found similar results in populations of *Lithobates yavapaiensis* in Arizona, USA, although Huang and Wilson (2013) did not find such a relationship when comparing amphibians in Georgia’s Blue Ridge and Piedmont provinces.

Acknowledgments.—Funding was provided by a Berry College Development of Undergraduate Research grant (to CBM and MBM) and a Richards Undergraduate Research Support grant (to RAM and JE). Amphibian sampling was conducted under a Berry College IA-CUC permit. Thanks to D. Gregory for facilitating access to Crockford-Pigeon Mountain WMA. For field assistance, we thank T. Moore and B. Pierson. J. Mendelson and L. Smith provided valuable guidance in initiating the study. We also thank C. Lillard, D. Olson, and an anonymous reviewer for helpful comments, and K. Clements for assistance in figure preparation.

**Literature Cited**


Two Ranaviruses-Associated Mass-Mortality Events among Larval Amphibians in Illinois, USA

**Lucas J. Kirschman***

Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901, USA

**John G. Palis**

Palis Environmental Consulting, P.O. Box 387, Jonesboro, Illinois 62952, USA

**Kelley A. Fritz**

**Kyle Althoff**

**Robin W. Warne**

Department of Zoology, Southern Illinois University, Carbondale, Illinois 62901, USA

*Corresponding author; e-mail: ljkirschman@alaska.edu

Growing evidence suggests that ranaviruses are globally distributed pathogens that infect a diversity of ectothermic vertebrates, and can cause sudden mass-mortality events, especially in larval amphibians (Brunner et al. 2004, 2015; Gray et al. 2009; Warne et al. 2016). Ranavirus epizootics in larval amphibians can also amplify viral titers in the environment (Hall et al. 2016) and potentially export sublethally infected metamorphs to the surrounding ecosystem, creating a possible transmission route to sympatric hosts (Brunner et al. 2007). Despite a growing awareness of disease threats to amphibians, ranavirus epizootics are likely undetected and underreported because they are often rapid and cryptic (Brunner et al. 2015). Given the broad range of hosts threatened by ranaviruses (Duffus
et al. 2015), documenting the geographic range, spread, and effects of the disease, particularly mass die-offs is important for conservation and management efforts.

Here, we report observations consistent with ranavirus-induced mass-mortality events in larvae of two amphibian species over two years (2015 and 2016) in southern Illinois, USA. These are the first reported ranavirus-related mass-mortality events among amphibians in the region, although ranavirus was previously detected on skin swabs of amphibians from Illinois and Wisconsin (Sekowska et al. 2014). We also report findings of water samples examined for ranavirus DNA collected from four water bodies in Illinois, including at the site of one of the observed mortality events, supporting the occurrence of ranavirus in the region prior to the die-off events.

We collected water samples in four water bodies in southern Illinois to sample for ranavirus environmental DNA (eDNA) in May and June of 2014 (Table 1; Fig. 1). Water was collected in sterile 500 mL containers and filtered through 0.22 μm micropore PVDF membrane filters (PVDF23050, Sterlitech, Kent, Washington, USA) via vacuum filtration to collect eDNA (Hall et al. 2016).

We also collected amphibian larvae from two die-offs. The first occurred among Eastern Spadefoot (Scaphiopus holbrookii) larvae in a natural sinkhole pond in Jonesboro, Union County, Illinois (Table 1; Fig 1). We first observed lethargic and moribund larvae with swollen hind limbs and erythema on 28 July 2015. We collected four recently-deceased larvae by dip net and stored them in 70% ethanol. Subsequently, we found hundreds of dead and dying metamorphs at the water’s edge on 29 and 30 July 2015. These metamorphosing larvae displayed gross lesions consistent with ranavirus, including erythema and ecchymosis on the ventral surface of the abdomen and hind limbs (Miller et al. 2015).

