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Authors: McCallum, Malcolm L., and Trauth, Stanley E.

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A FORTY-THREE YEAR MUSEUM STUDY OF NORTHERN CRICKET FROG (*ACRIS CREPITANS*) ABNORMALITIES IN ARKANSAS: UPWARD TRENDS AND DISTRIBUTIONS

Malcolm L. McCallum[1,3,4] **and Stanley E. Trauth**[2]

[1] Environmental Sciences Ph.D. Program, Arkansas State University, P.O. Box 847, State University, Arkansas 72467, USA

[2] Department of Biological Sciences, Arkansas State University, P.O. Box 599, State University, Arkansas 72467, USA

[3] Current address: Department of Biological Sciences, Louisiana State University at Shreveport, One University Place, Shreveport, Louisiana 71115, USA

[4] Corresponding author (email: mmccallu@astate.edu)

ABSTRACT: The northern cricket frog (*Acris crepitans*) is a resident of streams, rivers, and wetlands of eastern North America. We documented abnormalities in *A. crepitans* housed in the Arkansas State University Museum of Zoology Herpetology Collection. Abnormality frequency increased from 1957 to 2000 (χ^2=43.76, df=3, P<0.001). From 1957 through 1979 only 3.33% of specimens were unusual. This rate was 6.87% during the 1990s, and in 2000 it was 8.48%. High frequencies of abnormalities were identified in the following Ozark highland counties: Sharp, Lawrence, and Randolph. We observed 104 abnormalities among 1,464 frogs (7.10%). The differential abnormality frequencies observed between the Arkansas lowlands and highlands are striking. The Ozarks had significantly higher frequencies of abnormalities than other Arkansas regions (χ^2=59.76, df=4, P<0.001). The Ouachita Mountains had significantly higher frequencies than the Gulf Coastal Plain, Delta, or Arkansas River Valley (χ^2=13.172, df=3, P<0.01). There was no difference in abnormality frequency between the Gulf Coastal Plain, Delta, and Arkansas River Valley (χ^2=0.422, df=2, P>0.70). Proposed hypotheses for distributions include: 1) *A. crepitans* might possess naturally high abnormality levels, and land use practices of the Delta may reduce this variability; 2) an unknown xenobiotic may be in Ozark streams causing increased numbers of abnormalities; 3) the museum's collection effort may be skewed; 4) Delta habitat might be more favorable for green tree frogs (*Hyla cinerea*) allowing this species to drive out *A. crepitans* through competition; here, abnormal metamorphs are not detected because they are even less competitive than normal individuals.

Key words: Abnormalities, *Acris crepitans,* Arkansas, cricket frog, deformities, malformations, teratogen, teratology.

INTRODUCTION

Reports of isolated amphibian malformations are not new and have periodically appeared in the literature since 1920 (Hovelacque, 1920; Adler, 1958; McCallum, 1999). Early reports were very rare, but the frequency of these reports has increased dramatically since 1995 (Helgen et al., 1998), in part, due to better reporting methods. Discoveries of aberrant morphologies are now appearing throughout the continent as reported on the website of the North American Reporting Center for Amphibian Malformations (NARCAM; http://www.npsc.nbs.gov/narcam/reports/reports.htm). Up to 67% of Minnesota (USA) frogs possess malformations, but Minnesota, Quebec (Canada), and Vermont (USA) report average affliction rates near 8% (Kaiser, 1997).

Kaiser (1997) reviewed three hypotheses explaining naturally occurring amphibian teratogenesis. These were the parasite infestation hypothesis, the ultraviolet-B exposure hypothesis, and the water-borne contaminant hypothesis. The first hypothesis involves a parasitic trematode that embeds near the growth bud of the hind limbs in anuran larvae (Helgen et al., 1998; Johnson et al., 2001). Blaustein et al. (1997) provided evidence that exposure to UV-B radiation may induce mortality and increased incidences of abnormalities in the long-toed salamander (*Ambystoma macrodactylum*). This factor may result in malformations such as lateral flexure of the tail, edema, blisters, and increased susceptibility to fungal infections by interfering at the genetic level. No observations of extra limbs were reported in their study. Ouellet et al. (1997) suggested that hind limb malformations were associated with exposure to agricultural activities and, hypothetically, to agrochemical contaminant exposure. He found much higher incidence of all malformations in agronomic regions of Ontario, Canada as opposed to non-agronomic regions. Reeder et al. (1998a) provided further evidence of this hypothesis. They reported that expression of intersexuality (a type of hermaphroditism) by male northern cricket frogs (*A. crepitans*) might be associated with contaminant and agrochemical exposure. Reeder et al. (1998b) also found that historic expression of intersexuality by this species in Illinois (USA) was correlated with the use of organochlorine insecticides.

