AMPHIBIAN RESEARCH AND MONITORING INITIATIVE

SOUTHEASTERN UNITED STATES, PUERTO RICO, AND U.S. VIRGIN ISLANDS

U.S. Geological Survey

Florida Integrated Science Center

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STUDY PLAN

Title: Status and Trends of the Amphibians of the Southeastern United States, Puerto Rico, and U.S. Virgin Islands

Background and Justification

Amphibian populations and species are declining or disappearing from many regions and habitats world wide (Tyler, 1991; Blaustein et al., 1994; Blaustein and Wake, 1995; Waldman and Tocher, 1998; Alford and Richards, 1999; Houlahan et al., 2000; Semlitsch, 2003; Lannoo, 2005). In comparison with other vertebrates, amphibians seem to be more threatened than birds or mammals, a dubious condition shared with many other freshwater taxa (Abramovitz, 1996). No single cause has been demonstrated, although acid precipitation, environmental contaminants, introduction of nonindigenous species, disease agents, climate change, parasites, and the effects of UV-β light have been suggested as involved in amphibian declines (Corn, 2000; Blaustein and Kiesecker, 2002; Boone et al., 2003; Burkhart et al., 2003; Collins and Storfer, 2003; Westerman et al., 2003a; Lannoo, 2005 and papers therein). Indeed, several factors may interact in such a manner as to threaten species and populations locally or regionally (Carey and Bryant, 1995; Kiesecker et al., 2001). A single cause of environmental stressor can be identified in certain instances, such as mortality from road kills in the Austrian Alps

(Landmann et al., 1999) or a disease outbreak. Still, a major factor in the loss of amphibian populations world wide has been and continues to be the destruction and degradation of habitat (Bishop et al., 2003; Dodd and Smith, 2003).

In response to the decline and disappearance of amphibians, monitoring programs have been established throughout the world in order to track population changes and to differentiate between natural population fluctuation and anthropogenic causes of decline (Pechmann et al., 1991; Pechmann and Wilbur, 1994; Pechmann, 2003). In theory, research and management actions could then be taken to understand the causes of disappearances and declines should they be identified, and to prevent further decline. In practice, the causes of amphibian decline are probably also threatening the diversity of other taxa in the world (for example, Warren and Burr, 1994; Taylor et al., 1996; Gibbons et al., 2000; Lydeard et al., 2004), and stemming the loss of species, populations, and the habitats on which they depend is proving to be a daunting task.

The value of amphibians as indicator species of ecosystem function is another important reason to establish monitoring programs. Amphibian monitoring programs thus can be used to help indicate the success or failure of restoration or mitigation projects (Schwartze, 2002; Petranka et al., 2003). Also, the scientific merit of amphibian oriented monitoring programs is a key issue in land use planning and conservation mitigation because of the highly protected status of several species, which often results in giving amphibians importance in environmental impact assessments and in ecological risk assessment (Westerman et al., 2003b).

In response to concerns about amphibian population declines, the Department of Interior (DOI) received funding from Congress to institute long-term surveys of the status and trends of amphibians on DOI lands and to conduct research into the causes of their declines. This document describes the goals and objectives, organization of monitoring efforts, sampling methods, complicating factors, and the expected outcomes of this project in the southeastern United States, Puerto Rico, and the U.S. Virgin Islands. In the Southeast. these functions are performed by the Florida Integrated Science Center as part of the national Amphibian Research and Monitoring Initiative (ARMI).

ARMI is organized into seven regions throughout the country. Leadership and research direction is provided by project leaders in accordance with the national objectives. By combining integrated research programs involving inventories, standardized methodology in field data collection, data analysis, hydrology, sophisticated mapping tools, disease and contaminants studies, and a nationally centered database, ARMI researchers have embarked on a long-term, continent-wide study to assess amphibian populations and the environmental factors affecting the health and viability of species. Standard methods of data collection, analysis and management are outlined in Corn et al. (2005) and Muths et al. (2005).

The functional framework of ARMI may be envisioned as a pyramid with three levels (Hall and Langtimm 2001; Corn et al., 2005; Muths et al., 2005). The base of the pyramid is built on inventories that are geographically extensive (for example, national) in scope and collect necessarily coarse data. The mid-level of the ARMI pyramid involves monitoring amphibians at a moderate number of sites throughout the country, identified *a priori* (for example, individual National Parks or National Wildlife Refuges), within which amphibian habitats can be sampled and inference drawn about occurrence of a species within the site.

For mid-level monitoring, the main state variable is the proportion of area occupied (PAO) (MacKenzie, 2005; MacKenzie and Royle, 2004; MacKenzie et al., 2002, 2003, 2004, 2005) which can be thought of as the probability of occupancy. Estimating PAO and its variance requires multiple visits to sites within a particular time, so that detection probabilities can be estimated. Species richness is another state variable used in areas with high numbers of species, such as the southeastern United States. The specific objectives of mid-level monitoring are to provide spatial and temporal estimates of change in species occupancy or detection probability within the area of inference, to provide information for modeling amphibian and environmental stressor associations within the area of inference, and to map such associations at the regional level. Mid-level monitoring by ARMI provides a core framework for the program.

The apex of the ARMI pyramid represents intensive long-term monitoring at a small number of sites. These sites may also include a component of research, addressing a specific, small-scale question pertinent to that particular location. The research objectives and goals at apex-level sites vary considerably within and between regions, but generally involve the collection of demographic and life history data for select amphibian species, studies of relationships between environmental change and changes in demographic and life history characteristics of amphibian species over time, and development of monitoring protocols and techniques. More information can be found at ARMI's Internet site: http://www. mp2-pwrc.usgs.gov/armi/index.cfm.

Taxonomic Review

There are at least 6,022 amphibian species known in the world, of which 5,296 are Anura, 555 are Caudata and 171 are Gymnophiona (AmphibiaWeb, 2006), with many additional species likely to be described. Of these, 144 species are resident in the Southeast ARMI region. Within North America, the Southeast has the greatest regional amphibian species richness (Duellman and Sweet, 1999). In addition, a number of species await formal taxonomic description, particularly in the salamander families Plethodontidae, Proteidae and Sirenidae and possibly in the frog family Ranidae. Most of the native amphibians in the Southeast are salamanders, with 86 described species.

Order Caudata.- Of the seven salamander families in the southeastern United States, two (Amphiumidae and Sirenidae) are endemic to the region and two others (Ambystomatidae and Proteidae) have their greatest species richness in the Southeast. One of the three extant cryptobranchids occurs primarily in southern streams and rivers, whereas the remaining two species are found in Asia. The lungless salamanders, family Plethodontidae, are very diverse in the Southeast (57 species), although their greatest diversity occurs in the mountainous Neotropics of southern Mexico and Central America. The family Salamandridae is primarily Palearctic and Oriental in distribution, although two species of *Notophthalmus* are found in the Southeast. There are no salamanders in Puerto Rico or the US Virgin Islands.

The following salamander genera have their centers of distribution within or are endemic to the Southeastern United States: *Cryptobranchus, Necturus, Amphiuma, Siren, Pseudobranchus, Phaeognathus, Haideotriton*, and *Stereochilus*. Most or all species of *Desmognathus, Eurycea, Gyrinophilus, Notophthalmus, Plethodon*, and *Pseudotriton* also occur in the Southeast, although the ranges of individual species may extend substantially northward.

Order Anura.-There are no endemic families of frogs in the southeastern United States, and only two genera (*Acris* and *Pseudacris*) have centers of species richness within the region. The highest diversity (18 native species) of southeastern frogs occurs within the family Hylidae (treefrogs) followed by the Ranidae (true frogs: 11 species) and the Bufonidae (toads: 4 native species). *Gastrophryne* and *Scaphiopus* are represented by a total of two species. In addition to the native species of frogs, four non-indigenous species (*Bufo marinus, Eleutherodactylus coqui, E. planirostris, Osteopilus septentrionalis*) have established breeding populations (all in Florida).

In the Caribbean, there are 17 species of frogs in the family Leptodactylidae in Puerto Rico (Joglar, 1998; Rivero, 1998), 16 of which are in the direct-developing terrestrial and arboreal species-rich genus *Eleutherodactylus*. Several species are endemic to the island, including *Eleutherodactylus jasperi*, the only frog in the Western Hemisphere that gives birth to living young; development occurs entirely within the mother's body. Two species of hylids (*Osteopilus septentrionalis, Scinax rubra*), the toad *Bufo marinus*, the pig frog *Rana grylio* (Rios-López and Joglar, 2000), and the American bullfrog *Rana catesbeiana* have been introduced and have established breeding populations. The green treefrog *Hyla cinerea* was introduced in the past but there are no established breeding colonies.

There are eight species of frogs in the US Virgin Islands, of which three are introduced (*Bufo marinus, Osteopilus septentrionalis, Eleutherodactylus coqui*) (Maclean, 1982). Only the species *E. lentus* is endemic to the US Virgin Islands (on St. Croix, but introduced elsewhere).

Distribution and Habitats

Physiographic Regions and Centers of Speciation.-Amphibians are found in all physiographic regions of the southeastern United States. They are found from sea level to the tops of the highest Appalachian Mountains. Centers of species richness and endemism include the Appalachian Mountains, particularly at higher elevations (salamanders, especially the family Plethodontidae and the genus *Plethodon*), and the Atlantic and Gulf Coastal Plain (many salamanders and frogs, especially *Amphiuma, Siren, Pseudobranchus, Necturus,* and *Haideotriton*). Several of the frogs in Puerto Rico and the US Virgin Islands are endemic to the islands or have their primary centers of distribution within these islands.

In the following section, the types of habitats inhabited by southeastern and Caribbean amphibians are briefly discussed. A more comprehensive discussion with references to the published scientific literature is in Dodd (1997).

