

DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE
Southeast Region



**PRELIMINARY ASSESSMENT FOR ABNORMAL AMPHIBIANS
ON NATIONAL WILDLIFE REFUGES
IN THE SOUTHEAST REGION**

END OF YEAR REPORT:

Southeast Region

FY 2006

by

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Upper Ouachita NWR: Gypsy Gooding, Steph McCormick

D'Arbonne NWR: Gypsy Gooding, Steph McCormick

Executive Summary

Preliminary screening assessments for abnormal amphibians were initiated on national wildlife refuges (NWRs) in the southeast region in 2000, with additional refuges included in each subsequent year (2001-2006) (Table 1, Figure 1). Seven national wildlife refuges in the Southeast Region were assessed during 2006 (Figure 2). No new refuges were sampled this year because of the changes in the coordination of the program that occurred during the season. The four refuges initiating Year 2 surveys in 2006 were evenly split between Arkansas (Wapanocca and White River) and Florida (Lake Woodruff and St. Marks). One in refuge in Alabama (Wheeler) and two refuges in Louisiana (Upper Ouachita and D'Arbonne) conducted Year 3 surveys in 2006. Successful collections were conducted at Wheeler (n=5), Wapanocca (n=6), St. Marks (n=1), and D'Arbonne (n=1) NWRs, but not at White River, Lake Woodruff, or Upper Ouachita NWRs because of drought conditions.

Table 1. Summary of all results from Southeast Region NWRs assessed for abnormal amphibians from 2000 to 2006.

STATE	REFUGE	Range of Incidences of Abnormalities (%) found						
		2000	2001	2002	2003	2004	2005	2006
AL	Wheeler NWR					13-22 % ^F	3.9-13.9 % ^F	3.6-12.7 ^F
AR	Bald Knob NWR			0-11 % ^F	2-13.7% ^F	2.8-18 % ^F		
	Felsenthal NWR			3-25 % ^F	0-9.8 % ^F	0-3 % ^F		
	Overflow NWR			0-15 % ^F	3-7.7 % ^F	7-42 % ^F		
	Wapanocca NWR						0-9.3% ^F	0-3.0% ^F
	White River NWR						F, N	F, N
FL	Lake Woodruff NWR						0 % ^F	F, N
	Loxahatchee NWR				F, N			
	St. Marks NWR						0-5 % ^F	0 ^F
GA	Savannah NWR				0 % ^F	0-3.8 % ^F		
KY	Clarks River NWR		0 % ^F	D				
LA	Atchafalaya NWR	2.8 % ^F	0-3.6 % ^F	0-3 % ^F				
	Big Branch Marsh NWR		2-3 % ^F	2-4 % ^F				
	Black Bayou Lake NWR	F, C	0 % ^F	N				

Table 1 (continued). Summary of all results from Southeast Region NWRs assessed for abnormal amphibians from 2000 to 2006.

STATE	REFUGE	Range of Incidences of Abnormalities (%) found						
		2000	2001	2002	2003	2004	2005	2006
	Bogue Chitto NWR	F, N	F, N	1.7 % ^F				
	D'Arbonne NWR	F, C	F, N	0%				24% ^F
	Upper Ouachita NWR					0-2 % ^F	F, N	F, N
MS	Dahomey NWR		1-16.8% ^F	0-4.2 % ^F	3.7-9 % ^F	1-5.7 % ^V	V	
	Tallahatchie NWR		1.87 % ^F	0-6.4 % ^F	F, D		V	
	Sandhill Crane NWR				0-7.6 % ^F	0-6% ^F	0 % ^F	
	Yazoo NWR					0 % ^F	0-18.5 % ^F	
NC	Alligator River NWR				3.9-6 % ^F	0-6% ^{N, F}		
	Pocosin Lakes NWR				0-12 % ^F	0-9.8% ^F	F, N	
TN	Lake Isom NWR	9 % ^F	2 % ^F					
	Reelfoot NWR	F, N	3.3 % ^F					
	TN NWR		F, C	0-2.2 %				
	Big Sandy Unit							
	Hatchie NWR		F, C	0-5 %	0-12 % ^F	2.0-5.9% ^F		

Blank cell = refuge not funded or sampled during that year; F = funded; C = no sampling attempted this season due to late completion of contract;
D = sampling attempted but not achieved this season due to drought conditions; N = sampling attempted but not possible this season due to sparse number of metamorphs encountered; V = voluntary monitoring.

