Southeast Amphibian Research & Monitoring Initiative
2006 Annual Summary

Plethodon variolatus, Savannah National Wildlife Refuge. Photo William J. Barichivich

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Overview of Southeast ARMI Accomplishments in 2006

Inventory and Monitoring:

- St. Marks NWR – drift fence data manuscript completed and accepted for publication
- St. Marks NWR – completed initial phase of hurricane storm surge overwash study
- Harris Neck and Savannah NWRs – monitoring phase initiated
- Lower Suwannee NWR – preparated for monitoring with digital frogloggers

Research:

- Effects of storm surge overwash on amphibian communities in isolated wetlands at St. Marks NWR
- Completed analysis of tadpole health screening data from National Fish Hatcheries
- Continuation of project on population demography of *Siren* and *Amphiuma* species
- Evaluation of effects of fish predation on *Rana capito* and *R. sphenocephala* larvae
- Evaluation of baiting crayfish traps for sampling large aquatic salamanders

Publications:

- Seven ARMI-related scientific papers and book chapters
- 12 ARMI-related presentations at meetings and invited presentations
- Tadpoles of Southeastern United States Coastal Plain field guide
- National ARMI factsheet, with Hannah Hamilton (USGS-FISC)
- National ARMI 5-year report coauthored by Southeast ARMI personnel

Collaborations:

Southeast ARMI provided technical support and study design advice to two amphibian monitoring and research projects in Florida:

- Cedar Key Scrub State Preserve herpetofaunal inventory; Ron Black, Dan Dourson, and Jeff DiMaggio
- *Siren lacertina* radiotracking study, Merritt Island NWR; Trisha Craybill, Towson University, Maryland

Introduction

Amphibian Declines

At the First World Congress of Herpetology in 1989, participants presented scientific papers and exchanged personal accounts of amphibian declines and disappearances. Based on the magnitude and geographic extent of documented declines and local extinctions of amphibian populations, the herpetologists concluded that amphibian declines represented a potentially global environmental crisis. Research conducted by many scientists since the meeting has shown a variety of factors have contributed to the global amphibian decline, including acid precipitation, environmental contaminants, introduction of nonindigenous predators, disease agents, parasites, ultraviolet radiation, and unsustainable harvest and trade of amphibians. Habitat loss has been, and continues to be, a major factor contributing to declines and extinctions.

In the United States, amphibian declines of unknown origin were first identified in Puerto Rico and the western states, where many populations are small and isolated. In addition, malformed amphibians were observed in high numbers in the upper Midwest, Great Lakes region, and northern New England. Declines in the southeastern US have been linked directly to habitat loss. Although all regions of the US are not affected to the same degree, the scope of declines, disease, and malformations suggests vigilance is needed throughout the Nation to ensure the conservation of amphibian populations.

USGS Amphibian Research and Monitoring Initiative (ARMI)

In 1998, an international meeting of herpetologists convened by the National Science Foundation concluded that significant amphibian declines have occurred in protected areas not subjected to obvious changes in habitat, such as National Parks, National Wildlife Refuges, and wilderness areas. In 2000, the President of the United States and the Congress directed the Department of the Interior (DOI) agencies to develop a plan to initiate monitoring of trends in amphibian populations on DOI lands and conduct research into causes of declines. The DOI has stewardship responsibilities over extensive land holdings in the US, much of which is occupied by or is potential habitat for amphibians. The US Geological Survey (USGS), the science and research bureau for DOI, was given lead responsibility for planning and organizing this program, named the Amphibian Research and Monitoring Initiative (ARMI), in cooperation with the National Park Service (NPS), US Fish and Wildlife Service (USFWS), and Bureau of Land Management (BLM). The USGS is uniquely qualified to develop and provide scientific leadership for such an effort. USGS research scientists have pioneered studies on amphibian life history, sampling techniques, toxicology, and health-related issues, and the USGS has responsibility for many natural resource monitoring programs at regional, national, and continental scales.

ARMI Objectives

1) Establish a network designed to monitor the status and changes in the distributions and abundance of amphibian species and communities in the United States.

2) Identify and monitor environmental conditions known to affect amphibians and document their differences across the Nation.

3) Conduct research that identifies causes of amphibian population change and malformations.

4) Provide information to managers, policy makers and the general public in support of amphibian conservation.

ARMI Framework

Studies by USGS scientists concentrate on DOI and other federal lands, but ARMI also provides the framework for incorporating data collected on non-federal lands to encourage participation by states, universities, and non-governmental organizations. The framework can be conceptualized as a pyramid, with extensive and necessarily coarse measurements at many monitoring sites across the country (the base of the pyramid), mid-level efforts at a moderate number of sites to provide a regional perspective on the status of amphibians (the middle portions of the pyramid), and intensive research efforts at a relatively small number of index sites throughout the Nation (the top of the pyramid). Activities at the different levels of the framework are integrated by:

- Research on causes of change, which at all levels is guided by monitoring results
- Synthesis across ecological regions, scientific disciplines, and governmental and institutional boundaries
- Comparable protocols, analytical tools, training, and planning
- Common databases and reporting
- Ecological modeling

Monitoring efforts, the primary focus of ARMI, are conducted at all levels of the pyramid, although emphasis is placed on the middle level. Monitoring conducted at this level of the pyramid is carried out at mid-level monitoring areas, such as National Wildlife Refuges. Monitoring studies are designed to detect changes in the occurrence and abundance of species across the monitoring areas. The emphasis is on estimating well-defined parameters using statistical approaches applicable across species, monitoring areas, and ARMI regions. One such approach is to use detection/non-detection data to estimate a population level parameter, proportion of area occupied (PAO), on a species by species basis, as well as a community level parameter, species richness.

The Southeast ARMI (SEARMI) Program

For the purposes of the ARMI program implementation, the 50 States are divided into seven groups that are the focus of regional herpetological investigations. The Southeast Region encompasses Florida, Georgia, Alabama, Tennessee, South Carolina, and North Carolina, as well as Puerto Rico and the US Virgin Islands, an area approximately 1300 × 1660 km (800 × 1000 mi), excluding the Caribbean territories. Within this area, DOI land holdings are numerous, ranging in size from "postage stamp" historical sites to regional ecosystem-wide parks and preserves. Most lands are administered by the NPS and the USFWS; the BLM has certain responsibilities for oversight in coal mining areas (such as in northern Alabama), but there are no land holdings. SEARMI research and monitoring efforts are conducted from the Florida Integrated Science Center (FISC) office in Gainesville, Florida. Additional ARMI-related research has been conducted by USGS biologists with the FISC office in Ft. Lauderdale, Florida, although they have not received ARMI funding.

The southeastern US exceeds other regions of the Nation in the diversity and abundance of amphibians. With at least 144 species of amphibians, it has well over half of the species known from the US. Habitat diversity also is great, ranging from the high mountain peaks of the Great Smoky Mountains, to the humid forested lowlands of the coastal plain, and to the vast marshes of South Florida. Southeastern amphibians have diverse life histories, from fully aquatic salamanders, to a great variety of salamanders and frogs that spend portions of their life cycle in both aquatic and terrestrial environments, to fully terrestrial salamanders. Some species are widespread and abundant, whereas others are rare, localized, and highly vulnerable to extirpation.

As in other regions, SEARMI monitoring is based on a three-tiered approach involving sites with intensive research (apex sites), sites that form the basic areas for the core of monitoring activities (mid-level sites), and sites where inventories are conducted (base sites). With few exceptions, federal lands in the southeast have not been surveyed for amphibians or their habitats, thus requiring inventories prior to more intensive research. For this reason, most current activity focuses on mid-level and base sites. Another emphasis has been on the development of appropriate sampling techniques and in understanding the biases associated with their use. Information from SEARMI's inventory and monitoring program will be used to assess the status of amphibians on DOI lands using PAO analyses. By making probabilistic arguments, PAO uses an estimation of site occupancy rate to determine species detection probabilities. Details on PAO methodology appear in the scientific literature. SEARMI biologists are collecting extensive data on species and their habitats that will allow for an assessment of distribution patterns and trends, and the initiation of research on declines or problem areas should they be identified. Finally, we are developing partnerships and collecting data on amphibian distribution, available literature, and the extent of previous amphibian surveys on DOI lands.

Sampling Methods

Amphibian Sampling

Most amphibian species of the southeastern US coastal plain region breed in wetlands (e.g., ponds, streams, lakes), thus in 2006 we continued to focus our sampling in aquatic habitats to inventory amphibian species richness, identify appropriate sites for long-term monitoring, and determine the distribution of breeding sites. Focusing long-term amphibian monitoring at wetlands allows us to maximize species detection probabilities and identify important covariates (e.g., pH, fish predators) influencing species detection and proportion of area occupied. We used a variety of methods to sample amphibians at each refuge (Table 1). Aquatic sampling incorporated dip nets (Memphis Net and Twine Co., HDD-2 with 3/16" sq. Delta mesh), crayfish traps (Johnson and Barichivich, 2004), aural surveys (incidental observations and using automated frog call data loggers, Barichivich, 2003), and visual encounter observations. We also used terrestrial sampling techniques, including field searches (i.e., visual encounter surveys) for animals in the open and under cover objects (e.g., logs, rocks) and road cruises (i.e., making opportunistic observations while driving roads, typically at night), as part of our continued inventory efforts.

Reptile Sampling

Because the managers and biologists at our study sites are often interested in the status of reptile populations at those sites, and because most of the sampling methods we use are also effective for reptiles, we have included this taxon in our inventory efforts. The sampling methods used in 2006 are the same as those described above for amphibians.

Water Quality Data

We collected standard abiotic field parameters likely to influence amphibian species distribution at most of the wetlands sampled. Using a Hydrolab® Quanta® water-quality meter we measured water temperature (ºC), specific conductance (mS/cm), pH, dissolved oxygen (mg/L), and percent dissolved oxygen. More detailed water quality parameters were measured at selected wetlands in St. Marks, Harris Neck, and Savannah National Wildlife Refuges by USGS

Water Resources Discipline (WRD) personnel. Field parameters, major ions, nutrients, trace metals, and suspended/particulate organic carbon from these sites were analyzed by WRD.

<u>Voucher Specimens and Disease Monitoring</u>

We collected limited numbers of animals for voucher specimens, to confirm species identification, and for disease screening. When possible, voucher specimens were collected as dead-on-road (DOR) individuals. Because of the difficulty in positively identifying amphibian eggs and small larvae, live specimens were occasionally collected and reared at the USGS lab in Gainesville, Florida, to confirm species identifications. These specimens were preserved as vouchers once identifications were confirmed. All voucher specimens were (or will be) deposited in the herpetology collection of the Florida Museum of Natural History at the University of Florida, Gainesville. We collected amphibian larvae at three wetlands at St. Marks National Wildlife Refuge for disease screening by the USGS National Wildlife Health Center (NWHC), Madison, Wisconsin.

Inventory and Monitoring Activities: St. Marks National Wildlife Refuge

Background

Located in Florida's panhandle approximately 25 km south of Tallahassee (Fig. 1), St. Marks National Wildlife Refuge (SMNWR) encompasses 27,500 ha of diverse upland and wetland habitats (Fig 1). Established in 1931 to provide wintering habitat for migratory birds, SMNWR extends along the Gulf coast in Taylor, Jefferson, and Wakulla Counties (Fig. 2A). For management purposes the refuge is divided into three major units. The easternmost of these, the St. Marks Unit, is generally bounded by the Aucilla River to the east and the Wakulla and St. Marks Rivers to the west. The Wakulla Unit runs west from the Wakulla and St. Marks Rivers to Spring Creek Hwy (CR 365). The westernmost section, the Panacea Unit, extends west from Spring Creek Hwy to the Ochlockonee River and Ochlockonee Bay.

SMNWR has a diversity of upland and wetland habitats that potentially support 40 species of amphibians (21 frogs and 19 salamanders) and 68 species of reptiles (13 lizards, 34 snakes, 20 turtles, and one crocodilian). The Flatwoods Salamander (*Ambystoma cingulatum*), a federally threatened species, has been documented from many sites on SMNWR. In the late 1970s, data on presence of amphibians and reptiles were collected by the USFWS during a study that quantified the relationships among forestry management practices and diversity and abundance of non-game wildlife (USFWS, 1980). This previous study provided baseline data on herpetofaunal species richness and distributions, allowing for temporal comparisons with our more recent data (see Dodd et al., in press). SEARMI research at SMNWR began in May 2002.

In 2006, we focused on wetland sampling to evaluate the effects of storm surge overwash from Hurricane Dennis in 2005 on the amphibian communities of isolated wetlands in the Panacea Unit.

2006 Effort

Seven visits were made to SMNWR in 2006 as part of SEARMI (Table 2). Our WRD collaborators collected water samples during two visits. In Jan.-Feb. 2006, we assessed 71 sites as possible monitoring sites for the storm surge overwash effects study. Thirty sites were chosen based on specific conductance values and/or pressure transducer data (indicating whether or not the site was inundated with saltwater) and presence or absence of fish. The majority of 2006 sampling was focused at these sites (the exception was surveys focused on *Ambystoma cingulatum* larvae). In Mar. 2006, we prepared the 12 drift fence arrays for long-term closure, using sand to fill in all pit-fall traps.

Results

We have detected a total of 80 species of amphibians and reptiles at SMNWR to date (Table 3). This total includes captures and observations made with all the methods used, including incidental observations. The 31 amphibian species comprise 20 frog and 11 salamander species, and the 48 reptile species comprise 10 lizard, 27 snake, one crocodilian, and 11 turtle species (Table 3). In 2006, we sampled 91 wetland sites; 36 sites were visited for regular surveys (20 for regular surveys only, 16 for surveys and storm surge assessment) and 55 sites were visited for storm surge assessment only (Table 1). At these sites, we detected 14 frog, eight salamander, and 14 reptile species (six snakes, three lizards, four turtles, and one crocodilian) (Tables 5a, 5b). We confirmed the presence of *Ambystoma cingulatum* larvae in four ponds, representing the three populations on SMNWR, with David Cook (Florida Fish and Wildlife Conservation Commission) in Mar. 2006. We did not detect any previously unrecorded species during the 2006 sampling. It is probable we have recorded most species present on this refuge using the stated sampling methods, as indicated by the decreasing detection rate of new species (Figs. 3, 4).

Water Quality Data

In May and Aug. 2006, we assisted WRD personnel in the collection and field processing of water samples from 20 sites. Sampling was concentrated in the Panacea Unit at monitoring sites that are part of our study on the effects of storm surge overwash on the amphibian communities of isolated wetlands. Field parameters (pH, conductance, dissolved oxygen, temperature), major ions, nutrients, trace metals (including mercury), and suspended/particulate organic carbon were analyzed from all sites. The 2006 WRD water quality data for this refuge will be available at the USGS NWISWeb Data site (http://waterdata.usgs.gov/fl/nwis/qw). For more information on WRD water quality results, please contact W. Brian Hughes, Southeast Regional Hydrological Research Coordinator for ARMI (USGS, 3039 Amwiler Rd, Atlanta, GA 30360; 770-903-9162; wbhughes@usgs.gov).

The water quality field parameter data from all sites sampled in 2006 are shown in Table 5.

Disease monitoring

As part of our sampling activities at SMNWR, we look for diseased or malformed amphibians. During 2006, no animals exhibiting malformations or obvious disease symptoms were observed. In Jun. 2006, we submitted apparently healthy amphibian larvae from three sites (Jennifer's Sink, Talpoideum Pond, and Wpt 69) for routine disease screening by the NWHC. The submission comprised four *Rana catesbeiana*, five *Hyla femoralis*, one *Ambystoma talpoideum*, one *Hyla gratiosa*, and one *Acris gryllus* larvae. Results of the analyses of these specimens are pending.

Future Plans

The SEARMI drift fence arrays have been left in place, but will remain closed in FY 2007. Although long-term drift fence data are valuable, funding and personnel constraints prevent us from adequately sampling these fences every year. The drift fences may be opened and monitored on a 5-7 year cycle to continue to build a long-term dataset, depending on changing objectives and financial and logistic support. In FY 2007, ponds will be identified for long-term intensive monitoring using PAO analyses, and we will continue to collect and analyze data in connection with the storm surge overwash study. Further research plans are currently under review, pending the arrival of Dr. Susan Walls, the new SEARMI principal investigator, in Apr. 2007.

Inventory and Monitoring Activities: Lower Suwannee National Wildlife Refuge

Background

Located along Florida's Big Bend region on the Gulf of Mexico, approximately 80 km WSW of Gainesville (Fig. 1), Lower Suwannee National Wildlife Refuge (LSNWR) encompasses approximately 21,425 ha of upland and wetland habitats (Fig. 2B). The refuge stretches 42 km north to south in Levy and Dixie Counties, and was established in 1979 to preserve unique coastal, flood plain, and upland ecosystems along the lower reach of the Suwannee River. The refuge includes lands along both banks of the Suwannee River, from Yellow Jacket landing southwest to the Gulf of Mexico. The Dixie County portion extends north along the coast to Shired Island. The main Levy County portion extends south along the coast, almost to the mouth of Ericson Creek. The Shell Mound Unit of LSNWR is just south of Ericson Creek and adjacent to Cedar Keys NWR (administered and managed by LSNWR staff). Cedar Keys NWR is a complex of 13 islands (about 310 ha) around the municipality of Cedar Key and was established in 1929 as a refuge for colonial wading birds.

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. This refuge potentially supports 37 species of amphibians (21 frogs and 16 salamanders) and 66 species of reptiles (one crocodilian, one amphisbaenid, 15 lizards, 34 snakes, and 15 freshwater turtles). Historical information on the herpetofauna of the refuge is scant. Florida Museum of Natural History records included voucher specimens for only 18 species (three amphibians and 15 reptiles) from the refuge proper, most of which date from the 1970s or earlier. SEARMI research began at LSNWR in May 2002.

The results of our amphibian inventory efforts to date indicate we are detecting mostly anuran species in our wetland and drift fence sampling. Thus, we have decided to implement continual digital frog call data logger sampling at this refuge. The benefit of this method is it allows continuous and synchronous sampling, increasing the likelihood of detecting sporadic, explosively breeding anurans such as *Rana capito* and *Scaphiopus holbrookii*.

2006 Effort

One visit was made to LSNWR in Apr. 2006 as part of SEARMI. Our goal was to establish 20 long-term monitoring sites in the four most widespread habitat types (hydric hammock, bottomland hardwood, pine plantation, new tidal swamp). We selected sites in each habitat type in proportion to the area of the habitat on the refuge. Using ArcGIS, we calculated the area of each habitat and then allocated the number of sites to be located within each habitat based on its area. A 500 m grid was overlaid on each habitat type, and we randomly selected grid cells within each habitat in which to locate sampling stations. We then ground truthed each selected cell to confirm the habitat type and find an appropriate location for the digital froglogger. During this visit, we also measured water quality field parameters and conducted rapid amphibian surveys using dipnet, visual, and aural sampling where water was present. Our long-term monitoring program at LSNWR will begin as soon as viable digital frogloggers are available. Researchers from several ARMI regions, including SEARMI, are currently reviewing the available technology and evaluating prototype models and software.

Results

We have detected 61 species of amphibians and reptiles at LSNWR to date (Table 3). This includes captures and observations made with all methods used, including incidental observations. The 23 amphibian species comprise 19 frog and four salamander species. The 38 reptile species comprise eight lizard, 18 snake, one crocodilian, and 11 turtle species (Table 3). We did not detect any previously unrecorded species during our limited 2006 sampling. However, it is probable we have recorded most species present on this refuge we are likely to encounter using the stated sampling methods, as indicated by the decreasing detection rate of new species (Figs. 3, 4).

Future Plans

The drift fence arrays have been left in place, but will remain closed during completion of the analysis of data collected thus far. Although long-term drift fence data are valuable, funding and personnel constraints prevent us from adequately sampling these fences every year. Thus the fences may be opened and monitored on a 5-7 year cycle to continue to build a long-term dataset, depending on changing objectives and financial and logistic support. In FY 2007, ponds and river sites will be identified for long-term intensive monitoring using PAO analyses. Further research plans are currently under review, pending the arrival of Dr. Susan Walls, the new SEARMI principal investigator, in Apr. 2007.

Inventory and Monitoring Activities: Harris Neck and Savannah National Wildlife Refuges (Savannah Coastal Refuges complex)

Background

Harris Neck NWR

Harris Neck National Wildlife Refuge (HNNWR) is located about 46 km south of Savannah and 31 km north of Darien, in McIntosh County, Georgia (Fig. 1). The refuge comprises 1,255 ha of mostly coastal deciduous and oak woodlands, grasslands, former cropland, and some pine (Fig. 2C) and is surrounded by salt marshes and tidal creeks, limiting amphibian colonization. Harris Neck has a long history of human occupation (Amerindian, pre-Civil War plantations, a series of many small farms in the late 1800s-early 1900s, an airfield, a military base during World War II [Harris Neck Air Base], and under the ownership and management of several county, state, and federal agencies after the war (Sullivan, 1997), which certainly affected herpetofaunal species richness and distribution as a result of extensive habitat modification. Harris Neck became a NWR in 1962, and is managed primarily for waterfowl and wading birds, including the Wood Stork. Nearly all the wetlands at HNNWR are either anthropogenic impoundments, modified former tidal creeks, or ditches and borrow pits. Historical information on the herpetofauna of HNNWR is apparently nonexistent, as we have been unable to locate any museum specimens from the refuge. SEARMI research began at Harris Neck in Apr. 2004.

Savannah NWR

Savannah National Wildlife Refuge (SNWR) (Fig. 1) comprises 11,320 ha in Georgia and South Carolina, immediately upstream along the Savannah River from the city of Savannah (Fig. 2D). As with Harris Neck, it is part of the Savannah Coastal Refuges Complex. The refuge has an extensive history of human occupation and use, from Amerindian through the plantation era, when the bottomlands and freshwater tidal marshes were extensively diked and modified for rice production (constructed from the mid to late 1700s). Designated as a NWR in 1927, the refuge is primarily managed for waterfowl, and water levels within the former rice fields (1,364 ha) 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 that hold water for varying amounts of time. There is only one large pond on the refuge (Kingfisher Pond, = DT-2, 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 area, the Solomon Tract, is one of the most recent additions to SNWR. This is also the location for sampling in connection with the USFWS malformed frog survey. 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 SNWR in Apr. 2004.

<u>2006 Effort</u>

Two visits were made to Harris Neck and Savannah NWRs in 2006 as part of SEARMI (Table 2). Our WRD collaborators collected water samples during one visit.

<u>Results</u>

Harris Neck NWR

We have detected 36 species of amphibians and reptiles at HNNWR to date (Table 3). This includes captures and observations made with all methods, including incidental observations. The 13 amphibian species comprise 12 frog and one salamander species. The 23 reptile species comprise five lizard, 13 snake, one crocodilian, and four turtle species (Table 3). The reptile species include three snake species added in 2006; *Cemophora coccinea*, *Crotalus adamanteus*, and *Elaphe guttata*. In 2006, we sampled seven wetland sites, at which we detected 11 frog and one salamander species (Table 4a). We also detected eight reptile species (three snakes, three lizards, one turtle, and one crocodilian) at these sites (Table 4b).

Savannah NWR

We have detected 42 species of amphibians and reptiles at SNWR to date (Table 3). This includes captures and observations made with all methods, including incidental observations. The 23 amphibian species comprise 15 frog and eight salamander species. The 19 reptile species comprise three lizard, 11 snake, one crocodilian, and four turtle species (Table 3). The amphibian species include one possibly new record of the salamander, *Siren intermedia*, for 2006. Three individuals were regurgitated by a *Nerodia fasciata* captured in a crayfish trap at HQ-1, but because adult *S. intermedia* are difficult to distinguish from sub-adult *S. lacertina*, we must examine the specimens further to confirm the species identification. The reptile species include one snake, *Thamnophis sauritus*, and one turtle, *Trachemys scripta*, added in 2006. We sampled 10 wetland sites in 2006, at which we detected 10 frog and five (possibly six) salamander species (Table 4a). We also detected 11 reptile species (five snakes, two lizards, three turtles, and one crocodilian) at these sites (Table 4b).

Water Quality Data

Harris Neck NWR

We collected water quality field parameters at eight sites in 2006. We measured these parameters at four sites on more than one visit. Values for pH ranged from 4.95 to 7.58, with a mean among all samples of 6.04 and a median value of 6.00. Specific conductance was less than 0.700 mS/cm at all sites, with a mean of 0.219 mS/cm. Dissolved oxygen (DO) and %DO varied considerably, ranging from 0.27 mg/L and 3.70% to 10.30 mg/L and 137.90%. Water temperatures ranged from 21.81 °C to 35.92 °C (Table 5).

WRD personnel collected water samples at eight sites in Mar. 2006. Field parameters (pH, conductance, dissolved oxygen, temperature), major ions, nutrients, trace metals (including mercury), and suspended/particulate organic carbon were analyzed from all sites. The 2006 WRD water quality data for this refuge will be available at the USGS NWISWeb Data site (http://waterdata.usgs.gov/fl/nwis/qw). For more information on WRD water quality results, please contact W. Brian Hughes, Southeast Regional Hydrological Research Coordinator for ARMI (USGS, 3039 Amwiler Rd, Atlanta, GA 30360; 770-903-9162; wbhughes@usgs.gov).

Savannah NWR

We collected water quality field parameters at five sites in 2006, and we measured these parameters at each site on more than one visit. Values for pH ranged from 5.80 to 7.36, with a mean among all samples of 6.44 and a median value of 6.34. Specific conductance varied considerably, ranging from 0.032 to 1.035 mS/cm, with a mean of 0.483 mS/cm. Dissolved oxygen (DO) and %DO ranged from 0.18 mg/L and 2.20% to 1.94 mg/L and 22.80%. Water temperatures did not vary widely, ranging from 22.95 °C to 27.27 °C (Table 5).

