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Drew R. Davis
The University of Texas Rio Grande Valley

Jillian K. Farkas

Taylor R. Kruisselbrink

Jessa L. Watters

Elyse D. Ellsworth

See next page for additional authors

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Prevalence and Distribution of Ranavirus in Amphibians from Southeastern Oklahoma, USA

Drew R. Davis[1,2,5], Jillian K. Farkas[1], Taylor R. Kruisselbrink[1], Jessa L. Watters[3], Elyse D. Ellsworth[3,4], Jacob L. Kerby[1], and Cameron D. Siler[3,4]

[1]*Department of Biology, University of South Dakota, Vermillion, South Dakota 57069, USA*
[2]*School of Earth, Environmental, and Marine Sciences, University of Texas Rio Grande Valley, South Padre Island, Texas 78597, USA*
[3]*Sam Noble Oklahoma Museum of Natural History, University of Oklahoma, Norman, Oklahoma 73072, USA*
[4]*Department of Biology, University of Oklahoma, Norman, Oklahoma 73019, USA*
[5]*Corresponding author; e-mail:* drew.davis@utrgv.edu

Abstract.—**Several infectious diseases are threatening amphibian species worldwide and have resulted in mass-mortality events across the globe. An emerging group of viral pathogens (ranaviruses) are documented to cause die-offs in amphibian populations worldwide, including in several regions of the U.S. Unfortunately, large gaps remain in our understanding of the distribution of this systemic pathogen in the U.S., including within the state of Oklahoma. To address this gap in our understanding, we carried out surveys of this infectious pathogen across 14 sites in seven southeastern Oklahoma counties in spring 2015, screening 17 amphibian species from this region. Using liver and tail tissue samples collected from individual amphibians, we screened for the presence and infection load of ranavirus. Of the 390 samples, 84 (21.5%) tested positive for ranavirus, with infection prevalence varying among species surveyed. Notably, the family Bufonidae had no samples that tested positive for ranavirus, whereas the remaining families had an infection prevalence ranging from 14–50%. Despite an overall infection prevalence of 21.5%, we detected no clinical signs of ranavirosis and all sampled individuals appeared outwardly healthy. These results provide data on the geographic and host distribution of ranavirus in southeastern Oklahoma, as well as the first documented cases of the pathogen in three species of anurans: *Gastrophryne carolinensis* (Eastern Narrow-mouthed Toad), *G. olivacea* (Western Narrow-mouthed Toad), and *Pseudacris fouquettei* (Cajun Chorus Frog). With widespread ranavirus infection, there is potential for transmission from abundant, widespread species to more vulnerable, state-threatened amphibians**.

Key Words.—amphibian disease; frogs; infectious disease monitoring; salamanders

Introduction

During the past several decades, infectious diseases have contributed to declines in global amphibian populations (Collins and Storfer 2003; Wake and Vredenburg 2008). Despite many of these amphibian pathogens having broad, cosmopolitan distributions, large regions exist where little is known about their geographic and host distribution (Fisher et al. 2009; Duffus et al. 2015). Given that local and regional factors have been shown to influence pathogen transmission, pathogenesis, and both lethal and sublethal effects on individuals (Echaubard et al. 2010, 2014), detailed knowledge of pathogen distributions allow for targeted management actions and surveillance to reduce the frequency of anthropogenic-driven epizootic events, which can help mitigate population declines.

One major group of viral pathogens that has emerged alongside the global decline of amphibians is ranaviruses (Chinchar 2002), which have received considerably less attention than other amphibian pathogens (Duffus 2009). Amphibians infected with ranaviruses can display both behavioral (lack of equilibrium, erratic movements, lethargy) and physical signs (skin sloughing, erythema, lesions, swollen limbs) of infection, often culminating in organ necrosis and massive hemorrhaging (Gray et al. 2009a; Miller et al. 2011). Due to the ability of ranaviruses to impair normal behaviors of amphibians (Gray et al. 2009a), it is possible that many individuals die from predation prior to mortality from disease, reducing the likelihood of observing mortality in the field. Despite the difficulties of detecting mortality in the field, ranavirus-induced amphibian mass-mortality and population declines have been well documented across North America (e.g., Bollinger et al. 1999; Green et al. 2002; Davis and Kerby 2016) and Europe (Teacher et al. 2010; Price et al. 2014; Rosa et al. 2017). Whereas ranaviruses are believed to be widespread across both geographic areas and amphibian host species, many gaps in this knowledge exist (Duffus et al. 2015). Given current, widespread declines in amphibians (Wake and Vredenburg 2008), a better understanding of the geographic and host distribution of ranaviruses is needed to assess the risks posed to native amphibians.

