MONITORING PROGRAMS

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. Species and life stages are sometimes difficult to distinguish. 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. Amphibians have complex life cycles. 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. In the field, detectability of amphibians is likely influenced by the following variables, to a greater or lesser extent, depending on species. 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. <u>Species and populations occur in a landscape</u>. 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. <u>The human-based constraints on sampling, inventorying, and monitoring amphibian populations on Federal lands must to be considered at the outset.</u> These include:

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

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

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

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

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.

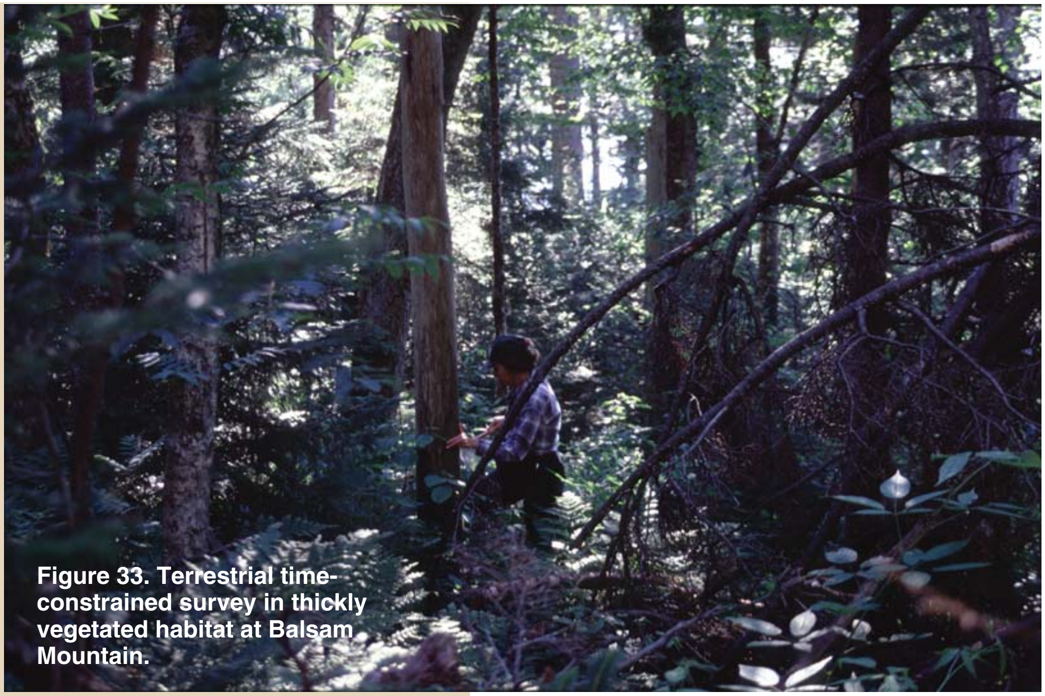


Figure 33. Terrestrial time-constrained survey in thickly vegetated habitat at Balsam Mountain.

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.

