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Batrachochytrium dendrobatidis and Ranavirus in Anurans Inhabiting Decorative Koi Ponds near Minneapolis, Minnesota, USA

Evidence of widespread chytrid infection in North American amphibian populations has been documented (Longcore et al. 2007; Ouelett et al. 2005; Woodhams et al. 2008) and *Batrachochytrium dendrobatidis* (*Bd*) has been reported from several locations in Minnesota (Rodriguez et al. 2009; Sadinski et al. 2010; Woodhams et al. 2008). Ranaviruses also have been linked to mass amphibian die-offs in North America (Daszak et al. 1999; Gray et al. 2009; Greer et al. 2005; Jancovich 2005), including Minnesota (Green et al. 2002; Vandenlangenberg et al. 2003). The geographic distribution of these pathogens in Minnesota is still unknown, however, and additional observations will aid assessment of the threat to local amphibian populations.

We report the presence of ranavirus and *Bd* in anurans inhabiting four decorative koi ponds in Burnsville, Minnesota, a suburb of Minneapolis (Fig. 1). The ponds were closely observed for indications of pathogenic activity following the discovery of dead Wood Frogs (*Lithobates sylvaticus*) floating on the surface during the last week of August 2011. Selected specimens were tested for *Bd* and ranavirus.

These four rubber-lined ponds were typical of those constructed by koi- and water-gardening enthusiasts. Their combined surface area was approximately 30 m^2 and their maximum depth was 1.05 m. Koi and goldfish were held in the ponds throughout the year. Total fish biomass was roughly 3.3 kg. The largest fish measured approximately 0.35 m, total length. Water was circulated through three of the four ponds by a pump connected to a simple filtering system employing fiberglass batting as filter material. A similar system was used to filter water in the fourth pond. Water lost via evaporation was replaced by precipitation and partial refilling from a tap connected to a chlorinated city water line.

The ponds were situated within a 260 m^2 area separated from a 970 ha park reserve by a short (100–150 m) strip of undeveloped land. The distance to the nearest permanent body of water was approximately 112 m.

From 25 August to 4 September 2011, three dead Wood Frogs were recovered from the ponds. All three specimens were fresh and exhibited significant reddening of the ventral surface. No signs of traumatic injury were observed and disease was determined to be a likely cause of death.

We visited the ponds daily from 7 September to 24 October 2011, following the initial frog deaths. During each visit, the ponds and immediate surroundings were visually surveyed for amphibians. Observations of dead and dying individuals were recorded and dead specimens were collected for examination, condition permitting. Anurans exhibiting unusual behavior were photographed.

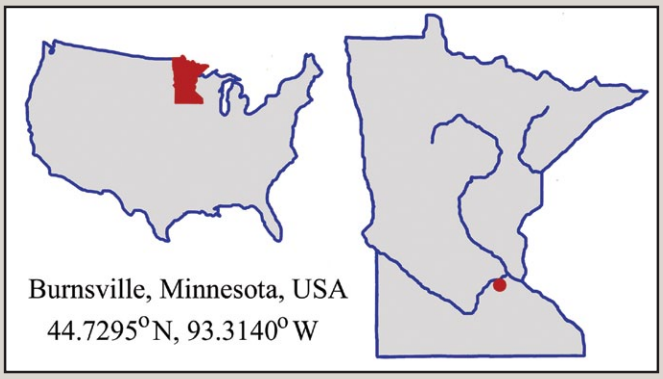

Fig. 1. Location of koi ponds in Minnesota, USA where frogs were found infected with *Batrachochytrium dendrobatidis* and ranavirus.

