Implementation of a Citizen Science Program to Assess Chytridiomycosis (*Batrachochytrium dendrobatidis*) Prevalence in Amphibians across Oklahoma, USA

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Abstract: Global amphibian populations are declining rapidly, due largely to infectious diseases such as chytridiomycosis caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). The Herpetology Department at the Sam Noble Museum has screened for *Bd* prevalence among amphibian communities across Oklahoma for over five years, providing ongoing data about the disease's prevalence and distribution. Recently, the museum partnered with other Oklahomans through a citizen science project allowing participants to sample their local amphibian communities for *Bd*. Our project targeted K–12 students in Oklahoma to promote curiosity in science and to foster an interest in pursuing career paths in science, technology, engineering, and mathematics (STEM). The multi-year baseline citizen science dataset we obtained shows a lower *Bd* prevalence compared to samples collected from trained researchers. In this study, we juxtapose the two datasets and make observations on the feasibility of the citizen science program. Results from the program suggest that kit return rates were average for a project of this scale, and many participants could correctly identify amphibian species. Our findings indicate that the citizen science initiative is successful in increasing statewide amphibian disease sampling range and heightening the public's awareness of this global amphibian epidemic.

Introduction

The global decline of amphibian populations is a growing concern to biologists (Stuart et al., 2004; O'Hanlon et al., 2018; Scheele et al., 2019). Multiple factors, such as habitat modification, environmental pollutants, and climate change are contributing to the observed population declines and recent extinction events, both individually and synergistically (Grant et al., 2020). Additionally, the continued

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spread of infectious diseases among amphibians is a known contributor to these population declines (Cheng et al., 2011; Berger et al., 2016). One of the most detrimental amphibian diseases is chytridiomycosis (often referred to as chytrid), which is caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*; Voyles et al., 2009). Amphibians contract *Bd* via direct contact with infected individuals or contaminated water, and the disease manifests as an epidermal infection (Voyles et al., 2009). Amphibian skin surfaces are crucial for osmotic regulation and respiration; therefore, when individuals become infected, death is often the result of cardiac arrest when respiration becomes interrupted by the presence of *Bd* on the epidermis (Voyles et al., 2007, 2009). Currently, this pathogen is prevalent worldwide and infects many different species of amphibian (Berger et al., 2016; Scheele et al., 2019); however, a full understanding of the patterns associated with disease transmission and prevalence in various species, habitats, and life stages is still lacking (Bienentreu and Lesbarrères, 2020).

As scientists continue to discover more information about the physiology of *Bd*, sampling has become standardized and disease monitoring has become a global priority. Pathogen presence and infection load can be sampled by means of a non-invasive skin swab, which removes *Bd* from the epidermis of amphibian skin (Piotrowski et al., 2004; Skerratt et al., 2008). Populations of amphibians can now be monitored frequently to determine the infection rate of the disease via established, regular sampling programs (Berger et al., 2016). With such method standardization, global sampling has become crucial for tracking the spread of *Bd* infection, and recent studies have targeted locations such as the United States, Europe, and Asia for increased sampling (Petersen et al., 2016; Kärvemo et al., 2018; Mutnale et al., 2018). In the United States, studies have focused primarily on the West and East coasts to date (Olson et al., 2013; Petersen et al., 2016). Although some *Bd* prevalence monitoring efforts have been carried out in Midwest states in the last decade, such as Illinois (Talley et al., 2015) and Oklahoma (Marhanka et al., 2017; Watters et al., 2018, 2019, 2021), there is still an incomplete picture of national *Bd* prevalence, especially in parts of the central region of the United States. As a result, increased and ongoing monitoring of central United States amphibian populations is critical to assess the health of these amphibian communities.

One method to help increase disease sampling efforts is the use of citizen science projects, which have been conducted in several biological fields to provide amateur scientists and volunteers an opportunity to gain a better understanding of the scientific process, and to raise general awareness of issues that affect the natural world by engaging them in practical, hands-on empirical contributions (Jordan et al., 2009, 2012; Dickinson et al., 2010). Citizen science programs use methods of data collection that involve the general public, and they have become increasingly popular in recent years (Catlin-Groves, 2012; Kosmala et al., 2016; McKinley et al., 2017; Maund et al., 2020). Some of the most successful and well-known citizen science projects in biology include Cornell Lab of Ornithology's eBird, Audubon's Christmas Bird Count, and iNaturalist (Butcher et al., 1990; Sullivan et al., 2009; Horn et al., 2018). Despite the recent success and more widespread use of several herpetology-specific citizen science projects, including HerpMapper and FrogID (Rowley et al., 2019; HerpMapper, 2020), these public-involvement programs have been implemented less often for the field of herpetology compared to other biological disciplines. Furthermore, few herpetology citizen science programs involve *Bd* monitoring (Ecoclub Amphibian Group et al., 2016; Julian et al., 2019; Nugent, 2020). To date, no citizen science projects have focused on *Bd* monitoring in Oklahoma, and the methods and target participants of existing *Bd* monitoring citizen science programs in the United States have varied greatly (Ecoclub Amphibian Group et al., 2016; Julian et al., 2019; Nugent, 2020).