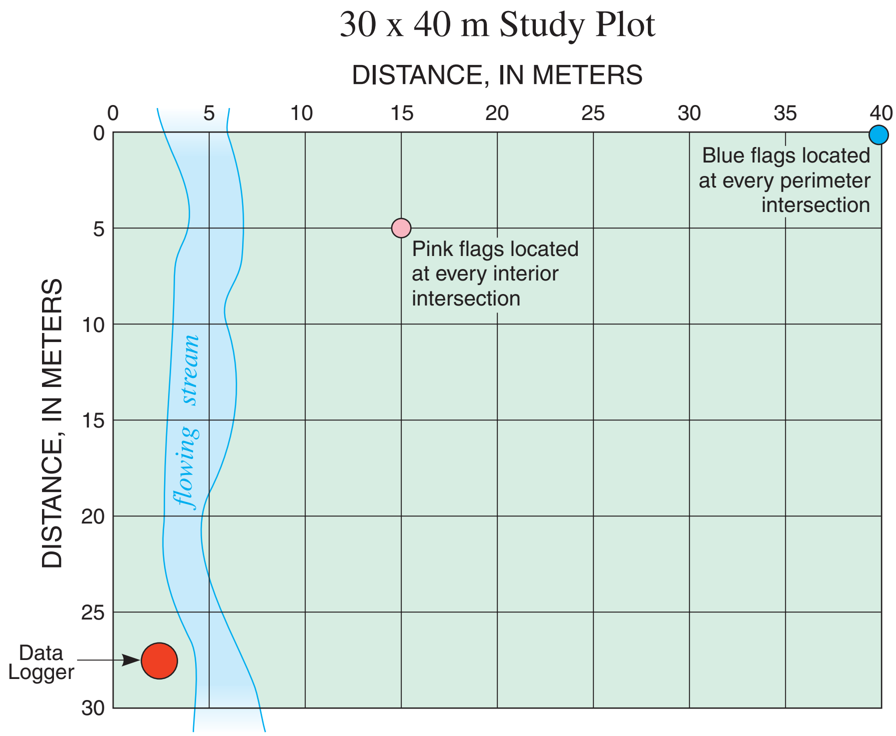
Example. Two persons search Gourley Pond for 67 minutes. The sampling effort is 2 x 67 = 134 person-minutes. Sample data might be: larval *A. opacum* (> 50 observed), 14 egg masses of *A. maculatum*, larval *R. sylvatica* (hundreds of tadpoles), 4 *P. crucifer* heard calling.

☐ **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.



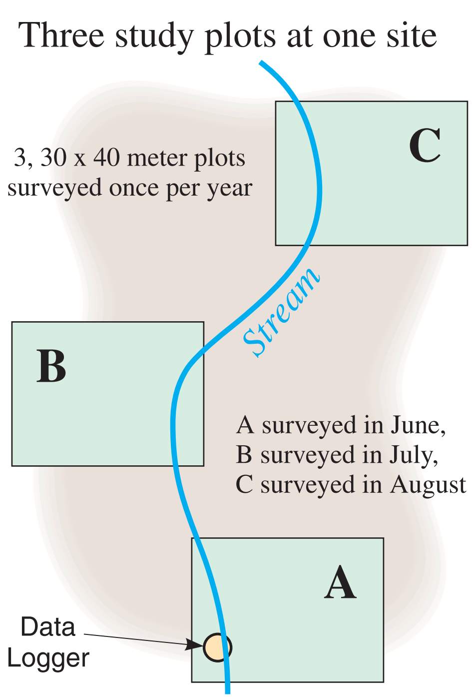


Figure 36. Diagram of the relationship of three 30 x 40-meter fixed sampling plots at a location. Plots need not be isolated. In this schematic, three plots are located along the course of a stream. Each plot is surveyed once per year during the summer, all in the same order (A in June; B in July; C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

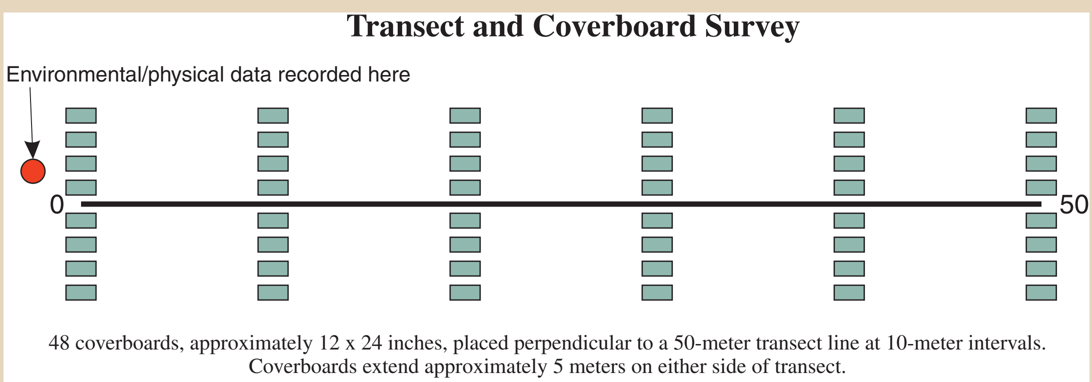
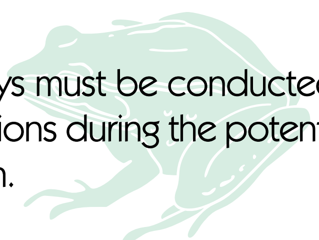


