FINAL PERFORMANCE REPORT

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Tracking the Emergence of Infectious Diseases Among Amphibian Species of Greatest Conservation Need

Oklahoma Department of Wildlife Conservation

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FINAL REPORT

State: Oklahoma **Grant Number:** F14F01225 (T-80-1)

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Grant Title: Tracking the Emergence of Infectious Diseases Among Amphibian Species of Greatest Conservation Need

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Project Leader:
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Executive Summary:
There are 16 amphibian species designated by the Oklahoma Department of Wildlife Conservation as "Oklahoma's Species of Greatest Conservation Need" (SGCN) (Oklahoma Comprehensive Wildlife Conservation Strategy). Among these, six priority species are listed as Tier I (Grotto Salamander, Kiamichi Slimy Salamander, Oklahoma Salamander, Rich Mountain Salamander, Ringed Salamander, and Sequoyah Slimy Salamander), and seven as Tier II (Crawfish Frog, Many-ribbed Salamander, Ouachita Dusky Salamander, Ozark Salamander, Southern Red-backed Salamander, Three-toed Amphiuma, Western Lesser Siren). The objectives of this study were to: 1) estimate the distribution and abundance of amphibian species in WMAs throughout the state through a series of focused field surveys across 21 counties; 2) track the presence and emergence of amphibian infectious disease among species of greatest conservation concern through the development of a novel, statewide monitoring program; and 3) establish a viable, long-term protocol for sustained monitoring of amphibian infectious diseases and forecasting of regions with the highest risk of outbreak through rapid state screening and web-based database developments. We conducted surveys of amphibian communities between March–July and September–October in 2015, 2016, and 2017 to screen wild populations for infectious amphibian diseases. Our surveys focused on counties in southeast Oklahoma in 2015, northeast Oklahoma in 2016, and southwest and northwest Oklahoma in 2017. These months were chosen due to peak breeding seasons for amphibians in the state. We sampled a total of 1,514 individual amphibians across 36 sites in 25 counties in the state. In total, we observed and screened populations of 30 species of amphibians (18 frogs, 12 salamanders). Although there is considerable variation across species and regions of the state, we observe significant disease prevalence in most surveyed sites, with a statewide average prevalence for chytrid fungus (*Bd*) of 28% and for Ranavirus (RV) of 16%. South and southeast Oklahoma had the highest prevalence of infectious disease for both pathogens (68% *Bd* and 21% RV), with northern, northwestern, and western populations having similar disease prevalence. Among anuran (frog) species

sampled, members of the family Bufonidae, Hylidae, and Ranidae showed the highest *Bd* infectious rates when summarizing data for families with significant sample sizes resulting from the study. All three of these families of frogs had representative species with *Bd* infection rates well above 50%. Interestingly, most frog species showed low infection rates for Ranavirus. Fewer salamander species had *Bd* infection rates close to or above 50%, although Ranavirus infection rates appear to be higher on average than those observed for frogs in the state. The results of this study provide a robust, baseline snapshot of disease prevalence at a statewide resolution. Unfortunately, these data represent a single point estimate in time for these populations, and does not provide data on disease spread, population health, or long-term impacts of infection on any of the sampled populations. Repeated surveys across several years will be needed to investigate trends in disease prevalence and load through time and assess risk factors for native amphibians in the state.

I. BACKGROUND AND NEED:

Amphibians represent perhaps the most threatened group of organisms on earth, with recent reports indicating that one-third of the world's species are facing extinction (Stuart et al. 2004; IUCN 2013). Although many factors are contributing to this global biodiversity crisis, including habitat alterations (Homan et al. 2004), the dominant concern for more than a decade has been the emergence of infectious diseases, particularly *Batrachochytrium dendrobatidis* (*Bd*), or chytrid fungus, and ranaviruses (RV; Daszak et al. 2003; Schloegel et al. 2010; Miller et al. 2011). Particularly concerning is how both infectious diseases are linked to amphibian population declines in pristine habitats (Wake & Vredenburg 2008).