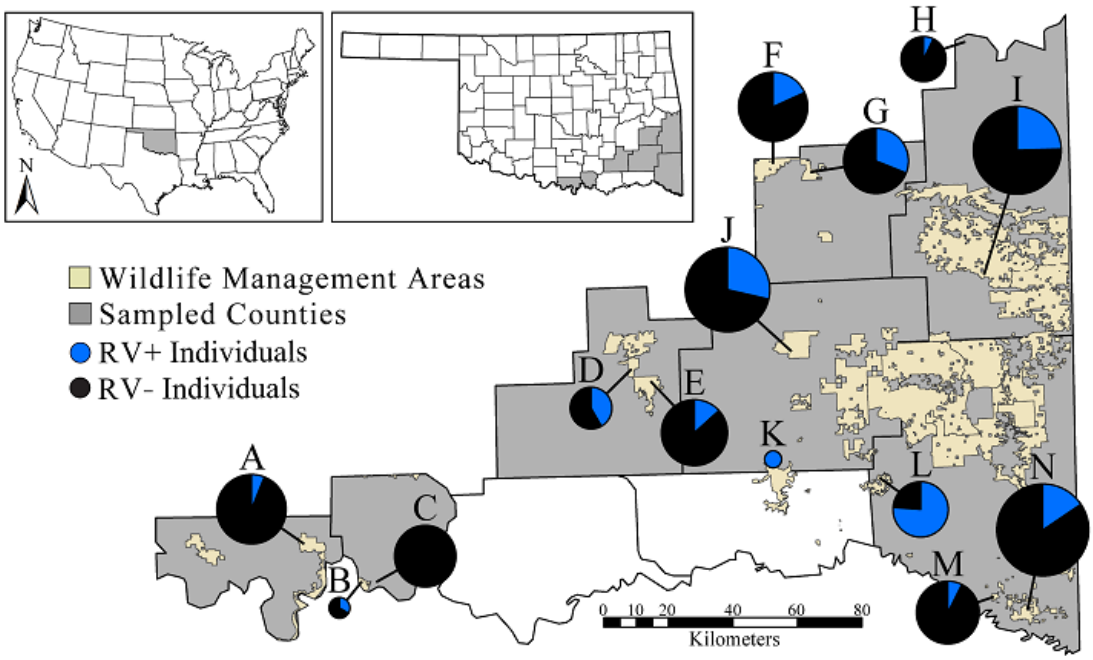

Figure 1. Map of southeastern Oklahoma, USA, showing sampled counties (grey) and localities (labeled with letters corresponding to those in Appendix). Pie chart size is scaled by the number of amphibians sampled at that locality (range, 3–58); pie chart illustrates the proportion of sampled individuals that tested positive for ranavirus (RV+; blue) and negative for ranavirus (RV-; black). Wildlife Management Areas of the Oklahoma Department of Wildlife Conservation (tan polygons) are included for reference.

One large geographic area where little is known about the distribution of ranaviruses, both across the landscape and among host species, is in the central U.S. (Duffus et al. 2015). Ranaviruses have been detected in amphibians from several states in the central U.S., including Colorado (Green et al. 2002), Nebraska (Davis and Kerby 2016), North Dakota (Green et al. 2002; Docherty et al. 2003), South Dakota (Davis et al. In press), and Texas (Torrence et al. 2010). In Oklahoma, limited data exists on the prevalence and distribution of ranaviruses (Watters et al. 2018; Smith et al. In press), and no published data on ranaviruses exists in the southeastern region of the state.

Given the importance of understanding the occurrence of amphibian pathogens at a regional level, there is a critical need for statewide pathogen surveillance and monitoring. Eleven amphibian species that are listed as Species of Greatest Conservation Need by the Oklahoma Department of Wildlife Conservation (ODWC) can be found in southeastern Oklahoma (ODWC. 2005. Oklahoma Comprehensive Wildlife Conservation Strategy. ODWC. Available from http://www.wildlifedepartment.com/CWCS.htm [Accessed 15 January 2019]). Due to the lack of host-specificity of ranaviruses, is it important to document and survey for ranaviruses across all amphibian species. With the information gained from these surveys, biologists and land managers can better understand ranavirus prevalence and infection load among native species. In this study, we surveyed 17 species of amphibians from 14 sites in southeastern Oklahoma for the presence of ranaviruses.

Materials and Methods

Sampling localities and methods.—From March to May 2015, we conducted six sampling trips to southeastern Oklahoma to collect amphibians and sample for ranavirus infection. Research occurred during this time period to coincide with the aggregation of amphibians around wetland sites during the breeding season. In total, we collected amphibian tissue samples from 14 sites across seven counties in southeastern Oklahoma: Atoka, Latimer, Le Flore, Love, Marshall, McCurtain, and Pushmataha (Fig. 1; Appendix). We collected larval, post-metamorphic, and adult amphibians by hand, dip net, or seine in ODWC Wildlife Management Areas (WMAs), and other public-use areas in the region. We kept amphibians in individual plastic bags in a cooler (< 72 h) until we euthanized them via submersion in an aqueous chloretone solution (Simmons 2015). Once euthanized, we measured mass and snout-vent length and determined the sex of individuals. We also examined them for clinical signs of ranavirus infection and took either liver tissue (adult or post-metamorphic individuals) or a tail clip (tadpoles) to screen for ranavirus infection. We stored all genetic samples in 95% ethanol at -20° C until

we later subsampled them in preparation for DNA extraction. We preserved all euthanized amphibians in 10% buffered formalin, transferred them to 70% ethanol for long-term storage, and subsequently deposited them at the Sam Noble Oklahoma Museum of Natural History (SNOMNH). We sterilized all collecting equipment (nets, seines, boots) with a 10% bleach solution between sites. Additionally, we used 95% ethanol to sterilize all tissue collection equipment (scissors, forceps) and wiped them with clean paper towels to prevent pathogen spread or contamination among samples (Gray et al. 2017).

