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### Toad (Anura: Bufonidae) Limb Abnormalities from an Aquatic Site in Scott, Pulaski County, Arkansas

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Running Title: Toad (Anura: Bufonidae) Limb Abnormalities from an Aquatic Site in Scott, Pulaski County, Arkansas

#### Abstract

We collected and examined 16 Fowler's toads, *Anaxyrus fowleri*, and one dwarf American toad, *Anaxyrus americanus charlesmithi*, in central Arkansas in 2011. Collection was initiated by observation of abnormal toads. Toads were euthanized, measured, photographed, and deposited in the Arkansas State University herpetological collection. Several toads were radiographed. We found various abnormalities in both forelimbs and hindlimbs and on both sides of the body. The causes of the abnormalities remain unknown and will require further studies to determine if the environment is imperiled.

#### Introduction

Studies on amphibian limb abnormalities have recently become common in the field of amphibian conservation. These abnormalities can vary from limb malformations to complete limb absence. Many stressors can cause similar responses and a singular cause that links the array of abnormalities is not known. Instead the several known causes of these abnormalities appear to have variable effects.

Although research has led to a better understanding of the abnormalities, there is a lack of knowledge of the causes and implications of the deformities (Blaustein and Johnson 2003). Lannoo (2008) believes many sources, both natural and artificial, ranging from fish excrement to overcrowding, are significant when pinpointing causes of the abnormalities.

Herein, we present an observational investigation of abnormalities in Fowler's toads (*Anaxyrus fowleri*) and dwarf American toads (*Anaxyrus americanus charlesmithi*) from a site in south central Arkansas. This is intended to describe the abnormalities and allow for future studies to determine potential causes.

#### **Materials and Methods**

Seventeen toads of varying sizes were collected by Scott. Arkansas (Pulaski County) hand in (34°38"03.19"N, 92°07'58.95"W) and sent to Arkansas State University for processing. The random collection was opportunistic and non-exhaustive. The site (Fig. 1) was around an in ground swimming pool in a residential neighborhood, bordered by several lakes with forest areas and many agricultural plots. The site was not chosen, and the collection was initiated by the property owner (D. Beard) when abnormal toads were observed. The toads were euthanized in a dilute chlorobutanol solution. Fixation was done in a 10% v/v neutral buffered formalin solution. All toads were then photographed, given museum tags, and placed in 70% v/v ethanol.



Figure 1. Map of collection site near Scott (Pulaski Co., Arkansas).

#### Results

Thirteen toads of the 17 collected exhibited some abnormality. Most (92.3%) abnormalities affected the limbs. A list of common abnormalities of the limbs adapted from Lannoo (2008) was used as a basis for identification of the abnormalities (Table 1). Limb abnormalities varied among the toads and were found

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in both adult and sub adult toads (maximum SVL was 75 mm; minimum SVL was 27 mm).

Table 1. Common amphibian limb abnormalities from Lannoo (2008).

Abnormality	Description
Amelia	Missing limb
Ectromelia	Missing limb segments
Polydactyly	Extra digits
Ectrodactyly	Complete absence of digit including metatarsal bone
Skin Webbing	Band of skin crossing a joint

Table 2 documents all abnormalities observed and gives a short description of location and affected area.

One of the 5 toads that did not exhibit any limb abnormalities did appear to have a slightly above average curvature of the sacral hump (seen in Fig. 1a), but it was not radiographed or considered suspicious. The other 4 toads that were collected did not exhibit any noteworthy malformation or abnormality and are not presented in any of the following tables or figures.

Photographs of toads can be seen in Figure 2. Figure 3 shows radiographs and photographs of some of the abnormal toads.

#### Discussion

The abnormalities presented do not have a known cause as of yet. We can speculate, however, to the causes of some of the abnormalities.

The trematode parasite *Ribeiroia ondatrae* is frequently associated with limb abnormalities in anurans, but usually affects the hind limbs. Johnson et al. (2001) found that infections of *R. ondatrae* in *Bufo* (*Anaxyrus*) boreas tadpoles induced severe limb malformations ranging from supernumerary limbs to complete limb absence. In 2002, Johnson et al. linked *R. ondatrae* infection to amphibian malformations in the western United States. In 9 species of amphibians from 4 states, they found that the parasite caused both forelimb and hind limb malformations of varying degrees. Although many believe the parasite is the culprit behind most abnormalities, others disagree.