Little information about the present or historical occurrences of abnormalities in Arkansas (USA) amphibians is available. No Arkansas record has been provided to NARCAM despite observations of abnormalities in bordering counties of Missouri, Tennessee, Oklahoma, and Mississippi (see NARCAM). This is surprising considering that a major component of the Arkansas economy is agricultural crops such as rice, cotton, and soybeans. These crops are intensively produced throughout the state's Mississippi Embayment (a.k.a. Delta) and the Arkansas River Valley; whereas timber and grazing are the primary activities in the Gulf Coastal Plain, Ouachita Mountains, and Ozark Plateau (Fig. 1).

The northern cricket frog (*Acris crepitans*) is a small hylid frog whose range extends across most of the eastern two-thirds of the continental United States (Conant and Collins, 1998). This species is of special concern in much of the northern part of its range, being listed as endangered or protected in Minnesota, Wisconsin, and Michigan (Lannoo, 1998) and may be declining in Illinois (Greenwell et al., 1996). It appears to be extirpated from Canada (Weller and Green, 1997) and in Colorado (USA; Hammerson and Livo, 1999). Based on our personal communications with conservation officials and review of non-peer reviewed literature accounts, this species may be declining in 17 states, extirpated from West Virginia (USA) and Mexico, and is listed as "vulnerable" by the International Union for the Conservation of Nature, Species Survival Commission (IUCN/SSC). The current status of *Acris crepitans* in parts of its range versus its previous abundance throughout that range provides questions as to why so few abnormalities have been reported in this frog.

Gray (2000) reported that *A. crepitans* collected statewide in Illinois between 1968 and 1971 possessed low frequencies of external morphologic abnormalities. This information was recorded while conducting unrelated studies (Gray, 2000). He recorded 39 abnormalities among 9,987 specimens (0.39% occurrence). Of these, 0.32% possessed amelia (missing limbs), and the remaining 0.07% of abnormalities consisted of polymelia (supernumerary limbs), deformed digits, and underdeveloped mouths. He also reported that collections in 1998 had similar frequencies, having recovered three fused/missing toes from a sample of 140 frogs (2.14%). His 1998 data represented a 669% increase from the rate observed in the early 1970s.

Gray (2000) also stated that evaluation of historic databases may show changes in the incidence of amphibian abnormalities and such research is both relevant and urgently needed given the current status of amphibian populations. The present investigation represents the only statewide, large-scale documentation of amphibian abnormalities from Arkansas. It is also one of a few extensive studies involving continuous, long-term (43 years), historic data sets regarding external amphibian abnormalities. Moreover, our study is the first large-scale historic and geographic examination of external abnormalities in *A. crepitans.* These considerations suggest the

