care guidelines and appropriate permits were obtained.

**Literature Cited**


Viruses in the genus *Ranavirus* are implicated as a possible contributing factor to global amphibian declines (Daszak et al. 1999). Previous studies have associated ranaviruses with mass amphibian die-offs throughout North America (Chinchar 2002 Greer et al. 2005; Williams et al. 2005) including Minnesota, USA (Green et al. 2002; Vandenlangenb erg et al. 2003). However, it is unclear whether ranavirus infections exist in wild anuran populations without causing die-offs. This study investigates the presence of ranavirus in six anuran species at the headwaters of the Mississippi River in Itasca State Park, Minnesota, USA.

**Methods.**—We collected recently metamorphosed and adult frogs and toads by hand and with nets from twenty-six sites in Itasca State Park, Clearwater County, Minnesota (47.2397°N, 95.2075°W) in June and July 2009. Sites were chosen for maximum species diversity and for accessibility. There have been no reports of mass amphibian die-offs in the park, which has been the site of a University of Minnesota field station since 1909 (D. Biesboer, pers. comm., 30 July 2009; C. Handrick, pers. comm., 3 Aug 2009). Both field-based and lab-based courses are taught during summer months and there are few places in the state that have had such a consistent and thorough presence of biologists during peak periods of amphibian activity. This steady stream of students and faculty provides some level of confidence that amphibian die-offs will be observed and reported. That said, there is no guarantee that amphibian die-offs, particularly of aquatic larvae, went unobserved.

To minimize contamination among sites, nets and waders were rinsed with 5% bleach solution between collecting events. Captured frogs were kept individually in separate plastic bags. Individuals were classified as either young-of-the-year (YOY) or adults by comparing their snout-vent length to published sizes of adult frogs (Oldfield and Moriarty 1994; Wright and Wright 1995). Frogs were returned to the laboratory and humanely euthanized with MS-222 (tricaine methanesulfonate) (Simmons 2002). Liver samples were stored in RNA later (Ambion, Austin, Texas, USA) or 99% ethanol at 4°C until processing. Vouchers were deposited in the Bell Museum of Natural History, University of Minnesota (JFBM).

We used disposable razor blades to subsample between 15–30 mg of liver tissues for DNA extraction using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Valencia, California, USA) follow-
Results.—We collected and tested 191 YOY and adult frogs of six different species. Four individuals (2%) from two species tested positive for ranavirus (Table 1): three *Lithobates sylvatica* (JFBM 16793, 16923, and 17116) and one *Lithobates pipiens* (JFBM 16932). Four frog species, *Lithobates septentrionalis*, *Pseudacris crucifer*, *Hyla versicolor*, and *Anaxyrus americanus*, had no positive samples. We captured infected frogs from three different sites within Itasca State Park (Fig. 1); the infected *L. pipiens* shared a site with one of the infected *L. sylvatica* (JFBM 17116). One *L. sylvatica* (JFBM 17116) and the infected *L. pipiens* were adults while the two remaining *L. sylvatica* were recent YOY. Aside from one dead ranavirus-negative *L. pipiens* (cause of death undetermined), all specimens appeared healthy at the time of capture.

DNA sequences (Genbank accession # GQ856477) from the four infected individuals were identical to each other and to other Frog Virus 3 (FV3) sequences from infected North American amphibians (Genbank FJ459783, GQ144408, FJ601916, AY548484, and U36913) and the Terrapene Virus 3 (TV3) sequence (U82553) from a turtle (Mao et al. 1997).

Discussion.—FV3 was originally isolated from a Northern Leopard Frog (*L. pipiens*) collected from Minnesota or Wisconsin, and proved to be fairly benign in metamorphosed amphibians in laboratory experiments (Granoff et al. 1966; Tweedell and Granoff, 1968; see Williams et al. 2005 for historical review), but since then amphibian ranaviruses have been primarily associated with mortality events, especially in larval amphibians (Gray et al. 2009, but see Gray et al. 2007). The presence of ranavirus in apparently healthy metamorphosed frogs at Lake Itasca State Park, an area with no reported amphibian die-offs, suggests that die-offs may not be an inevitable outcome of ranavirus infections and that ranavirus may be more common than previously thought.