Oklahoma is an important location to monitor for *Bd* infection given the diverse amphibian communities and the variety of ecosystems the state possesses. There are 49 recorded species of amphibians in Oklahoma, with 23

species of Anura (frogs) and 26 of Caudata (salamanders; Sievert and Sievert, 2021). Additionally, Oklahoma consists of 12 different ecoregions, which highlights the importance of sampling from many locations throughout the state to evaluate variation in infection dynamics among amphibian populations across complex landscapes (Oklahoma Forestry Services, 2020). The Herpetology Department at the Sam Noble Museum (SNM) has screened amphibian populations for *Bd* throughout Oklahoma since 2015 to determine the distribution and prevalence of *Bd* infection across the state (Marhanka et al., 2017; Watters et al., 2018, 2019, 2021). Despite these efforts, research conducted to date has been limited in scope due to both a focus on sampling public lands and time constraints, which has prevented sampling in some regions of the state.

To mitigate incomplete sampling in Oklahoma, the SNM Herpetology Department implemented a citizen science program beginning in 2016, called the Oklahoma Infectious Disease Citizen Science Project, or OKBD. This specific citizen science program intended to serve several purposes: (1) to supplement the ongoing scientific monitoring of regional amphibian populations in Oklahoma; (2) to increase public awareness of the threat *Bd* poses to amphibian populations, engaging them in the monitoring program and teaching them methods to reduce human-induced spread; and (3) to encourage K–12 student involvement in science, technology, engineering, and mathematics (STEM) activities by offering a free educational opportunity to contribute to a scientific research project. Additional benefits of the project are greater spatial sampling, increased taxonomic sampling, and a lower research budget due to public engagement. With citizen scientists able to revisit the same locations annually when scientific researchers cannot, the citizen science data can increase temporal sampling and raise awareness of regions in Oklahoma that require further sampling.

In recent years, the SNM Herpetology Department has pushed to understand *Bd* distribution across the state of Oklahoma (Marhanka et al., 2017; Watters et al., 2018, 2019, 2021). However, our understanding is limited due to the large number of ecoregions (Oklahoma Forestry Service, 2020), the diversity of amphibian species (Sievert and Sievert, 2021), and the restricted access to certain areas of the state (i.e., private land, federally protected areas). As a result, introducing a citizen science project in Oklahoma presented an excellent opportunity to simultaneously inform the public of *Bd* and its risk to amphibians, while also engaging participants in scientific research. We anticipated that many of the citizen scientists that participated in our project would be school-aged students, so a goal of the OKBD project was to provide the next generation with an opportunity to have a positive experience in nature and contribute meaningfully to science (Crall et al., 2012; Hiller and Kitsantas, 2014). The education experience that this program provided is especially important in Oklahoma, due to the current, typically low quality of K–12 education in the state; according to a 2019 study, Oklahoma ranks 45th in educational quality and 40th overall in K–12 education when compared with the rest of the United States (WalletHub, 2019).

As a result, the objective of the OKBD project is threefold, seeking to involve K–12 students in a STEM citizen science project and to raise broader public awareness of *Bd*, while simultaneously increasing the breadth of *Bd* sampling distribution in Oklahoma with citizen science data.

Methods

Citizen science participants

A variety of advertising methods informed the citizen science participants about the program. Based on the project aims, we focused on advertising specifically to teachers, homeschooling parents, and herpetology-interested educational groups of all ages within the state of Oklahoma. In January–February of each year, we contacted potential participants by: (1) sending direct emails to teachers who have participated in SNM activities (i.e. Science Institute or school field trips) and to

past participants in the project (after the first year of the program); (2) emailing newsletters to two local teacher listservs: Oklahoma Department of Wildlife Conservation (ODWC) school programs and Oklahoma Evolution/Climate News (Oklahomans for Excellence in Science Education); (3) advertising on the social media pages (Facebook and Twitter) of the Cameron Siler Lab, SNM, and ODWC; and (4) sending Facebook messages directly to local public schooling, homeschooling, and herpetology-enthusiast groups, including the Oklahoma Herpetological Society, Oklahoma Herpetology, OKSci Elementary, OKSci Middle School, OKSci High School, Oklahoma City Homeschool Association, Tulsa Homeschool, Oklahoma Homeschool, and Oklahoma Homeschool Science & Engineering Fair. Other advertising included a call for participants on the citizen science website, SciStarter.org (available year-round), and in-person advertising at the Oklahoma Association for Environmental Education Expo (OKAEEE) (in February each year) and BioBlitz! Oklahoma (in October each year). There were no limits on how many individuals or groups could apply online via a Microsoft Word document (2016–2017) or a Qualtrics form (2018–2019; Appendix I).

Once an Oklahoman citizen scientist requested to participate in the project, they were sent a kit with all the necessary supplies for disease sample collection. A standard kit included 10 rayon-tipped sterile swabs (Peel Pouch Dryswab Fine Tip [MWE 113], Corsham, Wiltshire, UK), 10 sterile 1.5 mL screw-top vials (various styles and manufacturers), a permanent marker, a Herpetology Department business card, an instruction sheet, a data sheet, and a homemade waterproof field guide of native Oklahoman frog species (participants outside the range of pick-up or drop-off at SNM also received a prepaid shipping label). The participants could access additional materials online at our citizen science homepage (https://cameronsiler.com/citizen-science/), such as lesson plans, state science standards, lecture slides, an elementary-level worksheet, and a secondary-level worksheet. Participants working with large groups had the option of receiving 20 swabs and vials instead of the standard 10. Citizen science kits could be requested between January and March every year during 2016–2019 and were available for pick up or mailing from late February–April. As a result, the citizen scientists had the opportunity to participate in the program between March and June of each year. This timeframe allowed participants to visit locations where frogs might be found during the most active breeding season of the year for most Oklahoma frog species (Sievert and Sievert, 2021).

Data collection

The citizen science groups typically consisted of educators who took small groups of students of various ages (elementary school through college and beyond) to a nearby body of water where amphibians could be found, such as a large pond. Participants were required to record GPS coordinates, physical location, and a description of the environment in the area on a data sheet (Appendix II). After the location was marked, the citizen scientists would find and catch frogs. Prior to collection, participants were instructed to bleach their field equipment and sterilize their hands with hand sanitizer, so that cross-site contamination could be minimized (Appendix III). Although the exact method at each sampling location is unknown, as citizen scientists were not supervised by us, participants likely captured frogs primarily by hand rather than by net. Participants attempted to identify and record the species that were caught, using the custom Oklahoma frog identification guide that came in the kit (modified with permission from Sievert and Sievert, 2021; Laurie Vitt and Janalee Caldwell, unpubl. data). Participants were also required to take several photographs (dorsal and ventral surfaces of body, and lateral surface of head) of the individual frogs that were caught, so that species' identifications could be confirmed by trained researchers at SNM. The citizen science participants then swabbed the skin of the frogs to collect disease samples using the provided instructions (Appendix III), which allowed for *Bd* screening at the SNM. To collect the sample, the participants followed a standardized, published protocol of swabbing five times each on the ventral, dorsal, hind legs, and webbing between the toes on the frog's skin,

as those are the regions where the most *Bd* fungus is located typically (Lannoo et al., 2011; Watters et al., 2018). Participants were then instructed to place these swabs into the provided sterile, screw-top 1.5 mL vial and use the permanent marker to write a label that also corresponded to the datasheet (Appendix II). At the conclusion of data collection, the participants released the frogs back into their original environments and returned the swab samples to the SNM where they were stored in a -20˚C freezer until DNA extraction could be performed by trained personnel. Before participants sent their swabs back, they stored the samples at room temperature for approximately 1–3 months. Additionally, participants were asked to respond to a post-completion survey, which we sent to all participants who returned swabs to the SNM, to allow for improvements in future years.

In the fall (August–December) of each year, we extracted the DNA from the citizen science samples at the SNM Genomics Core Facility, following the PrepMan Ultra (Life Technologies) protocol (Cheng et al., 2011; Watters et al., 2018). To prepare the samples for qPCR analysis, we diluted the extracted DNA samples 1:10 (Hyatt et al., 2007). We then used the qPCR protocol by Kerby et al. (2013) to quantify disease loads for all samples, running each sample in triplicate along with four standard dilutions of known pathogen gene copy number and one negative control of molecular grade water (Watters et al., 2016, 2018, 2021; Marhanka et al., 2017; Smith et al., 2019).

Raw values for each category were analyzed by year; however, summary percentages have been provided in the Results for easier comparisons. Due to small sample sizes and lack of standardization in frog collection and swabbing methods, we did not perform statistical analyses of *Bd* prevalence by county or species. Therefore, results are presented as summaries only.

Results

Our two resulting datasets included demographic information about participation in, and the success of, the OKBD project (Table 1), as well as *Bd* prevalence data based on screened citizen scientist swabs (Tables 2–4). Together, we used these datasets to assess the validity of this supplemental sampling and monitoring method.

The SNM Herpetology Department sent out a total of 362 kits from 2016–2019, with 96 kits returned in total (Table 1). The percentage of kits returned in 2018 (20%) was lower than in 2016, 2017, or 2019 (55.81%, 46.34%, and 42.86%, respectively; Table 1).

Citizen science participants collected a total of 807 amphibian swabs between the years 2016 and 2019 (Table 2). We found the highest overall *Bd* prevalence among samples collected in 2017 (21.60%), and the lowest overall *Bd* prevalence in 2019 (2.26%; Table 2). Between 10 and 34 counties were sampled each year (Table 3; Fig. 1). The highest county prevalence of *Bd*+ frogs was 86.70% in 2017 and the lowest was 16.67% in 2019, which follows the same

Table 1. Citizen science data from the project by year (2016–2019) and totaled, including rates of kit return, rates of kit completion, percentage of participants who submitted identification photos, average accuracy of participants making a correct species identification, and participation in the annual post-project survey.

	2016	2017	2018	2019	Total
Kits (no. sent)	43	41	100	77	261
Kits (no. returned)	24	19	20	33	96
Kits (% returned)	55.81%	46.34%	20.00%	42.86%	41.25%
Kits (% completed)	72.39%	69.50%	51.05%	69.53%	65.62%
Photographs provided	86.60%	89.50%	87.50%	90.90%	88.63%
Species identification accuracy	62.90%	71.30%	60%	57.08%	62.82%
Post-activity survey completion	54.50%	47.30%	38.89%	45.45%	46.54%

Table 2. *Bd* disease monitoring results from the citizen science project by year (2016–2019) and totaled, including swab and species numbers and percentage of sampled amphibians that were positive for *Bd* infection (*Bd* prevalence).

	2016	2017	2018	2019	Total
No. individuals swabbed	222	164	153	268	807
No. species swabbed	10	15	10	11	22
***Bd* prevalence**	12.60%	21.60%	11.10%	2.26%	11.89%

pattern of overall prevalence by year (Table 3). Sampling sites were concentrated near the urban areas of Lawton, Oklahoma City, and Tulsa, but more rural areas were also well represented, particularly in the eastern half of the state (Fig. 1). To date, frogs from 39 counties (out of 77 total in Oklahoma) have been sampled by citizen scientists, with eight sites sampled in multiple years (Fig. 1).

Citizen scientists sampled a total of 20 unique frog species over the duration of this project (Table 4). Although participants sampled some species more frequently than others across the years, those that were sampled in high percentages across all years were *Acris blanchardi*, *Anaxyrus woodhousii*, *Lithobates catesbianus*, and *L. sphenocephalus* (Table 4), all of which are considered common in Oklahoma (Sievert and Sievert, 2021). Although these species were consistently sampled in high frequencies across the duration of the project, the highest infection rate varied by species each year; 2016: *L. blairi/sphenocephalus* (66.67%; images could not be narrowed down between the two species); 2017: *A. blanchardi* (46.67%); 2018: *L. sphenocephalus* (13.64%); 2019: *A. blanchardi* (7.02%; Table 4). The percentage

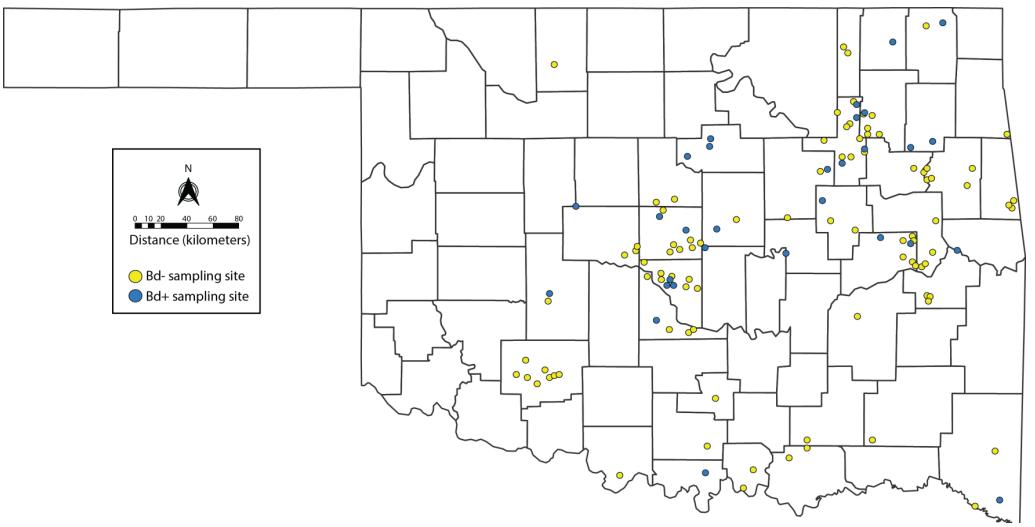

Figure 1. Map of Oklahoma representing sampling sites from participants in the Oklahoma Infectious Disease Citizen Science Project (2016–2019). County boundaries are outlined in black. Sites containing at least one positive individual (*Bd*+) are indicated in blue; sites with all negative individuals are indicated in yellow (*Bd*-). An interactive version of this map is available on the citizen science homepage (https://cameronsiler.com/citizen-science/), where hovering over each map point shows the participant's last name or organization as well as *Bd* prevalence.

Table 3. Breakdown of sample size (N) and *Bd*+ prevalence (%) by Oklahoma county and year (2016–2019).

	2016		2017		2018		2019	
County	**N**	***Bd*+ %**	**N**	***Bd*+ %**	**N**	***Bd*+ %**	**N**	***Bd*+ %**
Adair	0	N/A	0	N/A	0	N/A	12	0%
Atoka	0	N/A	0	N/A	0	N/A	5	0%
Bryan	0	N/A	0	N/A	0	N/A	5	0%
Caddo	0	N/A	0	N/A	0	N/A	10	10%
Canadian	31	9.67%	0	N/A	0	N/A	0	N/A
Carter	0	N/A	0	N/A	0	N/A	3	0%
Cherokee	1	0%	0	N/A	6	0%	26	0%
Cleveland	0	N/A	18	11.11%	52	11.54%	20	0%
Comanche	0	N/A	1	0%	26	0%	14	0%
Craig	10	20%	0	N/A	0	N/A	0	N/A
Creek	3	0%	0	N/A	0	N/A	8	0%
Delaware	0	N/A	0	N/A	0	N/A	7	0%
Haskell	3	0%	0	N/A	0	N/A	0	N/A
Jefferson	0	N/A	0	N/A	0	N/A	4	0%
Johnston	4	0%	0	N/A	0	N/A	0	N/A
Kay	N/A	N/A	N/A	N/A	N/A	N/A	6	N/A
Lincoln	9	77.78%	9	44.44%	0	N/A	0	N/A
Logan	0	N/A	0	N/A	0	N/A	10	0%
Love	6	16.67%	0	N/A	0	N/A	0	N/A
Marshall	9	0%	0	N/A	0	N/A	3	0%
Mayes	0	N/A	0	N/A	0	N/A	8	25%
McClain	0	N/A	16	43.75%	12	25%	16	0%
McCurtain	20	10%	0	N/A	0	N/A	0	N/A
McIntosh	0	N/A	11	9.09%	0	N/A	0	N/A
Murray	0	N/A	0	N/A	0	N/A	2	0%
Muskogee	0	N/A	17	5.88%	22	0%	0	N/A
Nowata	0	N/A	10	20%	0	N/A	0	N/A
Okfuskee	0	N/A	8	25%	0	N/A	0	N/A
Oklahoma	37	5.40%	12	8.33%	3	0%	10	10%
Okmulgee	8	0%	11	72.73%	1	0%	0	N/A
Osage	0	N/A	0	N/A	4	0%	0	N/A
Payne	0	N/A	20	35%	0	N/A	20	10%
Pittsburg	0	N/A	0	N/A	0	N/A	8	0%
Potawatomi	10	20%	0	N/A	0	N/A	0	N/A
Rogers	20	5%	5	0%	0	N/A	18	0%
Sequoyah	3	33.33%	0	N/A	0	N/A	0	N/A
Tulsa	48	12.50%	30	6.67%	22	18.18%	33	0%
Wagoner	0	N/A	0	N/A	0	N/A	9	0%
Washington	0	N/A	0	N/A	0	N/A	10	0%
Annual Totals	**16**	**62.50%**	**13**	**86.70%**	**10**	**40%**	**24**	**16.67%**

of sampled species that were *Bd*+ decreased dramatically in 2018 and 2019 (Table 4).

Discussion

Overall, the OKBD project accomplished our goals of increasing *Bd* sampling breadth in Oklahoma, raising public awareness (as determined by post-project surveys), and involving K–12 students in scientific research to promote STEM career paths. Although these goals were achieved, we did observe that the kit return rates, sample numbers, and *Bd* prevalence rates of this pilot project were lower than expected, and we ran into some difficulties with participants identifying the amphibian species incorrectly. There are several potential reasons why participants were not able to collect samples in any given year, such as poor weather conditions, failure to allocate enough time to the project, or unexpected changes in class schedules. One of the key concerns in the literature about implementing citizen science programs is that the data collected by citizen science participants might not be sufficiently reliable for scientific results (Goodchild, 2007; Catlin-Groves, 2012). However, we believe that our results support the importance of implementing citizen science programs such as the OKBD project and indicate the value of continuing this pilot program with implemented modifications, while still improving the breadth of knowledge about *Bd* in Oklahoma.

We gauged the participants' dedication to completing the project in the application form before sending the swabbing kit; however, some participants were likely unable to make collecting samples a priority. For example, the percentage of swabs returned per kit was between 51% and 72% by year, sometimes with participants returning only one or two samples (Table 1). Research modeling of the motivations of citizen science participants has suggested that projects with shorter commitments and shared results might have more success in retaining participants (Wiggins and Crowston, 2010; Nov et al., 2011; Eveleigh et al., 2014). Perhaps these suggestions can be incorporated into future citizen science programs to entice participants to continue and follow through with the project, such as annual updates sent to participants about summarized project progress. There were a handful of instances in which participants could not collect samples in the year they requested a kit, so they kept the kit and returned it the following year, while others participated in multiple years of the project, showing long-term commitment to the program. Upon receiving each submitted kit, we provided each participant with a post-project survey to assess the impacts of the program on the citizen scientists. However, we did not request Institutional Review Board (IRB) approval to analyze and publish the results of the post-project survey, as the project was still in the pilot phase. In future years of this project, we intend to request IRB permits to release the results of the survey, which will aid in determining methods for incentivizing participants and evaluating the degree to which *Bd* awareness was raised in Oklahoma.

Our results indicate that the number of kits sent and returned in 2018 was vastly lower from the other years of the project (Table 1). One possible explanation for this difference is that the field season for sample collection overlapped directly with the two-week Oklahoma teacher strike that occurred in the spring of 2018. As a result, it is possible that fewer kits were returned that year because fewer teachers were available to collect samples with their students, or that the slightly shortened school year required prioritizing other learning objectives. Despite unexpected events that led to a lower kit return rate in 2018, overall, the citizen science project had a return rate of 20–55%, which is comparable to a similar citizen science program by Warner et al. (2019) that sent their participants seafood testing kits and had a return rate of 33.4% on a national scale. Although their study was not in the field of herpetology, return rates are comparable with our own, as both projects used mailed citizen science kits, unlike the other published *Bd*-screening citizen science projects in herpetology, which involved in-person activities (Ecoclub Amphibian Group et al., 2016; Julian et al., 2019; Nugent, 2020).

When comparing disease data collected

Table 4. Breakdown of the number of amphibian species sampled (N) each year (2016–2019) and *Bd*+ prevalence (%) for the sampled individuals. When the specific species of amphibian could not be identified from photos, we labeled it as accurately as possible (*Anaxyrus* sp. represents the whole genus; *Lithobates blairi*/*sphenocephalus* and *Hyla chrysoscelis/versicolor* represent the two species that we were able to narrow identification down to within the genus).

	2016		2017		2018		2019	
Species Name	**N**	***Bd*+ %**	**N**	***Bd*+ %**	**N**	***Bd*+ %**	**N**	***Bd*+ %**
Bufonidae								
Anaxyrus americanus	8	0.00%	13	15.38%	8	0.00%	17	0.00%
Anaxyrus cognatus	0	N/A	0	N/A	0	N/A	3	0.00%
Anaxyrus woodhousii	48	4.17%	14	0.00%	12	0.00%	50	0.00%
Anaxyrus sp.	4	0.00%	0	N/A	2	0.00%	5	0.00%
Hylidae								
Acris blanchardi	59	28.81%	45	46.67%	61	18.03%	57	7.02%
Hyla cinerea	2	0.00%	0	N/A	6	0.00%	2	0.00%
Hyla chrysoscelis/versicolor	8	0.00%	16	6.25%	4	0.00%	7	0.00%
Gastrophryne carolinensis	0	N/A	0	N/A	2	0.00%	0	N/A
Gastrophryne olivacea	9	0.00%	4	25.00%	3	0.00%	0	N/A
Pseudacris crucifer	0	N/A	0	N/A	0	N/A	1	0.00%
Pseudacris clarkii	0	N/A	1	0.00%	0	N/A	0	N/A
Pseudacris fouquettei	0	N/A	1	0.00%	0	N/A	1	0.00%
Pseudacris streckerii	2	0.00%	0	N/A	0	N/A	1	0.00%
Ranidae								
Lithobates blairi/sphenocephalus	3	66.67%	0	N/A	0	N/A	1	0.00%
Lithobates catesbeianus	73	6.84%	43	9.30%	26	7.69%	52	0.00%
Lithobates clamitans	0	N/A	1	0.00%	2	0.00%	0	N/A
Lithobates sphenocephalus	3	33.33%	20	40.00%	22	13.64%	32	6.25%
Lithobates sylvaticus	0	N/A	1	0.00%	0	N/A	0	N/A
Scaphiopodidae								
Scaphiopus hurterii	0	N/A	1	0.00%	0	N/A	0	N/A
Spea bombifrons	0	N/A	0	N/A	1	0.00%	0	N/A

by citizen science participants and research conducted directly by the SNM Herpetology Department, we observed a discrepancy in *Bd* prevalence among samples collected by the two groups. Between 2015–2019, researchers recorded a yearly average *Bd* infection prevalence of approximately 50% in Oklahoma, with *Bd*+ individuals found in every county sampled to-date (Marhanka et al., 2017; Watters et al., 2018, 2019, 2021). In contrast, the samples collected by citizen science participants had an average total of only about 12% *Bd*+ across all four years (Table 2). One of the most likely explanations for the observed discrepancy is in sampling method. Previous studies of *Bd* in amphibians have indicated that the disease typically grows on the external skin of the amphibian, but that the rubbing motion of a swab on the dorsal side, ventral side, and appendages of the amphibian is sufficient to remove the fungus (Piotrowski et al., 2004; Skerratt et al., 2008; Lannoo et al., 2011). To standardize sampling methods, we provided an instruction sheet in each citizen science kit to minimize sampling error (Appendix III) and also linked a video to demonstrate the swabbing action. However, it is possible that participants were not swabbing the amphibian skin as thoroughly, and with enough pressure as necessary, to dislodge any potential fungal spores. As a result, some of the samples that came back negative might have been collected from frogs infected with *Bd*, but due to errors in swabbing technique, the fungus might not be sufficiently present in the swab sample to be detected (Shin et al., 2014). By using citizen science data, the goal is to allow scientists to collect data on a larger scale than before, but not at the cost of inaccurate data. Other citizen science programs suggest that projects with simpler instructions and tasks for participants are preferable to complex methodology, which would likely need additional training or guidance

to obtain accurate results (Bonney et al., 2009; Schmeller et al., 2009; Catlin-Groves, 2012). Because formal training could not be provided for each of the citizen science participants, the instructions that were provided with each citizen science kit were as concise and straightforward as possible for clarity (Appendix III). Additionally, it is possible that the sampling discrepancy was the result of the way participants stored their swabs before we received them. It is possible that some of the samples from participants were stored at or above room temperature for days or months before being processed at the SNM. Previous research has found that the DNA of *Bd* zoospores can become less detectable when stored in warm conditions for even temporary time periods such as seven days (Sluys et al., 2008), suggesting that temperature in which participants stored their samples could influence the DNA detectability of *Bd* zoospores from the samples. Further examination is necessary to determine why the participants in the OKBD project found a lower *Bd* infection rate than the SNM Herpetology Department measured over the same time period. Perhaps future adjustments to the citizen science program methods will continue to yield improving results in our monitoring of Oklahoma *Bd* prevalence, ultimately raising the public's awareness of global amphibian declines and amphibian infectious disease.

From the beginning, there were several key ways in which the OKBD project sought to minimize *Bd* detection error. As well as our swabbing instructions and video, at some events, such as BioBlitz! Oklahoma, an annual gathering of Oklahoman biologists and citizen scientists who record the biodiversity of an area over a weekend, we supervised the citizen science participants during the sample collection, ensuring that the protocol was followed closely. Additionally, we minimized error in disease screening by running each sample in triplicate, and then re-running samples that tested positive in <2 sample wells (Davis and Kerby, 2016). In the future, these measures should continue to be taken to mitigate future sampling discrepancies. However, in-person training for all statewide participants is not feasible at this time. Despite the limits to statewide training, adjustments can be made to the protocol to further increase the reliability of the participant results. For example, the phrasing of the instructions included with the kit can be altered to more emphasize the correct swabbing technique. Additionally, questions could be added to the post-project survey that ask participants to identify the portions of the project that were difficult or confusing to follow, to allow for ongoing adjustments to the protocol for more accurate results. In future years of this project, we can also reduce the possibility of participants potentially spreading *Bd* between sample sites. Although we included instructions in the protocol on how to properly clean all participants' hands before touching a frog, we can highlight the importance of handling frogs with clean hands at the beginning of the protocol, in addition to information regarding the cleaning of nets, boots, etc. after leaving the pond.

Data about *Bd* infection rates at the species level are important because earlier studies have suggested that the infection rate can vary depending on which species population has contracted it (Daszak et al., 2004; Garner et al., 2006; Gervasi et al., 2013; Ellison et al., 2014). Of the participants, 88.63% submitted photographs of the amphibians they sampled, and from that proportion, 62.82% of the participants were able to correctly identify the species using the customized Oklahoma species field guide (Table 1). Although most of the participants identified the amphibian species correctly by using the simple Oklahoma frog identification guide from the kit, experts checked these identifications to confirm the species. Previous literature has found that errors in species identification from photos can occur from both experts and non-experts (Austen et al., 2016), so the need for additional confirmation from experts in this project does not necessarily indicate unreliable results. Additionally, some of the photos were of poor quality and even the experts had a difficult time distinguishing the necessary species-specific characters, resulting in several genus-level identifications only (Table 3). Some researchers have suggested that customized field guides increase the probability that citizen scientists

will identify a species correctly, so the success rate of identification was likely higher than it would have been without the inclusion of our customized field guide (Silvertown, 2009).

Synthesis of scientific literature indicates a general upward trend in the publication of scientific articles that rely on citizen science results for data collection, which suggests that citizen science data are being increasingly trusted (Catlin-Groves, 2012; Biggs et al., 2015; Phillips et al., 2019). Overall, improvements can be made to the program, such as sending annual progress reports to project participants, rewording the protocol to highlight to necessity of pressure when participants swab the frogs, and receiving IRB approval to analyze the post-project survey to assess participant knowledge change. However, despite these necessary improvements, this citizen science project successfully met its goals of raising awareness about the effects of *Bd* on amphibian populations, engaging the public in scientific research with an emphasis on involving K–12 students in STEM research, and increasing sample breath in Oklahoma. Thus, this pilot project has paved the way for subsequent citizen science programs to continue monitoring *Bd* in Oklahoma and across the country.

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Appendix I: 2020 Participant Application Form

Name of applicant

Email of applicant

Phone number of applicant

Name of school or organization (If you are a homeschool family, please put N/A in this box)

Mailing address for the kit (school/organization address or home, as you prefer). Please type address on one line; no returns.

If you are located in central Oklahoma, are you willing to pick-up and drop-off your kit directly at the Sam Noble Museum in Norman? (You will need to schedule a day/time for pick-up at least two business days in advance). [Yes or No]

What grade(s) or classes(s) do you teach? (If you are a homeschool family or other participant, please list the ages of the participants)

How many total students in your classroom? (If you are a homeschool family or other participant, please list total number of participants)

How do you see this project fitting into your curriculum? (If you are a homeschool family, or other participant, please put N/A in this box)

How close are you to a pond/stream/lake that you can use for capturing frogs? Will it be easy for you to access?

What is your level of commitment for completing the full kit and sending the samples back to us by June 2020?

How did you find out about our program? (Select all that apply).

- ☐ Email received from the Herpetology Department, Sam Noble Museum
- ☐ Email forwarded from a friend or colleague
- ☐ Email from the Oklahoma Department of Wildlife Conservation (ODWC)
- ☐ Cameron Siler Lab website (http://cameronsiler.com)
- ☐ Cameron Siler Lab social media
- ☐ Sam Noble Museum, Herpetology Department website (http://samnoblemuseum.ou.edu/collections-and-research/herpetology/)
- ☐ Sam Noble Museum social media
- ☐ Science is OK (http://scienceisok.com)
- ☐ SciStarter (http://scistarter.com)
- ☐ Presentation by Herpetology Dept. (i.e. Environmental Education Expo, OKNRC, etc.)
- ☐ I was a previous participant

Date: _____ **Your Name(s):** _____

County: _____

Physical description of location: _____

GPS Coordinates & Elevation: _____

Habitat Description (and photo name): _____

Data for frogs:

Genus	Species	Common Name	Frog #	List of Photo #s for Frog

** Use a different datasheet for each sample site you visit!! **

Appendix III: Participant Instructions

INSTRUCTIONS FOR CITIZEN SCIENCE PROJECT

CATCHING FROGS:

1. Select the pond, creek, etc. that you plan to sample. Scope it out PRIOR to taking students.
2. Locate your location on a map to determine the GPS (latitude, longitude, elevation/altitude), using your smart phone or tablet. If you don't know how to do this, simply run a Google search using the make/model of your phone AND the phrase "how to find gps coordinates." Usually, this info can be found in some type of map app. *Record this info on the data sheet.*
3. Take a photo of the water body, with smartphone or camera. *Record this info on the data sheet.*
4. Before entering any water body, first thoroughly disinfect your shoes/boots and nets (if planning to use one). The easiest way to do this is to mix up a solution of 10% bleach in a spray bottle, and completely spray down the net(s) and shoes/boots. Allow them to dry in the sun for 5-10 minutes before entering the pond. The bleach will evaporate off. [Note: this is an extremely important step, because it is very easy for us to spread chytrid fungus from pond to pond on our shoes or nets!]
5. Wash your hands thoroughly with antibacterial soap or hand sanitizer, before attempting to touch any frogs (and between each frog if you catch more than one). This ensures that any chytrid fungus isn't transferred from frog to frog. If using hand sanitizer, allow the sanitizer to dry completely, then rinse your hands with water before you touch any frogs. The sanitizer can damage their skin!
6. Note: If you choose to visit more than one pond, creek, etc. you will need to use a **new data sheet** for each location. Download extra here: http://cameronsiler.com/citizen-science/

COLLECTING DATA:

1. Once you have caught a frog, identify the species using the provided identification guide for Oklahoma. *Record this info on the data sheet.*
2. Open one of the individually wrapped swabs. Do not set it down or allow it to touch any other surfaces. Swab the body of the frog with the swab tip, using the following techniques.
   Use as much pressure as would be necessary to thoroughly erase pencil from paper, using a pencil eraser. Otherwise, you will not dislodge the chytrid fungus from the skin. [You can also watch a video to learn the process here: https://www.youtube.com/watch?v=Ip-urLMLK9k]
   a. Rub the frog's belly, 5 times, back and forth
   b. Rub the frog's side, 5 times, back and forth
   c. Rub the frog's other side, 5 times, back and forth
   d. Rub down one hind leg, 5 times
   e. Rub down the other hind leg, 5 times
   f. Rub on the webbing in between each hind toe, 1 time per webbing

3. Carefully place the swab into one of the provided vials, without touching the outside. Break off the stick or cut it with scissors, so that the swab tip is fully contained within the vial. Screw the lid on tight. Label the vial (using the provided Sharpie marker) with your last name and a consecutive sample number starting with 1 (i.e. Watters #1, 2, etc.) for each frog that you swab. *Record this info on the data sheet.*
4. Take three pictures of the frog, which will allow us to confirm your ID. Label the photo file names in the same way as the frog, but with letters for each one (i.e. Watters #1a, 1b, 1c, 2a, 2b, 2c). *Record the photo names on the data sheet.*
    a. Close-up of the side view of the head
    b. The frog's back
    c. The frog's belly
5. Repeat these steps with all frogs you catch, up to 10 total.

RELEASE ALL FROGS: Release the frogs back where you found them. Do not attempt to relocate any frogs, even if they seem to be in a less than ideal location.

WHEN YOU ARE DONE:

1. Place data sheet(s) and vials in Ziploc bag; be sure it is sealed. Return all unused materials, and guidebook, to the box.
2. Contact us via email to let us know that you have completed sampling and plan to return the kit: camsiler@ou.edu or jwatters@ou.edu.
3. Notify us in the email to whether you plan to mail back the kit or drop it off in person (and when).
4. Upload all the pictures of frogs and habitat. Label them with your last name, sample number, and picture ID (i.e. Habitat, 1A, 1B, 1C, Frog 2A, 2B, 2C). Use the following upload link and create a new subfolder with your last name: [link changes each year]
5. If mailing, please use the provided prepaid shipping label. Be sure to add your name and address to the sender lines, in case of issues with mailing.
6. Drop-off at the Sam Noble Museum, 2401 Chautauqua Ave., Norman, OK 73072
    - Bring the kit to the small staff entrance to the left of the large main entrance – tell the security guard it is for Jessa Watters or Dr. Cameron Siler.
    - Availability: 7am–10pm, 7 days/week.