

OCCURRENCES OF LYMPHEDEMA IN ANURANS

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ABSTRACT: Lymphedema is a condition in which the lymph hearts fail to pump fluid from the lymph sacs of anurans and other amphibians. This causes the sacs to fill with fluid and provide the frog with balloon-like swellings or over-all appearance. The condition has previously been connected with various diseases including tadpole edema virus and chytrids. We observed lymphedema in six anuran species: Blanchard's Cricket Frog (*Acris blanchardi*)*, Fowler's Toad (*Anaxyrus fowleri*)*, Squirrel Treefrog (*Hyla squirella*)*, Illinois Chorus Frog (*Pseudacris illinoensis*)*, Wood Frog (*Rana sylvatica*), and the Southern Leopard Frog (*Rana sphenoccephala*)* (species with * are species records for lymphedema).

Key Words: lymphedema, *Batrachochytrium dendrobatidis*, *Flavobacterium indologenes*, chytrids, inflammation.

INTRODUCTION

Reports of lymphedema-like disorders in anurans are rare in the literature. This disorder, first described in 1915 (Moore, 1915) has been identified as one of the most common abnormalities in captive bred *Xenopus* (clawed frogs). This lymphatic dysfunction most commonly results before or just after metamorphosis. It is characterized by extreme inflammation of tissues giving the frog a ballooned appearance. Apparently, the lymph hearts fail to drain the lymph sacs and do not reach maturity (Elkan, 1976). This condition may be an inherited condition due to a recessive gene (Uehlinger, 1965, 1969). Subcutaneous lymph sac structure has been described in some anurans (Carter, 1979).

Gilbert (1942) reported abnormal inflation of the lymph sacs in a pair of male Wood Frogs (*Rana sylvatica*) from New York. Their submandibular, ventral, and lateral lymph sacs were filled with air causing them to float helplessly and be incapable of submerging. A similar problem occurs in Illinois Chorus Frogs (*Pseudacris illinoensis*) in Arkansas (McCallum et al., 2001b). This disorder can be confused with the build-up of fluid characteristic of lymphedema.

Captive African Clawed Frogs (*Xenopus laevis*) are known to develop a lymphedema termed "hydrops" when raised in water with low salinity (Schwabacher and Elkan,

1952). Tadpoles and froglets with hydrops possess inflamed dorsal, lateral, ventral, femoral, and tibial lymph sacs. Wild-caught hydropsic American Bullfrog (*Rana catesbeiana*) tadpoles have been reported with lymphedema of only the femoral and crural lymph sacs (Hintz, 1963). A similar condition was also reported in overwintering tadpoles and adults of Mountain Yellow-legged Frogs (*Rana muscosa*) (Bradford, 1984).

Iridoviruses known from amphibians are classified as polyhedral cytoplasmic amphibian viruses (PCAV). One of these groups, tadpole edema virus (TEV), mimics subcutaneous hydrops (Marcus, 1981). This virus is transmitted in the water and causes mortality in newly metamorphosed frogs. Its symptoms include subcutaneous edema, petechial hemorrhages, and necrosis in the liver, kidneys, gastrointestinal tract, and skeletal muscle (Faeh et al., 1998). Wolf et al. (1968) performed histopathologic analyses of edematous American Bullfrog tadpoles identifying a causal agent, the tadpole edema virus (TEV). They later isolated TEV from apparently healthy American Bullfrogs, though it could not be isolated from either *Anaxyrus* or *Scaphiopus* species from the original source pond. TEV was isolated from American Bullfrog tadpoles from Wisconsin, Alabama, West Virginia, North Carolina, and along the Mississippi River in Arkansas (Wolf et al., 1969). However, edema has also been attributed to the bacterium *Flavobacterium indologenes* (Ol-

son et al., 1992) and the fungus *Batrachochytrium dendrobatidis* (Martel et al., 2011).

