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 2007

by

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St. Vincent NWR: Terry McCall, Laura Jenkins, Peter "Gatorbait" Durkee, Monica Harris, Tom Lewis

Kentucky

Clarks River NWR: Michael Johnson, Jared Handley, Andy Eller

Mississippi

Noxubee NWR: Henry Sansing, David Richardson, Nicole Hodges, Amber Breland

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 (2001-2007) (Table 1, Figure 1). Six national wildlife refuges in the Southeast Region were assessed during 2007 (Figure 2). Three new refuges were sampled this year including Choctaw, Noxubee and St. Vincent NWRs. Clarks River NWR, Kentucky's only NWR, was sampled for the second funded season, but after a six year interval. Both St. Marks NWR in Florida and White River NWR in Arkansas completed a third year of sampling under severe drought conditions. Successful collections were conducted at Clarks River (n=8), Noxubee (n=3), and White River (n=1) NWRs, but not at St. Vincent, St. Marks or Choctaw NWRs because of drought conditions.

Table 1. Summary of all results from Southeast Region NWRs assessed for abnormal amphibians from 2000 to 2007.

STATE	REFUGE	Range of Incidences of Abnormalities (%) found							
		2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
AL	Wheeler NWR					13-22 % [F]	3.9-13.9 % [F]	3.6-12.7 [F]	
	Choctaw NWR								[D, 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]	0% [F]
FL	Lake Woodruff NWR						0 % [F]	[F, N]	
	Loxahatchee NWR			[F, N]					
	St. Marks NWR						0-5 % [F]	[D, F]	[D, F]
	St. Vincent NWR								[D, F]
GA	Savannah NWR				0 % [F]	0-3.8 % [F]			
KY	Clarks River NWR		0 % [F]	[D]					0-6.6% [F]
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 2007.

STATE	REFUGE	Range of Incidences of Abnormalities (%) found							
		2000	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**
	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		
	Noxubee NWR								2-13% F
	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 Big Sandy Unit		F, C	0-2.2 %					
	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.

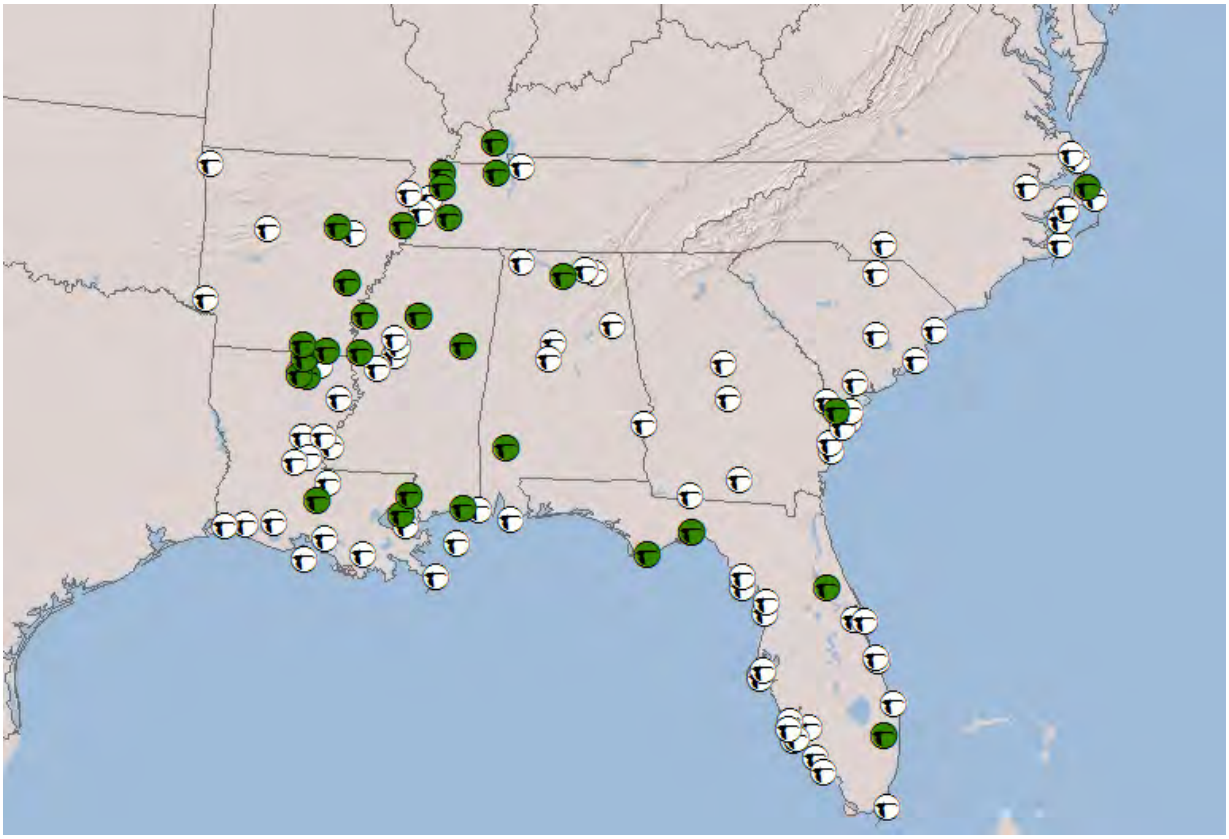

Figure 1: Locations of preliminary screening assessments for abnormal amphibians on national wildlife refuges (NWRs) in the southeast region from 2000 to 2007 (green icons) compared to refuges that have not been sampled in the Southeastern Region (white icons).

