Herpetological Review

Ranavirus Outbreaks in Amphibian Populations of Northern Idaho

DANELLE M. RUSSELL
Department of Biological Sciences,
University of Idaho, Moscow, Idaho 83844, USA
CAREN S. GOLDBERG
Department of Fish and Wildlife Resources
University of Idaho, Moscow, Idaho 83844, USA
LAURA SPRAGUE
Idaho Fish Health Center
P.O. Box 272 Orofino, Idaho 83544, USA
LISETTE P. WAITS
Department of Fish and Wildlife Resources
University of Idaho, Moscow, Idaho 83844, USA
D. EARL GREEN
U.S. Geological Survey, National Wildlife Health Center
6006 Schroeder Road Madison, Wisconsin 53711, USA
KRYSTEN L. SCHULER
U.S. Geological Survey, National Wildlife Health Center
6006 Schroeder Road Madison, Wisconsin 53711, USA
ERICA BREE ROSENBLUM*
Department of Biological Sciences,
University of Idaho, Moscow, Idaho 83844, USA

**Corresponding author: e-mail: rosenblum@uidaho.edu*

Herpetological Review, 2011, 42(2), 223–225.

Ranavirus Outbreaks in Amphibian Populations of Northern Idaho

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.

DANELLE M. RUSSELL
Department of Biological Sciences,
University of Idaho, Moscow, Idaho 83844, USA
CAREN S. GOLDBERG
Department of Fish and Wildlife Resources
University of Idaho, Moscow, Idaho 83844, USA
LAURA SPRAGUE
Idaho Fish Health Center
P.O. Box 272 Orofino, Idaho 83544, USA
LISETTE P. WAITS
Department of Fish and Wildlife Resources
University of Idaho, Moscow, Idaho 83844, USA
D. EARL GREEN
U.S. Geological Survey, National Wildlife Health Center
6006 Schroeder Road Madison, Wisconsin 53711, USA
KRYSTEN L. SCHULER
U.S. Geological Survey, National Wildlife Health Center
6006 Schroeder Road Madison, Wisconsin 53711, USA
ERICA BREE ROSENBLUM*
Department of Biological Sciences,
University of Idaho, Moscow, Idaho 83844, USA

*Corresponding author: e-mail: rosenblum@uidaho.edu

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

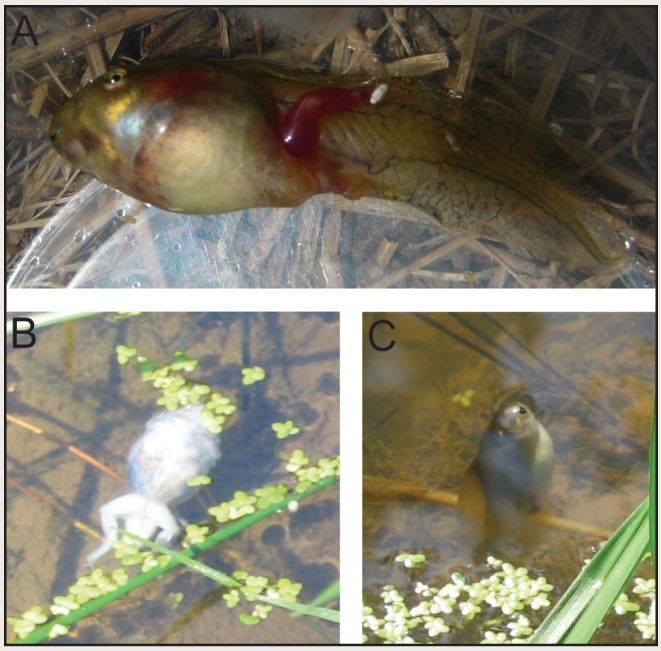

FIG. 1. Tadpoles with ranavirus infection, Latah County, Idaho, USA, 18 July 2009. (A) Columbia Spotted Frog with leg hemorrhage, (B) dead Sierran Treefrog, (C) moribund, bloated Columbia Spotted Frog.

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 totaling 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

TABLE 1. Dead amphibians collected from ponds and tested for ranavirus in Latah County, Idaho, following mass mortality events. Species are: Columbia Spotted Frog (CSF) and Sierran Treefrog (ST). Laboratory tests were conducted at the USGS National Wildlife Health Center (NWHC - full necropsy and viral cultures), the Idaho Fish Health Center (IFHC – viral cultures and PCR), and the Laboratory of Conservation and Ecological Genetics (LCEG - PCR and sequencing).