American Bullfrog tadpoles appear to be very sensitive to TEV (Wolf et al., 1969), but little is known about susceptibility of *Acris* species to this pathogen. Wolf et al. (1969) found that if the virus was added to water containing 18 one-day-old American Bullfrog tadpoles, that death ensued beginning after 5 days of exposure. Mortality peaked at 53% by the 7th day of this study. Forty-nine-day-old tadpoles showed first signs of mortality on the 5th day after exposure, with mortality peaking at 37% on the 9th day. All tadpoles were dead by the 13th day. Clear-cut histopathology included necrosis of the liver, kidneys, and digestive tract. Liver necrosis was identified by the second day after exposure. Kidney necrosis and edema were expressed after the 4th day. By the 5th day the digestive tract started to show necrosis.

Because lymphedema is closely associated with TEV it is important to document and report its occurrence in regions where this condition has not previously been reported.

MATERIALS AND METHODS

Observations of lymphedema in Anurans were recorded as encountered from 1997 - 2020. Most specimens were released, but others were anesthetized with dilute chlorbutone, fixed in 10% formalin, preserved in 70% ethanol and deposited in the Arkansas State University Museum of Zoology herpetological collection (ASUMZ), Illinois Natural History Survey collection (INHS), or the Louisiana State University at Shreveport Museum of Life

Sciences (LSUSMF).

RESULTS AND DISCUSSION

Incidence of lymphedema among wild-caught anurans was relatively low (Table 1), but was observed in 4 of 5 species in this study. Wood Frogs (*Rana sylvatica*) are a charismatic species of frog common in much of North America. They breed in explosive choruses over a few nights in late winter to early spring. The incidence in Wood Frogs was associated with a die-off of frogs during the breeding chorus in the Sylamore District of the Ozark National Forest in Arkansas (Trauth et al., 2000). In Wood Frogs, lymphedema ranged from the entire torso to localized single lymph sacs on the legs or patches on the back (Fig. 1).

The Illinois Chorus Frog (*P. illinoensis*) and Strecker's Chorus Frog (*Pseudacris streckeri*) are small fossorial frogs that occur in Arkansas and Oklahoma. The range of Illinois Chorus Frogs in Arkansas has severely contracted, most likely due to precision leveling of the farmland in which they reside (McCallum and Trauth, 2002; Trauth et al., 2006). We observed a single Arkansas frog with lymphedema of the right dorsal lymph sac (Fig. 1). This individual appeared normal in movements and activity although it did not appear to be calling or acting as a satellite to other calling males (McCallum et al., 2003). Illinois Chorus Frogs were also observed with frostbite scars (n = 10), red inguinal pustules (n = 2), a dysfunctional vocal sac (n = 1), and a missing forelimb (n = 1), (McCallum et al., 2006; McCallum et al., 2001b). No Strecker's Chorus Frogs from Oklahoma were observed with lymphedema.

