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PROCEEDINGS OF THE OKLAHOMA ACADEMY OF SCIENCE

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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

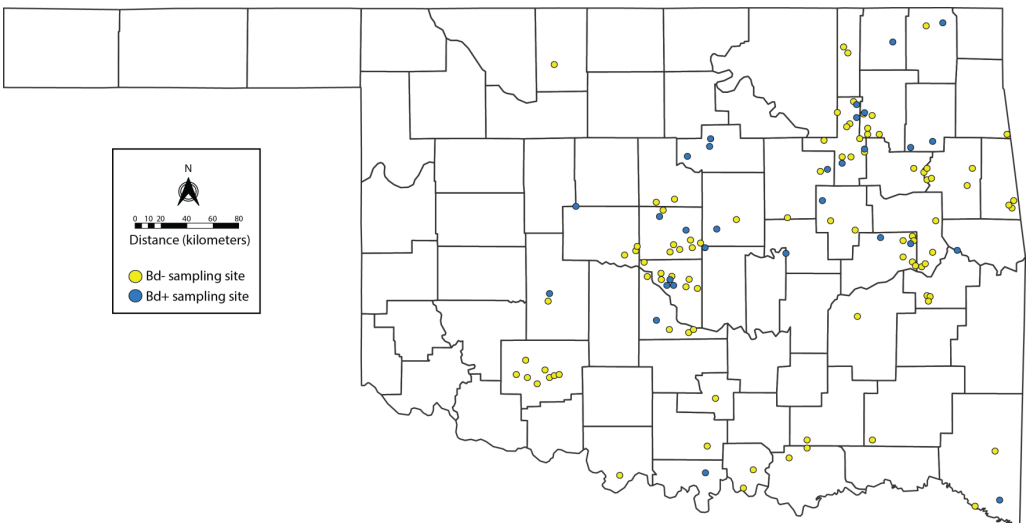

Figure 1. Map of Oklahoma representing sampling sites from participants in the Oklahoma Infectious Disease Citizen Science Project (2016–2019). County boundaries are outlined in black. Sites containing at least one positive individual (*Bd*+) are indicated in blue; sites with all negative individuals 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.

Population Characteristics of Channel Catfish in Meeker Reservoir, Oklahoma, a Small Impoundment

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Abstract: Channel Catfish are a popular sportfish throughout North America and agencies often support put-grow-take fisheries for Channel Catfish in small impoundments. The Oklahoma Department of Wildlife Conservation (ODWC) stocks impoundments throughout Oklahoma with Channel Catfish to provide anglers with catfishing opportunities. Meeker Reservoir (85.8 ha) is a small impoundment located in central Oklahoma that has been historically stocked with Channel Catfish. Uniquely, Meeker Reservoir also has a naturally reproducing population of Blue Catfish that is stunted. Sampling information for Meeker Reservoir is limited and preliminary analysis suggested that Channel Catfish were also stunted. Therefore, our objective was to describe the population characteristics of Channel Catfish in Meeker Reservoir using both 25-mm and 12.5-mm mesh tandem, baited hoop net sets. The Channel Catfish in Meeker Reservoir were stunted, slow growing, dominated by stock size individuals, and had relatively low mortality. Low reservoir productivity, ODWC Channel Catfish stocking rates, and high Channel Catfish and Blue Catfish abundances may be contributing to their slow growth. Additional research should focus on examining the interspecific competition of Blue Catfish and Channel Catfish, potential environmental factors that lead to Channel Catfish recruitment, and angler dynamics of Channel Catfish in small impoundments.

Introduction

Channel Catfish, *Ictalurus punctatus*, are a popular sportfish that inhabit many impoundments throughout the United States and elevated interest in catfish angling has led to increased research and management practices over recent decades (Bodine et al. 2013; Porath et al. 2022). Channel Catfish anglers tend to be harvest oriented, while some anglers desire trophy fishing opportunities (Michaletz and Dillard 1999, Wilde and Ditton 1999, Arterburn et al. 2002, Hutt et al. 2013). Put-grow-take fisheries for Channel Catfish are popular in small impoundments throughout North America; however, due to poor natural recruitment, agencies typically rely on continual stockings for sustainable fishing opportunities (Michaletz and Dillard 1999, Michaletz 2009). In Oklahoma, Channel Catfish are the third most pursued sportfish (York 2019) and the Oklahoma Department of Wildlife Conservation (ODWC) recognizes anglers' interest by stocking impoundments statewide to create and enhance Channel Catfish fishing opportunities.

Meeker Reservoir is a small impoundment (< 100 ha) located in central Oklahoma that supports many sportfish species such as Channel Catfish, Blue Catfish *Ictalurus furcatus*, Largemouth Bass *Micropterus salmoides*, sunfish species, and White Crappie *Pomoxis annularis* to promote fishing opportunities to anglers. ODWC began stocking Meeker Reservoir with Channel Catfish in 1972 with 6,000 fingerlings. Since the initial stocking in 1972, additional Channel Catfish have been stocked (~20,000 fingerlings/year from 1981 – 1989; ~10,000-20,000 fingerlings/year from 2009 – 2013) into Meeker Reservoir in hopes to enhance the fishery (ODWC unpublished data). Prior to this study, little sampling information existed on the Channel Catfish population in Meeker Reservoir. To assess the population, ODWC biologists set tandem, baited hoop nets (25-mm mesh) in the reservoir in 2019. However, catch rates were very low and preliminary age and growth statistics suggested that the population of Channel Catfish in Meeker Reservoir consisted of primarily old fish (up to age 11) at small lengths, which is characteristic of a stunted population. These preliminary results led to further sampling efforts to better understand the Channel Catfish population. The reservoir was then resampled with 12.5-mm mesh baited hoop nets (to target smaller size classes) and age and growth analysis was conducted to reevaluate this population.