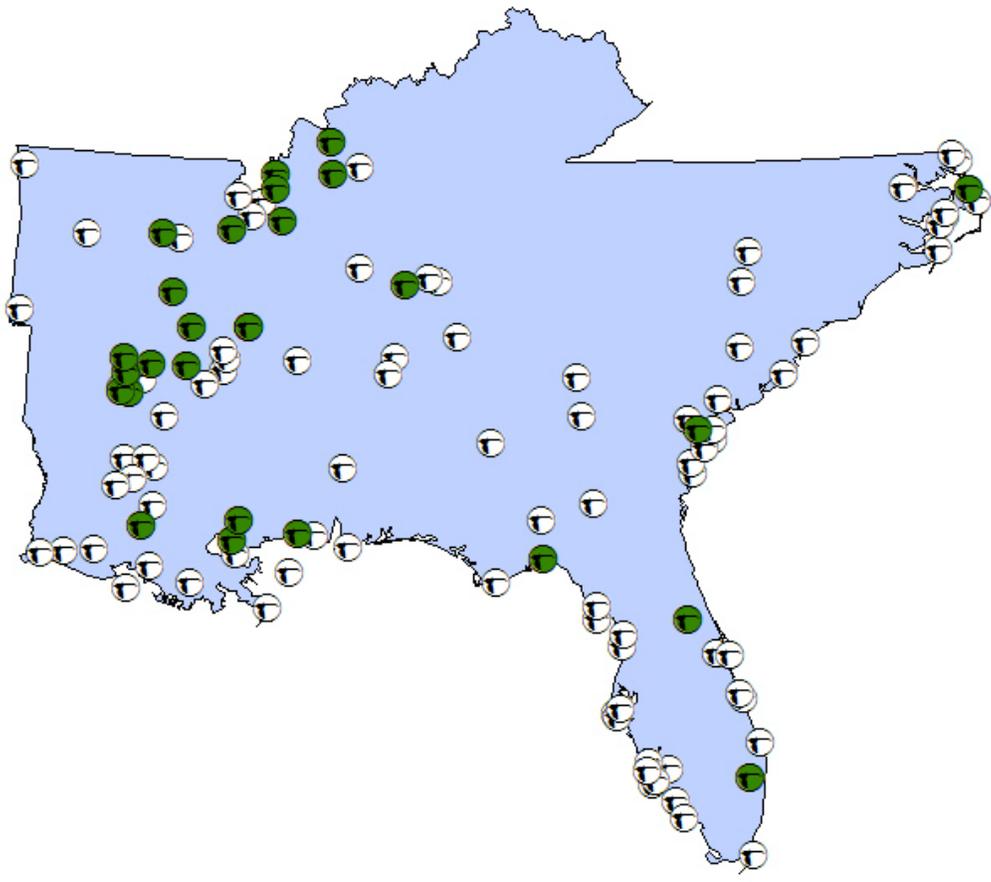


Figure 1: Locations of preliminary screening assessments for abnormal amphibians on national wildlife refuges (NWRs) in the southeast region from 2000 to 2006 (green icons) compared to refuges that have not been sampled in the Southeastern Region (white icons).

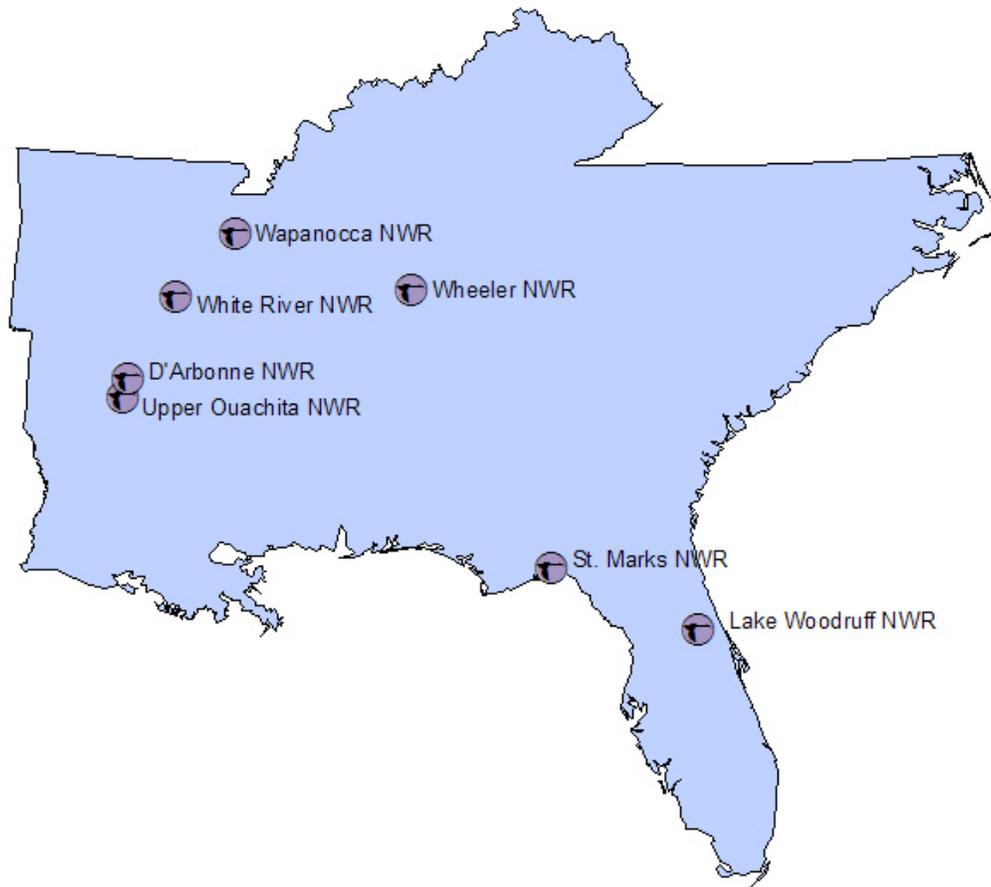


Figure 2: Seven national wildlife refuges in the Southeast Region assessed during the 2006 sampling season.

