

*DEPARTMENT OF THE INTERIOR
U.S. FISH AND WILDLIFE SERVICE*



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

END OF YEAR REPORT:

Southeast Region

FY 2008

by

Alicia C. Newberry and Jon M. Hemming

Panama City Field Office

Panama City, Florida

and

Bill Starkel

Regional Environmental Contaminants Coordinator

Southeast Regional Office

Atlanta, Georgia

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Mississippi

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

Coldwater River NWR: Becky Rosamond, Hal Mitchell, Mark Williams

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 (2001-2008) (Table 1). Seven national wildlife refuges in the Southeast Region were assessed during 2008 (Figure 1). Three new refuges were sampled this year including Coldwater River, Banks Lake and Okefenokee NWRs. Choctaw, Noxubee, and St. Vincent NWRs participated in their second year of sampling. Clarks River NWR, Kentucky's only NWR, completed its second consecutive year of sampling in 2008 and its third year overall. Successful collections were conducted at Clarks River (n=6), Coldwater River (n=3), Noxubee (n=5), and Okefenokee (n=5) NWRs, but not at Banks Lake, Choctaw, or St. Vincent NWRs because of a sparse number of metamorphs.

During 2008, the incidence of abnormalities exceeded background levels (3 percent; Dubois 1979, Johnson and Lunde 2001, Ouellet et al. 1997, Stocum 2000) in two of five samples taken from Noxubee NWR (3.8-3.9% abnormal) and one of three samples taken from Coldwater River NWR (8.9% abnormal). As with the previous reports, the abnormalities encountered consisted primarily of missing or clubbed digits, feet, or limbs. Other observations such as variant colorations, abrasions and similarly obvious soft tissue damages were not reported as abnormalities.

A total of 13 abnormal specimens were collected across the Southeast Region during 2008 during the collection of 1164 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 with the exception of the 2 samples from collection NXB12 (Fowler's toad) which were lost prior to submission for radiology. Examination and final determination for specimens examined by Dr. Lannoo have not yet been received. Interpretations of the pending

radiographs will lead to the final determinations on the field results. Samples of live metamorphs were not successfully collected for parasitological analysis this year.

The primary goal of the Abnormal Amphibian Monitoring Project for 2008 season was the completion of data validation and quality assurance to ensure the best possible evaluation of the cumulative data. This validation was performed to facilitate the analysis of the region's, as well as the nation's, cumulative survey data. The regional and national data have been analyzed and a draft report is currently in review.

Based on the outcome of the cumulative data analysis, the strategy for the upcoming 2009 season will include 3 main components. First, the national and region cumulative data review and analysis will be completed. Second, several refuges were determined to require follow up surveys based on the initial refuge results (high abnormality rates). Of these, both Overflow and Felsenthal will be able to perform follow up amphibian surveys in 2009. And finally, the program will continue to expand to include consecutive sampling years at more refuges in the Southeastern Region. Coldwater River NWR in Mississippi and Okefenokee and Banks Lake NWRs in Georgia will continue sampling in 2009 for their second year of sampling each. Relative geographic location of refuges to be sampled during the 2009 season is shown in Figure 2.

Table 1. Summary of all results from Southeast Region NWRs assessed for abnormal amphibians from 2000 to 2008.

State	Refuge	Range of Incidences of Abnormalities (%) found								
		2000	2001	2002	2003	2004	2005	2006	2007	2008
AL	Wheeler NWR					13-22 % ^F	3.9-13.9 % ^F	3.6-12.7 ^F		
	Choctaw NWR								D,F	F,N
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	F,N
GA	Banks Lake NWR									F,N
	Okefenokee NWR									0 % ^F
	Savannah NWR				0 % ^F	0-3.8 % ^F				
KY	Clarks River NWR		0 % ^F	D					0-6.6% ^F	0 % ^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 2008.

State	Refuge	Range of Incidences of Abnormalities (%) found								
		2000	2001	2002	2003	2004	2005	2006	2007	2008
	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	Coldwater River NWR									1.6-8.9% ^F
	Dahomey NWR		1-16.8% ^F	0-4.2 % ^F	3.7-9 % ^F	1-5.7 % ^V	V			
	Noxubee NWR								2-13% ^F	1.8-3.9% ^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.