In North America, chytrid fungus has been present for at least the last half century (Ouellet et al. 2005), and in the United States, both chytrid fungus and ranavirus have been documented at sites from sea level to high elevations (Lannoo et al. 2011). Sites with positive infectious disease samples have now been reported in most states across the US, with disease positive samples reported at numerous sites across states surrounding Oklahoma (*Bd*+: Arkansas, Colorado, Louisiana, Missouri, Nebraska, New Mexico, Texas; RV+: Colorado, Texas; Lannoo et al. 2011). Within Oklahoma, positive samples of chytrid have now been reported from three counties (Bd Maps 2018). In 2004, four non-SGCN frogs were screened at two National Wildlife Refuges (Wichita Mountains National Wildlife Refuge, Little River National Wildlife Refuge), with *Bd* + samples of *Lithobates sphenocephalus* reported in McCurtain County. In 2007, *Bd* + samples were reported from screened samples of *Acris crepitans* within the Packsaddle Wildlife Area in Ellis County. Then, in 2009, researchers screened non-SGCN species at two Department of Defense installations (Camp Gruber and Fort Still), reporting *Bd* + samples for species within Camp Gruber in Muskogee County.

Unfortunately, as of 2015, virtually nothing was known about disease prevalence among Oklahoma's species of conservation concern. The Oklahoma Department of Wildlife Conservation has designated 16 amphibian species as "Oklahoma's Species of Greatest Conservation Need" (SGCN) (Oklahoma Comprehensive Wildlife Conservation Strategy). Among these, six priority species are listed as Tier I (Grotto Salamander, Kiamichi Slimy Salamander, Oklahoma Salamander, Rich Mountain Salamander, Ringed Salamander, and Sequoyah Slimy Salamander), and seven as Tier II (Crawfish Frog, Many-ribbed Salamander,

Ouachita Dusky Salamander, Ozark Salamander, Southern Red-backed Salamander, Three-toed Amphiuma, Western Lesser Siren). Although past survey efforts have resulted in a basic understanding of the distribution of some of these species in Oklahoma, for most, comprehensive distribution information and status remain unknown, and vouchered collections and genetic resource samples are poorly represented. However, of greatest conservation concern is the near complete absence of any baseline information on the presence and prevalence of infectious diseases among amphibian populations in Oklahoma.

Despite the significant body of literature documenting the devastating impact that both chytrid and ranavirus have on many amphibian species worldwide (for review: Cheng et al. 2011), and the comprehensive datasets now available for monitoring disease emergence across the US (Lannoo et al. 2011; Bd Maps 2018), Oklahoma represents one of the few critical states remaining with little information available on its native species. To date, *a total of only five localities across Oklahoma have been screened for the presence of infectious disease in native amphibian populations* (Steiner & Lehtinen 2008; Lannoo et al. 2011). Furthermore, not only is there no information on the presence and prevalence of amphibian disease among the ODWC-designated amphibian SGCNs, but to our knowledge, no studies have ever screened for ranavirus in amphibian populations in the state.

Our project aimed to develop a baseline database on infectious amphibian diseases through a rapid, statewide monitoring program, with the goal of addressing the following core questions:

1. **Where are amphibian infectious diseases present across the state and what species are carriers of chytrid fungus and ranaviruses?**
2. **How do we monitor the vulnerability of Oklahoma's highest priority amphibians to disease-coupled decline?**
3. **How can we develop protocols for a sustained monitoring program of amphibian infectious diseases capable of identifying, as well as predicting, regions at highest risk of outbreak?**

Recognizing a number of factors can confound the measured impact of infectious disease on population declines (e.g., human disturbance, habitat alterations; Lannoo et al. 2011), we chose to focus baseline efforts on protected and managed habitats, employing Wildlife Management Areas (WMAs) around Oklahoma as starting sites to initiate a long-term, statewide monitoring program of amphibian distributions and population health throughout ODWC-managed habitat. The State of Oklahoma currently has 73 land holdings designated as WMAs, and with a few exceptions (i.e., USFWS refuges, several military bases) these WMAs represent some of the most pristine and undisturbed natural habitats throughout the state. From hunting and fishing to a wide variety of research and education activities, these areas are incredibly important to the public, both from a recreational standpoint and for research and educational endeavors. Furthermore, all SGCN amphibians have been documented within, or in close proximity to, WMA habitats.