During 2006, the incidence of abnormalities exceeded background levels (3 percent; Dubois 1979, Johnson and Lunde 2001, Ouellet et al. 1997, Stocum 2000) in all five samples taken from Wheeler NWR (3.6-12.7% abnormal) and one sample from D'Arbonne NWR (24% abnormal). As with the previous reports (Dubois 1979, Johnson and Lunde 2001, Ouellet et al. 1997, Stocum 2000), the abnormalities encountered consisted primarily of missing or clubbed digits, feet, or limbs. Other observations including excluding variant colorations, abrasions and other similarly obvious soft tissue damages were not reported as abnormalities. A total of 46 abnormal specimens were collected across the Southeast Region during 2006 during the collection of almost 900 individuals from full collections and over 300 from partial collections.

The 46 abnormal specimens collected in 2006 were preserved and shipped for radiographic analysis to Dr. Mike Lannoo at Indiana University School of Medicine, Terre Haute, Indiana. Examination and final determination for specimens examined by Dr. Lannoo are pending.

Samples of live metamorphs were shipped for parasitological analysis. Four refuges in the southeast were targeted for sampling including: Wheeler, St. Marks, Lake Woodruff, and Upper Ouachita. However, parasitology samples were only sent from St. Marks and Wheeler NWR during the 2006 season because of the low sampling success at both Lake Woodruff and Upper Ouachita NWRs.

The primary goal of the Abnormal Amphibian Monitoring Project for the 2007 season is database validation and quality assurance to ensure the best possible data evaluation in a five year review of the data. With that primary task in mind and if time and budget allow, two refuges are targeted for repeat sampling in 2007. These are the St. Marks and White River NWRs. This will conclude monitoring of St. Mark NWR and provide a final attempt at sampling White River NWR where sampling has met with limited success due to multiple challenges. New refuges to be sampled in 2007 include Noxubee (MS), Choctaw (AL), and St. Vincent (FL) NWRs. Refuges to be sampled during the 2007 season are shown in Figure 3.

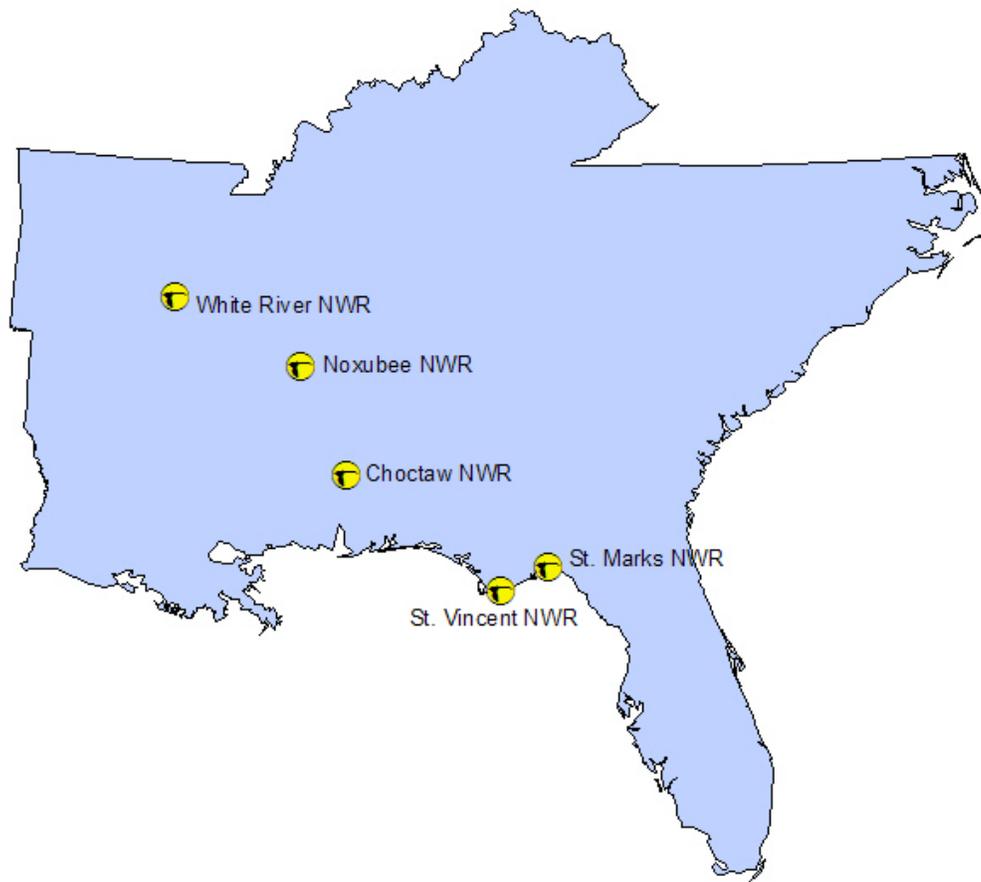


Figure 3: Five national wildlife refuges in the Southeast Region to be assessed during the 2007 sampling season.

The continued goal of this program is to expand sampling on refuges throughout the Southeast Region, in particular throughout the eastern states where limited sampling has been done to date.

Introduction

In response to the increasing number of amphibian abnormalities reported from sites throughout the United States, the U.S. Fish and Wildlife Service launched its National Abnormal Amphibian Initiative in February 2000. The purpose of the initiative is to screen national wildlife refuges for the incidence of abnormal amphibians and to subsequently investigate potential causes. This report summarizes the preliminary results for abnormal amphibian assessments conducted on refuges in the Southeast Region during the 2006 season.

Seven national wildlife refuges in the Southeast Region were assessed during 2006. No new refuges were sampled this year because of the changes in the coordination of the program that occurred during the season. The four refuges initiating Year 2 surveys in 2006 were evenly split between Arkansas (Wapanocca and White River) and Florida (Lake Woodruff and St. Marks). One in refuge in Alabama (Wheeler) and two refuges in Louisiana (Upper Ouachita and D'Arbonne) conducted Year 3 surveys in 2006.