Molecular detection methods.—We extracted total genomic DNA from liver or tail tissues using the high salt extraction method of Esselstyn et al. (2008) at the SNOMNH Genomics Core Facility. We then diluted DNA extracts 1:2 with 0.25× TE buffer and shipped frozen extracts to the Disease Testing Center at the University of South Dakota for analysis via quantitative PCR (qPCR). To determine ranavirus infection prevalence and to estimate the number of viral gene copies per sample, we followed the previously described protocol of Forson and Storfer (2006a). Each qPCR plate contained a negative control (water) and a standardized dilution series of gBlocks that contained the target sequence of DNA to use as a standard curve (1e2–1e5). We ran all samples in triplicate (20 µl reaction volume) and considered the sample positive (RV+) if amplification occurred in at least two of the three wells and the quantity was above 1.0 (Whitfield et al. 2012; Davis and Kerby 2016; Watters et al. 2018; Butterfield et al. 2019). We reran samples if there were two wells with quantities near 1.0, or if sample values differed by an order of magnitude. We quantified viral gene copies using StepOne software v2.3 (Applied Biosystems, Foster City, California, USA) by averaging gene copy numbers values from each RV+ well to create a mean gene copy number value for each sample.

Results

We collected 390 amphibian tissue samples (n = 325 Anura; n = 65 Caudata) from 14 sites in southeastern Oklahoma in 2015 (Fig. 1; Table 1; Appendix). We detected ranavirus in 84 (21.5%) of these samples (Fig. 1; Table 1; Appendix), which represents the first documented occurrence of ranavirus in southeastern Oklahoma. Of the 14 sites sampled for ranavirus, we detected ranavirus at 13 (Fig. 1; Appendix); we detected no ranavirus in samples from the University of Oklahoma Biological Station in Marshall County. We detected ranavirus in samples from 12 of the 17 (70.6%) species of amphibians sampled (Table 1), with the first reported detection of ranavirus in field collected *Gastrophryne carolinensis* (Eastern Narrow-mouthed Toad), *G. olivacea* (Western Narrow-mouthed Toad), and *Pseudacris fouquettei* (Cajun Chorus Frog). We did not detect ranavirus in three species: *Ambystoma texanum* (Small-mouthed Salamander), *P. streckeri* (Strecker's Chorus Frog), and *Rana blairi* (Plains Leopard Frog); however, it should be noted that few samples were available for these three taxa ($n \leq 2$). Additionally, we failed to detect ranavirus in two species of bufonids (*Anaxyrus americanus* [American Toad] and *A. woodhousii* [Woodhouse's Toad]), despite larger sample sizes ($n \geq 10$). The remaining families had an infection prevalence ranging from 14–50% (Table 1). Although the infection prevalence in some species and at some sites may be inflated artificially due to limited sample sizes, these data suggest that ranavirus infection is common and widespread within this region. In addition to detecting viral infection, we were also able to quantify viral gene copies within each sample. Average viral gene copies ranged across several orders of magnitude (103–106); observed average values were lowest in members of the family Ranidae (1.2×103 gene copies/sample) and highest in the family Hylidae (2.0×106 gene copies/sample; Table 1).

Discussion

To better understand the potential impact of ranavirus on individuals, populations, and species, both spatial and temporal understanding of the distribution and severity of infection is critical (Ostfeld et al. 2005). Our survey resulted in the first confirmed detection of ranavirus in southeastern Oklahoma amphibians and helps fill information gaps regarding host and geographic distribution of this pathogen in the central United States. We detected ranavirus in amphibians from all sites except one, the University of Oklahoma Biological Station, despite its geographic proximity (about 5 km) to Fobb Bottom WMA where we did detect ranavirus. Previously, Marhanka et al. (2017) found high overall prevalence (96%) of *Batrachochytrium dendrobatidis* in amphibians sampled from the University of Oklahoma Biological Station, but it remains unclear why we did not detect ranavirus there. Additionally, our efforts resulted in the first reported detection of ranavirus in *Gastrophryne carolinensis*, *G. olivacea*, and *Pseudacris fouquettei*, in the wild, though Hoverman et al. (2010, 2011) experimentally infected *G. carolinensis* during laboratory trials. The present study builds upon a growing understanding of both historic and modern occurrence of amphibian pathogens in Oklahoma (Watters et al. 2016, 2018, 2019; Marhanka et al. 2017). Overall, we detected ranavirus across most species and sites sampled in 2015, despite the lack of observed individuals with clinical signs of ranavirosis

Table 1. Amphibian species sampled for ranavirus (RV) infection by family in southeastern Oklahoma, USA. Total sample size (n), number of RV+ individuals (% prevalence), and mean RV gene copies per sample for infected amphibians (± 1 standard deviation [SD]) are indicated. When ≤ 1 individual per species tested RV+, SD is not presented (N/A).

Family, Species	n	RV+ (% prevalence)	Mean RV Gene Copies/Sample (± 1 SD)
Ambystomatidae	2	1 (50%)	3,855 (± N/A)
Ambystoma opacum (Marbled Salamander)	1	1 (100%)	0 (± N/A)
Ambystoma texanum (Small-mouthed Salamander)	1	0 (0%)	369 (± N/A)
Bufonidae	30	0 (0%)	0 (± N/A)
Anaxyrus americanus (American Toad)	20	0 (0%)	0 (± N/A)
Anaxyrus woodhousii (Woodhouse's Toad)	10	0 (0%)	0 (± N/A)
Hylidae	169	24 (14%)	2,046,502 (± 10,022,462)
Acris blanchardi (Blanchard's Cricket Frog)	93	13 (14%)	713 (± 659)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	41	4 (10%)	574 (± 590)
Hyla cinerea (Green Treefrog)	31	5 (16%)	755 (± 1,019)
Pseudacris crucifer (Spring Peeper)	1	1 (100%)	49,100,509 (± N/A)
Pseudacris fouquettei (Cajun Chorus Frog)	1	1 (100%)	203 (± N/A)
Pseudacris streckeri (Strecker's Chorus Frog)	2	0 (0%)	0 (± N/A)
Microhylidae	11	5 (45%)	2,410 (± 2,617)
Gastrophryne carolinensis (Eastern Narrow-mouthed Toad)	7	4 (57%)	2,888 (± 2,759)
Gastrophryne olivacea (Western Narrow-mouthed Toad)	4	1 (25%)	497 (± N/A)
Ranidae	115	40 (35%)	1,213 (± 4,714)
Rana blairi (Plains Leopard Frog)	1	0 (0%)	0 (± N/A)
Rana catesbeiana (American Bullfrog)	72	29 (40%)	1,416 (± 5,533)
Rana clamitans (Green Frog)	24	5 (21%)	547 (± 453)
Rana sphenocephala (Southern Leopard Frog)	18	6 (33%)	790 (± 919)
Salamandridae	63	14 (22%)	837,580 (± 3,131,528)
Notophthalmus viridescens (Eastern Newt)	63	14 (22%)	837,580 (± 3,131,528)
Total	390	84 (22%)	725,037 (± 5,492,612)