WRD personnel collected water samples at 12 sites in Feb. 2006. Additional information on this sampling is provided in the preceding section.

Future Plans

In FY 2007, ponds and other wetlands will be identified for long-term intensive monitoring using PAO analyses, and collections for disease screening will be conducted. Further research plans are currently under review, pending the arrival of Dr. Susan Walls, the new SEARMI principal investigator, in Apr. 2007.

Research Activities: The Potential for Fish Stocking to Spread Disease to Aquatic Amphibians

Background

Amphibian populations and species are declining or disappearing from many regions and habitats world wide (Stuart et al., 2004). 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-B radiation have been suggested as factors causing amphibian declines. Indeed, several factors may interact in such a manner as to threaten species and populations locally or regionally (Carey and Bryant, 1995). However, the negative effects of disease can be especially devastating, and declines due to disease, particularly from chytrid fungus and ranaviruses (Berger et al., 1998; Chinchar, 2002), are well documented from many disparate regions, including North America (Daszak et al., 2003; Kiesecker et al., 2004). In addition to the more well-known fungi and viruses, a disease of undetermined affinity (*Anuraperkinsus*) is now known to have had serious effects on populations of ranid frogs in the southeast (*Rana sevosa* in Mississippi; R. Seigel, C.K. Dodd, Jr., unpub. data). Of additional relevance, the spread of pathogens has been linked specifically with the introduction of nonindigenous amphibians (Mazzoni et al., 2003; Hanselmann et al., 2004; Beard and O'Neill, 2005; Jancovich et al., 2005).

The primary objective of this research was to determine whether diseases that could have a detrimental effect on southeastern amphibians (primarily chytrids, ranaviruses, *Anuraperkinsus*, saprolegniasis) were present in amphibian larvae living in warm-water National Fish Hatcheries (NFHs) in the southeastern US. Second, we wanted to make a preliminary assessment of the potential for amphibian larvae in stocking locations to have a higher incidence of disease than non-stocked locations. This research has provided a foundation for an ongoing assessment of the extent of disease in southeastern amphibians, and the role fish hatcheries may play in disease spread. An examination of hatchery records also allowed a preliminary assessment of the extent to which hatchery shipments could contribute to the mixture of amphibian genotypes from very different habitats on the southeastern coastal plain. Although the results have not demonstrated that hatchery shipments have spread disease in the past, they may help in the prevention of future disease outbreaks by allowing researchers to make recommendations to minimize such threats. To conduct this research, we partnered with the National Wildlife Health Center (NWHC) and various southeastern USFWS entities (NWRs and NFHs). Larvae were (and are being) screened at the NWHC for pathogens.

In the southeastern US, warm-water fish hatcheries supply NWRs and other land management agencies with stock for sport fishing, ecological restoration, and as food vital in endangered species management. Several million fish may be transported from one region to another across state lines in a single restocking event. For example, 3 million bluegills were stocked at HNNWR in 2004 to provide food for a nesting colony of endangered Wood Storks. A range of other aquatic invertebrates and vertebrates are associated with the fish, including tadpoles and potentially salamander larvae. Shipments are not screened for amphibian larvae or pathogens. Moving large numbers of non disease-screened amphibian larvae throughout a region has the potential to transfer disease pathogens quickly, and with serious consequences to resident amphibian populations. For example, chytrid fungi can remain virulent for seven days in contaminated water, thus offering the potential for disease transmission even without direct contact with infected amphibians (Johnson and Speare, 2003). In South Carolina, chytrid infections were first reported in bullfrogs in 1978 from the Savannah River Site, not far from the USFWS Orangeburg NFH, a source of fish stock for the southeastern Atlantic Coastal Plain. Chytrid infections in amphibians also have been reported from the coastal plain of North Carolina.

Methods

Our 2005 annual report (available in PDF format at http://cars.er.usgs.gov/armi /2005_Annual_Report/2005_annual_report.html) provides details on the methods used during this research.

Results

The complete final report of the NWHC analyses is provided in Appendix II. Findings of note include the presence of a potentially new microsporidian infection in four tadpoles (three *Hyla gratiosa*, one *Rana sphenocephala*) from Welaka NFH, Welaka, Florida, and oral chytridiomycosis infections in four of five *R. catesbeiana* tadpoles from Warm Springs NFH, Warm Springs, Georgia. The microsporidian from Welaka NFH has not been previously reported and is possibly a new species or was transmitted by fish present in the hatchery ponds. The following abstract is from the draft manuscript on this research currently in preparation (Green and Dodd, in prep.).

Presence and significance of chytrid fungus *Batrachochytrium dendrobatidis* and other amphibian pathogens at warm-water fish hatcheries in southeastern North America

Abstract: We conducted health screenings for emerging infectious diseases of amphibians at four warm-water fish hatcheries and one national wildlife refuge in the southeastern United States. We confirmed the presence of *Batrachochytrium dendrobatidis* (chytrid fungus) in *Rana catesbeiana* (American Bullfrog) from one hatchery, as well as potentially new species of microsporidian and myxozoan parasites infecting all 10 amphibian species sampled. No viruses or a virulent undescribed *Perkinsus*-like organism affecting amphibians were found. Tens of thousands of individual amphibians may breed in outdoor warm-water fish-rearing ponds. Although there are no reports of disease outbreaks at the sampling sites, the transmission of infectious diseases by larval amphibians, inadvertently or purposefully included when stocking fish, could have serious consequences for amphibian populations at recipient sites.

Research Activities: Evaluation of storm surge overwash effects on amphibians in isolated wetlands at St. Marks National Wildlife Refuge

Thirty ponds were selected for this study at St. Marks NWR based on preliminary sampling and specific conductance values and/or pressure transducer data (indicating whether or not the site was inundated with saltwater) after overwash by the storm surge associated with Hurricane Dennis in Jul. 2005. The selected ponds represent a gradient of conductivity from normal freshwater to brackish. SEARMI efforts included visits to 22 of the ponds during four visits in 2006; each visit consisted of amphibian sampling (dipnet, crayfish trap, froglogger, visual encounter, and aural surveys) and collection of standard water quality field parameters. Our WRD collaborators visited 20 of the sites on two sampling visits to conduct detailed water chemistry analyses.

The preliminary results indicate some of the ponds remained highly conductive for the duration of the study and fluctuations appeared to be associated with water level changes caused by drought and rainfall. Many wetlands remained highly conductive at the end of the study, and continued monitoring is necessary to determine the time scale over which these wetlands will return to pre-overwash conductivity levels. Although we initially observed a decrease in amphibian species richness and abundance at inundated wetlands, we subsequently detected several generalist species (particularly *Rana sphenocephala* and *Acris gryllus*) the following summer at many of these sites. We also documented the presence of fish (both freshwater and estuarine species) in some ponds where fish had previously not been observed.

Data analyses and manuscript preparation will be completed in 2007 in collaboration with WRD.

Research Activities: Effects of Fish Predation on Larvae of *Rana capito* and *Rana sphenocephala*

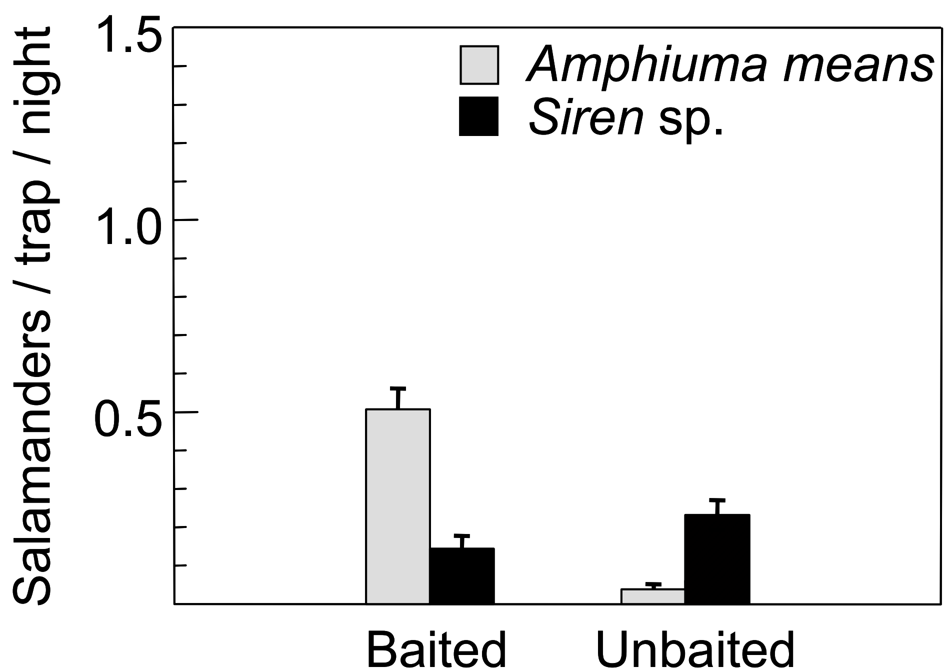
This research was conducted by Denise Gregoire, a biological science technician with the SEARMI program Mar. – Sep. 2006, in collaboration with Margaret Gunzburger. The following abstract is from the manuscript prepared from this research (D. Gregoire and M. Gunzburger, written commun. 2006).

Abstract: Southern Leopard Frogs, *Rana sphenocephala*, are habitat generalists occurring in virtually all aquatic habitats within their geographic range, whereas Gopher Frogs, *R. capito*, breed in temporary ponds and do not co-occur with fish. In order to evaluate the potential for predation by fish to influence the distribution of these species, we conducted a randomized factorial experiment to examine the survival rate and behavior of tadpoles when exposed to warmouth sunfish, *Lepomis gulosus*, banded sunfish, *Enneacanthus obesus*, and eastern mosquitofish, *Gambusia holbrooki*. We then conducted a choice experiment to examine the survival rate of the two tadpole species when a predator is given a choice of both species simultaneously. *Lepomis gulosus* consumed the most tadpoles and ate significantly more *R. capito* tadpoles than *R. sphenocephala* larvae. *Gambusia holbrooki* injured the most tadpoles, especially *R. capito*. *Enneacanthus obesus* did not have an effect on behavior or survival of either anuran species. Tadpoles of both anurans increased hiding when in the presence of *L. gulosus* and *G. holbrooki*, but a greater proportion of *R. capito* hid than *R. sphenocephala*. The results suggest that *R. capito* are more vulnerable to predation by fish than *R. sphenocephala*. The introduction of fish may play a role in population declines of certain anurans breeding in normally fish-free wetlands, and even small fish, such as mosquitofish, may have significant negative effects on *R. capito* tadpoles.

Research Activities: Evaluation of the Influence of Baiting on Trapping Success for Large Aquatic Salamanders

This research was conducted by Clinton Smith, a student technician for the SEARMI program Apr. – Sep. 2006, in collaboration with Margaret Gunzburger and Denise Gregoire. The following abstract is from the manuscript prepared from this research that will be submitted to a scientific journal for peer-review and publication (Smith et al., written commun. 2006).

Abstract: Trapping is a commonly used technique for sampling aquatic salamanders. However, the potentially differential effects of using baited and unbaited traps when sampling these amphibians are largely unstudied. We conducted an experiment comparing the numbers of sirens and amphiumas captured in baited and unbaited modified crayfish traps at Lake Suggs, a blackwater lake located in Putnam County, Florida. Our results demonstrated that baiting has a mixed effect on capture, with a relatively higher number of *Amphiuma means* captured in baited traps and a relatively higher number of *Siren* sp. captured in unbaited traps. These results suggest that researchers must carefully consider the objectives of their study to determine whether baited or unbaited traps are appropriate.



Mean (with standard errors) of catch per unit effort (number of captures/trap/night) of *Amphiuma means* and *Siren* sp. in baited and unbaited crayfish traps.

Research Activities: *Siren* and *Amphiuma* Population Demography

Background

Apex-level studies that include research on population estimates, demographic rates, and other long-term research on focal species are critical components of the ARMI program. As part of SEARMI, an apex mark-recapture study was initiated in 2005 on the large aquatic salamanders *Siren lacertina* and *Amphiuma means*. These salamanders are often abundant in aquatic ecosystems in the southeastern US, but their life histories are poorly known.

This study is being conducted at Lake Suggs on the Ordway-Swisher Biological Station, a property jointly owned by the Nature Conservancy and the University of Florida and managed by the University of Florida Department of Wildlife Ecology and Conservation. The project is a continuation of an earlier study conducted Aug. 2001 – Jul. 2002 by former ARMI biologist Kristina Sorensen (Sorensen, 2004). Although 58 *A. means* and 66 *S. lacertina* were marked using Passive Integrated Transponder (PIT) tags in the earlier study, low recapture rates prevented calculation of growth rates

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 were collected at Lake Suggs using 20 mesh-lined crayfish traps (see Johnson and Barichivich, 2004) set 5 m apart on permanent trap poles for four nights each month. During Feb. 2006, traps were set an additional two nights; in May 2006 an additional five traps were set; and in Jun. and Jul. 2006 an additional 20 traps were set. Data collected for all captured sirens and amphiumas included snout vent length (SVL), total length (TL), and mass. Animals larger than approximately 150 mm TL were marked by injecting a PIT tag into the lateral tail muscle. Data were also collected on the number of other animals captured in each trap, including fish and invertebrates.

Results

ARMI began this project in Jun. 2005 and conducted sampling all but one month through Dec. 2006 (18 months), for a total of 1,470 trap nights. We made 278 captures of sirens and amphiumas (including one capture of an unidentified *Siren* sp.). Most of the animals were relatively large, though we did catch several smaller sirens. The recapture rate of sirens was lower than that of amphiumas. A summary of the data collected to date is shown in the following table.

	Amphiuma means	*Siren lacertina*	*Siren intermedia*
Number of captures (including recaptures)	125	146	6
Number of marked individuals	71	115	4
Number of individuals recaptured from 2001-2002 study	3	2	N/A
Total number of individuals recaptured at least once	34	15	0
$\bar{x}$ Snout-vent length ± standard deviation (mm)	459 (±60)	285 (±99)	130 (±48)
$\bar{x}$ Total length ± standard deviation (mm)	627 (±81)	418 (±141)	209 (±77)
$\bar{x}$ Mass ± standard deviation (g)	301 (±119)	328 (±311)	44 (±41)

<u>Future Plans</u>

This study will continue through FY 2007 and beyond as time and funds are available. We are encouraged by recaptures from the 2001-2002 study, which provide new data on the longevity of these species in nature.

Collaboration: *Siren lacertina* Radio-Tracking Study

This research was conducted by Trisha Craybill, a graduate student at Towson University, using SEARMI crayfish traps. The following abstract is from the presentation of this research at the 2006 Joint Meeting of Ichthyologists and Herpetologists, New Orleans, Louisiana.

Abstract: With the continued reduction of wetlands habitat in the United States, the use of incidental and artificial habitats by wildlife may become increasingly important. Roadside ditches are prevalent in many areas and commonly used by a variety of wildlife. However, a number of characteristics may cause some ditches to function as "ecological traps", including frequent drying and saltwater intrusion in coastal areas. We examined the suitability of roadside ditches by comparing populations of greater sirens (*Siren lacertina*) in roadside ditches to those in natural wetlands on the Kennedy Space Center (KSC) in east-central Florida. Sirens were captured using commercial crayfish and wire-mesh minnow traps, and all animals over 10 g were pit-tagged. To assess habitat suitability, we compared reproduction, body condition, and survivorship of sirens between ditches and natural wetlands. Survivorship was estimated using program MARK and supplemented with radio telemetry of sirens in each habitat type. Results of these comparisons will be reported as well as recommendations for managing the roadside ditches as suitable habitats for sirens and other amphibians.

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Southeast ARMI research and monitoring is conducted under the following permits and protocols:

- SMNWR: USFWS Special Use Permit 41640-02011
- LSNWR: USFWS Special Use Permit 41515-06-004
- Savannah Coastal Refuges complex (HNNWR and SNWR): USFWS Special Use Permit 41620-04016
- Ordway-Swisher Biological Station: University of Florida Research Permit OR-05-07
- State of Florida: Florida Fish and Wildlife Conservation Commission Special Purpose Permit WX06079
- USGS/FISC IACUC Protocols 2006-04 and 2006-05

Table 1. Coordinates for sites and methods used by Southeast ARMI at St. Marks, Lower Suwannee, Harris Neck, and Savannah National Wildlife Refuges during 2006. Coordinates are projected in WGS84 datum.

Map code	General Location	**UTM E**	**UTM N**	Aural	Crayfish trap	Dip-net	Froglogger	Funnel trap	Visual	Overwash	WRD grab sample
	St. Marks NWR										
1	Biggins Pond	746570	3323072	X	X	X	X		X	X	X
2	Chicky Pond	750336	3324395	X	X	X	X		X		X
3	Cingulatum Pond	774260	3338757			X					
4	Corner Pond	747034	3327586		X	X	X		X		X
5	Ditched Pond	741490	3322532							X	
6	Fat Nerodia Pond	746057	3324407	X	X	X	X		X	X	
7	Goose Pond	748788	3322398	X	X	X	X		X	X	X
8	Goose Pond 2	747611	3322197							X	
9	Headquarters Pond	773370	3332137							X	
10	Jennifer's Sink	747990	3327929	X	X	X	X		X		X
11	Kingfisher Pond	747354	3322146	X	X	X	X		X	X	X
12	ORSP Borrow	742592	3322592							X	
13	ORSP Sink	741811	3321768							X	
14	Otter Lake	748835	3323966		X	X	X		X		X
15	Perched Pond	743245	3324488							X	
16	Plum Orchard Pond	774826	3338857							X	
17	Pond 2	749184	3322169							X	
18	Pond 7	749608	3322158							X	
19	Pond 10	749834	3323024							X	
20	Printiss Pond	744431	3325997	X	X	X	X		X	X	
21	Printiss 2 Pond	741688	3322542						X	X	
22	Ring Pond	751315	3326573	X	X	X	X		X	X	X
23	Small Prairie Pond	746380	3324168	X	X	X	X		X	X	X
24	SMNWR0102	776846	3338267			X		X			
25	SMNWR0109	776637	3337627			X			X		
26	SMNWR0111	776394	3337408			X					
27	SMNWR0130	780775	3337464			X			X		
28	SMNWR0132	780769	3337393			X					
29	SMNWR1001	780769	3337393	X		X					
30	SMNWR1004	780789	3337201			X					
31	SMNWR1009	776605	3337997			X		X			
32	SMNWR1014	774584	3339506			X					
33	SMNWR2001	777211	3337641			X			X		
34	SMNWR3001	774151	3338785			X					
35	SMNWR3004	774616	3339140			X					
36	SPC Prairie	750401	3323961	X	X	X	X		X	X	X
37	Spike Buck Pond	744780	3326461	X		X			X	X	
38	Talpoideum Pond	747248	3327146	X	X	X	X		X		X
39	Temp Wpt 202	746702	3323625	X					X	X	
40	Temp Wpt 203	746713	3323334						X	X	

Map code	General Location	**UTM E**	**UTM N**	Aural	Crayfish trap	Dip-net	Froglogger	Funnel trap	Visual	Overwash	WRD grab sample
41	Temp Wpt 204	746612	3322575	X						X	
42	Temp Wpt 205	749998	3322190							X	
43	Temp Wpt 206	750212	3322374							X	
44	Temp Wpt 207	750175	3321629	X						X	
45	Temp Wpt 208	750234	3321576	X						X	
46	Temp Wpt 209	750213	3321744	X						X	
47	Temp Wpt 210	746003	3323986							X	
48	Temp Wpt 211	746223	3323928	X					X	X	
49	Temp Wpt 212	746484	3322878							X	
50	Temp Wpt 213	746480	3322710							X	
51	Temp Wpt 214	746676	3322742						X	X	
52	Temp Wpt 215	746767	3322831							X	
53	Temp Wpt 216	746865	3322486						X	X	
54	Temp Wpt 217	746814	3322392							X	
55	Temp Wpt 218	746955	3322481							X	
56	Temp Wpt 219	747303	3322377						X	X	
57	Temp Wpt 220	747663	3321511							X	
58	Temp Wpt 222	747506	3323222	X					X	X	
59	Temp Wpt 223	747474	3323249							X	
60	Temp Wpt 301	741659	3322182						X	X	
61	Temp Wpt 302	742849	3321496						X	X	
62	Temp Wpt 303	743059	3321737						X	X	
63	Temp Wpt 304	743533	3322227						X	X	
64	Temp Wpt 305	743529	3322216							X	
65	Temp Wpt 306	743657	3324760							X	
66	Temp Wpt 307	751183	3326362	X					X	X	
67	Temp Wpt 308	751392	3327020	X						X	
68	Temp Wpt 309	751405	3327008						X	X	
69	Temp Wpt 310	751835	3326558							X	
70	Temp Wpt 311	752008	3325789							X	
71	Temp Wpt 312	752235	3326638						X	X	
72	Temp Wpt 313	752611	3326609						X	X	
73	Temp Wpt 314	752637	3326925	X					X	X	
74	Temp Wpt 315	752531	3326929						X	X	
75	Temp Wpt 317	753381	3327465							X	
76	Temp Wpt 318	753107	3327467	X					X	X	
77	WBF Pond	744806	3325943			X	X		X	X	
78	Wpt 16	750412	3321028	X						X	
79	Wpt 19	750312	3321070	X	X	X	X		X	X	X
80	Wpt 68	750151	3322421	X	X	X	X		X	X	X
81	Wpt 69	747479	3322471	X	X	X	X		X	X	X
82	Wpt 79	749872	3322969	X	X	X	X		X	X	X
83	Wpt 103	745998	3324492	X	X	X	X		X	X	X
84	Wpt 127	753471	3326208							X	
85	Wpt 128	753012	3326212							X	
86	Wpt 150	746677	3327269	X	X	X	X		X		X

Map code	General Location	UTM E	UTM N	Aural	Crayfish trap	Dip-net	Froglogger	Funnel trap	Visual	Overwash	WRD grab sample
87	Wpt 151	751940	3327248						X	X	
88	Wpt 192	752419	3330376	X	X	X	X		X		X
89	Wpt 201	745998	3324655			X			X	X	
90	Wpt 221	747553	3322795	X	X	X	X		X	X	X
91	Wpt 316	753007	3327665	X	X	X	X		X	X	X
	Harris Neck NWR										
	Borrow Pond	473461	3499861	X	X	X			X		
	Goose Pond	474660	3500905	X	X				X		X
	Greenhead	474587	3500632								X
	Lucas Borrow	473751	3498331		X	X			X		
	Lucas Pond	473590	3498216	X	X				X		X
	Snipe Pond 1	473990	3500432	X	X				X		X
	Snipe Pond 2	473990	3500432	X	X				X		X
	Wigeon Pond	475034	3499460	X	X				X		X
	Woody Pond	473713	3499346	X	X				X		X
	Woody Swamp	473609	3499391								X
	Savannah NWR										
	DT-2	492492	3561681	X	X				X		X
	DT-4	491846	3562316						X		
	HQ-1	488878	3559112	X	X				X		X
	ND-1	490757	3560655								X
	ND-2	490685	3561563								X
	ND-3	490076	3561532	X	X				X		X
	ND-4	489667	3560003	X	X				X		X
	ST-1	484422	3564331						X		
	ST-3	484456	3563367	X		X			X		
	ST-4	483936	3563874	X					X		
	ST-6	484106	3562910	X					X		
	WD-1	489800	3557575								X
	WD-2	491164	3557083								X
	WD-3	491572	3558678	X	X				X		X
	WD-6	489240	3558398								X
	WD-7	490416	3556912								X
	WD-8	491129	3556549								X
	Total number of observations			49	35	39	23	2	65	71	32

Table 2. Southeast ARMI 2006 sampling dates and effort by sampling site at St. Marks, Harris Neck, and Savannah National Wildlife Refuges.