Methods

Assessments for abnormal amphibians on refuges are being conducted using the following SOPs that were implemented in March 2003 (available on the Service Intranet (SII):

<http://sii.fws.gov/r9dec/>). A collection consists of between 50-100 metamorphs of a single species, collected from the same cohort from a single site. Optimally, two collections should be made from each of two sites per refuge, for two consecutive seasons (i.e., four collections/refuge/season). A minimum of one collection per refuge for two consecutive seasons is desired to meet program goals. Ideally, the sampling sites should include both reference and potentially impacted sites. In order to successfully sample at least two sites, typically four to six sites should be selected per refuge for initial monitoring since weather conditions, water levels, and frog breeding activity are unpredictable. Sites should be assigned a unique identification code using standard refuge codes followed by two digit sequential numbers (i.e., BLD01). Historical site names may not necessarily conform to this naming convention so, when available, the site “alias” is included so that site data can be correlated with previous reports. Site descriptions should include pertinent locality information (i.e., latitude/longitude), habitat type, descriptions of known or suspected contaminant sources, and surrounding land uses. Refuge information should be derived from refuge fact sheets and communication with refuge staff. Contaminant concerns may be documented for those refuges where a Contaminant Assessment Process (CAP) has been completed and is available through the on-line CAP database (<http://ecos.fws.gov/ecapreport/>). Site characterization should include size of wetland area, average water depth, and any notable changes that occurred over the course of the season. Digital photographs should be taken of each sampling site throughout the season to document changes in water levels or vegetation.

Selected sites should be monitored regularly until tadpoles are observed and visited weekly until development is nearly complete. Sites should then be visited every other day until late stage metamorphs (Gosner stages 44-46) can be collected. Collecting earlier staged metamorphs should be avoided since bones may not be calcified sufficiently for radiography. Two collections are to be made from each site. Collections can be satisfied by either 50-100 individuals per species for two different species collected at the same time from the same site, or 50-100 individuals of one species collected from two distinct cohorts during the season. Collections can be completed by one or two person teams by dip-netting metamorphs from wetland margins or by sweeping through pools. Collection of metamorphs by hand or seine may

be more productive at some sites. Depending on conditions at the site; such as water levels, amount of solar heating, and rate of emergence of metamorphs; two or three day sampling periods may be required to achieve the minimum required sample size of 50 individuals. When collections are to be made over an extended period, metamorphs must be held in large containers and kept cool and moist with site water to eliminate resampling. To minimize stress to metamorphs during holding periods, every effort should be made to complete a collection within a week.

Once sufficient numbers of appropriately aged animals are collected and processed, the data are recorded on standard data collection forms. Body measurements should include snout to vent length (SVL) and tail length (TAL). Species identification and Gosner stage (GS) for each individual should also be recorded. When positive species identification is not possible, genus should be noted and representative individuals retained and preserved for identification. Animals that qualify as metamorphs (i.e. all four legs emerged) should be recorded and processed, even if fewer than 50 individuals are collected. Each metamorph should be inspected for abnormalities using the standard checklist on each form. When reporting the incidence of abnormalities, data should not be combined for different species or cohorts and only collections of a minimum of 50 metamorphs should be reported as a percent. Results should be reported as raw data when fewer than 50 metamorphs are processed. All normal metamorphs should be released back to the capture site as soon as possible by randomly placing specimens throughout the entire site to avoid unnatural predation events. Abnormal animals should be properly euthanized using dilute chloretone or MS-222 solution. Documentation of abnormal specimens includes assignment of unique identification numbers using the system detailed on standard data sheets that incorporates region, site identification, collection date, species code, and specimen number (i.e., R4-BLD01-071803-RASP-0001). Occasionally, normal specimens will be tracked, either as vouchers or normal individuals submitted for parasitological analysis. Normal specimens should be tracked similar to abnormal specimens, with the exception that the specimen number should lack one digit, and end in "N" (i.e., R4-BLD01-071803-RASP-001N). Digital photographs should be taken immediately following euthanasia and all abnormalities detailed by hand on standard forms. Proper positioning of specimens for preservation is prone with all limbs, feet, and digits extended flat. Specimens should be positioned in wax lined containers with tight fitting lids and either pinned or taped in place for fixation in 95% EtOH for a minimum of 48 hours, then placed in 70% EtOH for storage.

The incidence of abnormalities reported from field collections are considered preliminary and require confirmation through radiographic or other diagnostic analyses. The fate of abnormal specimens will be assigned on a case-by-case basis, depending on historical data from the respective collection sites and availability of diagnostic funds. Typically, all abnormal specimens collected during the initial season will be targeted for radiography, with parasitological studies added for select sites during the second season.

Abnormal specimens collected in 2006 have been preserved and shipped for radiographic analysis to Dr. Mike Lannoo at Indiana University School of Medicine, Terre Haute, Indiana. Examination for skeletal abnormalities will be made by Dr. Lannoo. Samples of live metamorphs were shipped for parasitological analysis to Dr. Pieter Johnson at the University of Colorado in Boulder, Colorado care of his student Don Larson in Fairbanks, Alaska. Four refuges in the southeast were targeted for sampling: Wheeler, St. Marks, Lake Woodruff, and Upper Ouachita. Results will follow examination by Dr. Johnson. All specimens selected for parasitological analyses will be forwarded to Dr. Lannoo for follow-up radiography.

