Ranavirus Outbreaks in Amphibian Populations of Northern Idaho

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Ranavirus outbreaks, caused by pathogens in the genus *Ranavirus* (Family Iridoviridae), were the largest single cause of reported amphibian mass mortality events in the United States from 1996–2001 (Green et al. 2002). Mortality events associated with ranaviruses have been documented on five continents and throughout the latitudes and elevations where amphibians occur (Gray et al. 2009). However, the threat of ranaviruses to amphibian and reptile populations in specific regions is still largely unknown (Chinchar 2002; Gray et al. 2009).

In Idaho, ranavirus was first documented as the cause of death for Tiger Salamanders (*Ambystoma tigrinum*) in 2000 near Yellowstone National Park (USGS 2001); however mass mortality events have not been reported since by the USGS National Wildlife Health Center (NWHC) in Idaho or the eastern parts of neighboring Washington and Oregon. In the Palouse region of north Idaho, multiple species of amphibians and aquatic reptiles, including Long-toed Salamanders (*A. macrodactylum*), Tiger Salamanders, Columbia Spotted Frogs (*Rana luteiventris*), Sierran Treefrogs (*Pseudacris sierra*), Western Toads (*Anaxyrus boreas*), Rough-skinned Newts (*Taricha granulosa*), and Painted Turtles (*Chrysemys picta*), co-occur at small man-made ponds where ranavirus could potentially be a threat. Here we report ranavirus outbreaks in two amphibian species (Columbia Spotted Frogs and Sierran Treefrogs) at two ponds in north Idaho during the summer and fall of 2009.

In 2009, we visually-surveyed and trap-surveyed seven ponds in Latah County, Idaho, USA for amphibians during three seasons (spring, summer, and fall). All footwear and field equipment were disinfected using 10% bleach solution between pond visits to inactivate pathogens and prevent transmission of disease between ponds (Bryan et al. 2009). During our survey period, we observed mortality events at two of the seven ponds, one documented on 17 July 2009 at Latah Trail Pond (46.4423°N, 116.4818°W) and one documented on 4 October 2009 at Mort Pond (46.4811°N, 116.5838°W).

We collected 14 dead amphibians (Table 1) for disease diagnostics. A new pair of nitrile gloves was used to handle and place each specimen separately in a new container. Specimens were preserved within 1 h of collection; those analyzed using PCR were preserved in 95% ethanol, and those used for histological analysis were preserved in 10% buffered formalin for 25 hours and then transferred into 70% alcohol. Unpreserved samples for inoculating cell lines were processed on the day of collection.

Dead specimens were analyzed by one of three laboratories to employ multiple methods for diagnostics and obtain independent evidence for causes of death. The NWHC
analyzed five specimens using histology. At the NWHC, diagnostics for ranavirus were based on characteristic histological changes in the livers, spleens, mesonephroi, and blood vessels of the frogs. The U.S. Fish and Wildlife Service Idaho Fish Health Center (IFHC) analyzed five specimens using cell culture and PCR. At the IFHC, liver tissue was removed aseptically, ground with a Hank’s balanced salt solution (HBSS), spun down, and incubated for 24 hours. Samples were then plated on *Epithelioma papulosum cyprini* (EPC), fathead minnow (FHM), and CHSE-214 (derived from Chinook Salmon) cell lines to culture virus. Ranavirus infection was confirmed using PCR with nested FV3 primers (Bollinger et al. 1999; Kattenbelt et al. 2000). The Laboratory of Conservation and Ecological Genetics (LCEG) at the University of Idaho analyzed three specimens using PCR and sequencing. To confirm the strain of ranavirus, DNA was extracted from tail-clips of three tadpole samples using a DNeasy Tissue Kit (Qiagen, Inc., Valencia, CA). DNA was amplified using MCP4 and MCP5 primers (Mao et al. 1997), and the resulting 508 base pair PCR product was sequenced with the amplifying primers using a 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Resulting sequences were compared with those in the GenBank database.

On the day before the mortality event was first observed at Latah Trail Pond, we had set 15 minnow traps throughout the pond overnight. We repeated trap sampling on 24 July 2009 at this location to compare amphibian captures seven days after the mortality event was first documented. In addition to trapping, we conducted visual surveys at this pond every other day during the mortality event where one person walked the perimeter of the pond for 5 minutes.

