PREVALENCE AND DISTRIBUTION OF RANAVIRUS IN AMPHIBIANS FROM SOUTHEASTERN OKLAHOMA, USA

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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.
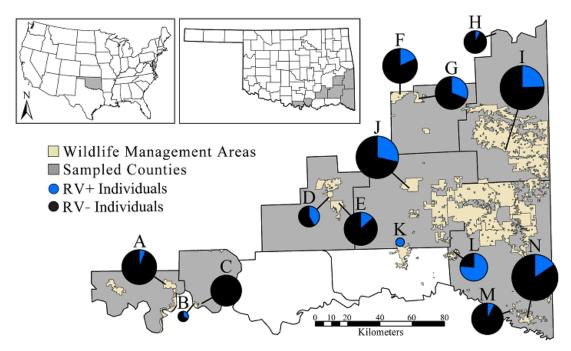
Figure 1. Map of southeastern Oklahoma, USA, showing sampled counties (grey) and localities (labeled with letters corresponding to those in Appendix). Pie chart size is scaled by the number of amphibians sampled at that locality (range, 3–58); pie chart illustrates the proportion of sampled individuals 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 ≤ 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 ≥ 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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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 ($\pm$ 1 standard deviation [SD]) are indicated. When $\leq$ 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 ($\pm$ 1 SD)
(A) Hickory Creek WMA, Love County	33	2 (6%)	181 ($\pm$ 89)
Anaxyrus americanus (American Toad)	11	0 (0%)	0 ($\pm$ N/A)
Anaxyrus woodhousii (Woodhouse's Toad)	5	0 (0%)	0 ($\pm$ N/A)
Acris blanchardi (Blanchard's Cricket Frog)	6	1 (17%)	243 ($\pm$ N/A)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	6	0 (0%)	0 ($\pm$ N/A)
Gastrophryne olivacea (Western Narrow-mouthed Toad)	3	0 (0%)	0 ($\pm$ N/A)
Rana sphenocephala (Southern Leopard Frog)	2	1 (50%)	118 ($\pm$ N/A)
(B) Fobb Bottom WMA, Marshall County	3	1 (33%)	497 ($\pm$ N/A)
Acris blanchardi (Blanchard's Cricket Frog)	1	0 (0%)	0 ($\pm$ N/A)
Gastrophryne olivacea (Western Narrow-mouthed Toad)	1	1 (100%)	497 ($\pm$ N/A)
Rana sphenocephala (Southern Leopard Frog)	1	0 (0%)	0 ($\pm$ N/A)
(C) Univ. Oklahoma Biological Station and Vicinity, Marshall County	26	0 (0%)	0 ($\pm$ N/A)
Anaxyrus americanus (American Toad)	5	0 (0%)	0 ($\pm$ N/A)
Anaxyrus woodhousii (Woodhouse's Toad)	5	0 (0%)	0 ($\pm$ N/A)
Acris blanchardi (Blanchard's Cricket Frog)	10	0 (0%)	0 ($\pm$ N/A)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	3	0 (0%)	0 ($\pm$ N/A)
Rana blairi (Plains Leopard Frog)	1	0 (0%)	0 ($\pm$ N/A)
Rana sphenocephala (Southern Leopard Frog)	2	0 (0%)	0 ($\pm$ N/A)
(D) Stringtown WMA, Atoka County	12	5 (42%)	413 ($\pm$ 367)
Acris blanchardi (Blanchard's Cricket Frog)	4	0 (0%)	0 ($\pm$ N/A)
Rana catesbeiana (American Bullfrog)	6	5 (83%)	413 ($\pm$ 367)
Notophthalmus viridescens (Eastern Newt)	2	0 (0%)	0 ($\pm$ N/A)
(E) McGee Creek WMA, Atoka County	30	4 (13%)	459 ($\pm$ 318)
Acris blanchardi (Blanchard's Cricket Frog)	4	0 (0%)	0 ($\pm$ N/A)
Rana catesbeiana (American Bullfrog)	6	3 (50%)	463 ($\pm$ 390)
Rana clamitans (Green Frog)	9	1 (11%)	447 ($\pm$ N/A)
Notopthalmus viridescens (Eastern Newt)	11	0 (0%)	0 ($\pm$ N/A)
(F) James Collins WMA, Latimer County	33	6 (18%)	666 ($\pm$ 370)
Acris blanchardi (Blanchard's Cricket Frog)	12	2 (17%)	751 ($\pm$ 760)
Hyla chrysoscelis/versicolor (Gray Treefrog complex)	11	1 (9%)	544 ($\pm$ N/A)
Rana catesbeiana (American Bullfrog)	2	0 (0%)	0 ($\pm$ N/A)
Rana sphenocephala (Southern Leopard Frog)	1	0 (0%)	0 ($\pm$ N/A)
Notophthalmus viridescens (Eastern Newt)	7	3 (43%)	650 ($\pm$ 197)
(G) Robbers Cave WMA, Latimer County	29	9 (31%)	1,305,964 ($\pm$ 3,904,431)
Anaxyrus americanus (American Toad)	1	0 (0%)	0 ($\pm$ N/A)
Acris blanchardi (Blanchard's Cricket Frog)	4	0 (0%)	0 ($\pm$ N/A)
Rana catesbeiana (American Bullfrog)	6	1 (17%)	30,157 ($\pm$ N/A)
Rana clamitans (Green Frog)	2	0 (0%)	0 ($\pm$ N/A)
Notophthalmus viridescens (Eastern Newt)	16	8 (50%)	1,465,440 ($\pm$ 4,142,558)
(H) Arkansas River at Robert S. Kerr Lock & Dam 15, Le Flore Co.	14	1 (7%)	203 ($\pm$ 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)