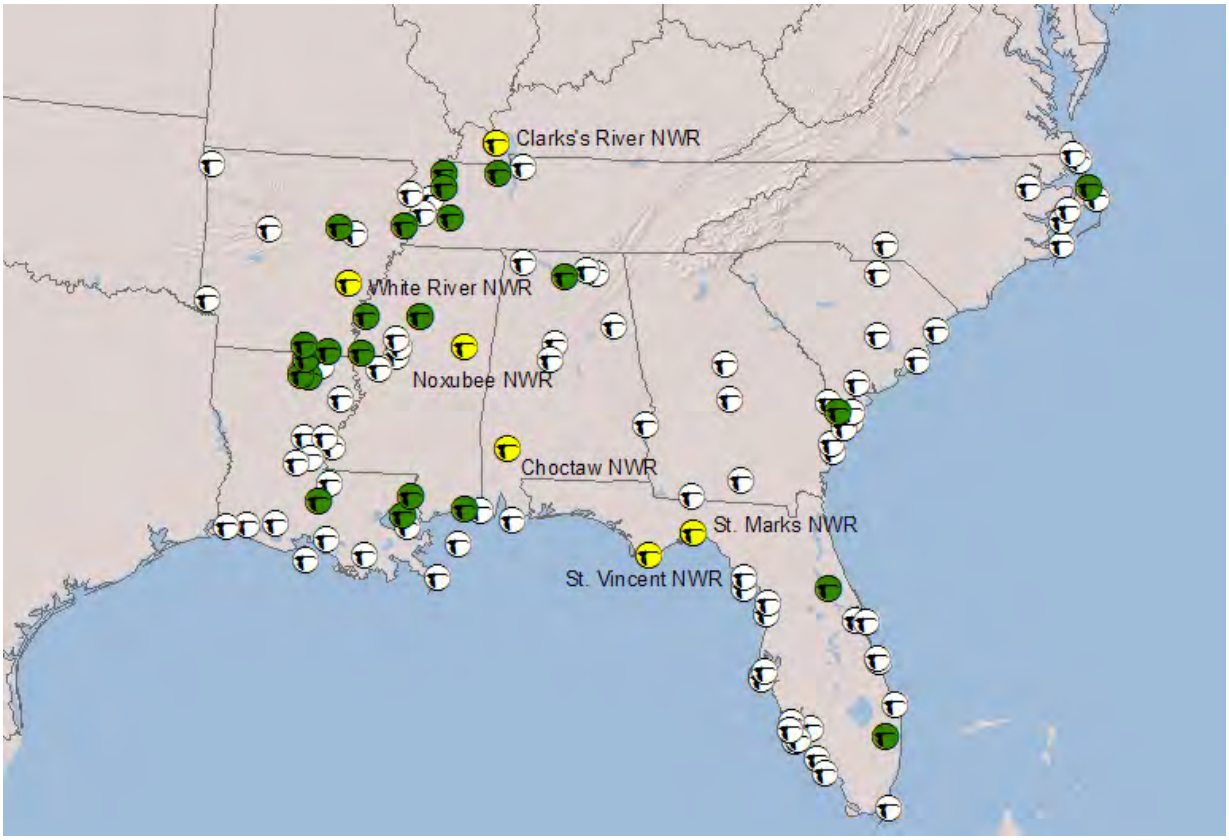

Figure 2: Six national wildlife refuges in the Southeast Region assessed during the 2007 sampling season highlighted in yellow and labeled by National Wildlife Refuge (NWR).

During 2007, the incidence of abnormalities exceeded background levels (3 percent; Dubois 1979, Johnson and Lunde 2001, Ouellet et al. 1997, Stocum 2000) in three of eight samples taken from Clarks River NWR (5.6-6.6% abnormal) and two samples taken from Noxubee NWR (8 and 13% 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, as well as asymmetrical eyes. Other observations such as variant colorations, abrasions and similarly obvious soft tissue damages were not reported as abnormalities.

A total of 28 abnormal specimens were collected across the Southeast Region during 2007 during the collection of 700 individuals from full collections and several hundred more from partial collections.

The abnormal specimens collected 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 have been received. Interpretations of the radiographs lead to final determinations on the field results.

Samples of live metamorphs were shipped for parasitological analysis. Four refuges in the southeast were targeted for sampling including St. Marks and Clarks River NWRs. Samples were sent from both refuges despite no complete collections being made at St. Marks NWR. Results are pending.

The primary goal of the Abnormal Amphibian Monitoring Project for the 2008 season is the completion of validation and quality assurance to ensure the best possible evaluation of the cumulative data. With that primary task in mind and if time and budget allow, four refuges are targeted for repeat sampling in 2008. These are the Choctaw, Clarks River, Noxubee and St. Vincent NWRs. Coldwater River NWR in Mississippi and Okefenokee and Banks Lake NWRs in Georgia are new refuges expected to be sampled in 2008. Relative geographic location of refuges to be sampled during the 2008 season is shown in Figure 3.