We observed a mass mortality event involving approximately 200 Columbia Spotted Frog and Sierran Treefrog tadpoles, at least five Long-toed Salamander larvae, and at least seven adult Columbia Spotted Frogs at Latah Trail Pond on 17 July 2009. Another mortality event involving at least 12 adult Columbia Spotted Frogs was observed at Mort Pond on October 4, 2009. Due to the extremely small population sizes of the Columbia Spotted Frog in this system (Davis and Verrell 2005), we also consider the Mort Pond event a mass mortality. The probable cause of death in both cases was infection by ranavirus (Table 1). At Latah Trail Pond, dead and dying amphibians had clinical signs of ranavirus disease, specifically bloated abdomens, leg hemorrhaging, and irregular swimming (Bollinger et al. 1999; Docherty et al. 2003; Jancovich et al. 1997; Fig. 1). Histological analyses indicated characteristic changes in livers, spleen, mesonephros, and blood vessels of frogs. DNA sequence for the major capsid protein gene was identical to several frog virus 3 (FV3) sequences (Tan et al. 2004), including one from a Northern Leopard Frog (*R. pipiens*; Holopainen et al. 2009) and the *Terrapene carolina* ranavirus (TV3, Mao et al. 1997).

The mass mortality event at Latah Trail Pond lasted at least seven days, with the majority of carcasses found on the first day of observations. By the third day, the majority of amphibian carcasses were gone. Minnow traps at Latah Trail on 17 July captured 63 Long-toed Salamander larvae, 40 Sierran

![Fig. 1. Tadpoles with ranavirus infection, Latah County, Idaho, USA, 18 July 2009. (A) Columbia Spotted Frog with leg hemorrhage, (B) dead Sierran Treefrog, (C) moribund, bloated Columbia Spotted Frog.](image-url)
Ranaviruses (family Iridoviridae): emerging threats to amphibian populations across North America

A. Ranaviruses (family Iridoviridae): emerging threats to amphibian populations across North America

This study describes two mass mortality events due to ranavirus in north Idaho. Diagnostic tests indicated that this infectious disease was the cause of mass mortality of Columbia Spotted Frog tadpoles and adults. The presence of sick and dead Long-toed Salamanders and a moribund Painted Turtle during the outbreak suggests that the virus may have affected additional species, however, we did not conduct diagnostic tests on these species. We identified the north Idaho ranavirus as FV3 using sequence from the major capsid protein. Ranaviruses identified as FV3 are multi-host pathogens with a large geographic range in North America.

Our trapping data indicate that the summer ranavirus outbreak we observed may have removed much of the reproductive output for Latah Trail Pond in 2009. While the drop in trap captures could have been due to metamorphosis during the week-long mortality event, several tadpoles were stages from metamorphosis at the beginning of the outbreak (Fig. 1), making this unlikely. As long as ponds in the area do not experience mass mortality events simultaneously or continuously, the high amount of gene flow between sites (Goldberg and Waits 2010) should help maintain population persistence for Columbia Spotted Frogs. Gene flow, however, may also indicate high potential for pathogen transmission. Columbia Spotted Frogs in this region experience a range of stressors and have low numbers of breeding adults (Davis and Verrell 2005). Local populations are subject to aerial spraying of pesticides, disturbance by cattle [which has been shown to increase prevalence of FV3 in Green Frog (Rana clamitans) tadpoles (Gray et al. 2007)], and infection by Bd (since at least 2004 for Latah Trail and 2005 for Mort; CSG and LPW, unpublished data), among other factors. Additional population monitoring will be required to detect any long-term impacts of the observed mass mortality events on these populations.

Our observations underscore the difficulties of detecting ranavirus outbreaks. Due to variation in timing of metamorphosis and the short time frame in which the visual evidence of mortality was gone (ca. 2 days), it could be difficult to distinguish a mass mortality event from a metamorphosis and dispersal event. Ranavirus outbreaks are therefore likely underreported and may represent a greater threat to native amphibians than currently recognized.

Acknowledgments.—This research was conducted in compliance with an Idaho Department of Fish and Game research collecting permit and a University of Idaho Animal Care and Use Protocol (#2008-55). We thank Erin Gomez for assistance with fieldwork. We thank Hon Ip and Renee Long, USGS National Wildlife Health Center, Madison, Wisconsin, for confirmation of ranaviral infections by culture isolations. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited


