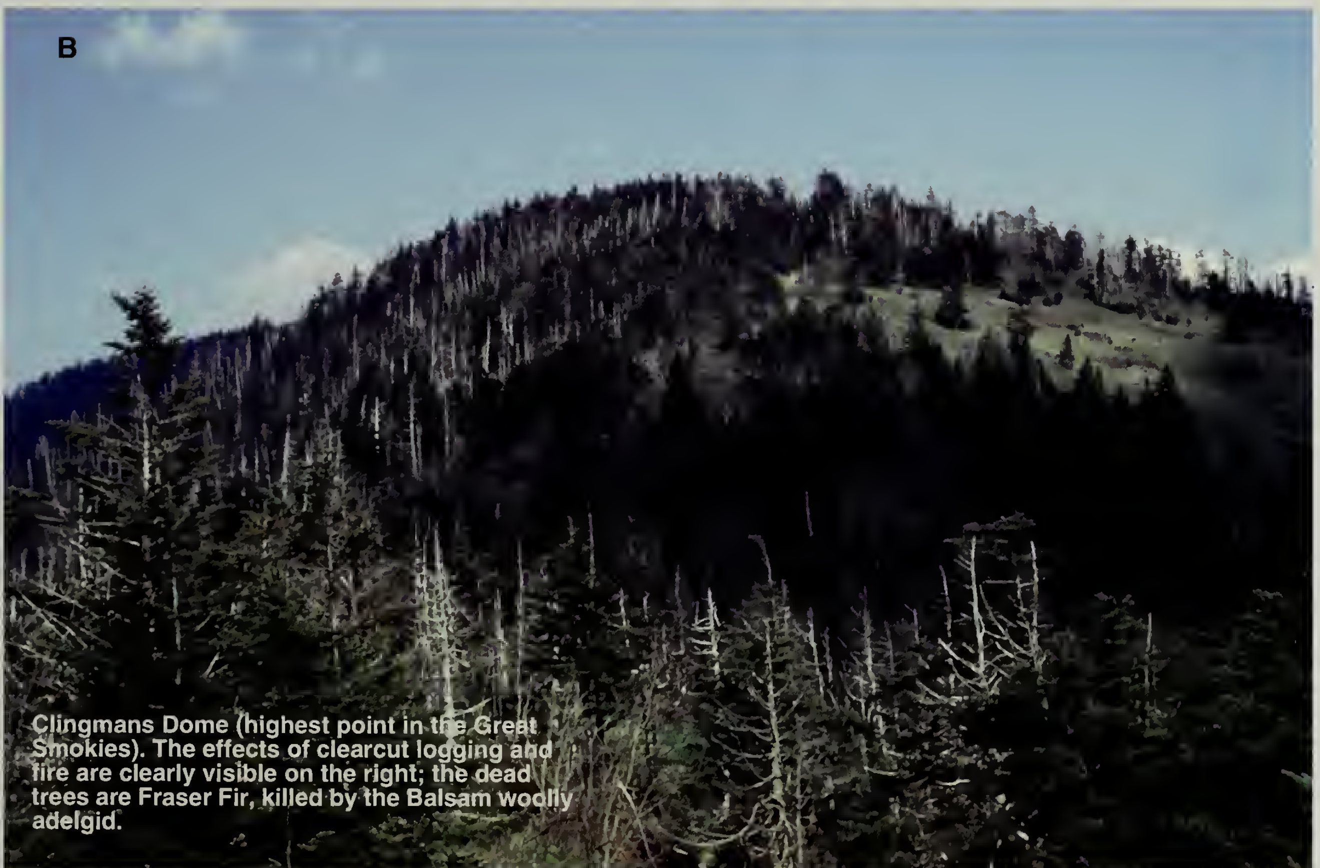
Mt. LeConte.

**PLATE 4**



A

Top of Andrews Bald, view toward Thunderhead Mountain.



B

Clingmans Dome (highest point in the Great Smokies). The effects of clearcut logging and fire are clearly visible on the right; the dead trees are Fraser Fir, killed by the Balsam woolly adelgid.

PLATE 5

A

Cades Cove, view to southeast.

B

Cades Cove, view toward Gregorys Bald (hidden in clouds). Friendly resident.

