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Observation of *Ribeiroia ondatrae* (Price, 1931) Infection in a Malformed American Bullfrog, *Rana catesbeiana* Shaw, 1802, in Kane County, Illinois (United States)

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Amphibian limb malformations attributed to *Ribeiroia ondatrae,* a teratogenic trematode, have been reported in the United States and Canada (Johnson and Sutherland, 2003; Lunde and Johnson, 2012; Roberts and Dickinson, 2012). The complex parasitic relationship between *R. ondatrae* and North American amphibians is summarized by Johnson and Sutherland (2003). Parasitism of larval anurans by *R. ondatrae* cercariae can lead to the development of supernumerary limbs or other malformations as animals metamorphose. These malformations are hypothesized to enhance the anuran's vulnerability to predation, primarily by predatory birds that act as definitive hosts in which the trematode reproduces sexually and is spatially dispersed. Although *R. ondatrae* is native to North America (Johnson and McKenzie, 2008), anthropogenic factors such as increased aquatic eutrophication can bolster algal growth and populations of the freshwater snails that serve as first intermediate hosts for the parasite (Johnson et al., 2007).

Beginning in the 1990s, widespread reports of malformed frogs prompted considerable public concern and scientific controversy over the causes and implications of such malformations (e.g., Souder, 2000; Lannoo, 2008; Johnson et al., 2010). Although such malformations have been detected in American bullfrogs (*Rana catesbeiana*), reports are relatively uncommon in natural populations (see Johnson et al., 2001; Haas et al., 2018), and experimental studies indicate that bullfrogs have an especially low susceptibility to *Ribeiroia* infection and the malformations it induces (e.g., Johnson et al., 2012). For instance, among the 13 amphibian species exposed to *Ribeiroia ondatrae* under controlled conditions, *Rana catesbeiana* was among the least susceptible to infection and exhibited a low frequency of malformations and mortality even following high levels of exposure. Particularly little is known about geographical patterns of infection and malformations within the native range of bullfrogs. Although *Ribeiroia* infection has been detected within the native range of bullfrogs, including Illinois (Johnson, personal communication), little is known about its impact on amphibians in the Chicago region.

Here, we describe an instance of *Ribeiroia ondatrae* parasitism and severe limb deformities of an American bullfrog (*Rana catesbeiana*) from southern Kane County, Illinois (41.72792, -88.36985). At 12:30 P.M. on 09 July 2020, a malformed metamorphic American bullfrog (*Rana catesbeiana*) (Figure 1; HM 324718; HerpMapper, 2020) was observed leaping erratically on a dry asphalt parking lot (sunny, 33.3°C; 757 mmHg; 55% humidity). The surface temperature of the asphalt was not measured but likely exceeded 38°C. The nearest habitat was a marsh approximately 250 meters east of the parking lot located between a residential neighborhood, an industrial park, and an agricultural field, the latter of which likely contributes runoff to the marsh.

Figure 1.

In addition to its four normal limbs, two smaller, supernumerary limbs had developed anterior of the left hind leg, hindering normal movement (see Figures 1 and 2). The supernumerary limbs were non-functioning and paler in color, although blood vessels were clearly visible while the frog was alive. The super-

Figure 2.

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Table 1. Morphometric measurements of the malformed bullfrog.

Morphometric parameter	Length (mm)
Snout–vent length	51.2
Left normal hind limb length	63.5
Right normal hind limb length	63.5
Anterior supernumerary hind limb length	38.1
Posterior supernumerary hind limb length	51.2

numerary limbs were ventral, which is consistent with previous limb malformations in bullfrogs associated with *Ribeiroia ondatrae* infection (e.g., Johnson et al., 2001).

The frog was collected and an attempt was made to maintain the frog in captivity. However, it expired one week later. Immediately upon its death, the snout–vent and various hind limb lengths were measured (Table 1). The snout–vent length was measured from the tip of the nose to the vent and hind limb lengths were measured from the vent (or other point of attachment) to tip of the longest toe (toe IV) while extended (i.e., combined thigh, tibia, tarsus, and foot lengths).