General Location	January		February		March			May				June				July	August		Number of visits
St. Marks NWR																			
Biggins Pond		31				21	22		2	3		19	20			31	1		9
Chicky Pond												19	20				1	2	4
Cingulatum Pond	16			2	6														3
Corner Pond						21	22		2	3			20	21		31	1		8
Ditched Pond		31																	1
Fat Nerodia Pond													20	21					2
Goose Pond		31						1	2				20	21			1	2	7
Goose Pond 2			1																1
Headquarters Pond				2															1
Jennifer's Sink						21	22		2	3			20	21		31	1		8
Kingfisher Pond		31				21	22	1	2			19	20			31	1		9
ORSP Borrow		31																	1
ORSP Sink		31																	1
Otter Lake									2	3			20	21			1	2	6
Perched Pond		31																	1
Plum Orchard Pond				2															1
Pond 2		31																	1
Pond 7		31																	1
Pond 10		31																	1
Printiss Pond		31												21	22				3
Printiss 2 Pond		31																	1
Ring Pond			1							3	4		20	21		31	1		7
Small Prairie Pond			1						2	3		19	20			31	1		7
SMNWR0102	16		1	2															3
SMNWR0109				2															1
SMNWR0111	16																		1
SMNWR0130					6														1
SMNWR0132					6														1

General Location	January		February		March				May		June			July	August		Number of visits
SMNWR1001	16			2	6												3
SMNWR1004					6												1
SMNWR1009	16		1	2	6												4
SMNWR1014				2													1
SMNWR2001				2	6												2
SMNWR3001					6												1
SMNWR3004				2													1
SPC Prairie		31				20	21		3	4	19	20			1	2	9
Spike Buck Pond		31											21				2
Talpoideum Pond							21	22	3	4		20	21	31	1		8
Temp Wpt 202		31															1
Temp Wpt 203		31															1
Temp Wpt 204		31															1
Temp Wpt 205		31															1
Temp Wpt 206		31															1
Temp Wpt 207		31															1
Temp Wpt 208		31															1
Temp Wpt 209		31															1
Temp Wpt 210			1														1
Temp Wpt 211			1														1
Temp Wpt 212			1														1
Temp Wpt 213			1														1
Temp Wpt 214			1														1
Temp Wpt 215			1														1
Temp Wpt 216			1														1
Temp Wpt 217			1														1
Temp Wpt 218			1														1
Temp Wpt 219			1														1
Temp Wpt 220			1														1
Temp Wpt 222			1														1
Temp Wpt 223			1														1
Temp Wpt 301		31															1

General Location	January	February	March		May				June				July	August		Number of visits
Temp Wpt 302	31															1
Temp Wpt 303	31															1
Temp Wpt 304	31															1
Temp Wpt 305	31															1
Temp Wpt 306	31															1
Temp Wpt 307		1														1
Temp Wpt 308		1														1
Temp Wpt 309		1														1
Temp Wpt 310		1														1
Temp Wpt 311		1														1
Temp Wpt 312		1														1
Temp Wpt 313		1														1
Temp Wpt 314		1														1
Temp Wpt 315		1														1
Temp Wpt 317		1														1
Temp Wpt 318		1														1
WBF Pond	31										21	22				3
Wpt 16	31															1
Wpt 19	31		20	21	1	2			19	20				1	2	9
Wpt 68	31		20	21					19	20				1	2	7
Wpt 69		1	20	21	1	2			19	20			31	1		9
Wpt 79	31		20	21	1				19	20				1	2	8
Wpt 103	31						3			20	21			1	2	6
Wpt 127	31															1
Wpt 128	31															1
Wpt 150				21			3			20	21			1	2	6
Wpt 151	31															1
Wpt 192							3	4		20	21		31	1		6
Wpt 201	31						3									2
Wpt 221		1			1	2			19	20			31	1		7
Wpt 316		1									21			1	2	4

General Location	January		February		March				May									June				July	August							Number of visits
Harris Neck NWR																														
Borrow Pond													22	23											14	15	16			5
Goose Pond													22	23	24										14	15	16			6
Lucas Borrow													22	23											14	15	16			5
Lucas Pond													22	23	24										14	15	16			6
Snipe Ponds 1, 2													22	23	24										14	15	16			6
Wigeon Pond													22	23	24										14	15	16			6
Woody Pond													22	23	24										14	15	16			6
Savannah NWR																														
DT-2															24	25	26											16	17	18	6
DT-4																													17		1
HQ-1															24	25	26											16	17	18	6
ND-3															24	25	26											16	17	1	6
ND-4															24	25	26											16	17	1	6
ST-1															24	25															2
ST-3															24	25															2
ST-4															24	25															2
ST-6															24	25															2
WD-3															24	25	26											16	17	18	6
Number of sites visited	6	39	33	11	9	6	12	6	7	11	13	5	7	8	15	10	6	11	21	15	3		21	11	8	8	13	7	6	

Table 3. Amphibian and reptile species documented by Southeast ARMI at St. Marks, Lower Suwannee, Harris Neck, and Savannah National Wildlife Refuges through 2006. Counts including subspecies in parentheses.

Scientific Name	Common Name	SMNWR	LSNWR	HNNWR	SNWR
Frogs					
Acris gryllus dorsalis	Florida Cricket Frog	X	X	X	
Acris gryllus gryllus	Coastal Plain Cricket Frog				X
Bufo fowleri	Fowler's Toad				X
Bufo quercicus	Oak Toad	X	X		
Bufo terrestris	Southern Toad	X	X	X	X
Eleutherodactylus planirostris	Greenhouse Frog (introduced)	X	X		
Gastrophryne carolinensis	Eastern Narrow-mouthed Toad	X	X	X	X
Hyla avivoca ogechiensis	Eastern Bird-voiced Treefrog				X
Hyla chrysoscelis	Cope's Gray Treefrog	X	X		X
Hyla cinerea	Green Treefrog	X	X	X	X
Hyla femoralis	Pine Woods Treefrog	X	X	X	X
Hyla gratiosa	Barking Treefrog	X	X	X	
Hyla squirella	Squirrel Treefrog	X	X	X	X
Osteopilus septentrionalis	Cuban Treefrog (introduced)		X[1]		
Pseudacris crucifer bartramiana	Southern Spring Peeper	X	X	X	
Pseudacris crucifer crucifer	Northern Spring Peeper				X
Pseudacris nigrita nigrita	Striped Southern Chorus Frog	X	X		
Pseudacris ocularis	Little Grass Frog	X	X	X	
Pseudacris ornata	Ornate Chorus Frog	X			
Rana catesbeiana	American Bullfrog	X	X	X	X
Rana clamitans clamitans	Bronze Frog	X	X		X
Rana grylio	Pig Frog	X	X	X	X
Rana heckscheri	River Frog	X			X
Rana sphenocephala sphenocephala	Florida Leopard Frog	X	X		
Rana sphenocephala utricularia	Southern Leopard Frog			X	X
Scaphiopus holbrookii	Eastern Spadefoot	X	X		
Number of frog species observed		20	19	12	15
Salamanders					
Ambystoma cingulatum	Flatwoods Salamander	X			
Ambystoma opacum	Marbled Salamander				X
Ambystoma talpoideum	Mole Salamander	X			
Amphiuma means	Two-toed Amphiuma	X	X		X
Desmognathus auriculatus	Southern Dusky Salamander	X			X
Eurycea quadridigitata	Dwarf Salamander	X	X		X
Notophthalmus viridescens ssp.	Eastern Newt		X		
Notophthalmus viridescens louisianensis	Central Newt	X		X	X
Plethodon grobmani	Southeastern Slimy Salamander	X			
Plethodon variolatus	South Carolina Slimy Salamander				X
Pseudobranchus striatus spheniscus	Slender Dwarf Siren	X			
Siren intermedia intermedia	Eastern Lesser Siren	X			X
Siren lacertina	Greater Siren	X	X		X

Scientific Name	Common Name	SMNWR	LSNWR	HNNWR	SNWR
Siren sp. (undescribed species)	Undescribed siren	X			
Number of salamander species observed		11	4	1	8
Number of amphibian species observed		31	23	13	23
Lizards					
Anolis carolinensis carolinensis	Northern Green Anole	X	X	X	X
Aspidoscelis sexlineata sexlineata (=Cnemidophorus sexlineatus sexlineatus)	Eastern Six-lined Racerunner	X	X	X	
Eumeces egregius similis	Northern Mole Skink	X			
Eumeces fasciatus	Common Five-lined Skink	X	X		
Eumeces inexpectatus	Southeastern Five-lined Skink	X	X	X	X
Eumeces laticeps	Broad-headed Skink	X	X		
Ophisaurus attenuatus longicaudus	Eastern Slender Glass Lizard	X			
Ophisaurus ventralis	Eastern Glass Lizard	X	X	X	
Sceloporus undulatus	Eastern Fence Lizard	X	X		
Scincella lateralis	Little Brown Skink	X	X	X	X
Number of lizard species observed		10	8	5	3
Snakes					
Agkistrodon contortrix contortrix	Southern Copperhead			X	X
Agkistrodon piscivorus ssp.	Cottonmouth				X
Agkistrodon piscivorus conanti	Florida Cottonmouth	X	X		
Cemophora coccinea copei	Northern Scarletsnake	X	X	X	
Coluber constrictor priapus	Southern Black Racer	X	X	X	X
Crotalus adamanteus	Eastern Diamond-backed Rattlesnake	X	X	X	
Crotalus horridus	Timber Rattlesnake			X	
Diadophis punctatus punctatus	Southern Ring-necked Snake	X	X		X
Drymarchon couperi	Eastern Indigo Snake		X		
Elaphe alleghaniensis (=*E. obsoleta*)[2]	Eastern Ratsnake	X	X	X	X
Elaphe guttata	Red Cornsnake	X			X
Farancia abacura abacura	Eastern Mudsnake	X[1]	X		
Heterodon platirhinos	Eastern Hog-nosed Snake	X			
Heterodon simus	Southern Hog-nosed Snake		X		
Lampropeltis getula getula	Eastern Kingsnake	X[1]		X	
Lampropeltis triangulum elapsoides	Scarlet Kingsnake	X	X		
Masticophis flagellum flagellum	Eastern Coachwhip	X		X	
Micrurus fulvius	Harlequin Coralsnake	X			
Nerodia clarkii clarkii	Gulf Saltmarsh Snake	X			
Nerodia erythrogaster erythrogaster	Red-bellied Watersnake				X
Nerodia fasciata ssp.	Southern Watersnake		X		
Nerodia fasciata fasciata	Banded Watersnake	X		X	X
Nerodia floridana	Florida Green Watersnake	X			
Nerodia taxispilota	Brown Watersnake	X			X
Opheodrys aestivus	Rough Greensnake	X		X	
Pituophis melanoleucus mugitus	Florida Pinesnake	X			
Regina alleni	Striped Crayfish Snake	X	X		

Scientific Name	Common Name	SMNWR	LSNWR	HNNWR	SNWR
Regina rigida rigida	Glossy Crayfish Snake	X	X		
Seminatrix pygaea pygaea	Northern Florida Swampsnake	X			
Sistrurus miliarius barbouri	Dusky Pygmy Rattlesnake	X	X		
Storeria dekayi wrightorum	Midland Brownsnake	X			
Storeria occipitomaculata ssp.	Red-bellied Snake				X
Storeria occipitomaculata obscura	Florida Red-bellied Snake		X		
Storeria victa (=*Storeria dekayi victa*)	Florida Brownsnake		X		
Thamnophis sauritus nitae	Blue-striped Ribbonsnake	X	X		
Thamnophis sauritus sackenii	Peninsula Ribbonsnake	X		X	
Thamnophis sirtalis similis	Blue-striped Gartersnake	X	X		
Thamnophis sirtalis sirtalis	Eastern Gartersnake	X		X	
Virginia valeriae valeriae	Eastern Smooth Earthsnake	X			
Number of snake species observed		27(29)	18	12	11
Crocodilians					
Alligator mississipiensis	American Alligator	X	X	X	X
Number of crocodillian species observed		1	1	1	1
Turtles					
Apalone ferox	Florida Softshell	X	X		
Chelydra serpentina osceola	Florida Snapping Turtle	X	X		
Deirochelys reticularia reticularia	Eastern Chicken Turtle	X	X	X	
Gopherus polyphemus	Gopher Tortoise	X	X	X	
Kinosternon baurii	Striped Mud Turtle	X	X		
Kinosternon subrubrum subrubrum	Eastern Mud Turtle	X		X	X
Malaclemys terrapin macrospilota	Ornate Diamond-backed Terrapin	X			
Pseudemys concinna concinna	Eastern River Cooter	X			
Pseudemys concinna floridana	Coastal Plain Cooter	X			
Pseudemys nelsoni	Florida Red-bellied Cooter		X		
Pseudemys peninsularis	Peninsula Cooter		X		
Pseudemys suwanniensis	Suwannee Cooter		X		
Sternotherus odoratus	Stinkpot	X	X		X
Terrapene carolina bauri	Florida Box Turtle		X		
Terrapene carolina carolina	Eastern Box Turtle				X
Terrapene carolina major	Gulf Coast Box Turtle	X			
Trachemys scripta scripta	Yellow-bellied Slider	X	X	X	X
Number of turtle species observed		11(12)	11	4	4
Number of reptile species observed		49(52)	38	22	19
Total number of species observed		80(83)	61	35	42

1 – record for this species from near, not within, refuge boundary
2 – *Elaphe alleghaniensis = E. obsoleta obsoleta, E. o. quadrivitatta, E. o. spiloides*

Table 4a. Amphibian species documented by Southeast ARMI during wetland and time constrained surveys at St. Marks, Harris Neck, and Savannah National Wildlife Refuges during 2006.

Species Name	*Acris gryllus*	*Ambystoma cingulatum*	*Ambystoma talpoideum*	*Amphiuma means*	*Bufo quercicus*	*Bufo terrestris*	*Eurycea quadridigitata*	*Gastrophryne carolinensis*	*Hyla chrysoscelis*	*Hyla cinerea*	*Hyla femoralis*	*Hyla squirella*	*Hyla gratiosa*	*Notophthalmus viridescens*	*Plethodon variolatus*	*Pseudacris crucifer*	*Pseudacris nigrita*	*Pseudacris ocularis*	*Pseudacris ornata*	*Pseudobranchus striatus*	*Rana catesbeiana*	*Rana clamitans*	*Rana grylio*	*Rana heckscheri*	*Rana sphenocephala*	*Siren intermedia*	*Siren lacertina*	*Siren* sp. (undescribed)	Number of amphibian species detected at site
St. Marks NWR																													
Biggins Pond	X				X	X			-		X	-			-	-		X					X	-	X		-		7
Chicky Pond	X				X	X			-		X	-			-	-							X	-	X		-		6
Cingulatum Pond							X		-			-			-	-								-			-		1
Corner Pond	X		X			X			-		X	-	X		-	-		X						-	X		-		7
Fat Nerodia Pond					X				-		X	-			-	-		X						-			-		3
Goose Pond	X					X			-			-			-	-							X	-	X		-		4
Jennifer's Sink	X								-			-			-	-					X	X		-	X		-		4
Kingfisher Pond	X								-	X	X	-			-	-							X	-	X	X	-	X	7
Otter Lk	X			X		X			-	X		-			-	-							X	-	X		-		6
Printiss Pond	X							X	-		X	-			-	-		X						-			-		4
Printiss 2 Pond	X								-			-			-	-								-			-		1
Ring Pond	X				X				-		X	-			-	-							X	-	X		-		5
Small Prairie Pond	X					X			-		X	-			-	-				X			X	-	X		-		6
SMNWR0102		X					X		-			-		X	-	-								-	X		-		4
SMNWR0109							X		-	X		-		X	-	-								-			-		3
SMNWR0130		X							-			-			-	-								-			-		1
SMNWR1001	X	X					X		-			-			-	-								-			-		3
SMNWR1009							X		-			-			-	-								-	X		-		2
SMNWR1014							X		-			-			-	-								-			-		1
SMNWR2001							X		-			-		X	-	-								-			-		2
SMNWR3001		X					X		-			-			-	-								-			-		2
SMNWR3004							X		-			-			-	-								-			-		1
SPC Prairie	X								-			-			-	-								-	X		-		2
Spike Buck Pond					X				-			-			-	-		X						-			-		2
Talpoideum Pond	X		X		X				-		X	-	X		-	-			X					-	X		-		7

Species Name	*Acris gryllus*	*Ambystoma cingulatum*	*Ambystoma talpoideum*	*Amphiuma means*	*Bufo quercicus*	*Bufo terrestris*	*Eurycea quadridigitata*	*Gastrophryne carolinensis*	*Hyla chrysoscelis*	*Hyla cinerea*	*Hyla femoralis*	*Hyla squirella*	*Hyla gratiosa*	*Notophthalmus viridescens*	*Plethodon variolatus*	*Pseudacris crucifer*	*Pseudacris nigrita*	*Pseudacris ocularis*	*Pseudacris ornata*	*Pseudobranchus striatus*	*Rana catesbeiana*	*Rana clamitans*	*Rana grylio*	*Rana heckscheri*	*Rana sphenocephala*	*Siren intermedia*	*Siren lacertina*	*Siren* sp. (undescribed)	Number of amphibian species detected at site
Temp Wpt 202	X								-			-			-	-								-			-		1
Temp Wpt 203	X								-			-			-	-								-			-		1
Temp Wpt 204	X								-			-			-	-								-			-		1
Temp Wpt 207	X								-			-			-	-								-			-		1
Temp Wpt 208	X								-			-			-	-								-			-		1
Temp Wpt 209	X								-			-			-	-								-			-		1
Temp Wpt 211	X								-			-			-	-								-	X		-		2
Temp Wpt 214									-			-			-	-								-	X		-		1
Temp Wpt 216	X								-			-			-	-								-			-		1
Temp Wpt 219	X								-			-			-	-								-			-		1
Temp Wpt 222	X								-			-			-	-								-			-		1
Temp Wpt 301							X		-			-			-	-								-			-		1
Temp Wpt 302	X								-			-			-	-								-			-		1
Temp Wpt 303	X								-			-			-	-								-			-		1
Temp Wpt 304	X								-			-			-	-								-			-		1
Temp Wpt 307	X								-			-			-	-								-			-		1
Temp Wpt 308	X								-			-			-	-								-			-		1
Temp Wpt 309	X								-			-			-	-								-			-		1
Temp Wpt 312									-			-			-	-								-			-		0
Temp Wpt 313	X								-			-			-	-								-			-		1
Temp Wpt 314	X								-			-			-	-								-			-		1
Temp Wpt 315	X								-			-			-	-								-			-		1
Temp Wpt 318									-			-			-	-								-	X		-		1
WBF Pond	X					X			-			-			-	-		X						-			-		3
Wpt 16	X								-			-			-	-								-			-		1
Wpt 19	X			X			X		-		X	-		X	-	-		X					X	-	X		-		8
Wpt 68	X		X		X			X	-		X	-	X		-	-	X	X					X	-	X		-		10
Wpt 69	X		X						-		X	-	X		-	-		X					X	-	X		-	X	8
Wpt 79	X		X		X				-		X	-			-	-		X					X	-	X		-		7

Species Name	*Acris gryllus*	*Ambystoma cingulatum*	*Ambystoma talpoideum*	*Amphiuma means*	*Bufo quercicus*	*Bufo terrestris*	*Eurycea quadridigitata*	*Gastrophryne carolinensis*	*Hyla chrysoscelis*	*Hyla cinerea*	*Hyla femoralis*	*Hyla squirella*	*Hyla gratiosa*	*Notophthalmus viridescens*	*Plethodon variolatus*	*Pseudacris crucifer*	*Pseudacris nigrita*	*Pseudacris ocularis*	*Pseudacris ornata*	*Pseudobranchus striatus*	*Rana catesbeiana*	*Rana clamitans*	*Rana grylio*	*Rana heckscheri*	*Rana sphenocephala*	*Siren intermedia*	*Siren lacertina*	*Siren* sp. (undescribed)	Number of amphibian species detected at site
Wpt 103	X							X	-		X	-	X		-	-		X						-	X		-		6
Wpt 150	X		X			X			-		X	-			-	-		X			X			-	X		-		7
Wpt 151	X								-			-			-	-								-			-		1
Wpt 192	X				X	X			-		X	-			-	-						X		-	X		-		6
Wpt 201	X								-			-			-	-								-	X		-		2
Wpt 221	X								-			-			-	-							X	-			-		2
Wpt 316	X				X				-		X	-			-	-		X						-	X		-		5
Number of sites	46	4	6	2	10	9	11	3	-	3	17	-	5	4	-	-	1	13	1	1	2	2	12	-	25	1	-	2	
Naive occupancy	75.41	6.56	9.84	3.28	16.39	14.75	18.03	4.92	-	4.92	27.87	-	8.20	6.56	-	-	1.64	21.31	1.64	1.64	3.28	3.28	19.67	-	40.98	1.64	-	3.28	

Species Name	*Acris gryllus*	*Ambystoma cingulatum*	*Ambystoma talpoideum*	*Amphiuma means*	*Bufo quercicus*	*Bufo terrestris*	*Eurycea quadridigitata*	*Gastrophryne carolinensis*	*Hyla chrysoscelis*	*Hyla cinerea*	*Hyla femoralis*	*Hyla squirella*	*Hyla gratiosa*	*Notophthalmus viridescens*	*Plethodon variolatus*	*Pseudacris crucifer*	*Pseudacris nigrita*	*Pseudacris ocularis*	*Pseudacris ornata*	*Pseudobranchus striatus*	*Rana catesbeiana*	*Rana clamitans*	*Rana grylio*	*Rana heckscheri*	*Rana sphenocephala*	*Siren intermedia*	*Siren lacertina*	*Siren* sp. (undescribed)	Number of amphibian species detected at site
Harris Neck NWR																													
Borrow Pond	X	-	-	-	-		-	-	X	X	X	X	-	X	-		-	X	-	-		-	X	-	X	-	-	-	9
Goose Pond		-	-	-	-		-	-		X			-		-		-		-	-	X	-	X	-	X	-	-	-	4
Lucas Borrow	X	-	-	-	-		-	-					-	X	-	X	-		-	-		-		-	X	-	-	-	4
Lucas Pond	X	-	-	-	-		-	-		X			-	X	-		-		-	-	X	-	X	-	X	-	-	-	6
Snipe Ponds 1&2		-	-	-	-		-	-		X		X	-		-		-	X	-	-		-		-		-	-	-	3
Wigeon Pond		-	-	-	-		-	-		X			-	X	-		-		-	-		-		-	X	-	-	-	3
Woody Pond		-	-	-	-	X	-	-		X			-		-		-		-	-	X	-	X	-	X	-	-	-	5
Number of sites	3	-	-	-	-	1	-	-	1	6	1	2	-	4	-	1	-	2	-	-	3	-	4	-	6	-	-	-	
Naive occupancy	42.86	-	-	-	-	14.29	-	-	14.29	85.71	14.29	28.57	-	57.14	-	14.29	-	28.57	-	-	42.86	-	57.14	-	85.71	-	-	-	
Savannah NWR																													
DT-2	X	-	-		-				X		-	-	-			-	-	-	-	-	X		X	X				-	5
DT-4	X	-	-		-	X		X			-	-	-			-	-	-	-	-		X						-	4
HQ-1		-	-	X	-						-	-	-			-	-	-	-	-			X		X	X	X	-	5
ND-3		-	-		-						-	-	-			-	-	-	-	-			X		X		X	-	3
ND-4		-	-	X	-						-	-	-			-	-	-	-	-			X				X	-	3
ST-1		-	-		-						-	-	-			-	-	-	-	-								-	0
ST-3		-	-		-					X	-	-	-	X		-	-	-	-	-								-	2
ST-4		-	-		-	X	X		X		-	-	-	X	X	-	-	-	-	-		X						-	6
ST-6		-	-		-		X		X	X	-	-	-		X	-	-	-	-	-		X						-	5
WD-3	X	-	-		-	X					-	-	-			-	-	-	-	-		X	X	X	X			-	6
Number of sites	3	-	-	2	-	3	2	1	3	2	-	-	-	2	2	-	-	-	-	-	1	4	5	2	3	1	3	-	
Naive occupancy	30.00	-	-	20.00	-	30.00	20.00	10.00	30.00	20.00	-	-	-	20.00	20.00	-	-	-	-	-	10.00	40.00	50.00	20.00	30.00	10.00	30.00	-	
Total number of sites (all refuges) where species was observed	52	4	6	4	10	13	13	4	4	11	18	2	5	10	2	1	1	15	1	1	6	6	21	2	34	2	3	2	

Table 4b. Reptile species documented by Southeast ARMI during wetland and time constrained surveys at St. Marks, Harris Neck, and Savannah National Wildlife Refuges during 2006.