Pond	Date	Species	Age	N	Laboratory	Result
Latah Trail	18-Jul-09	CSF	Tadpole	3	LCEG	Ranavirus
Latah Trail	20-Jul-09	CSF	Metamorph	2	IFHC	Ranavirus
Latah Trail	20-Jul-09	ST	Tadpole	2	IFHC	Ranavirus
Latah Trail	20-Jul-09	CSF	Adult	2	IFHC	Ranavirus
Latah Trail	20-Jul-09	CSF	Adult	2	NWHC	Ranavirus
Mort	4-Oct-09	CSF	Adult	2	NWHC	Ranavirus
Mort	4-Oct-09	CSF	Adult	1	NWHC	Reproductive tract disease

Treefrog tadpoles, and 16 Columbia Spotted Frog tadpoles. Resampling on July 24 yielded only one tadpole and one adult Columbia Spotted Frog. In addition to amphibian mortality, one Painted Turtle at Mort Pond was found alive but lethargic, had difficulty breathing, and was unresponsive to handling, which are consistent with signs of ranaviral disease in turtles with TV3 (De Voe et al. 2004).

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 and Sierran Treefrog tadpoles. 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 (Schock et al. 2008).

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 weeklong mortality event, most tadpoles were several 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 Erim 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

Bollinger T. K., J. H. Mao, D. Schock, R. M. Brigham, and V. G. Chinchar. 1999. Pathology, isolation, and preliminary molecular characterization of a novel iridovirus from tiger salamanders in Saskatchewan. J. Wildl. Dis. 35:413–429.

Bryan L., C. A. Baldwin, M. J. Gray, and D. L. Miller. 2009. Efficacy of select disinfectants at inactivating *Ranavirus*. Dis. Aquat. Org. 84:89–94.

Chinchar, V. G. 2002. Ranaviruses (family Iridoviridae): emerging cold-blooded killers. Arch. Virol. 147: 447–470.

Davis, A. B., and P. A. Verrell. 2005. Demography and reproductive ecology of the Columbia spotted frog (*Rana luteiventris*) across the Palouse. Can. J. Zool. 83:702–711.

De Voe, R., K. Geissler, S. Elmore, D. Rotstein, G. Lewbart, and J. Guy. 2004. Ranavirus-associated morbidity and mortality in a group of captive eastern box turtles (*Terrapene carolina carolina*). J. Zoo Wildl. Med. 35:534–543.

Docherty D. E., C. U. Meteyer, J. Wang, J. Mao, S. Case, and V. G. Chinchar. 2003. Diagnostic and molecular evaluation of three iridovirus-associated salamander mortality events. J. Wildl. Dis. 39:556–566.

Goldberg C. S., and L. P. Waits. 2010. Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. Mol. Ecol. 19:3650–3663.

Gray, M. J., D. L. Miller, and J. T. Hoverman. 2009. Ecology and pathology of amphibian ranaviruses. Dis. Aquat. Org. 87:243–266.

———, ———, A. C. Schmutzer, and C. A. Baldwin. 2007. Frog virus 3 prevalence in tadpole population inhabiting cattle-access and non-access wetlands in Tennessee, USA. Dis. Aquat. Org. 77:97–103.

Green, D. E., K. A. Converse, and A. K. Schrader. 2002. Epizootiology of sixty-four amphibian morbidity and mortality events in the USA, 1996–2001. Ann. New York Acad. Sci. 969:323–339.

Holopainen R., S. Ohlemeyer, H. Schütze, S. M. Bergmann, and H. Tapiovaara. 2009. Ranavirus phylogeny and differentiation based on major capsid protein, DNA polymerase and neurofilament triplet H1-like protein genes. Dis. Aquat. Org. 85:81–91.

Jancovich, J. K., E. W. Davidson, J. F. Morado, B. L. Jacobs, and J. P. Collins. 1997. Isolation of a lethal virus from the endangered tiger salamander *Ambystoma tigrinum stebbinsi*. Dis. Aquat. Org. 31:161–167.

Kattenbelt, J. A., A. D. Hyatt, and A. R. Gould. 2000. Recovery of ranavirus dsDNA from formalin-fixed archival material. Dis. Aquat. Org. 39:151–154.

Mao, J., R. P. Hedrick, and V. G. Chinchar. 1997. Molecular characterization, sequence analysis, and taxonomic position of newly isolated fish iridoviruses. Virology 229:212–220.

Schock, D. M., T. K. Bollinger, V. G. Chinchar, J. K. Jancovich, and J. P. Collins. 2008. Experimental evidence that amphibian ranaviruses are multi-host pathogens. Copeia 2008:133–143.

Tan,W. G., T. J. Barkman, G. V. Chinchar, and K. Essani. 2004. Comparative genomic analyses of frog virus 3, type species of the genus Ranavirus (family Iridoviridae). Virology 323:70–84.

United States Geological Survey (USGS). 2001. Quarterly report, 1st quarter. http://www.nwhc.usgs.gov/publications/quarterly_reports/2001_qtr_1.jsp, accessed 7/23/10.