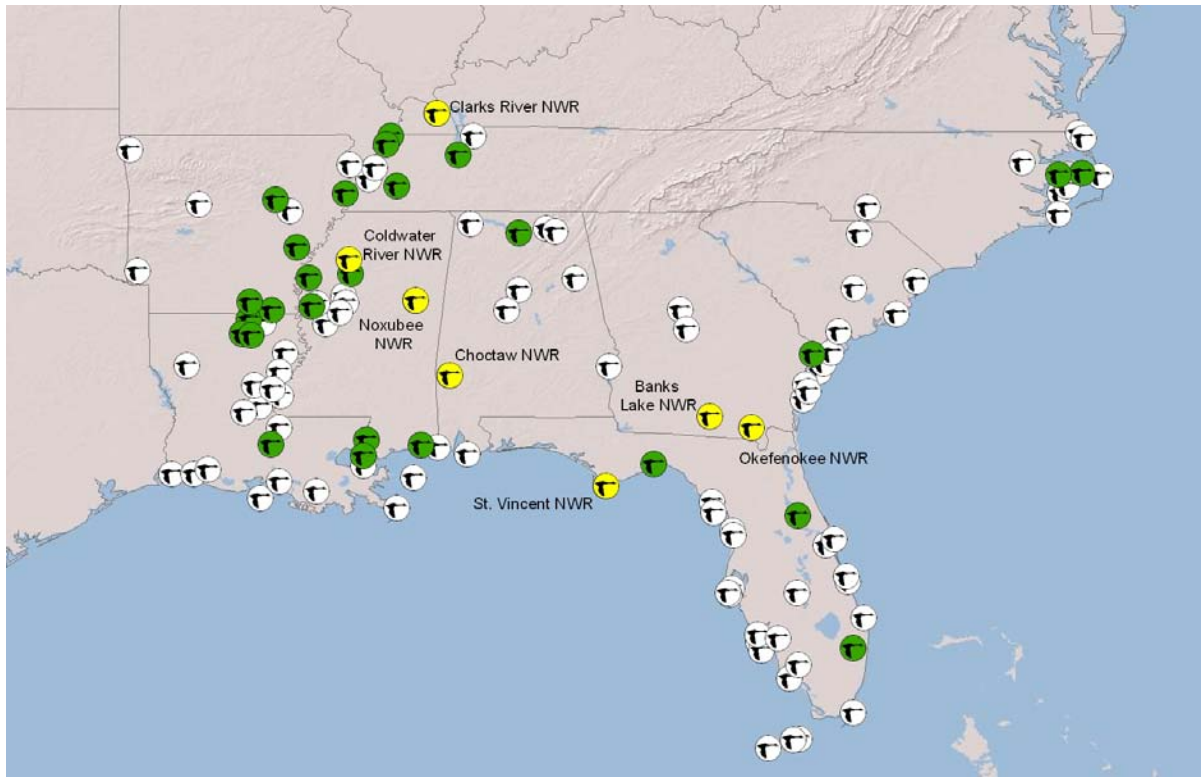


Figure 1. Locations of preliminary screening assessments for abnormal amphibians on national wildlife refuges (NWRs) in the southeast region from 2000 to 2007 (green icons), refuges that have not been sampled (white icons), and the 7 NWRs assessed during the 2008 sampling season highlighted in yellow and labeled.