Species Name	*Agkistrodon contortrix*	*Alligator mississippiensis*	*Anolis carolinensis*	*Apalone ferox*	*Aspidoscelis sexlineata*	*Coluber constrictor*	*Deirochelys reticularia*	*Diadophis punctatus*	*Elaphe alleghaniensis*	*Kinosternon subrubrum*	*Nerodia erythrogaster*	*Nerodia fasciata*	*Nerodia floridana*	*Nerodia taxispilota*	*Ophisaurus ventralis*	*Regina alleni*	*Sceloporus undulatus*	*Scincella lateralis*	*Sistrurus miliarius*	*Sternotherus odoratus*	*Terrapene carolina*	*Thamnophis sauritus*	*Trachemys scripta scripta*	Number of reptile species detected at site
St. Marks NWR																								
Biggins Pond	-			X		-	-	-	-		-				-			-				X	-	2
Chicky Pond	-					-	-	-	-		-				-			-					-	0
Cingulatum Pond	-					-	-	-	-		-				-			-					-	0
Corner Pond	-	X			X	-	-	-	-		-				-		X	-					-	3
Fat Nerodia Pond	-					-	-	-	-		-				-			-					-	0
Goose Pond	-					-	-	-	-		-				-			-					-	0
Jennifer's Sink	-					-	-	-	-		-	X			-			-					-	1
Kingfisher Pond	-					-	-	-	-		-				-			-				X	-	1
Otter Lake	-	X	X			-	-	-	-		-	X		X	-			-		X			-	5
Printiss Pond	-					-	-	-	-		-				-			-					-	0
Printiss 2 Pond	-					-	-	-	-		-				-			-			X		-	1
Ring Pond	-					-	-	-	-		-				-			-				X	-	1
Small Prairie Pond	-		X			-	-	-	-		-	X			-			-					-	2
SMNWR0102	-					-	-	-	-		-				-			-					-	0
SMNWR0109	-					-	-	-	-		-				-			-					-	0
SMNWR0130	-					-	-	-	-		-				-			-					-	0
SMNWR1001	-					-	-	-	-		-				-			-					-	0
SMNWR1009	-					-	-	-	-		-				-			-					-	0
SMNWR1014	-					-	-	-	-		-				-			-					-	0
SMNWR2001	-					-	-	-	-		-	X			-			-					-	1
SMNWR3001	-					-	-	-	-		-				-			-					-	0
SMNWR3004	-					-	-	-	-		-				-			-					-	0
SPC Prairie	-					-	-	-	-		-	X			-			-					-	1
Spike Buck Pond	-					-	-	-	-		-				-			-					-	0
Talpoideum Pond	-					-	-	-	-	X	-	X			-			-					-	2

Species Name	*Agkistrodon contortrix*	*Alligator mississippiensis*	*Anolis carolinensis*	*Apalone ferox*	*Aspidoscelis sexlineata*	*Coluber constrictor*	*Deirochelys reticularia*	*Diadophis punctatus*	*Elaphe alleghaniensis*	*Kinosternon subrubrum*	*Nerodia erythrogaster*	*Nerodia fasciata*	*Nerodia floridana*	*Nerodia taxispilota*	*Ophisaurus ventralis*	*Regina alleni*	*Sceloporus undulatus*	*Scincella lateralis*	*Sistrurus miliarius*	*Sternotherus odoratus*	*Terrapene carolina*	*Thamnophis sauritus*	*Trachemys scripta scripta*	Number of reptile species detected at site
Temp Wpt 202	-	X				-	-	-	-		-				-			-					-	1
Temp Wpt 203	-					-	-	-	-		-				-			-					-	0
Temp Wpt 204	-					-	-	-	-		-				-			-					-	0
Temp Wpt 207	-					-	-	-	-		-				-			-					-	0
Temp Wpt 208	-					-	-	-	-		-				-			-					-	0
Temp Wpt 209	-					-	-	-	-		-				-			-					-	0
Temp Wpt 211	-					-	-	-	-		-				-			-					-	0
Temp Wpt 214	-					-	-	-	-		-				-			-					-	0
Temp Wpt 216	-					-	-	-	-		-				-			-					-	0
Temp Wpt 219	-					-	-	-	-		-				-			-					-	0
Temp Wpt 222	-					-	-	-	-		-				-			-					-	0
Temp Wpt 301	-					-	-	-	-		-				-			-					-	0
Temp Wpt 302	-					-	-	-	-		-				-			-					-	0
Temp Wpt 303	-					-	-	-	-		-				-			-					-	0
Temp Wpt 304	-					-	-	-	-		-				-			-					-	0
Temp Wpt 307	-					-	-	-	-		-				-			-					-	0
Temp Wpt 308	-					-	-	-	-		-				-			-					-	0
Temp Wpt 309	-					-	-	-	-		-				-			-					-	0
Temp Wpt 312	-			X		-	-	-	-		-				-			-					-	1
Temp Wpt 313	-					-	-	-	-	X	-				-			-					-	1
Temp Wpt 314	-					-	-	-	-		-				-			-					-	0
Temp Wpt 315	-					-	-	-	-		-				-			-					-	0
Temp Wpt 318	-					-	-	-	-		-				-			-			X		-	1
WBF Pond	-					-	-	-	-		-				-			-					-	0
Wpt 16	-					-	-	-	-		-				-			-					-	0
Wpt 19	-					-	-	-	-		-	X			-			-	X				-	2
Wpt 68	-					-	-	-	-		-		X		-			-					-	1
Wpt 69	-	X		X		-	-	-	-		-		X		-	X		-					-	4
Wpt 79	-					-	-	-	-		-				-			-					-	0

Species Name	*Agkistrodon contortrix*	*Alligator mississippiensis*	*Anolis carolinensis*	*Apalone ferox*	*Aspidoscelis sexlineata*	*Coluber constrictor*	*Deirochelys reticularia*	*Diadophis punctatus*	*Elaphe alleghaniensis*	*Kinosternon subrubrum*	*Nerodia erythrogaster*	*Nerodia fasciata*	*Nerodia floridana*	*Nerodia taxispilota*	*Ophisaurus ventralis*	*Regina alleni*	*Sceloporus undulatus*	*Scincella lateralis*	*Sistrurus miliarius*	*Sternotherus odoratus*	*Terrapene carolina*	*Thamnophis sauritus*	*Trachemys scripta scripta*	Number of reptile species detected at site
Wpt 103	-					-	-	-	-		-				-			-		X			-	1
Wpt 150	-					-	-	-	-		-	X			-			-					-	1
Wpt 151	-					-	-	-	-		-				-			-					-	0
Wpt 192	-					-	-	-	-		-				-			-				X	-	1
Wpt 201	-					-	-	-	-		-				-			-					-	0
Wpt 221	-	X				-	-	-	-		-		X		-			-		X			-	3
Wpt 316	-					-	-	-	-		-				-			-					-	0
Number of sites	-	5	2	3	1	-	-	-	-	2	-	8	3	1	-	1	1	-	1	3	2	4	-	
Naive occupancy	-	8.20	3.28	4.92	1.64	-	-	-	-	3.28	-	13.11	4.92	1.64	-	1.64	1.64	-	1.64	4.92	3.28	6.56	-	
Harris Neck NWR																								
Borrow Pond	-	X		-			X	-		-		X	-	-		-	-	-	-	-	-	-	-	3
Goose Pond	-			-		X		-		-			-	-		-	-	-	-	-	-	-	-	1
Lucas Borrow	-			-	X			-		-			-	-		-	-	-	-	-	-	-	-	1
Lucas Pond	-	X	X	-				-		-			-	-		-	-	-	-	-	-	-	-	2
Snipe Ponds 1&2	-	X		-				-		-			-	-		-	-	-	-	-	-	-	-	1
Wigeon Pond	-			-		X		-		-		X	-	-	X	-	-	-	-	-	-	-	-	3
Woody Pond	-	X	X	-		X		-	X	-			-	-		-	-	-	-	-	-	-	-	4
Number of sites	-	4	2	-	1	3	1	-	1	-	0	2	-	-	1	-	-	-	-	-	-	-	-	
Naive occupancy	-	57.14	28.57	-	14.29	42.86	14.29	-	14.29	-	0.00	28.57	-	-	14.29	-	-	-	-	-	-	-	-	
Savannah NWR																								
DT-2				-	-	-	-		-	-			-	-	-	-	-		-			X		1
DT-4	X			-	-	-	-		-	-			-	-	-	-	-	X	-					2
HQ-1				-	-	-	-		-	-		X	-	-	-	-	-		-					1
ND-3		X		-	-	-	-		-	-			-	-	-	-	-		-				X	2

Species Name	*Agkistrodon contortrix*	*Alligator mississippiensis*	*Anolis carolinensis*	*Apalone ferox*	*Aspidoscelis sexlineata*	*Coluber constrictor*	*Deirochelys reticularia*	*Diadophis punctatus*	*Elaphe alleghaniensis*	*Kinosternon subrubrum*	*Nerodia erythrogaster*	*Nerodia fasciata*	*Nerodia floridana*	*Nerodia taxispilota*	*Ophisaurus ventralis*	*Regina alleni*	*Sceloporus undulatus*	*Scincella lateralis*	*Sistrurus miliarius*	*Sternotherus odoratus*	*Terrapene carolina*	*Thamnophis sauritus*	*Trachemys scripta scripta*	Number of reptile species detected at site
ND-4			X	-	-	-	-		-	-			-	-	-	-	-		-	X				2
ST-1			X	-	-	-	-		-	-			-	-	-	-	-	X	-		X			3
ST-3			X	-	-	-	-		-	-	X		-	-	-	-	-	X	-					3
ST-4				-	-	-	-	X	-	-			-	-	-	-	-	X	-					2
ST-6				-	-	-	-		-	-			-	-	-	-	-		-					0
WD-3		X		-	-	-	-		-	-			-	-	-	-	-		-				X	2
Number of sites	1	2	3	-	-	-	-	1	-	-	1	1	-	-	-	-	-	4	-	1	1	1	2	
Naive occupancy	10.00	20.00	30.00	-	-	-	-	10.00	-	-	10.00	10.00	-	-	-	-	-	40.00	-	10.00	10.00	10.00	20.00	
Total number of sites (all refuges) where species was observed	1	11	7	3	2	3	1	1	1	2	1	11	3	1	1	1	1	4	1	4	3	5	2	

Table 5. Abiotic field parameters of wetlands sampled by Southeast ARMI at St. Marks, Harris Neck, and Savannah National Wildlife Refuges in 2006. Measurements were made with a Hydrolab® Quanta handheld meter.

Date	General Location	Water Temperature (°C)	SpC (ms/cm)	DO (mg/L)	pH	% DO
SMNWR						
1/31/2006	Biggins Pond	16.02	5.770	8.11	3.84	83.90
3/21/2006		25.47	5.340	2.40	4.65	29.90
5/3/2006		20.93	7.370	2.10	5.11	24.20
6/19/2006		39.52	3.630	8.12	4.25	126.10
8/1/2006		34.21	4.190	6.02	3.89	86.80
6/20/2006	Chicky Pond	24.75	0.047	2.75	4.09	33.20
8/1/2006		25.21	0.035	2.17	4.14	-
2/2/2006	Cingulatum Pond	9.71	0.061	2.60	6.91	22.70
3/21/2006	Corner Pond	23.97	0.030	7.58	3.59	89.90
5/3/2006		26.75	0.042	4.73	4.10	57.80
6/20/2006		35.13	0.032	6.06	4.11	86.80
8/1/2006		32.39	0.032	3.41	4.16	46.60
6/21/2006	Fat Nerodia Pond	24.55	0.037	1.96	3.88	23.60
1/31/2006	Goose Pond	17.57	0.037	9.22	3.93	94.50
5/2/2006		26.02	0.042	6.62	3.87	81.30
6/21/2006		28.55	0.042	1.53	5.17	19.60
8/1/2006		31.45	0.049	3.63	4.18	49.10
2/1/2006	Goose Pond 2	16.51	0.039	8.31	3.97	84.40
2/2/2006	Headquarters Pond	13.82	10.070	4.32	6.75	43.20
3/21/2006	Jennifer's Sink	19.87	0.026	5.48	6.03	60.30
5/3/2006		21.21	0.027	1.78	4.95	20.00
6/20/2006		25.88	0.035	0.43	4.68	5.40
8/1/2006		27.90	0.032	1.57	4.66	20.10
1/31/2006	Kingfisher Pond	15.85	2.770	6.43	4.33	67.00
3/21/2006		22.96	2.200	2.24	4.28	26.30
5/2/2006		19.90	2.820	0.34	5.46	3.80
6/19/2006		31.99	1.098	3.07	5.51	43.70
8/1/2006		28.30	1.290	0.15	5.50	2.00
1/31/2006	ORSP Borrow	16.08	6.180	8.91	5.91	92.40
5/3/2006	Otter Lake	25.03	0.975	7.83	6.30	94.60
6/21/2006		29.29	1.341	4.53	6.18	59.70
8/2/2006		30.13	1.006	3.70	6.28	49.20
2/2/2006	Plum Orchard Pond	11.91	0.106	3.61	7.46	33.00
1/31/2006	Pond 2	15.91	0.043	7.90	4.01	80.50
1/31/2006	Pond 7	18.32	0.048	7.88	3.96	83.00
1/31/2006	Pond 10	16.36	0.031	8.93	4.09	90.80
1/31/2006	Printiss Pond	16.54	0.932	7.78	5.05	79.90
1/31/2006	Printiss 2 Pond	12.10	0.046	4.72	4.92	44.10
6/21/2006		26.06	0.245	0.75	4.85	9.30
2/1/2006	Ring Pond	11.89	0.367	7.19	5.15	64.30
5/3/2006		23.23	0.351	1.72	3.69	20.10
6/20/2006		35.74	0.375	5.37	3.93	78.00
8/1/2006		28.25	0.093	3.71	4.27	47.80

Date	General Location	Water Temperature (°C)	SpC (ms/cm)	DO (mg/L)	pH	% DO
2/1/2006	Small Prairie Pond	11.17	0.040	5.92	3.80	53.30
5/3/2006		21.75	0.045	1.14	4.74	12.90
6/19/2006		35.15	0.040	6.58	4.81	94.40
8/1/2006		29.71	0.033	8.24	4.74	109.00
1/31/2006	SPC Prairie	19.59	8.680	8.42	4.39	96.20
3/21/2006		22.52	7.870	3.36	4.94	39.90
5/3/2006		33.97	1.141	6.62	4.76	97.20
6/20/2006		29.64	8.680	3.53	5.03	47.90
8/1/2006		32.26	9.130	2.93	5.61	42.20
1/31/2006	Spike Buck Pond	15.08	0.079	4.20	4.28	40.90
6/21/2006		28.37	0.083	2.07	3.87	26.60
3/21/2006	Talpoideum Pond	27.39	0.030	4.54	3.52	57.30
5/3/2006		29.23	0.045	4.30	3.88	55.80
6/20/2006		31.08	0.036	7.93	3.83	109.10
8/1/2006		29.16	0.028	6.51	3.99	84.90
1/31/2006	Temp Wpt 202	14.83	0.237	7.22	3.89	71.50
1/31/2006	Temp Wpt 203	16.18	4.440	9.02	3.91	91.50
1/31/2006	Temp Wpt 204	15.99	11.090	8.73	4.37	91.70
1/31/2006	Temp Wpt 205	18.65	6.370	7.16	4.59	78.60
1/31/2006	Temp Wpt 206	18.72	4.120	8.25	4.35	86.80
1/31/2006	Temp Wpt 207	13.52	0.040	8.72	4.54	82.40
1/31/2006	Temp Wpt 208	18.17	4.450	7.52	4.18	79.70
1/31/2006	Temp Wpt 209	17.00	0.042	10.32	4.03	105.40
2/1/2006	Temp Wpt 210	7.97	1.630	5.68	3.65	47.80
2/1/2006	Temp Wpt 211	7.92	0.074	6.66	3.19	56.10
2/1/2006	Temp Wpt 212	9.58	4.900	7.08	4.17	62.70
2/1/2006	Temp Wpt 213	16.29	12.970	4.21	4.61	40.80
2/1/2006	Temp Wpt 214	12.20	3.060	9.61	4.42	90.00
2/1/2006	Temp Wpt 215	11.18	1.425	9.01	3.93	82.10
2/1/2006	Temp Wpt 216	10.63	3.700	7.08	3.90	63.80
2/1/2006	Temp Wpt 217	9.25	7.545	7.57	3.48	70.00
2/1/2006	Temp Wpt 218	10.01	2.320	13.55	3.74	119.50
2/1/2006	Temp Wpt 219	12.65	4.330	4.61	4.35	43.60
2/1/2006	Temp Wpt 220	11.04	5.140	3.80	4.26	35.10
2/1/2006	Temp Wpt 222	16.27	0.045	5.52	4.15	55.50
2/1/2006	Temp Wpt 223	11.00	0.043	4.61	4.42	41.40
1/31/2006	Temp Wpt 302	13.60	0.706	8.48	4.43	86.20
1/31/2006	Temp Wpt 303	14.68	0.749	4.51	4.77	44.70
1/31/2006	Temp Wpt 304	15.64	0.854	3.08	5.39	31.30
1/31/2006	Temp Wpt 305	15.24	5.300	8.80	4.05	89.60
1/31/2006	Temp Wpt 306	13.04	0.297	6.19	7.12	58.60
2/1/2006	Temp Wpt 307	11.31	0.031	6.63	4.73	59.90
2/1/2006	Temp Wpt 308	12.03	0.369	7.86	4.28	72.60
2/1/2006	Temp Wpt 309	9.21	0.834	8.11	4.77	70.30
2/1/2006	Temp Wpt 310	13.10	5.295	4.02	5.06	37.40
2/1/2006	Temp Wpt 311	14.46	11.400	6.52	5.95	66.20
2/1/2006	Temp Wpt 312	12.88	3.550	9.27	4.14	87.60
2/1/2006	Temp Wpt 313	11.78	0.323	7.02	4.62	64.40

Date	General Location	Water Temperature (°C)	SpC (ms/cm)	DO (mg/L)	pH	% DO
2/1/2006	Temp Wpt 314	13.06	0.065	8.39	4.44	79.50
2/1/2006	Temp Wpt 315	15.14	0.057	7.93	4.31	78.10
2/1/2006	Temp Wpt 317	17.41	5.050	10.58	5.68	110.60
2/1/2006	Temp Wpt 318	10.74	0.885	9.93	6.08	90.20
1/31/2006	WBF Pond	17.48	0.048	5.90	3.83	61.60
6/21/2006	WBF Pond	26.35	0.033	2.78	3.99	33.50
1/31/2006	Wpt 16	17.00	10.490	7.72	4.20	82.30
1/31/2006	Wpt 19	16.42	1.409	5.51	3.87	55.30
3/21/2006		20.29	1.193	1.70	3.96	18.40
5/2/2006		22.19	1.281	0.28	3.75	3.20
6/20/2006		30.08	0.835	8.56	3.87	114.20
8/2/2006		26.79	0.481	4.23	4.21	53.00
1/31/2006	Wpt 68	19.78	3.380	4.50	4.42	49.00
3/21/2006		23.17	1.329	3.59	4.20	42.00
6/20/2006		29.61	0.580	6.07	4.98	80.00
8/2/2006		31.15	0.202	5.68	5.49	78.50
2/1/2006	Wpt 69	12.17	0.036	6.30	3.76	58.70
3/21/2006		21.19	0.034	3.09	4.15	34.30
5/2/2006		19.08	0.046	2.27	4.05	24.40
6/19/2006		33.75	0.037	4.12	4.12	57.80
8/1/2006		27.17	0.033	1.57	4.36	19.90
1/31/2006	Wpt 79	16.06	0.061	7.22	3.86	72.20
3/21/2006		21.23	0.054	3.77	3.59	42.50
6/20/2006		26.34	0.064	3.41	3.96	43.10
8/1/2006		30.46	0.044	3.86	3.92	51.10
1/31/2006	Wpt 103	14.18	0.057	4.08	3.68	40.40
5/3/2006		22.85	0.057	1.92	4.83	22.20
6/21/2006		28.15	0.037	2.29	4.45	29.30
8/2/2006		29.63	0.038	5.04	4.32	66.40
1/31/2006	Wpt 127	17.39	2.940	10.17	4.62	107.20
1/31/2006	Wpt 128	13.62	1.359	7.60	4.46	73.30
3/21/2006	Wpt 150	24.87	0.026	0.51	4.22	4.30
6/20/2006		27.92	0.027	4.50	4.37	57.70
8/2/2006		29.13	0.019	2.71	4.61	35.30
1/31/2006	Wpt 151	16.95	0.043	8.25	3.85	84.70
5/3/2006	Wpt 192	18.54	0.047	5.50	3.42	58.60
6/21/2006		31.84	0.041	8.30	3.89	113.40
8/1/2006		25.88	0.045	2.34	4.00	-
1/31/2006	Wpt 201	12.45	0.071	4.20	3.62	38.70
2/1/2006	Wpt 221	14.98	2.620	6.56	4.34	64.50
5/2/2006		27.21	2.830	3.56	3.97	45.70
6/19/2006		36.98	2.420	6.77	4.21	100.40
8/1/2006		30.00	1.345	1.80	5.47	24.10
2/1/2006	Wpt 316	14.20	0.334	6.41	4.98	62.00
6/21/2006		36.76	0.310	-	5.01	-
8/1/2006		26.78	0.108	2.98	5.15	37.40
HNNWR						
5/23/2006	Borrow Pond	28.13	0.034	4.24	5.08	54.30

Date	General Location	Water Temperature (°C)	SpC (ms/cm)	DO (mg/L)	pH	% DO
8/15/2006		26.25	0.026	4.02	4.95	49.60
5/23/2006	Goose Pond	30.82	0.099	7.55	6.47	101.70
5/23/2006	Lucas Borrow	25.44	0.071	1.64	5.35	20.10
8/15/2006		25.53	0.046	1.48	5.49	17.50
5/23/2006	Lucas Pond	21.81	0.049	0.76	5.89	8.40
8/15/2006		28.01	0.057	1.01	5.49	12.40
5/23/2006	Snipe Pond 1	35.92	0.566	0.27	6.96	3.70
5/23/2006	Snipe Pond 2	30.24	0.673	10.30	7.58	137.90
5/23/2006	Wigeon Pond	22.97	0.344	0.36	6.11	4.20
5/23/2006	Woody Pond	26.35	0.304	0.47	6.69	6.00
8/15/2006		27.31	0.363	0.30	6.44	4.10
SNWR						
5/25/2006	DT-2	23.76	0.032	1.25	5.98	14.30
8/16/2006		26.11	0.060	0.18	5.80	2.20
5/25/2006	HQ-1	26.90	0.609	1.18	6.27	14.90
8/16/2006		27.15	0.555	0.71	6.71	8.60
5/25/2006	ND-3	24.21	0.273	1.56	6.35	18.90
8/16/2006		27.27	0.429	0.68	6.91	8.30
5/25/2006	ND-4	25.25	0.449	1.94	6.33	22.80
8/16/2006		24.24	0.560	0.62	6.40	7.40
5/25/2006	WD-3	22.95	1.035	1.51	6.30	14.10
8/16/2006		24.46	0.826	0.67	7.36	7.80

Table 6. Amphibian and reptile specimens collected at St. Marks, Lower Suwannee, Harris Neck, and Savannah National Wildlife Refuges and Ordway-Swisher Biological Station by Southeast ARMI during 2006.

Date	USGS #	Species	St.	Co.	UTM-E	UTM-N	Zone	Locality	Collector(s)	Notes
9-Feb-06	live	*Rana capito*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Collected as egg for tadpole predation experiment
11-Apr-06	13984	*Rana clamitans*	FL	Putnam	401870	3284681	17	Ordway-Swisher Biological Station; Lake Suggs	DRG	Collected as tadpole; preserved as metamorph 14Jun06
14-Apr-06	12683	*Siren intermedia*	FL	Putnam	401870	3284681	17	Ordway-Swisher Biological Station; Lake Suggs	DRG, JMB	Found dead in crayfish trap, possibly killed by *Nerodia fasciata* captured in same trap
24-Apr-06	12785	*Rana sphenocephala*	FL	Levy	302284	3243154	17	Lower Suwannee NWR; USGS-ARMI site Hardwood Hammock 03	DRG, MSG, JSS, WJB	Collected as tadpole to photograph; preserved 14Jun06
24-Apr-06	12786	*Rana catesbeiana*	FL	Levy	301767	3251630	17	Lower Suwannee NWR; USGS-ARMI site Hardwood Hammock 46, near the boardwalk	DRG, MSG, JSS, WJB	Collected as tadpole to photograph; preserved 14Jun06
2-May-06	live	*Ambystoma talpoideum* (2)	FL	Wakulla	747479	3322471	16	St Marks NWR; Panacea Unit, USGS-ARMI site Wpt 69	MSG, JSS, DRG	Collected as larvae to photograph
2-May-06	live	*Siren intermedia*	FL	Wakulla	747479	3322471	16	St Marks NWR; Panacea Unit, USGS-ARMI site Wpt 69	MSG, JSS, DRG	Collected as larva to photograph
2-May-06	12787	*Rana catesbeiana*	FL	Wakulla	747990	3327929	16	St Marks NWR; Panacea Unit, USGS-ARMI site Jennifer's Sink	DRG	Collected as tadpole to photograph; preserved 11Jun06
9-May-06	live	*Hyla gratiosa*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Collected as tadpole to photograph
10-May-06	live	*Hyla gratiosa* (2)	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Collected as eggs to confirm ID
10-May-06	12794	*Rana catesbeiana* (6)	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Collected as eggs to confirm ID
12-May-06	12686	*Siren lacertina*	FL	Putnam	401870	3284681	17	Ordway-Swisher Biological Station; Lake Suggs	MSG, DRG	Caught in crayfish trap, being eaten by *Dolomedes* sp. (fishing spider)
24-May-06	12692	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12693	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12694	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12695	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12696	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID

Date	USGS #	Species	St.	Co.	UTM-E	UTM-N	Zone	Locality	Collector(s)	Notes
24-May-06	12697	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12698	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12699	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12700	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12701	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12702	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
24-May-06	12703	*Rana sphenocephala*	FL	Putnam	404004	3287119	17	Ordway-Swisher Biological Station; Blue Pond	DRG	Tadpoles from egg masses collected 9Feb06 for predation experiment; raised to confirm ID
25-May-06	12740	*Siren lacertina*	SC	Jasper	488878	3559168	17	Savannah NWR; USGS-ARMI site HQ-1	WJB, CPS	Killed by *Nerodia fasciata* in crayfish trap
25-May-06	12741	*Siren lacertina*	SC	Jasper	488878	3559168	17	Savannah NWR; USGS-ARMI site HQ-1	WJB, CPS	Killed by *Nerodia fasciata* in crayfish trap
26-May-06	12742	*Siren* sp.	SC	Jasper	488878	3559168	17	Savannah NWR; USGS-ARMI site HQ-1	WJB, CPS	Found dead in crayfish trap
21-Jun-06	12758	*Coluber constrictor*	FL	Wakulla			16	St Marks NWR; Panacea Unit, DOR on RR330 just S of jct w/ RR316	WJB, MSG, DRG	
21-Jun-06	12770	*Pseudacris ocularis* (2)	FL	Wakulla	753007	3327665	16	St Marks NWR; Panacea Unit, USGS-ARMI site Wpt 316	DRG	Collected as tadpoles to photograph; preserved 3Jul06
21-Jun-06	12771	*Gastrophryne carolinensis* (2)	FL	Wakulla	744431	3325997	16	St Marks NWR; Panacea Unit, USGS-ARMI site Printiss Pond	DRG	Collected as tadpoles to photograph; preserved as metamorphs 15 and 17Aug06
21-Jun-06	12791	*Gastrophryne carolinensis*	FL	Wakulla	745998	3324492	16	St Marks NWR; Panacea Unit, USGS-ARMI site Wpt 103	WJB, MSG, DRG	Collected as tadpole to photograph; preserved as metamorph
6-Jul-06	12759	*Siren lacertina*	FL	Putnam	401870	3284681	17	Ordway-Swisher Biological Station; Lake Suggs	DRG, CPS	Found dead in crayfish trap
1-Aug-06		*Acris gryllus*	FL	Wakulla	746380	3324168	16	St Marks NWR; Panacea Unit, USGS-ARMI site Small Prairie Pond	WJB, DRG	Collected as tadpole to photograph
1-Aug-06		*Hyla gratiosa* (2)	FL	Wakulla	747479	3322471	16	St Marks NWR; Panacea Unit, USGS-ARMI site Wpt 69	WJB, DRG	Collected as tadpoles to photograph
2-Aug-06	12772	*Pseudacris ocularis*	FL	Wakulla	749872	3322969	16	St Marks NWR; Panacea Unit, USGS-ARMI site Wpt 79	DRG	Collected as tadpole to photograph; preserved as metamorph 24Aug06

