

*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 2009

by

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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 (2001-2009, Table 1). Five national wildlife refuges in the Southeast Region were assessed during 2009 (Figure 1). Three refuges were sampled for the second time this year including Coldwater River, Banks Lake and Okefenokee NWRs. Successful full collections were conducted at Felsenthal (n=1) and Coldwater River (n=3) NWRs, but not at Okefenokee, Banks Lake, Choctaw, or Overflow NWRs because of a sparse number of metamorphs.

During 2009, the incidence of abnormalities exceeded background levels (3 percent; Dubois 1979, Johnson and Lunde 2001, Ouellet et al. 1997, Stocum 2000) in the collection made at Felsenthal (9.2% abnormal) and in two of the three collections made at Coldwater River NWR (3.6 and 4.5% abnormal). As with the previous reports, the abnormalities encountered consisted primarily of missing or clubbed digits, feet, or limbs, however observations of multiple limbs or missing eyes were also made. Other irregularities such as variant colorations, abrasions and similarly obvious soft tissue damages were not reported as abnormalities.

The abnormal specimens collected were preserved for shipment for radiographic analysis via Dr. Mike Lannoo at Indiana University School of Medicine, Terre Haute, Indiana. 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 2009 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 for the first ten years of the program (2000-2009). The data collected through 2009 are currently under review

and analysis. This complete data set will be subjected to exploration and the findings will be summarized in a pending report.

Based on the outcome of the cumulative data analysis described above, the strategy for the upcoming 2010 season will include 3 main components. First, the national and region cumulative data review and analysis will be completed. Second, both Felsenthal and Overflow NWR will continue follow up surveys in 2010 based on the initial refuge results (high abnormality rates). 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 will continue sampling in 2010 for their third year of sampling.

Table 1. Summary of all results from Southeast Region NWRs assessed for abnormal amphibians from 2000 to 2009.

State	Refuge	Range of Incidences of Abnormalities (%) found									
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
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					9.2 % ^F
	Overflow NWR			0-15 % ^F	3-7.7 % ^F	7-42 % ^F					F, N
	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	F, N
	Okefenokee NWR									0 % ^F	F, N
	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 2009.

State	Refuge	Range of Incidences of Abnormalities (%) found									
		2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
	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	1.7-4.5% ^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.