The frog was subsequently frozen and sent to the University of Colorado, Boulder, to test for the presence and abundance of *R. ondatrae* metacercariae (the encysted stage of the parasite). Upon necropsy, approximately 50 *R. ondatrae* metacercariae were detected within the frog, which were concentrated within the tail reabsorption site and around the hips. The cysts were melanized and dark gold-brown in color. Additionally, one adult worm belonging to the genus *Haematoloechus* was found in the left lung and 65 echinostome metacercariae were found in the kidneys (likely of the genus *Echinostoma* or *Echinoparyphium*). Sixty-four of the cysts were in the right kidney while one cyst was in the left kidney, consistent with the sidedness that is observed in infections of *Echinostoma* (Johnson et al., 2014).

Various aquatic gastropod shells were later collected from the marsh and identified. Among several species found was the marsh ram's-horn snail (*Helisoma* [*Planorbella*] *trivolvis*), which is an important first intermediate host of *Ribeiroia ondatrae* (see Johnson et al., 2004). Alongside the juvenile stage of the bullfrog, this observation helps support the hypothesis that the animal developed within the marsh, although no other amphibians were inspected for malformations or infection.

The presence of *R. ondatrae*, its relatively high abundance within the specimen (~50 metacercariae), and the consistency of the observed malformations with those known to be induced by *Ribeiroia* under experimental conditions all support the hypothesis that the abnormalities were parasite-induced. Examination of additional amphibians both from this wetland and the surrounding area would be informative for better understanding the distribution of *R. ondatrae* and such malformations. Why bullfrogs appear to be less susceptible to *R. ondatrae* infection and the malformations it induces remains an open question. Thus far, however, few studies have explored the prevalence of *Ribeiroia* or limb malformations among bullfrogs in their native range. As part of a continental study of amphibian malformations on National Wildlife Refuges (NWRs) in the USA, Haas et al. (2018) detected *R. ondatrae* infections in bullfrogs from three NWRs within their native range: Crab Orchard NWR in Illinois (3 sites), Oxbow NWR in Massachusetts (2 sites), and Erie NWR in Pennsylvania (1 site). The average and maximum infection loads were 14.4 and 52 (Crab Orchard NWR; n = 52), 14.1 and 115 (Oxbow NWR, n = 29), and 26.0 and 58 (Erie NWR, n = 11). Malformations were noted in three of the individuals dissected.

The implications that can be drawn from the presence of *R. ondatrae* in the immediate area may be that there are high levels of bird definitive host activity, consistent with the location along the Mississippi bird flyway (see also Hartson et al., 2011). While the parasite is native to North America, its infection levels—and the resultant malformations in amphibians—can become amplified in response to elevated levels of nitrogen and phosphorus-based fertilizers, often used in agriculture, which can cause eutrophication. Eutrophication can increase density or size of *P. trivolis* and therefore optimize and increase the transfer of trematode from snail to anuran larvae (Johnson et al., 2007). Other factors hypothesized to enhance the transmission or abundance of *R. ondatrae* include amphibian biodiversity losses, which increase the proportion of highly suitable hosts (see Johnson et al., 2013), and progressive warming as an effect of climate change, which can amplify trematode production within aquatic snails (see Paull and Johnson, 2011). The year 2020 was the hottest year in Chicago since record-keeping began in 1873 (US Department of Commerce, NOAA, 2020), and regional climate models predict increased warming in the 21st century (Zhang et al., 2020).

Globally, anurans are threatened by a multitude of fungal, bacterial, viral, and parasitic diseases. Many of these diseases are at least indirectly promoted by anthropogenic factors, such as accelerated climate change and enhanced pollution that can disrupt the natural life processes of anurans, increasing susceptibility to established or emerging pathogens. The continued use of agricultural chemicals, especially near anuran habitat, has the potential to reshape local wetland ecology, countering conservation initiatives geared toward improving biodiversity. We should re-evaluate our use of currently applied fertilizers to decrease their negative effects on aquatic ecosystems and anuran populations.

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