Date	USGS #	Species	St.	Co.	UTM-E	UTM-N	Zone	Locality	Collector(s)	Notes
8-Aug-06	12769	*Siren lacertina*	FL	Putnam	401870	3284681	17	Ordway-Swisher Biological Station; Lake Suggs	DRG, CPS	Killed by alligator in crayfish trap

Collectors:
WJB - W.J. Barichivich, USGS
DRG - D.R. Gregoire, USGS
MSG - M.S. Gunzburger, USGS
CPS - C.P. Smith
JSS - J.S. Staiger, USGS

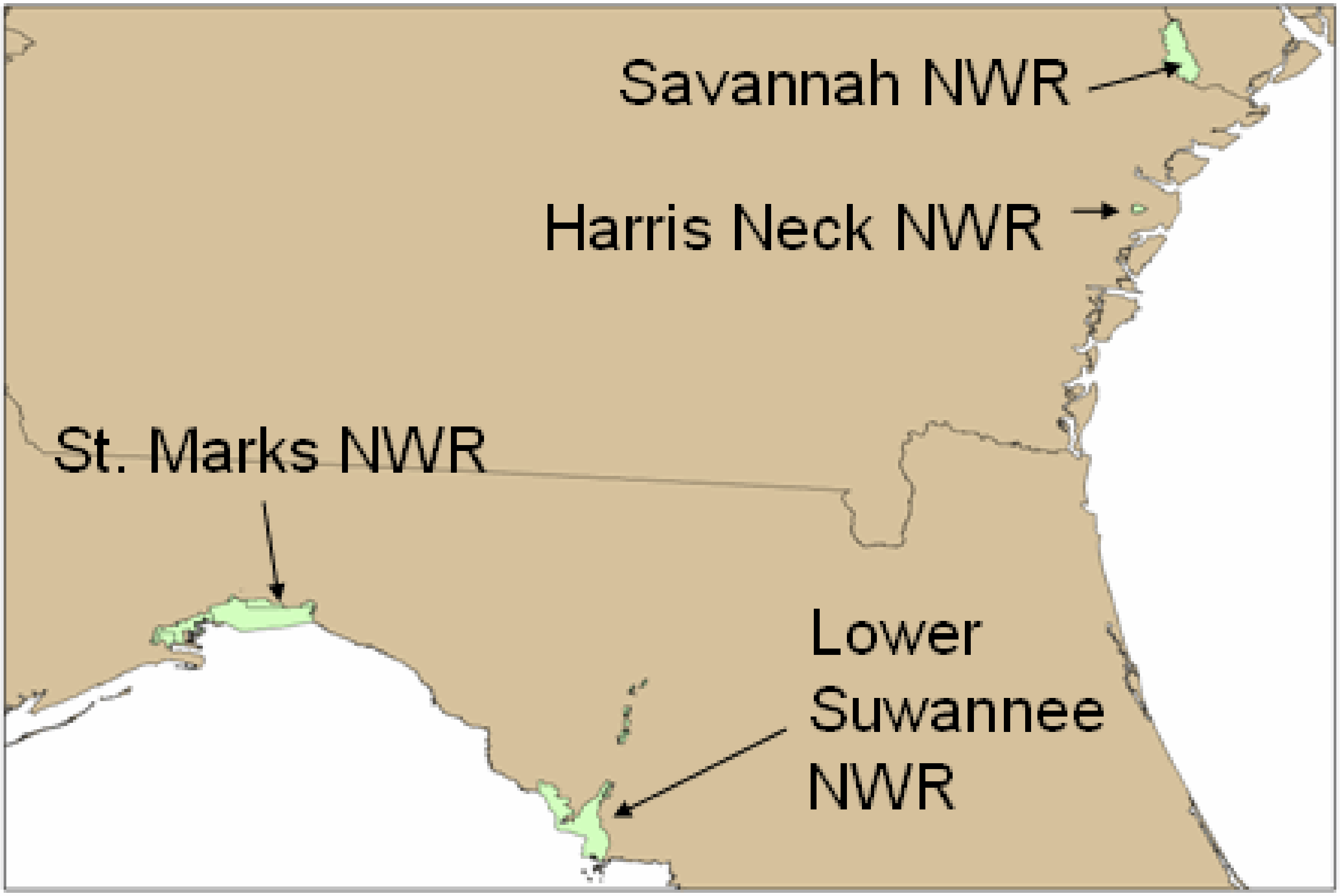

Fig. 1. General locations of St. Marks, Lower Suwannee, Harris Neck, and Savannah National Wildlife Refuges.

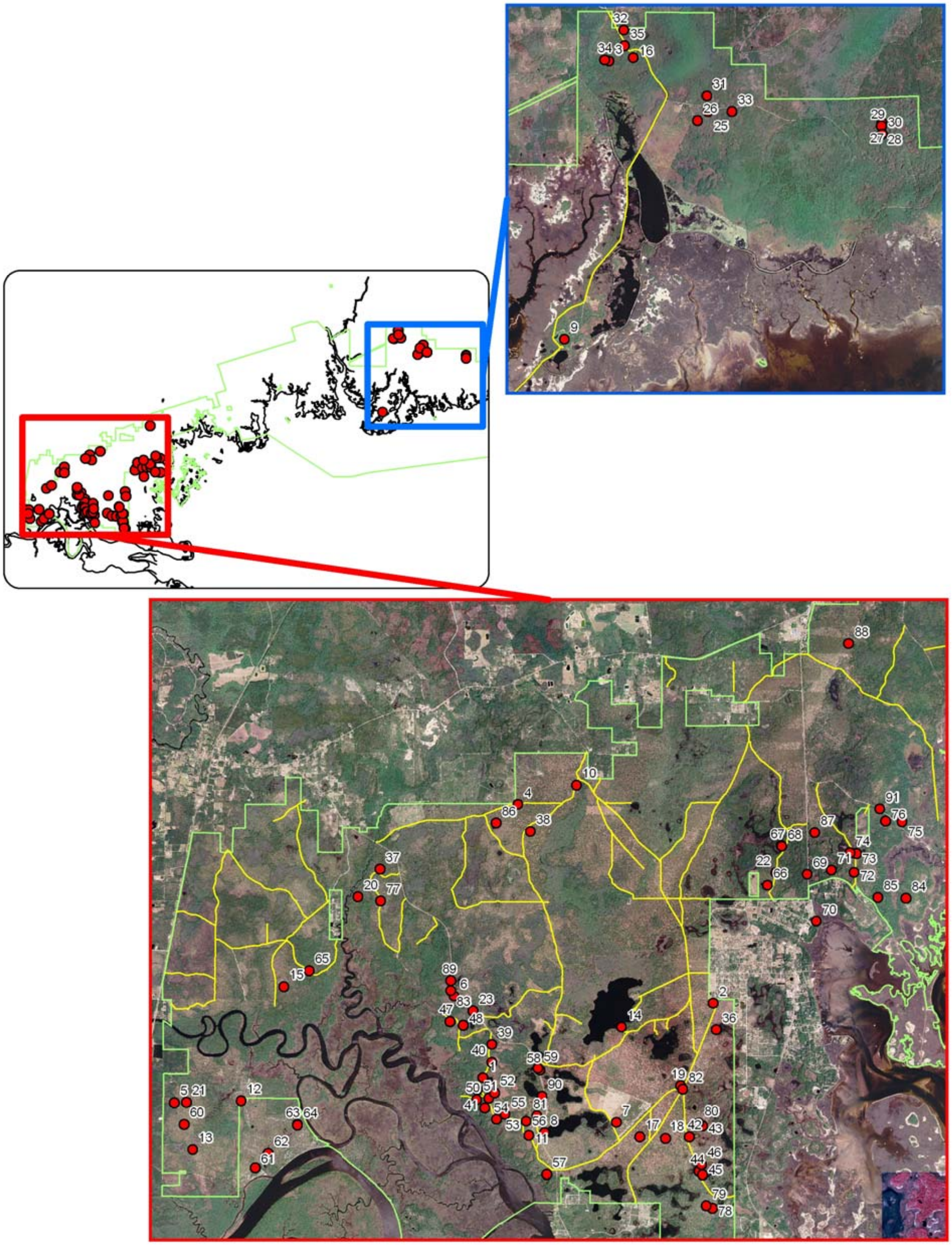

Fig. 2A. Locations of 2006 Southeast ARMI sampling sites at St. Marks NWR. Site numbers correspond to Table 1.

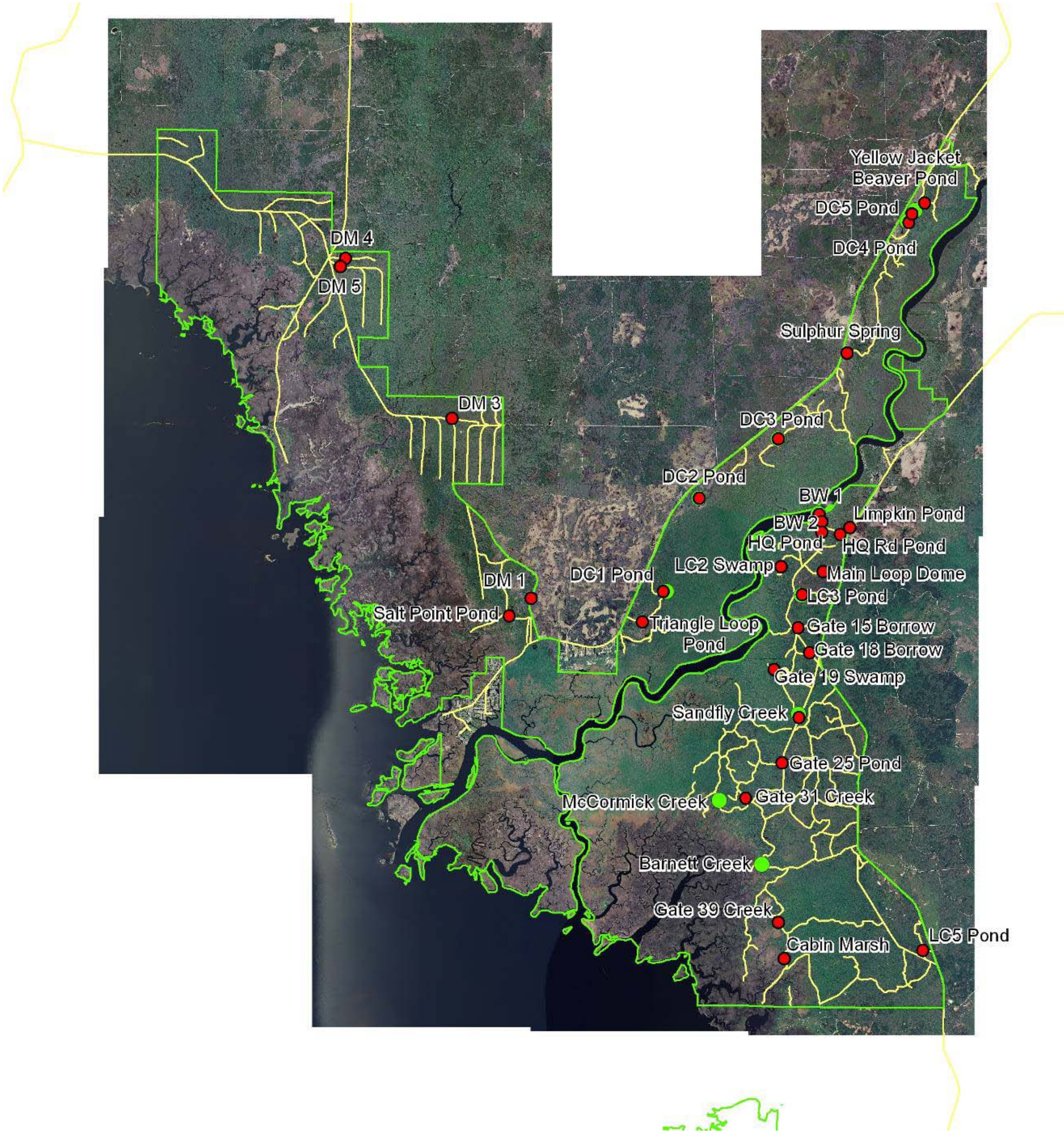

Fig. 2B. Locations of Southeast ARMI sampling sites at Lower Suwannee NWR.

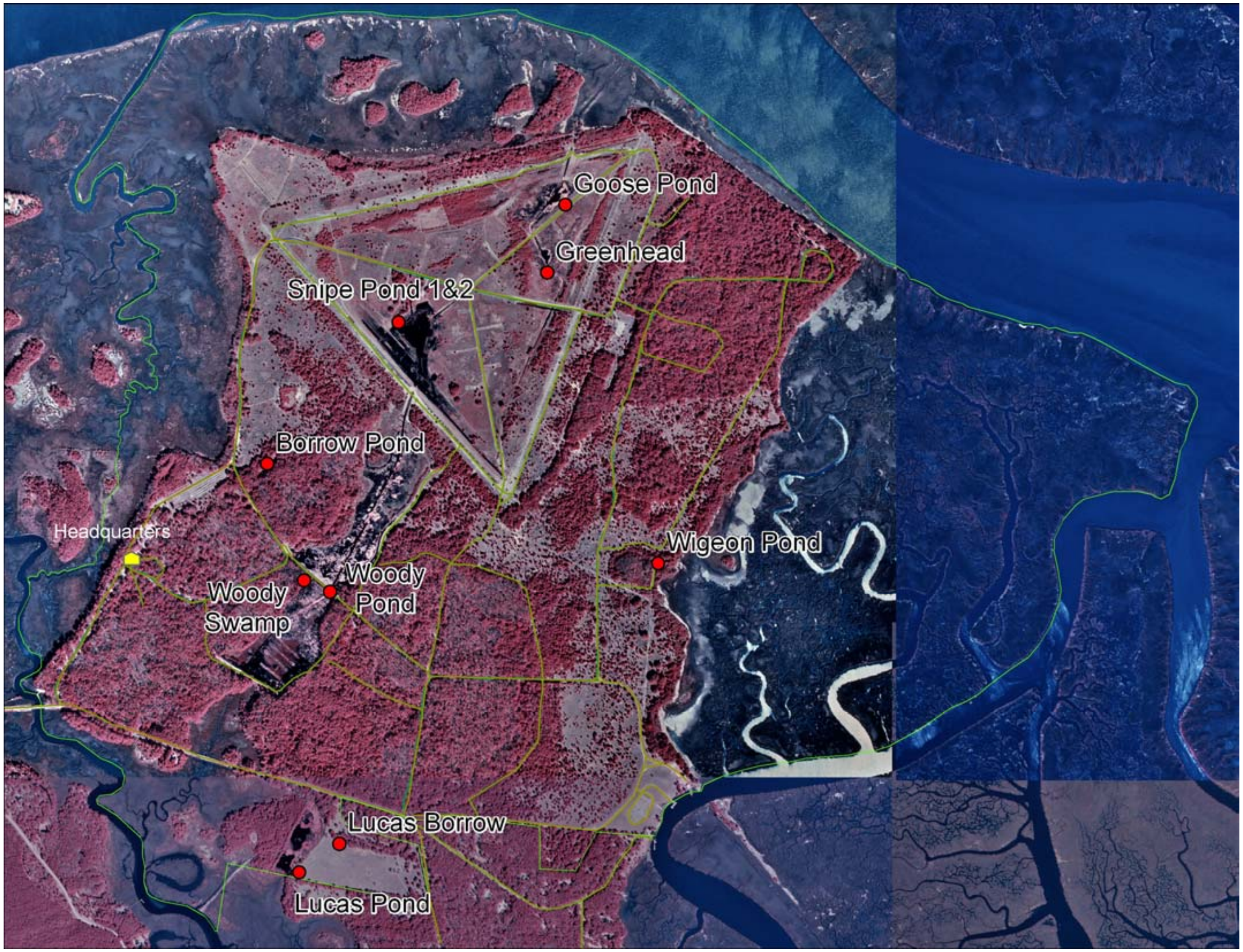

Fig. 2C. Locations of 2006 Southeast ARMI sampling sites at Harris Neck NWR.

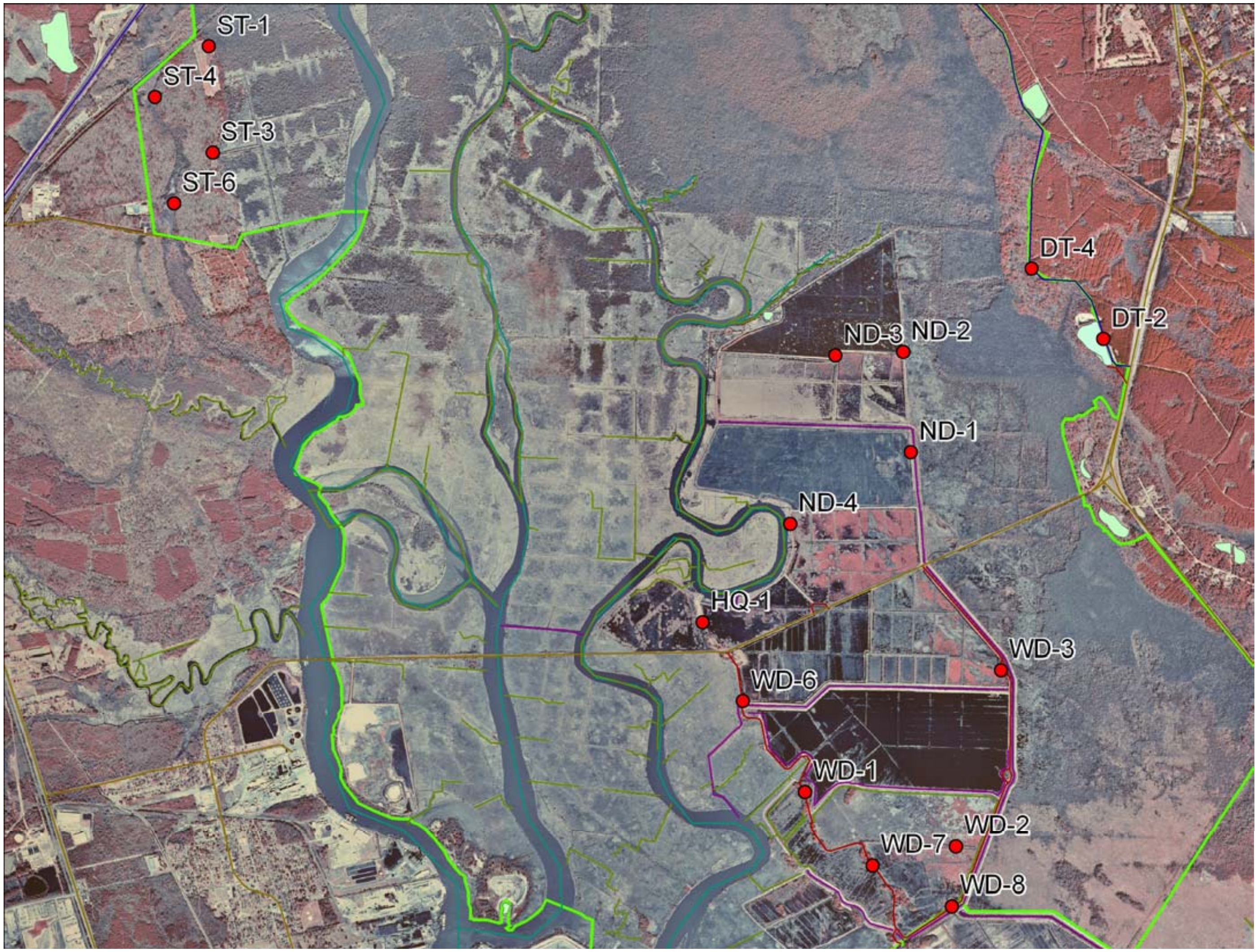

Fig. 2D. Locations of 2006 Southeast ARMI sampling sites at Savannah NWR.

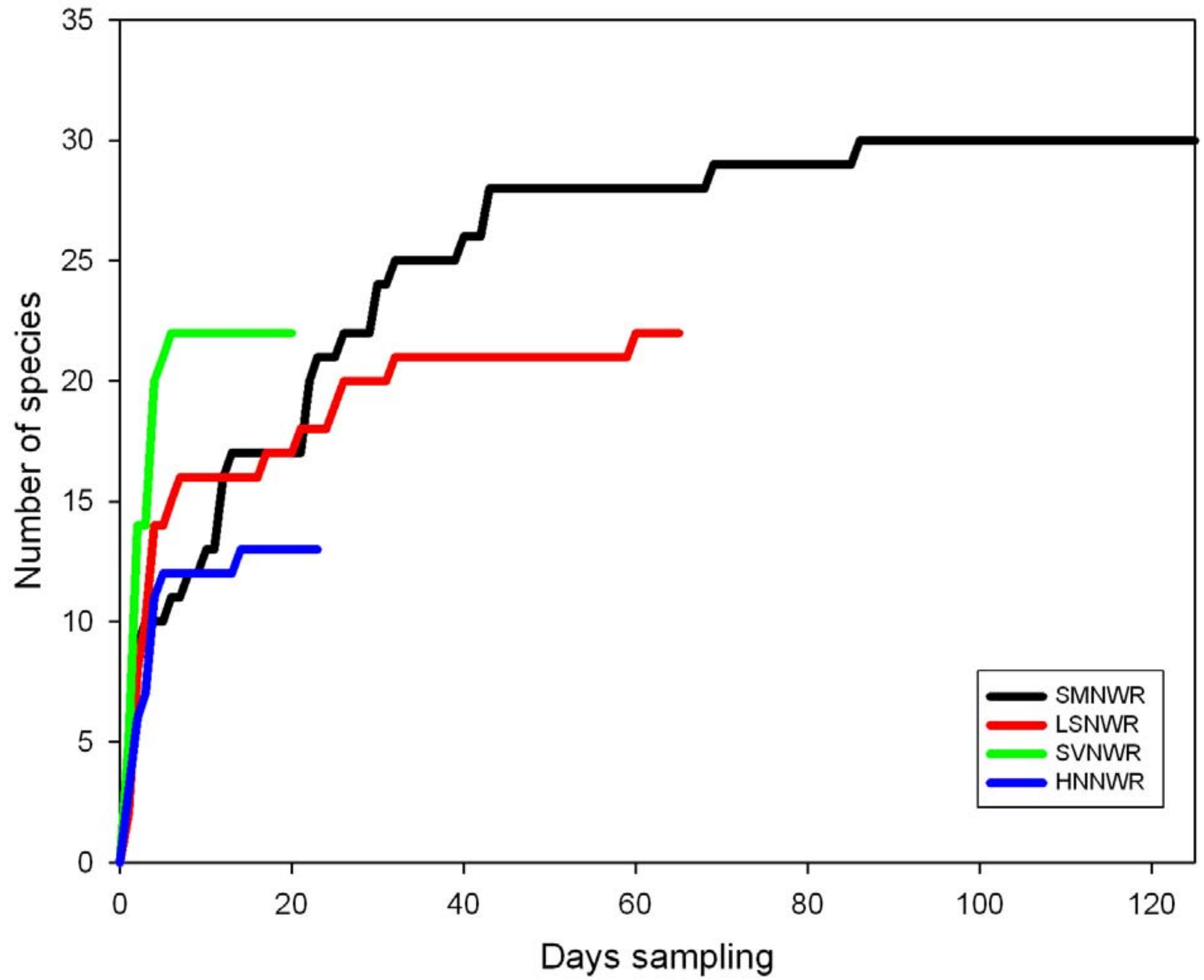

Fig. 3. Cumulative number of species of amphibians detected at each refuge relative to sampling effort (total number of days).