PLATE 6

# *Larvae and Tadpole Mouthparts*

A


Spotted Salamander (*Ambystoma maculatum*)

B


Marbled Salamander (*Ambystoma opacum*)

C


Mole Salamander (*Ambystoma talpoideum*)

**PLATE 7**

A


Hellbender (*Cryptobranchus alleganiensis*)

B


Spotted Dusky Salamander (*Desmognathus conanti*)

C


Imitator Salamander (*Desmognathus imitator*)

D


Shovel-nosed Salamander (*Desmognathus marmoratus*)

E


Seal Salamander (*Desmognathus monticola*)

**PLATE 8**

A


Ocoee Salamander (*Desmognathus ocoee*)

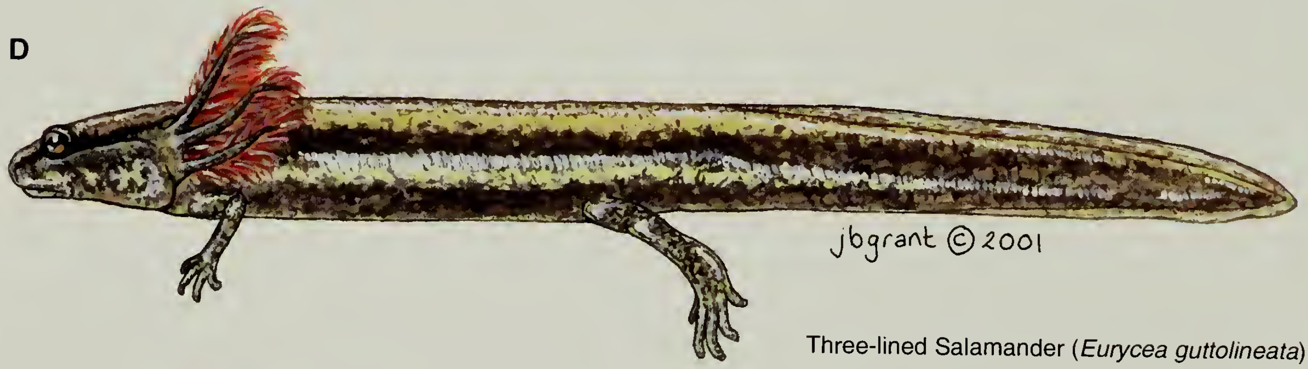
B


Black-bellied Salamander (*Desmognathus quadramaculatus*)

C


Santeetlah Salamander (*Desmognathus santeetlah*)

D




Three-lined Salamander (*Eurycea guttolineata*)

E


Junaluska Salamander (*Eurycea junaluska*)

**PLATE 9**

**A**


Long-tailed Salamander (*Eurycea longicauda*)

**B**


Cave Salamander (*Eurycea lucifuga*)

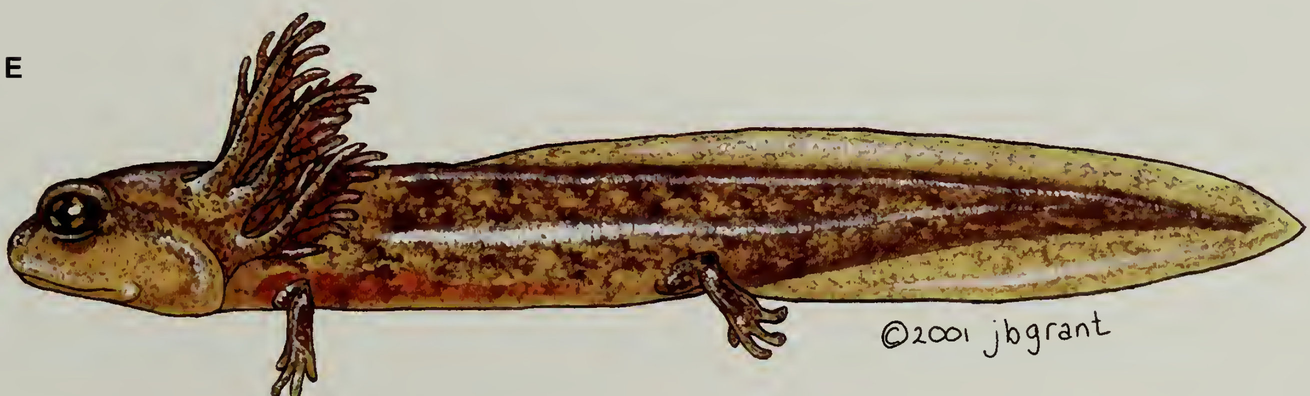
**C**


Blue Ridge Two-lined Salamander (*Eurycea wilderae*)