Aquatic Habitats.-Amphibians are found in all aquatic wetland types except those associated with the saline waters of the coast. Even there, however, some species occasionally are found in brackish habitats. Southeastern aquatic environments include temporary ponds, wetlands in pine flatwoods, saturated forested wetlands, cypress domes, bayheads, large swamps, wet prairies, lakes, streams, rivers, and man-made aquatic habitats including borrow pits and ponds at former mine sites. Much information on amphibian use of aquatic habitats is contained in state or regional books (e.g. Wright and Wright, 1932) and in numerous accounts of species in need of conservation.

Large fully-aquatic salamanders (*Cryptobranchus, Necturus*) are typically found in larger rivers and streams, whereas small aquatic salamanders (*Desmognathus, Eurycea*) frequent small streams and seeps. In these latter genera, larval development occurs within the stream and, after metamorphosis, adults live along the wet stream sides or among the gravelly substrate. The salamanders *Siren, Pseudobranchus*, and *Amphiuma* inhabit various types of vegetated ponds and mucky swamps. Newts and most *Ambystoma* species require temporary ponds to complete metamorphosis, and premature pond drying is an ever present threat to their development. Of course, even salamanders that do not require water to breed need moist environments to prevent desiccation.

As with salamanders, frogs use a variety of wetlands for reproduction. Most frog species have tadpoles which develop within ponds, lakes, wet prairies or other lentic waters. Fewer species use streams, rivers, or swift flowing waters (e.g., *Rana heckscheri* in rivers, streams, and oxbows in addition to lentic waters). Some frogs are very habitat specific, such as *Rana capito* and *Hyla gratiosa*, which require fishless temporary ponds for reproduction. Some species, such as *Bufo terrestris*, breed in a wide variety of wetland habitats.

Terrestrial Habitats.-Although amphibians are usually associated with water, most species spend a substantial amount of time in terrestrial habitats. Individuals of some species often can be found at great distances from the nearest breeding ponds (Dodd, 1996). Franz et al. (1988) recorded a gopher frog (*Rana capito*) at a tortoise burrow 2 km from where the frog was marked. Such long distance movements probably are not unusual. Greenberg (1993) captured southern toads (*Bufo terrestris*), eastern narrow-mouthed toads (*Gastrophryne carolinensis*), and eastern spadefoot toads (*Scaphiopus holbrooki*) in Florida sand pine scrub between 5 and 6 km from the nearest known water source.

Terrestrial refugia include caves, burrows of tortoises, pocket gophers, crayfish (especially by *Rana capito*) and other invertebrates, tree roots, rock crevices, surface debris, and probably many other subterranean habitats. Treefrogs often use arboreal retreats. Selected references on the use of terrestrial habitats by amphibians that require water to breed are found in Dodd (1997).

Tropical Habitats. - In the Caribbean, much of the lowlands have been modified for agriculture and urbanization, although certain frogs have adapted well to human presence. Amphibian species richness is greatest in the high-elevation forests of the interior mountains of Puerto Rico, where frogs are found in habitats from the forest floor litter to the forest canopy. Certain species require bromeliads (e.g., the recently extinct *E. jasperi*), whereas others live in boulder caves (*E. cooki*) or in torrential streams (e.g., the recently extinct *E. karlschmidti*). All *Eleutherodactylus* require moist places to deposit their eggs.

Aquatic Amphibian Life History

In North America, many amphibians have a biphasic life cycle consisting of an egg and larval stage in water, metamorphosis into a terrestrial adult, and remigration back to water as adults to breed and lay eggs. The time between metamorphosis and first breeding varies among species, although it is usually from 1-4 years (Duellman and Trueb, 1986). The life span of wild individuals also varies. For example, *Gastrophryne carolinensis* may live 4 or more years whereas the entirely aquatic hellbender may live >25 years. Generally, salamanders live longer than frogs, and larger species live longer than smaller species (Duellman and Trueb, 1986). Duellman and Trueb (1986) discussed life history variations and the factors that affect reproduction, life cycles, and other facets of amphibian biology.

There are exceptions to the "typical" amphibian life cycle. All non-hemidactyliine salamanders of the family Plethodontidae (i.e., *Aneides, Plethodon*), two species of *Desmognathus* (*D. aeneus* and *D. wrighti*), and *Phaeognathus hubrichti* have no free-living aquatic larval stage. Instead, eggs are laid on land in moist environments, the larval stage is passed within the egg, and the hatchling resembles a miniature adult.

Several salamanders, including all *Siren* spp., *Pseudobranchus* spp. and *Necturus* spp. some *Eurycea* spp., and *Haideotriton wallacei* and *Cryptobranchus alleganiensis*, are entirely aquatic and never leave the water or boggy wetlands. Eggs are deposited in vegetation, debris, or under rocks, young usually pass through a larval stage, and adults often retain larval features, such as exposed gills. *Amphiuma* spp. generally are aquatic, although eggs may be deposited on land near water. Other species (*Ambystoma talpoideum, Notophthalmus* spp.) have individuals or populations that are facultative paedomorphs (that is, they never transform as long as permanent water remains, and they become reproductively active while otherwise retaining larval phenotypes).

All native southeastern frogs, as well as most of the non-indigenous species in Puerto Rico, have a "typical" amphibian life cycle. All of the *Eleutherodactylus* spp. are direct developers with no aquatic life stage, except for the now presumably extinct ovoviviparous *E. jasperi*.

Terrestrial Amphibian Life History

All members of the Tribe Plethodontinii in the salamander family Plethodontidae, several salamanders of the subfamily Desmognathinae, and most members of the tropical frog family Leptodactylidae are entirely terrestrial and do not use standing water for reproduction. All deposit their eggs in moist situations, however, and the young develop within the eggs and hatch as miniature adults. For most salamanders, this is thought to occur in underground retreats or deep within large rotting logs; the eggs of some of these species have never been found under natural conditions. For the small *Desmognathus aeneus* and *D. wrighti*, nests may be placed in seeps or crevices near wet areas. For the native Puerto Rican and Virgin Island *Eleutherodactylus*, eggs are deposited in moist leaf litter on the forest floor, in tree cavities, in boulder caves, in rotten logs, or arboreally in bromeliads, depending on species.

Terrestrial amphibians have a three or four-dimensional spatial life history pattern which may vary seasonally. During favorable environmental conditions, they are active under surface debris and on the surface of the ground. Some of the tropical Puerto Rican frogs and some of the Appalachian salamanders are or become arboreal, taking to vegetation to feed. However, during unfavorable conditions, animals may retreat underground to inaccessible locations. Likewise, some of the species retreat underground or to specialized places to deposit eggs. A terrestrial life history activity pattern does not imply continuous surface activity.

Federal status

The following species are protected by the Endangered Species Act of 1973, as amended within the region of Southeast ARMI.

Species	**Common Name**	**Range**
Ambystoma cingulatum	Flatwoods salamander	AL, GA, FL, SC
Phaeognathus hubrichti	Red Hills salamander	AL
Eleutherodactylus cooki	Guajón	PR
Eleutherodactylus jasperi	Golden coqui	PR
Peltophryne (=Bufo) lemur	Puerto Rican crested toad	PR

Listing of the Junaluska salamander (*Eurycea junaluska*) has been found "warranted but precluded" by more pressing agency priorities within the U.S. Fish and Wildlife Service. The striped newt (*Notophthalmus perstriatus*) also is a likely candidate for federal protection. Unfortunately, only the Junaluska salamander and possibly the Puerto Rican crested toad among these species occur on DOI lands to any extent (in the Great Smokies and Virgin Islands N.P., respectively). The Red Hills Salamander occurs primarily on privately-owned properties and also on a very small portion of land owned by the U.S. Army Corps of Engineers on the Alabama River.

Although not federally protected, substantial declines of a number of amphibians have occurred in the southeastern states, including the Blue Ridge Escarpment populations of *Aneides aeneus* (green salamander; Corser, 2001), *Desmognathus auriculatus* (southern dusky salamander; Dodd, 1998; Means and Travis, in press), *Pseudacris brachyphona* (Mountain chorus frog), and *Rana c. capito* (Carolina gopher frog; Braswell, 1993). Whereas some of these declines are the result of habitat destruction or alteration, the cause of the declines of others (green salamander, southern dusky salamander) remain unknown and speculative. The status of southeastern amphibians was reviewed by Dodd (1997); the status of Puerto Rican frogs was reviewed by Joglar and Burrowes (1996) and Joglar (1998).

Objectives

The objectives of this initiative are to determine the status and trends of amphibian populations on DOI lands in the southeastern United States, Puerto Rico, and the U.S. Virgin Islands, and to provide information useful in determining causes of declines should they be discovered. This project will not determine the status and trends of most species of amphibians throughout their ranges nationally or in the southeast. For a number of reasons, DOI lands comprise an inadequate sampling frame to assess range-wide trends of many species. DOI lands may be too small, may not include appropriate habitats, or some species may not occur on DOI land. However, larger tracts of DOI land, such as the Great Smoky Mountains National Park, Okefenokee National Wildlife Refuge, and the large parks of south Florida, should be of sufficient size to allow the determination of species trends, particularly of localized endemics. Projects focusing on large land tracts will be supplemented by data recorded on smaller DOI parks and refuges scattered throughout the geographic region assigned to SE ARMI. Particular emphasis will be placed on lands with previous completed inventories, sensitive species, or where potential threats to amphibians are known or suspected.

The scope of this initiative is sufficiently complex that several objectives will need to be achieved for success:

1. Study sites will be established in a manner that allows for statistically valid estimates of the status of amphibians within the boundaries of individual DOI lands and changes in the abundance and distribution of selected amphibian species in larger landscapes centered on large DOI lands.