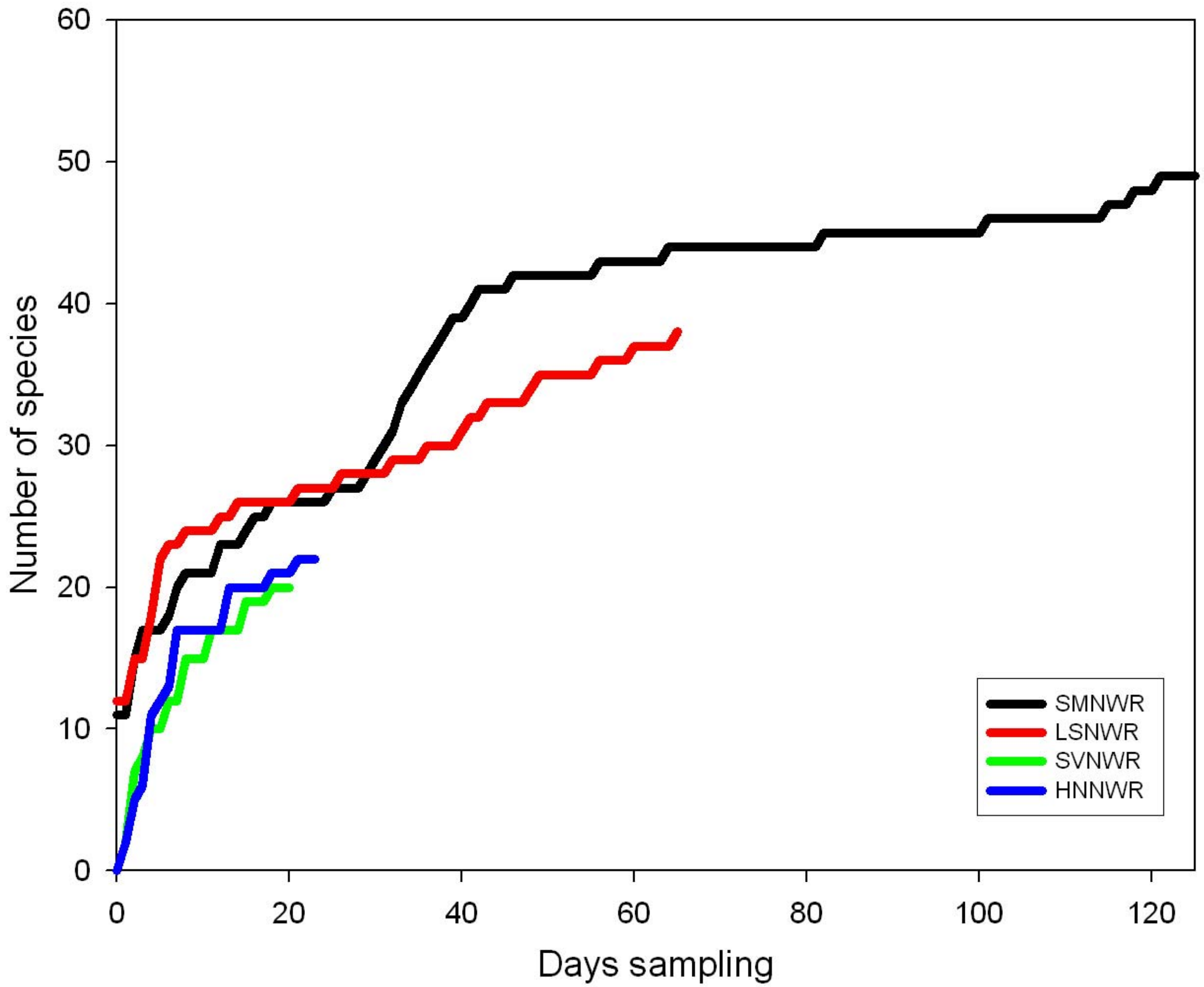

Fig. 4. Cumulative number of species of reptiles detected at each refuge relative to sampling effort (total number of days).

Appendix I. Publications and presentations by Southeast ARMI researchers in 2006.

Publications of Herpetology Project:

Aresco, M.J., M.A. Ewert, M.S. Gunzburger, G.L. Heinrich, and P.A. Meylan. 2006. *Chelydra serpentina* - Snapping Turtle, *in* P.A. Meylan, ed., Biology and conservation of Florida turtles. Chelonian Research Monographs 3:44-57.

Dodd, C.K., Jr. 2006. Remembering 'common' species during restoration: lessons from Egmont Key (Florida). Restoration Ecology 24:211-212.

Dodd, C.K., Jr., A. Ozgul, and M.K. Oli. 2006. The influence of disturbance events on survival and dispersal rates of Florida box turtles. Ecological Applications 16(5):1936-1944.

Dodd, C.K., Jr., and W.J. Barichivich. *In press*. Movements of large snakes (*Drymarchon*, *Masticophis*) in north-central Florida. Florida Scientist.

Farrell, T.M., C.K. Dodd, Jr., and P.G. May. 2006. *Terrapene carolina* – Eastern Box Turtle, *in* P.A. Meylan, ed., Biology and conservation of Florida turtles. Chelonian Research Monographs 3:235-248.

Redder, A.J., C.K. Dodd, Jr., and D. Keinath. 2006. Ornate Box Turtle (*Terrapene ornata ornata*): a technical conservation assessment. USDA Forest Service, Rocky Mountain Region, 54 pp. Available: http://www.fs.fed.us/r2/projects/scp/assessments/ornateboxturtle.pdf. Web published 16 May 2006.

Richardson, J.M.L., M.S. Gunzburger, and J. Travis. 2006. Variation in predation pressure as a mechanism underlying differences in numerical abundance between populations of the poeciliid fish *Heterandria formosa*. Oecologia 147:596-605.

Schmid, J.R., and W.J. Barichivich. 2006. *Lepidochelys kempi* –Kemp's Ridley, *in* P.A. Meylan, ed., Biology and conservation of Florida turtles. Chelonian Research Monographs 3:128-141.

Steen, D.A., M.J. Aresco, S.G. Beilke, B.W. Compton, E.P. Condon, C.K. Dodd, Jr., H. Forrester, J.W. Gibbons, J.L. Greene, G. Johnson, T.A. Langen, M.J. Oldham, D.N. Oxier, R.A. Saumure, F.W. Schueler, J.M. Sleeman, L.L. Smith, J.K. Tucker, and J.P. Gibbs. 2006. Relative vulnerability of female turtles to road mortality. Animal Conservation 9(3):269-273.

SEARMI:

Dodd, C.K., Jr., J. Loman, D. Cogălniceanu, and M. Puky. *In press*. Monitoring Amphibian Populations. pp. – *in* H.H. Heatwole and J.W. Wilkenson, eds., Conservation and Decline of Amphibians, Amphibian Biology, Volume 9A. Surrey Beatty & Sons, Chipping Norton, New South Wales, Australia.

Dodd, C.K., Jr., and W.J. Barichivich. *In press*. Establishing a baseline and faunal history in amphibian monitoring programs: The amphibians of Harris Neck, Georgia, USA. Southeastern Naturalist.

Dodd, C.K., Jr., W.J. Barichivich, S.A. Johnson, and J.S. Staiger. *In press*. Changes in a northwestern Florida Gulf Coast herpetofaunal community over a 28-year period. American Midland Naturalist.

Gunzburger, M.S. 2006. Reproductive ecology of the green treefrog (*Hyla cinerea*) in Northwestern Florida. American Midland Naturalist 155:309-316.

Kuzmin, S.L. and C.K. Dodd, Jr. *In press*. Commonwealth of Independent States (Former Soviet Union), pp. – *in* H.H. Heatwole and J.W. Wilkenson, eds., Regional Assessment of Decline in Amphibians, Amphibian Biology, Volume 9B. Surrey Beatty & Sons, Chipping Norton, New South Wales, Australia.

Muths, E., A.L. Gallant, E.H. Campbell Grant, W.A. Battaglin, D.E. Green, J.S. Staiger, S.C. Walls, M.S. Gunzburger, and R.F. Kearney. 2006. The Amphibian Research and Monitoring Initiative (ARMI): 5-Year Report. USGS Scientific Investigations Report 2006-5224, 77 pp.

Smith, K.G. 2006. Keystone predators (eastern newts, *Notophthalmus viridescens*) reduce the impacts of an aquatic invasive species. Oecologia 148:342-349.

Smith, L.L., W.J. Barichivich, J.S. Staiger, K.G. Smith, and C.K. Dodd, Jr. 2006. Detection probabilities and site occupancy estimates for amphibians at Okefenokee National Wildlife Refuge. American Midland Naturalist 155:149-161.

Submitted for USGS Review:

Gregoire, D.R. A guide to the tadpoles of the southeastern United States. USGS Circular.

Gregoire, D.R., and M.S. Gunzburger. Effects of predatory fish on survival and behavior of larval Gopher Frogs (*Rana capito*) and Southern Leopard Frogs (*Rana sphenocephala*). Journal of Herpetology.

Oral Presentations:

Barichivich, W.J., and J.S. Staiger. 2006. USGS herpetological research at Lower Suwannee National Wildlife Refuge. Invited presentation, Friends and Volunteers of Refuges regular meeting, Cedar Key, Florida. Nov. 18.

Dodd, C.K., Jr. 2006. Declining amphibians and emerging disease issues. Invited lecture, Warm Springs Regional Fishery Center, Warm Springs, Georgia. Jan. 12.

Dodd, C.K., Jr. 2006. Tracking amphibians across the southern landscape, with a side trip to some tadpole farms [aka fish hatcheries]. National Conservation Training Center, Shepherdstown, West Virginia. Feb. 8.

Dodd, C.K., Jr. 2006. USGS Amphibian Research and Monitoring Initiative. National Conservation Training Center, Shepherdstown, West Virginia. Feb. 8.

Dodd, C.K., Jr., and W.J. Barichivich. 2006. Changes in a northwestern Florida Gulf Coast herpetofaunal community over a 28-year period. SE PARC annual meeting, Andalusia, Alabama. Feb. 24.

Dodd, C.K., Jr., W.J. Barichivich and S.A. Johnson. 2006. Changes in a northwestern Florida Gulf Coast herpetofaunal community over a 28-year period. Joint Meeting of Ichthyologists and Herpetologists, New Orleans, Louisiana. Jul. 15.

Dodd, C.K., Jr. 2006. Developing a national program for monitoring amphibians. Invited lecture, Society of Wetland Scientists (South Atlantic chapter) and Florida chapter of the Wildlife Society annual meeting, Palatka, Florida. Oct. 5.

Dodd, C.K., Jr. 2006. Amphibians, emerging infectious diseases, and warm-water fish hatcheries in the southeast. Invited lecture, Gopher Tortoise Council annual meeting, Valdosta, Georgia. Oct. 28-29.

Dodd, C.K., Jr., M.S. Gunzburger, W.J. Barichivich and J.S. Staiger. 2006. The Southeastern Amphibian Research and Monitoring Initiative: Activities in FY 2006. USGS/ARMI annual meeting, Point Reyes, California. Nov. 8.

Dodd, C.K., Jr. 2006. Efficacy of wildlife barriers and underpass systems in reducing highway-related wildlife mortality: lessons from Florida. Invited seminar, Department of Forestry and Natural Resources, Purdue University, West Lafayette, Indiana. Nov. 28.

<u>Poster Presentations:</u>

Gregoire, D.R. 2006. Effects of predatory fish on *Rana capito* and *Rana sphenocephala* larvae. Joint Meeting of Ichthyologists and Herpetologists, New Orleans, Louisiana. Jul. 2006.

Gunzburger, M.S. 2006. Evaluation of seven aquatic sampling methods for amphibians and other aquatic fauna. Joint Meeting of Ichthyologists and Herpetologists, New Orleans, Louisiana. Jul. 2006.

Staiger, J.S., and W.J. Barichivich. 2006. Terrestrial herpetofaunal community structure along a faunal break in north central Florida. Joint Meeting of Ichthyologists and Herpetologists, New Orleans, Louisiana. Jul. 2006.

Appendix II: National Wildlife Health Center Pathology Reports

NATIONAL WILDLIFE HEALTH CENTER
6006 SCHROEDER ROAD
MADISON, WI 53711-6223
PHONE: (608) 270-2400 FAX: (608) 270-2415

science for a changing world

FINAL PATHOLOGY & HISTO-PATHOLOGY REPORT on AMPHIBIANS from NATIONAL FISH HATCHERIES and NATIONAL WILDLIFE REFUGES in SOUTHEASTERN UNITED STATES, 2005

Submitter:
Ken Dodd & Jamie Barichivich
USGS Florida Caribbean Science Center
7920 Northwest 71st Street
Gainesville FL 32653

Date of Report: 31 August 2006

Case No.: 4912 (Animal No. 012-187)

Capture Dates: May & June 2005

Specimens:

State	Refuge or Hatchery	Capture Date	Species	n=	Life Stage	NWHC Acsn No.	Primary Diagnoses
FL	St Marks	8 June 05	*A. gryllus*	5	Larva	173-177	Nephrocalcinosis in 2 tadpoles
FL	St Marks	7 June 05	*B. quercicus*	1	Adult	163	Scarring of skin
FL	St Marks	8 June 05	*H. femoralis*	5	Larva	164-168	Nephrocalcinosis in 3 tadpoles
FL	St Marks	8 June 05	*H gratiosa*	4	Larva	169-172	Nephrocalcinosis in 2 tadpoles
FL	Welaka	26 May 05	*H. cinerea*	2	Larva	096-097	*Myxidium* sp. in 2 gall bladders
FL	Welaka	26 May 05	*H. gratiosa*	5	Larva	035-039	**Microsporidia** in 3 spinal cords *Myxidium* sp. in 3 gall bladders
FL	Welaka	26 May 05	*H. squirella*	30	Larva	040-049, 076-095	*Myxidium* sp. in 14 gall bladders
FL	Welaka	26 May 05	*R. sphenocephala*	48	Larva	012-034, 050-075	*Myxidium* sp. in 2 gall bladders **Microsporidia** in 1 brain *Myxidium* sp. in 18 gall bladders
GA	Harris Neck[1]	26 July 05	*R. catesbeiana*	5	Larva	178-182	**Metacercariae in thyroids**
GA	Harris Neck[2]	26 July 05	*R. catesbeiana*	5	Larva	183-187	Skin & muscle metacercariae *Myxidium* sp. in 4 gall bladders
GA	Warm Springs	13 June 05	*B. terrestris*	5	Larva	128-132	Gill protozoa in 5
GA	Warm Springs	13 June 05	*H. cinerea*	5	Larva	123-127	Adult trematodes in 1 intestine
GA	Warm Springs	13 June 05	*R. catesbeiana*	5	Larva	153-157	**Oral chytridiomycosis** in 4 *Myxidium* sp. in 3 gall bladders
GA	Warm Springs	13 June 05	*R. clamitans*	5	Larva	133-137	Skin metacercariae in 1
NC	Edenton	15 June 05	*B. terrestris*	5	Larva	118-122	Gill protozoa in 4 *Myxidium* sp. in 2 gall bladders
NC	Edenton	15 June 05	*H. cinerea*	5	Larva	143-147	Gill protozoa in 4
NC	Edenton	15 June 05	*R. catesbeiana*	10	Larva	138-142, 148-152	*Myxidium* sp. in 4 gall bladders
SC	Orangeburg	14 June 05	*B. terrestris*	5	Larva	113-117	Skin & muscle metacercariae in 2 *Myxidium* sp. in 1 gall bladder
SC	Orangeburg	14 June 05	*G. carolinensis*	5	Larva	103-107	Skin & muscle metacercariae in 2
SC	Orangeburg	14 June 05	*H. cinerea*	5	Larva	098-102	Gill protozoa in 3
SC	Orangeburg	14 June 05	*H. femoralis*	5	Larva	108-112	Skin & muscle metacercariae in 2
SC	Orangeburg	14 June 05	*R sphenocephala*	5	Larva	158-162	Gill protozoa in 4

Abbreviations. 1, Goose Pond at Harris Neck NWR; 2, Snipe Pond at Harris Neck NWR.

Final Histological Diagnoses:

1. Florida

a. **St Marks NWR** [5 larval *Acris gryllus*, 1 adult *Bufo quercus*, 5 larval *Hyla femoralis* and 4 larval *H. gratiosa*]

1) Colon: Verminous colopathy due to *Gyrinicola*-like pinworms in 1 *A. gryllus*, 1 *H. femoralis* and 1 *H. gratiosa*.

2) Mesonephroi: Tubular calcification (nephrocalcinosis) of unknown etiology in 2 *A.gryllus*, 3 *H. femoralis* and 2 *H. gratiosa*

b. **Welaka NFH** [2 larval *Hyla cinerea,* 5 larval *H. gratiosa*, 30 *H. squirella*, and 48 larval *R. sphenocephala*]

1) Brain: Mild microsporidial encephalitis suggestive of *Spraguea* nov. sp. in 1 *R. sphenocephala*.

2) Spinal cord: Mild or moderate microsporidial myelitis suggestive of *Spraguea* nov. sp. in 2 *H. gratiosa*.

3) Cranial & spinal ganglia: Mild or moderate microsporidial ganglioneuritis suggestive of *Spraguea* nov. sp. in 3 *H. gratiosa*.

4) Mesonephroi: Slight microsporidial glomerulitis in 2 *H. gratiosa*.

5) Gills: Ectoparasites suggestive of Epistylis or Trichodina in 2 *H. gratiosa,* 16 *H. squirella*, and 23 *R.. sphenocephala*.

6) Gall bladder: Myxozoan cholecystopathy typical of *Myxidium* sp. in 8 *H. squirella*, and 7 *R. sphenocephala*

7) Esophagus: Encysted unidentified metacercaria in 1 *H. squirella*

8) Wall of gill chamber: Encysted unidentified metacercaria (or granulomas) in 2 *H. squirella*

9) Dermis ("subcutis"): Encysted unidentified metacercaria in 3 *R. sphenocephala*

10) Dermis ("subcutis"): Encysted unidentified immature cestode (sparganum) in 1 *R. sphenocephala*

11) Body cavity: Non-encysted unidentified metacercaria (or mesocercaria) in 1 *H. squirella*

12) Colon: Verminous colopathy due to *Gyrinicola*-like pinworms in 2 *H. gratiosa*, 5 *H. squirella*.

13) Liver: Mild hepatocellular necrosis of undetermined etiology in 4 *R. sphenocephala*.

14) Liver: Hepatocellular calcification & degeneration of undetermined etiology in 1 *R. sphenocephala*

15) Lung: Suppurative pneumonia of undetermined etiology in 1 *R. sphenocephala*

2. Georgia, Harris Neck NWR & Warm Springs NFH

a. **Harris Neck NWR** (Goose Pond) [n= 5 larval *R. catesbeiana*]

1) Thyroids: Metacercarial granulomas (or cysts) in 2 of 2 tadpoles

2) Spleen: Non-specific mild focal necrosis of undetermined etiology in 1 of 5 tadpole

3) Stomach: Focal minimal verminous submucosal gastritis due to unidentified larval nematode in 1 tadpole

4) Colon: Verminous colopathy due to *Gyrinicola*-like pinworms in 2 tadpoles

b. **Harris Neck NWR** (Snipe Pond) [n= 5 larval *R. catesbeiana*]

1) Wall of gill chamber: Metacercarial granulomas in 2 of 5 tadpoles

2) Gills: Ectoparasites suggestive of Epistylis or Trichodina in 5 of 5 tadpoles

3) Colon: Verminous colopathy due to *Gyrinicola*-like pinworms in 1 tadpole

4) Mesonephroi: Mild metacercarial granulomas in 3 of 5 tadpoles

5) Eye: Retrobulbar metacercarial granuloma in 1 of 5 tadpoles

c. **Warm Springs NFH** [5 *B. terrestris,* 5 *H. cinerea*, 5 *R. catesbeiana* and 5 *R. clamitans*]

1) Oral disc (jaw sheaths or toothrows): Mycotic rhamphothecitis or stomatitis due to ***Batrachochytrium dendrobatidis*** in 4 *R. catesbeiana*.

2) Intestine: Unidentified adult trematodes in 1 *H. cinerea*.

3) Gills: Ectoparasites suggestive of Epistylis or Trichodina in 5 *B. terrestris,*1 *H. cinerea* and 4 *R. clamitans*

4) Liver: Caseous micro-granuloma of undetermined etiology in 1 *R. catesbeiana*.

5) Pancreas: Caseous micro-granuloma of undetermined etiology in 1 *R. clamitans*.

6) Dermis: Encysted unidentified metacercaria in 2 *R. clamitans*.

3. North Carolina, Edenton NFH

1) Gills: Ectoparasites suggestive of Epistylis or Trichodina in 4 *B. terrestris*, 4 *H. cinerea* and 2 *R. catesbeiana*.

4. South Carolina, Orangeburg NFH

1) Gills: Ectoparasites suggestive of Epistylis or Trichodina in 4 *B. terrestris*, 1 *G. carolinensis*, 3 *H. cinerea*, 5 *H. femoralis* and 4 *R. sphenocephala*.

2) Tail: Verminous granuloma due to unidentified encysted metacercariae in 2 *B. terrestris*, 2 *G. carolinensis* and 2 *H. femoralis*.

3) Pancreas: Verminous caseous granuloma due to unidentified metacercariae 1 *G. carolinensis*.

4) Dermis: Encysted unidentified metacercaria in 2 *G. carolinensis*, 1 *H. femoralis* and 1 *R. sphenocephala*.

5) Esophagus: Nodular serosal esophagitis of undetermined etiology in 1 *B. terrestris*.

Summary of Histological Findings:
The most important finding in histological examinations was oral chytridiomycosis (infection by *Batrachochytrium dendrobatidis*) in 4 of 5 larval bullfrogs from Warm Springs NFH, GA (Fig 3 – 6). This pathogenic fungus was **not** detected in histological examinations in any other species of amphibians from Warm Springs NFH, nor any amphibians from the other 3 hatcheries and 2 refuges (Fig 1 & 2).

The second significant finding in these amphibians was a potentially new, previously unreported microsporidian infection of 4 tadpoles from Welaka NFH, FL. The infections occurred in the brain, spinal cords, spinal ganglia and renal glomeruli of 3 of 5 *Hyla gratiosa* (Fig 9 & 10) and 1 of 48 *Rana sphenocephala*. The other 2 species of amphibians from the same pond and hatchery had no histological evidence of microsporidia. The microsporidial organisms were present in neurons of the brain, spinal cord and ganglia. The size of the microsporidial cysts and their tropism for neurons suggests these microsporidia may belong to the genera, Glugea or Spraguea. Spraguea has a strong tropism for neurons of the brain, spinal cord and spinal ganglia but is reported only from marine fish (*Lophius* spp.). It is possible the microsporidia in these tadpoles are a new species or were transmitted from fish in the pond. Since no fish were submitted or examined from the pond, it is unknown whether this is an endemic or epizootic disease of fish and amphibians, or the infection is limited to certain species of amphibians.

A variety of internal helminthic parasites and external ectoparasites were found in the tadpoles from all 4 fish hatcheries and one of 2 national wildlife refuges. However, no encysted metacercariae were found in the 20 tadpoles (of 4 species) from Edenton NFH, NC. The internal parasites consisted mostly of the common innocuous tadpole pinworm, *Gyrinicola batrachiensis*, and multiple species of encysted immature trematodes (metacercariae); the significance of metacercariae in amphibians is usually negligible but indicates that aquatic snails (first intermediate hosts) and fish/amphibian predators (final hosts) were present in the ponds. The protozoan ectoparasites were found in the chambers of the mouth, pharynx and gills (Fig 7) and on the ventral skin; these probably were the innocuous genera, Epistylis and Trichodina. About 10% of larval *R. sphenocephala* from Welaka NFH had *Gyrodactylus* sp. (monogean trematode) on the skin of their bodies; these parasites were not detected histologically but were observed during dissections. Myxozoan infections of the gall bladders were observed histologically in several more tadpoles; this infection was reported previously in the preliminary pathology report (14 Oct 2005).

Background:
A total of 177 larval anuran amphibians and one adult toad from 4 national fish hatcheries and 2 national wildlife refuges were submitted in 2005 for diagnostic examinations. Histological examinations were attempted on 169 of these frogs and toads. Nine tadpoles were not submitted for histology because they were either saved whole as frozen vouchers or they were too decomposed. Ten larval bullfrogs from Harris Neck NWR are included in this report because it is likely the amphibians were transported to the refuge from Orangeburg NFH with the stocking of bluegill fish.
The main purposes of this study were to determine whether any diseases of fish could be detected in amphibians from fish hatcheries and whether hatchery-associated amphibians had any important amphibian diseases that might be transported to new sites when hatchery-raised fish were shipped. The 4 national fish hatcheries, amphibian species, capture dates and numbers of amphibians are shown above.

Histological Findings (by site):

I. Florida

St Marks NWR. A total of 20 amphibians were collected on 26 May 2005 from this refuge: 5 larval *Acris gryllus*, 1 adult *Bufo quercicus*, 5 larval *Hyla femoralis* and 4 larval *H. gratiosa*. Histological examinations were performed on 19 animals.

No serious infectious diseases were detected histologically in these 19 amphibians. Virus cultures on all 20 amphibians were negative and histological abnormalities typical of virus infection were not found. Bacterial cultures on 4 amphibians failed to isolate any bacteria considered to be pathogenic in fish or amphibians.

Parasites were detected histologically in intestines of 3 tadpoles; the parasites were typical of the common, innocuous pinworm of tadpoles, *Gyrinicola batrachiensis*. Interestingly, no ectoparasitic protozoa were found on the skin or gill chambers of the 19 tadpoles from this refuge.

An unusual abnormality of the mesonephroi ("kidneys") was detected in 2 of 5 *A. gryllus*, 3 of 5 *H. femoralis* and 2 of 3 *H. gratiosa*. It is possible many more tadpoles actually were affected, but tissue sections of the mesonephroi were not present for all tadpoles. The abnormality was diagnosed as nephrocalcinosis and was characterized by death of cells in the renal tubules with accumulation of calcium-like material in the dead cells; no infectious diseases or agents were seen in the numerous minute foci of necrosis. The cause of this abnormality remains unknown, but it suggests the tadpoles were stressed by the presence of high levels of cations in the surface water, such as salt water, calcium or other ions. The abnormality could be due to other chemicals in the surface water, such as oxalates and ethylene glycol, and it also is possible the abnormality was due to an undetected infectious agent such as a new virus; however, virus cultures on the mesonephroi of all 20 amphibians from this site were consistently negative. In summary, the precise cause of the suspected dystrophic nephrocalcinosis in this group of tadpoles was not determined.

Welaka NFH. A total of 85 larvae were collected from one pond (Beecher Unit) on 26 May 2005: 2 *H. cinerea*, 5 *H. gratiosa*, 30 *H. squirella* and 48 *Rana sphenocephala*. Histological examinations were performed on 82 larvae and all histological findings are listed on page 2.