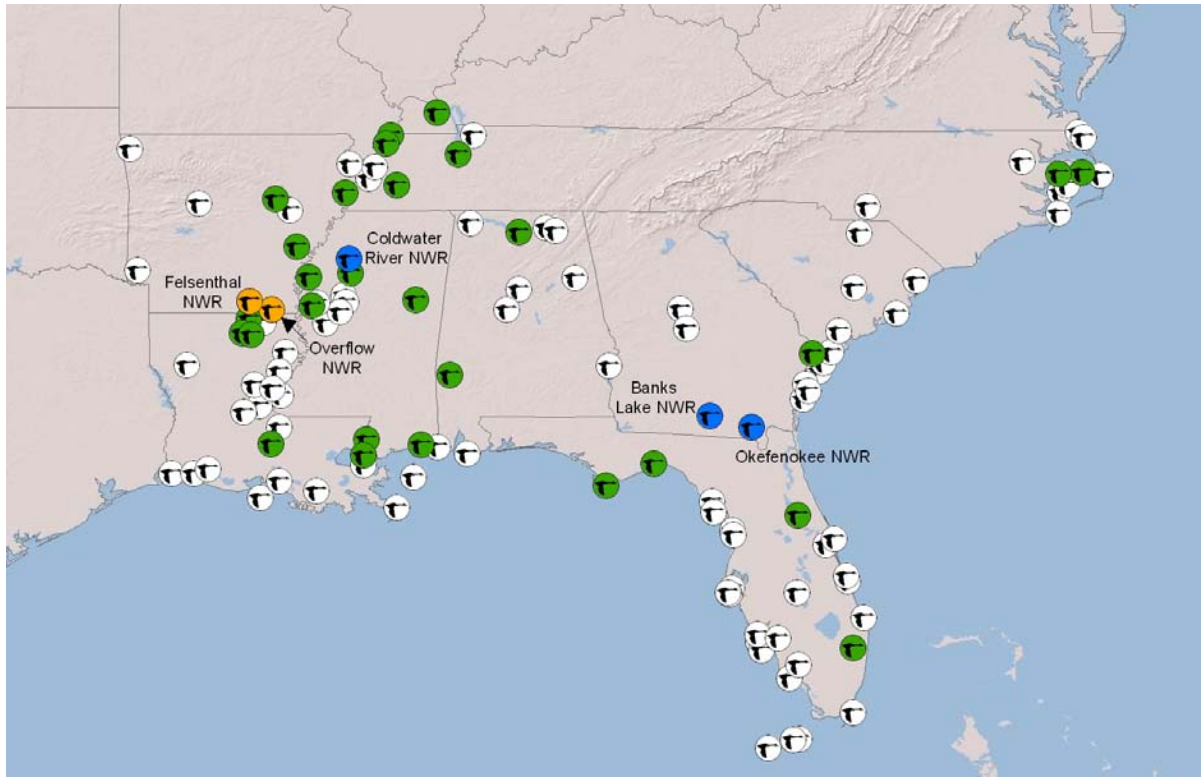


Figure 2. The five national wildlife refuges in the Southeast Region to be assessed during the 2009 sampling season are highlighted and labeled. Blue indicates a continuation of consecutive year sampling while orange indicates a follow up survey on return sampling refuges. Their relative geographic location is 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 2008 season.

Seven national wildlife refuges in the Southeast Region were assessed during 2008. Three new refuges were sampled this year including Coldwater River, Banks Lake and Okefenokee NWRs. Choctaw, Noxubee, and St. Vincent NWRs participated in their second year of sampling. Clarks River NWR, Kentucky's only NWR, completed its second consecutive year of sampling in 2008 and its third year overall. Successful collections were conducted at Clarks River (n=6), Coldwater River (n=3), Noxubee (n=5), and Okefenokee (n=5) NWRs, but not at Banks Lake, Choctaw, or St. Vincent NWRs because of the sparse number of the metamorphs encountered.

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. Sites were selected based on several criteria including: 1) presence of anurans, particularly *Rana* species; 2) documented or suspected occurrence of environmental contaminants; 3)

availability of sites with suitable and accessible habitat; 4) results from previous sampling and/or documented occurrences of abnormal frogs; and 5) availability of refuge staff for survey participation, especially in the pre-season monitoring of tadpoles. Potential amphibian breeding areas were identified based on site visits and discussions with refuge personnel and local experts. All sites were located within refuge boundaries. When possible, sites were selected to represent areas of the refuge affected by human development as well as areas free from known human disturbance.

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 are 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 shipped for parasitological analysis are sent to Dr. Pieter Johnson at the University of Colorado in Boulder, Colorado. Results will follow examination by Dr. Johnson. All specimens selected for parasitological analyses will be forwarded to Dr. Lannoo for follow-up radiography.

