

Investigating Ranavirus Prevalence in Central Oklahoma, USA, Amphibians

Ranaviruses are viral pathogens that cause systemic problems in the liver, digestive tract, and kidneys of amphibians (Daszak et al. 1999) and are among the infectious diseases implicated in the global decline of amphibian populations (Kiesecker 2002; Blaustein et al. 2003; Harp and Pentranka 2006; Lips et al. 2006; Raffel et al. 2006). To date, little is known about ranavirus (RV) prevalence among wild amphibian populations in the central United States (but see Duffus et al. 2015; Kirschman et al. 2017). Although the historical and recent patterns of pathogen prevalence of another amphibian pathogen, *Batrachochytrium dendrobatidis* (*Bd*), have been documented throughout Oklahoma, USA (Steiner and Lehtinen 2008; Lannoo et al. 2011; Watters et al. 2016, 2018; Marhanka et al. 2017), few studies have focused on RV in the state. In this study, we provide the first assessment of RV infection among wild amphibian populations in central Oklahoma.

We conducted repeated sampling trips to survey and screen amphibians across a five-month period from four sites in Cleveland and Oklahoma counties, in central Oklahoma (Fig. 1). Sampling occurred during Spring (March and May), Summer (June and August), and Fall (October) in 2015. At each site, all amphibian species encountered were captured and sampled, regardless of life stage. Ten anuran species (Tables 1, 2) were collected for a total of 198 adults, 16 juveniles, 2 metamorphs,

and 23 tadpoles. We categorized adults as full-grown anurans; juveniles as metamorphosed, but smaller, individuals with four limbs and no tail; metamorphs as individuals with four limbs and a reduced tail; and tadpoles as individuals with full tails and fewer than four limbs. In Oklahoma County, we surveyed a site adjacent to a stream outflow of Northeast Lake near the Oklahoma City Zoo (Fig. 1: Site 1), and a wetland site located within a residential neighborhood at NE 50th St. and N. Bartell Rd. (Fig. 1: Site 2). We sampled two sites in Cleveland County: Sutton Urban Wilderness Park (Fig. 1: Site 3) and Lexington Wildlife Management Area (WMA; Fig. 1: Site 4). The surveys were conducted on either the second or fourth week of each focal month. When different sites were surveyed in the same month, site assessments were conducted within ten days of each other; however, Sutton Urban Wilderness Park (Site 3) was the only site surveyed twice in one month. In addition, Sutton Urban Wilderness Park (Site 3) was not sampled in May and the wetland in the residential neighborhood (Site 2) was not sampled in March, August, or October, due to low water levels and the inability to find amphibians. Between ponds, all equipment, waders, and boots were decontaminated using a 10% bleach solution to ensure pathogens would not be spread among sample sites (Gray et al. 2017).

Animals were collected in the field at night, by hand, dip net, or seine. Adult, juvenile, and metamorph anurans were placed in individual clean collection bags. If multiple tadpoles of the same species were collected at a site, they were held in one collection bag for up to 24 h before being euthanized and processed as a tadpole lot, with 5–10 individuals subsampled for RV from each group. Tadpoles that were not sampled for RV were vouchered as a group representing a developmental series for an unrelated study. We did not encounter or sample any individuals that were

SIERRA N. SMITH*

Sam Noble Oklahoma Museum of Natural History,
 University of Oklahoma, 2401 Chautauqua Ave.,
 Norman, Oklahoma 73072, USA; Department of Biology,
 University of Oklahoma, 730 Van Vleet Oval, Room 314,
 Norman, Oklahoma 73019, USA

JESSA L. WATTERS

Sam Noble Oklahoma Museum of Natural History,
 University of Oklahoma, 2401 Chautauqua Ave.,
 Norman, Oklahoma 73072, USA

ELIZABETH C. MARHANKA

Oklahoma State University, Center for Veterinary Health Sciences,
 112 McElroy Hall, Stillwater, Oklahoma 47078, USA

SHELBY L. McMILLIN

Oklahoma State University, College of Osteopathic Medicine,
 1111 W. 17th St., Tulsa, Oklahoma 74107, USA

DREW R. DAVIS

School of Earth, Environmental, and Marine Sciences,
 University of Texas Rio Grande Valley, 100 Marine Lab Dr.,
 South Padre Island, Texas 78597, USA

JILLIAN K. FARKAS

Department of Biology, University of South Dakota, 414 E. Clark St.,
 Vermillion, South Dakota, 57069, USA

CAMERON D. SILER

Sam Noble Oklahoma Museum of Natural History,
 University of Oklahoma, 2401 Chautauqua Ave.,
 Norman, Oklahoma 73072, USA; Department of Biology,
 University of Oklahoma, 730 Van Vleet Oval, Room 314,
 Norman, Oklahoma 73019, USA

*Corresponding author; e-mail: sierrasmith@ou.edu

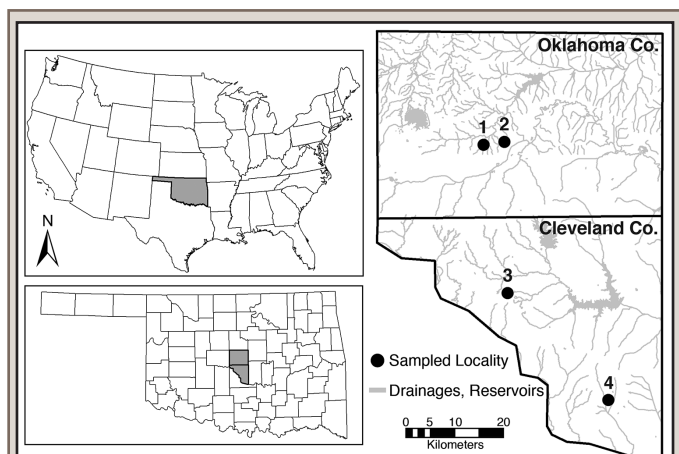


FIG. 1. Map of samples sites for amphibians tested for ranavirus in central Oklahoma, USA. Black circles represent the sampled sites: near the Oklahoma City Zoo (Site 1: 35.51705°N, 97.47129°W); intersection of NE 50th St. and N. Bartell Rd. in Oklahoma City (Site 2: 35.52229°N, 97.43267°W); Sutton Urban Wilderness Park (Site 3: 35.24266°N, 97.42689°W); Lexington Wildlife Management Area (Site 4: 35.04437°N, 97.24004°W).

TABLE 1. Prevalence of ranavirus (RV) in amphibian populations in central Oklahoma, USA, summarized per site across sampling dates, including species, life stage, number of individuals who tested positive out of the total number sampled (RV+/total), Mean Viral Load, and Infection Load Standard Deviation (Std. Dev.). We report high RV prevalence among *Lithobates catesbeianus* tadpoles collected from Site 1 on 21 March 2015; however, because multiple tadpoles of the same species were held in one bag after collection, we removed these individuals from all statistical summaries because of the potential for false positives (Brunner et al. 2007; Gray et al. 2018).

Site	Date (2015)	Species	Life stage	Tissue type	Number RV+/total	Mean viral load	Infection load Std. Dev.	
Oklahoma City Zoo (Site 1)	21-Mar	<i>Lithobates catesbeianus</i>	Tadpole	Tail	14/19	412	240	
	12-May	<i>Acris blanchardi</i>	Adult	Liver	0/6	N/A	N/A	
	24-Jun	<i>Acris blanchardi</i>	Adult	Liver	0/4	N/A	N/A	
		<i>Anaxyrus woodhousii</i>	Adult	Liver	1/2	2,221	N/A	
		<i>Lithobates catesbeianus</i>	Adult	Liver	0/1	N/A	N/A	
	22-Aug	<i>Lithobates sphenoccephalus</i>	Adult	Liver	0/3	N/A	N/A	
		<i>Acris blanchardi</i>	Adult	Toe	0/1	N/A	N/A	
		<i>Lithobates catesbeianus</i>	Adult	Toe	0/3	N/A	N/A	
				Tadpole	Tail	0/1	N/A	N/A
	29-Oct	<i>Acris blanchardi</i>	Adult	Toe	2/18	151	42	
		<i>Lithobates catesbeianus</i>	Adult	Toe	0/2	N/A	N/A	
	50th & Bartell, Oklahoma City (Site 2)	12-May	<i>Acris blanchardi</i>	Adult	Liver	0/3	N/A	N/A
			<i>Anaxyrus americanus</i>	Adult	Liver	0/1	N/A	N/A
<i>Anaxyrus woodhousii</i>			Adult	Liver	0/6	N/A	N/A	
24-June		<i>Gastrophryne olivacea</i>	Adult	Liver	1/3	1,264	N/A	
		<i>Hyla chrysocelis/versicolor</i>	Adult	Liver	0/10	N/A	N/A	
		<i>Lithobates catesbeianus</i>	Juvenile	Liver	0/1	N/A	N/A	
		<i>Pseudacris clarkii</i>	Adult	Liver	0/2	N/A	N/A	
		<i>Pseudacris fouquettei</i>	Adult	Liver	0/1	N/A	N/A	
		<i>Hyla cinerea</i>	Adult	Liver	1/2	753	N/A	
		<i>Lithobates catesbeianus</i>	Adult	Liver	1/3	295,920	N/A	
			Metamorph	Tail	0/1	N/A	N/A	
Sutton Urban Wilderness Park (Site 3)		12-Mar	<i>Lithobates sphenoccephalus</i>	Tadpole	Tail	1/1	1,060	N/A
			<i>Acris blanchardi</i>	Adult	Liver	1/7	283	N/A
	23-Mar	<i>Lithobates catesbeianus</i>	Tadpole	Tail	1/1	927	N/A	
		<i>Acris blanchardi</i>	Adult	Liver	0/6	N/A	N/A	
		<i>Lithobates catesbeianus</i>	Adult	Liver	0/13	N/A	N/A	
	23-June			Toe	0/1	N/A	N/A	
		<i>Acris blanchardi</i>	Adult	Liver	0/9	N/A	N/A	
		<i>Lithobates sphenoccephalus</i>	Adult	Liver	0/1	N/A	N/A	
				Juvenile	Liver	0/10	N/A	N/A
	21-Aug			Tadpole	Tail	0/1	N/A	N/A
				Metamorph	Tail	1/1	245	N/A
		<i>Acris blanchardi</i>	Adult	Toe	5/8	851	894	
		<i>Gastrophryne olivacea</i>	Adult	Liver	0/1	N/A	N/A	
<i>Hyla chrysocelis/versicolor</i>		Adult	Toe	1/2	238	N/A		
<i>Lithobates sphenoccephalus</i>		Adult	Toe	1/12	210	N/A		
15-Oct		<i>Acris blanchardi</i>	Adult	Toe	2/6	626	13	
	<i>Lithobates catesbeianus</i>	Adult	Toe	0/6	N/A	N/A		
			Juvenile	Toe	0/3	N/A	N/A	
Lexington WMA (Site 4)	11-Mar	<i>Lithobates sphenoccephalus</i>	Adult	Toe	2/11	322	141	
		<i>Acris blanchardi</i>	Adult	Toe	2/6	634	162	
		<i>Lithobates sphenoccephalus</i>	Adult	Toe	0/1	N/A	N/A	
	10-May	<i>Acris blanchardi</i>	Adult	Liver	1/2	109	N/A	
		<i>Gastrophryne olivacea</i>	Adult	Liver	0/1	N/A	N/A	
		<i>Hyla chrysocelis/versicolor</i>	Adult	Liver	0/1	N/A	N/A	
		<i>Lithobates sphenoccephalus</i>	Adult	Liver	0/1	N/A	N/A	
	22-June	<i>Acris blanchardi</i>	Adult	Liver	2/10	925	969	
		<i>Hyla chrysocelis/versicolor</i>	Adult	Liver	0/3	N/A	N/A	
		<i>Lithobates sphenoccephalus</i>	Juvenile	Liver	1/1	634	N/A	
	20-Aug	<i>Lithobates catesbeianus</i>	Adult	Liver	0/1	N/A	N/A	
		<i>Lithobates sphenoccephalus</i>	Adult	Liver	1/2	910	N/A	
	19-Oct	<i>Acris blanchardi</i>	Adult	Toe	1/12	349	N/A	
<i>Anaxyrus americanus</i>		Adult	Liver	0/1	N/A	N/A		
<i>Hyla chrysocelis/versicolor</i>		Juvenile	Liver	0/1	N/A	N/A		
<i>Lithobates sphenoccephalus</i>		Adult	Liver	1/3	342	N/A		

TABLE 2. Comparison of ranavirus (RV) prevalence by host amphibian species from Oklahoma, USA, including sample size (N), number of individuals who tested negative (RV-), number of individuals who tested positive (RV+), Mean Viral Load and Infection Load Standard Deviation (Std. Dev.). We report the abnormally high infection load for a liver sample collected from a single *Lithobates catesbeianus*; however, this sample was not included in any Mean Viral Load calculations to avoid potential biases. Additionally, the 19 *L. catesbeianus* tadpoles collected from Site 1 in March were removed from the total calculations reported here because of the potential for false positives, see text for full explanation.

Species	N	Tissue type	RV-	RV+	RV%	Mean viral load	Infection load Std. Dev.
Bufonidae							
<i>Anaxyrus americanus</i>	2	Liver	2	0	0%	N/A	N/A
<i>Anaxyrus woodhousii</i>	8	Liver	7	1	12.5%	2,221	N/A
Hylidae							
<i>Acris blanchardi</i>	47	Liver	43	4	8.5%	560	704
	51	Toe	39	12	23.5%	619	603
<i>Hyla chrysoscelis/versicolor</i>	15	Liver	15	0	0%	N/A	N/A
	2	Toe	1	1	50%	238	N/A
<i>Hyla cinerea</i>	2	Liver	1	1	50%	753	N/A
<i>Pseudacris clarkii</i>	2	Liver	2	0	0%	N/A	N/A
<i>Pseudacris fouquettei</i>	1	Liver	1	0	0%	N/A	N/A
Microhylidae							
<i>Gastrophryne olivacea</i>	5	Liver	4	1	20%	1,264	N/A
Ranidae							
<i>Lithobates catesbeianus</i>	19	Liver	18	1	5.3%	295,920	N/A
	3	Tail	2	1	33.3%	927	N/A
	15	Toe	15	0	0%	N/A	N/A
<i>Lithobates sphenoccephalus</i>	21	Liver	18	3	14.3%	628	284
	3	Tail	1	2	66.7%	653	577
	24	Toe	21	3	12.5%	285	119
TOTAL/MEAN	220		190	30	13.6%	815	245

dead or showed outward signs of RV infection. After collection, specimens were either: (1) euthanized in aqueous chloroform (Simmons 2015) and a piece of liver tissue was collected from the individual, or; (2) non-destructively sampled, with a toe or tail clip taken immediately after capture (only for common species sampled during the Fall). To minimize cross-contamination, dissection equipment was cleaned and sterilized, using 95% ethanol, and gloves were changed between each individual (Gray et al. 2017). Tissue samples (liver or toe) were preserved in 95% ethanol (St-Amour and Lesbarrères 2007). DNA from tissue subsamples was extracted via a high salt extraction method (Esselstyn et al. 2008), with extracts stored at -20°C until used for pathogen screening.

Prior to quantitative PCR (qPCR) analysis at the Disease Testing and Sequencing Facility at the University of South Dakota, DNA extracts were diluted 1:2 with a 0.25 TE buffer solution to reduce the effects of potential inhibitors, following standardized published protocols (Watters et al. 2018). Primers used for the RV assay target a ~70bp fragment of the Major Capsid Protein (MCP) gene as described by Forson and Storfer (2006). Hall et al. (2016) demonstrated that this specific qPCR primer set is likely to detect all ranaviruses known to circulate in North American amphibian populations. All qPCR assays of samples were run in triplicate (three reactions per sample) for 50 cycles, with standard dilutions of known pathogen gene copy number (DNA quantities) based on customized, double-stranded DNA fragments (qBlocks; Integrated DNA Technologies) from the MCP gene (Watters et al. 2018). The 750-base-pair qBlocks contain known copy numbers

of the PCR fragment target for RV, which allowed us to quantify the gene copy number amplified in each sample. Four standards were created for the RV pathogen gBlock by serially diluting the gBlock stock of known DNA quantities into concentrations ranging from $1e^1$ – $1e^4$. As with study samples, each gBlock standard was run in triplicate on each qPCR assay plate, with the exception of the $1e^4$ standard which was run in duplicate next to a single negative control sample (sterile, molecular grade water). Infection loads were quantified using a StepOnePlus Real-Time PCR System and StepOnePlus software version 2.3 (Applied Biosystems, Inc. [ABI]), following the protocols of Davis and Kerby (2016) and Watters et al. (2018), with 3 μ L of DNA added to a 7 μ L cocktail solution (TaqMan Fast Advanced Master Mix, ABI; forward primer; reverse primer; TaqMan probe, ABI; and molecular grade water). Samples were considered positive for ranavirus (RV+) if (1) at least two of the three (triplicate) wells were amplified and (2) the resulting mean gene copy number was above 1.0 (Davis and Kerby 2016; Watters et al. 2016, 2018; Marhanka et al. 2017). To determine final infection load per sample, RV gene copy numbers were multiplied by the dilution factor (two for RV) and then were multiplied again by a value in order to reach the total original extraction volume for each sample (Davis and Kerby 2016; Watters et al. 2018).

Out of the 220 amphibians screened, 30 (13.64%) tested positive for RV infection across all sites (Table 2). The highest prevalence of RV+ amphibians was observed during the month of August (25.81%), followed by June (15.09%). The months of March, May, and October had lower prevalence of RV (8.82%,

5.26%, and 12.7%, respectively). The high prevalence of RV infection in August was driven by *Acris blanchardi* individuals from Site 3 (Table 1). We report high RV prevalence among *Lithobates catesbeianus* tadpoles collected near the Oklahoma City Zoo in March (73.68%; Table 1); however, because multiple tadpoles of the same species were held in one bag after collection, we removed these individuals from statistical summaries to account for potential false positives as a result of infected animals shedding virus into the water in which groups of tadpoles were held (Brunner et al. 2007; Gray et al. 2018). When we summarized RV prevalence by season, Summer (19%) and Fall (13%) had the highest number of RV+ individuals followed by Spring (8.22%). Looking at the summaries by site, we found the highest percentage of RV+ individuals at Site 4 (19.57%), followed by Site 3 (14.14%). Sites 1 and 2 had lower percentages of RV prevalence (7.32% and 11.76%, respectively). Additionally, the average RV viral infection load differed between months. Specimens collected in June had the highest viral infection load (966.23 mean gene copies/sample), followed by August (701.76 mean gene copies/sample). March, May, and October had lower viral infection loads (619.59, 686.71, and 361.25 mean gene copies/sample, respectively). The average viral infection load for the month of June does not include the abnormally high liver sample collected from a single *L. catesbeianus* individual from Site 2 (Table 1). A previous study that utilized identical field and molecular techniques found higher average RV viral infection loads in northeastern Oklahoma (Watters et al. 2018), while another found very similar infection loads in southeastern Oklahoma (Davis et al. 2019).

We found the highest number of infected individuals came from the family Hylidae (*A. blanchardi*, 16.33%; *Hyla chrysoscelis/versicolor*, 5.88%) followed closely by Ranidae (*L. catesbeianus*, 5.41%; *L. sphenoccephalus*, 16.27%). Comparing the different tissue types and developmental stages sampled, we observed higher prevalence of RV infection in the tail tissues obtained from *L. sphenoccephalus* tadpoles (Tables 1, 2). Additionally, our study screened individuals from species that are not often reported in RV manuscripts (Table 2). Despite smaller sample sizes for *Anaxyrus woodhousii* and *Hyla cinerea*, we found a low level of RV infection among these species (Table 2). Both species of *Pseudacris* tested were negative for RV; however, our sample sizes do not allow for definitive reporting on whether these species are negative for RV in central Oklahoma, especially considering *P. clarkii* has been reported as RV+ in other studies (Torrence et al. 2010). Additionally, this is one of the first reports of RV infection in the Microhylidae species *Gastrophryne olivacea* (Table 2; Davis et al. 2019). Although RV is fairly well documented in the United States, Oklahoma remains largely understudied (but see Watters et al. 2018; Davis et al. 2019). More robust surveys are needed to determine the threat of this pathogen to amphibian communities in the state.

Acknowledgments.—This research was supported by grants from the Research Council of the University of Oklahoma Norman Campus to CDS, and the Oklahoma Department of Wildlife Conservation (F14F01225 [T-80-1]) and the Oklahoma City Zoo and Botanical Gardens Conservation Action Now (CAN) grants to CDS and JLW. Additionally, we thank the University of Oklahoma Honors College Undergraduate Research Opportunities Program for funding SLM, the University of Oklahoma McNair Scholars program for funding ECM, and the University of Oklahoma Mentored Research Fellowship for funding SNS. All amphibians were collected under applicable ODWC Scientific Collecting Permits in 2015 (CDS: 6147; JLW:

6148) and research was approved by the University of Oklahoma Institutional Animal Care and Use Committee (#R14-026). The authors thank the members of the Siler Lab for fieldwork and DNA extraction assistance. We thank Stacey Sekscienski, Joe Branham, and the herpetology department staff at the Oklahoma City Zoo and Botanical Gardens for their assistance in sample collection. Also, we thank the five anonymous reviewers for their critical evaluations of this manuscript.

LITERATURE CITED

- BLAUSTEIN, A. R., J. M. ROMANSIC, J. KIESECKER, AND A. C. HATCH. 2003. Ultraviolet radiation, toxic chemicals, and amphibian population declines. *Divers. Distrib.* 9:123–140.
- BRUNNER, J. L., D. M. SCHOCK, AND J. P. COLLINS. 2007. Transmission dynamics of the amphibian ranavirus *Ambystoma tigrinum* virus. *Dis. Aquat. Organ.* 77:87–95.
- DASZAK, P., L. BERGER, A. A. CUNNINGHAM, A. D. HYATT, D. E. GREEN, AND R. SPEARE. 1999. Emerging infectious diseases and amphibian population declines. *Emerg. Infect. Dis.* 5:735–748.
- DAVIS, D. R., J. K. FARKAS, T. R. KRUISELBRINK, J. L. WATTERS, E. D. ELLSWORTH, J. L. KERBY, AND C. D. SILER. 2019. Prevalence and distribution of ranavirus in amphibians from southeastern Oklahoma, USA. *Herpetol. Conserv. Biol.* 14:360–369.
- , AND J. L. KERBY. 2016. First detection of ranavirus in amphibians from Nebraska, USA. *Herpetol. Rev.* 47:46–50.
- DUFFUS, A. L. J., T. B. WALTZEK, A. C. STÖHR, M. C. ALLENDER, M. GOTESMAN, R. J. WHITTINGTON, P. HICK, M. K. HINES, AND R. E. MARSCHANG. 2015. Distribution and host range of ranaviruses. In M. J. Gray and V. G. Chinchir (eds.), *Ranaviruses: Lethal Pathogens of Ectothermic Vertebrates*, pp. 9–57. Springer International Publishing, New York, New York.
- 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. *J. Mammal.* 89:815–825.
- FORSON, D. D., AND A. STORFER. 2006. Atrazine increases ranavirus susceptibility in the tiger salamander, *Ambystoma tigrinum*. *Ecol. Appl.* 16:2325–2332.
- 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, AND D. L. MILLER. 2017. Pathogen surveillance in herpetofaunal populations: guidance on study design, sample collection, biosecurity, and intervention strategies. *Herpetol. Rev.* 48:334–351.
- , J. A. SPATZ, E. D. CARTER, C. M. YARBER, R. P. WILKES, AND D. L. MILLER. 2018. Poor biosecurity could lead to disease outbreaks in animal populations. *PLoS ONE* 13:e0193243.
- HALL, E. M., E. J. CRESPI, C. S. GOLDBERG, AND J. L. BRUNNER. 2016. Evaluating environmental DNA-based quantification of ranavirus infection in wood frog populations. *Mol. Ecol. Resour.* 16:423–433.
- HARP, E. M., AND J. W. PETRANKA. 2006. Ranavirus in wood frogs (*Rana sylvatica*): potential sources of transmission within and between ponds. *J. Wildl. Dis.* 42:307–318.
- KIESECKER, J. M. 2002. Synergism between trematode infection and pesticide exposure: a link to amphibian limb deformities in nature? *Proc. Natl. Acad. Sci.* 99:9900–9904.
- KIRSCHMAN, L. J., J. PALIS, K. FRITZ, K. ALTHOFF, AND R. WARNE. 2017. Two ranavirus associated mass mortality events among larval amphibians in Illinois. *Herpetol. Rev.* 48:779–782.
- LANNOO, M. J., C. PETERSEN, R. E. LOVICH, P. NANJAPPA, C. PHILLIPS, J. C. MITCHELL, AND I. MACALLISTER. 2011. Do frogs get their kicks on Route 66? Continental U.S. transect reveals spatial and temporal patterns of *Batrachochytrium dendrobatidis* infection. *PLoS ONE* 6:e22211.
- LIPS, K. R., F. BREM, R. BRENES, J. D. REEVE, R. A. ALFORD, J. VOYLES, C. CAREY, L. LIVO, A. P. PESSIER, AND J. P. COLLINS. 2006. Emerging infectious disease and the loss of biodiversity in a Neotropical amphibian community. *Proc. Natl. Acad. Sci. USA* 103:3165–3170.

- MARHANKA, 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. *Herpetol. Rev.* 48:70–74.
- RAFFEL, T. R., J. R. ROHR, J. M. KIESECKER, AND P. J. HUDSON. 2006. Negative effects of changing temperature on amphibian immunity under field conditions. *Funct. Ecol.* 20:819–828.
- SIMMONS, J. E. 2015. *Herpetological Collecting and Collections Management*. Third Edition. Society for the Study of Amphibians and Reptiles, Salt Lake City, Utah. 153 pp.
- ST-AMOUR, V., AND D. LESBARRÈRES. 2007. Genetic evidence of ranavirus in toe clips: an alternative to lethal sampling methods. *Conserv. Genet.* 8:1247.
- STEINER, S., AND R. LEHTINEN. 2008. Occurrence of the amphibian pathogen *Batrachochytrium dendrobatidis* in Blanchard's cricket frog (*Acris crepitans blanchardi*) in the U.S. Midwest. *Herpetol. Rev.* 39:193–196.
- 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. *J. Aquat. Anim. Health.* 22:65–72.
- 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 Oklahoma, USA. *Herpetol. Rev.* 47:214–220.
- , D. R. DAVIS, T. YURI, AND C. D. SILER. 2018. Concurrent infection of *Batrachochytrium dendrobatidis* and ranavirus among native amphibians from northeastern Oklahoma, USA. *J. Aquat. Anim. Health* 30:291–301.