**D**


Spring Salamander (*Gyrinophilus porphyriticus*)

**E**




Four-toed Salamander (*Hemidactylium scutatum*)

**PLATE 10**

A


Common Mudpuppy (*Necturus maculosus*)

B


Eastern Red-spotted Newt (*Notophthalmus viridescens*)

C


Mud Salamander (*Pseudotriton montanus*)

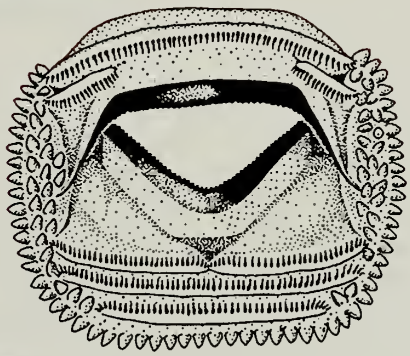
D


Black-chinned Red Salamander (*Pseudotriton ruber*)

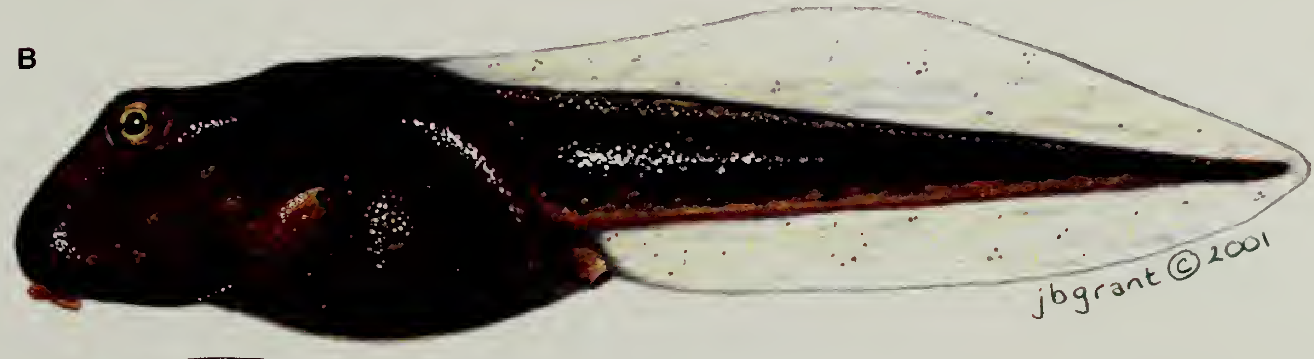
**PLATE 11**

A

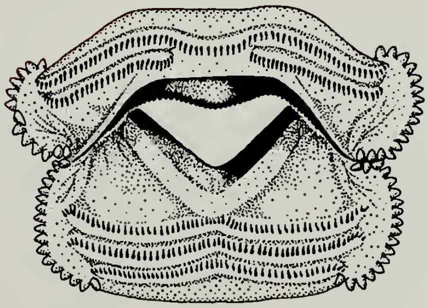

Northern Cricket Frog (*Acris crepitans*)



B




American Toad (*Bufo americanus*)

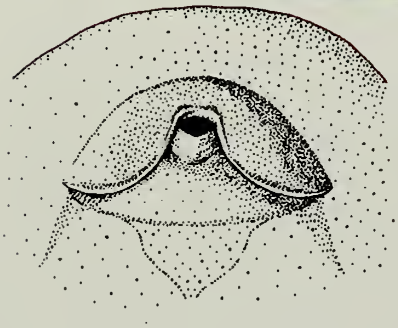


Fowler's Toad (*Bufo fowleri*)

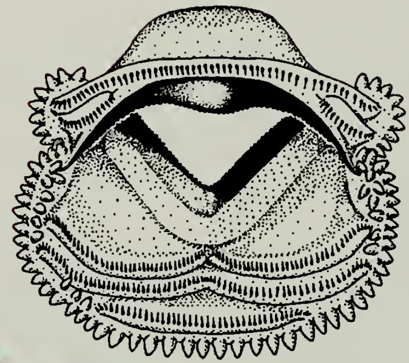
C


**PLATE 12**

A

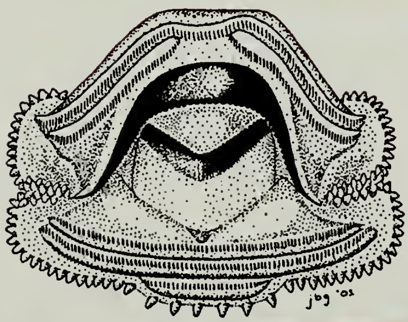



Eastern Narrow-mouthed Toad
(*Gastrophryne carolinensis*)