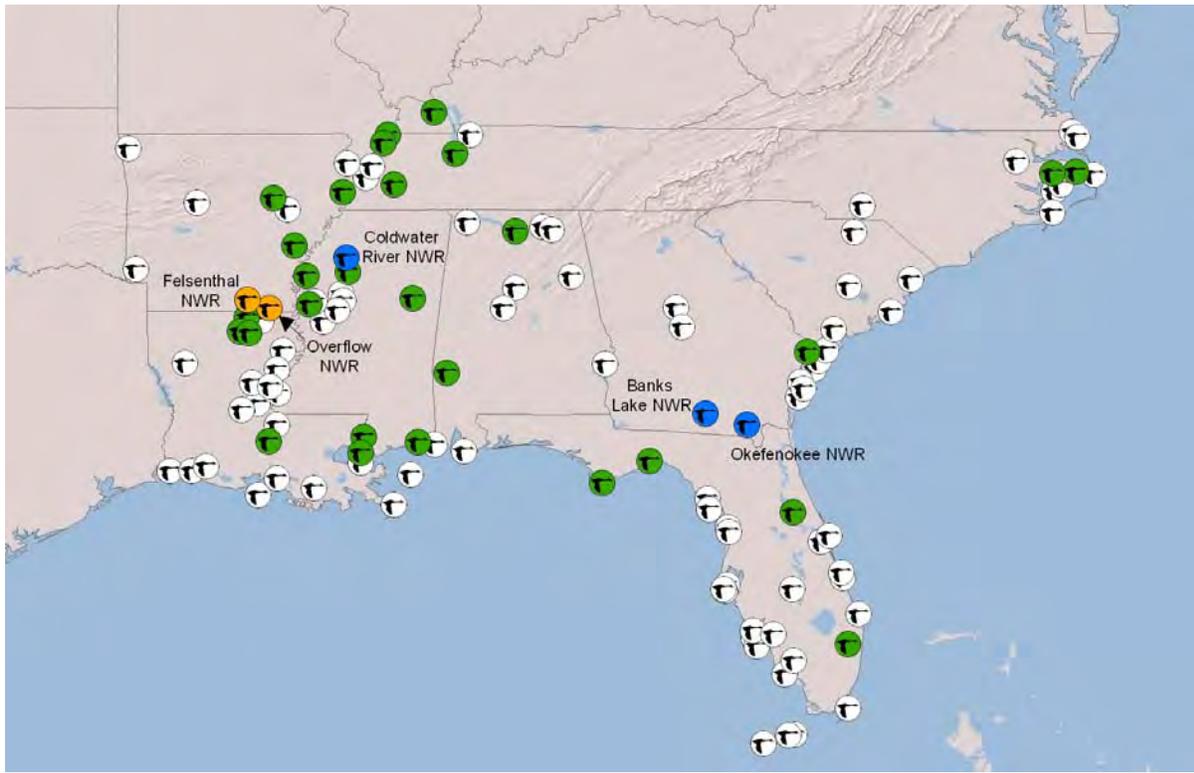


Figure 1. The five national wildlife refuges in the Southeast Region assessed during the 2009 sampling season are highlighted and labeled. Blue indicates a continuation of consecutive year sampling while orange indicates a follow up survey on refuges requiring additional sampling based upon initial findings. Their relative geographic location is presented with 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 2009 season.

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 of a single species between the Gosner stages of 42 and 46) are documented in these tables.

A. Arkansas Refuges

1. Felsenthal National Wildlife Refuge

Felsenthal NWR is located in Ashley, Union, and Bradley Counties of southern Arkansas at the confluence of the Ouachita and Saline Rivers. It contains 40,000 acres of bottomland

hardwood forest, 15,000 acres of open water areas, and 10,000 acres of upland with mixed pine-hardwood forest. Felsenthal NWR lies in a watershed where elevated mercury, chlorides, and petroleum constituents are contaminants of concern. The predominant suspected source is the extensive oil production within the northern refuge boundary. This refuge was selected to be resampling as a result of the high incidence of abnormalities observed in preliminary sampling in 2002, 2003, and 2004.

As a result of the far greater normal rainfall, only one suitable sampling site was located at Felsenthal NWR. Precipitation brought the water level up 2.5-3.5 feet across the refuge and completely submerged nearly all previously sampled sites (2002-2004). Photographs of the site were taken before and after the water rose (Figures 2 and 3).

This year's sampled site (FSL04; N 33.11159 , W 92.17722) was a natural pond on Shallow Lake Road without known sources of contamination. The 15,200 m² pond is surrounded by fields (food plots) and natural forest. Numerous tadpoles were observed at this site early in the sampling season prior to flooding. Potential predators and wildlife observed at this site were large-mouth bass (*Micropterus salmoides*), cottonmouths (*Agkistrodon piscivorus*), western ribbon snakes (*Thamnophis proximus*), crayfish (*Cambarus spp.*), broad-banded water snakes (*Nerodia fasciata*), diamondback water snakes (*Nerodia rhombifer*), and numerous wading birds. There were two species of frogs found at this site. The first was the green frog (*Rana clamitans*). This species was found when the water was low; however, a full collection was not obtained, though three frogs with abnormalities were found. The other species found was the Northern cricket frog (*Acris crepitans*). This species was found later in

the season when the pond was flooded and was abundant around the pond's edge. A full collection of Northern cricket frogs was made (N=55) and 6 abnormalities were observed (Table 2). The abnormalities found on these six individuals included: loss of pigments on a limb, a clubbed foot (Figure 4, defined as "other skeletal malformation"), a missing appendage (Figure 5, amelia), a missing eye (Figure 6, anophthalmia), and an additional leg and foot formed on one individual for a total of five (Figure 7, polymelia).