Figure 39. Schematic of a combined transect/coverboard survey design. A series of eight coverboards are located at the origin and thereafter at 10-meter intervals along a 50-meter transect. In this design, the boards are placed perpendicular to the transect. This survey design could be combined with 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

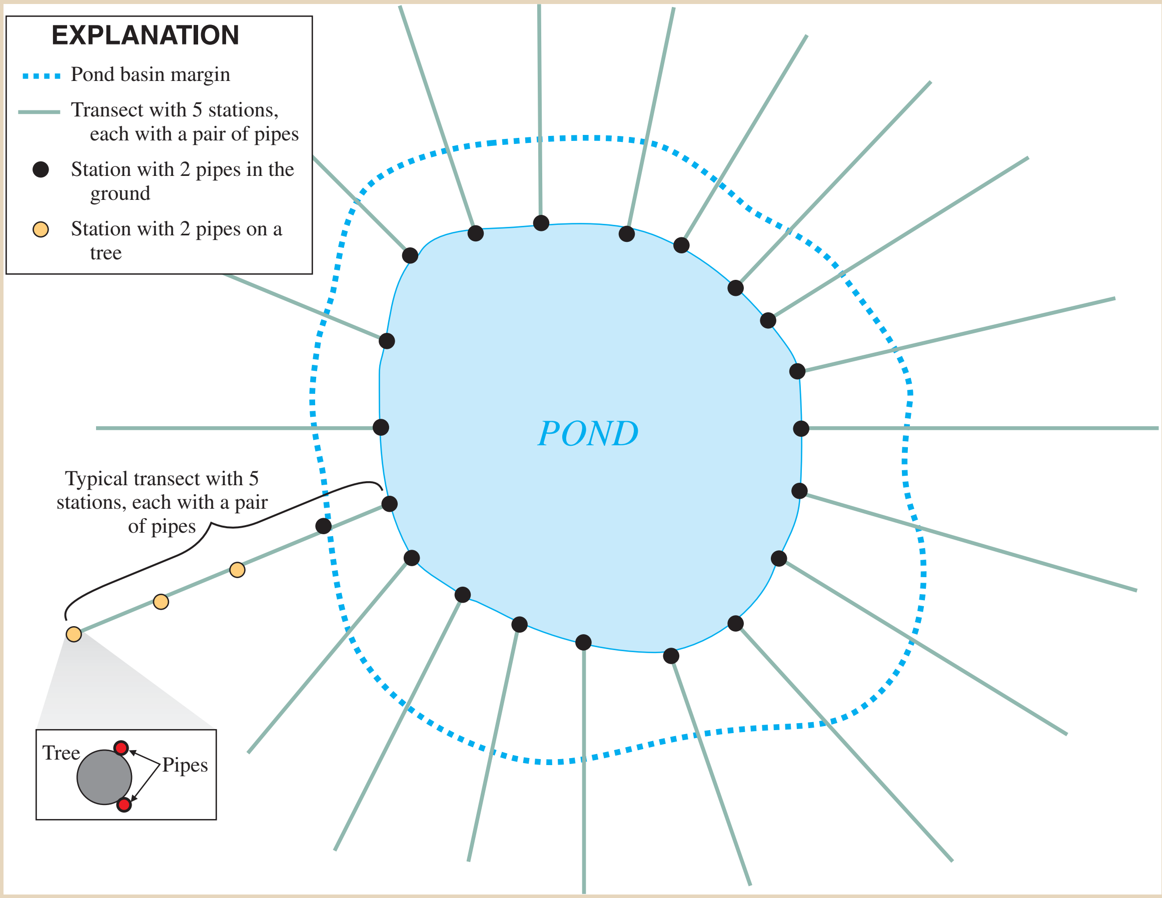


Figure 42. Schematic of a survey design using paired PVC pipes located at 10-meter intervals around a pond's perimeter. The first set of pipes is located at the pond's margin, and thereafter at 5- or 10-meter intervals perpendicular to the pond. The second set of pipes is located at the margin of the 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.