Meeker Reservoir is unique because it also contains a naturally recruiting population of Blue Catfish which is uncharacteristic of small impoundments (Waters et al. 2020a). The Blue Catfish population in Meeker Reservoir exhibits high longevity (fish aged up to 29), slow growth, low annual mortality, sexual maturity at small sizes, high abundance, and is stunted (Waters et al. 2020a). Stunted catfish populations are understudied in the literature and to our knowledge, no studies have examined the population characteristics of a Channel Catfish population residing with a stunted Blue Catfish population in a small impoundment. Additionally, Channel Catfish growth in reservoirs is variable, and stocked populations can exhibit slow growth and poor size structure in reservoirs, creating limited management options for biologists (Michaletz 2009, Tyszko et al. 2021a). Therefore, the objective of our study was to describe the population characteristics (age and size structure, condition, growth, mortality, and age and size at maturity) of Channel Catfish in Meeker Reservoir using tandem baited hoop nets.

Methods

Meeker Reservoir is an 85.8 ha impoundment located 2.3 miles southwest of Meeker, Oklahoma in Lincoln County (35º 29′ 46.4″N, 96º 56′ 10.2″W; Waters et al. 2020a; Figure 1). Meeker Reservoir was formed in 1970 with the primary purposes being municipal water supply, flood control, and recreation. Meeker Reservoir has 8 km of shoreline, a maximum depth of 7.4 m, and a mean depth of 2.8 m (OWRB 2009). The river reservoir interface of Quapaw Creek is silted in, reducing the surface area of the lake by 21% (109.3 ha in 1970; OWRB 2009; Waters et al. 2020a). The reservoir consists mostly of open

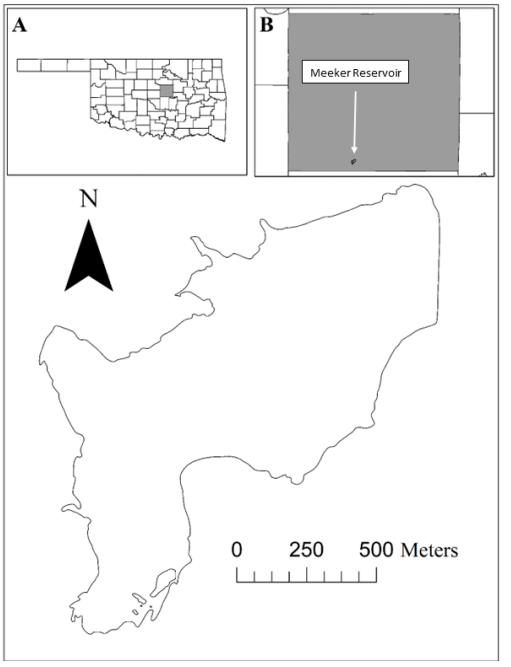

Figure 1. Map of Meeker Reservoir located in Lincoln County, Oklahoma (A) and location of the reservoir within Lincoln County (B).

water with areas of emergent aquatic vegetation, limited submerged or exposed standing timber, rock, coarse gravel, and clay or sand substrate. The reservoir is considered mesotrophic, is extremely turbid (mean secchi depth of 10 cm), has a salinity range of 0.10 to 0.11 ppt, and is neutral to slightly alkaline (7.33 - 8.37 pH; OWRB 2009).

Study Design

Channel Catfish were collected from Meeker Reservoir using tandem baited hoop nets (25-mm mesh and 12.5-mm mesh) during May and June 2020 (correlates with Channel Catfish spawning season in Oklahoma; Miller and Robison 2004). Tandem, baited hoop nets were rigged following ODWC standardized sampling protocols. Specifically, each tandem net set consisted of three 3.4-m-long hoop nets (25-mm and 12.5-mm bar mesh; Miller Net Company, Inc., Memphis, Tennessee) containing seven fiberglass hoops, with the lead hoop being about 0.8 m in diameter and each subsequent hoop gradually decreasing in diameter toward the cod end. Each net included a throat on the second hoop and a restricted throat on the fourth hoop and was baited with fish food (Sportsman's Choice Trophy Fish Feed, Multi-species Formula, Cargill Animal Nutrition, Minneapolis, MN). Nets were fished parallel to shore at depths of 1–3 m for 72 hours. Temperature (°C) and dissolved oxygen concentration (mg/L) were recorded with a YSI meter (model Pro 2030, Yellow Springs Instruments, Yellow Springs, OH) just above bottom at each net set to ensure that dissolved oxygen was ≥ 4 mg/L.

All fish were measured for total length (TL; mm) and weighed (g). Twenty Channel Catfish per 10-mm TL group were collected for age estimation and sex determination. Fish kept for age estimation and sex determination were euthanized using a 1:1 ice to water slurry (Blessing et al. 2010) and processed at the Oklahoma Fishery Research Laboratory in Norman, Oklahoma. Fish were re-measured for total length (TL; mm), weighed (g), sex determined, and lapilli otoliths were removed for age estimation.

Fish were assigned a maturity status (immature or mature) following methods of Davis and Posey (1958) and Perry and Carver (1972). Immature Channel Catfish were those showing no signs of gonadal development. The ovaries and testes of these immature fish are classified as barely distinguishable or are readily distinguishable but not developed. Fish were classified as mature female if they had well developed ovaries that contained yellowish to creamy-yellow eggs or their ovaries were spent (the eggs deposited). Males were classified as mature if their testes were enlarged and white in color.

Lapilli otoliths were extracted from each fish (Long and Stewart 2010) and placed into an individually numbered envelope and allowed to dry for ≥ 24 h prior to processing (Secor et al. 1992, Hull et al. 2022). Once dried, otoliths were processed following methods from Buckmeier et al. (2002) and Waters et al. (2020b). After processing, otoliths were viewed using a stereo microscope (capable of 130x magnification) with a fiber optic filament attached to an external light source to illuminate annuli (Buckmeier et al. 2002, Waters et al. 2020b). Each otolith was estimated in concert by two readers, however if the readers disagreed on the age of the fish, then that otolith was put aside and later viewed again (Hoff et al. 1997). If an otolith was unreadable, the second otolith's age was estimated, however if that otolith was also unreadable, the fish was removed from the study. Each otolith was evaluated in random order with no reference of TL, weight, or sex (Hoff et al. 1997).

Analysis

Size structure of the Meeker Reservoir Channel Catfish population was described with 20-mm bin length frequency histograms of all fish captured and proportional size distribution (PSD, stock ≥ 280mm, quality ≥410 mm, preferred ≥610; Gabelhouse 1984). PSDs were calculated using psdcalc function in the Fisheries Stock Analysis (FSA) package (Ogle 2022) in R (R Core Team 2022, version 1.4.1103). A simple linear regression was used to describe the relationship between log10(weight):log10(length). The relationship

of Channel Catfish length to weight was also used to evaluate fish condition by calculating relative weight (Wr) using the wrAdd function in Ogle's FSA package (2022) in R (R Core Team 2022, version 1.4.1103). Separate length-frequency histograms were created for each hoop net mesh size (12.5-mm and 25-mm) using 20-mm length bins. A Fisher's exact test using the fisher.test() function in R (R Core Team 2022, version 1.4.1103) was used to determine if 20-mm bin length frequencies from each hoop net mesh size differed (P value of ≤ 0.05 was considered significant).

A logistic regression model was used to determine the relationship between maturity at age for male and female Channel Catfish using binary variables (0 = immature, 1 = mature). Mean length at age was calculated for male and female Channel Catfish. These data were then log transformed to linearize the relationship, and differences in growth between sexes were tested using analysis of covariance (ANCOVA) with the aov() function in R (R Core Team 2022, version 1.4.1103). Because prior analysis of growth between sexes was similar ($F_1 = 1.19$, $P = 0.28$), all fish were combined to estimate growth rates using a von Beralanffy growth model.

Growth trajectories and instantaneous mortality rates for Channel Catfish were estimated using a von Betalanffy growth model fit to total length and age estimates using the FSA package (Ogle 2022) in R (R Core Team 2022, version 1.4.1103). Instantaneous mortality rates (Z) were estimated via a weighted catch curve fit to estimated ages using the catchCurve() function in the FSA package (Ogle 2022) in R (R Core Team 2022, version 1.4.1103). Total annual mortality (A) was also estimated for each structure using $1\text{-}e^{-z}$ (Ricker 1975). Channel Catfish < age-2 were not fully recruited to the sampling gear and were removed from catch-curve analysis.

Results

Of the 850 Channel Catfish collected, 298 fish were kept for age estimation and population assessment (Figure 2). Fish used for age analysis ranged from age 1- to age-11 and 70 - 456 mm TL. Male (51%) add female (49%) fish were similarly represented in the sample, with male Channel Catfish reaching 100% maturity by age-8 and female by age-10 (Figure 3). The earliest that male Channel Catfish reached maturity was age-2, 50-75% of all male fish reached maturity

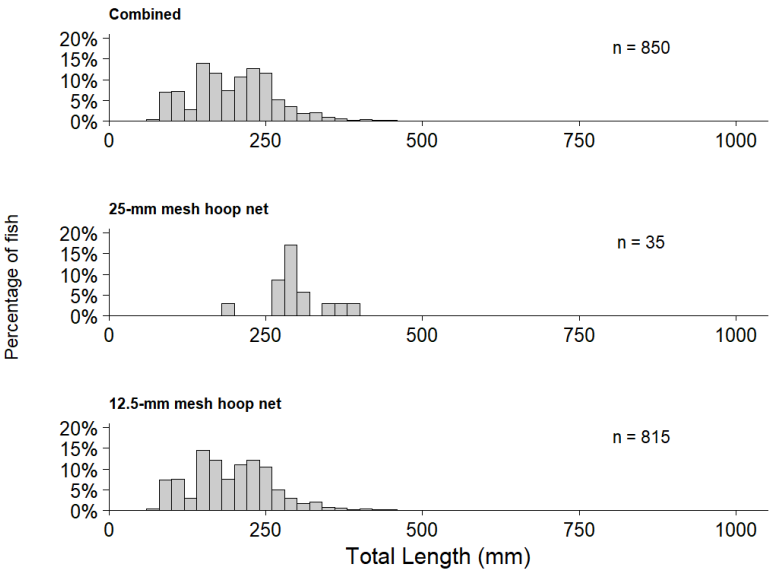

Figure 2. Length Frequency histogram using 20-mm bins of Channel Catfish from Meeker Reservoir, Oklahoma caught using tandem baited hoop nets with 25-mm and 12.5-mm mesh.

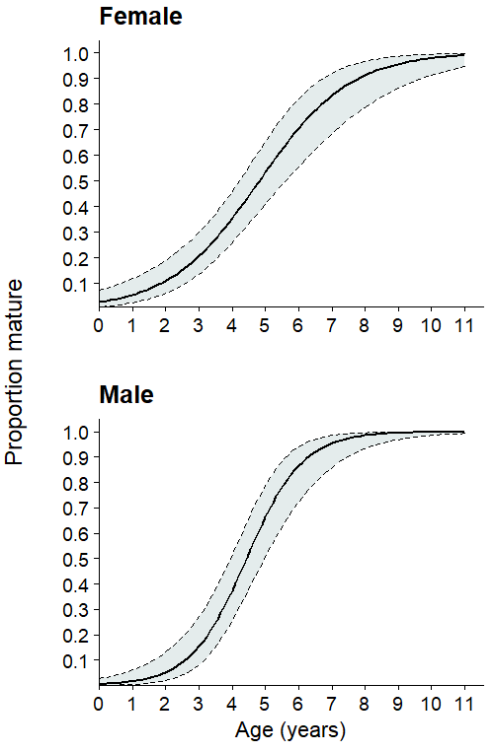

Figure 3. Results of logistic regression analysis displaying the proportion of mature female (top) and male (bottom) Channel Catfish by age. Grey dashed lines represent 95% confidence intervals.

by age-5, and 100% by age-8. The earliest that female Channel Catfish reached maturity was age-2, 50% of all female fish reached maturity by age-5, 75% by age-6, and 100% by age-10.

This population was dominated by sub-stock (90.2%) and stock (9.3%) sized Channel Catfish (Table 1). As a result, PSD was low (PSD-Q = 2), and no fish exceeded PSD-Q size (>410mm). Stock length fish had a mean age of 7, and quality length fish had a mean age of 8.25 (Table 1). The weight-length relationship ($\log_{10}(W) = 3.32(\log_{10}(TL)) - 13.66$) was significant ($r^2 = 0.98$, $P < 0.01$; Figure 4). This relationship resulted in a mean Wr of 93.23 (range of 93-96) (Table 1), which is average (near the 75th percentile) for Channel Catfish in Oklahoma. Length frequencies derived from the catch rates of 25-mm and 12.5-mm hoop net mesh sizes differed significantly (Fisher's exact P value ≤ 0.01).

The Von Bertalanffy growth model indicates that Channel Catfish approach L_∞ (L_∞ = 393.7 mm TL; predicted maximum total length) slowly (k = 0.21), with individuals in the population reaching approximately 50% of the L_∞ by age-3 and 75% of L_∞ by age-7 (Figure 5). The estimated instantaneous mortality was 0.32 and the total annual mortality rate was 27.2% (Figure 6).

Discussion

Channel Catfish in Meeker Reservoir exhibit slow growth and have dense populations of sub-stock size (< 280mm fish) individuals. Our results are consistent with Tyszko et al. (2021a), where they found that Channel Catfish growth was slow in small reservoirs <101 ha. The Channel Catfish population in Meeker Reservoir is exceptionally slow growing compared to other reservoirs throughout Oklahoma. For example, the predicted maximum length for Channel Catfish (393.7 mm) in Meeker Reservoir are in the 5th percentile (451 mm) compared to other Oklahoma reservoirs (OFAA 2022). Additionally, lengths in Meeker Reservoir

Table 1. Proportional size distribution (PSD; 95% confidence interval (CI)), mean age (range), and relative weight (Wr; 95% CI) of Channel Catfish by size class collected from Meeker Reservoir, Oklahoma.

Size Category	*n*	PSD Value	Mean Age (range)	W_r (95% CI)
Sub-Stock (≤ 280 mm)	767	N/A	2.8 (1 - 11)	93 (92.9-93.1)
Stock (≥ 280 mm)	79	95 (88 – 99)	7 (4 -10)	94.5 (94.4 - 94.6)
Quality (≥ 410 mm)	4	5 (1 - 12)	8.3 (7 -10)	95.2 (95 - 95.4)
Overall	850	-	3.8 (1 -11)	93.23

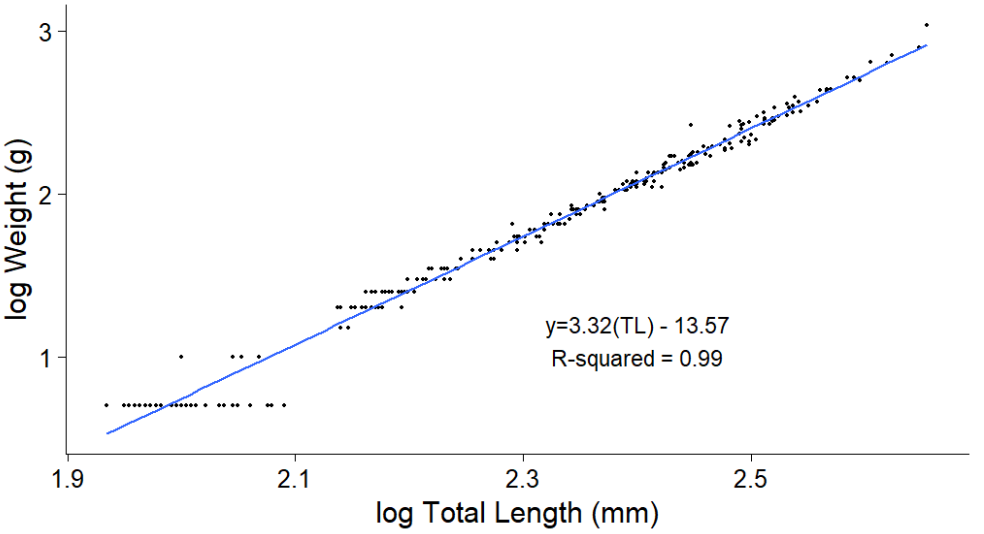

Figure 4. Weight-length relationship for 287 Channel Catfish collected from Meeker Reservoir, Oklahoma. The logarithmically transformed weight-length equation is log10 (W) = 3.32(log10 TL) – 13.57.

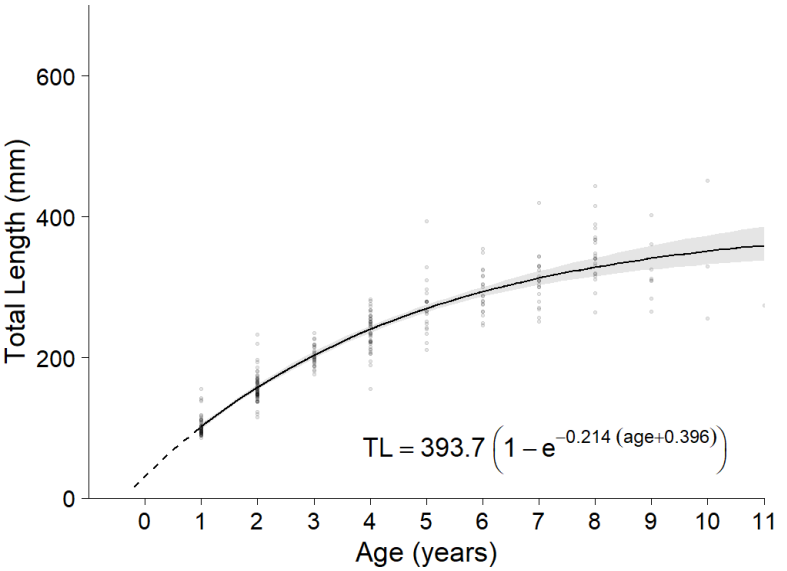

Figure 5. Von Bertalaffy growth curve calculated from 276 lapilli otolith age estimates for Channel Catfish collected from Meeker Reservoir, Oklahoma using tandem baited hoop nets.

generally fall between the 5th and 10th percentile of mean lengths from North American Channel Catfish populations up to 10 years of age (Hubert 1999). Other states have shown variable growth and population densities across regions (Tyszko et al 2021a). Therefore, a more comprehensive understanding of the growth rates of Channel Catfish across Oklahoma would be beneficial for managing populations statewide.

The slow growth of Meeker Reservoir Channel Catfish may be driven by various factors including low reservoir productivity. Meeker Reservoir is classified as mesotrophic

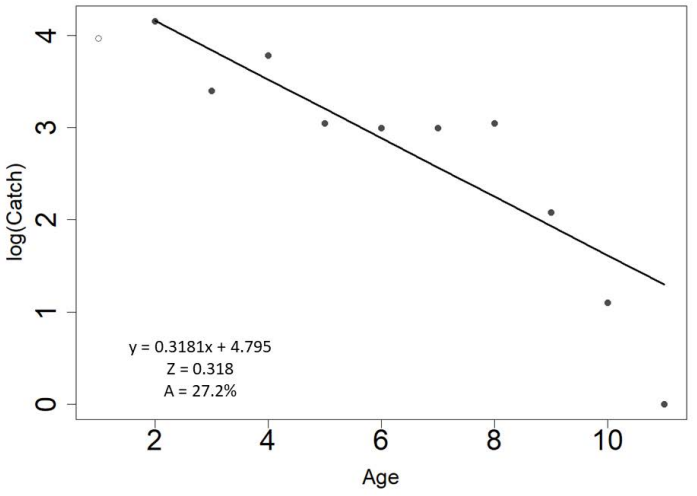

Figure 6. Weighted catch curve regression used to estimate total annual mortality (A) for Channel Catfish collected from Meeker Reservoir, Oklahoma. Age-1 fish are excluded from the catch curve regression. Z = instantaneous total mortality and S = total annual survival.

(OWRB 2009), having low to moderate productivity. The competition for food could be higher in less productive reservoirs because of decreased food availability, likely negatively impact on Channel Catfish growth rates (Shoup et al. 2007, Michaletz 2009). For example, in Missouri reservoirs, Michaletz (2009) found that Channel Catfish growth was slow when lake productivity (indexed by chlorophyll-a concentration) was low. In Illinois reservoirs, Shoup et al. (2007) noted that Channel Catfish growth was positively correlated with available forage abundances (i.e., the more productive a reservoir, the higher abundance of forage present). Also, in Ohio reservoirs, Channel Catfish growth generally decreased as reservoir productivity decreased, but the effect of productivity was less clear in describing the precision of variability in Channel Catfish growth models (Tyszko et al. 2021a). Future research should examine how lake productivity and forage abundance impacts Channel Catfish growth and size structure in impoundments throughout Oklahoma. Additionally, their slow growth may also be driven by interspecific competition between high abundances of both Channel and Blue Catfish, resulting in density-dependent processes attributing to slow Channel Catfish growth (Michaletz 2009; Flammang et al. 2011, Waters et al. 2020a). Future research should examine forage competition and energetic requirements of Blue Catfish and Channel Catfish in small impoundments.

Additionally, ODWC stocking rates may have been too high in Meeker Reservoir, artificially inflating densities leading to slow growth of Channel Catfish (Michaletz 2009). Furthermore, Tyszko et al. (2021a) found that larger reservoirs ($\geq$ 406 ha) were more suited to support preferable-sized Channel Catfish to anglers (i.e., populations with fast growth and larger size structure), citing that smaller reservoirs (< 101 ha) may be unsuitable for sustaining fishable Channel Catfish populations through continued agency stocking. Therefore, ODWC biologists should consider reexamining stocking rates in Meeker Reservoir and develop alternative protocols for future stockings throughout Oklahoma. The reevaluation of stocking rates across Oklahoma will not only benefit put-grow-take fisheries and create higher quality fishing opportunities but will also improve the allocation of hatchery resources and efficiency (i.e., less time and money spent on Channel Catfish). Additional research that identifies more effective stocking rates for small impoundments will be prudent for agencies in

the future.

Habitat and environmental variables that affect reproduction and recruitment may also be affecting the high population densities of Channel Catfish in Meeker Reservoir. Waters et al. (2020a), noted that Blue Catfish are successfully reproducing in Meeker Reservoir, likely leading to high Blue Catfish densities. The increased density of Channel Catfish in Meeker Reservoir may in part be attributed to successful utilization of available spawning habitat leading to increased recruitment. Stocking has not occurred in Meeker Reservoir since 2013 and the presence of 1-year old fish in the reservoir suggests that natural reproduction is occurring. The successful spawning and recruitment of both species in the reservoir is a likely culprit of increased densities and competition, perhaps leading to small size structures. Tyszko et al. (2021a) found that Channel Catfish densities were highest where natural recruitment and reproduction occurs, and stocking does not. However, catfish recruitment in small impoundments is understudied and additional research on environmental factors such as available habitat, annual temperatures, and hydrology are needed to better understand their effect on catfish densities.

Total annual mortality was relatively low for the Meeker Channel Catfish population (27.2%), possibly due to low angler exploitation and harvest rates. Angler exploitation is typically correlated with increased annual mortality (Parrett et al. 1999). However, there may be low angler exploitation and harvest in Meeker Reservoir due to small size structure and resulting angler disinterest (Hutt et al. 2013). Previous studies have documented a decrease in Channel Catfish angling effort and exploitation in general (Parrett 1999, Michaletz et al. 2008, Tyszko et al. 2021a). Creel surveys and exploitation studies would be beneficial for managers to meet angler's preferences and better understand the harvest of Channel Catfish in small impoundments throughout Oklahoma. Therefore, additional information regarding the exploitation and harvest of stunted Channel Catfish populations is warranted.

We found that the number of Channel Catfish caught using standardized tandem baited hoop nets (25.4-mm mesh) differed from the number caught using smaller-sized mesh tandem baited hoop nets (12.5-mm; Figure 2). We were able to sample smaller size classes (< 170 mm) and catch more individuals using the 12.5-mm sized mesh. Tandem, baited hoop nets efficiently sample Channel Catfish (Bodine et al. 2013); however, underrepresentation of smaller size classes of fish have also been documented in the literature. For example, Buckmeier and Schlechte (2009) observed underrepresentation of fish ≤ 250 mm in 25.4-mm mesh tandem hoop nets. Tyszko et al. (2021b) found that juvenile fish (< 400 mm) in tandem baited hoop nets with 25-mm mesh did not fully recruit to the gear. Additional research should incorporate and develop a standardized sampling protocol using tandem baited hoop nets with smaller mesh sizes for sampling juvenile and smaller size structures of Channel Catfish. Understanding juvenile catfish population characteristics such as recruitment, reproduction, and size structure will allow managers to better develop strategies to manage these populations.

Put-grow-take fisheries are popular in Oklahoma and provide valuable catfish angling opportunities (York 2019). However, it is imperative for management agencies to optimize stocking practices and create fisheries that are valued by catfish anglers. Therefore, developing a standardized approach for stocking small impoundments is essential for managing quality catfishing opportunities for anglers. Additional research should focus on developing standards to assess stocked Channel Catfish populations and the variables that influence their population characteristics. Furthermore, understanding angler dynamics in small impoundments will be beneficial to managers trying to develop management strategies that match angler preference and behavior to provide quality fishing experiences. Identifying and understanding variables that attribute to stunted populations will allow for more effective fisheries management on small impoundments that support Channel Catfish populations.

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An Investigation into the Effects of Elevated Water Hardness on Channel Catfish Egg Viability

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Abstract: Channel catfish (*Ictalurus punctatus*) are a popular sportfish across the United States and are often stocked to enhance fishing opportunities. There has been increased research into their life history, management, and population characteristics over recent decades. In a study conducted on channel catfish recruitment in Thunderbird Reservoir, Oklahoma, researchers found that recruitment was negatively associated with total annual water hardness, hypothesizing that larval fish survival decreased when water hardness was ≥ 170 mg/L. To test this hypothesis, we investigated the effects of water hardness on channel catfish egg hatch rates to determine if total water hardness impacts the survivability of larva. Fertilized eggs were obtained from the Holdenville State Fish Hatchery, Oklahoma and transferred to the Oklahoma Fishery Research Laboratory. Eggs were divided and placed in tanks of seven water hardness levels (78 [control], 100, 200, 300, 500, 1000, 3000 mg/L $CaCo_3$). Overall survival, hatch rate, and larval abnormalities were recorded and analyzed for differences between hardness levels and fish. Water hardness did not influence survival or growth early in life in our study. However, we did observe that the spawning matrix deteriorated in higher hardness concentrations (≥ 500 mg/L). Future studies should investigate the effects of water hardness on channel catfish survival post yolk-sac abortion to determine if mortality increases later in life and determine if water quality optima vary between catfish populations at smaller spatial extents. Future work examining the effects of varying water chemistry levels on egg/larval fish survival can replicate our methods, providing additional insight into the early life history of Channel Catfish or other catfish species.

Introduction

Channel catfish (*Ictalurus punctatus*) are popular among anglers, generally abundant, long-lived, commercially important species found throughout the United States (Bouska et al. 2011). Original distribution of Channel catfish included the Mississippi River basin and Gulf States, north into southern Canada, and south into Mexico, but they can now be found across the Atlantic basin and west of the Rocky Mountains (Wellborn 1988). Federal and state agencies manage and stock channel catfish to enhance fishing opportunities for anglers

(Bodine et al. 2013).

There has been an increase in channel catfish aquaculture research and management literature in recent decades because of their popularity and commercial importance (Porath et al. 2021). Aquaculture studies have yielded information on pond preparation guidelines (Steeby and Brunson 1997), broodfish and hatchery care (Steeby and Avery 2005), and reproductive physiology (Tucker and Hargreaves 2004). Management focused studies have investigated ageing techniques (Hubert 1999, Buckmeier et al. 2002,), growth rates (Holland and Peters 1992, Shephard and Jackson 2006;), and overall population dynamics in Oklahoma and across the country (Shrader et al. 2003, Barada 2009, Bouska et al. 2011, Griffin et al. 2022,).

The Oklahoma study was completed on Thunderbird Reservoir, where channel catfish exhibited slow growth and recruitment was negatively associated with annual water total hardness, seemingly reducing year class strength when water hardness values exceeded 170 mg/L (Griffin et al. 2022). Other studies have found that comparatively higher concentrations of water hardness (150mg/L vs 70mg/L) lead to lower survival of larval fish (*Rhamdia quelen*; Silva et al. 2005) and hatching abnormalities occur at hardness levels ≥ 300mg/L (*Clarias gariepinus*; Molokwu and Okpokwasili 2002). For channel catfish, Tucker and Steeby (1993) tested varying levels of water hardness, up to 100 mg/L, and recommended that hatchery water should contain a minimum of 10 mg/L for the best survival of embryos.

Because a gap exists between better survival of channel catfish embryos exposed to water hardness values ≥ 10mg/L (Tucker and Steeby 1993) and reduced recruitment when water hardness values were ≥ 170mg/L in Thunderbird Reservoir, Oklahoma (Griffin et al., 2022), further investigation was warranted. Therefore, the objectives of this study were to 1) investigate the effects of varying concentrations of water hardness on channel catfish egg hatch rates and 2) determine if higher total water hardness levels impact the survivability of larva.

Methods

Fertilized channel catfish eggs from three individual fish were obtained from the Holdenville State Fish Hatchery in Holdenville, Oklahoma. Eggs were produced in accordance with the methods listed in Steeby and Avery (2005). After incubating for ~24 hours at the hatchery, eggs were transferred to the Oklahoma Fishery Research Laboratory, divided, placed in experimental tanks of seven water hardness levels (78 [control], 100, 200, 300, 500, 1000, 3000 mg/L $CaCo_3$), and brought up to and held at 28 °C for the remainder of the experiment. Overall survival, hatch rate, and larval abnormalities were recorded and analyzed for differences between hardness levels.

Study Design

Twenty-one 19-liter aquariums (Aqueon Standard Glass Aquarium Tank, Aqueon Products, Franklin, WI) were placed on top of seven 68 L coolers (Igloo Marine Ultra 72, Igloo Products Corp., Katy, TX) in three rows of seven (rows A, B, and C; Figure 1). Each

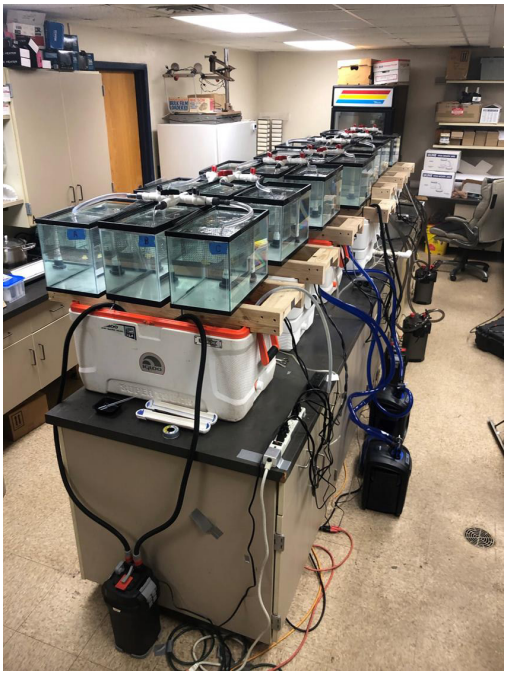

Figure 1. Image of tank setup showing seven treatment levels (cooler/tank complex) and three replicates (A, B, and C).

individual aquarium and cooler were filled with 19 and 57 L of water, respectively, for a total of 114 L of water per system. Pre-soaked baskets built with galvanized hardware cloth (Galvanized Steel Hardware Cloth, 6.35 mm square mesh, Blue Hawk, Lowe's) were used to support egg masses to help ensure adequate circulation (Figure 2). Treatment water for each hardness level was aerated, heated, and filtered in a cooler then pumped through a manifold into three individual aquariums to circulate (Figure 1). We used one heater (ViaAqua 300-Watt Quartz Glass Submersible Heater), filter (Fluval 207 Performance Canister Filter, Rolf C. Hagen Corp., Mansfield, MA; Top Fin CF60 Canister Filter, United Pet Group, Earth City, Missouri; Marineland Magnum Polishing Internal Canister Filter and Marineland Magniflow 220 Canister Filter, Spectrum Brands Pet, LLC, Blacksburg, VA), and water pump (Ecoplus Eco-396 Submersible Pump, Hawthorne Gardening Company, Vancouver, WA) per cooler. We used one aerator (Sweetwater Model SL56, Aquatic Eco-Systems Inc., Apopka, FL) with a manifold that split to each of the seven coolers for aeration. Total hardness (mg/L), pH, dissolved oxygen (mg/L), ammonia (ppm), salinity (ppt), specific conductivity (µS/cm), and temperature (C) were monitored throughout the experiment to ensure water quality was adequate and that treatment levels remained consistent (Table 1). Water was added or changed as needed to maintain a constant volume and to sustain negligible ammonia levels (≤ 3.8 ppm; see Colt and Tchobanoglous 1976).

Figure 2. Image showing galvanized mesh egg basket.

We used City of Norman, Oklahoma tap water as source water and treated at a rate of 10 ml per 114 L to remove chloramines, chlorine, and detoxify heavy metals (API Tap Water Conditioner, Mars Fishcare North America, Inc. Chalfont, PA). Treated water was stored in six clean 208 L plastic drums (with lids) and mixed accordingly with 70:30 Ca to Mg stock solution (the naturally occurring ratio in our source water) to achieve the desired total hardness level (Table 1). Our stock solution was created by adding 700 g of calcium chloride and 300 g of magnesium carbonate (Reagent Grade, Innovating Science, Aldon Corp., Avon, NY) to 20 L of deionized water, mixing, then boiling for 30 minutes. This stock solution was allowed to cool then added at an incremental rate to achieve the desired hardness level for each treatment (Figure 3). Unmixed treated tap water was used for the control (Table 1). Sand shiners (*Notropis stramineus*, n = 60) were held 48 hours in six aquariums with baskets in place to ensure the effectiveness of the tap water conditioner and determine if the galvanized basket material had a negative effect. No deaths were observed after 48 hours and fish were released.

Eggs from three different individuals (replicates, tank rows A, B, C) were split and placed into tanks according to treatment level (Figure 1; Tucker and Steeby 1993, Molokwu and Okpokwasili 2002,). An initial test was aborted early due to substantial die off, likely caused by an overabundance of eggs. For the second trial, a mass of approximately 50 eggs was weighed, number estimated, then placed in baskets in each tank. Dead eggs were counted and removed as needed. The duration of incubation (hours), number of hatched eggs, number of larval abnormalities, mean length

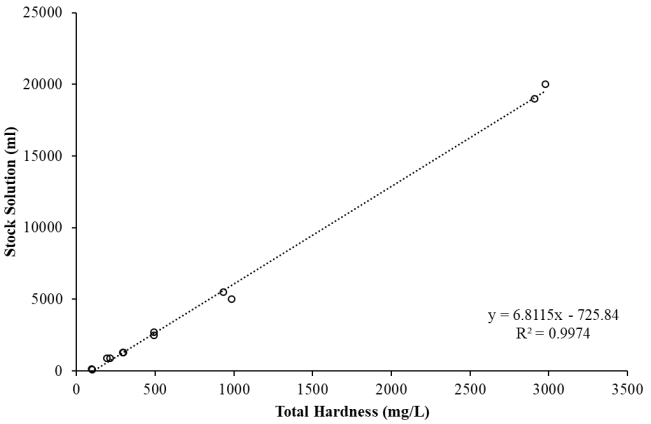

Figure 3. Ratio of stock solution to total hardness (mg/L) level when the stock solution is mixed with 208 L of source water (total hardness = 87 mg/L).

second day post-hatch, and final mean length was recorded for each tank. Throughout the entire process, waste was removed via siphoning to prevent ammonia build up and reduce stress.

Analysis

Three growth intervals were determined similar to Molokwu and Okpokwasili (2002): the egg interval (initial placement in tanks to beginning of hatch), hatching interval (duration in which eggs were hatching), and yolk-sac interval (post-hatch until yolk-sacs were fully absorbed). For each growth interval, percent survival was determined. Lengths of larva (subset of 10 from each tank) were measured two days post-hatch and at final yolk sac absorption. Percent mortality ($\sin^{-1}\sqrt{}$% transformed) during the egg interval, hatch interval, and duration of the experiment (overall) along with growth (length at yolk sac absorption – larval length two days post-hatch; $\log_e$-transformed) were analyzed using two-way analysis of variance (ANOVA) without replication (Zar 1999). The assumptions of each ANOVA were assessed using a Shapiro-Wilk's normality test (Shapiro and Wilk 1965) and a Bartlett's test for homogeneity of variances (Bartlett 1937). If ANOVA detected a significant difference within either of our treatment groups (i.e., fish, hardness), we used a Duncan's multiple range test (DMRT; Tucker and Steeby 1993) *post hoc* to determine which means were significantly different. All statistical tests were conducted using program R 4.2.1 (R Core Team 2022) and *post hoc* tests were performed via the "bartlett.test()" function in the agricolae package (Mendiburu 2021). The threshold for statistical significance was $\alpha = 0.05$ for all tests.