The purpose of the funded project was to implement an unprecedented, statewide monitoring program for tracking the presence and emergence of amphibian infectious disease across all six Oklahoma Wildlife Action Plan Ecoregions, focusing on species of greatest conservation need as identified in the Oklahoma Comprehensive Wildlife Conservation Strategy, Appendix E.

II. OBJECTIVE(S):

***Objective 1*—**To estimate the distribution and abundance of amphibian species in WMAs throughout the state through a series of focused field surveys across 21 counties. ***Objective 2*—** To track the presence and emergence of amphibian infectious disease among species of greatest conservation concern through the development of a novel, statewide monitoring program. ***Objective 3*—**To establish a viable, long-term protocol for sustained monitoring of amphibian infectious diseases and forecasting of regions with the highest risk of outbreak through rapid state screening and web-based database developments.

III. SUMMARY OF PROGRESS

Methods

Field data collection.—We conducted surveys in Spring and Fall from 2015–2017 for herpetological biodiversity and amphibian infectious disease in 34 public use sites (i.e. Wildlife Management Areas [WMA], Oklahoma State Parks [SP], National Wildlife Refuges [NWR]) in 25 counties. Each site was sampled for 24–48 hours, including both day and nighttime sampling, and overnight aquatic trapping. All reusable equipment was sterilized between water bodies via 10% bleach, as recommended by Grey et al. (2017). Animals were then tagged and kept in individual plastic bags until such time as they were swabbed and euthanized at the Sam Noble Oklahoma Museum of Natural History (SNOMNH). In the case of amphibians, their skin was swabbed for *Bd* fungal spores on the ventral, lateral, and dorsal portions of the trunk, on the hind limbs (five swipes per region), and between hind limb toe webbing, where there is often the highest concentration of *Bd* zoospores (Lannoo et al. 2011). Animals were then euthanized via submersion in a chlorobutanol solution prior to the dissection of genetic material (liver tissue, preserved in 95% ethanol) for RV screening. Vouchered specimens were then fixed in 10% buffered formalin, and eventually transferred to 70% ethanol. All vouchered material is deposited at SNOMNH in either the Herpetology Collection or the Oklahoma Collection of Genomic Resources. Throughout swabbing and tissue collection, sterile techniques were employed to prevent cross-contamination (Grey et al. 2017).

DNA extraction and disease screening.—Extraction of DNA from *Bd* swabs employed the PrepMan Ultra (Life Technologies) reagent and protocol (Cheng et al. 2011); DNA was extracted from tissue samples via a high salt extraction method (Esselstyn et al. 2008). Swab and tissue genetic extracts from each amphibian were then stored at -20°C until used for pathogen screening. DNA extracts have been archived in the SNOMNH Herpetology Collection. Quantitative PCR (qPCR) techniques were utilized to determine the presence/absence of *Bd* or RV in collected samples and to estimate the number of gene copies (infection load) per sample (Kerby et al. 2013; Davis and Kerby 2016). Prior to qPCR analysis, DNA extracts were diluted 1:10 for *Bd* swab samples and 1:2 for RV tissue samples. Dilution methodologies for both pathogens follow standardized, published protocols (Davis and Kerby 2016; Watters et al. 2016; Marhanka et al. 2017). Pathogen screening of 2015 field samples was completed on an Applied Biosystems StepOnePlus system at the Disease Testing and Sequencing Facility at the University of South Dakota, while 2016–2017 samples were screened on an Applied Biosystems QuantStudio 3 system at the Genomics Core Facility at the SNOMNH. Each qPCR plate

contained triplicate DNA extract samples, a negative control (ddH_2O), and four standards using diluted gBlocks of known DNA quantities ($1e^1$–$1e^4$) for each pathogen to create a standard curve, which allows us to analyze gene copy number. Disease loads were quantified using StepOne software v2.3 (Applied Biosystems) in 2015 and QuantStudio Design and Analysis Software (Applied Biosystems) in 2016–2017. A sample was considered positive for a pathogen if at least two of the three wells were amplified and the resulting mean gene copy number was above 1.0 (Kerby et al. 2013).

RESULTS

During the course of the project, we visited 36 distinct WMAs, state parks, and wildlife refuges in 25 counties, collecting a total of 1,936 vouchered specimens (306 reptiles, 1,630 amphibians). The sampled populations represented 72 distinct species (44 reptiles, 28 amphibians). Of these, we selected a large set of the sampled amphibians to screen for two infectious amphibian diseases (chytrid fungus [*Bd*] and Ranavirus [RV]). For disease screening, we sampled a total of 1,514 individual amphibians across 36 sites in 25 counties in the state (Tables 1–4), representing 30 species (18 frogs, 12 salamanders; Tables 1–4).

STATEWIDE PATTERNS

We observe significant disease prevalence in most surveyed sites, with a statewide average prevalence for chytrid fungus (*Bd*) of 28% and for Ranavirus (RV) of 16% (Table 1). South and southeast Oklahoma had the highest prevalence of infectious disease for both pathogens (68% *Bd* and 21% RV), with northern, northwestern, and western populations having similar disease infection rates (Table 1).

Among the counties visited, those with the highest rates of *Bd* infection included: Comanche (83%), Ellis (93%), Love (100%), Marshall (90%), Muskogee (79%), and Pushmataha (71%) (Table 2). We recorded positive *Bd* infection in greater than 70% of all individual amphibians screened from these counties (Table 2). In comparison, the counties with the highest rates of RV infection were Cherokee (33%), Cimarron (50%), and Pushmataha (31%; Table 2). Interestingly, we recorded higher *Bd* infection rates than RV infection rates in all but two counties (Cimarron and Pontotoc). For both of these counties, RV prevalence was higher than *Bd* prevalence (Table 2). We also observed counties with higher *Bd* prevalence to also have lower RV infection rates. In fact, Love, Marshall, and Muskogee counties all had RV infection rates below 7%, while Comanche and Ellis counties had RV rates below 15% (Table 2).

SITE PATTERNS

We screened for infectious diseases at 36 distinct sites in the state, including 25 Wildlife Management Areas (WMAs), three state parks, six National Wildlife Refuges (NWRs), and two public and/or private localities (Arkansas River at Robert S. Kerr Lock & Dam 15; University of Oklahoma Biological Station). Across all sampled sites, the average chytrid infection rate was 48%, with 16 sites observed to have more than one-half of sampled individuals infected with *Bd* (≥ 50%), including two NWRs (Wichita Mountain, Ozark Plateau), both public/private sites, one state park (Osage Hills), and 11 WMAs (Hickory Creek, Osage Hills, Packsaddle, Red Slough, Camp Gruber, Grassy Slough, Cookson Hills, McGee Creek, Robbers Cave, James Collins, and Pushmataha) (Table 3). Several sites had extremely high infection rates approaching 100%, including the OU Biostation (96% *Bd* positive individuals), Hickory Creek and Osage Hills

WMAs (both 100% *Bd* positive individuals), and Packsaddle WMA (93% *Bd* positive individuals; Table 3). However, all four of these sites had low RV prevalence (<14%), with two of the sites (OU Biostation and Osage Hills WMA) having no positive RV samples (0%; Table 3).

Overall, RV infection rates were considerably lower than *Bd* rates observed in our study, with the average statewide prevalence observed to be 20% (Table 3). However, less than one-half of the sites were observed to have RV infection rates above 15%, and 16 sites had RV infection rates below 10%, including 10 sites where we did not detect any RV-infected individuals (Table 3). The four sites with the highest RV infection rates were Hugo (100% RV+) and Pine Creek (76% RV+) WMAs, and the Osage Hills (60% RV+) and Black Mesa (50% RV+) state parks. A unique pattern observed for Hugo, Pine Creek and Black Mesa was the higher RV prevalence coupled with lower observed *Bd* infection rates (Table 3). A similar pattern was observed at Fort Supply WMA and Great Plains State Park, where both had moderate RV infection rates (25% and 20%, respectively), but no observed *Bd* infection. However, it should be noted that five of the six of these aforementioned sites had smaller sample sizes available for this study (≤10 individuals), which may be a factor in these results.

SPECIES PATTERNS

During the course of this project, we were able to screen 30 species of amphibians for infectious disease (18 frogs, 12 salamanders), with an average of 51 individuals screened per species (Table 4). Several species were rarely encountered in the wild, and therefore, we were unable to screen more than a handful of individuals for disease. Unfortunately, even with lower samples sizes, many of these species had significant numbers of individuals infected with either *Bd*, RV, or both pathogens (i.e., *Ambyostoma annulatum*, *A. opacum*, *Gastrophryne carolinensis*, *Pseudacris fouqueteii*, and *P. streckeri*; Table 4).

Among anuran (frog) species sampled, members of the family Bufonidae, Hylidae, and Ranidae showed the highest *Bd* infectious rates when summarizing data for families with significant sample sizes resulting from the study. All three of these families of frogs had representative species with *Bd* infection rates well above 50% (Table 4). Interestingly, most frog species showed low infectious rates for Ranavirus. Fewer salamander species had *Bd* infection rates close to or above 50%, although Ranavirus infection rates appear to be higher on average than those observed for frogs in the state (Table 4).

One of the goals of this study was to investigate disease prevalence among the state's SGCN amphibian species. Of the 16 SGCN species recognized currently in Oklahoma, we were able to encounter five during our surveys, including three Tier 1 SGCN taxa (*Ambystoma annulatum*, *Eurycea spelaea*, and *E. tynerensis*) and two Tier 2 SGCN taxa (*Eurycea multiplicata* and *Plethodon angusticlavius*) (Table 5). Although sample sizes for three of these species (*Ambystoma annulatum*, *Eurycea multiplicata*, and *Eurycea spelaea*) were lower, some important patterns should be noted. First, the Ringed Salamander (*Ambystoma annulatum*) and the Many-ribbed Salamander (*Eurycea multiplicata*) had significant levels of disease infection observed despite small sample sizes. Among the eight individuals of *Ambystoma annulatum* screened for infection, six were positive for *Bd* and all eight were positive for RV (Table 5). Similarly, of the four *Eurycea multiplicata* screened, one was *Bd*+ and two were RV+ (Table 5). Only two individuals of *Eurycea spelaea* were observed in the wild; however, both were negative for pathogen infection.

RESULTING PUBLICATIONS

Watters, J. L., D. R. Davis, T. Yuri, and C. D. Siler. *In review*. Concurrent infection of *Batrachochytrium dendrobatidis* and ranavirus among native amphibians from northeastern Oklahoma, USA. Journal of Aquatic Animal Health.

Davis, D. R., J. K. Farkas, T. R. Kruisselbrink, J. L. Watters, E. D. Ellsworth, J. L. Kerby, and C. D. Siler. *In review*. Prevalence and distribution of ranavirus in amphibians from southeastern Oklahoma. Herpetological Conservation and Biology.

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. Herpetological Review 48:70–74.

IV. RECOMMENDATIONS

PRIORITY 1

Over the last decade, research has shown that infectious diseases are impacting more than just amphibian communities worldwide. In fact, turtles are at risk for systemic infection by ranaviruses (Johnson et al. 2008; Chinchar et al. 2009; Chinchar & Waltzek 2014; Huang et al. 2009; Perpiñan et al. 2016). Currently, nine native turtles in Oklahoma are designated as SGCN species, including one Tier I (Alligator Snapping Turtle), one Tier II (Chicken Turtle), and seven Tier III (False Map Turtle, Northern Map Turtle, Ouachita Map Turtle, Razor-backed Musk Turtle, River Cooter, Smooth Softshell Turtle, Spiny Softshell Turtle) species. However, no comparative studies to date have screened for Ranavirus in turtle populations in the state. We feel it is critical that a baseline study be conducted on Oklahoma's native turtle populations for viral infection. In addition to field-based survey techniques, such a study could take advantage of existing, vouchered tissue collections in natural history museums to screen populations surveyed historically for the presence of RV+ individuals. This would provide the necessarily preliminary data to make more informed decisions on monitoring native aquatic and terrestrial turtles in the state.

PRIORITY 2

The results of this study provide a robust, baseline snapshot of disease prevalence at a statewide resolution. Unfortunately, this data represents a single point estimate in time for each these populations, and does not provide data on disease spread, population health, or long-term impacts of infection on any of the sampled populations. Amphibians and reptiles are ectothermic organisms, and so seasonal fluctuations impact their ability to respond to external stressors. For example, among amphibians, studies have shown that extreme temperature variation can cause immune suppression and weakened immune responses (Maniero & Carey 1997; Rollins-Smith et al. 2002; Raffel et al. 2006). Given the recognized relationship between climatic variation and immune response, investigating seasonal patterns of pathogen infection among amphibian and turtle populations in Oklahoma is of critical importance to conservation assessments and strategic planning. Repeated surveys across several years will be needed to investigate trends in disease prevalence and load through time and assess risk factors for native species in the state. This is particularly true for the state's SGCN species. We observed infected individuals for four of the five SGCN taxa we encountered during surveys, and often our sample sizes were quite small. Regular and repeated screening of populations of Tier 1 and 2 species (turtles and

amphibians) is important for monitoring whether pathogen intensities are increasing or stable through time.

PRIORITY 3

Although Ranavirus prevalence was lower among our screened samples than *Bd* prevalence, it is known that RV infection in amphibians is often greatest in pre-metamorphic (and aquatic) larval stages of development. Adult amphibians that survive RV infection during early development more often show lower infection loads than pre-metamorphic individuals. The majority of our screening efforts for this project focused on post-metamorphic individuals (often adults or subadults). Therefore, the lower observed RV infection rates across sites and species may be an artifact of the developmental stage we screened during the project. It is important that future studies attempt to screen more lots of pre-metamorphic individuals among populations of native Oklahoma amphibians to determine if RV infection is having a greater impact than observed. If higher rates of infection are observed in tadpole and larval salamanders than adults, this could indicate that either species are capable of tolerating and surviving RV infection to some extent, or there are more pre-metamorphic die-offs than we understand currently.

PRIORITY 4

Public outreach and education plays a major role in conservation efforts. This is particularly true for wildlife disease awareness. We highly recommend increasing awareness about wildlife diseases and appropriate steps that can be taken by state park and WMA managers, wildlife biologists, students and the public to minimize the spread of infectious disease and invasive species of plants and animals across Oklahoma. For infectious disease spread prevention efforts, it is critical that all individuals clean all nets, waders, boots, etc. between sites and at the end of each day's activities in any aquatic or semi-aquatic habitat across the state by one of the following two methods:

1. Spray down the equipment with 10% bleach solution and allow it to dry for one hour prior to putting the equipment away.
2. Allow the equipment to dry in the sun for a full day.

Additionally, all individuals should avoid the use of felt-bottomed waders, which can trap disease spores, microbes and invasive plant material and inadvertently transfer these threats across aquatic habitats. In addition to increased communication and education campaigns with wildlife managers, state biologists, park rangers, etc., we feel adding posters or information signs and/or boards at park and WMA entrances and headquarters and notices on websites would contribute to improved awareness.

V. SIGNIFICANT DEVIATIONS

There have been no significant deviations.

VI. EQUIPMENT

No equipment exceeding $5,000 in cost was purchased for this project.

VII. PREPARED BY:

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VIII. APPROVED BY:

Fisheries Division Administration
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TABLE 1: Disease screening summary by large-scale region of the state (2015–2017).*

Oklahoma Region	***Bd*** **Sample size**	***Bd*** **Prevalence (%)**	***RV*** **Sample size**	***RV*** **Prevalence (%)**
South/southeast (2015)	373	68%	390	21%
North/northeast (2015–2016)	563	49%	534	17%
Central (2015, 2017)	461	28%	463	11%
West (2017)	113	57%	112	14%
TOTAL	**1514**	**28%**	**1493**	**16%**

*Please note that some of the samples were funded by non-ODWC sources, such as the Oklahoma City Zoo, The Nature Conservancy, and the University of Oklahoma.

TABLE 2: Disease screening summary by Oklahoma county (2015–2017).*

Oklahoma County	***Bd*** **Sample size**	***Bd*** **Prevalence (%)**	***RV*** **Sample size**	***RV*** **Prevalence (%)**
Adair	28	36%	27	0%
Atoka	43	56%	43	21%
Cherokee	155	57%	165	33%
Choctaw	1	0%	0	0%
Cimarron	4	25%	10	50%
Cleveland	167	28%	163	16%
Comanche	58	83%	51	14%
Delaware	125	42%	123	5%
Ellis	14	93%	14	14%
Johnston	177	13%	179	0%
Kiowa	23	4%	23	4%
Latimer	48	56%	63	24%
Le Flore	69	49%	67	21%
Love	35	100%	33	6%
Marshall	30	90%	29	3%
Mayes	37	22%	37	8%
McCurtain	108	69%	106	25%
Muskogee	28	79%	28	4%
Nowata	56	48%	54	2%
Oklahoma	99	58%	94	22%
Osage	66	68%	43	28%
Pontotoc	38	5%	39	10%
Pushmataha	49	71%	51	31%
Sequoyah	68	34%	57	28%
Woodward	14	7%	14	7%

*Please note that some of the samples were funded by non-ODWC sources, such as the Oklahoma City Zoo, The Nature Conservancy, and the University of Oklahoma.

TABLE 3: Disease screening summary by sampling site (i.e. Wildlife Management Areas [WMA], Oklahoma State Parks [SP], National Wildlife Refuges [NWR], etc.). All summarized data supported strictly by ODWC grant F14F01225 (T-80-1).

Sample Site	*Bd* Sample size	*Bd* Prevalence (%)	*RV* Sample size	*RV* Prevalence (%)
South/southeast Oklahoma (2015)				
Arkansas River at Robert S. Kerr Lock & Dam 15	14	64%	14	7%
Fobb Bottom WMA	3	33%	3	33%
Grassy Slough WMA	27	74%	27	7%
Hickory Creek WMA	35	100%	33	6%
Hugo WMA	3	33%	2	100%
James Collins WMA	26	54%	33	18%
McGee Creek WMA	29	61%	30	13%
Ouachita WMA	54	46%	53	25%
Pine Creek WMA	16	31%	21	76%
Pushmataha WMA	47	51%	49	29%
Red Slough WMA	58	83%	58	16%
Robbers Cave WMA	22	59%	29	31%
Stringtown WMA	12	42%	12	42%
University of Oklahoma Biological Station & Vicinity	27	96%	26	0%
North/northeast Oklahoma (2015–2016)				
Camp Gruber WMA	28	75%	28	4%
Cherokee WMA	14	0%	14	0%
Cookson Hills WMA	142	63%	151	36%
Hulah WMA	11	36%	3	0%
McClellan-Kerr WMA	30	27%	19	21%
Oologah WMA	62	42%	53	0%
Osage Hills State Park	35	74%	20	60%
Osage Hills WMA	14	100%	20	0%
Ozark Plateau NWR, Hamby Unit	40	35%	38	11%
Ozark Plateau NWR, in vicinity of Night Train Farm	14	50%	13	8%
Ozark Plateau NWR, Looney Unit	47	49%	47	0%
Ozark Plateau NWR, Sallybull Unit	20	30%	19	0%
Sequoyah NWR	28	36%	28	43%
Spavinaw WMA	69	25%	70	4%
Tenkiller WMA/State Park	4	25%	4	0%
West Oklahoma (2017)				
Wichita Mtn NWR	57	84%	51	14%
Packsaddle WMA	14	93%	14	14%