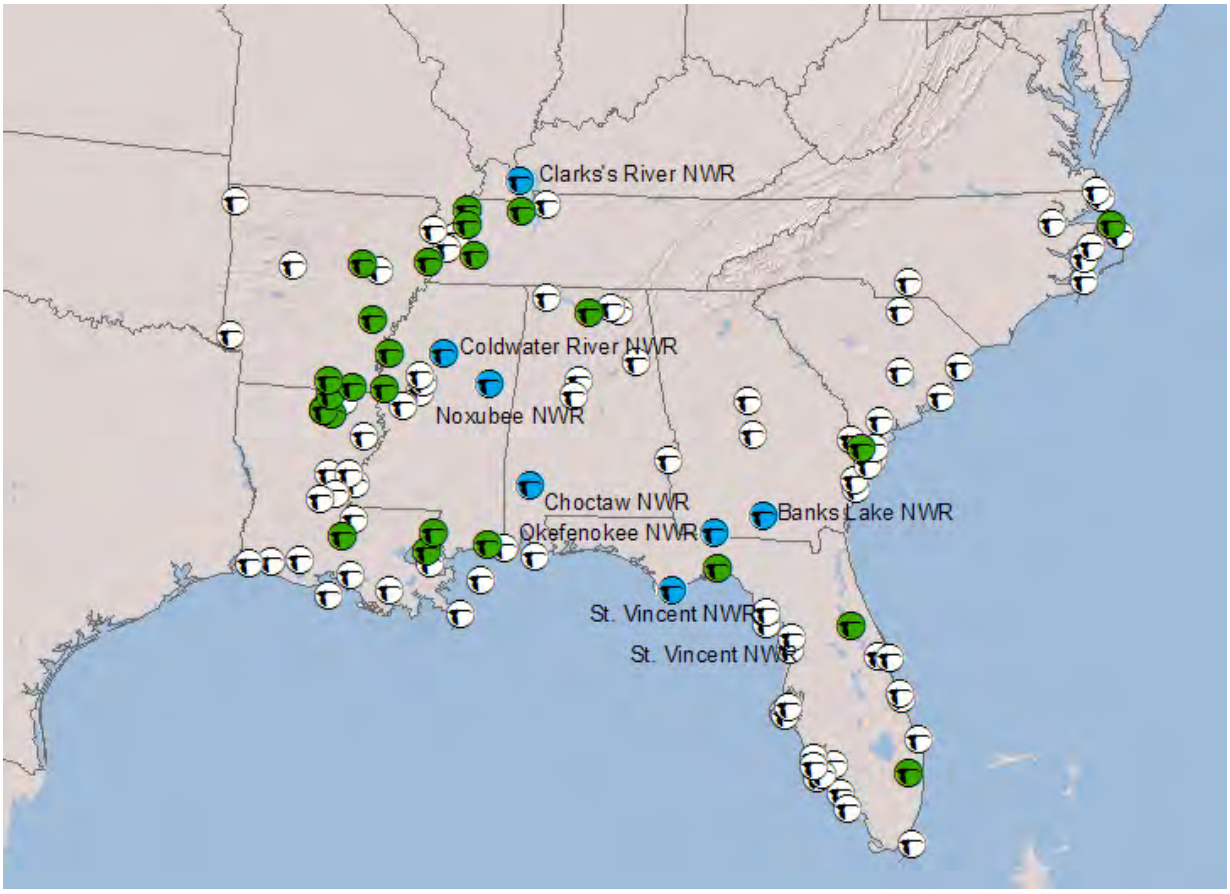

Figure 3: The seven national wildlife refuges in the Southeast Region to be assessed during the 2008 sampling season (blue icons) compared to refuges already sampled (green icons) and refuges where sampling has not yet been attempted (white icons).

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 2007 season.

Six national wildlife refuges in the Southeast Region were assessed during 2007. Three new refuges were sampled this year including Choctaw, Noxubee and St. Vincent NWRs. Clarks River NWR, Kentucky's only NWR, was sampled for the second funded season, but after a six year interval. Both St. Marks NWR in Florida and White River NWR in Arkansas completed a third year of sampling under severe drought conditions. Successful collections were conducted at Clarks River (n=8), Noxubee (n=3), and White River (n=1) NWRs, but not at St. Vincent, St. Marks or Choctaw NWRs because of drought conditions.

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): https://intranet.fws.gov/contaminants/amphibians.htm). 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 CAP database. 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. St. Marks NWR and Clarks River NWR were targeted for sampling this season with little success due to limited sampling. 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.

A. Alabama Refuges

1. Choctaw National Wildlife Refuge

The Choctaw National Wildlife Refuge is located in southwest Alabama along the Tombigbee River approximately 80 miles north of Mobile. The Refuge was established in 1964 on lands acquired by the Corp of Engineers in conjunction with the Coffeeville Lock and Dam project. The 4,218 acre refuge encompasses approximately 1,802 acres of lakes, sloughs, and creeks, 2,265 acres of bottomland hardwoods, and 151 acres of croplands and moist soil units.
The refuge is divided into three units by Okatuppa and Turkey Creeks. This makes a large portion of the refuge accessible only by boat. Due to its location along the Tombigbee River, the entire refuge is subject to annual Spring flooding.

The primary purpose of the refuge is to provide wood duck brood habitat and serve as a protected wintering area for waterfowl. Up to 200 broods of wood ducks are produced annually in the refuge's artificial nest boxes, and wintering waterfowl numbers can exceed 10,000. In addition, numerous neotropical migrant and wading birds benefit from management activities. Following a successful bald eagle hacking program in the early 1990's the refuge has played host to a nesting pair of eagles each winter. During the summer months wood storks can be found resting and feeding in the back-water sloughs and moist soil units. Resident wildlife includes white-tailed deer, gray squirrels, turkey, raccoons, opossum, American alligator, and beaver

Abnormal amphibian sampling was initiated on Choctaw NWR in 2007. Ten study sites were designated for sampling during our initial visit to the refuge (Table 2). All ten sites were found dry shortly later and were not viable collection sites for the study. Those sites that persisted were able to maintain adequate water levels because they were attached to larger bodies of water like

rivers and swamps. Sampling attempts were made three times. Despite observations of tadpoles at early gosner stages, no metamorphs were observed or collection. Drought conditions severely impeded collection efforts. Adequate habitat exists and will provide for successful collections, precipitation providing.

Table 2 . Results for sampling efforts on Choctaw NWR.

	YEAR 1 (2007)			
Site Name	Sample Date	Species Code	% abnormal	Fate
CHC01	n/a			
CHC02	n/a			
CHC03	n/a			
CHC04	n/a			
CHC05	n/a			
CHC06	n/a			
CHC07	n/a			
CHC08	n/a			
CHC09	n/a			
CHC10	n/a			

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

B. Arkansas Refuges

1. 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 2007, a total of seven sample locations were monitored on White River NWR for a third time (Table 3). Some sites included multiple adjacent ponds. The majority of the sites sampled were within the Jacks Bay Unit of the refuge. Most of the amphibian surveys were initiated at approximately 10:00 am each morning. These surveys continued until late May at which time all monitoring sites dried, despite having more precipitation than in previous years.