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.

Efforts to collect metamorphs continued to be difficult at Choctaw NWR during the 2008 sampling season, the refuge's second year of participation in the abnormal amphibian survey. Choctaw NWR is managed by Noxubee NWR which is approximately 170 miles away. Site visits to Choctaw were conducted on a weekly basis to review water conditions and observations of pools with eggs and tadpoles, with an emphasis on locating leopard and bronze frog eggs. This was the second year that both species could not be located despite knowledge where egg masses have been found in recent years. Water was extremely abundant in the early portion of the spring but efforts to obtain complete samples were unsuccessful. Future efforts should still consider sampling at this refuge due in part to its connection with the Tennessee-Tombigbee River.

Table 2 . Results for sampling efforts on Choctaw NWR.

Site Name	YEAR 1 (2007)				YEAR 2 (2008)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
CHC01	n/a				n/a			
CHC02	n/a				n/a			
CHC03	n/a				n/a			
CHC04	n/a				n/a			
CHC05	n/a				n/a			
CHC06	n/a				n/a			
CHC07	n/a				n/a			
CHC08	n/a				n/a			
CHC09	n/a				n/a			
CHC10	n/a				n/a			

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

B. Florida Refuges

1. St. Vincent's National Wildlife Refuge

St. Vincent National Wildlife Refuge was established in 1968 in Franklin County, Florida. The refuge is a triangular, undeveloped barrier island just offshore from the mouth of the Apalachicola River in the Gulf of Mexico. The island is dissected by dune ridges that are remnant beaches created by fluctuating sea levels over the last 5,000 years. Many of the sloughs and marshes along the inter-dune zone along the perimeter of the island are influenced by tidal flows and do not support amphibian populations. Small freshwater lakes and seasonal ponds found in the inner island provide suitable habitat for 10 species of anurans. 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 amphibians were assessed for the first time at St. Vincent NWR in 2007 without success due to drought conditions. In 2008, four additional collection sites were added to the 2007 sites to produce a more robust sample. The additional sites had sufficient aquatic vegetation and appeared to be able to hold adequate water to allow full development of metamorphs. All stations were sampled bimonthly from February through August. While water volume in all isolated ponds was dramatically reduced in the summer months, all sites held water through August which likely allowed full metamorphosis. As in 2007, tadpoles were infrequently collected from any site, and less than ten metamorphs were sampled overall.

Table 3. Results for sampling efforts on St. Vincent NWR.

Site Name	YEAR 1 (2007)				YEAR 2 (2008)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
STV01	n/a				n/a			
STV02	n/a				n/a			
STV03	n/a				n/a			
STV04	n/a				n/a			
STV05	n/a				n/a			
STV06	n/a				n/a			
STV07	n/a				n/a			
STV08	n/a				n/a			

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

C. Georgia Refuges

1. Banks Lake National Wildlife Refuge

Banks Lake National Wildlife Refuge is located in Lanier County, near Lakeland, GA, as part of a much larger backwater system. The Refuge was established in 1985 for the protection and conservation of this unique environment as well as migratory and resident wildlife. Banks Lake is a natural pocosin or sink of ancient geologic origin. The refuge contains a variety of habitat types including 1,500 acres of marsh, 1,549 acres of cypress swamp, and 1,000 acres of open water. It is neither funded nor staffed as a refuge. All work is done by staff and volunteers from Okefenokee NWR, 125 miles away.

The 2008 sampling season was Banks Lake NWR's first year of participation in the Service's abnormal amphibian survey. It was difficult to locate good sites that remained wet throughout the sampling season, but two sites were ultimately selected at Banks Lake that were not expected to dry up. Both sites were sampled from June through September. Two

species were targeted for collection, the southern leopard frog and the southern cricket frog. One large site (BLM02) contained many frogs of the correct Gosner stage and abundant numbers of the southern cricket frog were observed, but a full collection was never made. The other site was not a productive area for anurans. It was located around the boat basin in the lake and may have been heavily influenced by boat traffic.