Figure 2. Sampling site FSL04 on Felsenthal NWR during the 2009 sampling season prior to flooding.



Figure 3. Sampling site FSL04 on Felsenthal NWR during the 2009 sampling season after flooding rains elevated water levels 2.5 to 3.5 feet resulting in strong flow through ponded areas.



Figure 4. Northern cricket frog collected during the 2009 sampling season on Felsenthal NWR possessing a clubbed foot (other skeletal malformation, R4-FSL04-100209-ACCR-07).



Figure 5. Northern cricket frog collected during the 2009 sampling season on Felsenthal NWR with a missing appendage (Amelia, R4-FSL04-100209-ACCR-53).



Figure 6. Northern cricket frog collected during the 2009 sampling season on Felsenthal NWR with a missing eye (Anolphthalmia, R4-FSL04-100209-ACCR-54)



Figure 7. Northern cricket frog collected during the 2009 sampling season on Felsenthal NWR exhibiting polymelia, an additional leg and foot formed on one individual for a total of five limbs, R4-FSL04-100209-ACCR-28).

Despite searching for and monitoring additional sampling sites, very few additional metamorphs were found. The metamorphs that were found were normal (without notable abnormalities).

Table 2 . Results for sampling efforts on Felsenthal NWR.

Site Name	YEAR 1 (2002)				YEAR 2 (2003)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
FSL01	14 May 03	RASP	18.6%	R	9 Jul 03	GACA	7.3%	P, I
FSL01	4 Jun 03	RASP	5.5%	R				
FSL01	4 Jun 03	BUFO	3%	S				
FSL02	14 May 03	RASP	25%	R	9 Jul 03	BUFO	1.8%	P
FSL03	n/a				n/a			
FSL04	21 May 03	RASP	9.8%	R	n/a			
FSL05	5 Jun 03	RASP	13.8%	R	24 Apr 03	PSFE	7%	R
FSL05					1 Jul 03	HYCH	8%	P
FSL06	n/a				n/a			
FSL07	n/a				10 Jun 03	RASP	0%	--
FSL08	n/a				n/a			
FSL09	--				24 Apr 03	PSFE	4.8%	R
FSL10	--				21 Apr 03	PSFE	9.8%	R, I

Table 2 continued: Results for sampling efforts on Felsenthal NWR.

Site Name	YEAR 3 (2004)				Year 4 (2009)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
FSL01	23 Apr 04	PSFE	3%	R	n/a			
FSL02	n/a				n/a			
FSL03	n/a				n/a			
FSL04	7 Jul 04	BUFO	0%		10 Oct 09	ACCR	9.2%	R
FSL05	n/a				n/a			
FSL06	n/a				n/a			
FSL07	7 Jun 04	BUFO	0%		n/a			
FSL08	n/a				n/a			
FSL09	n/a				n/a			
FSL10	n/a				n/a			
FSL11	n/a				n/a			

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. (--) indicates that a site was not monitored. “ * # ” indicates a collection with animals excluded from the % abnormal calculation. P = parasitology, R = radiography, S = storage of preserved specimens not suitable for current diagnostic analyses, or I = invertebrate predator studies.

2. Overflow National Wildlife Refuge

Overflow NWR is located in Ashley County near the southeast corner of the state. It contains 9,427 acres of bottomland hardwood forest, 2,620 acres of agricultural fields, and 200 acres of upland pine-hardwood forest. Its western boundary follows the 110 foot elevation contour along the Mississippi Alluvial Valley escarpment with an abrupt rise in elevation separating the Mississippi River Delta from the Gulf Coastal Plain. Overflow NWR has historically been farmed intensively for cotton, rice, soybeans, winter wheat, and

milo. The refuge is bounded on the west by logged timber and by active agriculture (primarily soybeans and cotton) on the north, east, and south. The potential for drift of currently used pesticides (CUP's) onto much of the refuge is present. Existing natural wetlands on Overflow NWR are limited due to the extensive historical alteration of natural hydrology for irrigation. Most sites contain fast draining soils that dry completely within weeks after water control structures are pulled to drain winter flooded fields. Crop rotations and the associated changes in irrigation practices present great difficulty in successfully sampling amphibians from the same areas year after year. This lack of ponding water created repeat sampling difficulties in the initial sampling years (2002-2004).