Our daily visits produced 202 sightings of six species (Table 1). Two dead Wood Frogs, one dead Green Frog (*Lithobates clamitans*), and two dead American Toads (*Anaxyrus americanus*, formerly *Bufo americanus*) were recovered from 7 September to 24 October 2011. Most of the dead specimens were retrieved from the ponds. The lone exception was an American Toad found within 0.25 m of a pond. Detailed examination of the Green Frog and one of the toads was precluded by advanced decomposition. The other toad was recovered in very good condition and transported

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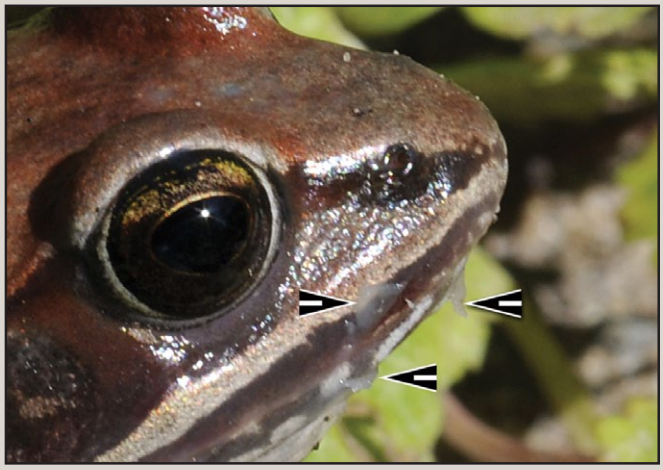

FIG. 2. Wood Frog (*Lithobates sylvaticus*) observed near koi ponds in Burnsville, Minnesota, USA, during the final 24 h of life. Arrows indicate sloughing of the epidermis bordering the mouth.

FIG. 3. Wood Frog (*Lithobates sylvaticus*) observed near koi ponds in Burnsville, Minnesota, USA, during the final 24 h of life.

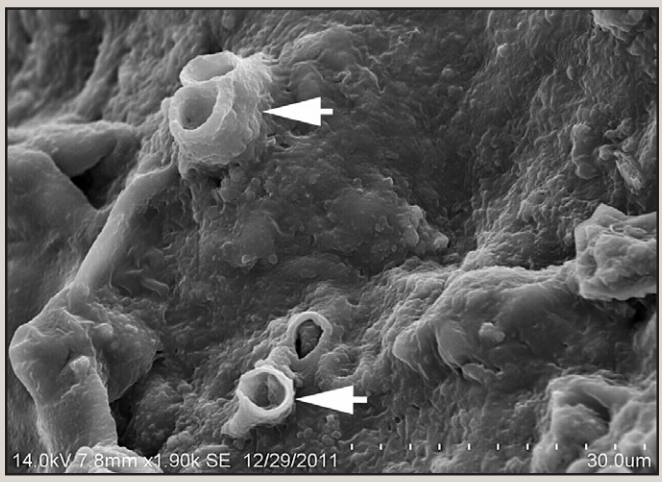

FIG. 4. Scanning Electron Microscope image of skin removed from the groin of a dead Wood Frog (*Lithobates sylvaticus*) collected from a koi pond in Burnsville, Minnesota, USA. Arrows indicate the presence of zoosporangia of *Batrachochytrium dendrobatidis*.

to the United States Geological Survey's National Wildlife Health Center (NWHC) in Madison, Wisconsin (USA). Chytrid infection was confirmed in this immature female by histological examination. The two dead Wood Frogs were observed during the final 24 h of life and photographed. Both were unusually sluggish and unresponsive to stimulation of the hindquarters. They were first observed in exposed locations, more than one meter from water. One of these frogs exhibited obvious sloughing of the epidermis bordering the mouth (Fig. 2). The other rested with the legs splayed out posteriorly (Fig. 3). The former specimen was found dead approximately 24 h after first being observed. The latter specimen was collected for observation and placed in a 300-gallon livestock-watering tank with moist soil and leaves. It died within 5 h of first being observed. Reddening of the ventral surface and legs was apparent in both specimens.

Over a four-day period, beginning on 24 October, the ponds were drained to inhibit possible pathogen dispersal to a nearby wildlife refuge. All anurans encountered in the process of draining the ponds were collected for examination. This included 27 Green Frogs and two Northern Leopard Frogs (*Lithobates pipiens*). Sixteen of the Green Frogs exhibited significant reddening of the ventral skin consistent with ranavirus and/or *Bd* infection. One adult Leopard Frog, seven adult Green Frogs, and five immature Green Frogs were submitted to the NWHC for ranavirus testing via PCR using 4/5 capsid protein primers (Mao et al. 1997). The Leopard Frog tested negative, but one immature and one adult Green Frog tested positive, as did a single pooled sample from two adult Green Frogs.