In a study of wood frogs, Eaton et al. (2004) found abnormalities associated with *R. ondatrae*, but found

no evidence, i.e. cysts, linking the parasite with the abnormalities and instead suggested that sublethal predation was a primary cause for the abnormalities. Because limbs develop outside the body, abnormalities in hind limbs of anurans ranging from missing digits to missing limbs may be linked to sublethal predation (Ballengée and Sessions 2009, Bowerman et al. 2010). Ballengée and Sessions (2009) attribute the abnormalities to dragonfly nymphs and Bowerman et al. (2010) attribute abnormalities to sticklebacks. Others believe pollution is to blame.

Table 2. Observed abnormality and description

Abnormality	Description
Superimposition	Third toe on left foot perpendicular to 2nd toe
Ectromelia	Right forelimb and hindlimb missing distal elements
Oligodactyly	Left hand missing 4th toe and distal portion of 3rd toe
Oligodactyly and skin webbing	Right foot missing 3 medial toes and two lateral toes fused
Ectromelia	Elements below proximal half of right radioulna absent
Adactyly	All toes on right foot absent
Ectrodactyly	All metatarsals and phalanges on left hand absent
Skin webbing	Medial toes on right hand fused
Skin webbing and oligodactyly	Second, third, and fourth toe fused on right hand and 2nd and 3rd toe missing distal phalanges
Amelia	Entire right forelimb missing
Bilateral Ectromelia	Distal portions of both forelimbs missing
Bilateral Ectromelia	Elements distal to proximal portion of humeri missing
Kyphosis	Above average convex curvature of the sacral hump

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Pesticides. herbicides and other chemical compounds used for agriculture have been suggested as the direct or indirect cause of some amphibian malformations (Ouellet et al. 1997, McCallum 1999, Taylor et al. 2005). Reeves et al. (2008) found that skeletal abnormalities increased with proximity to roads in wood frogs in Alaska and suggested that multiple factors from vehicles, pollution, and predator community shifts may cause the increase in abnormalities. Reeves et al. (2010) discussed multiple stressors that may increase likelihood of abnormalities such as radiation, pollution, and predation. The cutaneous fusion observed in some of the toads may be linked to the trematode parasite Ribeiroia that has been found by Johnson et al. (2001) to cause skin webbing in western toads. Missing digits and kyphosis could be caused by injury from sublethal predation. Other abnormalities such as partial limbs or ectromelia of the hind limbs may also be caused by sublethal predation. Ouellet et al. (1997) and Lannoo (2008) believe hindlimb deformities can be attributed to agricultural chemicals such as pesticides and fertilizers.

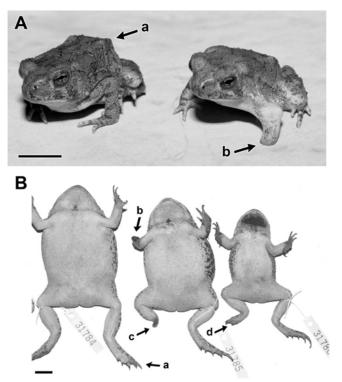


Figure 2. Toads in A with abnormal curvature of sacral hump (a) and ectrodactyly of right hand (b). Toads in B with superimposed toe (a), ectromelia of right forelimb (b) and right hindlimb (c), and oligodactyly (d)

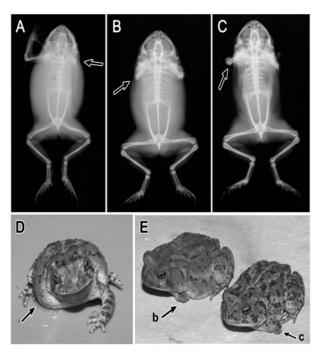


Figure 3. Radiographs showing amelia (A), and bilateral ectromelia (B and C). Photographs D and E are the corresponding toads for radiographs A,B, and C. Missing and partial limbs shown in photographs D and E (b and c).

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