Cope's Gray Treefrog (*Hyla chrysoscelis*)

B


Spring Peeper (*Pseudacris crucifer*)

C

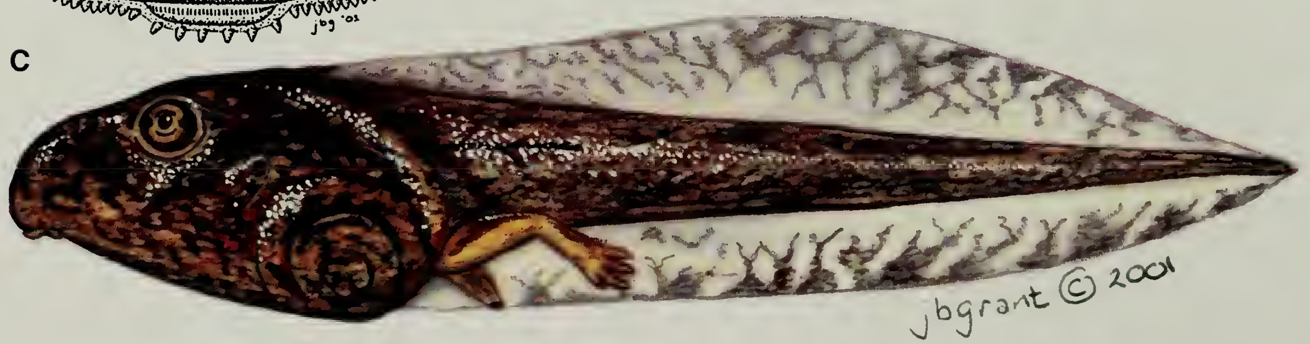
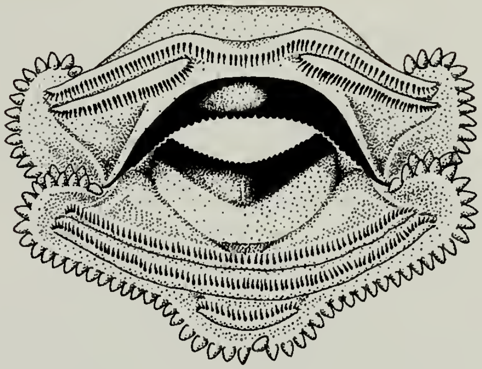
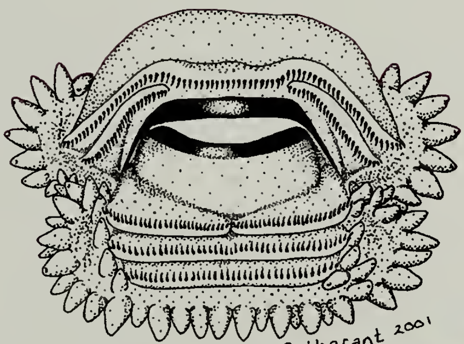



**PLATE 13**

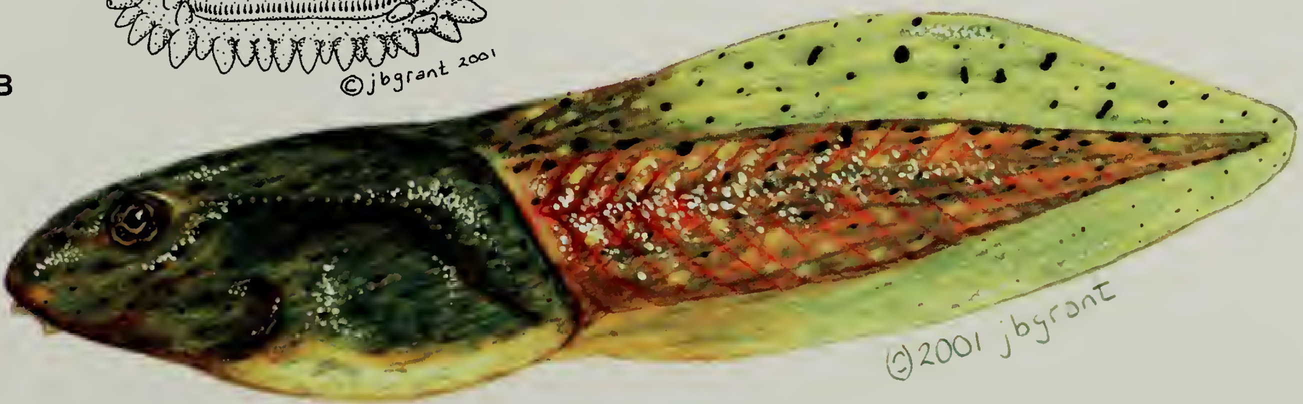
**A**


Upland Chorus Frog (*Pseudacris feriarum*)