One full collection was made at White River NWR during the 2007 sampling season. The sample was taken from WHR07, "Jacks Bay Levee B Low Water Crossing." The sample consisted of 73 southern leopard frogs (*Rana sphenocephala*) that were mostly in Gosner stage 43. No abnormalities were observed in the sample. During this time, incomplete samples of southern leopard frogs were made, ranging from 13 to 25 individuals among five attempts.

This was only complete collection made during three years of sampling. Lack of water resulting from the prolong drought created conditions that appeared favorable in the early spring each year, but dried before the metamorph developmental stage was reached (Figure XX). This third year was attempted because the significant planning and sampling effort put forth by the refuge staff each year to obtain successful collections. This was the third and final year of sampling at White River NWR. No abnormalities were observed at this refuge.

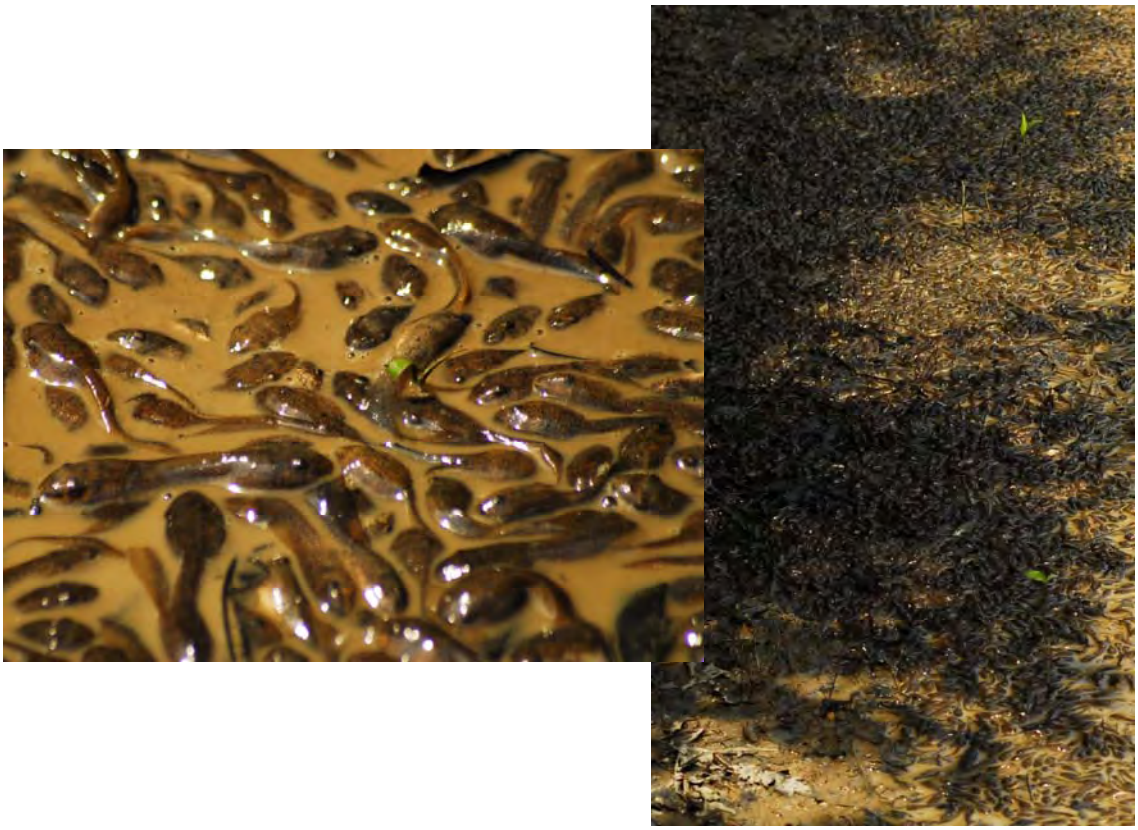

Figure 4: Typical fate of frog larvae on White River NWR during abnormal amphibian monitoring from 2005 to 2007.

Table 3. Results for sampling efforts on White River NWR.

	YEAR 1 (2005)				YEAR 2 (2006)			
Site Name	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			

	YEAR 3 (2007)			
Site Name	Sample Date	Species Code	% abnormal	Fate
WHR01	n/a			
WHR02	n/a			
WHR03	n/a			
WHR04	n/a			
WHR05	n/a			
WHR06	n/a			
WHR07	05/26/2007	RASP	0%	

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

C. Florida Refuges

1. 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 nearly 70,000 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.

This was the third and final year of participation for St. Marks NWR in the Service's Abnormal Amphibian Program (Table 4). In addition to the five sites that were monitored over the past two years, numerous other sites were monitored (n=27) between April and August 2007 in an attempt to improve sampling success during this prolong drought. However, all sites dried before tadpoles reached the metamorph stage. One incomplete sample of bronze frogs (*Rana clamitans clamitans*) was obtained and sent for parasitological evaluation via Dr. Larson. Results are pending.

Table 4. Results for sampling efforts on St. Marks NWR.

	YEAR 1 (2005)				YEAR 2 (2006)			
Site Name	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

	YEAR 3 (2007)			
Site Name	Sample Date	Species Code	% abnormal	Fate
SMK01	n/a			
SMK02	n/a			
SMK03	n/a			
SMK04	n/a			
SMK05	n/a			

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

2. St. Vincent National Wildlife Refuge

St. Vincent National Wildlife Refuge was established in 1968 in Franklin County, Florida. The refuge is an undeveloped barrier island just offshore from the mouth of the Apalachicola River, in the Gulf of Mexico. The refuge is managed to preserve ten separate habitat types, including: tidal marsh; freshwater lakes and streams; dunes dominated by live oak/mixed hardwood understory; scrub oaks; relatively pure stands of cabbage palm; and four different slash pine communities, each with its own unique understory species.