Conversely, severe flooding on Overflow NWR inundated all wetlands across the refuge and negatively affected our ability to collect samples during this 2009 season. As a result no collections were successfully made on Overflow NWR (Table 3).

Table 3. Results for sampling efforts on Overflow NWR.

Site Name	YEAR 1 (2002)				YEAR 2 (2003)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
OVF01	1 May 02	RASP	1.9%	R	n/a			
OVF02	14 May 02	BFOW	2.3%	S	n/a			
OVF03	n/a				1 Jul 03	BUFO	3.3%	S, I
OVF04	25 Jun 02	HYCI	15.4%	R	n/a			
OVF05	14 May 02	BFOW	5.6%	S	13 May 03	RASP	7.7%	R
OVF05	24 Jun 03	HYCI	0%					
OVF06	--				n/a			
OVF07	--				13 May 03	BUFO	6%	R, I

Table 3 continued. Results for sampling efforts on Overflow NWR.

Site Name	YEAR 3 (2004)				YEAR 4 (2009)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
OVF01	--				n/a			
OVF02	n/a				n/a			
OVF03	--				n/a			
OVF04	--				n/a			
OVF05	n/a				n/a			
OVF06	--				n/a			
OVF07	05 May 04	RASP	42%	R	n/a			
OVF07	11 May 04	RASP	30%	R	n/a			
OVF07	18 May 04	RASP	18.3%	R	n/a			
OVF07	07 Jun 04	RASP	7%	P, R	n/a			

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. (--) indicates that a site was not monitored. “*#” indicates a collection with animals excluded from the % abnormal calculation. P = parasitology, R = radiography, S = storage of preserved specimens not suitable for current diagnostic analyses, or I = invertebrate predator studies.

B. Georgia Refuges

1. Banks Lake National Wildlife Refuge

Banks Lake National Wildlife Refuge is located in Lanier County, near Lakeland, as part of a much larger blackwater 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. Bank Lake NWR is managed by the staff of Okefenokee NWR.

The 2009 sampling season was Banks Lake NWR's second year of participation in the Service's abnormal amphibian survey. Two sites were monitored on Banks Lake this year (Table 5). The first site (BLM01) was along the periphery of the boat basin on the refuge. Very few tadpoles, no metamorphs, and no adults frogs were found at this site. It was suspected that the boat traffic and surface turbulence made the site unsuitable for amphibian breeding. It is also possible that the deeper waters adjacent to the site made amphibians susceptible to predatory pressures from fish.

The second site monitored on Banks Lake NWR (BLM02) was located at the northern boundary of the lake along a flooded nature trail. This area is inundated during high water conditions, but even small amounts of rain resulted in standing water on the site. This expansive, shallow sampling site possessed ample submerge aquatic vegetation. Amphibian activity was observed consistently here and the species present included the program's target species (ranids). Although this site was very productive and samples were collected, a full collection (50-100 individuals) in the proper Gosner stage (44-46, 4 legs) was not achieved. However, no abnormal individuals were observed in the collections of southern leopard frogs or cricket frog species.

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

Site Name	YEAR 1 (2008)				Year 2 (2009)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
BLM01	n/a				n/a			
BLM02	n/a				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 second consecutive year in 2009. Five sites were monitored this year (Table 6). Okefenokee's first site (OKE01, Figure 8) was located on the east side of the swamp adjacent to Refuge Headquarters. The 2009 sampling began at this site on May 9. This was a shallow grassy pond about four acres in size with an average depth of about 2.5 feet deep. The entire pond had a sandy bottom and emergent grass throughout. Conditions at this site were thought to be near optimal for metamorph collection based on its depth and permanent waters. Numerous

tadpoles and adult amphibians were found throughout. The last day that we sampled the site was on August 28, 2009.