2. For species and habitats where existing methods are inadequate to collect data on trends, research will be conducted to develop sampling protocols and appropriate methods to analyze data, detect trends, and make predictions concerning status.

3. Ancillary biological and physical data will be collected so that correlations with changes in abundance and distribution of amphibians can be determined.

4. Should emergency situations be detected, such as the presence of disease or malformations, research will assist in the determination of cause and methods of containment. In this regard, research and field personnel will work closely with the USGS National Wildlife Health Center.

5. Data collection will be coordinated within USGS, among DOI and other federal agencies, and partners (such as State agencies, university researchers, and non-governmental organizations).

6. Information will be made available to cooperating agencies, the scientific community, and the public.

Procedures and methods

Historical Information. - Information on southeastern amphibians may be contained in numerous publications (e.g., scientific and popular literature and agency publications), unpublished reports, and museum collections. As part of initiating amphibian monitoring programs in the southeastern U.S., USGS personnel will determine the extent of information available on amphibians within DOI-administered lands. Initially, we will focus on lands selected for intensive sampling and monitoring, but through time we will assemble a database on all DOI lands in the southeastern US and Caribbean. In addition to published literature, agencies, museums, and Natural Heritage Programs will be contacted to determine historical species presence and the types of habitats where species are found. Data collection will be coordinated with the Database Management Program administered by the Patuxent Wildlife Research Center (see below).

Inventories. - Although the distribution of amphibians is reasonably well understood on certain DOI lands, such as in the Great Smoky Mountains National Park (Dodd, 2004), this is not the case for most units of the National Park Service and National Wildlife Refuges. This study plan recognizes that there is a need to survey amphibian species richness and distribution patterns on many DOI lands prior to selecting species to be monitored and sampling protocols. Some of the inventory techniques available to sample amphibians are the same as those used to monitor populations (see below). However, inventory protocols will be used to determine species presence rather than long-term population trends. Thus, sampling will occur over a large area to include all habitat types; multiple sampling techniques may be employed; and sampling may be restricted by time (that is, it may be extensive rather than site intensive).

Inventories are part of the Resource Survey component of the conceptual framework developed by USGS in connection with ARMI. Inventories will be conducted on a variety of DOI lands, including National Park Service administered lands (within the NPS Regional Networks framework) and on U.S. Fish and Wildlife Service administered National Wildlife Refuges. Inventories will be conducted on Index Sites when such data are not already available.

All sampling techniques employ some variation of a time constraint approach whereby search or trap effort is quantified and results are expressed in catch per unit effort of sampling. In all cases, characteristics of the environment/habitat, sampling conditions, and the number of animals observed or captured by species are recorded. Examples of such techniques include: visual searches while walking predetermined paths or transects; searching cave walls; searches of terrestrial leaf litter and under surface debris (logs, rocks, coarse woody debris); searches of aquatic habitats by moving stream debris or by using dipnets to look for adults and larvae; snorkeling for large salamanders; road surveys during favorable activity periods (e.g., during rain near breeding ponds).

Frogs may be inventoried by listening for calls during the breeding season, although not all frogs are easily sampled this way. Some frogs call very softly and may not be heard except in their immediate vicinity. When conducting inventories using calls, it may be necessary to categorize the numbers of animals calling categorically, for example: 1 (1 calling), 2 (2-5 calling), 3 (6-10 calling), 4 (>10 calling), 5 (large chorus). This is because it is often very difficult to determine how many frogs are calling when a chorus is in progress. In southern wet prairies, literally hundreds or thousands of males may call simultaneously.

In certain instances, it may be desirable to inventory amphibians using specialized techniques, such as by employing traps (e.g., soft or hard minnow traps), modified crayfish traps

(Johnson and Barichivich, 2004), coverboards, PVC pipe (Zacharow et al., 2003), debris bags (Pauley and Little, 1998; Waldron et al., 2003) or drift fences with pitfalls. Each of these techniques has sampling biases (see below), although they are useful to determine the presence of some species in certain habitats (techniques discussed in Heyer et al., 1994). Dodd (2003) and Dodd et al. (in press) have reviewed amphibian sampling and monitoring techniques, the biases associated with the techniques, and what types of data the techniques provide to researchers.

DOI Resource Survey and Index Sites (with acreage) of major importance are as follows:

National Park Service

Everglades	1,506,539
Big Cypress	729,000
Great Smoky Mountains	521,000
Big South Fork	116,000
Timucuan	46,000
Little River Canyon	14,000
Congaree Swamp	11,000
Chickamauga	8,200

Fish and Wildlife Service

Okefenokee	396,000
St. Marks	68,000
Cape Romain	64,229
Lower Suwannee	52,935
Wheeler	34,500
Savannah	24,904
Lake Woodruff	19,400
Harris Neck	2,761

Monitoring Amphibians

Habitat/Environmental Data. - At permanent study sites, we will use data loggers to monitor the air, substrate, and water temperature, barometric pressure, relative humidity, and rainfall. During surveys, we will record weather conditions (sun, cloud cover, precipitation, wind), rainfall, soil and water pH, and a variety of other variables related to habitat quality where appropriate (e.g., conductivity, nitrates). Vegetation structure and composition, slope, elevation, aspect, ground and canopy cover, size, shape, and approximate depth of water bodies, and aquatic substrate characteristics will be described (see Corn and Bury, 1990; Bury and Corn, 1991; Heyer et al., 1994; Fellers and Freel, 1995; Olson et al., 1997; Droege et al., 1997 for habitat characteristics and typical data sheets). These data will help assess the effect of habitat (both biotic and physical) and weather variables on capture results and assist in data interpretation. Data will be stored in Excel formats for later analysis.

Site selection. - In general, all habitats will be surveyed to determine species richness (species present/not detected). Distributional sampling site selection will vary with terrain, physical access, vegetation type, and amphibian habitats present. These variables will also influence sites selected for mid-level monitoring and protocol evaluation, in addition to the presence of species targeted for evaluation. When necessary, sites for distributional sampling and intensive monitoring will be determined based on consultation with biologists familiar with the areas to be sampled. Although the number of sites to be sampled or monitored must be determined separately for each park or refuge (see MacKenzie and Royle, 2004), a sufficient number will be selected to minimize the effects of small sample size. In some cases, it may be feasible to sample all available habitats (e.g., ponds in Great Smoky Mountains National Park); in other habitats, however, it will not be possible to sample all sites, nor may it be possible to sample sites in a strictly random pattern (e.g., because of access over a huge area). In such cases, sites will be selected based on a stratified sampling procedure, such as by narrowing locations to be examined by elevation, drainages, past or present land use, road access, vegetation, or a combination of these factors.

Terrestrial Surveys. - Many species of lungless salamanders inhabit terrestrial situations, often far from the nearest water. Members of the genus *Plethodon*, in particular, require moist habitats but not standing water. Eggs are deposited in moist locations, sometimes deep underground, and development is direct. Individuals are seasonally active (at cooler times of the year), but surface activity patterns vary with moisture conditions and elevation. High elevation species are active throughout the summer as long as moisture conditions are favorable. The presence of terrestrial plethodontids is often detected using some form of time constrained sampling within a defined area either at night or diurnally during or immediately following (preferably) rainfall. Salamanders may be observed on the ground surface or arboreally on vegetation and tree trunks, by searching leaf litter in plots of predetermined size (e.g., 30 x 40 meters) (see Dodd and Dorazio, 2004), or by turning surface objects such as logs, rocks, and coarse woody debris. Terrestrial plethodontids also have been sampled using artificial coverboards made of various types of material (seasoned untreated wood, shingles, metal sheets, bricks, plastic, etc); coverboards may be placed in grid patterns or evenly spaced singly or in groups along transect lines of varying lengths.

Monitoring terrestrial salamanders has proved far more difficult than determining presence, and every technique tested thus far has serious biases and limitations (in this case, permanent large study plots sampled by annual litter and debris searches; removal sampling; coverboards, night searches, and visual transect searches). Results from studies on terrestrial salamanders in the Great Smoky Mountains and nearby Nantahala Mountains suggest that no one technique will be sufficient to monitor all species (Smith and Petranka, 2000; Petranka and Murray, 2001; Hyde and Simons, 2001). Indeed, some authors have suggested that "community surveys are simply not a viable option for rigorous monitoring under current technology" (Thompson et al., 1998), and this caution seems apropos to species-rich terrestrial salamander communities in the southern mountains. Nothing is known about how to monitor terrestrial salamanders in the Piedmont and Coastal Plain where fewer species are present during more restricted times of year, and where individual densities are not as great as in the mountains. Amphibian monitoring programs in the southeast will likely, of necessity, concentrate efforts on certain species or groups of species while developing protocols for others.

Stream Surveys. - Many amphibians use small streams for all of a portion of their life cycle. Within the southeast, small streams vary considerably in physical characteristics, from high mountain trickles and torrents in the Southern Appalachians to the slow-moving coastal plain streams of Florida. The techniques used to sample and monitor amphibians will vary accordingly. Smaller streams may be sampled by using time constrained visual searches, by removal sampling (i.e., by blocking a section of stream and removing all animals within the section), by quadrat sampling (such as by measuring a series of 5 m quadrats spaced evenly or randomly along a stream section and counting all amphibians within the series of quadrats), or by trapping (using soft or hard mesh traps, which come in many designs, or by using porous bags containing debris; Pauley and Little, 1998; Waldron et al., 2003). The efficacy of these techniques has largely been untested in the southeast (but see Bruce, 1995, concerning temporary removal sampling of aquatic salamanders in a Southern Appalachian stream). The type of aquatic funnel trap (metal vs. plastic) has been shown to influence capture efficiencies of certain larval salamanders (Fronzuto and Verrell, 2000).