Abnormal Amphibian Survey sampling was conducted for the first time at St. Vincent NWR in 2007 (Table 6). As a result of an ongoing drought in the region, few suitable locations for sampling were found (Figures 4 to 7). Two (SV03 and SV04) of the four sites found suitable for sampling early in the season, dried early without successful samples taken. A third site (SV01) retained water throughout the sampling, however very few adult and larval leopard frogs were captured there. A final site (STV02) also retained water throughout the spring and summer. A large number of adult leopard frogs were observed at this location, but not tadpoles or metamorphs were found among the omnipresent American alligators. Predation of tadpoles was suspected (Figure 8).

Ten adult leopard frogs were collected from this final site (SV02) for parasitological evaluation despite full collections of metamorphs not being possible. Results are pending.

Table 5. Results for sampling efforts on St. Vincent NWR.

	YEAR 1 (2007)			
Site Name	Sample Date	Species Code	% abnormal	Fate
STV01	n/a			
STV02	n/a			
STV03	n/a			
STV04	n/a			

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

Figure 5: St. Vincent NWR Site 1 (STV01) located at N29.65323 and W 85.15543, looking east followed by west of the access road (B4) that runs north-south through this wetland.

Figure 6: St. Vincent NWR Site 2 (STV02) located at N29.64754 and W 85.14219, looking east followed by west of the access road (B5) that runs north-south through this wetland.

Figure 7: St. Vincent NWR Site 3 (STV03) located at N29.63393 and W 85.11167, looking north of the access road that runs east-west through across the island.

Figure 8: St. Vincent NWR Site 4 (STV04) located at N29.63533 and W 85.10816, looking north of the access road that runs east-west through across the island.

Figure 9: American alligators (*Alligator mississipiensis*) in sampling sites at St. Vincent National Wildlife Refuge and a couple of brave sampling souls.

D. Kentucky Refuges

1. Clarks River National Wildlife Refuge

Clarks River National Wildlife Refuge is a bottomland hardwood forest located in western Kentucky. The refuge lies along the East Fork of the Clarks River and is seasonal home to over 200 different species of migratory birds. The bottom lands are dominated with overcup oaks, bald cypress, and tupelo gum, and the slightly higher, better drained areas, are covered with willow oak, swamp chestnut oak, red oak, sweet gum, sycamore, ash and elm.

In May of 2007 wetlands on the refuge were evaluated to determine suitable survey sites. Nine sites were selected and monitored for tadpoles and/or egg masses. Sites were monitored from May through August when the drought preempted further sampling. Many of the original

proposed sites went dry before tadpoles could reach the proper Gosner stage for sampling. Although there were many sites monitored initially, only nine sites were able to be sampled (Figures 9 to 17). The sites were labeled CLK01 through CLK09. The drought also limited repeat samples from all but one of the sites.

Figure 10: Clarks River NWR Site 1 (CLK01) located at N36º 58 50.2 and W 88º 33’ 42.0’’, from center of “water hole” also known as “Blizzard 2”.

Figure 5: Clarks River NWR Site 2 (CLK02) located at N36º 53 25.9 and W 88º 22’ 47.4’’, backwater flood plain also known as “Castleberry”.

Figure 6: Clarks River NWR Site 3 (CLK03) located at N36º 52 36.5 and W 88º 20’ 51.3’’, a backwater floodplain also known as “Chambers”.

Figure 7: Clarks River NWR Site 4 (CLK04) located at N36º 55 38.8 and W 88º 27’ 30.3’’, a roadside ditch also known as “Elva 1”.

Figure 8: Clarks River NWR Site 5 (CLK05) located at N36º 55 46.9 and W 88º 27’ 17.6’’, a man-made pond also known as “Elva 2”.

Figure 9: Clarks River NWR Site 6 (CLK06) located at N36º 55 41.7 and W 88º 26’ 58.6’’, an impoundment or cultivated field also known as “Elva 3”.

Figure 16: Clarks River NWR Site 7 (CLK07) located at N36º 51 26.0 and W 88º 19’ 52.5’’, a man-made pond also known as “Shop Impoundment Pond”.

Figure 10: Clarks River NWR Site 8 (CLK08) located at N 36º 58 46.8 and W 88º 33’ 51.5’’, a man-made pond also known as “Blizzard 1”.

Figure 18: Clarks River NWR Site 9 (CLK09) located at N36º 56 03.6 and W 88º 28’ 35.1’’, an impoundment also known as “Redhead”.

One collection was made at each of the following sites: CLK02, CLK03, CLK05, CLK06, CLK08. Two full collections were made at site CLK09. Field observations of abnormalities ranged from 0 to 6.6 percent (Table 7). Radiographic specimens were sent from collections with abnormalities to Dr. Michael Lannoo as described previously.

Table 6. Results for sampling efforts on Clarks River NWR.

	YEAR 1 (2001)				YEAR 2 (2007)			
Site Name	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate, Confirm%
CLK01	n/a				n/a			
CLK02	n/a				05/24/07	RASP	6.6	R, 5.2
CLK03	08/15/01	BFOW	0		05/29/07	BFOW	2.4	R, 0.0
CLK04	n/a				n/a			
CLK05	06/27/01	BFOW	0		06/01/07	BFOW	0	
CLK06	n/a				06/07/07	BFOW	5.6	R, 2.8
CLK07	n/a				06/08/07	BFOW	1.4	R, 1.4
CLK08	n/a				06/12/07	RASP	5.8	R, 1.9
CLK09	n/a				06/20/07	BFOW	0	
					03/30/07	ACBL	0	

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

A number of mole salamanders were collected at CLK01 but were not used in the because of uncertainty about how to determine Gosner stage (Figure 18). However, many were found to have missing feet (front, back or a combination of both). Seventy-six southern leopard frogs were examined at site CLK02 with five abnormalities observed including missing extremities and optical asymmetry (Figure19). Two individual Fowler's toads were found with a missing hind foot out of the 84 that were examined at CLK03 (Figure 20). Another 61 Fowler's toads were examined at CLK05 and no abnormalities were observed. Seventy-one more Fowler's toads were examined at site CLK06 where five individuals were found to have missing feet and one

with optical asymmetry (Figure 21). One noteworthy abnormality was found in the 73 Fowler's toads examined at site CLK07, a nub-like appendage where the tail was expected to be re-adsorbed (Figure 22).Southern leopard frogs were collected (n=52) at CLK08 and three individuals were observed with shortened digits, a missing forelimb and an enlarged red calf, respectively (Figure 23). Two collections were made at site CLK09 including 56 Fowler's toad, and 50 Blanchard's cricket frogs (*Acris crepitans blanchardi*).