No serious infectious diseases were detected histologically in these 82 amphibians. Virus cultures were attempted on 65 larvae; no viruses were isolated and histological abnormalities typical of virus infection were not found in the 82 larvae. Bacterial cultures on internal organs of 10 amphibians failed to isolate any bacteria considered to be pathogenic in fish or amphibians. Special bacterial cultures for *Salmonella* spp. were attempted on the cloacas of 21 tadpoles; no salmonellae were isolated. Oral chytridiomycosis was not detected histologically in any tadpoles from this one pond at this hatchery.

A new, unreported infectious disease of the brains, spinal cords and spinal ganglia was found in 4 tadpoles from this hatchery. The infectious disease was diagnosed as neuronal microsporidiosis. The infection rates in the 4 amphibian species were 60% (3 of 5) in *H. gratiosa*, 2.2% (1 of 46) in *R. sphenocephala*, and 0% each in 2 *H. cinerea* and 20 *H. squirella*. It is probable that if additional histological slides ("recuts" or "step sections") were made of each amphibian from this hatchery then additional infected tadpoles would be found; hence, these infection rates are considered estimates and probably minimal infection rates in the tadpoles. Because no fish were examined from this pond or any of the other 3 hatcheries, it is unknown whether this new disease is endemic in amphibians or epizootic in fish and amphibians. The marked variation in infection rates in tadpoles from the same pond suggests this microsporidian parasite has a strong predilection for barking treefrogs with only rare infections in other anurans. But because 2 genera of tadpoles were found with the infection, it is possible many other amphibian species also are susceptible.

The microsporidial infections in 3 of 5 *H. gratiosa* were not slight or mild infections. Numerous ganglia and neurons were affected in each tadpole. Such infections could impair alertness and locomotion of the tadpoles, making them more susceptible to predation. Because no metamorphs or post-metamorphic amphibians were examined from the hatcheries, it is not possible to predict the impact of this disease on tadpole longevity, metamorphosis, survival and recruitment.

The tissue tropism (neurons of the ganglia, spinal cord and brain) of this parasite is similar to the microsporidian, *Spraguea lophii*, of angler fish (*Lophius* spp.). However, angler fish are marine, hence, it is unlikely this microsporidian parasite was transmitted directly from this species of fish, unless tissues of angler fish were incorporated into the feed given to the fish in this freshwater pond. Other genera of microsporidia, such as Glugea, also may infect the neurons of fish, so the precise identity of the parasite in these tadpoles is uncertain. Transmission electron microscopy will be necessary on the tiny spores to determine precise taxonomy.

Internal parasites and ectoparasitic protozoa were detected histologically in each of the species of tadpoles except the 2 *H. cinerea*; it is likely that if more *H. cinerea* had been submitted and examined, similar internal and external parasites would have been found. External parasites consisted of protozoa on the ventral skin and within the mouth and gill chamber; most of these protozoa were tentatively identified as the genera, Epistylis and Trichodina. No disease has been associated with infestations of tadpoles by these protozoa; they are superficial ectoparasites and do not invade cells or tissues. The occurrence of the myxozoan parasite, *Myxidium* sp., in the gall bladders of these tadpoles was reported in the previous Preliminary Pathology Report (14 Oct 2005). This parasite was detected histologically in the gall bladders of an additional 8 *H. squirella* and 7 *R. sphenocephala*. Because the lifecycles of all myxozoan parasites of amphibians remain unknown, especially what life-stage infects the tadpole and how the parasite enters the gall bladder, the full impact of this parasite on tadpoles is difficult to estimate; however, the presence of spore-producing stages in the lumen of the gall bladder with essentially no inflammatory cell reaction by the hosts suggests the parasite is mostly innocuous in these amphibian hosts. It is curious that a second common and nationally distributed myxozoan parasite of the mesonephroi ("kidneys") of tadpoles, *Sphaerospora* (*Leptotheca*) *ohlmacheri*, was not detected in any tadpoles from this hatchery or the other 3 hatcheries. This suggests that Myxidium and Sphaerospora may utilize different final (invertebrate) hosts and that suitable invertebrate hosts for only Myxidium are present in the hatcheries.

A variety of helminthic parasites were detected in tadpoles from Welaka NFH. These included multiple genera and species of encysted metacercariae, an encysted immature cestode (sparganum) and a few pinworms (*Gyrinicola batrachiensis*). The immature trematodes (metacercariae) and cestode were not further identified. The pinworms were detected histologically in 2 *H. gratiosa* and 5 *H. squirella*; the same parasites were detected during dissections in 1 *H. squirella* and 6 *R. sphenocephala*. Because the intestines were not opened or flushed in every tadpole nor was the entire intestinal tract submitted for histology, the prevalence of pinworms in each species of tadpole cannot be determined. The significance of the encysted metacercariae in these tadpoles is that it indicates the presence of first intermediate hosts (aquatic snails) in the pond, and predatory final hosts had defecated in the pond. Final hosts for these trematodes parasites are a variety of birds (eg, herons, bitterns, cormorants, gulls, etc) and some mammals (eg, raccoons).

Miscellaneous abnormalities of the livers and lung were found in 6 larval *R. sphenocephala*. Four tadpoles had very mild necrosis of liver cells of undetermined cause. While liver necrosis is commonly associated with ranaviral infections in tadpoles, no ranaviruses were isolated in cultures from tadpoles at this site. The most likely cause of the liver necrosis was migration tracts of metacercariae or other immature helminthic parasites. The lungs of less than 50% of submitted tadpoles were examined histologically and only one larval *R. sphenocephala* had mild pneumonia of undetermined cause.

II. Georgia

Harris Neck NWR (Goose Pond). A total of 5 larval *R. catesbeiana* were collected on 26 May 2005 from this pond. Histological examinations were performed on all 5 animals.

No lethal amphibian diseases were detected histologically in these 5 larval bullfrogs. Virus cultures on the 5 tadpoles were negative and histological abnormalities typical of virus infection were not found. Bacterial cultures on 4 amphibians failed to isolate any bacteria considered to be zoonotic or pathogenic in fish or amphibians.

Parasites were detected histologically in thyroids, stomach and intestines of all 5 tadpoles. The intestinal parasites in all 5 tadpoles were typical of the common, innocuous pinworm of tadpoles, *Gyrinicola batrachiensis*. One tadpole also had an unidentified larval nematode in its stomach. The most interesting parasites in tadpoles from this pond were unidentified encysted metacercariae in the thyroids of 2 larvae. These encysted metacercariae were not observed at dissections but were incidental histological findings (Fig 8). Because no other encysted metacercariae were found in other organs of these 5 tadpoles, this suggests the unidentified trematode has a strong tropism for the thyroids. While normal-appearing thyroid follicles (containing normal-appearing colloid) were still present in both tadpoles, suggesting these specific animals had no impairment of thyroid function, much heavier parasitic infections could result in marked impairment of thyroid function. Inasmuch as thyroid hormones are essential for metamorphosis, this raises the interesting possibility that this unidentified parasite could prevent or seriously delay metamorphosis in some infected tadpoles. Whether this possible delayed metamorphosis makes the tadpole more susceptible to predation by the final host(s) of this parasite (for example, tadpoles become more visible to predators as the pond dries) is purely speculative, but certainly is worthy of further investigations. Interestingly, no ectoparasitic protozoa were found on the skin or gill chambers of the tadpoles from this pond.

Harris Neck NWR (Snipe Pond). A total of 5 larval *R. catesbeiana* were collected on 26 May 2005 from this pond. Histological examinations were performed on all 5 animals.

No lethal amphibian diseases were detected histologically in these 5 larval bullfrogs. Virus cultures on all 5 tadpoles were negative and histological abnormalities typical of virus infection were not found. Bacterial cultures on each of the 5 amphibians failed to isolate any bacteria considered to be zoonotic or pathogenic in fish or amphibians.

Parasites were detected histologically in gills, skin, mesonephroi ("kidneys") and intestines of all 5 tadpoles. Intestinal parasites typical of the common, innocuous pinworm of tadpoles, *Gyrinicola batrachiensis*, were found histologically in 1 tadpole; these parasites were observed during dissection of this tadpole and 2 others from this site and were reported previously. All 5 tadpoles from this pond had protozoan ectoparasites on the skin and in their gill chambers; the protozoa were not identified by a protozoologist but were considered typical of the innocuous genera, Epistylis and Trichodina. Encysted metacercariae, probably of 2 or more genera, were found in tissue sections in the skin (dermis), adjacent to the eye, and in the mesonephroi ("kidneys"). The renal metacercariae lacked characteristic spines and were too large to be the common renal parasite, *Echinostoma* sp. The identity of the renal metacercariae was not determined. The metacercariae adjacent to the eye of one tadpole and in the dermis of 2 others also were not identified. All trematode and pinworm infections at this site were considered mild infections. Tissue sections of the thyroids of these 5 tadpoles were not present, so it remains unknown whether these larval bullfrogs had metacercarial infections of their thyroids, as were detected in the other larval bullfrogs from Goose Pond.

There was no evidence of oral chytridiomycosis in the 10 larval bullfrogs from Harris Neck NWR in gross and histological examinations.

Warm Springs NFH. A total of 20 amphibian larvae of 4 species (3 genera) were collected on 13 June 2005 from this hatchery. The amphibians were 5 *Bufo terrestris*, 5 *H. cinerea*, 5 *R. catesbeiana* and 5 *R. clamitans*. Histological examinations were performed on 18 of 20 tadpoles from this hatchery.

The important amphibian disease, chytridiomycosis (infection by ***Batrachochytrium dendrobatidis***), was detected histologically in 4 of 5 larval bullfrogs. Chytridiomycosis was **not** detected in histological examinations in the other 13 tadpoles of 3 other species. Virus cultures were attempted on all 5 larval bullfrogs and 3 each of the other 3 species of tadpoles from this hatchery; no viruses were isolated in cultures and abnormalities typical of viral infection were not detected histologically. No other fungal infections (eg, microsporidia, *Saprolegnia* spp.) were detected in these 20 tadpoles. Bacterial cultures on 3 tadpoles failed to isolate any bacteria considered to be zoonotic or pathogenic in fish or amphibians. All identified bacteria were isolated from the intestines of the tadpoles and all bacteria are common in the intestinal tracts of amphibians and ubiquitous in aquatic surface waters. No bacteria were isolated from the normally sterile livers of 2 tadpoles.

Infections by *Batrachochytrium dendrobatidis* (BD) in 4 of 5 larval bullfrogs were limited to the oral disc (jaw sheaths and toothrows) as is typical in tadpoles (Fig 3 – 6). Because the oral disc of tadpoles is the only body region to produce keratin (the body skin produces keratin only after metamorphosis is complete), this chytrid infection is not considered life-threatening or lethal in tadpoles. The portions of the oral disc that contain keratinized cells also contain marked amounts of melanin pigment; hence, the loss of black pigmentation on the oral discs (Fig 3) closely mirrors the loss of keratinized cells due to BD infection. This loss of black pigmentation (depigmentation) of the oral discs was evident in all 5 live larval bullfrogs at dissection. All 5 larval *B. terrestris* also had partial depigmentation of their toothrows, but no chytrid organisms were detected histologically. It is not clear whether the abnormal toothrows in the larval toads were due to undetected infections by BD or some other factor (eg, mechanical injury). In 4 larval bullfrogs, the diagnosis of oral chytridiomycosis was based on 1 or 2 histological sections through the mouth of each animal; it is likely additional chytrid infections would have been found if multiple sections (ie, multiple histology slides) of the oral disc of each tadpole had been produced and examined. Hence, the BD infection rate of 22.2% (4 of 18 tadpoles) at this site is considered a minimal detection rate rather than a true infection rate; additional histology slides of each tadpole probably would have resulted in additional BD-positive diagnoses.

Protozoan and helminthic parasites were detected histologically in gills, skin and intestines of at least one individual of each species of tadpole. Protozoan ectoparasites on the skin and in the gill chamber were detected in 13 of 18 (72%) tadpoles (1 to 5 individuals of each species). The protozoa were not identified by a protozoologist but were considered typical of the innocuous genera, Epistylis and Trichodina. Encysted metacercariae were found in tissue sections in the skin (dermis) of only 2 larval *R. clamitans*; the metacercariae were not identified. The significance of these few metacercariae is that suitable intermediate hosts (aquatic snails) and final hosts (fish-eating and amphibian-eating birds or mammals) were present in the pond. These first intermediate and final hosts generally are suitable hosts for numerous genera of trematodes. However, no metacercariae were detected in the thyroids and mesonephroi ("kidneys") of the amphibian larvae from this hatchery pond. One larval *H. cinerea* had unidentified minute adult trematodes in its intestine and the common, innocuous pinworm of tadpoles, *Gyrinicola batrachiensis*, was found in larval *R clamitans*. In general however, the tadpoles from this site had few internal helminthic parasites.

Minute abscesses or micro-granulomas were found histologically in the liver and pancreas of one larval *R. catesbeiana* and 1 larval *R. clamitans*, respectively. The cause of these minute foci of inflammation was not detected. Similar micro-granulomas are fairly common in larval and post-metamorphic amphibians nationwide; it is assumed that most such nodules are dead metacercariae or the migration tracts of larval helminthic parasites.

III. North Carolina

Edenton NFH. A total of 20 amphibian larvae of 3 species (3 genera) were collected on 15 June 2005 from this hatchery. The amphibians were 5 *Bufo terrestris*, 5 *H. cinerea* and 10 *Rana catesbeiana*. Histological examinations were performed on 17 of 20 tadpoles from this hatchery, including 9 of 10 larval bullfrogs.

Tadpoles from this hatchery showed no evidence of viral infection, pathogenic bacteria, fungal infections, encysted metacercaria or other helminthic parasites. However, 2 larval *H. cinerea* and 4 larval *R. catesbeiana* had myxozoan parasites within their gall bladders that were identified by a parasitologist as *Myxidium* sp. (these myxozoan infections were previously reported on 10 Oct 2005). All 3 species of tadpoles had protozoan ectoparasites in their gill chambers and on their skin. The protozoa were not identified by a protozoologist but were considered typical of the innocuous genera, Epistylis and Trichodina (Fig 7). These ectoparasites were detected in 10 of 17 (59%) tadpoles; 2 to 4 individuals of each amphibian species was infested.

Larval bullfrogs of 2 age groups were submitted from this hatchery. One group had body weights of 0.5 to 1.2 grams, and the other group weighed 2.5 to 3.3 grams. The smaller tadpoles had no evidence of protozoan ectoparasites and lacked myxozoa in their gall bladders. This suggests the smaller larval bullfrogs were recently hatched individuals and may have been too young to develop parasitic infections.

There was no evidence of infectious disease or inflammation in the internal organs of tadpoles from this hatchery.

IV. South Carolina

Orangeburg NFH. A total of 25 amphibian larvae of 5 species (4 genera) were collected on 14 June 2005 from this hatchery. The amphibians were 5 *Bufo terrestris*, 5 *Gastrophryne carolinensis*, 5 *Hyla cinerea*, 5 *H. femoralis* and 5 *Rana sphenocephala*. Two larval *H. cinerea* were dead on arrival with ruptured body cavities; these 2 tadpoles were discarded and no cultures or tests were done. Histological examinations were performed on 21 of the remaining 23 tadpoles from this hatchery.

No lethal amphibian diseases were detected histologically in these 21 larvae. Virus cultures were negative on 14 tadpoles (2 or 3 larvae of each species), and histological abnormalities typical of viral infections were not found. Bacterial cultures on 6 amphibians failed to isolate any bacteria considered to be zoonotic or pathogenic in fish or amphibians. All bacteria were isolated from the intestines of the 6 tadpoles; no bacteria were isolated from the normally sterile livers. No fungal infections (eg, BD) were detected histologically in these 21 larvae.

A variety of protozoan, myxozoan and trematodal parasites were detected in the skin, gill chambers, muscles, gall bladder and pancreas of these tadpoles. All protozoan parasites were ectoparasites on the skin and in the gill chambers; the parasites were not identified by a protozoologist but were considered typical of the common and innocuous genera, Epistylis and Trichodina. There was no histological evidence of tissue invasion or inflammation associated with these ectoparasites. Myxozoa typical of *Myxidium* sp. were detected in the gall bladder of one larval *R. sphenocephala* at dissection by an experienced parasitologist (previously reported on 10 Oct 2005) and no additional infections were detected in histological examinations.

Encysted metacercariae with varying amounts of host inflammatory cell reaction (ie, granulomas) were detected histologically in the skin (and dermis), muscles (and tail) and pancreas in 10 tadpoles of 4 species (no metacercaria were detected grossly and histologically in the larval *H. cinerea*). The number of tadpoles of each species with histologically detected encysted metacercariae is listed above (page 3). Three tadpoles (one *R. sphenocephala*, one *G. carolinensis* and one *H. femoralis*) had encysted metacercariae in their skin and dermis; in two of these tadpoles, the size, shape and tissue location of the metacercariae were suggestive of the malformation-inducing trematode, *Ribeiroia ondatrae*; however, the identity of the encysted metacercariae was not confirmed by a parasitologist. Seven tadpoles also had small encysted metacercariae within muscles of the head, body and tail. Tadpoles with muscle metacercariae included 2 *B. terrestris*, 2 *G. carolinensis*, and 3 *H. femoralis*; the identity of the metacercariae within muscles was not determined. Encysted metacercariae of a third genus or species were found at dissection in the pancreas of one *G. carolinensis*, but the parasites were not detected histologically. A fourth genus of metacercariae were seen at dissection in the mesonephroi ("kidneys") of 2 *B. terrestris*, but the parasites were not seen histologically; the size, shape, color and location of these renal metacercariae are consistent with the genus, *Echinostoma*, which is widespread and fairly common in tadpoles nationwide. Hence, encysted metacercariae of at least 4 different genera or species were observed at dissection in 9 tadpoles of 4 species; metacercariae were detected histologically in one additional tadpole. The significance of these metacercariae is that suitable intermediate hosts (aquatic snails) and final hosts (fish-eating and amphibian-eating birds or mammals) were present in the pond. These first intermediate and final hosts generally are suitable hosts for numerous genera of trematodes capable of infecting fish and amphibians.

No adult nematodes, trematodes, cestodes and acanthocephalans were detected at dissection of the tadpoles from this hatchery. The intestines of 16 tadpoles were examined histologically, and no adult helminths and acanthocephalans were found. This lack of adult helminths is considered unusual, because even the common tadpole pinworm, *Gyrinicola batrachiensis*, was not found in larvae at this hatchery.

One larval **B. terrestris** had an unusual minute cyst adjacent to its esophagus; the cyst contained unidentified amorphous organisms suggestive of protozoa or a cluster of bacteria.

Summary of Diseases.

Viruses. No viruses were isolated in cultures and there was no histological evidence of viral infection in these tadpoles and one adult toad.

Bacteria. No pathogenic bacteria were isolated in cultures of the internal organs.

a. *Salmonella* spp. Cultures for *Salmonella* spp. were negative in all 23 tadpoles in which special (selective media) cultures were attempted. Routine aerobic cultures of the cloacas of an additional 29 tadpoles also were negative for *Salmonella* spp.

b. Other pathogenic bacteria. No pathogenic bacteria were isolated in routine aerobic cultures of the livers, bile, spleens, mesonephroi and body cavity of tadpoles from these 4 hatcheries and 2 refuges. Recognized bacterial fish pathogens, such as *Yersinia* spp, *Edwardsiella* sp., *Flavobacterium* spp, *Vibrio* spp. and *Aeromonas salmonicida* were not isolated from these tadpoles. Coliform (sewage) bacteria such as *Escherichia coli* were not isolated from these tadpoles. The bacterium, *Aeromonas hydrophila*, which may cause secondary or opportunistic infections in fish and amphibians, was isolated from the intestines only (not from livers) of 10 of 21 tadpoles; this bacterium was isolated from the intestines of at least one tadpole from each of the 4 fish hatcheries. Because *A. hydrophila* was not isolated from the livers of any tadpoles, it is likely the organism was a normal, non-pathogenic inhabitant of the amphibian gut. *Aeromonas hydrophila* is ubiquitous in wetlands and has been isolated frequently from the intestines of normal-appearing amphibians nationwide.

Fungi. The important, pathogenic chytrid fungus, *Batrachochytrium dendrobatidis* (BD), was detected in histological examinations in 4 larval *R. catesbeiana* from Warm Springs NFH (Fig 3 – 6). Although several tadpoles of multiple species from all 4 hatcheries had macroscopic (gross) changes in their jaw sheaths and toothrows (ie, loss of black pigment or depigmentation) suggestive of chytridiomycosis, the pathogenic chytrid fungus was not detected histologically in any tadpoles from the other 3 hatcheries and 2 refuges (Fig 1 & 2). Also, oral BD infections were not detected in 15 other tadpoles of 3 different species from the same pond at Warm Springs NFH. The size of the BD-infected larval bullfrogs (6.0 to 7.7 g body weight) suggests they had over-wintered in the pond at least once (winter of 2004-2005), while the other 3 species of tadpoles probably had hatched from eggs that were laid in the Spring of 2005. (Their body weights were 0.1 to 2.0 g.) Hence, results of this study suggest that >1 year-old larval bullfrogs (*R. catesbeiana*) may be the best amphibian species for screening tests for this disease. Newer diagnostic tests, such as polymerase chain reaction (PCR) for *Batrachochytrium dendrobatidis*, may have detected more BD-infected tadpoles.

Microsporidia. A new, previously unreported microsporidian infection of the brain, spinal cord, cranial & spinal ganglia and glomeruli was detected histologically in 3 of 5 larval *H. gratiosa* and 1 of 48 larval *R. sphenocephala* from one pond (Beecher Unit) at Welaka NFH. The infection was not detected in 2 larval *H. cinerea* and 30 larval *H. squirella* from the same pond. All infections were considered mild, but some spinal ganglia had extensive numbers of organisms within neurons (Fig 9 & 10). It is not clear whether this new infectious disease causes morbidity or mortality in amphibians, and it is unknown whether this organism is endemic to amphibians or was acquired by the amphibians from infected fish in the pond. No fish from the pond were submitted or examined. Based only on tissue and cellular tropism of this new microsporidian parasite, it is suspected the organism is the genus, Spraguea. However, this genus is described from marine fish only. It is improbable that the microsporidia in these 4 tadpoles was transmitted directly from a marine fish, unless the fish food used at this pond contained marine fish. It is more likely this microsporidian is endemic in amphibians and simply has not been seen previously. Some, but not all, microsporidian parasites are rather host specific, so there is a small possibility that this microsporidian also infects freshwater fish. Diagnostic examinations of fish from this pond for this parasite are warranted. Precise identification of this new microsporidian will require transmission electron microscopy, and these studies will be conducted at a later date. Because this parasite is considered new, the range of susceptible amphibian hosts is unknown and its potential impact on amphibian populations cannot be estimated.

Protozoa. No significant protozoan infections were detected in amphibians from the 4 hatcheries and 2 refuges. Ectoparasitic protozoa were present on the majority of amphibians from all 4 hatcheries. These protozoa were not identified by a protozoologist but were considered typical of the common and widespread genera, Epistylis and Trichodina (Fig 7).

Disease and mortality has not been associated with these ectoparasites in amphibians. One larval *B. terrestris* from Orangeburg NFH had a minute cyst near its esophagus which contained an unidentified protozoan-like organism; only the one cyst was found in the tadpole and no other amphibians from the hatchery were affected.

Myxozoa. Two common and geographically widespread myxozoan parasites are found in larval (and post-metamorphic) amphibians. These are *Myxidium* spp. in the gall bladder and *Sphaerospora* (formerly *Leptotheca*) *ohlmacheri* in the mesonephroi ("kidneys"). Illness and death have not been associated with renal and gall bladder infections by these myxozoa, however, 1) the final hosts of these parasites are unknown (amphibians are considered an intermediate host), 2) the life-stage of the organism that invades the amphibian is unknown, and 3) the routes the infective organisms take to enter the gall bladder and mesonephroi are unknown, and therefore, the impact on amphibians of the initial unknown infective stage of these organisms is unknown.

The best-known and best studied myxozoan parasite is *Myxobolus cerebralis*, which causes the important disease in trout called whirling disease. The final host for *Myxobolus* spp. are various mudworms (fish and amphibians are actually the intermediate hosts for myxozoa, because myxozoan sexual reproduction occurs only in the invertebrate host). *M. cerebralis* is not known to infect amphibians. There was no histological evidence of any myxozoan parasites in the brains and skulls of amphibians from the 4 hatcheries and 2 refuges. The final hosts and infective stages for all parasitic myxozoans of amphibians are unknown and unstudied. Although *Sphaerospora* (formerly *Leptotheca*) *ohlmacheri* was not found in any amphibians from the hatcheries and refuges in this report, *Myxidium* sp. was found in at least one amphibian from all 4 hatcheries and both refuges. The widespread presence of one myxozoan parasite and absence of the other suggests these parasites may utilize different final invertebrate hosts, and that only the final host for *Myxidium* spp. was present at the hatcheries and refuges. Again, the identities of the final (invertebrate) hosts for all myxozoa that infect amphibians are unknown and these parasites are not known to cause morbidity or mortality.

Gall bladders were examined for myxozoa by one of 2 methods: submission of the whole bile-filled gall bladder to a parasitologist for direct microscopic examinations, or histology. Mesonephroi ("kidneys") were examined for

Table 1. Gall bladder infection rates by *Myxidium* sp.