Mountain Park WMA	18	6%	18	0%
Great Plains State Park	5	0%	5	20%
Fort Supply WMA	4	0%	4	25%
Cooper WMA	10	10%	10	0%
Black Mesa State Park	4	25%	10	50%

TABLE 4: Disease screening summary by species (2015–2017).*

Family	Genus	Species	*Bd* Sample size	*Bd* Prevalence (%)	*RV* Sample size	*RV* Prevalence (%)
ANURA (FROGS)						
Bufonidae	*Anaxyrus*	*americanus*	40	65%	39	8%
Bufonidae	*Anaxyrus*	*woodhousii*	22	59%	21	5%
Hylidae	*Acris*	*blanchardi*	363	54%	356	10%
Hylidae	*Hyla*	*chrysoscelis/versicolor*	115	37%	108	7%
Hylidae	*Hyla*	*cinerea*	40	37%	38	16%
Hylidae	*Pseudacris*	*clarkii*	16	19%	15	0%
Hylidae	*Pseudacris*	*crucifer*	12	67%	12	8%
Hylidae	*Pseudacris*	*fouqueteii*	2	100%	2	50%
Hylidae	*Pseudacris*	*maculata*	8	88%	7	0%
Hylidae	*Pseudacris*	*streckeri*	2	100%	2	0%
Microhylidae	*Gastrophryne*	*carolinensis*	8	100%	8	50%
Microhylidae	*Gastrophryne*	*olivacea*	21	43%	28	7%
Ranidae	*Lithobates*	*blairi*	40	83%	40	10%
Ranidae	*Lithobates*	*catesbeianus*	319	40%	317	29%
Ranidae	*Lithobates*	*clamitans*	44	43%	44	11%
Ranidae	*Lithobates*	*palustris*	14	86%	13	8%
Ranidae	*Lithobates*	*sphenocephalus*	178	36%	180	16%
Scaphiopodidae	*Spea*	*bombifrons*	3	0%	3	0%
CAUDATA (SALAMANDERS)						
Ambystomatidae	*Ambystoma*	*annulatum*	8	75%	4	100%
Ambystomatidae	*Ambystoma*	*maculatum*	7	0%	7	0%
Ambystomatidae	*Ambystoma*	*opacum*	2	0%	1	100%
Ambystomatidae	*Ambystoma*	*texanum*	5	80%	3	33%
Plethodontidae	*Eurycea*	*longicauda*	39	41%	73	11%
Plethodontidae	*Eurycea*	*lucifuga*	21	14%	22	18%
Plethodontidae	*Eurycea*	*multiplicata*	4	25%	6	50%
Plethodontidae	*Eurycea*	*spelaea*	2	0%	2	0%

Plethodontidae	*Eurycea*	*tynerensis*	40	18%	39	8%
Plethodontidae	*Plethodon*	*albagula*	31	16%	37	30%
Plethodontidae	*Plethodon*	*angusticlavius*	13	15%	15	13%
Salamandridae	*Notophthalmus*	*viridescens*	110	81%	118	11%

*Please note that some of the samples were funded by non-ODWC sources, such as the Oklahoma City Zoo, The Nature Conservancy, and the University of Oklahoma.

TABLE 5: Disease prevalence recorded for SGCN species observed during study (2015–2017). Significant infection rates shaded and in bold for emphasis.

Genus	**Species**	**SGCN Tier**	***Bd* Sample size**	***Bd* Prevalence (%)**	***RV* Sample size**	***RV* Prevalence (%)**
Ambystoma	*annulatum*	Tier 1	8	**75%**	4	**100%**
Eurycea	*multiplicata*	Tier 2	4	**25%**	6	**50%**
Eurycea	*spelaea*	Tier 1	2	0%	2	0%
Eurycea	*tynerensis*	Tier 1	40	18%	39	8%
Plethodon	*angusticlavius*	Tier 2	13	15%	15	13%