Large Stream/River Surveys.- Few large streams or rivers within the southeast contain resident amphibians. However, several frogs breed in or along larger rivers, and certain permanently aquatic salamanders (hellbenders [*Cryptobranchus*] and mud puppies [*Necturus*]) reside in them; larval salamanders use pools along large streams, depending on the physical characteristics of the banks of the stream or river. The presence of frogs can be determined by visual or acoustic surveys, and larvae can be sampled using dipnets. Larger salamanders are observed by systematically turning large river rocks, by snorkeling or along transects (Peterson, 1987), by trapping using soft or hard mesh nets or minnow traps, and by intensively seining or dipnetting large leaf beds, especially in the autumn or winter. Smaller salamanders are observed visually or by searching bottom rocks and debris using small nets.

Pond Surveys. - In the southeastern United States, significant amphibian breeding occurs in isolated ponds. Ponds range in size from large and permanent to very small and temporary. Different amphibian assemblages use ponds of various sizes and hydroperiods. Small temporary ponds are particularly difficult to monitor since hydroperiods may vary considerably both annually and seasonally. Ponds may be used throughout the year, as different species breed at different times, from winter through late autumn. The sampling and monitoring protocols chosen must recognize the variation in timing in which ponds are used and the different species assemblages that use them.

Ponds may be surveyed and monitored using visual shoreline and shallow water searches, by dipnetting randomly or at predetermined intervals along the shoreline, by trapping using a variety of trap designs and configurations, and by call surveys. Eggs, larvae, and adults may be sampled or monitored, depending on season. However, monitoring a specific life history stage may mask certain population trends. For example, counting egg masses says nothing about recruitment, although it provides an index of the numbers of breeding females. In addition, many southeastern ponds are difficult to survey because of tannin stained water, thick floating mats of vegetation, and unstable and dangerous substrates (e.g., deep layers of mud and muck). Sampling in or near water may be supplemented by sampling nearby surface debris or by placing coverboards on or near the shoreline.

Automated data loggers have been used successfully to determine the presence of calling frogs at breeding sites (Barichivich, 2003). They can be set to record at variable time intervals for various amounts of time throughout the entire day, or they can be programmed 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 or digital recordings, although this may be a very time consuming process, and it is sometimes possible to gain an index of calling intensity, provided large choruses are not involved. Automated frog call data loggers provide information on: 1) species presence (but not absence) 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), and what environmental influences affect calling, and 3) a relative index of the number of males calling.

Although species can be easily identified, categorizing abundance may be very difficult in even moderately sized choruses because of call-overlapping interference. It is also often not possible to separate individual callers, allowing the possibility that a single calling male could be counted multiple times. 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. Frog call surveys using automated data loggers are best implemented where researchers have limited access by road, sample along rivers or other extensive water bodies accessible by boat, or when rare species are suspected.

Whereas automated frog call data loggers are relatively easy to assemble (Barichivich, 2003) or can be purchased already assembled, they are somewhat expensive (ca. $250 in 2006 for new digital models), although prices can be expected to drop. Researchers must listen to tapes/digital recordings and manually record the results, a somewhat tedious exercise. In SE ARMI, we have used two observers independently listening to the tapes as a measure to reduce and quantify observer bias. Automated data loggers must be well hidden in order to reduce theft and vandalism, and this may limit their effectiveness. Curious large and small mammals also may investigate and damage the data loggers.

Freshwater Marshes and Swamps. - Significant questions remain about sampling large wetlands (e.g., extensive swamps in the southeastern U.S. at Okefenokee National Wildlife Refuge or Everglades National Park). These habitats are difficult to sample and describe, and it may be extremely difficult to delineate exactly what constitutes an amphibian population. Standard methods developed for discrete ponds or small wetlands may not be especially useful, and additional research may be necessary to determine effective ways to sample these habitats. In certain large wetland systems, a combination of call surveys, systematic visual searches (either randomly selected or along pre-determined transects of specified lengths), intensive manual sampling (using nets or Goin dredges), and passive trapping (using minnow traps or PVC pipes; Boughton, 1997; Boughton et al., 2000; Zacharow et al., 2003) will be necessary.

Specialized Habitats. - Specialized habitats in the southeast and the Caribbean may contain unusual or unique amphibian species. Examples of such habitats include caves and cave entrances, the granitic boulder caves of Puerto Rico, the axils of bromeliads, gopher tortoise or crayfish burrows (inhabited by gopher frogs, *Rana capito* and *R. areolata*), crevices or high on trees (*Aneides aeneus*), and deep mucks (inhabited by *Amphiuma pholeter*). Sampling methodology either has not been developed or has not been tested for species in these habitats. In Parks and Refuges where such habitats and species are found, sampling/monitoring protocols will have to be developed and tested on a case by case basis.

Data Handling, Analysis and Interpretation

Determining the status and tracking trends of populations that comprise the amphibian fauna of the southeast is a daunting task. The number and diversity of amphibians makes monitoring every single species difficult, if not impossible. Nonetheless, amphibian diversity is a hallmark of ecosystems in the southeastern U.S. and changes in ecosystems through disturbance, human development, 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, rather than only identifying a subset of species for intensive monitoring and extrapolating from those few species.

The main hindrance to making valid 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. Invariable some species will be missed, biasing the estimates (Boulinier et al., 1998). Methods however are now available which account for variation in detection probabilities and which estimate species richness, standard error, and 95% Confidence Intervals (Nichols and Conroy, 1996; MacKenzie et al., 2005). These methods have been extended to estimate several important vital rates in animal communities that bear on amphibian status: rates of local species extinction, turnover, and colonization (Nichols et al., 1998a). And they have been used to test hypotheses concerning factors affecting temporal (Boulinier et al., 1998) and spatial variation (Nichols et al., 1998b.) in species richness.

The application of these estimation methods to amphibian survey data is promising not only because they can address important questions, but they may easily be applied to inventory surveys, intensive monitoring at index sites, and extensive surveys initiated by partners at other sites. These methods however need to be evaluated, particularly with regard to proposed field protocols and issues of scale.

Estimation of species richness is only one analytical tool to assess amphibian status. Important target species (relatively common but vulnerable to a specific type of stressor) and species of special concern (rare and possible threatened) will be intensively monitored at index sites. Based on the results of initial inventories we will identify species for intensive monitoring, determine the best available field protocols for those species, and then in conjunction with our advisory committee of biometricians choose the best sampling design and analytical methods from which to draw inferences from the data. Because amphibian populations fluctuate dramatically over time and across the landscape (Alford and Richards, 1999), it is important that we monitor not only areas of known abundance of target species, but also areas where the species may have been absent or in low numbers during inventories. We are currently considering a dual frame design (Haines and Pollock, 1998), which was developed to specifically address this issue.

Field Data

Field data should be recorded immediately when taken. Data may be recorded on data sheets, preferably in pencil on waterproof paper, or if financial conditions permit, using pre-programmed palm pilots. The following is an example of the data that may be recorded at sampling sites: date (month/day/ year), site code (a unique identifying site number or alpha-numeric identifier), personnel (initials or names of those persons conducting the survey), weather, altitude, wind (categorical judgement of wind speed 1 m above sampling area, or measured using the Beaufort Scale; see http://www.crh.noaa. gov/lot/webpage/beaufort/), general location, specific location (using GPS), 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), air temperature, water temperature, substrate temperature, relative humidity, pH, conductivity, habitat type, vegetation, canopy (a categorical assessment of canopy cover, especially important at wetland sites), slope aspect, and drainage direction. The amphibians observed, their sex (if discernible), life stage (adult, juvenile), number of individuals, and other notes (for example, reproductive condition, missing limbs, malformations) 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. Photographs can provide much information, especially if the animal is photographed with a ruler for scale.

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 associated with placement. The distance an animal is captured or observed from a transect's origin and baseline helps indicate spatial distribution. 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.

The number of observers (exclusive of the person recording data, unless that person is also sampling animals) x the amount of time sampling or the number of dipnet sweeps gives a measure of sampling effort. 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 rare or unusual plants and animals.

Spreadsheets and Databases

Data from field data sheets should be transferred into a database 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. 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 using Abundance Data

Regardless of methodology, the objective of monitoring amphibians is to detect population trends or adverse environmental perturbations 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, depending on the extent of the variance and the power to detect the trends, and therefore reflect changes in conservation status. Most monitoring programs have assumed that counts obtained during periodic surveys provide an index of population size, and thus indirectly to status. Such assumptions have rarely been tested, and there seems little reason to believe that counts by themselves offer a measure of status because of the many factors which influence species detection. Indeed, every study thus far that has examined detection probabilities among sites suggests a wide amount of variance can be expected (for example, Jung et al., 2002; Dodd and Dorazio, 2004).

A possible exception to the above is a small closed population drawn from a localized area, where nearly every individual can be observed or captured. Such situations probably are uncommon and, indeed, even in mesocosm studies it is difficult to capture every individual (Altwegg, unpublished). In addition, the large variance associated with most count data might make the power to detect biologically significant trends rather difficult (Pechmann et al., 1991; Reed and Blaustein, 1995, 1997: Hayes and Steidl, 1997; Thomas, 1997).