Diagnostic results for all abnormal specimens are entered into the national online amphibian database. After quality assurance checks on this process during the 2007 season, this will link historical data with diagnostic analyses and provide a user friendly way to track each specimen over time. Result tables and the associated data entry forms are current being evaluated. All historic data for the southeast region has been uploaded into the database which will facilitate regional, and eventually national analysis of amphibian data on refuges.

Results from sampling efforts on each refuge will be evaluated each season. Selected refuges will typically be sampled for a minimum of two consecutive years, unless habitat proves to be unsuitable or other factors prevent successful sampling. If the percent incidence of abnormalities encountered exceeds 3 percent in either of the first two seasons, that refuge will be selected for additional, more intensive sampling during a third season. Three percent is a conservatively low background level derived from limited published amphibian deformity studies and expert opinion.

Refuge Data

This section contains general discussions for each refuge sampled this year and the current status of refuge sampling. Each refuge section contains a brief narrative, followed by a table of cumulative results for each sampling site. Only full collections (samples of >50 individuals) are documented in these tables. Sampling site maps, descriptions, photographs, data sheets, and abnormality forms for each refuge can also be found in the appendices.

A. Alabama Refuges

1. Wheeler National Wildlife Refuge

Wheeler NWR is located between Decatur and Huntsville in the Tennessee River Valley of northern Alabama. It consists of 35,000 acres of bottomland hardwoods, moist soil units, riparian woodlands, back water embayments, pine uplands, and croplands. Sampling was initiated on Wheeler NWR in 2004. Some baseline herpetofaunal inventory work has been conducted on the refuge, including breeding call surveys following the USGS North American Amphibian Monitoring Program (NAAMP), but no historical surveys of suitable ephemeral areas for larval amphibians have been conducted to date. Seventeen sites were selected by refuge staff for monitoring from a large number of known amphibian sites. Many sites dried early in the season, prior to resident anurans completing metamorphosis, so no complete collections were possible at 13 of these sites.

Complete collections for FY06 included 391 metamorphs from 5 collections. The species were southern leopard frogs (*Rana sphenocephala*) in four collections and northern spring peepers (*Pseudacris crucifer*) in the fifth collection. Abnormality rates for these five collections in FY06 ranged from 3.6 to 12.7%. Abnormalities (excluding variant colorations, abrasions and similar soft tissue observations) included shortened, irregular, or missing digits (Figure X). All abnormal individuals are prepared for shipment for radiographic examination.

Sampling results from all three sampling seasons (2004-2006) on Wheeler NWR indicated that at least six areas on the refuge have higher than background rates of abnormalities (>3 percent, Table 2). Abnormalities most often included shortened or missing digits. Depending on the

radiological evaluation of these observed field abnormalities, further evaluation of the amphibian populations on Wheeler NWR may be needed and investigation into cause may be required.

Table 2. Results for sampling efforts on Wheeler NWR.

Site Name	YEAR 1 (2004)				YEAR 2 (2005)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
WLR01	n/a				n/a			
WLR02	n/a				n/a			
WLR03	n/a				n/a			
WLR04	n/a				n/a			
WLR05	n/a				n/a			
WLR06	n/a				n/a			
WLR07	n/a				5/5/2005	RASP	13.9	R
WLR08	n/a				n/a			
WLR09	n/a				n/a			
WLR10	06/15/04	RASP	13.3	R	n/a			
WLR11	n/a				n/a			
WLR12	n/a				n/a			
WLR13	n/a				n/a			
WLR14	05/23/04	RASP	15.1	R	05/27/05	RASP	12.5	D/R
	06/03/04	RASP	22	R	06/02/05	RASP	4.0	R
	n/a				06/14/05	RASP	3.9	R
WLR15	n/a				05/13/05	RASP	6.8	R
WLR16	n/a				n/a			

	YEAR 3 (2006)			
Site Name	Sample Date	Species Code	% abnormal	Fate
WLR01	05/09/06	RASP	3.6	R
WLR02	n/a			
WLR03	n/a			
WLR04	n/a			
WLR05	n/a			
WLR06	n/a			
WLR07	n/a			
WLR08	n/a			
WLR09	n/a			
WLR10	n/a			
WLR11	n/a			
WLR12	n/a			
WLR13	n/a			
WLR14	n/a			
WLR15	05/17/06	RASP	8.8	R
	05/30/06	RASP	6.8	R
	06/09/06	RASP	12.7	R
WLR16	04/27/06	PSCR	4.5	R

Note: Common and scientific names for the standard species codes used in Tables 2-13 can be found in Appendix B. n/a indicates that full collections were not feasible. R = radiography.

B. Arkansas Refuges

1. Wapanocca Wildlife Refuge

Wapanocca NWR is located 20 miles northwest of Memphis, Tennessee, in Crittenden County, Arkansas. The refuge was established in 1961. The refuge is located four miles west of the Mississippi River and protected from the river by the river levee. The refuge is composed of diversity of habitat including agricultural lands, bottomland hardwood forest, early stage reforested hardwoods, open water and flooded cypress/willow swamp. Additionally, 30 small field impoundments totaling 190 acres have been developed for waterfowl in the agricultural area.

