DATA HANDLING

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 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)

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

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

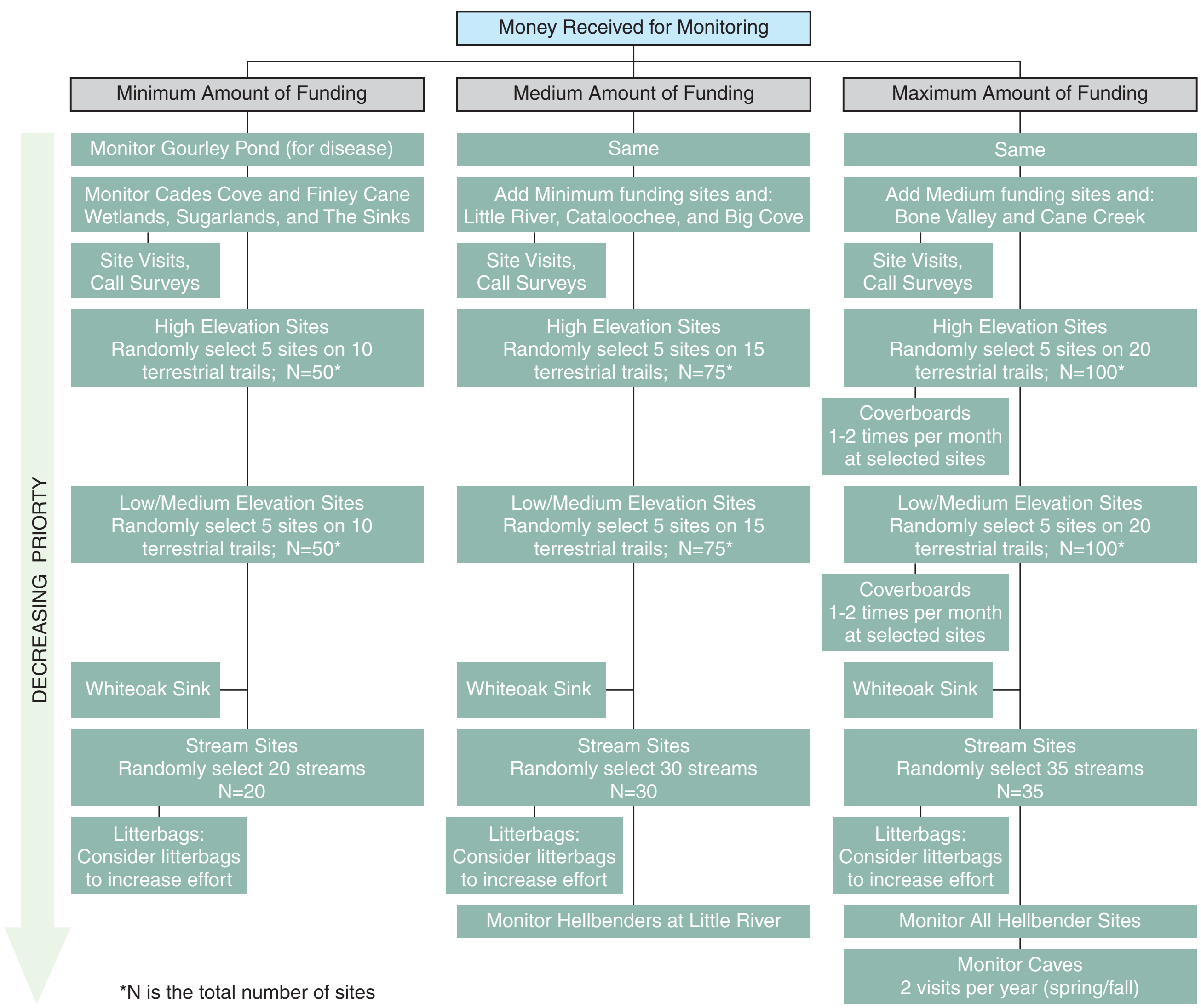

Figure 47. Decision path for helping design an amphibian monitoring program at Great Smoky Mountains National Park based on three levels of funding (see **Conclusion**).

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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