or mortality.

Ranaviruses have been associated with mass-mortality events and population declines in amphibians (Green et al. 2002; Price et al. 2014). Mass-mortality events are considered to be triggered by abiotic and/or biotic factors to which amphibians are exposed (Gray et al. 2009a). Anthropogenic stressors such as environmental contaminants (Forson and Storfer 2006b; Kerby et al. 2011), cattle access to wetlands (Gray et al. 2007), reduced water quality (Greer and Collins 2008), and land-use change (Daszak et al. 2001) have all been linked to increased ranavirus susceptibility and virulence. Within sampled WMAs, anthropogenic threats such as cattle-use of aquatic habitats and overall land-use change are unlikely to be of major concern due to current management practices, although anthropogenic threats such as environmental contaminants and reduced water quality may threaten amphibian populations. For example, crude oil pumpjacks were present in the majority of the WMAs sampled, some in close proximity to sampled amphibian habitats, and may be a source of environmental contaminants. Further, high vehicle and human traffic due to recreational hunting and fishing is known to have negative effects on aquatic and terrestrial communities and can reduce overall water quality (Trombulak and Frissel 2000). With widespread occurrence of ranavirus in both sites and amphibian species sampled, land managers should work to reduce the effects of anthropogenic stressors on these habitats (i.e., reduce contaminant runoff or restrict fisherman access), which are considered to have stronger effects on ranavirus than natural stressors (Reeve et al. 2013).

In other regions of the central U.S., there is major concern over human-mediated movement of amphibians and ranavirus (Picco and Collins 2008; Davis and Kerby 2016). The commercial trade and use of bait amphibians (e.g., larval *Ambystoma mavortium* [Western Tiger Salamander]) is one major source of pathogen pollution that may spread ranavirus around North America. This movement of amphibians may

introduce strains of ranavirus to regions where native amphibians have little resistance to this non-local strain and as a result, pathogen virulence is expected to be greater (Storfer et al. 2007; Picco and Collins 2008). Although Oklahoma was not examined by Picco and Collins (2008), the transport of bait amphibians, and subsequently the ranavirus strains that infect them, from other regions and states may continue to threaten native Oklahoma amphibians. In our study, amphibians were primarily sampled in habitats containing fish, and with fishing allowed in most ODWC WMAs, the release or escape of non-native bait amphibians may provide a way for novel strains of ranavirus to be introduced to these habitats. If the introduction of novel strains of ranavirus is of concern to land managers, new regulations preventing the sale of imported bait amphibians should be established.

Although we did not detect any of the 11 amphibian species listed by ODWC as Species of Greatest Conservation Need (SGCN) as part of this study, targeted surveys for these species to determine ranavirus prevalence should remain a priority. Seven of these SGCN amphibian species are present in aquatic habitats for at least part of the year, during the breeding season or as aquatic larvae (i.e., *Ambystoma annulatum* [Ringed Salamander], *Desmognathus brimleyorum* [Ouachita Dusky Salamander], *Eurycea multiplicata* [Many-ribbed Salamander], *Hyla avivoca* [Bird-voiced Treefrog], *Rana areolata* [Crawfish Frog]), or year-round (i.e., *Amphiuma tridactylum* [Three-toed Amphiuma], *Siren intermedia* [Lesser Siren]). These SGCN aquatic species use similar habitats as species screened in this study and may encounter infected individuals, allowing ranavirus transmission to occur. The remaining four SGCN species are plethodontid salamanders (*Plethodon kiamichi* [Kiamichi Slimy Salamander], *P. ouachitae* [Rich Mountain Salamander], *P. sequoyah* [Sequoyah Slimy Salamander], *P. serratus* [Southern Red-backed Salamander]) and do not use aquatic environments for reproduction due to direct developing larvae. Although transmission of ranavirus in aquatic environments is unlikely to involve *Plethodon*, previous studies have detected low levels of ranavirus in plethodontids, which suggests that these species may also be susceptible to ranavirus (Gray et al. 2009b; Hamed et al. 2013; Duffus et al. 2015; Watters et al. 2018).

To better understand the potential effects of ranavirus on Oklahoma amphibians, continued efforts to survey for the pathogen across localities, hosts, and through time are needed. This study resulted in widespread detection of ranavirus in both sampled hosts and sites in southeastern Oklahoma. Further, the identification and elimination of anthropogenic stressors on the landscape as well as limiting the use and release of bait amphibians at sites should be key management tools in order to prevent ranavirus-driven mass mortality events in Oklahoma.

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Literature Cited

Bollinger, T.K., J. 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. Journal of Wildlife Diseases 35:413–429.