Table 4. Results for sampling efforts on Banks Lake NWR.

	YEAR 1 (2008)			
Site Name	Sample Date	Species Code	% abnormal	Fate
BLM01	n/a			
BLM02	n/a			

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

2. Okefenokee National Wildlife Refuge

The Okefenokee National Wildlife Refuge was established in 1937 and is located in Ware, Charlton, and Clinch Counties, Georgia and Baker County, Florida. The Refuge comprises over 402,000 acres and is the largest National Wildlife Refuge in the eastern United States. The Refuge contains the majority of the Okefenokee Swamp, a 438,000 acre bog contained inside a saucer shaped depression that was once part of the ocean floor. It supports a variety of wildlife including 39 fish, 37 amphibian, 64 reptile, 234 bird, 50 mammal, and 621 plant species. The Okefenokee is a rainfall-dependent system, and when periods of drought occur, the area becomes susceptible to wildfire. A 20-30 year cycle of drought and fire has allowed the Okefenokee to exist as the unique wetland it is. These periods cause changes in the abundance of certain plants, the nesting success of certain wading birds, and the location of some species of wildlife.

Okefenokee NWR participated in the abnormal amphibian program for the first time in 2008. Deeper, more permanent bodies of water were sought out as sites so that they would not be affected by drought. As at Banks Lake NWR, two species were targeted for collection, the

southern leopard frog and the southern cricket frog. Six sites were sampled from May through September, yielding four full collections at three sites. Site OKE01 contained impressive numbers of the river frog and collectors were able to make two full collections at this site (n=105 and n=154). A full collection of one of the targeted species, the southern leopard frog, was made at OKE8C (n=50). One full collection was also made of the little grass frog at site OKE14A (n=58). Although the other targeted species, the southern cricket frog, was observed in large numbers at many of the sites, no full collection was made. No abnormalities were observed at any of the sites.

Table 5. Results for sampling efforts on Okefenokee NWR.

	YEAR 1 (2008)			
Site Name	Sample Date	Species Code	% abnormal	Fate
OKE01	8/1/08	RAHE	0	
	9/14/08	RAHE	0	
OKE8A	n/a			
OKE8B	n/a			
OKE8C	9/7/08	RASP	0	
OKE14A	7/3/08	PSOC	0	
OKE15H	n/a			

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

D. Kentucky Refuges

1. Clark's River National Wildlife Refuge

Clarks River National Wildlife Refuge, Kentucky's only NWR, was established in 1997 and is an 8,500 acre 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.

Clark's River NWR was sampled for the second consecutive year during the 2008 season, its third year overall. Beginning in May, 11 sites were selected and monitored for tadpoles and/or egg masses, including two new sites to supplement the 2007 sites. Six complete collections were made at four of the sites. The site known as CLK09, a water impoundment, was a very productive area for metamorphs, generating two full collections of Fowler's toad (n=52 and n=56) and one collection of the southern leopard frog (n=58). Two additional collections of Fowler's toad were made at CLK07 (n=50) and CLK10 (n=75). One full collection of the upland chorus frog was made at CLK11 (n=76). No abnormalities were observed at any of the sites.

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

Site Name	YEAR 1 (2001)				YEAR 2 (2007)			
	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	n/a				05/29/07	BFOW	2.4	R, 0.0
CLK04	n/a				n/a			

CLK05	n/a				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	
					06/30/07	ACBL	0	
CLK10	n/a				n/a			
CLK11	n/a				n/a			

	YEAR 3 (2008)			
Site Name	Sample Date	Species Code	% abnormal	Fate
CLK01	n/a			
CLK02	n/a			
CLK03	n/a			
CLK04	n/a			
CLK05	n/a			
CLK06	n/a			
CLK07	6/16/08	BFOW	0	
CLK08	n/a			
CLK09	6/2/08	BFOW	0	
	6/4/08	RASP	0	
	6/9/08	BFOW	0	
CLK10	5/30/08	BFOW	0	
CLK11	6/6/08	PSFE	0	

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

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 km²) of land, approximately 44,500 acres (180 km²) consists of bottomland and upland forest which support the federally endangered red-cockaded woodpecker. Four green-timber reservoirs, two major lakes, 16 small impoundments, and assorted wetland areas provide important habitat for the wood stork, American alligator, bald eagle and wintering waterfowl.