The 2006 sampling season represented Wapanacca NWR's second year of participation in the Abnormal Amphibian Monitoring Program. Six full collections were made from four sites on the refuge. The collections were comprised of 402 metamorphs. All collections were of southern leopard frogs (*Rana sphenocephala*). Abnormalities in 2006 included shortened or missing digits for most, but one specimen was missing a hind right foot. No areas sampled in 2006 yielded abnormality rates higher than background rates of abnormalities (>3 percent, Table 3).

Over the two years of sampling, sixteen full collections were made. Of these, three showed abnormality rates higher than background (3%). All three of these samples were taken in 2005 at one site. The only sample taken from this site in 2006 showed no abnormalities. Because of the nature of the abnormalities being limited to potential injury related events, the overall low incidence of abnormalities, and the lack of any sites having samples above background during the 2006 season, a third funded sampling season is not recommended.

Further evaluation of the site showing abnormalities in 2005 may be necessary. It is recommended that a study be conducted by refuge biologists to further examine potential differences between this site and other sites sampled on this refuge. Continued voluntary sampling by refuge staff and volunteers will be important to contributing to a database capable of identifying trends in amphibian populations on our National Wildlife Refuges.

Table 3. Results for sampling efforts on **Wapanocca** NWR.

	YEAR 1 (2005)				YEAR 2 (2006)			
Site Name	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
WPN01	n/a				n/a			
WPN02	n/a				n/a			
	YEAR 1 (2005)				YEAR 2 (2006)			
WPN03	n/a				n/a			
WPN04	06/09/05	BUFO	2.7	R	n/a			
	06/09/05	RASP	0	n/a	n/a			
WPN05	n/a				n/a			
WPN06	05/27/05	RASP	1.8	R	06/02/06	RASP	1.8	R
	06/10/05	RASP	0	n/a	06/28/06	RASP	0	n/a
WPN07	06/13/05	RASP	0	n/a	06/14/06	RASP	3.0	R
	05/09/05	RASP	2.0	R	06/29/06	RASP	0.9	R
WPN08	n/a				n/a			
WPN09	06/06/05	RACL	7.4	n/a	06/15/06	RASP	0	n/a
	05/20/05	RASP	5.4	n/a	n/a			
	06/06/05	RASP	9.1	n/a	n/a			
WPN10	n/a				n/a			
WPN11	06/15/05	RASP	0	n/a	n/a			
WPN12	n/a				n/a			
WPN13	n/a				06/16/06	RASP	0	n/a

Note: n/a indicates that full collections were not feasible. R = radiography.

2. White River Wildlife Refuge

White River National Wildlife Refuge was established in 1935. The refuge lies in the floodplain of the White River. The long, narrow refuge is a large area of bottomland hardwood forests in the Mississippi River Valley. The refuge hosts three hundred lakes and associated are streams, sloughs, and bayous.

In 2006, a total of seven sample locations were monitored on White River NWR. Some sites included multiple adjacent ponds. The majority of the sites sampled were within the Jacks Bay Unit of the refuge. The majority of the work was centered within this unit due to time constraints and additional commitments needed in conducting and/or assisting with the Ivory-billed Woodpecker (IBWO) surveys during early morning hours. Following IBWO surveys, the majority of amphibian surveys were initiated at or about 10 am each morning. These surveys continued until late May when drought conditions became significant. The drought eliminated all sample sites at Jacks Bay. Additional and sporadic sampling was conducted in the North Unit in order to have some alternate sites in case of drought. High water had “refilled” most of the sloughs and ditches on the lower end of the North Unit. The North Unit sites dried as did those at Jacks Bay. The Farm Unit moist soil impoundments were sampled and were providing excellent results, however in order to plant the impoundments for waterfowl food production, refuge staff members drained these impoundments before a collection could be made.

For the second consecutive year, southeastern Arkansas continued in a severe drought. In 2005, all sites were dry by June. In 2006, the trend continued with all sites drying up by early May (Table 4). As a reference, several oxbow lakes which had not been dry since the late 1930s were dry this summer. Ultimately, no collections were possible on White River NWR for the second year of participation in the Abnormal Amphibian Project due to the persisting drought.

White River NWR has expressed interest in participating in the monitoring effort during the 2007 season. To improve sampling success during 2007 season, measures have been proposed by the refuge. The refuge proposed that 1) all efforts be concentrated on moist soil impoundments on the Farm Unit of the refuge; 2) refuge maintenance staff will be informed to leave residual

water for amphibian production and 3) the sites at Jacks Bay and the North Unit will receive additional monitoring as a comparison, if sufficient water is present.

Table 4. Results for sampling efforts on White River NWR.

Site Name	YEAR 1 (2005)				YEAR 2 (2006)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
WHR01	n/a				n/a			
WHR02	n/a				n/a			
WHR03	n/a				n/a			
WHR04	n/a				n/a			
WHR05	n/a				n/a			
WHR06	n/a				n/a			
WHR07	n/a				n/a			

Note: n/a indicates that full collections were not feasible

C. Florida Refuges

1. Lake Woodruff National Wildlife Refuge

Lake Woodruff National Wildlife Refuge was established in 1964 on a 21,574 acre area in Western Volusia County and Lake Counties, FL and an additional two conservation easements totaling 652 acres in Putnam County, FL. The refuge surrounds the 2,200-acre Lake Woodruff, 1,800-acre Lake Dexter, and numerous other waterways including the St. Johns River which forms the western boundary.

Initial success in the 2005 sampling year was not found during the 2006 sampling season at Lake Woodruff National Wildlife Refuge. In 2006, seven sites were monitored. Only two sites

selected retained water throughout the project. Three partial collections were made, but no full collections were possible. No abnormalities were observed in the incomplete samples.