Butterfield, M.M., D.R. Davis, J.D. Madison, and J.L. Kerby. 2019. Surveillance of *Ranavirus* in False Map Turtles (*Graptemys pseudogeographica*) along the lower Missouri River, USA. Herpetological Review 50:76–78.

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

Collins, J.P., and A. Storfer. 2003. Global amphibian declines: sorting the hypotheses. Diversity and Distribution 9:89–98.

Daszak, P., A.A. Cunningham, and A.D. Hyatt. 2001. Anthropogenic environmental change and the emergence of infectious diseases in wildlife. Acta Tropica 78:103–116.

Davis, D.R., K.J. Ferguson, M.S. Schwarz, and J.L. Kerby. In press. Effects of agricultural pollutants on stress hormones and viral infection in larval salamanders. Wetlands.

Davis, D.R., and J.L. Kerby. 2016. First detection of ranavirus in amphibians from Nebraska, USA. Herpetological Review 47:46–50.

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

Duffus, A.L.J. 2009. Chytrid blinders: what other disease

risks to amphibians are we missing? EcoHealth 6:335–339.

Duffus, A.L.J., T.B. Waltzek, A.C. Stohr, M.C. Allender, M. Gotesman, R.J. Whittington, P. Hick, M.K. Hines, and R.E. Marschang. 2015. Distribution and host range of ranaviruses. Pp. 9–57 *In* Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates. Gray, M.J., and V.G. Chinchar (Eds). Springer International Publishing, New York, New York, USA.

Echaubard, P., J. Leduc, B. Pauli, V.G. Chinchar, J. Robert, and D. Lesbarrères. 2014. Environmental dependency of amphibian-ranavirus genotypic interactions: evolutionary perspectives on infectious diseases. Evolutionary Applications 7:723–733.

Echaubard, P., K. Little, B. Pauli, and D. Lesbarrères. 2010. Context-dependent effects of ranaviral infection on Northern Leopard Frog life history traits. PLoS ONE 5:1–7. https://doi.org/10.1371/journal.pone.0013723.

Esselstyn, J.A., H.J.D. Garcia, M.G. Saulog, and L.R. Heaney. 2008. A new species of *Desmalopex* (Pteropodidae) from the Philippines, with a phylogenetic analysis of the Pteropodini. Journal of Mammalogy 89:815–825.

Fisher, M.C., T.W.J. Garner, and S.F. Walker. 2009. Global emergence of *Batrachochytrium dendrobatidis* and amphibian chytridiomycosis in space, time, and host. Annual Review of Microbiology 63:291–310.

Forson, D., and A. Stofer. 2006a. Atrazine increases ranavirus susceptibility in the Tiger Salamander, *Ambystoma tigrinum*. Ecological Applications 16:2325–2332.

Forson, D., and A. Storfer. 2006b. Effects of atrazine and iridovirus infection on survival and life-history traits of the Long-toed Salamander (*Ambystoma macrodactylum*). Environmental Toxicology and Chemistry 25:168–173.

Gray, M.J., A.L.J. Duffus, K.H. Haman, R.N. Harris, M.C. Allender, T.A. Thompson, M.R. Christman, A. Sacerdote-Velat, L.A. Sprague, J.M. Williams, et al. 2017. Pathogen surveillance in herpetofaunal populations: guidance on study design, sample collection, biosecurity, and intervention strategies. Herpetological Review 48:334–351.

Gray, M.J., D.L. Miller, and J.T. Hoverman. 2009a. Ecology and pathology of amphibian ranaviruses. Diseases of Aquatic Organisms 87:243–266.

Gray, M.J., D.L. Miller, and J.T. Hoverman. 2009b. First report of *Ranavirus* infecting lungless salamanders. Herpetological Review 40:316–319.

Gray, M.J., D.L. Miller, A.C. Schmutzer, and C.A. Baldwin. 2007. *Frog virus 3* prevalence in tadpole populations inhabiting cattle-access and non-access wetlands in Tennessee, USA. Diseases of Aquatic Organisms 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. Annals of the New York Academy of Sciences 969:323–339.

Greer, A.L., and J.P. Collins. 2008. Habitat fragmentation as a result of biotic and abiotic factors controls pathogen transmission throughout a host population. Journal of Animal Ecology 77:364–369.

Hamed, M.K., M.J. Gray, and D.L. Miller. 2013. First report of ranavirus in plethodontid salamanders from the Mount Rogers National Recreational Area, Virginia, USA. Herpetological Review 44:455–457.

Hoverman, J.T., M.J. Gray, and D.L. Miller. 2010. Anuran susceptibilities to ranaviruses: role of species identity, exposure route, and a novel virus isolate. Diseases of Aquatic Organisms 89:97–107.

Hoverman, J.T., M.J. Gray, N.A. Haislip, and D.L. Miller. 2011. Phylogeny, life history, and ecology contribute to differences in amphibian susceptibility to ranaviruses. EcoHealth 8:301–319.

Kerby, J.L., A.L. Hart, and A. Storfer. 2011. Combined effects of virus, pesticide, and predator cue on the larval Tiger Salamander (*Ambystoma tigrinum*). EcoHealth 8:46–54.

Markanka, E.C., J.L. Watters, N.A. Huron, S.L. McMillin, C.C. Winfrey, D.J. Curtis, D.R. Davis, J.K. Farkas, J.L. Kerby, and C.D. Siler. 2017. Detection of high prevalence of *Batrachochytrium dendrobatidis* in amphibians from southern Oklahoma, USA. Herpetological Review 48:70–74.