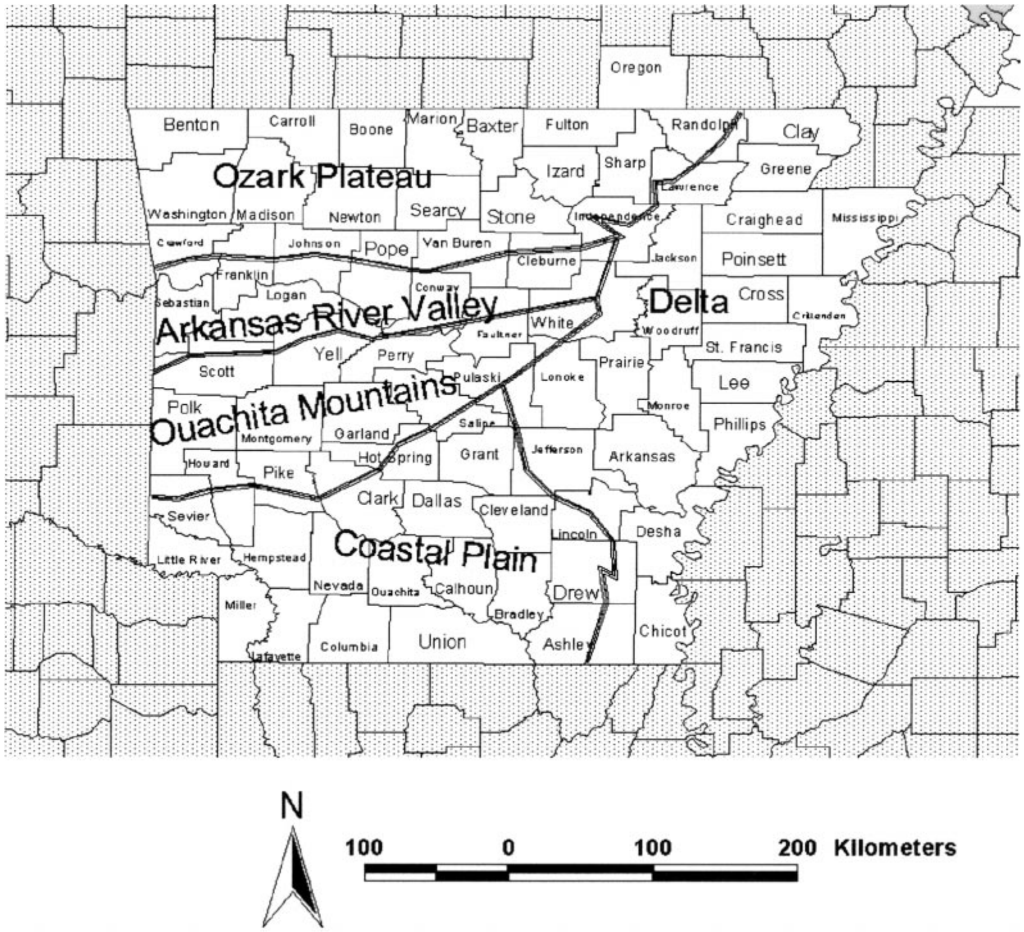

FIGURE 1. Major ecoregions of Arkansas.

abnormality information, herein, should be of great interest to the wildlife disease and herpetological communities.

MATERIALS AND METHODS

Between 1957 and 2000 adult and post-metamorphic northern cricket frogs (*A. crepitans*) were collected from 49 Arkansas counties and a single Missouri county (Table 1). These specimens were euthanized by immersion in a dilute aqueous chloretone solution (Etheridge, 1958), transferred to 10% formalin for 48–72 hr, preserved in 70% ethanol, and deposited in the Arkansas State University Museum of Zoology (ASUMZ) herpetology collection. All specimens were examined thoroughly for external abnormalities. These were identified according to Meteyer (2000), documented, and cross-referenced by specimen number, year, and collection locality. Because animals were not dissected, certain abnormalities were lumped (e.g., ectrodactyly and brachyphagy). Individuals were tallied as having retained tails (NARCAM website) if they were obviously beyond the Gosner 43–45 stages due to the date they were collected (e.g., winter) or if their body size was beyond that of a recent metamorph (unpubl. data). Frequency of abnormalities was then categorized into four groups according to years from 1957–1979, 1980–1989, 1990–1999, and 2000. Differences between time periods and between ecoregions were calculated by hand using Chi-square. The Arkansas ecoregion map was produced using ArcView GIS 3.2 (Environmental Systems Research Institute, Inc.). Statewide frequency of each kind of abnormality was tallied and recorded Limited point and transect population counts were implemented at selected locations. These were performed for 1 hr intervals at each locality and at least once a month at each location.

TABLE 1. Distribution and frequency of *Acris crepitans* abnormalities by county in Arkansas and in Oregon County, Missouri.