It has been argued that moderate to high repeatability (that is, precision) and a measure of power together form a valid basis to detect trends in area-constrained terrestrial salamander populations (Smith and Petranka, 2000). However, other authors have argued that abundance indices based on point counts alone cannot be used to monitor amphibians unless some form of detection probability is incorporated into the model (Jung et al., 2000; Schmidt, 2003; Dodd and Dorazio, 2004; Schmidt, 2004, 2005). Marsh (2001) noted that coefficients of variation of abundance estimates could vary 2 to 10-fold even with time series in excess of five years of sampling. The power to detect small, negative (< -5%) population trends in Appalachian salamanders increases with the number of years (minimum of 10-40) and sites sampled (Smith and Petranka, 2000; Hyde and Simons, 2001). However, small population changes may have nothing to do with the question of immediate threats to amphibians, and surveying many populations intensively for a long time in order to detect trends is impractical. As a consequence, most ongoing monitoring programs are capable of revealing only dramatic changes in amphibian populations (that is, extinction or changes of orders of magnitude), but not small changes. Although the detection of drastic or sudden changes in amphibian populations is critically important, it is these minor changes which may be the usual pattern under natural conditions, with the exception of changing abundance in highly fluctuating environments.

In studies on stream-dwelling and terrestrial salamanders, Jung et al. (2000) estimated population sizes using three methods: an index (mean number of captures per day), total counts, and capture-mark-recapture. Recapture rates were very low. They reported that the proportion of salamanders detected (p) varied among plots when comparing results from capture-recapture to indexes and counts, necessitating the use of “adjusted” population estimates. These were less precise than population indices, which in turn were not likely to be efficient in predicting the effects of environmental covariates on abundance. They recommended using capture-recapture models to verify consistency in *p*’s among sites in order to estimate bias among sites. They also found congruent patterns of abundance among four different sampling methods at nine monitoring sites, suggesting that relative biases among methods were similar. Pollock et al. (2002) recommend computing detection probabilities at a subset of sites if it is impossible to estimate them at all sampling sites.

Even determining population numbers of tadpoles in relatively simple desert pools can prove difficult. Using visual and dip net indices and double observer, removal, and red dye capture-recapture protocols, Jung et al. (2002) demonstrated that many techniques underestimated tadpole numbers, and that detection rates decreased with increasing pool volume and area. In addition, detection rates varied among pools indicating that the techniques did not sample the proportion of tadpoles within pools consistently.

It is thus unlikely that surveys based on counts on a relatively small number of sites will be effective at monitoring population changes in amphibians, regardless of estimator used (Schmidt, 2003, 2004). This is especially true when detection probabilities are not provided. Without some measure of detection probability, it is impossible to determine the meaning of count data and associated spatial and temporal biases (Jung et al., 2000). Even with such data, the wide range of variance associated with both the detection probabilities and resulting abundance estimates might preclude detection of small but important population trends.

There are four ways to determine amphibian abundance accurately.

Capture-mark-recapture

The most commonly used method to estimate abundance is to individually mark animals or cohorts of individuals release them, 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 to an estimate of actual population size within a certain degree of confidence. Although it is beyond the scope of this chapter to discuss the nuances, theory, and assumptions of mark-recapture analysis, there is substantial literature available on this subject (Pollock et al., 1990; Nichols, 1992; Thompson et al., 1998; Williams et al., 2002). Capture-recapture studies have been used to monitor certain populations of amphibians, including some extending over a long time span (Semb-Johansson, 1992).

It is not logistically easy to use mark-recapture techniques when studying populations of amphibians. Amphibians are not easy to mark 'permanently.' Various methods, such as toe clipping, knee tagging, elastomer implants, photographic ID, and the injection of Passive Integrated Transponder (PIT) tags (Gibbons and Andrews, 2004; Arntzen et al., 2004) have been used, although each technique has limitations. Amphibians lose toes naturally and re-grow clipped toes (although this can be prevented by treating clipped toes with $AgNO_3$ or $BeNO_3$); knee-tagging cannot be used on amphibians whose knee region is not distinctively narrower than their upper and lower legs; elastomers are time consuming to apply and are difficult to read under field conditions; photographic ID is not practical when hundreds or thousands of animals are involved or when animals are uniformly or un-patterned (Arntzen et al., 2004), or if pattern changes with time (Arntzen and Teunis, 1993); and PIT tags are too large for some species or costly when large numbers of animals must be tagged. Observer error is an ever-present bias. In most instances, very few recaptures are recorded in relation to the number of amphibians marked. In such cases, the variance confidence intervals of the population estimate can become quite large, thus negating the value of the estimate.

Distance sampling

Distance sampling involves walking a transect line and measuring the perpendicular distance from the line to an observed animal, and the linear distance from the transect's origin to the perpendicular line. Four assumptions are critical to obtaining accurate estimates of abundance using line transects: animals directly on the survey line will not be missed, no animals will be counted twice, perpendicular distances are measured accurately, and sightings of animals are independent events. Some of these assumptions may be violated during amphibian surveys, especially when monitoring secretive species (salamanders may be underground, frogs may be in amplexus). Line transect distance sampling to determine abundance has not been popular in monitoring programs (but see Dodd, 1990; Funk et al., 2003). Additional discussions of distance sampling using line transects are found in Burnham et al. (1979, 1980, 1985).

Removal Sampling

Removal sampling may be used to derive abundance estimates (Blower and Bishop, 1981; Bruce, 1995; Petranka and Murray, 2001; Salvidio, 2001), but it has not been used to monitor amphibian populations because of the logistical constraints of time and manpower. Temporary removal sampling might prove useful in comparing abundance estimates derived from other methods during monitoring programs (Petranka and Murray, 2001), or when studing amphibians at small ponds during the breeding season.

Simultaneous Estimation of Abundance and Detection Probabilities

Royle (2004) devised a statistical technique whereby abundance estimates and detection probabilities may be derived from temporally and spatially replicated count data. Dodd and Dorazio (2004) adapted this model to estimate these parameters for salamanders sampled over a six year period in area-constrained plots in Great Smoky Mountains National Park. Estimates of salamander abundance varied among years, but annual changes in abundance did not vary uniformly among species. Except for one species, abundance estimates were not correlated with site covariates (elevation, soil and water pH, conductivity, air and water temperature). The uncertainty in the estimates was so large as to make correlations ineffectual in predicting which covariates might influence abundance. Detection probabilities also varied among species and sometimes among years for the six species examined. Dodd and Dorazio (2004) found such a high degree of variation in counts and in estimates of detection among species, sites, and years as to cast doubt upon the appropriateness of using count data to monitor population trends using a small number of area-constrained survey plots. Still, the model provided reasonable estimates of abundance that could make it useful in estimating population size from count data.

Analysis using Percent of Area Occupied

This is the preferred estimator to be used in ARMI analyses. The number and diversity of amphibians within a region often makes monitoring every species difficult, if not impossible. Nonetheless, amphibian high species richness is characteristic of ecosystems in southeastern North America, temperate China, and in the tropical regions of the world. 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 because of the low recapture probabilities in mark-recapture studies of amphibians (MacKenzie, 2005; MacKenzie and Royle, 2004; MacKenzie et al., 2002, 2003, 2004, 2005).

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. Invariably, some species will be missed, thus biasing the estimates (Boulinier et al., 1998). However, methods are available which account for variation in detection probabilities, and which estimate species richness, standard error, and 95% 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 et al., 1998a). They also have been used to test hypotheses concerning factors affecting temporal (Boulinier et al., 1998) and spatial variation (Nichols et al., 1998b) in species richness.

The application of PAO methods to amphibian inventory data is promising, not only because these methods can address important questions, but also because they may easily be applied to inventories, intensive monitoring at pre-selected sites, and in extensive inventories (MacKenzie et al., 2002, 2003, 2004, 2005). Furthermore, detection of a change in species richness can alert biologists and managers to potential problems that may require more focused study. On the other hand, Strayer (1999) has noted that many factors (sample size, clustering of sites, number of repeat visits, decrease in spatial variance associated with population density) influence the statistical power of presence-absence data to detect anything but the steepest population declines.

PAO should be used when monitoring discreet sites, rather than extensive area-based grids. PAO estimates are useful when derived from survey data recorded from many repeat visits to many sampling locations throughout the geographic range of a species. For example, a series of ponds or terrestrial locations can be visited several times a year for several years in order to derive PAO estimates. Through time, a pattern of changes in distribution coupled with changes in site-based detection probabilities then could be used to determine changes in the status of amphibian populations. Estimates of occupancy (*psi*) and detection probability (*p*) can also be used in determining the number of independent visits to be conducted per site (Table 1), and the number of sites that need to be visited in order to achieve a desired level of precision. MacKenzie (2005) and MacKenzie and Royle (2004) provide detailed discussions of the factors to be considered using occupancy to estimate trends in a monitoring program. We have carefully considered these papers when designing our sampling regime at the various refuges, and we have tried to follow their guidance as far as logistics and financial/personnel considerations allow.

Table 1. Number of visits per site within a season needed for a probability of occupancy (Ψ) given a certain level of detection probability (*p*). See MacKenzie and Royle (2004).

	Ψ								
p	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0.1	14	15	16	17	18	20	23	26	34
0.2	7	7	8	8	9	10	11	13	16
0.3	5	5	5	5	6	6	7	8	10
0.4	3	4	4	4	4	5	5	6	7
0.5	3	3	3	3	3	3	4	4	5
0.6	2	2	2	2	3	3	3	3	4
0.7	2	2	2	2	2	2	2	3	3
0.8	2	2	2	2	2	2	2	2	2
0.9	2	2	2	2	2	2	2	2	2

Species Accumulation Curves

One measure of the extent of biodiversity in a region is the rate at which new species are added to a species inventory (Soberón and Llorente, 1993). If regular surveys are undertaken using standardized techniques, the rate at which species are detected and the point at which detection of new species levels off gives an indication of the number of species within an area. Such information is useful when little or nothing is known *a priori* concerning species richness, and the results can be incorporated into a monitoring program to assess the effectiveness of sampling.