Figure 8. Sampling site OKE01 on Okefenokee NWR during the 2009 sampling season.

Site OKE8a was sampled for the first time on May 1, 2009. This wetland was located on “Pocket Road” on the west side of the swamp adjacent to Stephen Foster State Park. The site was a helicopter dip site used to provide water to firefighting helicopters. Although the previous season’s sampling was successful here, the higher than average rains increased the depth (>5 feet) making it less suitable for tadpole development because the deeper areas dominated the scant littoral zones resulting from steep banks. Sampling at this site was wholly unsuccessful this year. The site was last sampled on 18, 2009.



Figure 9. Sampling site OKE8a on Okefenokee NWR during the 2009 sampling season.

Another site located off “Pocket Road” was OKE 8c. We began monitoring this pond on May 1, 2009. This three acre pond covered with water lilies, was deep at its center but possessed a shallow shelf around the edge. Regrettably, it also possessed an aggressive female alligator that moved into the pond and built a nest along the edge of it. She required our attention while sampling this site. Both adult and tadpoles were found at this location. The last day that we sampled the site was on August 28, 2009.



Figure 10. Sampling site OKE8b on Okefenokee NWR during the 2009 sampling season.

The third site monitored on “Pocket Road” was OKE8d. We began monitoring this site on May 1, 2009. This location was also a helicopter dip site with a grassy littoral zone. Like the other dip tank site (OKE8a), the depth was believed to be too great to provide suitable habitat for tadpole development due to relatively insufficient littoral zones. Although early sampling revealed numerous adult frogs, the remainder of the sampling attempts were not productive. The last sampling for this site was on June 19, 2009.



Figure 11. Sampling site OKE8d on Okefenokee NWR during the 2009 sampling season.

Beginning May 1, 2009 we began monitoring a long shallow grassy ditch along “Crooked Sapling Road.” The site was referred to as OKE 14 (Figure 12). The first time this site was sampled, we found some southern leopard frogs and the southern cricket frogs. However, in mid-May lack of rain caused the site to dry for several weeks. Rain returned in early June. Once water levels increased, we started finding small cricket frog tadpoles. No metamorphs were found. The last day we sampled the site was on September 3, 2009. At this time we found some adult cricket frogs in the grass along the ditch.



Figure 12. Sampling site OKE14 on Okefenokee NWR during the 2009 sampling season.

Sampling at Okefenokee NWR targeted collections of southern leopard frogs and southern cricket frogs. Five sites were sampled from May through September and monitoring allowed for many collection attempts. Most collections came from OKE01 and were of river frogs. Collections of southern leopard frogs were made at OKE8C. And some little grass frogs were collected at OKE14. However, very few of the collected specimens were at an adequate stage of development (Gosner stages 42-46) to be included in this survey according to program protocol. Nevertheless, no abnormalities were observed at any site.

Table 5. Results for sampling efforts on Okefenokee NWR.

Site Name	YEAR 1 (2008)				YEAR 2 (2009)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
OKE01	8/1/08	RAHE	0		n/a			
	9/14/08	RAHE	0		n/a			
OKE8A	n/a				n/a			
OKE8B	n/a				n/a			
OKE8C	9/7/08	RASP	0		n/a			
OKE14A	7/3/08	PSOC	0		n/a			
OKE15H	n/a				n/a			

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

E. Mississippi Refuges

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 second abnormal amphibian assessment in 2009. Four sites were focused on for sampling this year, although more were evaluated (Table 8). A

riparian corridor with a history of recent pesticide applications was defined as CLD02 (Figure 13). This 500² meter area had water collected primarily in shallow (<3 feet) ruts. It was sprayed with UltraBlazer herbicide (acifluorfen) to kill coffeeweed in the summer of 2008 as part of a US Geological Study (ARMI) study. Previous pesticide applications were also reported. The area now is heavily vegetated with grasses and smartweed, but also contains some small clumps of willow saplings.



Figure 13. Sampling site CLD02 on Coldwater River NWR during the 2009 sampling season.