Miller, D., M. Gray, and A. Storfer. 2011. Ecopathology of ranaviruses infecting amphibians. Viruses 3:2351–2373.

Ostfeld, R.S., G.E. Glass, and F. Keesing. 2005. Spatial epidemiology: an emerging (or re-emerging) discipline. Trends in Ecology and Evolution 20:328–336.

Picco, A.M., and J.P. Collins. 2008. Amphibian commerce as a likely source of pathogen pollution. Conservation Biology 22:1582–1589.

Price, S.J., T.W.J. Garner, R.A. Nichols, F. Balloux, C. Ayres, A. Mora-Cabello del Alba, and J. Bosch. 2014. Collapse of amphibian communities due to an introduced *Ranavirus*. Current Biology 24:2586–2591.

Reeve, B.C., E.J. Crespi, C.M. Whipps, and J.L. Brunner. 2013. Natural stressors and ranavirus susceptibility in larval Wood Frogs (*Rana sylvatica*). EcoHealth 10:190–200.

Rosa, G.M., J. Sabino-Pinto, T.G. Laurentino, A. Martel,

F. Pasmans, R. Rebelo, R.A. Griffiths, A.C. Stöhr, R.E. Marschang, S.J. Price, et al. 2017. Impact of asynchronous emergence of two lethal pathogens on amphibian assemblages. Scientific Reports 7:43260. https://doi.org/10.1038/srep43260

Simmons, J.E. 2015. Herpetological Collecting and Collections Management. 3rd Edition. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah, USA.

Smith, S.N., J.L. Watters, E.C. Marhanka, S.L. McMillin, D.R. Davis, J.K. Farkas, and C.D. Siler. In press. Investigating ranavirus prevalence in central Oklahoma, USA, amphibians. Herpetological Review.

Storfer A., M.E. Alfaro, B.J. Ridenhour, J.K. Jancovich, S.G. Mech, M.J. Parris, and J.P. Collins. 2007. Phylogenetic concordance analysis shows an emerging pathogen is novel and endemic. Ecology Letters 10:1075–1083.

Teacher, A.G.F., A.A. Cunningham, and T.W.J. Garner. 2010. Assessing the long-term impact of *Ranavirus* infection in wild Common Frog populations. Animal Conservation 13:514–522.

Torrence, S.M., D.E. Green, C.J. Benson, H.S. Ip, L.M. Smith, and S.T. McMurry. 2010. A new ranavirus isolated from *Pseudacris clarkii* tadpoles in playa wetlands in the Southern High Plains, Texas. Journal of Aquatic Animal Health 22:65–72.

Trombulak, S.C., and C.A. Frissell. 2000. Review of ecological effects of roads on terrestrial and aquatic communities. Conservation Biology 14:18–30.

Wake, D.B., and V.T. Vredenburg. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. Proceedings of the National Academy of Sciences USA 105:11466–11473.

Watters, J.L., D.R. Davis, T. Yuri, and C.D. Siler. 2018. Concurrent infection of *Batrachochytrium dendrobatidis* and ranavirus among native amphibians from northeastern Oklahoma, USA. Journal of Aquatic Animal Health 30:291–301.

Watters, J.L., R.L. Flanagan, D.R. Davis, J.K. Farkas, J.L. Kerby, M.J. LaBonte, M.L. Penrod, and C.D. Siler. 2016. Screening natural history collections for historical presence of *Batrachochytrium dendrobatidis* in anurans from Oklahoma, USA. Herpetological Review 47:214–220.

Watters, J.L., S.L. McMillin, E.C. Marhanka, D.R. Davis, J.K. Farkas, J.L. Kerby, and C.D. Siler. 2019. Seasonality in *Batrachochytrium dendrobatidis* detection in amphibians in central Oklahoma, USA. Journal of Zoo and Wildlife Medicine 50:492–497.

Whitfield, S.M., M.A. Donnelly, E. Geerdes, and J. Kerby. 2012. Ranavirus infection in native amphibians at La Selva Biological Station, Costa Rica: first report of ranavirus in Central America. Herpetological Review 43:425–427.

Drew R. Davis is an Associate Research Scientist at the University of Texas Rio Grande Valley, South Padre Island, USA, studying the ecology and distribution of Black-spotted Newts (*Notophthalmus meridionalis*) and sirens (*Siren* sp.) in south Texas. Previously, he completed a Ph.D. in Biological Sciences at the University of South Dakota, Vermillion, USA, a M.S. in Population and Conservation Biology at Texas State University, San Marcos, USA, and a B.S. in Biology at the University of Texas at Austin, USA. His research interests involve amphibian ecology, particularly how biotic and abiotic stressors influence individuals and populations. (Photographed by Cameron Siler).

Jillian K. Farkas is a Government Relations Associate with the University Corporation for Atmospheric Research in Washington, D.C., USA. She has previously been a Knauss Sea Grant Fellow, a fellow in the Directorate Fellowship Program of the U.S. Fish and Wildlife Service, and worked for the Michigan Department of Natural Resources. She has a B.A. in Biology from Hope College, Holland, Michigan, USA, and recently finished her M.S. thesis on the effects of agriculture on fish and wetlands at the University of South Dakota, Vermillion, USA. Her research interests center on conservation, disease ecology, and anthropogenic stressors on wetland fauna. (Photographed by Janan Evans-Wilent).