Thompson and Withers (2003) have shown that the shape of a species accumulation curve is influenced by both abundance and diversity. If rare species are present, or if there are few species with high abundance, accumulation curves have low shoulders and long trajectories to the asymptote. Conversely, areas with large numbers of abundant species have steep trajectories and reach asymptotes quickly. Diversity is positively correlated with the initial slope of the trajectory of the accumulation curve. Species accumulation curves also can provide an index of the amount of sampling required to assess local and regional biodiversity, that is, if the curve has not reached its asymptote, then sampling probably has been inadequate. In studies in Western Australia, the shape of the accumulation curve indicates that some sampling regimes may be undertaken for too short a period of time in order to accurately gauge regional biodiversity (Thompson et al., 2003). An example of species accumulation curves based on intensive pitfall and other sampling techniques for amphibians is shown in Dodd et al. (submitted).

The number of species within a community, species richness, is the simplest way to describe local and regional diversity (Magurran, 1988). Although species richness is a natural measure of biodiversity, it is also an elusive quantity to measure properly (May, 1988; Gotelli and Colwell, 2001). Observed richness based on species counts over limited time periods often underestimates actual richness and shows sample size dependency (Smith and van Belle, 1984). In species-rich communities, if the site of interest is sampled repeatedly, the number of new species recorded is usually largest in the initial sample and decreases as sampling proceeds, but new species are still detected if sampling is continued (Cam et al., 2002). The sampling effort is considered sufficient if the species accumulation curve reaches an asymptote, indicating that no additional species are to be found. Since the number of species in any community is finite, if the sampling effort continues, the curve will eventually reach an asymptote at the actual community richness.

Maintaining cost-effectiveness in species inventories requires that sampling efforts be redirected to more productive sites, methods, or time periods as the expected effectiveness of further sampling declines. Statistical estimates of the richness of the species pool or the number of additional species expected in the next samples can aid this process (Keating et al. 1998). A variety of methods of estimation of species richness that allow the reduction of the underestimation associated with incomplete sampling have been developed. The different estimation methods can be grouped in those using extrapolation, that is, inferring species richness based on sub-samples, and those using interpolation, that is, inferring species richness based on comparisons with other areas or datasets (Cogălniceanu, 2003). The former methods are widely employed, with three distinct classes of statistical approaches used to estimate species sampling data (Chazdon et al. 1998):

(1) extrapolation of either species-accumulation curves or species-area curves to an asymptotic value;
(2) fitting the data on the relative abundance of species in a single sample to a parametric distribution (e.g. log-series, log-normal, Poisson log-normal);
(3) non-parametric estimators.

Usually the failure to detect rare species can dramatically underestimate the true local species richness. However, if a limited fraction of a specific taxonomic group is sampled quantitatively, sampling bias can theoretically be reduced by using statistical extrapolation to estimate species richness (Colwell and Coddington, 1994). Estimating the true number of classes (either species or individual types) in a statistical population from a random sample of classifiable objects (in this case, individuals) is a classical problem in statistics. Applications in ecology include not only the estimation of species richness, but also the estimation of population size from mark-recapture records. The situations are equivalent as capture probabilities differ among individuals in a population as the relative abundance of species varies in a community.

Several non-parametric estimators have either been developed specifically for estimating species richness from samples, have been adapted to do so from mark-recapture applications, or were developed for the general class-estimation problem (Colwell and Coddington, 1994). These non-parametric estimators only require the number of samples in which each species is found, rather than any parametric information about their abundance (Brose et al., 2003). Some of them can be reduced to a very simple form:

$S_{estimated} = S_{observed} + R$, where R is an estimate based on the presence/absence from samples of the rare species. Overall, non-parametric estimators appear to be less biased and more precise than the other two approaches. The program EstimateS does most computations required for species-accumulation curves and non-parametric analyses of species richness.

There are few applications of these methods dealing with amphibians. Pineda and Halffter (2004) have used them to verify the completeness of inventories at both local and regional scale and determined the sampling effort needed for reaching the plateau. Heyer et al. (1999) have tested the utility of museum collections for conservation decisions and have focused, among others on frogs of the genus *Leptodactylus* from Amazonia. The results indicated that, at least for amphibians, the data set was adequate in terms of sampling effort and useful for conservation decisions.

Software

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 re-trapped or re-sighted), 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 sub-set, 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 re-capture 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 or at www.phidot.org/software.

Program PRESENCE

In order to facilitate PAO analyses in amphibian monitoring studies (see *Analysis using Percent of Area Occupied*), USGS researchers have developed Program PRESENCE (version 2). This program is available free at: http://www.mbr-pwrc.usgs.gov/ software.html#presence. Researchers can record a capture history for each species at each location through time. 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, detection probabilities can be determined for each species. Trends then can be observed by examining changes in the percent of area occupied (PAO) by a species and by changes in detection probabilities.

Program EstimateS

EstimateS (version 6) computes randomized species accumulation curves, statistical estimators of true species richness (S), and a statistical estimator of the true number of species shared between pairs of samples, based on species-by-sample (or sample-by-species) incidence or abundance matrices. For comparative purposes, EstimateS also computes Fisher's alpha and the Shannon and Simpson diversity indexes for each sample, as well as the Jaccard,Morisita-Horn, and Sørensen (both incidence-based and abundance-based) indexes of biotic similarity between samples. EstimateS can be downloaded free of charge at: http://viceroy.eeb.uconn.edu/EstimateS.

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 et al., 1999; Kiesecker et al., 2004). 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 will be necessary in areas with known disease problems.

Biosecurity Protocol

Protective Wear & Equipment	Disinfecting & Sanitizing Methods
non-permeable boots or waders	rinse in bleach solution immediately after leaving each study site[3]
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 of after each use
needles & syringes (for blood extraction)	properly dispose of 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 et al., 2001).

2. Place only one specimen per bag.

3. Pre-mixed 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 pum.

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 for10 minutes

Additional Precautions

1. Avoid contact between used and unused protective wear and equipment.
2. House specimens separately.
3. Avoid contact between gloved hands and face, especially the area of the nose.
4. DO NOT urinate in or near ponds and streams.
5. Wash hands thoroughly with soap and water, or use a sanitary wipe, after urinating.
6. Wash hands thoroughly with soap and water, or use a sanitary wipe, after handling specimens known or suspected of being diseased or contaminated.
7. Wash hands thoroughly with soap and water, or use a sanitary wipe, after leaving each site.
8. 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 2 March 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% formalin (or 70-75% 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

1. Eggs. Place eggs in heavy 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.

2. Tadpoles, Larvae & 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. It is important to assure enough air is 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.

3. 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

1. About half the dead amphibians should be immediately placed into 10% buffered neutral formalin or 75% ethanol for histological 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.
2. 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.
3. 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 those that consist of just 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

1. Shipping container. Use a picnic cooler or styrofoam-lined cardboard box.

2. 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.

3. 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.

4. 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 so as to avoid crushing of the sample during transport.

5. 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 of samples in plastic bags. 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 in order to minimize leaks and moisture condensation into the cardboard box.

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

7. 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.

8. Overnight couriers should be used for sick, live and frozen amphibians.

9. 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 delivery vans in hot weather over the weekend. A significant percentage of packages mailed by overnight courier on Thursdays, do not arrive in 24 hrs, and these also may be held over the weekend in freezing or very hot delivery vans.

10. 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, you need to 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 usually causes problems for most couriers. 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 or arms and legs, missing eyes, and deformed jaws (Meteyer, 2000). Several hypotheses have been tested as causes, including parasite-induction during development (Morrell, 1999; Johnson et al., 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 the Southeastern United States to any great extent. 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 during ARMI monitoring, these protocols should be followed.

Database management and technology transfer

The Patuxent Wildlife Research Center (PWRC) is developing and will manage centralized databases and the electronic transfer of information to cooperators, land managers, the scientific community, and the general public. In addition to creating relational databases for all monitoring activities associated with ARMI, PWRC will develop appropriate metadata and make it available through the National Biological Information Infrastructure (NBII). Data management activities at Patuxent will include: 1) creation of web-based data entry pages to allow for the prompt electronic entry of data by all cooperating scientists, 2) development of all necessary quality assurance/quality control functions and appropriate security measures, 3) creation of web-based pages to allow for the easy retrieval of processed data, and 4) data analysis and interpretation. Related responsibilities include the creation of web pages providing general information about ARMI and linking these pages to other appropriate amphibian-related web sites, developing an inventory of research and monitoring activities on DOI lands, and completing development of an electronic national atlas of amphibian distribution information. The specific tasks required to successfully accomplish the overall data collection and management goals of ARMI are described in the **Database Management Plan** developed by PWRC.

Data will be collected on amphibians on DOI lands in the southeast in such a manner as to be compatible with centralized databases assembled by PWRC. Data will be transferred to PWRC in a timely and efficient manner so as to be made available through electronic transfer to ARMI partners and cooperators. Data collected by Southeast ARMI will be used in the development of the national amphibian distribution atlas.

Monitoring and Research Plan 2006

Monitoring Overview

SEARMI plans to continue inventory and monitoring of four National Wildlife Refuges in Florida and Georgia. Intensive long-term monitoring, based on previous inventories, will be initiated this year. Detailed information on sampling techniques, methods, and data collected thus far from these refuge may be found in our previous annual reports (http://cars.er.usgs.gov/armi/).

St Marks NWR

Located in Florida's panhandle approximately 25 km south of Tallahassee, St. Marks National Wildlife Refuge (SMNWR) encompasses 27,500 hectares of diverse upland and wetland habitats. Established in 1931 to provide wintering habitat for migratory birds, SMNWR extends along the Gulf coast in Taylor, Jefferson, and Wakulla Counties. SMNWR has a diversity of upland and wetland habitats and potentially supports 40 species of amphibians (21 frogs and 19 salamanders) and 68 species of reptiles (13 lizards, 34 snakes, 20 turtles, and 1 crocodilian). The Flatwoods salamander (*Ambystoma cingulatum*), a federally threatened species, has been documented from many sites on the SMNWR. In the late 1970's, data on presence of amphibians and reptiles were collected by the USFWS during a study which quantified the relationships among forestry management practices and diversity and abundance of non-game wildlife (USFWS, 1980). This study included 14 upland drift fence arrays which were monitored for 2 years.