Figure 11: Mole salamanders collected at site CLK01 at Clarks River NWR for the abnormal Amphibian Survey during 2007. These specimens were not used in the survey because of uncertainty about how to determine Gosner stage.

Figure 20: Southern leopard frogs collected at site CLK02 at Clarks River NWR for the abnormal Amphibian Survey during 2007.

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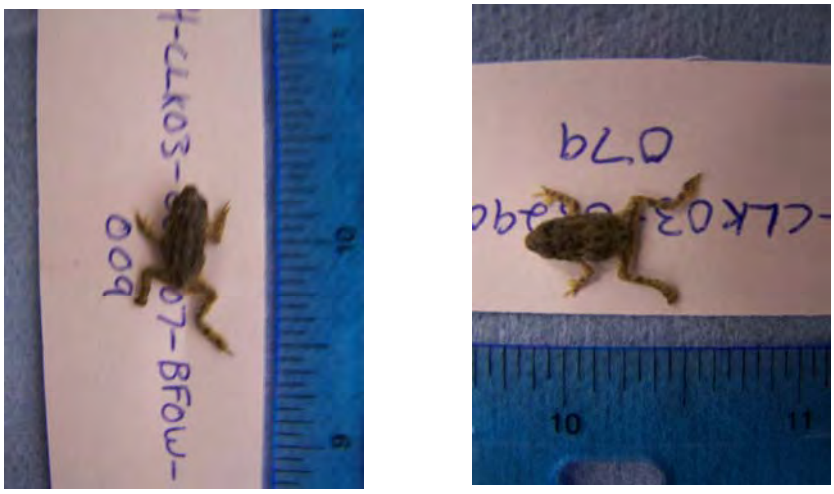

Figure 21: Fowler's toads collected at site CLK03 at Clarks River NWR for the abnormal Amphibian Survey during 2007.

Figure 12: Fowler's toads collected at site CLK06 at Clarks River NWR for the abnormal Amphibian Survey during 2007.

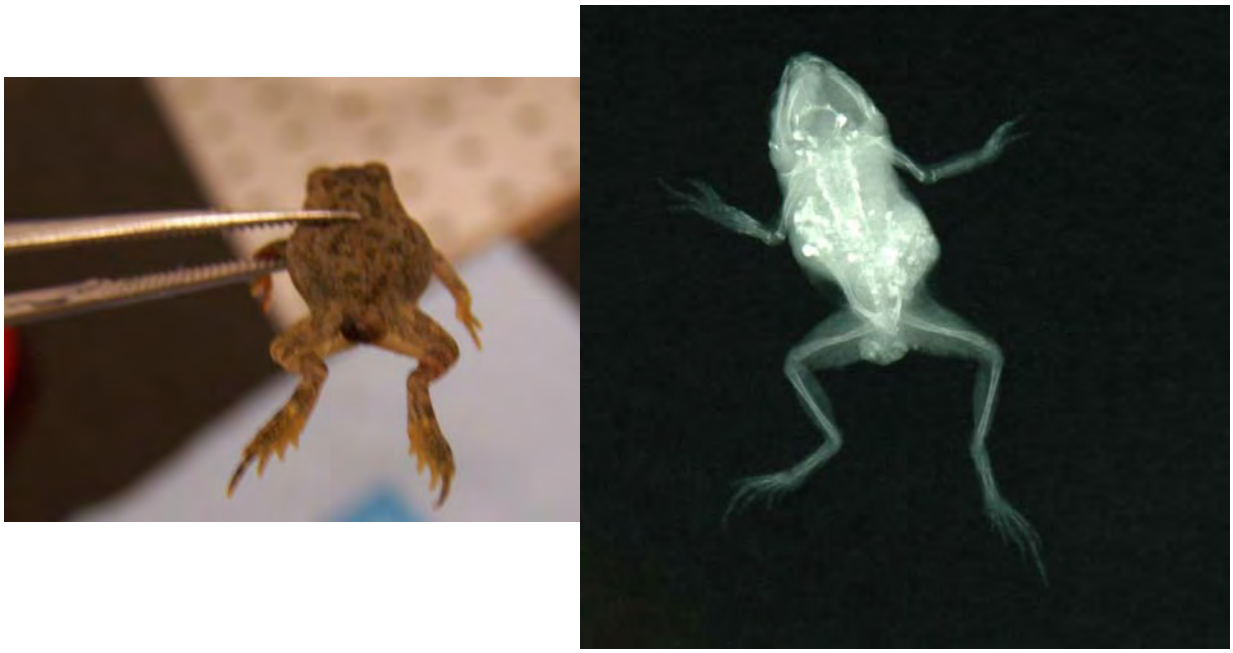

Figure 13: Fowler's toads collected at site CLK07 at Clarks River NWR for the abnormal Amphibian Survey during 2007.

Figure 14: Southern leopard frogs collected at site CLK08 at Clarks River NWR for the abnormal Amphibian Survey during 2007.