Taylor R. Kruisselbrink recently obtained a B.S. in Cell, Physiology, and Molecular Biology from the University of South Dakota, Vermillion, USA, and is planning to pursue a M.S. in Physician Assistant Studies. She has previously worked with the Avera McKennan Molecular Oncology Lab, Sioux Falls, South Dakota, USA, studying new immunotherapy options in triple-negative breast cancer, and was involved in undergraduate research on topics including disease ecology and colon cancer. (Photographed by Sydney Esselink).

Jessa L. Watters is the Collection Manager of the Herpetology Collection at the Sam Noble Museum, University of Oklahoma, Norman, USA. She received her M.S. from the University of New Hampshire, Durham, USA, in 2006 and her B.A. from Earlham College, Richmond, Indiana, USA, in 2001. She is actively involved in numerous education outreach programs, maintaining the integrity of the herpetological specimens and their associated data and archives, supervising undergraduate and graduate volunteers and employees, assisting in various research projects, and leading field research surveys around the state of Oklahoma. (Photographed by Lara Souza).

Elyse D. Ellsworth is a Master's student who first joined Cameron Siler's lab at the University of Oklahoma, Norman, USA, as a Research Technician in Spring 2015. During her time as a Research Technician she was involved in a range of projects both locally in Oklahoma and abroad. She surveyed for amphibian infectious disease throughout Oklahoma and spent time in the Philippines investigating squamate functional morphology and development. These questions lead to her interests in conservation in tropical climates. Elyse is now studying spatial ecology and conservation of an endangered freshwater turtle in Belize. (Photographed by Joseph Brown).

Jacob L. Kerby is Associate Professor in the Department of Biology at the University of South Dakota, Vermillion, USA. His research focuses on anthropogenic impacts to amphibians and reptiles. He serves on the Board of Governors for the American Society of Ichythology and Herpetology and is co-chair of the Bsal Task Force focused on preventing the spread of *Batrachochytrium salamandrivorans* (Bsal). (Photographed by Jacob Kerby).

Cameron D. Siler is the Curator of the Herpetology Collection at the Sam Noble Museum and an Associate Professor of Biology at the University of Oklahoma, Norman, USA. He received his Ph.D. from the University of Kansas, Lawrence, USA, and his B.S. from the University of Texas at Austin, USA. His lab studies patterns of diversity, spatial ecology, disease ecology, and microbial diversity of amphibians and reptiles. Additionally, his lab works closely with state and federal agencies in Oklahoma to monitor amphibian and reptile population health and develop new techniques for wildlife surveys and management. (Photographed by Cameron Siler).

Appendix. Amphibian species sampled for ranavirus (RV) infection at sample sites, primarily Oklahoma Department of Wildlife Conservation Wildlife Management Areas (WMAs), in southeastern Oklahoma, USA. Letters in parentheses indicate the site code used in Fig. 1. Total sample size (n), number of RV+ individuals (% prevalence), and mean RV gene copies per sample for infected amphibians (± 1 standard deviation [SD]) are indicated. When ≤ 1 individual per species, or site tested RV+, SD is not presented (N/A).

Sample Site, Species	n	RV+ (% Prevalence)	Mean RV Gene Copies/ Sample (± 1 SD)
(A) Hickory Creek WMA, Love County	33	2 (6%)	181 (± 89)
Anaxyrus americanus (American Toad)	11	0 (0%)	0 (± N/A)
Anaxyrus woodhousii (Woodhouse's Toad)	5	0 (0%)	0 (± N/A)
Acris blanchardi (Blanchard's Cricket Frog)	6	1 (17%)	243 (± N/A)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	6	0 (0%)	0 (± N/A)
Gastrophryne olivacea (Western Narrow-mouthed Toad)	3	0 (0%)	0 (± N/A)
Rana sphenocephala (Southern Leopard Frog)	2	1 (50%)	118 (± N/A)
(B) Fobb Bottom WMA, Marshall County	3	1 (33%)	497 (± N/A)
Acris blanchardi (Blanchard's Cricket Frog)	1	0 (0%)	0 (± N/A)
Gastrophryne olivacea (Western Narrow-mouthed Toad)	1	1 (100%)	497 (± N/A)
Rana sphenocephala (Southern Leopard Frog)	1	0 (0%)	0 (± N/A)
(C) Univ. Oklahoma Biological Station and Vicinity, Marshall County	26	0 (0%)	0 (± N/A)
Anaxyrus americanus (American Toad)	5	0 (0%)	0 (± N/A)
Anaxyrus woodhousii (Woodhouse's Toad)	5	0 (0%)	0 (± N/A)
Acris blanchardi (Blanchard's Cricket Frog)	10	0 (0%)	0 (± N/A)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	3	0 (0%)	0 (± N/A)
Rana blairi (Plains Leopard Frog)	1	0 (0%)	0 (± N/A)
Rana sphenocephala (Southern Leopard Frog)	2	0 (0%)	0 (± N/A)
(D) Stringtown WMA, Atoka County	12	5 (42%)	413 (± 367)
Acris blanchardi (Blanchard's Cricket Frog)	4	0 (0%)	0 (± N/A)
Rana catesbeiana (American Bullfrog)	6	5 (83%)	413 (± 367)
Notophthalmus viridescens (Eastern Newt)	2	0 (0%)	0 (± N/A)
(E) McGee Creek WMA, Atoka County	30	4 (13%)	459 (± 318)
Acris blanchardi (Blanchard's Cricket Frog)	4	0 (0%)	0 (± N/A)
Rana catesbeiana (American Bullfrog)	6	3 (50%)	463 (± 390)
Rana clamitans (Green Frog)	9	1 (11%)	447 (± N/A)
Notopthalmus viridescens (Eastern Newt)	11	0 (0%)	0 (± N/A)
(F) James Collins WMA, Latimer County	33	6 (18%)	666 (± 370)
Acris blanchardi (Blanchard's Cricket Frog)	12	2 (17%)	751 (± 760)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	11	1 (9%)	544 (± N/A)
Rana catesbeiana (American Bullfrog)	2	0 (0%)	0 (± N/A)
Rana sphenocephala (Southern Leopard Frog)	1	0 (0%)	0 (± N/A)
Notophthalmus viridescens (Eastern Newt)	7	3 (43%)	650 (± 197)
(G) Robbers Cave WMA, Latimer County	29	9 (31%)	1,305,964 (± 3,904,431)
Anaxyrus americanus (American Toad)	1	0 (0%)	0 (± N/A)
Acris blanchardi (Blanchard's Cricket Frog)	4	0 (0%)	0 (± N/A)
Rana catesbeiana (American Bullfrog)	6	1 (17%)	30,157 (± N/A)
Rana clamitans (Green Frog)	2	0 (0%)	0 (± N/A)
Notophthalmus viridescens (Eastern Newt)	16	8 (50%)	1,465,440 (± 4,142,558)
(H) Arkansas River at Robert S. Kerr Lock & Dam 15, Le Flore Co.	14	1 (7%)	203 (± N/A)

Appendix (continued). Amphibian species sampled for ranavirus (RV) infection at sample sites, primarily Oklahoma Department of Wildlife Conservation Wildlife Management Areas (WMAs), in southeastern Oklahoma, USA. Letters in parentheses indicate the site code used in Fig. 1. Total sample size (n), number of RV+ individuals (% prevalence), and mean RV gene copies per sample for infected amphibians (± 1 standard deviation [SD]) are indicated. When ≤ 1 individual per species, or site tested RV+, SD is not presented (N/A).

Sample Site, Species	n	RV+ (% Prevalence)	Mean RV Gene Copies/ Sample (± 1 SD)
Anaxyrus americanus (American Toad)	2	0 (0%)	0 (± N/A)
Acris blanchardi (Blanchard's Cricket Frog)	2	0 (0%)	0 (± N/A)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	7	0 (0%)	0 (± N/A)
Pseudacris fouquettei (Cajun Chorus Frog)	1	1 (100%)	203 (± N/A)
Pseudacris streckeri (Strecker's Chorus Frog)	2	0 (0%)	0 (± N/A)
(I) Ouachita WMA, Le Flore County	53	13 (25%)	586 (± 692)
Anaxyrus americanus (American Toad)	1	0 (0%)	0 (± N/A)
Acris blanchardi (Blanchard's Cricket Frog)	15	5 (33%)	638 (± 507)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	10	1 (10%)	155 (± N/A)
Hyla cinerea (Green Treefrog)	10	4 (40%)	880 (± 1,132)
Rana catesbeiana (American Bullfrog)	4	1 (25%)	254 (± N/A)
Rana clamitans (Green Frog)	6	2 (33%)	251 (± 47)
Notophthalmus viridescens (Eastern Newt)	7	0 (0%)	0 (± N/A)
(J) Pushmataha WMA, Pushmataha County	49	14 (29%)	1,305 (± 1,789)
Acris blanchardi (Blanchard's Cricket Frog)	11	4 (36%)	1,056 (± 914)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	4	2 (50%)	797 (± 876)
Hyla cinerea (Green Treefrog)	2	1 (50%)	255 (± N/A)
Gastrophryne carolinensis (Eastern Narrow-mouthed Toad)	7	4 (57%)	2,888 (± 2,759)
Rana catesbeiana (American Bullfrog)	1	0 (0%)	0 (± N/A)
Rana clamitans (Green Frog)	4	0 (0%)	0 (± N/A)
Notophthalmus viridescens (Eastern Newt)	20	3 (15%)	217 (± 140)
(K) Hugo WMA, Pushmataha County	2	2 (100%)	460 (± 444)
Rana catesbeiana (American Bullfrog)	2	2 (100%)	460 (± 444)
(L) Pine Creek WMA, McCurtain County	21	16 (76%)	3,069,333 (± 12,274,980)
Pseudacris crucifer (Spring Peeper)	1	1 (100%)	49,100,509 (± N/A)
Rana catesbeiana (American Bullfrog)	12	10 (83%)	420 (± 199)
Rana sphenocephala (Southern Leopard Frog)	8	5 (63%)	925 (± 960)
(M) Grassy Slough WMA, McCurtain County	27	2 (7%)	302 (± 267)
Acris blanchardi (Blanchard's Cricket Frog)	3	0 (0%)	0 (± N/A)
Hyla cinerea (Green Treefrog)	18	0 (0%)	0 (± N/A)
Rana catesbeiana (American Bullfrog)	2	2 (100%)	302 (± 267)
Rana sphenocephala (Southern Leopard Frog)	4	0 (0%)	0 (± N/A)
(N) Red Slough WMA, McCurtain County	58	9 (16%)	414 (± 363)
Acris blanchardi (Blanchard's Cricket Frog)	21	1 (5%)	105 (± N/A)
Hyla cinerea (Green Treefrog)	1	0 (0%)	0 (± N/A)
Rana catesbeiana (American Bullfrog)	31	5 (16%)	293 (± 92)
Rana clamitans (Green Frog)	3	2 (67%)	893 (± 626)
Ambystoma opacum (Marbled Salamander)	1	1 (100%)	369 (± N/A)
Ambystoma texanum (Small-mouthed Salamander)	1	0 (0%)	0 (± N/A)
Total	390	84 (22%)	725,037 (± 5,492,612)