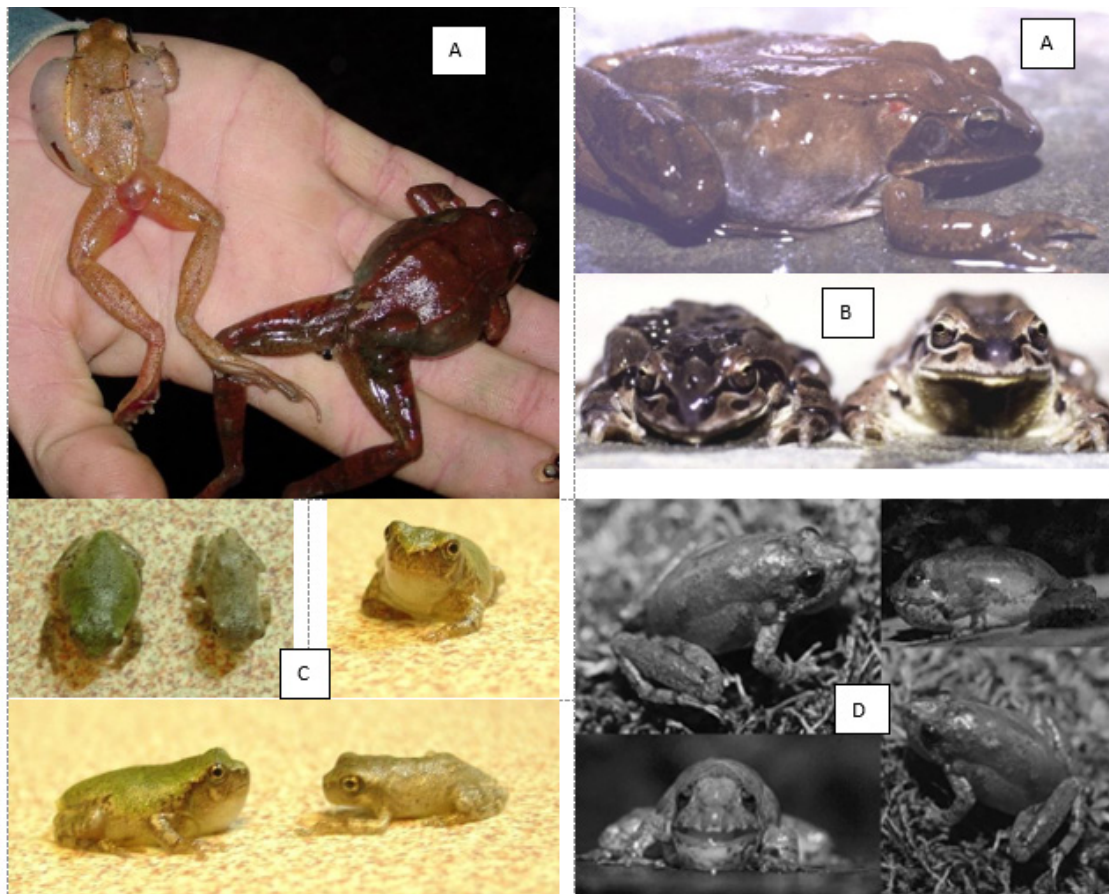


Figure 1. Lymphedema in wild-caught anurans. A. Wood Frog (*Rana sylvatica*) note: USGS Wildlife Disease Laboratory found no sign of disease (Green Pers. Comm). B. Illinois Chorus Frog (*Pseudacris illinoensis*). C. Squirrel Tree Frog (*Hyla squirella*). D. Blanchard's Cricket Frog (*Acris blanchardi*).

The Southern Leopard Frog (*Rana sphenoccephala*) is a generic frog species easily confused with several other species of leopard frogs with whose ranges they overlap. They are common to creeks, grasslands, forests, and ponds throughout the southeastern United States. Among 66 post-metamorphic Southern Leopard Frogs that were examined, a few (est. 3-4) were observed with lymphedema. However, the exact numbers were not recorded among malformed frogs in that study (McCallum, 1997), nor were possible incidences of partial lymphedema noticed.

Fowler's Toad (*Anaxyrus fowleri*) is an average sized toad occurring across the eastern half of the United States. A single metamorph had lymphedema. It seemed

otherwise normal, except that it had difficulty moving with the disorder. No Spring Peepers (*Pseudacris crucifer*) or Gray Treefrogs (*Hyla versicolor* or *H. chrysoscelis*) were observed with the condition despite occurring in locations where other species had it (Wood Frogs, Southern Leopard Frogs, Blanchard's Cricket Frogs).

The Squirrel Treefrog (*Hyla squirella*) is a common arboreal frog of the Southeastern United States, although it was unknown to the Shreveport, Louisiana area at the time of this observation. We collected five near-metamorphosed tadpoles at Loggy Bayou Wildlife Management (Bowie Co., Louisiana, USA) and placed them in a 20 L aquarium containing water from the collection site. They appeared morphologically normal. Upon metamor-

Table 1. Surveys of amphibians with incidences of lymphedema.