Noxubee NWR was sampled for the second year in the 2008 sampling season. Beginning in May, collectors focused their efforts on the Refuge in areas previously known to have eggs and tadpoles through the summer. Sweep netting of deeper pools was done to determine the possible presence of leopard, bronze and bullfrog tadpoles. However, no sites were found that would likely have resulted in a collection. Overall, it appeared that during the past 2 sampling years, these three frogs species have had very limited reproductive success despite a relatively high occurrence of winter and spring water for breeding. Efforts were therefore centered on making collections of Fowler's toad, Cope's gray tree frog, green tree frog, and cricket frog. These 4 species have extended breeding periods in the central part of Mississippi and readily breed in ephemeral pools.

Approximately 50 sites were located along refuge roads containing water either in the ditches proper, small stock ponds, or tire ruts. By far the most abundant species located was Cope's gray tree frogs which repeatedly laid eggs through the summer. However, summer conditions quickly reduced the available pool waters resulting in substantial death to amphibian larvae prior to a Gosner stage necessary for collection. Sampling efforts at Noxubee were completed by mid-August. The refuge was ultimately able to make 5 complete collections consisting of 2 Fowler's toad at NXB11 and NXB12 (n=51, n=52), 2 Cope's gray

tree frog at NXB01 and NXB05 (n=53, n=50), and 1 cricket frog (n=50) at NXB10. Four collections had 1-5 abnormal frogs in each. The complete collection at NXB05 (Cope's gray tree frog) did not have any abnormal metamorphs (Table 7). Radiographic specimens were sent from collections with abnormalities to Dr. Michael Lannoo as described previously. However, the 2 samples from collection NXB12 (Fowler's toad) were lost prior to submission for radiology.

Field observations of abnormalities included a mixture of surficial and skeletal abnormalities. Surficial abnormalities such as cysts, bruising or lesions were not counted as abnormalities for the purposes of this evaluation because they cannot be confirmed radiographically. For example, abnormalities observed in the two fowlers toad collections included skeletal abnormalities such as a missing front limb and a missing rear foot on another individual, but also surficial abnormalities like pink cyst-like growths (2), a red lesion (1), and dark bruising on another's torso. In this case, only the skeletal abnormalities would be included for the abnormality reporting in the table below. Similarly, the cricket frog with a disfigured foot would be included as abnormal, but the potential bruising of another individual would not. And finally, the Cope's tree frog with asymmetry and bruising of the right hindlimb would be included only as skeletal asymmetry.

Table 7. Results for sampling efforts on Noxubee NWR.

Site Name	YEAR 1 (2007)				YEAR 2 (2008)			
	Sample Date	Species Code	% abnormal	Fate, Confirm%	Sample Date	Species Code	% abnormal	Fate, Confirm%
NXB01	07/02/07	BFOW	2.0	R, 0.0	5/22/08	HYCH	1.8	
NXB02	n/a				n/a			
NXB03	07/23/07	HYCH	13.0	R, 1.9	n/a			
NXB04	07/25/07	ACCR	8.0	R, 0.0	n/a			
NXB05	n/a				6/12/08	HYCH	0	
NXB06	n/a				n/a			
NXB07	n/a				n/a			
NXB08	n/a				n/a			
NXB09	n/a				n/a			
NXB10	n/a				6/23/08	ACCR	1.9	
NXB11	n/a				6/24/08	BUFO	3.9	
NXB12	n/a				6/26/08	BUFO	3.8	

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

2. Coldwater River National Wildlife Refuge

Coldwater River National Wildlife Refuge is a 2,508 acre refuge located in Tallahatchie and Quitman counties, Mississippi. The refuge is currently managed by the North Mississippi Refuges complex in Grenada, MS. It was established in 1991 to provide habitat for migratory waterfowl, shorebirds and wadingbirds and to provide fallow field habitat for wintering grassland birds. All refuge lands were agricultural lands upon acquisition and one thousand acres of the property was planted in hardwood seedlings in 2000. Much of the refuge is inaccessible during the winter months due to backwater flooding of the Tallahatchie River.