There are several possible explanations for our observations of ranavirus in the absence of large-scale mortality events. These include: genetic resistance in these anuran populations (Green et al. 2002; Pearman et al. 2004; Teacher et al. 2009); a less virulent strain of FV3-like virus; the absence of some environmental conditions necessary to cause mass die-offs (Gahl and Calhoun 2008; Pearman et al. 2004; Teacher et al. 2009); a less virulent strain of FV3-like virus; the absence of some environmental conditions necessary to cause mass die-offs (Gahl and Calhoun 2008; Pearman et al. 2004; Teacher et al. 2009); a less virulent strain of FV3-like virus; the absence of some environmental conditions necessary to cause mass die-offs (Gahl and Calhoun 2008; Pearman et al. 2004; Teacher et al. 2009); or that die-offs occurred but went unnoticed or unreported, particularly since mortality in early, aquatic life history stages would be difficult to observe.

Table 1. Ranavirus infections in six frog species collected in Itasca State Park, Minnesota, USA, in 2009.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Adults positive / tested</th>
<th>YOY positive / tested</th>
<th>All individuals positive / tested</th>
<th>Prevalence in all individuals (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Lithobates sylvatica</em></td>
<td>Ranidae</td>
<td>1/21</td>
<td>2/47</td>
<td>3/68</td>
<td>4.4 (2.01–12.69)</td>
</tr>
<tr>
<td><em>Lithobates pipiens</em></td>
<td>Ranidae</td>
<td>1/68</td>
<td>—</td>
<td>1/68</td>
<td>1.5 (0.01–8.63)</td>
</tr>
<tr>
<td><em>Lithobates septentrionalis</em></td>
<td>Ranidae</td>
<td>0/27</td>
<td>—</td>
<td>0/27</td>
<td>0 (0–10.85)</td>
</tr>
<tr>
<td><em>Pseudacris crucifer</em></td>
<td>Hylidae</td>
<td>0/3</td>
<td>0/2</td>
<td>0/5</td>
<td>0 (0–40.10)</td>
</tr>
<tr>
<td><em>Hyla versicolor</em></td>
<td>Hylidae</td>
<td>0/7</td>
<td>0/2</td>
<td>0/9</td>
<td>0 (0–26.93)</td>
</tr>
<tr>
<td><em>Anaxyrus americanus</em></td>
<td>Bufonidae</td>
<td>0/3</td>
<td>0/11</td>
<td>0/14</td>
<td>0 (0–19.08)</td>
</tr>
</tbody>
</table>
Other studies have confirmed that five of the frog species we examined are susceptible to ranavirus (Duffus et al. 2008; Green et al. 2002). Although the susceptibility of *A. americanus* to ranavirus remains unknown, ranavirus has been identified in other bufonids such as *Anaxyrus fowleri*, *Bufo bufo*, and *Rhinella marina* (Chinchar 2002; Cunningham et al. 2007; Zupanovic et al. 1998). Given our small sample sizes and the potential for false negatives (Greer and Collins 2007), we cannot eliminate the possibility that species we identified as negative were also infected at low levels.

It is important to document the baseline prevalence of ranavirus infections in amphibian populations in order to understand its epidemiology and transmission in wild populations. In much of the ranavirus literature, amphibians are sampled and tested only after or during die-offs. While this response-based sampling is valuable, it offers a skewed estimate of ranavirus prevalence and perhaps virulence in amphibian populations. Future studies should expand the limited documentation of baseline ranavirus levels in randomly-sampled wild populations and also investigate the factors that may cause sublethal infections to become virulent. Such research would allow scientists to better evaluate the causes and consequences of ranavirus infections in amphibian communities.

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