After draining, the ponds were partially refilled at various intervals by precipitation. We continued visiting the ponds on an irregular basis through the end of December. On 4 December, one dead Spring Peeper (*Pseudacris crucifer*), two dead Green Frogs, and two dead Wood Frogs were collected from several inches of water in one partially frozen pond. Their appearance followed an 11-day stretch of relatively warm weather (e.g., mean high 6.1°C) accompanied by approximately 8 mm of precipitation. None of the frogs showed signs of traumatic injury. One of the Wood Frogs was in particularly good condition and skin from the legs and groin was removed for electron microscopy. *Bd* infection was confirmed by the presence of zoosporangial discharge tubes (Fig. 4).

Bd has been reported in Minnesota anurans, but we believe this is the first confirmed report of *Bd* and ranavirus in a Minneapolis/St. Paul suburban area and the first report of *Bd* and ranavirus in decorative koi ponds.

Decorative koi ponds and water gardens, which are extremely simplified ecosystems, may be especially conducive to *Bd* propagation and viral transmission. Cladocerans and other grazers, which have been shown to significantly reduce zoospore densities (Buck et al. 2011; Hamilton et al. 2012; Woodhams et al. 2011), are likely to be absent from simple koi ponds and water gardens. The water in koi ponds and water gardens is seldom replaced during the summer and zoospores are very unlikely to be removed by the filtration systems popular with hobbyists.

It is clear from our observations that anuran densities in rubber-lined koi ponds and water gardens can be impressive and certainly adequate to facilitate disease transmission. We recorded an anuran density of approximately 1.04 frogs/m^2 of pond surface at the time of draining. In addition to creating conditions that might facilitate the transmission of important anuran pathogens, koi enthusiasts and water gardeners may be fostering the spread of *Bd* and ranavirus by importing infected

TABLE 1. Summary of koi-pond amphibian observations made 25 August to 31 December 2011 in Burnsville, Minnesota, USA. Live sightings = total observations of live animals among days, may count individuals multiple times; Minimum number of individuals was established by the number of specimens collected (*B. americanus, L. clamitans, L. pipiens, L. sylvaticus*) or the maximum number of unique individuals observed during a single observation period (*Hyla* sp., *P. crucifer*); *Bd* = *Batrachochytrium dendrobatidis*; Rv = Ranavirus. * One Ranavirus-positive animal came from a pooled sample, and one or both specimens from the pool could have been Ranavirus-positive.

Species	No. live sightings	Minimum No. individuals	No. known mortalities	No. specimens collected (No. *Bd*-symptomatic)	No. *Bd*-examined (No. *Bd*-confirmed)	No. Rv-examined (No. Rv-confirmed)
Anaxyrus (*Bufo*) *americanus*	4	2	2	2 (1)	1 (1)	0
Hyla sp.	7	4	0	0 0	0	
Lithobates clamitans	174	30	3	30 (16)	0	12 (3-4)*
L. pipiens	6	3	0	3 (0)	0	1 (0)
L. sylvaticus	10	7	7	7 (7)	1 (1)	0
Pseudacris crucifer	1	1	1	1 (0)	0	0

pond organisms. Bullfrogs (*Lithobates catesbeianus*), for example, are marketed to koi enthusiasts and water gardeners to control algae (https://www.pondmegastore.com/shop/home.php?cat=322; accessed 2 April 2012) and have been linked to *Bd* and ranavirus dispersal (Daszak et al. 2004; Garner et al. 2006; Miller et al. 2007). Water hyacinth was introduced to the ponds surveyed in this study from a company that advertises that its plants are grown organically, and that tadpoles are used to control algae (http://stores.ebay.com/HELENS-PERENNIAL-WATER-GARDENS/BIO-ECO-FRIENDLY-ORGANIC-GROWN.html; accessed 15 January 2012). Research clearly demonstrates that *Bd* can survive for several days in moist packing materials and it has been shown that the fungus can attach itself to plant material (Johnson and Speare 2005). The possibility that koi ponds and water gardens serve as loci for *Bd* dispersal would seem to warrant greater attention.

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