During both years of sampling at Lake Woodruff National Wildlife Refuge no abnormalities were observed (Table 5). The refuge will therefore not be selected for further funded monitoring at this time. Voluntary sampling of the refuge is uncertain.

Table 5. Results for sampling efforts on Lake Woodruff NWR.

Site Name	YEAR 1 (2005)				YEAR 2 (2006)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
LWD01	n/a				n/a			
LWD02	6/14/2005	BUQU	0.0		n/a			
LWD03	n/a				n/a			
LWD04	7/14/2005	HYFE	0.0	n/a	n/a			
LWD05	6/2/2005	BUQU	0.0	n/a	n/a			
LWD06	7/21/2005	RASP	0.0	n/a	n/a			
LWD07	7/26/2005	HYFE	0.0	n/a	n/a			

Note: n/a indicates that full collections were not feasible

2. St. Marks National Wildlife Refuge

St. Marks National Wildlife Refuge was established in 1931 and is one of the oldest refuges in the National Wildlife Refuge System. It encompasses 68,000 acres within Wakulla, Jefferson, and Taylor counties along the Gulf Coast of northwest Florida. Refuge habitat includes coastal marshes, islands, and tidal creeks and estuaries.

The 2006 sampling season was St. Marks NWR’s second year of participation in the Service’s Abnormal Amphibian Program, In 2006, five sites were monitored on St. Marks NWR. Like many areas in the Southeastern Region, the sampling on St. Marks suffered significantly in 2006 from the drought (Table 6). Only one complete sample was achieved at St. Marks this year, compared to three in year one (2005). The metamorphs were identified as bullfrogs (*Rana catesbeiana*) in this sample and one other incomplete sample.

The one observed abnormality at St. Marks NWR in 2006 was from an incomplete sample (n=21). The observation was of a forelimb that failed to emerge from the body. It appeared to be a fully developed arm beneath the epidermis of the ventral thorax. It was described by the sampling biologists as an “ingrown right foreleg.” This specimen, along with nine normal individuals, constituted the parasitology sample (bullfrogs, *Rana catesbeiana*).

The St. Marks NWR biologists and volunteers have expressed interest in participating in a third year of sampling to facilitate obtaining more complete collections. Only four complete collections have been made in the first two survey years. Only one collection was possible in year two (2006) because of low water conditions due to the drought. If resources allow, it is recommended that the refuge receive funding for a third year in 2007 to accomplish obtaining more complete collections.

Table 6. Results for sampling efforts on St. Marks NWR.

Site Name	YEAR 1 (2005)				YEAR 2 (2006)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
SMK01	05/18/05	BUFO	0	n/a	n/a			
	015/18/05	PSOC	2.7	R	n/a			
SMK02	06/07/05	ACGR	5.0	R	n/a			
SMK03	n/a				n/a			
SMK04	n/a				n/a			
SMK05	n/a				09/11/06	RACA	0	n/a

Note: n/a indicates that full collections were not feasible..

D. Louisiana Refuge

1. Upper Ouachita National Wildlife Refuge

Upper Ouachita NWR is located approximately six miles north of West Monroe, in north central Louisiana. It consists of over 40,000 acres; with more than 14,500 acres of bottomland hardwood forest, 5,000 acres of upland forest, 3,000 acres of shrub/wooded swamp, 16,000 acres of reforested farmland, and 2,000 acres of open water. Approximately 80 percent of the refuge is subject to flooding typically from December through May. The refuge is in a watershed with elevated mercury. Elevated dioxin levels have been detected in wildlife samples from Upper Ouachita NWR. A potential source is a large wood processing plant, Georgia-Pacific in Crossett, Arkansas. The cultivated acreage on the refuge is planted primarily in rice. Additionally, the refuge lies in a largely agricultural landscape, therefore the potential for aerial drift or runoff of pesticides onto the refuge is likely.

This 2006 sampling season was the third funded abnormal amphibian sampling season for Upper Ouachita NWR. For the second consecutive year low water conditions caused by the drought prevented successful sampling on the refuge despite monitoring of eight sites throughout the season (Table 7). After three years of sampling, only two complete collections have been made and evaluated. Both of these collections possessed below background abnormality rates (0 and 1.96%). Further funded sampling and evaluation on Upper Ouachita NWR is not thought to be warranted at this time. If conditions improve for sampling success or new pressing information is provided through voluntary monitoring on the refuge, it will then be considered for future funded participation in the program.

Table 7. Results for sampling efforts on Upper Ouachita NWR.

	YEAR 1 (2004)				YEAR 2 (2005)			
Site Name	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
UOC01	n/a				n/a			
UOC02	n/a				n/a			
UOC03	05/10/04	RASP	0 %	n/a	n/a			
UOC03	05/11/04	RASP	1.96 %	R	n/a			
UOC04	n/a				n/a			
UOC05	n/a				n/a			
UOC06	n/a				n/a			
UOC07	n/a				n/a			
UOC08	n/a				n/a			
	YEAR 3 (2006)							
Site Name	Sample Date	Species Code	% abnormal	Fate				
UOC01	n/a							
UOC02	n/a							
UOC03	n/a							
UOC03	n/a							
UOC04	n/a							
UOC05	n/a							
UOC06	n/a							
UOC07	n/a							
UOC08	n/a							

Note: (/a) indicates that full collections were not feasible. R = radiography.