Site CLD03 was a shallow man-made pond with grasses and rushes emerging from the waters (Figure 14). Historic pesticide use was reported for the areas surrounding this site. The site included a number of noteworthy species including: sirens (juvenile), crayfish, mosquitofish, predaceous diving beetles, giant water bugs, larval dragonflies, larval diving beetles, and fishing spiders.



Figure 14. Sampling site CLD03 on Coldwater River NWR during the 2009 sampling season.

The third site monitored on Coldwater River NWR during the 2009 season was another shallow (<3 feet), man-made pond (CLD04, Figure 15). Recent pesticide use was reported for this area. This pond covered approximately 17 acres, but only a segment of about 1,000 m² was monitored. The abundant emergent vegetation was predominately water primrose (*Ludwigia*) with some dense clumps of 2 - 5 year old willow. However, the area with permanently standing water had little to no vegetation.



Figure 15. Sampling site CLD04 on Coldwater River NWR during the 2009 sampling season.

The fourth monitoring site on Coldwater River NWR was another man-made pond (CLD05, Figure 16). Historic pesticide use was reported for areas surrounding this site. The pond was reportedly less than 3 feet deep throughout.



Figure 16. Sampling site CLD04 on Coldwater River NWR during the 2009 sampling season.

During this season, staff at the North Mississippi Refuges Complex collected three complete samples and two partial samples of metamorphs. Although most abnormalities were missing portions of digits or other foot abnormalities, one northern cricket frog was collected with a missing eye (Figure 17) and one southern leopard frog was found with a lesion at the base of its tail (not easily seen in digital image). Abnormal specimens were sent to Dr. Mike Lannoo for radiography.

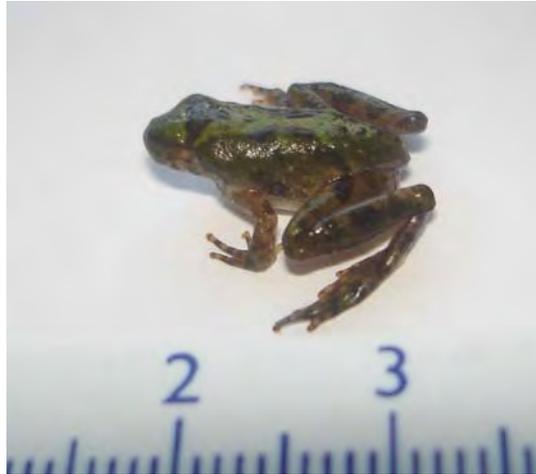


Figure 17. Northern cricket frog collected during the 2009 sampling season on Coldwater River NWR with a missing eye (Anolphthalmia, R4-CWR05-070809-ACCR-046)

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

Site Name	YEAR 1 (2008)				YEAR 1 (2009)			
	Sample Date	Species Code	% abnormal	Fate	Sample Date	Species Code	% abnormal	Fate
CLD01	6/12/08	BFOW	8.9	R	n/a			
	6/24/08	BFOW	0		n/a			
CLD02	6/12/08	BFOW	1.6	R	05/27/09	BFOW	1.7	R
CLD03	n/a				n/a			
CLD04	n/a				06/18/09	BFOW	4.5	R
CLD05	n/a				07/08/09	ACRI	3.6	R

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

Diagnostic Results

A. 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 2010 to obtain these samples for analysis.

B. Radiography

Abnormal specimens collected during 2009 were preserved and held for shipment to Dr. Michael Lannoo. The specimens are in the care of the Southeastern Regional Coordinator of the program at the Panama City Field Office. Examination for skeletal abnormalities will be performed by Dr. Lannoo after contract specifications have been defined. Radiographic results are therefore pending.

Discussion

Nine years of sampling on refuges in the Southeast Region has revealed 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 prepared for summarization in a “ten-year review” for the Southeastern Region Abnormal Amphibian Monitoring Project. The results of this summary will be submitted for inclusion in the Abnormal Amphibian Program’s National Ten Year Review. Decisions about the future of the program in the southeast will be based on the information provided in the ten-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 2011 survey season.

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