State	NFH or NWR	Species	Number Examined	Infection Rate
FL	St. Marks	*A. gryllus*	3	67%
FL	St. Marks	*B. quercicus*	1	100%
FL	St. Marks	*H. femoralis*	2	100%
FL	St. Marks	*H. gratiosa*	2	100%
FL	Welaka	*H. cinerea*	2	100%
FL	Welaka	*H. gratiosa*	3	100%
FL	Welaka	*H. squirella*	9	100%
FL	Welaka	*R. sphenocephala*	13	77%
GA	Harris Neck[1]	*R. catesbeiana*	4	0
GA	Harris Neck[2]	*R. catesbeiana*	4	50%
GA	Warm Springs	*B. terrestris*	2	50%
GA	Warm Springs	*R. catesbeiana*	3	100%
GA	Warm Springs	*R. clamitans*	2	50%
NC	Edenton	*B. terrestris*	2	0
NC	Edenton	*R. catesbeiana*	6	67%
SC	Orangeburg	*B. terrestris*	2	0
SC	Orangeburg	*R. sphenocephala*	1	100%

(1, Goose Pond; 2, Snipe Pond)

Sphaerospora sp. by histology only. Not all gall bladders from all amphibians were submitted for histology or direct examinations by a parasitologist. The gall bladders of many tadpoles were too small to be removed at dissection. Infection rates by *Myxidium* sp. at the hatcheries and refuges are arranged by location and host species in Table 1, below. In addition, infections by *Myxidium* sp. were detected by histological examinations of the gall bladders in 13 more tadpoles of 3 species from Welaka NFH (data **not** shown in table). Additional gall bladder infections were not detected histologically in amphibians from the other 3 hatcheries and 2 refuges.

Many of the myxozoa shown in Table 1 were preserved in ethanol and submitted to a myxozoan expert (Dr. Spall, Idaho State Univ.) for molecular analyses in order to confirm they are the genus, *Myxidium*, and to potentially identify the parasites to species. It is possible that one or more suspected *Myxidium* spp. from these amphibians will be new, undescribed species. Results of the molecular analyses are not expected until late in 2006 or 2007.

Helminths. A variety of immature and adult cestodes, trematodes and nematodes were detected in amphibians from the hatcheries and refuges during dissections and by histology. Representative live adult helminths were submitted to a parasitologist for identification, but most immature, encysted metacercariae were not identified. No helminths were detected in any of the 20 tadpoles from Edenton NFH. This suggests the sampled pond may have been newly constructed or recently drained and dried to eliminate aquatic snails. It also is possible that an anthelminthic drug was added to the pond water or fish food. Another possibility is that the pond was enclosed and successfully had excluded predators (small mammalian carnivores and fish-eating birds) that are final hosts for many helminths of fish and amphibians and whose egg-laden feces are necessary to begin the lifecycle in aquatic snails.

Cestodes. Immature cestodes (tapeworms) were detected histologically in only one larval *R. sphenocephala* from Welaka NFH. The identity of the parasite was not determined. No other immature or adult cestodes were detected in amphibians from the other 3 hatcheries and 2 refuges.

Metacercariae. Encysted immature trematodes (metacercariae) were detected in at least one tadpole from 3 hatcheries and both refuges. The metacercariae cannot be identified to species in histology and by parasitologists because of their immaturity. However, their size, features of some organs and their location in the amphibian tissues often offers a clue to their identity. Many genera of metacercariae have a tropism for specific organs or body locations. Hence, small encysted metacercariae in the mesonephroi ("kidneys") of amphibians usually are the genus, Echinostoma. Small encysted metacercariae in the skin (dermis) around the vent often are the genus, Ribeiroia. The identity of other encysted metacercariae in the body cavities, thyroids, esophagi, pancreases and skeletal muscles is largely unknown. Encysted renal metacercariae, probably *Echinostoma* spp., were observed at dissection and histologically in only 7 tadpoles (4 *R. catesbeiana* from Harris Neck NWR, 1 *H. squirella* from Welaka NFH and 2 *B. terrestris* from Orangeburg NFH). Encysted Ribeiroia-like metacercariae were observed in the ventral skin of 7 tadpoles (1 *R. sphenocephala* and 1 *H. squirella* from Welaka NFH, 1 *R. clamitans* from Warm Springs NFH, 1 *H. femoralis* and 1 *R. sphenocephala* from Orangeburg NFH, and 2 *R. catesbeiana* from Harris Neck NWR). Unidentified encysted metacercariae were found in the skeletal muscles of 14 tadpoles (2 *R. clamitans* from Warm Springs NFH, 1 *H. gratiosa* and 2 *R. sphenocephala* from Welaka NFH; 2 *B. terrestris*, 3 *G. carolinensis*, and 4 *H. femoralis* from Orangeburg NFH). Unidentified encysted metacercariae in the body cavities of tadpoles were found in 7 hylid larvae from Welaka NFH only. Two larval *R. catesbeiana* from Harris Neck NWR had noteworthy, often large, encysted metacercariae in their thyroids (Fig 8); the thyroids of the other 8 larval bullfrogs from this refuge were not present in histological sections, so the precise incidence of this parasite is uncertain. It is suspected that the metacercariae in the thyroids of these bullfrogs is a distinct species; the parasites were not suspected or observed at dissection, and were considered an incidental histological finding. However, given the importance of thyroid hormones in amphibian development and metamorphosis, this unusual parasite could have clinical significance to infected individuals by delaying or impeding metamorphosis. This thyroidal parasite has been detected once previously in larval *R. catesbeiana* from Fort Stewart, GA, hence, at present, this novel parasite appears to have limited geographic distribution. Because the identity of the trematode is unknown, the intermediate and final hosts also are unknown. Because at present, this thyroidal parasite appears to have a limited geographic distribution in the Coastal Plain of eastern Georgia, the impact of its spread to other amphibians in other states cannot be predicted.

Ribeiroia-like metacercariae were detected in 7 tadpoles from 3 hatcheries and one refuge. This parasite already has nationwide distribution. Its significance is that it induces striking limb malformations in recently metamorphosed frogs and toads if, and only if, the tadpole is infected by the parasite at very specific stages of development. No limb malformations were detected in the infected tadpoles, but all tadpoles were too immature to express the possible range of malformations that occur once metamorphosis is complete. It is possible that high rates of limb malformations will occur at these sites in future years when release of cercariae from aquatic snails coincides with appropriately susceptible age-classes (ie, Gosner stages) of tadpoles. This parasite has a second significant potential impact on tadpoles; in experimental conditions, as few as 3 metacercariae killed very young larval *R. pipiens*. Hence, die-offs of very young tadpoles could occur at these sites in future years due to infection by this parasite. Because numerous wide-ranging amphibian-eating birds are the final hosts for *Ribeiroia* spp., and because the parasite already is geographically wide-spread in the USA, it is unlikely this parasite poses a significant risk to amphibian populations if it were to be transported from hatcheries to other sites.

Trematodes (adult flukes). The monogean ectoparasite, *Gyrodactylus* sp., was detected on the skin of 9 larval *R. sphenocephala* from Welaka NFH and 2 *H. cinerea* and 1 *R. clamitans* from Warm Springs NFH. Not all tadpoles from these 2 hatcheries were subjected to thorough body skin examinations under a dissecting microscope while submerged in water, hence, many more tadpoles from these 2 hatcheries probably were infested. Rates of infection in each species of amphibian and each hatchery were not determined, because examinations were halted after 1-9 tadpoles in each group were found to be infested. *Gyrodactylus* spp. are principally ectoparasites of fishes, but have been reported repeatedly on the skin of tadpoles. Hence, it remains uncertain whether this parasite was transmitted to the tadpoles from infested fish in the ponds, or whether this non-host-specific parasite would have been present on the tadpoles even if fish were absent from the ponds. The significance of this ectoparasite to amphibian populations is unknown, but the apparent impact on the affected tadpoles in this study was negligible.

Other Adult Trematodes. Endo-parasitic adult trematodes were rare in amphibians from the 4 hatcheries and 2 refuges. In general, larval amphibians have many more immature trematodes (metacercariae) than adult trematodes. One *H. cinerea* from Warm Springs NFH had two tiny adult trematodes in its intestine; these small parasites were not observed at dissection but were found histologically. The identity of the flukes was not determined. One tiny adult trematode was flushed from the colon of one larval *R. catesbeiana* from Harris Neck NWR; the parasite was submitted for identification by a parasitologist; results are still pending. Adult trematodes of similar size, shape, and organ location in larval bullfrogs have been seen nationwide; most have been identified as *Megalodiscus* sp.

Helminths. Oxyurids ("pinworms") were the only nematode found in the tadpoles from the hatcheries and refuges. The common and geographically widespread pinworm of tadpoles is *Gyrinicola batrachiensis*. The vast majority of pinworms, regardless of vertebrate host, are considered innocuous. Each infected tadpole had 1 to 32 pinworms. Only a few of the pinworms from the tadpoles were submitted for identification by a parasitologist. Parasites are still being examined and re-examined by parasitologists. Oxyurids were found in 20 tadpoles of 3 species from Welaka NFH, one *R. clamitans* from Warm Springs NFH, 2 tadpoles from St Marks NWR and 8 larval *R. catesbeiana* from Harris Neck NWR. Nematodes were not detected in larval amphibians from Edenton and Orangeburg NFHs.

Leeches, anchorworms, chiggers, fish-lice and oligochaetes were not detected in amphibians from the 4 hatcheries and 2 refuges in 2005.

Conclusions.

1. Important Amphibian Diseases. The only serious and lethal amphibian disease that was found in amphibians from the 4 hatcheries and two refuges was oral chytridiomycosis (infection by *Batrachochytrium dendrobatidis*) in 4 of 5 larval bullfrogs from Warm Springs NFH. This disease agent was not found in the other 3 hatcheries and 2 refuges. The pond from which these bullfrog tadpoles were collected must be considered contaminated with this serious disease agent and all fish, amphibians and water from this pond also must be considered contaminated and potentially infectious.

There are no reports of successful eradication of this pathogenic chytrid fungus from contaminated sites, but well designed studies to eradicate this organism from field sites do not appear to have been attempted. Two other serious and lethal infectious diseases of amphibians, namely ranaviruses and a Perkinsus-like organism, were not found in any of the amphibians from the hatcheries and refuges. However, this does not mean that the sampled ponds, hatcheries and refuges will remain free of these infectious diseases in future years.

2. Potentially Significant Amphibian Diseases. Metacercariae of the malformation-inducing parasite, Ribeiroia, and a new undescribed microsporidian parasite of the brain, spinal cord and ganglia are two infectious diseases from the hatcheries that may cause morbidity, mortality and malformations in amphibians in the hatcheries in the future. In addition, these 2 diseases could have adverse impacts on free-living amphibian populations should infected hatchery animals be released into naïve populations or among other amphibian species not sampled in this study (eg, gopher frogs [*R. capito*], flatwoods salamanders [*Ambystoma cingulatum*]). In addition, the unidentified metacercariae in the thyroids of 2 larval *R. catesbeiana* from Goose Pond, Harris Neck NWR, GA, may be worthy of further investigations because an infection of larval thyroids may result in hypothyroidism and impaired metamorphosis.
3. Zoonotic Diseases. Infectious diseases of potential threat to humans (eg, *Salmonella* spp., enterotoxigenic *E. coli*, etc) were not detected in amphibians from the hatcheries and refuges.

4. Diseases of Epizootiological Importance. The only disease that was likely to have been transmitted from fish to amphibians was the ectoparasite, *Gyrodactylus* sp. However, no fish were examined in this study, so the source of this skin parasite remains speculative. Likewise, it is unknown whether the microsporidian infection of the brain, spinal cord and ganglia of a few larval amphibians from Welaka NFH was acquired from fish in the pond; the microsporidian parasite may or may not be host-specific for amphibians. Also, it is possible but unlikely that the myxozoan parasites (*Myxidium* spp.) in the gall bladders of the tadpoles are capable of infecting both fish and amphibians, but until further molecular and morphological identifications are made, it remains unknown whether these parasites are specific for amphibians or capable of infecting multiple classes of vertebrates.

Recommendations.
1. Chytrid fungus. Because this disease agent is the single most important and serious pathogen of amphibians in the Western Hemisphere (and other regions), and is considered the proximate cause of the extinction of multiple species of amphibians and the cause of multiple extirpations of amphibian populations, serious discussions among hatchery biologists, consumers of hatchery fish/amphibians, refuge managers and disease experts to control the spread of this disease are warranted. Some important topics to be considered include:

a. Methods to control entry of breeding amphibians into outdoor fish ponds in the hatcheries
b. Design of studies to eradicate chytrid fungus from contaminated ponds
c. Methods to remove all amphibians from shipments of hatchery-raised fish
d. Minimal volumes of water needed to flush contaminated pond water from shipments of fish
e. Development of a disease monitoring program in amphibians from hatchery ponds for chytrid fungus and other serious infectious diseases, such as ranaviruses and the Perkinsus-like organism
f. Incorporation of molecular methods (ie, PCR testing) for detection of infectious agents in follow-up and future surveys for diseases in amphibians
g. Develop a method to assess the threat- or hazard-potential for introduction of amphibian diseases on native amphibian populations at fish release sites. This could involve a ban on release of hatchery fish at any site or watershed known to contain listed species of fish or amphibians, but also should consider those sites harboring known remnant populations of species affected by serious population declines (eg, gopher frogs).

2. Microsporidian infection at Welaka NFH. This new infectious disease needs to be investigated further with transmission electron microscopy to characterized and identify the organism. This new disease is worthy of publication in peer-reviewed journal. In addition, fish from this pond should be examined for this parasite, and follow-up sampling of larval and post-metamorphic amphibians from the pond is desirable to determine the persistence of the organism at a site and its host-range. Because the only other described microsporidian (Spraguea) of the brain, spinal cord and ganglia occurs in marine fish, a review of fish food used at this pond for the presence of marine fish in the fish-meal is desirable.

3. Myxozoa. Studies of the myxozoa in the gall bladders of these larval amphibians are still in progress. As the parasites are characterized molecularly and morphologically, it may be necessary to request additional live amphibians from the hatchery ponds in order to obtain fresh gall bladders and then optimally preserve the myxozoa for morphological studies and comparisons. Results of pending studies may be worthy of a separate publication in a peer-reviewed journal.

4. Metacercariae in thyroids. Collection of additional larval amphibians from Harris Neck NWR may be warranted in order to obtain fresh live specimens of the parasite for identification. Follow-up surveys may be warranted to determine whether there are any malformed amphibians emerging from the pond or whether impaired metamorphosis is evident at the site. "Impaired metamorphosis" might present as 1) giantism in larval bullfrogs or 2) persistence of larvae of other amphibian species long after their cohorts have completed metamorphosis.

Report prepared & written by:
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FIGURES: Gross and histological photographs

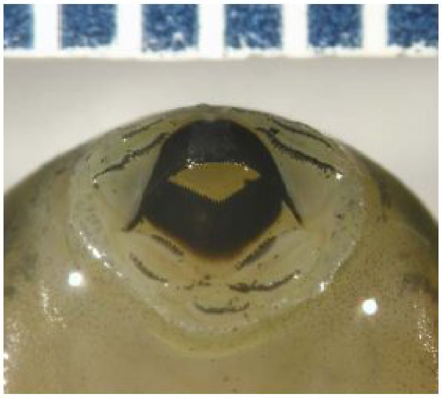

Figure 1. Close-up photograph of 4912-035, larval *Hyla gratiosa*, oral disc. Note the normal heavy black pigment of the jaw sheaths and the interrupted (abnormal) black pigment of the toothrows. Etiology was not determined. 25X (scale bar at top in millimeters)

Figure 2. Close-up photograph of 4912-114, larval *Bufo terrestris*, oral disc. Note complete loss of black pigment on lower jaw sheath and interrupted (abnormal) black pigment of the toothrows. Diagnosis: depigmentation of unknown etiology. 46X

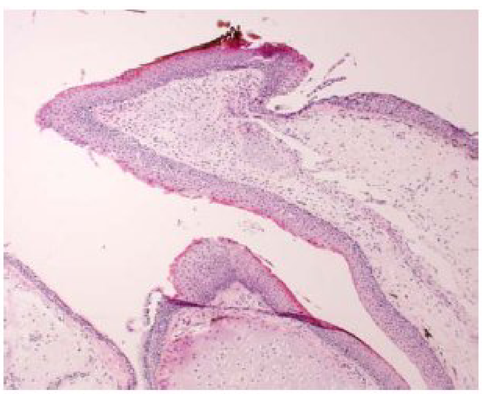

Figure 3. Photomicrograph of 4912-155, larval *Rana catesbeiana*, oral disc. Note the extensive loss of brown/black pigment at the tips of the jaw sheaths compared to pigment in Fig 4. Diagnosis: chytridiomycosis. H&E stain, 40X.

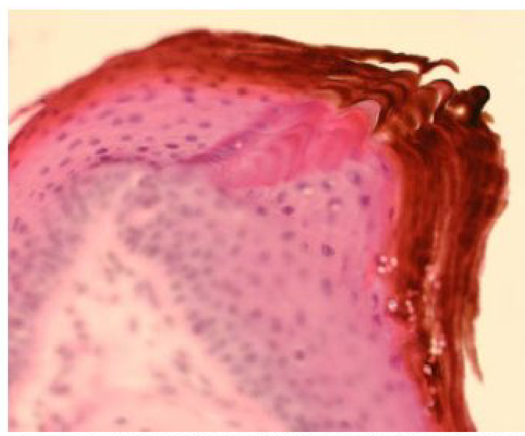

Figure 4. Photomicrograph of 4912-156, larval *R. catesbeiana*, tip of one jaw sheath. Note only mild loss of brown/black pigment at tip, rounding of the tip, and tiny clear vacuoles (chytrid thalli) in lower right. Diagnosis: chytridiomycosis. H&E stain, 200X.

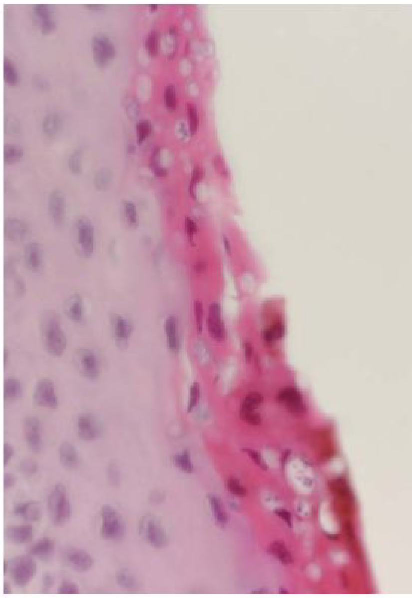

Figure 5. Photomicrograph of 4912-155, larval *R. catesbeiana*, jaw sheath. Chytrid thalli are the circular and ovoid clear vacuoles in the red keratinized cells at right; some chytrid thalli contain wispy, pale blue zoospores. H&E stain, 400X

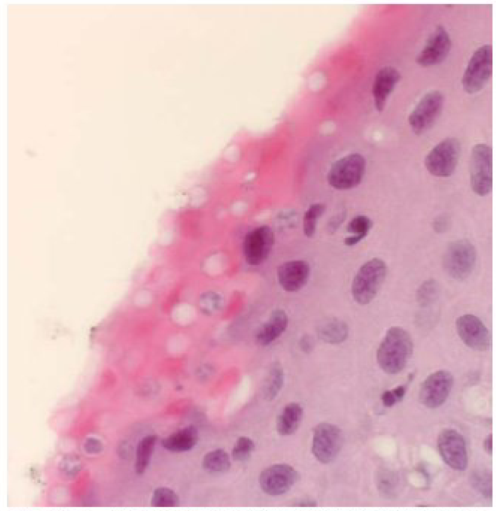

Figure 6. Photomicrograph of 4912-156, larval *R. catesbeiana*, jaw sheath. The clear and pale blue circular vacuoles in the surface (red) cells at left are chytrid thalli.
Diagnosis: chytridiomycosis. H&E stain, 400X

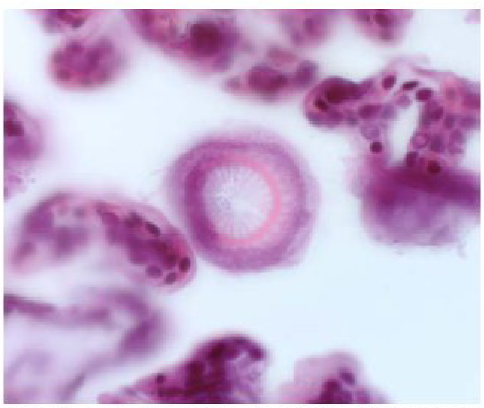

Figure 7. Photomicrograph of 4912-118, larval *Bufo terrestris*, gills. In the center of the image is a roughly circular protozoan ecto-parasite with distinct radiating spokes of its rotor, typical of *Trichodina* spp. H&E, 400X

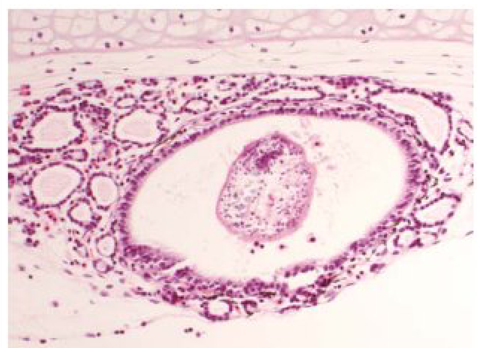

Figure 8. Photomicrograph of 4912-179, larval *R. catesbeiana*, thyroid. One prominent metacercaria is present in a dilated cystic thyroid follicle; the parasite was not identified. H&E stain, 100X

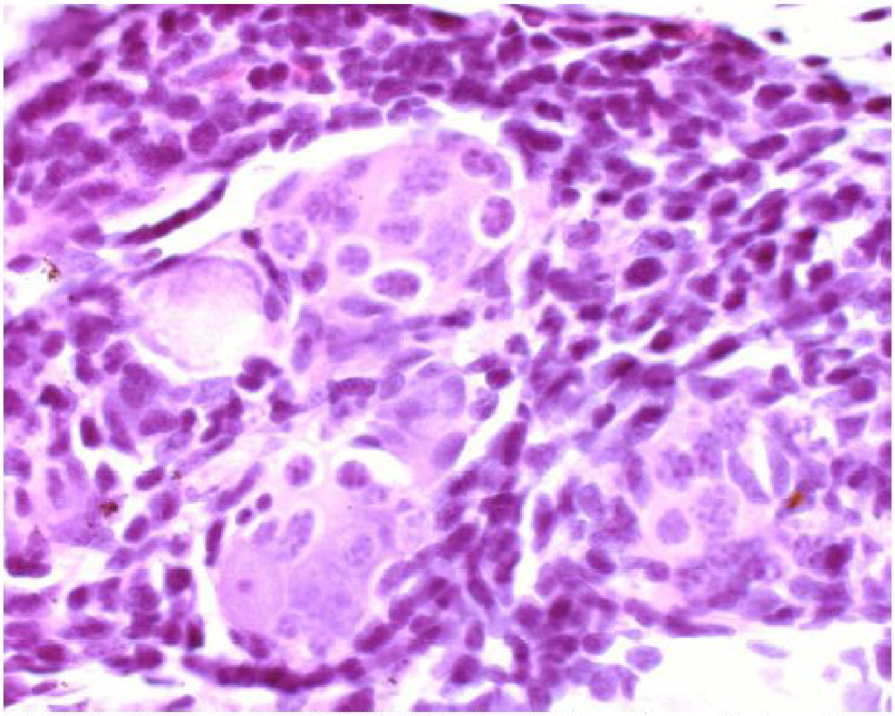

Figure 9. Photomicrograph of 4912-035, larval *Hyla gratiosa*, spinal ganglion. Marked infiltrates of lymphocytes are present in the ganglion and several neurons have microsporidia in their perikaryon (cytoplasm). H&E stain, 400X

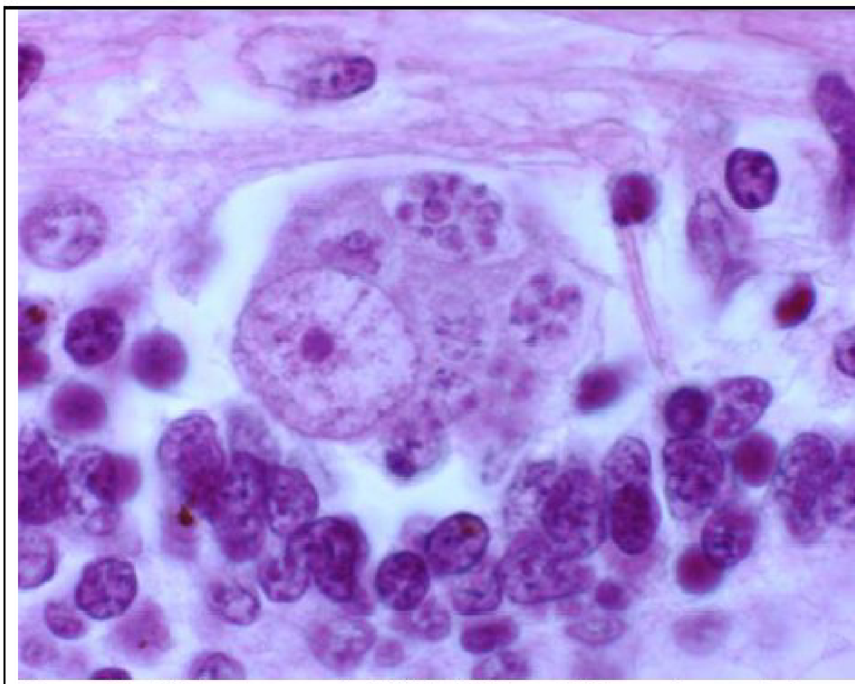

Figure 10. Photomicrograph of 4912-038, larval *Hyla gratiosa*, spinal cord. The one large neuron in the center of the figure has multiple clusters of microsporidia in its perikaryon. H&E stain, 1000X (oil immersion).