2. D'Arbonne National Wildlife Refuge

D'Arbonne NWR consists of over 11,000 acres of bottomland hardwood forest, 3,000 acres of upland forest, 1,000 acres of moist soil habitat, and 2,000 acres of permanent water. This refuge is located in Ouachita and Union Parishes in northeast Louisiana. Approximately 75% of the refuge is subject to annual flooding from December through May. The central physical feature includes 21.7 km of Bayou D'Arbonne, which meanders through a 3.3 – 6.7 km wide floodplain within the refuge. Mercury is a major contaminant of concern due to mercury spills near gas well sites on D'Arbonne NWR. Elevated mercury levels have been found in fish, aquatic insects, and wading birds on this refuge.

Surveys were conducted at four locations in 2006 on D'Arbonne NWR. The refuge had previously been sampled in 2001 and 2002. The minimum collection of 50 individuals was achieved only once in 2002, but not in 2001. Again, only one complete collection of Fowler's toads (*Bufo fowleri*) was made in 2006 (Table 8). Numerous other samples were attempted but the numbers were sparse.

Because of the difficulty in obtaining complete collections (n=2 for 3 sampling years) the D'Arbonne NWR will not be targeted for further funded surveys at this time. Alternative funding for further investigation of this site may be sought because of the high abnormality rate for the 2006 sample (24%). The decision will be based on the radiographic evaluation of these abnormalities. The abnormalities were described in the field as numerous cases of missing hind legs and one individual with a missing foreleg. This sample was taken from a gas well pit adjacent to gas tanks. As with all sites selected on D'Arbonne NWR, heavy metals and petroleum product sources were potential contaminants of concern.

Table 8. Results for sampling efforts on D'Arbonne NWR.

	YEAR 1 (2001)				YEAR 2 (2002)			
Site Name	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
DAR01	n/a							
DAR02	n/a							
DAR03	n/a				5/15/2002	BFOW	0	n/a
DAR04	n/a							
	YEAR 3 (2006)							
Site Name	Sample Date	Species Code	% abnormal	Fate				
DAR01	n/a							
DAR02	05/22/06	HYCH	24	R				
	YEAR 3 (2006)							
DAR03	n/a							
DAR04	n/a							

Note: (n/a) indicates that full collections were not feasible. R = radiography.

Diagnostic Results

A. Radiography

Abnormal specimens collected during 2006 were preserved and held until approval for shipment for radiographic analysis was received from the National Program Coordinator. Abnormal specimens collected in 2006 have been preserved and shipped for radiographic analysis to Dr. Mike Lannoo. Examination for skeletal abnormalities will be made by Dr. Lannoo.

Radiographic diagnoses for the 2001 and 2002 seasons have been summarized in the 2004 annual report. These data together with all more recent data will be summarized, after data review, in the five year review of the data from the Southeastern Region Abnormal Amphibian Monitoring Project later this year.

B. Parasitology

Samples of live metamorphs were shipped for parasitological analysis to Dr. Pieter Johnson at the University of Colorado in Boulder, Colorado care of his student Don Larson in Fairbanks, Alaska. Four refuges in the southeast were targeted for sampling: Wheeler, St. Marks, Lake Woodruff, and Upper Ouachita. Results will follow examination by Dr. Johnson. All specimens selected for parasitological analyses will be forwarded to Dr. Lannoo for follow-up radiography.

Parasitological diagnoses for the 2001, 2002 and 2003 seasons have been summarized in the 2004 annual report. These data together with all more recent data will be summarized, after data review, in the five year review of the data from the Southeastern Region Abnormal Amphibian Monitoring Project later this year.

Discussion and Conclusions

Six years of sampling on refuges in the Southeast Region, indicate that frog abnormality rates can vary both within and among refuges as well as temporally. The level of variability seen in the southeast appears to be consistent with those reported for other Service regions. However, the field-identified abnormalities rates found on Southeast Region refuges to date are among the highest in the program nationally. The highest rates (>10 percent) found in the Southeast Region have been noted primarily on refuges with active agriculture (e.g, Dahomey, Bald Knob, Overflow, Pocosin Lakes, Hatchie, and Wheeler NWRs) and active or historic oil production (e.g., Felsenthal and D'Arbonne NWR). While there is no direct evidence linking these activities with elevated frog abnormality rates, further evaluation may be warranted.

All available data from field inspections and from radiographic and parasitological diagnoses will be inspected for quality during the 2007 season. Qualified data will be evaluated in light of the nature of the abnormalities and summarized in a five-year review for the Southeastern Region Abnormal Amphibian Monitoring Project, to be reported in the fall of 2007. Decisions about the need for chytrid sampling on NWRs, parasitological evaluation needs, and other needs

for the 2008 survey season will be based on the information provided in the five-year data review.

Planned training

In order to ensure consistency in data collection and to maximize the use of limited resources, training is recommended for any new refuge selected to participate in the abnormal amphibian program. A regional training workshop will be held for staff from new and continuing refuges.

Three new refuges are scheduled to begin sampling during the 2007 season. Individuals performing sampling at St. Vincent, Choctaw and Noxubee NWRs will participate in a training hosted by Choctaw NWR. The workshop will include both classroom instruction and demonstration of field techniques. The objective of the workshop is to ensure that all parties became familiar with standard data collection requirements and to provide training for use of the online database. Topics covered at the workshop will include field collecting techniques, species identification, site selection, and proper specimen preservation and shipping.

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Appendices