SEARMI research at SMNWR began in May 2002 and I&M has been conducted through drift fence surveys, wetland sampling (aquatic funnel traps, dipnets, automated audio recorders), water quality sampling, disease screening, and visual encounter surveys (see Dodd et al., submitted).

Lower Suwannee NWR

Located along Florida's Big Bend region on the Gulf of Mexico, approximately 80 km WSW of Gainesville, Lower Suwannee National Wildlife Refuge (LSNWR) encompasses approximately 21,425 hectares of upland and wetland habitats. Established in 1979 to preserve unique coastal, flood plain, and upland ecosystems at the lower reach of the Suwannee River, the refuge stretches 42 km north to south in Levy and Dixie Counties. LSNWR protects a diversity of aquatic and upland habitats including floodplain forest, salt marsh, hardwood swamp, cypress swamp, cabbage palm hammock, sandhill, scrub, and pine flatwoods. LSNWR potentially supports 37 species of amphibians (21 frogs and 16 salamanders) and 66 species of reptiles (1 crocodilian, 1 amphisbaenid, 15 lizards, 34 snakes, and 15 turtles - excluding sea turtles). Historical information on the herpetofauna of the refuge is scant. Florida Museum of Natural History records included voucher specimens for only 18 species (3 amphibians and 15 reptiles) from the refuge proper, most of which dated from the 1970's or earlier.

SEARMI research began at LSNWR in May 2002 and I&M has been conducted through drift fence surveys, wetland sampling (aquatic funnel traps, dipnets, automated audio recorders), water quality sampling, and visual encounter surveys.

Harris Neck NWR

Harris Neck NWR is located ca. 46 km south of Savannah and 31 km north of Darien, in McIntosh County, Georgia. The refuge comprises 1,255 hectares of mostly coastal deciduous and oak woodlands, grasslands, former cropland, and some pine. The refuge is surrounded by salt marshes and tidal creeks, limiting amphibian colonization. Harris Neck has a long history of human occupation which certainly affected herpetofaunal species richness and distribution as a result of extensive habitat modification. Harris Neck became a National Wildlife Refuge in 1962, and is managed primarily for waterfowl. Nearly all the wetlands at Harris Neck are man-made impoundments, modified former tidal creeks, or ditches and borrow pits.

This refuge supports 13 amphibians (11 species of frogs and 1 species of salamander) and at least 17 species of reptiles (1 crocodilian, 5 lizards, 7 snakes, and 4 turtles - excluding sea turtles). It is likely that additional reptiles, particularly snakes, occur on the refuge. Historical information on the herpetofauna of the refuge is apparently nonexistent, as we have been unable to locate any museum specimens from Harris Neck. SEARMI research began at Harris Neck in April 2004 (see Dodd and Barichivich, submitted)..

Savannah NWR

Savannah National Wildlife Refuge comprises 11,320 hectares in Georgia and South Carolina immediately upstream along the Savannah River from the city of Savannah. As with Harris Neck, it is part of the Savannah Coastal Refuges Complex. The refuge has an extensive history of human occupation and use and freshwater tidal marshes were extensively diked and modified for rice production (constructed from the mid to late 1700's). Designated in 1927, the refuge is primarily managed for waterfowl, and water levels within the former rice fields (1,364 hectares) are carefully controlled. The refuge occasionally clears vegetation from the impounded areas, resulting in a variety of marsh habitats of different depths, vegetation structure, and species composition.

The northern part of the refuge (upstream from the freshwater tidal marshes) consists mostly of extensive islands of bottomland hardwoods (cypress, gum, maple) that may or may not be periodically flooded. These islands contain creeks and an extensive number of woodland pools and channels which hold water for varying amounts of time. There is only one large pond on the refuge (Kingfisher Pond, an old borrow pit) not associated with the bottomlands. River bluffs and upland terraces on the refuge are few, as the refuge boundary often terminates at the base of the river bluff. However, some uplands and slope are present along Dodge Tram Road on the north side of the river, and more extensive upland and swamp habitats are found on the south side of the river east of O'leary (as marked on the USGS 7.5' Port Wentworth topographical map). This tract is called the Solomon Tract, and is one of the most recent additions to SVNWR. This is also the location for sampling in connection with the USFWS malformed frog survey.

To date, 21 species of amphibians (15 species of frogs and 6 species of salamanders) and at least 11 species of reptiles (1 crocodilian, 2 lizards, 5 snakes, and 3 turtles) have been reported from SVNWR. Undoubtedly, many more species will be found as sampling continues, especially among the reptiles. We are currently examining historical information on the herpetofauna of the refuge, as well as the field notes from early collectors. SEARMI research began at Savannah National Wildlife Refuge in April 2004.

Additional Sites

In addition to our four DOI monitoring sites, we plan to expand I&M to several parks and refuges not under DOI management, such as the Katharine Ordway Preserve-Swisher Memorial Sanctuary in Melrose, Florida. Partnering with state, county, and non-governmental organizations is critical to achieving ARMI goals in the southeastern United States because a large proportion of conservation lands in this region are not in DOI or Federal ownership.

Southeast ARMI Research and Monitoring Plan: 2006

Overall Goals

Based on National ARMI goals and objectives, SEARMI will conduct amphibian research and monitoring at multiple scales:
Apex-level monitoring (research): Katharine Ordway Preserve
Mid-level monitoring: SMNWR, LSNWR, HNNWR, SNWR, Katharine Ordway Preserve
Base-level Inventory: initiating at least one new inventory of a NWR each year

Monitoring Methods

Wetland surveys (Mid-level Monitoring and Base-level Inventory)

Evaluation of amphibian occurrence data at wetland sites will be conducted using multiple sampling methods, including dipnetting (constrained number of sweeps), crayfish traps, automated tape and digital frog call recorders, visual-encounter surveys, and aural sampling. In order to meet the assumptions of PAO analysis, wetland sampling will be conducted at least twice a year at each site, focusing on the summer breeding season. Occupancy data from all sampling methods will be pooled to provide list of species detected at each site on each visit. The count of amphibian individuals in each crayfish trap and for each individual observer for each visit will be recorded separately in order to refine occupancy estimates. Covariates (i.e., possible factors influencing occupancy and detection probabilities) will include water chemistry data and co-occurring species (fish, invertebrates).

Three different levels of wetland sample will be conducted:
1) intensive samples which require visiting the site on 2 consecutive days (crayfish traps, dipnet, automated tape and digital frog call recorders, aural, visual)

2) basic samples which can be conducted in one visit (dipnet, visual, aural)
3) automated tape and digital frog call recorders placed at a site continuously

Wetland sites will be selected in two ways depending on the refuge sampled and the goal of monitoring (see MacKenzie, 2005). Sites will not be completely randomly selected, but rather sites will be selected based on a stratification protocol designed to either minimize or maximize wetland habitat diversity sampled. First, researchers may select wetland sites that are a representative sample of the habitat types at a particular refuge (LSNWR, HNNWR, SNWR, i.e. to include several wetlands of each habitat type in order to ensure that all wetland types on the refuge are being monitored and thus allow an area of inference to be an entire refuge). Second, wetland sites may be selected to reduce habitat variability in order to evaluate occupancy in a particular habitat type (SMNWR, i.e., if all wetlands sampled are isolated ponds or lakes, then the area of inference will only be similar habitats). Stratification will also allow for logistical constraints to be factored into the sampling protocol, such as access by road or boat.

St Marks National Wildlife Refuge

Wetland Amphibian Monitoring

SEARMI will focus on wetland sampling for amphibians at SMNWR. In 2006, wetland sampling will be concordant with a study of the effects of saltwater overwash from hurricane storm surge at SMNWR. In subsequent years, some wetland sites may be discontinued from the monitoring program if they are determined to be unsuitable for amphibians during 2006 sampling. Pressure transducers to monitor pond water levels were placed in 10 ponds in 2004 and will be maintained as necessary.

We plan to visit at least 30 sites approximately every six weeks to collect amphibian and water chemistry data. Sites will be selected using a stratified approach: first by limiting samples to the Panacea (Western) portion of SMNWR; second by including partially overwashed and non overwashed sites; third by including sites with and without fish. This design will be difficult to achieve because most overwashed sites will have fish, whereas most non-overwashed sites will be without fish, thus confounding these covariates. Our goal for this study is to determine what effects the saltwater overwash of Hurricane Dennis had on amphibian species richness and abundance, to evaluate the change in salinity of wetlands through time, and to monitor the potential recovery of amphibians at sites affected by overwash.

Intensive wetland samples (at least 3 visits each year): name (overwashed/not overwashed, fish/no fish)

Wpt 150 (no, no)
Chicky Pond (no, no)
Jennifer Sink (no, yes)
Talpoideum Pond (no, no)
Corner Pond (no, no)
Wpt 69 (no, no)
Wpt 192 (no, no?)
Perkinsus Pond (no, no)
Streetlight Pond (no, no)
Wpt 103 (no?, yes?)
Small Prairie Pond (no, yes?)
WBF Pond (no, no?)
Goose Pond (no?, yes)
Wpt 57 (no, no?)
Wpt 79 (no, yes?)
Fat Nerodia (no, no)
Wpt 222 (no, yes?)
Wpt 316 (no, yes?)
Otter Lake (no, yes)
Ring Pond (yes?, yes)
Printiss Pond (yes, yes?)
Wpt 19 (yes, yes)
Kingfisher (yes, yes)
Wpt 128 (yes, yes)
Wpt 68 (yes, no)
Wpt 317 (yes, yes)
Biggins (yes, yes)
SPC Prairie (yes, yes)
Wpt 127 (yes, yes)

Disease Monitoring

SEARMI researchers will begin repeated, systematic collections of apparently healthy tadpoles for disease analysis from at least five sites (Perkinsus Pond, Jennifer Sink, Wpt 69, Talpoideum Pond, Wpt 79) during at least two visits each year. In addition, SEARMI will continue to collect samples of dead and dying tadpoles from any sites at SMNWR where disease events are observed. This protocol will result in a more complete dataset on the dynamics of disease occurrence on this refuge.