After radiologic examination, the following descriptions were provided. These descriptions were interpreted to differentiate between field observed abnormalities and true developmental malformations. Field observation at CLK02 of 6.6% was shown radiographically to represent a 5.2% malformation rate, CLK03 field of 2.4% were all determined to be trauma, CLK06 5.6% field observation was found to be 2.8% malformed, CLK08 5.8% field observation was found to be 1.9% malformed, and finally the field observation at CLK07 of 1.4% was confirmed and noteworthy. It was described as a growth on the rump of one toad and was confirmed radiographically as rudiments of multiple limbs (Figure 13).

Radiological Evaluation and Interpretation by Dr. Michael Lannoo

R4-CLK02-052407-RASP-013 Missing right hind foot. Femur of affected limb normal. Tibio-fibula shortened. Position of nutrient artery (density in the middle of a bone) proximal compared to normal limb, suggesting abnormal growth, therefore developmental problem.

R4-CLK02-052407-RASP-021 Missing left hind foot/ small eye. Unresorbed tail, which may be normal. Small eye, but skull ossification is symmetrical. In leg with missing foot, tibio-fibula shortened, thin, not completely ossified. No bones present beyond this point. Foot is fleshy. In affected limb, ventral portion of knee, there is more pigment present than on normal leg; do not know whether this is abnormal or within the normal range of asymmetry. The combination of no bones in foot, affected eye, possibly affected tail, and possibly affected pigment, suggests this is a developmental problem. Otherwise this animal is extremely unlucky.

R4-CLK02-052407-RASP-022 Missing digits on right hindfoot. This animal has unresorbed tail. Both eyes present and symmetrical. On leg with missing digits, tibio-fibula shortened, incompletely ossified and lightly ossified bones present distally. In normal limb, tibio-fibula is irregularly ossified. Difficult to determine whether this is trauma or developmental. Unlikely, though not impossible, that trauma could affect both tibio-fibulas, resulting in missing foot in one limb and irregular bone in the other. Presence of several

animals that look like this from this population suggests a developmental origin rather than trauma, although this is clearly an open discussion.

R4-CLK02-052407-RASP-030 Missing entire calf on right hind leg. Radiographically, femur short and irregularly ossified. Dorsal surface of affected limb exhibits abnormal pigment compared to opposite side; therefore appears to be a developmental problem.

R4-CLK02-052407-RASP-047 Digits missing from right hind foot. Tibio-fibulare short; distal bones weakly ossified. Could be trauma or developmental, but I'm leaning towards trauma.

R4-CLK03-052907-BFOW-009 Missing left hindfoot. Femur and tibio-fibula normal. Proximal portions of tibio-fibulare in stump. Looking at the specimen, this could be either developmental or trauma, with me leaning towards trauma.

R4-CLK03-052907-BFOW-079 Missing left hindfoot. In affected limb, femur and tibio-fibula normal sized. Ossification of feet not well developed, therefore difficult to see bones. Looking at the specimen, this could be either developmental or trauma, with me leaning towards trauma.

R4-CLK04-060207-RASP-028 Missing right foot. Bones of tibio-fibulare are present, shortened, lightly ossified. Bones proximal are normal length, normal ossification, normal curvature. Difficult to determine whether this is trauma or developmental.

R4-CLK06-060707-BFOW-002 Missing right front foot. Proximal portions of limb may be present, but shortened. No bone as long as the humerus can be seen in radiograph. Clearly a developmental issue. This is not a simple failure of, or delay in, the limb to emerge.

R4-CLK06-060707-BFOW-015 Small left eye. Skull bones developed normally and symmetrically, therefore cannot determine whether this is trauma or development.

R4-CLK06-060707-BFOW-047 Missing right hind foot. Tibio-fibula also small. No ossified bones revealed distal to this. I suspect this is not trauma, but it might be.

R4-CLK06-060707-BFOW-052 Missing right hindfoot. Tibio-fibula shortened. Appears to be terminal expansion at point where limb goes missing. If so, would suggest a developmental problem. Affected limb segment also abnormally pigmented, suggesting developmental problem.

R4-CLK07-060807-BFOW-020 Growth on rump. Two tiny ossification sites revealed in radiograph. Suggests that this is not simply an unresorbed tail, but maybe the rudiments of multiple limbs. Not trauma.

R4-CLK08-061207-RASP-007 Right calf enlarged and red. Nothing diagnostic radiographically. Redness could be due to trauma, or to bacterial infection redleg which can occur in stressed animals.

R4-CLK08-061207-RASP-014 Digits shortened on left hind foot. This may be trauma. Digits are missing in a linear fashion, suggesting a shearing trauma. Remaining bones of the foot not well ossified.

R4-CLK08-061207-RASP-048 Missing right front limb (skin still attached). What's unusual is that the forelimbs emerged, but the tail hasn't begun to be resorbed. Because of the presence of the tadpole spiracle, the left forelimb emerges first through the spiracle; right forelimb emerges later. Proximal portion of affected forelimb appears normal. Distal bones appear variously ossified, perhaps wavy. Comparing skull ossification to other animals, it appears that tail resorption is indeed late.

Clarks River NWR, Kentucky's only NWR, was sampled for the second funded season, but after a six year interval. We intend to sample this refuge again in during the 2008 sampling season to provide two consecutive years of survey.

E. Mississippi Refuges

1. Noxubee National Wildlife Refuge

Noxubee NWR is a 48,000 acre refuge located in central Mississippi. Land for the Noxubee NWR was obtained in the 1930s through the Resettlement Administration. The refuge was established in 1940 to ensure the wetland protection as a means of providing migratory bird species and other animals a safe haven. Of the 48,000 acres (194 km^2) of land, approximately 44,500 acres (180 km^2) consists of bottomland and upland forest.