County	Sample size	Abnormal	County	Sample size	Abnormal
Arkansas	77	2 (2.6%)	Monroe	1	0 (0%)
Baxter	12	0 (0%)	Montgomery	12	2 (17%)
Benton	1	0 (0%)	Nevada	1	0 (0%)
Bradley	3	0 (0%)	Newton	1	0 (0%)
Carroll	2	0 (0%)	Oregon (MO)	72	7 (10%)
Clay	14	0 (0%)	Ouachita	5	1 (20%)
Cleveland	4	0 (0%)	Perry	9	1 (11%)
Cleburne	7	0 (0%)	Phillips	12	0 (0%)
Conway	33	0 (0%)	Pike	3	0 (0%)
Craighead	177	0 (0%)	Poinsett	7	0 (0%)
Crittenden	1	0 (0%)	Polk	2	0 (0%)
Cross	10	0 (0%)	Pope	1	0 (0%)
Faulkner	6	0 (0%)	Prairie	4	0 (0%)
Fulton	33	0 (0%)	Pulaski	1	0 (0%)
Garland	44	0 (0%)	Randolph	482	71 (14.5%)
Greene	26	0 (0%)	St. Francis	7	1 (14%)
Hot Spring	13	0 (0%)	Saline	5	0 (0%)
Izard	56	0 (0%)	Scott	3	1 (33%)
Jackson	68	0 (0%)	Sevier	1	0 (0%)
Jefferson	2	0 (0%)	Sharp	44	10 (23%)
Lawrence	83	6 (7.2%)	Stone	16	4 (25%)
Logan	7	0 (0%)	Union	2	0 (0%)
Marion	6	0 (0%)	Van Buren	4	0 (0%)
Miller	9	0 (0%)	White	18	1 (6%)
Mississippi	4	0 (0%)	Yell	12	1 (8%)
			TOTAL	1464	104 (7.1%)

RESULTS

Abnormalities were documented in 12 of 49 Arkansas counties and in Oregon County, Missouri. Sample size, number of abnormalities observed, and percent frequency of abnormalities are given in Table 1. Among the counties we sampled, there were abnormalities in five of 13 Ozark, zero of three Arkansas River Valley, five of 10 Ouachita Mountain, one of six Gulf Coastal Plain, and two of 14 Delta counties. Hot spots for abnormalities were identified in Sharp (10/44, 23%), Lawrence (6/83, 7%), and Randolph (71/482, 14.5%) counties from Arkansas and Oregon County (7/72, 10%) in Missouri. We observed 113 abnormalities in 104 of the 1,464 specimens (7.1%) examined. This included 46 (40.7% of abnormalities) missing (ectrodactyly)/fused toes, 37 (32.7%) retained tails, 17 (15.0%) with amelia, nine incidents of lymphedema (8.0%), three (2.7%) with overbites (brachygnathia), one (1.0%) reduced eye (microphthalmia), and one (1.0%) missing eye (anophthalmia). The Ozarks had significantly higher frequencies of abnormalities than other Arkansas regions (χ^2=59.76, df=4, P<0.001). The Ouachita Mountains possessed a significantly higher frequency of abnormalities than the Gulf Coastal Plain, Delta, or Arkansas River Valley (χ^2=13.172, df=3, P<0.01). There was no difference in abnormality frequencies between the Gulf Coastal Plain, Delta, and Arkansas River Valley (χ^2=0.422, df=2, P>0.70).

Frequencies of abnormalities in the ASUMZ herpetologic collection from 1957 through 2000 are provided in Table 2. During the period from 1957 through 1979 only 90 specimens were collected, and 3% were abnormal. From 1980 through 1989, 398 specimens were deposited with 22

TABLE 2. Changes in percent *Acris crepitans* abnormalities in the Arkansas State Museum of Zoology herpetology collection (1957–2000).

	1957–1979	1990–1989	1990–1999	2000	Total
Number[1]	90	398	233	743	1464
Abnormal[2]	3	22	16	63	104
Percent[3]	3.33	5.53	6.87	8.48	7.10

[1] Number of *Acris crepitans* collected during indicated time period.
[2] Number of abnormal individuals observed in the sample.
[3] Percent of the sample that was abnormal.

(5.5%) possessing abnormalities. This represents a 60.2% rise from the previous collection period. During the 1990s, 16 (6.9%) of the 233 *A. crepitans* deposited possessed abnormalities, a 19.5% increase in frequency from the previous period. In 2000, 63 (8.59%) of the 743 specimens deposited at ASU MZ were abnormal. This represented a 19.0% rise from the 1990s, a 34.8% increase since the 1980s, and a 60.7% increase since the period from 1957 through 1979. This is a significant increase over the four periods (Table 2, χ^2=43.76, df=3, P<0.001).