The second die-off occurred among Wood Frog (Lithobates sylvaticus [Rana sylvatica]) larvae in a wetland in the Cave Creek Valley of Jackson County, Illinois (Table 1; Fig. 1). On 17 April 2016, we observed some moribund larvae on the water’s surface, displaying no visible lesions. We collected seven of the moribund larvae by dip net and stored them in 70% ethanol. The moribund Wood Frog larvae were present alongside hundreds of apparently healthy conspecifics, as well as larvae of four other taxa: Spring Peeperers (Pseudacris crucifer), Trilling Chorus Frogs (Pseudacris triseriata complex), Spotted Salamanders (Ambystoma maculatum), and Marbled Salamanders (Ambystoma opacum), none of which exhibited signs of disease. Upon return to the site less than 36 h later to conduct a broader survey for a potential epizootic event, we did not observe or capture a single larvae or carcass of any species despite extensive searching and core (enclosure) sampling. All larvae in the pond were in early

Table 1. Average ranavirus titer in plaque forming unit equivalents (PFU) per sample and location of amphibian die-offs.

<table>
<thead>
<tr>
<th>Species or eDNA sample</th>
<th>County</th>
<th>GPS coordinates</th>
<th>Sample date</th>
<th>Number positive / tested</th>
<th>Log10 Mean Viral Load ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scaphiopus holbrookii</td>
<td>Union</td>
<td>37.459350°N 89.268647°W</td>
<td>28 July 2015</td>
<td>4 / 4</td>
<td>7.80 ± 0.22</td>
</tr>
<tr>
<td>Lithobates sylvaticus</td>
<td>Jackson</td>
<td>37.641145°N 89.340451°W</td>
<td>17 April 2016</td>
<td>7 / 7</td>
<td>7.37 ± 0.26</td>
</tr>
<tr>
<td>eDNA Sample (500 mL)</td>
<td>Jackson</td>
<td>37.639368°N 89.353640°W</td>
<td>16 May 2014</td>
<td>1 / 1</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89.172319°W</td>
<td>14 June 2014</td>
<td>1 / 1</td>
<td>2.03</td>
</tr>
<tr>
<td>eDNA Sample (500 mL)</td>
<td>Williamson</td>
<td>37.703788°N 89.130864°W</td>
<td>16 May 2014</td>
<td>0 / 2</td>
<td>1.61 ± 1.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89.010335°W</td>
<td>14 June 2014</td>
<td>0 / 1</td>
<td>2.19</td>
</tr>
<tr>
<td>eDNA Sample (500 mL)</td>
<td>Williamson</td>
<td>37.668570°N 89.010335°W</td>
<td>16 May 2014</td>
<td>0 / 1</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>89.147505°W</td>
<td>14 June 2014</td>
<td>1 / 1</td>
<td>2.13</td>
</tr>
</tbody>
</table>
mechanisms contributing to the emergence of ranavirus.

Days to perhaps a week (see also Wheelwright et al. 2014). With ranavirus is restricted to a narrow time window of just a few site, suggests that identifying a mass mortality event associated with ranavirus is restricted to a narrow time window of just a few days to perhaps a week (see also Wheelwright et al. 2014).


Acknowledgments. —Collections were authorized by the U.S. Forest Service, Illinois Department of Natural Resources permit NH16.5988, and a State of Illinois fishing license. Wood Frog euthanization was approved by the Institutional Animal Care and Use Committee at Southern Illinois University (16-012). Thank you to Jessica Fulgoni for making the map.

The reservoirs for ranavirus and the ecological factors driving outbreaks are generally poorly understood. In the Eastern Spadefoot case, the ranavirus outbreak may have been related to near-complete drying of the sinkhole by 2100 h on 19 July 2015 when thousands of larvae were confined to a 1.3-cm deep, 50 cm × 60 cm pool, followed by refilling during a thunderstorm starting at ca. 0100 h on 20 July 2015. By contrast, we detected no obvious environmental factors that could explain the second outbreak and putative die-off at the Cave Creek site. Ranavirus eDNA was detected in a nearby pond in this wetland matrix two years prior to the observed die-off, suggesting the virus was present in the area, but again, no die-offs were observed.

Wood Frogs are also highly susceptible to infection and ranavirus-induced mortality and may act as amplification hosts for ranaviruses (Brunner et al. 2015). Indeed, they have been shown to increase community level mortality when they are the first species exposed to ranavirus (Brenes 2013). These findings suggest that community composition and dynamics, potentially in conjunction with abiotic factors (e.g., thermal stress, hydroperiod, pollution), may interact to influence the spread and likelihood of ranavirus epizootics (St-Amour et al. 2008; Echaubard et al. 2010).