Coldwater River NWR completed its first abnormal amphibian assessment in 2008. Beginning in May, staff monitored multiple ponds at the refuge for the presence of tadpoles to monitor development. Very few tadpoles were observed initially, due to the large size of the ponds and concentration of metamorphs. As the ponds began drying, metamorphs were more concentrated, making collections easier. During a visit on June 12, numerous metamorph Fowler's toads were observed on two of the drying units, CWR01 and CWR02, and a collection was made at each site (n=56, n=60). Additionally, at site CWR01, metamorph southern leopard frogs were observed. However, many of these were beyond the maximum size allowed by the sampling protocol. A second collection of Fowler's toad was made at CWR01 on June 24 (n=58). Several more visits were made to the units through July, but no other samples were collected.

Two collections had abnormal frogs or toads. Site CWR01, a drying man-made pond, had one collection of Fowler's toad on June 24 (n=58) that did not contain any abnormal metamorphs (Table 8). The collection of CWR01 on June 12 (n=56) contained 5 abnormal specimens of Fowler's toad and one abnormal southern leopard frog. Fowler's toad abnormalities at this site included shortened or missing digits on hind limbs and one instance of a missing foot. The abnormal southern leopard frog was part of an incomplete collection at this site and was missing the left hind foot and all tissue from the calf with the tibia and fibula bones still present. Site CWR02, also characterized as a drying man-made pond, contained only one abnormal amphibian, a Fowler's toad with a rear clubbed foot. No contaminants were suspected at either site, and the surrounding land use was a waterbody or

riparian corridor. Radiographic specimens were sent from collections with abnormalities to Dr. Michael Lannoo as described previously.

Based on the presence of leopard frog metamorphs, it appears that monitoring began too late to collect leopard frogs. Monitoring in 2009 should begin earlier to attempt collecting a sample of this species. Additionally, the refuge supports a large population of green treefrogs and numerous young of the year are seen during the summer. However, they are usually seen some distance from the breeding sites and exceed the maximum size for this survey. Based on anecdotal information, sampling for this species may need to occur at night.

Table 8. Results for sampling efforts on Coldwater River NWR.

	YEAR 1 (2008)			
Site Name	Sample Date	Species Code	% abnormal	Fate
CWR01	6/12/08	BFOW	8.9	
	6/24/08	BFOW	0	
CWR02	6/12/08	BFOW	1.6	

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

Diagnostic Results

A. Radiography

Abnormal specimens collected during 2008 were preserved and sent directly to Dr. Michael Lannoo from the NWRs. Examination for skeletal abnormalities were be made by Dr. Lannoo. During the 2008 season, only Noxubee (n=4) and Coldwater River (n=6) NWRs sent specimens for radiological evaluation by Dr. Lannoo. Radiographic results are pending.

B. Parasitology

No samples of live metamorphs were shipped for parasitological analysis to Dr. Pieter Johnson at the University of Colorado in Boulder. All refuges were invited to send parasitology samples, however none were able to collect samples for various reasons (lack of metamorphs, timing etc.). We will make efforts again in 2009 to obtain these samples for analysis.

Discussion

As reported last year, eight years of sampling on refuges in the Southeast Region, has shown 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 is warranted. However, it should also be noted that radiographic confirmation of field-identified abnormalities as developmental errors is often lacking. As a result many of these abnormalities are ultimately categorized as *injuries* or *abnormalities of unclear origin*.

After extensive database review over the past year, qualified data have been evaluated and summarized in a “five-year review” for the Southeastern Region Abnormal Amphibian Monitoring Project. The results of this summary have been submitted for inclusion in the Abnormal Amphibian Program’s National Five Year Review. Decisions about future of the program in the southeast will be based on the information provided in the five-year data review. These decisions will include whether there is a need for chytrid sampling on NWRs, further parasitology evaluation needs, which refuges warrant being revisited for secondary surveys, as well as other resource allocation choices for the 2009 survey season

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