Flatwoods Salamander Research and Monitoring

SMNWR supports a relatively large population (at least 44 breeding sites) of the federally threatened Flatwoods Salamander (*Ambystoma cingulatum*). Management recommendations for *A. cingulatum* include burning of uplands with growing season fires and increasing the herbaceous edge vegetation around breeding ponds. SEARMI will begin monitoring the status of *A. cingulatum* on SMNWR and assisting managers at SMNWR in evaluating the effects of management activities on *A. cingulatum* populations. SEARMI will conduct annual dipnet surveys, when winter weather conditions are appropriate, of all known breeding sites to evaluate the proportion of area occupied by *A. cingulatum* at SMNWR. Dependent on water levels and larval density, SEARMI will evaluate larval habitat use in ponds using funnel traps to determine the extent to which *A. cingulatum* larvae use the sawgrass interior of ponds relative to the herbaceous edge area. Potential future research includes evaluation of upland habitat use by juvenile and adult *A. cingulatum*.

Upland Amphibian Monitoring (Drift Fences)

Our drift fence arrays will be left in place but will remain closed during completion of the analysis collected thus far. Long-term drift fence data is valuable, but funding and personnel constraints prevent ARMI from adequately sampling these fences every year. Thus the drift fences may be opened and monitored on a 5-7 year cycle to build a long-term dataset without having to monitor the fences every year.

Lower Suwannee National Wildlife Refuge

Wetland sampling

Wetland sampling at LSNWR will focus on anuran species, as we have only detected four caudate species at this refuge. In addition, transitioning to anuran surveys using automated tape and digital frog call recorders will allow ARMI to monitor this refuge in the winter, when access is restricted during hunting season. Sites will be selected based on a stratified design based on habitat type, in which the four main freshwater aquatic habitat types will be monitored (tidal swamp, hydric hammock, bottomland hardwood, isolated ponds in pine uplands). The number of sites of each habitat type will be proportional to the amount of these four habitat types on the refuge. The goal for this refuge is to achieve continual monitoring using automated tape and digital frog call recorders, in combination with twice-yearly samples of water quality and dipnet surveys for anuran larvae and species which may be covariates (fish and invertebrates). SEARMI will identify 20 total sites for monitoring. Automated tape and digital frog call recorders will be set at the same site continually and data downloads and maintenance will be performed monthly. One of the benefits of this design is that it probably increases the chances of detecting explosive-breeding species, such as *Scaphiopus holbrooki* and *Rana capito*, which are stimulated to breed immediately after heavy rain events. Having automated tape and digital frog call recorders in place at wetlands even when the sites are dry ensures that short-term breeding events will be recorded.

Automated tape and digital frog call recorders wetland sites:

Tidal 1	Bottomland Hardwood 1
Tidal 2	Bottomland Hardwood 2
Tidal 3	Bottomland Hardwood 3
Tidal 4	Bottomland Hardwood 4
Hydric Hammock 1	Bottomland Hardwood 5
Hydric Hammock 2	Bottomland Hardwood 6
Hydric Hammock 3	Pine Plantation 1
Hydric Hammock 4	Pine Plantation 2
Hydric Hammock 5	Pine Plantation 3
Hydric Hammock 6	Pine Plantation 4

Drift Fence Sampling

Our drift fence arrays will be left in place but will remain closed during completion of the analysis collected thus far. Long-term drift fence data is valuable, but funding and personnel constraints prevent ARMI from adequately sampling these fences every year. Thus the drift fences may be opened and monitored on a 5-7 year cycle to build a long-term dataset without having to monitor the fences every year.

Harris Neck National Wildlife Refuge

Wetland Amphibian Monitoring

At least 2 visits will be made to HNNWR each year during the summer breeding season.

Wetland sites at HNNWR that will be intensively sampled on each sampling trip:

Borrow Pond	Goose Pond

Greenhead Pond	Snipe Pond 1 & 2
Lucas Borrow	Widgeon Pond
Lucas Pond	Woody Pond

Additional wetland sites will be visited for basic samples as time and water levels permit:

Church Ditch	Red Maple Swamp
Culvert Pond	Snake Bog
Goose Meadow	Snipe Pond 3
Lucas Seepage	Teal Pond
N Runway Ditch	Woody Swamp
Plantation Fountain	

Disease Monitoring

SEARMI will begin repeated, systematic collections of apparently healthy tadpoles for disease analysis from at least three sites (Goose Pond, Snipe Pond, Borrow Pit Pond) during at least one visit each year. In addition, SEARMI will collect samples of dead and dying tadpoles from any sites at HNNWR where disease events are observed. This protocol will result in a more complete dataset on the dynamics of disease occurrence on this refuge.

Savannah National Wildlife Refuge

Wetland Amphibian Monitoring

At least 2 visits will be made to SNWR each year, during the summer breeding season. Wetland sites at SNWR that will be intensively sampled on each sampling trip:

DT-2	WD-3
HQ-1	WD-6
ND-3	WD-7
ND-4	WD-8

Additional wetland sites will be visited for basic samples as time and water levels permit:

ND-5	ST-5
ST-2	ST-6
ST-3	WD-1

WD-2
WD-5
WD-4

At least one visit per year will be made to Bear Island by boat to conduct basic wetland surveys and visual encounter surveys along FWS transects across the island, or at other appropriate sites, water levels permitting (BI-1, BI-4, BI-5, BI-6, BI-7).

Disease Monitoring

ARMI will begin repeated, systematic collections of apparently healthy tadpoles for disease analysis from at least three sites (DT-2, WD-3, ND-3) during at least one visit each year. In addition, ARMI will collect samples of dead and dying tadpoles from any sites at HNNWR where disease events are observed. This protocol will result in a more complete dataset on the dynamics of disease occurrence on this refuge.

Katharine Ordway Preserve (Ordway)

In conjunction with monthly visits to the Ordway Preserve for apex-level siren and amphiuma monitoring and research, basic wetland samples will be conducted in 10 wetland sites.

Blue Pond
One-shot Pond
Anderson Que Pond (N)
Smith Lake
Pine Lodge Pond
Goose Lake
Lake Rowan
Lake Barco
Lake Suggs
Fox Pond
Breezeway Pond (when water is present)

Apex-level Monitoring & Research Activities: *Siren* and *Amphiuma* Population Demography

Background

Apex-level studies are critical components of the ARMI program which include research on population estimates, demographic rates, and other long-term research on focal species. In 2005 SEARMI began an apex mark-recapture study on the large aquatic salamanders *Siren* *lacertina* and *Amphiuma means*. These large aquatic salamanders are often abundant in aquatic ecosystems in the southeast, but their life history is poorly known.

This study is being conducted at Lake Suggs on the Katharine Ordway Preserve-Swisher Memorial Sanctuary, a property jointly owned by the Nature Conservancy and the University of Florida and managed by the UF Department of Wildlife Ecology and Conservation. This research project is a continuation of a project conducted from Aug 2001 – Jul 2002 by then ARMI biologist Kristina Sorensen (Sorensen, 2004). In the initial study 58 *A. means* and 66 *S. lacertina* were marked using Passive Integrated Transponder (PIT) tags. Due to the low recapture rate, calculation of growth rates in the initial study was difficult.

The objectives of the current study are to: (1) evaluate the population size and demographic structure for *A. means* and *S. lacertina* at Lake Suggs, (2) obtain growth rate and survival estimates for each species, and (3) understand activity and movement patterns of these species.

Methods

Sirens and amphiumas are collected at Lake Suggs using 20 mesh-lined crayfish traps which are set 5 m apart on permanent trap poles for four nights each month. Data collected for all captured sirens and amphiumas includes snout vent length (SVL), total length (TL), weight, and any injuries or bite marks are noted and described. Animals larger than approximately 150 mm TL are marked by injecting a PIT tag into the lateral tail muscle. Data are also collected on the number of other animals captured in each trap, including fish and invertebrates.

Other Research

In addition to our monitoring efforts, we plan to continue to perform experiments evaluating the factors that contribute to amphibian population dynamics using our laboratory and outdoor mesocosm facility at FISC.

Work Schedule

ARMI is a Congressionally supported program, and there is no specified end date. We anticipate that data collection, analysis and research will continue as long as Congress perceives there is a need for monitoring amphibian populations. Field work continues year-round within the

Southeast.

Four National Wildlife Refuges will be monitored in 2006, with visits mostly during the summer anuran breeding season.

Expected Products

Publications and presentations on baseline inventories of amphibians on DOI lands

Reports to DOI agencies and peer-reviewed publications on the efficacy of various amphibian sampling protocols for use in southeastern National Parks and National Wildlife Refuges

Presentations, reports and publications, as appropriate, on the status and trends of amphibian populations on DOI lands within the southeastern United States and Caribbean. Reports to DOI agencies are usually due at the end of the calendar year.

WEB-based and outreach information on the distribution, identification, life history, and status and trends of southeastern amphibians coordinated through PWRC and through the FISC/CARS web sites (see above)

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