**B**

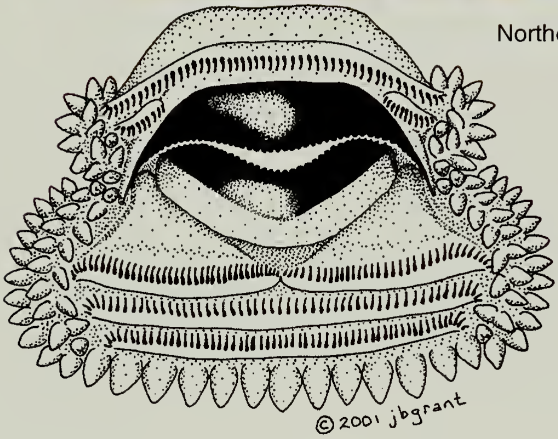


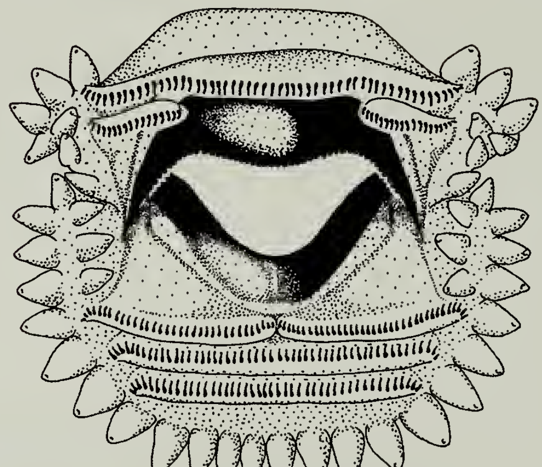

American Bullfrog (*Rana catesbeiana*)

**PLATE 14**

**A**


Northern Green Frog (*Rana clamitans*)



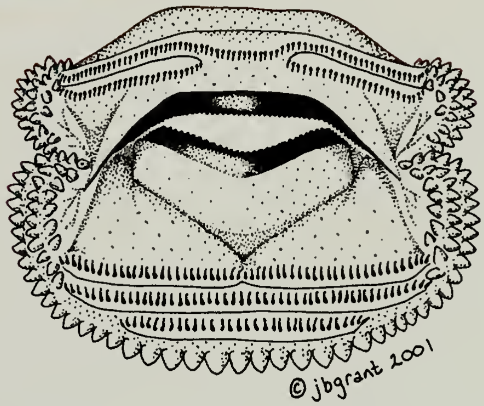
Pickerel Frog (*Rana palustris*)

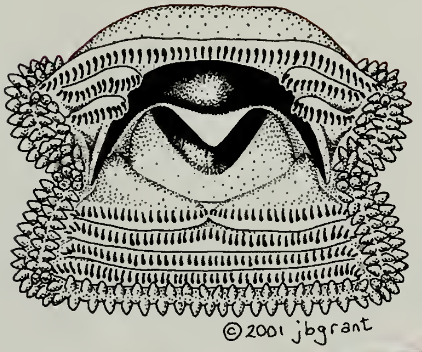
**B**


**PLATE 15**

A

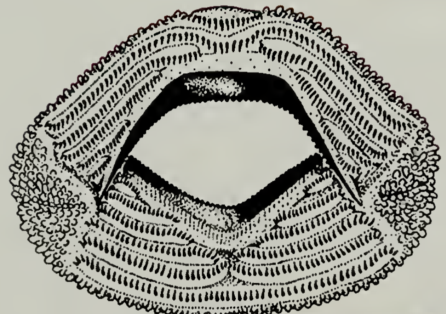

Northern Leopard Frog
(*Rana pipiens*)




Wood Frog
(*Rana sylvatica*)

B

Eastern Spadefoot (*Scaphiopus holbrooki*)

C


PLATE 16

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