Noxubee NWR was sampled for the first time during the 2007 season by the same participants that sampled the Choctaw NWR this year. As with many areas sampled during this dry season, several sites did contain anuran egg masses or larvae, but populations were not adequate for complete samples or dried before tadpoles reached the required stage. Ultimately, ten sites were selected and consistently monitored for anuran larvae development. Some of these sites dried early in the monitoring season. Three successful collections were achieved, including fifty Fowler's toads at site NXB01, 54 grey tree frogs (*Acris crepitans*) at NXB03, and 50 northern cricket frogs (*Hyla versicolor*) at site NXB04. Each of these 3 collections was difficult to obtain and required 2-3 days of sampling at the same site to acquire a full collection. Field abnormality rates for these collections were 2% (NXB01), 13% (NXB03), and 8% (NXB04), respectively (Table 8). Radiographic specimens were sent from collections with abnormalities to Dr. Michael Lannoo as described previously.

Noxubee NWR field results were compared with radiographic results for 21 individuals. Descriptions of these evaluations are provided below. Many of the field abnormality observations were omitted from the rates reported here. They were skin associated observations such as red bumps or irregular warts. The only radiographically confirmed abnormality was for

NXB03, a roadside ditch. The missing eye appeared to be a developmental error. This made the confirmed abnormality rate 1.9% compared to the 13% abnormal observation from the field.

Radiological Evaluation and Interpretation by Dr. Michael Lannoo

R4-NXB01-070207-BFOW-
042 Nothing shows up radiographically; may be due to failed predation or trauma.

003 Toes missing but because of the position in which animal was preserved with toes adducted, and arms tucked under the animal, impossible to determine from radiograph. Animals need to be preserved in an anatomical position so bones can be visualized unambiguously. Especially important with smaller animals. Appears to have ectoparasites attached to ventral surface of abdomen. Appear as small open cases attached to abdominal skin. Considered that these may be seed pods or ostracods incidentally attached, but these are adherent and so this interpretation is unlikely.

005 Preserved animal does not appear to be abnormally bloated or bruised. No signs of edema or hygroma. There are signs of the ectoparasite-like effect mentioned in the 003 description.

009Red bumps are associated with warts. They are unusual, but since they're not ossified, do not show up in radiographs. Suspect some sort of skin infection, perhaps viral or fungal, but best course of action would be to contact David E. Green at the National Wildlife Health Lab and arrange to ship live specimens to him. This diagnosis holds for all other animals in this series including: 031, 018, 050, 041,017, 016, 035

R4-NXB03-072307-HYCH-
024 Missing eye and socket on right side. Examined radiograph under microscope; skull elements associated with eye not formed. No scarring or pigment issues; appears to be developmental. If due to trauma or accident, happened very early, or immediately post-hatching.

042 Tail shortened. Might be due to predation, but probably no long term consequences, because tail is resorbed at metamorphosis.

028 Notched tail. Tail also kinked, but probably no long term consequences because tail is resorbed at metamorphosis.

046 Tail shortened. Might be due to predation, but probably no long term consequences, because tail is resorbed at metamorphosis.

004 Missing eye. This appears to be due to trauma, because skin is irregular at the position of the eye and underlying skull bones are present and symmetrical. Compare this radiograph to radiograph of 024 and you see clear differences.

002 Cannot see this in radiograph because affected arm was preserved in a position under the body. Proximal portion of hand appears expanded. Unable to determine whether this is trauma or developmental.

039 Tail shortened. Might be due to predation, but probably no long term consequences, because tail is resorbed at metamorphosis.

R4-NXB04-072507-ACCR-

023 Broken tail; might be due to predation, but probably no long term consequences, because tail is resorbed at metamorphosis.

002 Right hind limb missing proximal and distal portions of foot. Some remaining foot elements are normal sized. This abnormality is consistent with predation.

047 Tail broken or shortened; might be due to predation, but probably no long term consequences, because tail is resorbed at metamorphosis.

036 Right leg missing foot. Left leg bent tibio-fibula; missing/shortened foot elements. No pigment abnormalities. Both legs look traumatized. Left tibio-fibula bends at right angle; know of no developmental anomalies like this. I suspect trauma.

Noxubee was sampled for the first time this season. We intend to sample this refuge again in during the 2008 sampling season to provide two consecutive years of survey.

Table 7. Results for sampling efforts on Noxubee NWR.

	YEAR 1 (2007)			
Site Name	Sample Date	Species Code	% abnormal	Fate, Confirm%
NXB01	07/02/07	BFOW	2.0	R, 0.0
NXB02	n/a			
NXB03	07/23/07	HYCH	13.0	R, 1.9
NXB04	07/25/07	ACCR	8.0	R, 0.0
NXB05	n/a			
NXB06	n/a			
NXB07	n/a			
NXB08	n/a			
NXB09	n/a			
NXB10	n/a			

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

Diagnostic Results

A. Radiography

Abnormal specimens collected during 2007 were preserved and sent directly to Dr. Michael Lannoo from the NWRs. Examination for skeletal abnormalities were be made by Dr. Lannoo. Comparisons with field observations are discussed separately within the results for each refuge submitting specimens for examination. During the 2007 season, only Clarks River (n=16) and Noxubee (n=21) NWRs sent specimens for radiological evaluation by Dr. Lannoo.

Previous radiograph results and discussions are being reconciled with the field observations reported in the database. They 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: St. Marks, Clarks River, St. Vincent and Choctaw. Samples were sent from St. Marks and Clarks River. Results will follow examination by Dr. Johnson. All specimens selected for parasitological analyses will be forwarded to Dr. Lannoo for follow-up radiography.

Discussion

Seven years of sampling on refuges in the Southeast Region, indicate that frog abnormality rates can vary 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, Noxubee, 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.

However, it should also be noted that radiographic confirmation of field-identified abnormalities as developmental errors is often lacking.

All available data from field inspections and from radiographic and parasitological diagnoses will be inspected for quality during the 2007-2008 seasons. 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 2008. Decisions about the need for chytrid sampling on NWRs, parasitological evaluation needs, and other needs for the 2009 survey season will be based on the information provided in the five-year data review.

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