Location (Date)	Positive (N)	Inspected (N)	Species	Life Stage/s
Clay Co., Arkansas (Feb 2001)	1	40	Illinois Chorus Frog (<i>Pseudacris streckeri</i>)	Adults
Craighead Co., Arkansas (Aug 1999 – May 2003)	0	>1,000	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults and Juveniles
Hot Springs Co., Arkansas (June 2002)	1	11	Fowler's Toad (<i>Anaxyrus fowleri</i>)	Juveniles
Jane's Creek, Randolph Co., Arkansas (Aug 1999 – May 2003)	6	>1,000	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Post-metamorphic juveniles and one adult
Sharp Co., Arkansas (Feb 2000)	1	10	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults
Stone Co., Arkansas (Feb 2000)	5	140	Wood Frog (<i>Rana sylvatica</i>)	Adults
Stone Co., Arkansas (Feb 2000)	0	>500	Spring Peeper (<i>Pseudacris crucifer</i>)	Adults
Madison Co., Illinois (Summer 1997)	1	1	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults
Madison Co., Illinois (July 1997)	4	66	Southern Leopard Frog (<i>Rana sphenoccephala</i>)	Post-metamorphic juveniles
McLean Co., Illinois (Feb – Apr 1990)	0	89	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults
Bossier Parish, Louisiana (Aug 2004)	1	5	Squirrel Treefrog (<i>Hyla squirella</i>)	Metamorphs
Ozark Co., Missouri (Feb 2002)	2	70	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults
Johnson Co., Missouri (Feb 2012 – June 2016)	0	>5,000	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults, juveniles, metamorphs
Mammoth Spring, Oregon Co., Missouri (Feb 2000 – May 2003)	0	>1,000	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults, juveniles, metamorphs, tadpoles
Bowie Co., Texas (May 2003 – Dec 2011)	0	>1,000	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults, juveniles, metamorphs, tadpoles
Logan and Payne Counties, Oklahoma (July 2016 – Oct. 2020)	0	>5,000	Blanchard's Cricket Frog (<i>Acris blanchardi</i>)	Adults, juveniles, metamorphs, tadpoles.
Logan and Payne Counties, Oklahoma (July 2016 – Oct. 2020)	0	24	Strecker's Chorus Frog (<i>Pseudacris streckeri</i>)	Juveniles

phosis, one developed lymphedema, the rest were normal. This frog died seven days later.

Blanchard's Cricket Frog (*Acris blanchardi*) whose taxonomic status is subject to debate (McCallum 2003; McCallum and Trauth 2006; Gamble et al. 2008), is a small, non-arboreal hylid of the eastern half of North America that can reach extraordinarily high populations on stream banks in the Ozarks and elsewhere (McCallum and Trauth, 2004; McCallum et al., 2011), which facilitates some of its unique anti-predator behaviors (McCallum, 1999; McCallum, 2011). Anatomical abnormalities in Blanchard's Cricket Frogs from Arkansas have increased over the past half century (McCallum and Trauth, 2003) and its reproductive and growth strategies (McCallum and Trauth, 2007; McCallum et al., 2011) appear to place it at threat from climate change (McCallum, 2010). We observed large numbers of living Blanchard's Cricket Frogs in six different states (Arkansas, Illinois, Louisiana, Missouri, Oklahoma and Texas). Additionally, we inspected hundreds of preserved frogs from South Dakota, Nebraska, Georgia and Florida during other studies (McCallum, 2003; McCallum et al., 2011) although it was difficult to assess lymphedema in preserved specimens that had not also been seen alive. We observed more cases of lymphedema in Blanchard's Cricket Frog (Fig. 1) than in other species, but this may be an artifact of sampling effort.

Lymphedema was relatively rare (Table 1). Afflicted frogs of all species were more sedentary compared to non-afflicted individuals (as previously seen in malformed Southern Leopard Frogs from southern Illinois [McCallum, 1997]), which would reduce the effectiveness of their escape behavior (McCallum, 2011), thus increasing the threat of predation, including from adult conspecifics (McCallum et al., 2001a).

Except in cases with minor lymphedema consisting of single or partial lymph sac inflammation, the disorder can be very debilitating. Frogs with extreme swelling have difficulty moving, jumping, and often remain motionless much of the time. If the disorder is caused by TEV or chytrids then it would constitute a possible method of dissemination if metamorphs emerged unable to escape hungry adult frogs of the same and other species. Although the condition of lymphedema has been known for many years, it is surprising that more studies have not been conducted among wild populations considering its association with amphibian pathogens and the rapidly accelerating extinction of amphibians we have already observed (McCallum, 2007; McCallum 2015).

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