Acris crepitans occurs in extraordinary numbers at Arkansas Post National Memorial (near Guillett, Arkansas County, Arkansas), Jane's Creek (near Ravenden Springs, Randolph County, Arkansas), and the Spring River in the vicinity of Mammoth Spring (Fulton County, Arkansas). Catch-per-unit-effort (cpu) at these sites was 66 cricket frogs/man-hour (cf/mh). Shoreline transects at Whaley Slough Ditch (an agricultural drainage ditch surrounded by rice and soybean operations) in the vicinity of its intersection with US Highway 63 (Bono, Craighead County, Arkansas) only resulted in 3.5 cf/mh. Nighttime surveys in rice fields (vicinity of Jonesboro, Craighead County, Arkansas) between 1 February 2000 and 1 June 2000 produced only 5 cf/mh.

DISCUSSION

The portion of the population of *A. crepitans* observed with abnormalities was higher than the 2% rate usually considered to be normal (Ouellet et al., 1997; Gardiner and Hoppe, 1999; Sessions et al., 1999). While fused toes were usually obvious, partially fused toes were sometimes difficult to identify, making some cases mildly subjective. Only the 4th and 5th toes were ever fused. Fusion ranged from complete to partial and emanated from the angle between these two toes. If fused toes are ignored entirely, the rate of abnormalities is still 4.2%, more than twice the maximum expected rate.

Discovery of high frequencies of abnormalities in northcentral Arkansas and Oregon County, Missouri was surprising. These animals were collected from watersheds dominated by coldwater streams and springs, with little agronomic activity nearby. We expected a low frequency there; whereas we expected a high frequency in the Delta because of its domination by rice and cotton farming. Ouellet et al. (1997) observed positive associations between agronomic activity and anuran malformations in Ontario. Our data suggest a negative association between these factors in *A. crepitans* from Arkansas. In Craighead County 177 specimens were collected from flooded rice fields and adjacent habitats. Despite obvious direct and indirect exposure to agronomic chemicals, no abnormalities were observed there. We observed two (2.6%) abnormalities among 77 specimens from the Arkansas Post National Memorial within the Delta. This national park is confined to a peninsula bound to the north by rice farming which may influence this park's aquatic habitats.

Despite comparatively low populations of *A. crepitans* in rice fields, males were heard calling in moderate numbers from virtually every rice field and agricultural ditch visited. Green tree frogs (*Hyla cinerea*) also were observed breeding in rice fields. These fields produced such large numbers of green tree frog metamorphs by late summer that adjacent roads might become hazardous for driving due to the quantities of froglets on the pavement. Explosive recruitment of *A. crepitans* metamorphs within rice fields was not observed, although dramatic population growth appears to be commonplace each summer in Ozark streams. While our data regarding apparent differences in *A. crepitans* populations between agronomic and non-agronomic counties are limited at this time, they imply rice field populations are much smaller than those found along Ozark streams. Large populations of *A. crepitans* also appear more likely to have comparatively higher frequencies of abnormalities.

We recognize four hypotheses that must be considered to explain the distribution of abnormalities as follows: 1) *A. crepitans* might possess naturally high abnormality levels, and land use practices of the Delta may reduce this variability; 2) an unknown xenobiotic may be in Ozark streams causing increased numbers of abnormalities; 3) the museum's collection effort may be skewed; 4) Delta habitat might be more favorable for green tree frogs allowing this species to drive out *A. crepitans* through competition. The abnormal metamorphs may not be observed because they are even less competitive than normal individuals.

Our study is not extensive enough to draw conclusions about the above hypotheses. The roles that each of these hypotheses contributes to abnormality ratios may provide insight into the declines of this species in other regions. Currently there is little knowledge about the proliferation and ecologic/evolutionary role of abnormalities in amphibian populations. Because we lack this background, it is essential that studies addressing abnormality ratios be implemented. Furthermore, additional historic museum studies should be conducted to better understand population-level incidence and significance of abnormalities.

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