The work of Hall et al. (2016) and our eDNA analysis suggest that surveillance using water sampling can potentially increase the probability of detecting ranavirus and potential epizootics. We detected ranavirus eDNA from water at multiple sites, including a pond at the Cave Creek site two years prior to the observed mortality event (Table 1, Fig. 1). Although these eDNA data do not provide insight into the reservoirs or factors that could lead to a potential outbreak, they do suggest that ranavirus may be present throughout southern Illinois. Three of our sites showed eDNA viral titers above 100 PFU, a level which is near the median lethal dose for Wood Frogs (Warne et al. 2011) and associated with ranavirus die-offs in Connecticut, USA (Hall et al. 2016). Furthermore, these results suggest repeated eDNA surveillance may provide a means to more efficiently identify the prevalence and potential threat of ranavirus across time and space (Johnson and Brunner 2014; Hall et al. 2016). At a minimum, these data suggest more intensive surveillance is necessary to ascertain the prevalence of the pathogen and severity of the threat to amphibians and other ectothermic vertebrates.

Acknowledgments. —Collections were authorized by the U.S. Forest Service, Illinois Department of Natural Resources permit NH16.5988, and a State of Illinois fishing license. Wood Frog euthanization was approved by the Institutional Animal Care and Use Committee at Southern Illinois University (16-012). Thank you to Jessica Fulgoni for making the map.

Literature Cited


---

**The Columbia Spotted Frog (Rana luteiventris)—Another Species Persisting with Batrachochytrium dendrobatidis Infection**

---

**HEATHER L. ARAOS**  
Department of Biology, Brigham Young University, Provo, Utah 84602, USA

**KELLEY L. KROFT**  
Department of Biology, Long Island University Post, Brookville, New York 11548, USA

**ROBERT M. BOGARDUS**  
Department of Biology, Brigham Young University, Provo, Utah 84602, USA; Division of Mathematics and Sciences, McCook Community College, McCook, Nebraska 69001, USA

**YUAN-MOU CHANG**  
Department of Biology, Brigham Young University, Provo, Utah 84602, USA; Department of Ecoscience and Ecotechnology, National University of Tainan, Tainan, Taiwan 70005

**KERRI R. DONOHUE**  
Department of Biology, Long Island University Post, Brookville, New York 11548, USA

**DANIEL HANLEY**  
Department of Biology, Long Island University Post, Brookville, New York 11548, USA

**KENT A. HATCH**  
Department of Biology, Brigham Young University, Provo, Utah 84602, USA; Department of Biology, Long Island University Post, Brookville, New York 11548, USA

**KRISSEY W. WILSON**  
Utah Division Wildlife Resources, Salt Lake City, Utah 84116, USA

*Corresponding author, e-mail: kent.hatch@liu.edu

Small, isolated populations of amphibians are of particular concern because they may be especially vulnerable to the effects of pathogens (Smith et al. 2009). The Columbia Spotted Frog (Rana luteiventris) is an explosive breeder (Bull and Shepherd 2003; Greene and Funk 2009) found across much of the American northwest, as well as British Columbia and Alberta, Canada (Funk et al. 2008). In Utah, USA R. luteiventris is a species of concern because within the state it exists in small, relatively isolated populations (Bailey et al. 2006). An emergent pathogen that may affect R. luteiventris is the chytrid fungus Batrachochytrium dendrobatidis (hereafter, Bd), which causes the amphibian disease chytridiomycosis. This fungus has been implicated in the decline of hundreds of species of amphibians worldwide, including some populations of ranid frogs (Skerratt et al. 2007; Voolhard et al. 2016; Vredenburg et al. 2010), whereas individuals in numerous populations and from numerous species have been found to coexist with Bd (e.g., Muths et al. 2008; Pearl et al. 2009; Briggs et al. 2010; Pilleiod et al. 2010; Puschendorf et al. 2011; Tobler et al. 2012; Scheele et al. 2014).

Bd was first detected among Utah’s Columbia Spotted Frogs in 2001 in the Heber Valley population of the Wasatch Range region (Wilson and Olsen 2002). We investigated Bd infection of spotted frogs from small, isolated populations in two regions: north-central Utah along the Wasatch Range and the West Desert bordering Nevada (Fig. 1). Also, we used long-term data for a preliminary exploratory analysis of the potential adverse effects of Bd on reproductive effort. We predicted that at sites where Bd...