Results

The initial number of eggs per tank ranged from 43-64 (Table 2). Hatching began five days post placement and took ~48 hours to complete across all tanks. Mean hatch and final survival ranged from 96.7-99.3 and 85-94.7 percent, respectively across treatment levels (Table 2). Mean larval total length measured at two days post hatch and after complete absorption of the yolk sac ranged 10.9-11.7 mm and 13.3-14.3 mm, respectively (Table 3). Two larval abnormalities were observed. One fish in treatment group 100 A and one in 200 B hatched roughly 48 hours prior to the rest of the fish (regardless of treatment level) and both died. Interestingly, we observed that the spawning matrix in higher treatment levels (≥ 500 mg/L, but particularly at 3000 mg/L) began breaking down early (two days prior to the beginning of the hatch period) to the point where eggs were spread out and some were lost through the basket and laying in the bottom of the tank (A-spawning matrix intact, B-spawning matrix deteriorated; Figure 4).

ANOVA results revealed no significant differences for egg interval mortality or growth

Table 1. Mean (SD) values of water quality parameters for each total water hardness (mg/L) treatment level.

	Parameters						
Treatment Level	Total Hardness (mg/L)	pH	Dissolved Oxygen (mg/L)	Ammonia (ppm)	Salinity (ppt)	Specific Conductivity (µS/cm)	Temperature (C)
Control	87 (3)	7.48 (0.41)	7.01 (0.46)	0.25 (0.21)	0.3 (0.05)	617 (106)	27.6 (0.3)
100	103 (5)	7.91 (0.19)	7.11 (0.18)	0.73 (0.23)	0.23 (0.02)	483 (33)	27.8 (0.5)
200	238 (12)	7.91 (0.12)	6.99 (0.25)	0.34 (0.29)	0.4 (0.02)	829 (47)	27.8 (0.4)
300	293 (13)	7.98 (0.07)	7.01 (0.23)	1.36 (0.65)	0.51 (0.03)	1035 (57)	27.6 (0.6)
500	506 (19)	7.89 (0.16)	6.9 (0.22)	0.68 (0.36)	0.8 (0.04)	1607 (80)	27.8 (0.5)
1000	1008 (68)	7.93 (0.15)	6.98 (0.28)	0.27 (0.17)	1.4 (0.07)	2712 (133)	27.4 (0.7)
3000	3044 (158)	7.41 (0.29)	6.85 (0.22)	0.89 (0.2)	4.24 (0.19)	7715 (329)	27.9 (0.2)

within fish (df = 2, F range = 2.18 – 2.42, all $P > 0.05$) or hardness level (df = 6, F range = 1.56 – 1.71, all $P > 0.05$) groups. Hatch interval and overall mortality were determined to be significantly different between fish (df = 2, F range = 5.62 – 8.11, all $P < 0.05$) but similar across hardness levels (df = 6, F range = 0.63 – 1.81, all $P > 0.05$). DMRT results suggested that Fish B (mean = 0.19) had significantly higher hatching mortality than fish C (mean = 0.02); however, both mortality rates were similar to fish A (mean = 0.11; Figure 5). Interestingly, DMRT results suggested that Fish B (mean = 0.36) had significantly higher overall mortality than fish A (mean = 0.20); however, both mortality rates were similar to Fish C (mean = 0.28; Figure 5). Shapiro-Wilk's normality test confirmed transformed-residuals were normally distributed in all ANOVA models (W range = 0.43 – 0.98, all $P > 0.05$). Bartlett's test confirmed homogeneity of variance for transformed mortality and growth rates between fish (df = 2, K^2 range = 0.45 – 3.71, all $P > 0.05$) and hardness (df = 6, K^2 range = 0.73 – 9.62, all $P > 0.05$) groups.

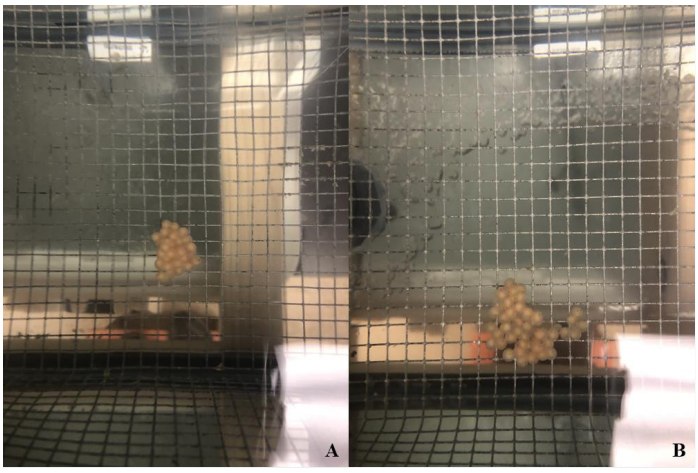

Figure 4. Image (A) shows a clumped egg mass indicative of control tanks with a water hardness of 87 mg/L and image (B) illustrates the observed breakdown of the egg mass spawning matrix in the treatment tanks with water hardness ≥500 mg/L.

Table 2. Survival of channel catfish eggs and larva incubated in different treatments of total water hardness (mg/L).

	Egg interval						Hatch interval			Yolk sac interval		
	Initial # of eggs			Survival # to hatch (percent)			Survival # to end of hatch (percent)			Final survival (percent)		
Treatment Level	A	B	C	A	B	C	A	B	C	A	B	C
Control	48	56	50	48 (100)	52 (93)	49 (98)	47 (98)	48 (92)	49 (100)	47 (98)	46 (82)	47 (94)
100	54	64	48	53 (98)	50 (78)	41 (85)	53 (100)	48 (96)	41 (100)	51 (94)	48 (75)	41 (85)
200	49	47	51	46 (94)	44 (94)	46 (90)	44 (96)	42 (96)	46 (100)	44 (90)	41 (87)	46 (90)
300	47	51	55	44 (94)	50 (98)	55 (100)	44 (100)	47 (94)	54 (98)	44 (94)	46 (90)	54 (98)
500	51	52	48	51 (100)	48 (92)	48 (100)	51 (100)	47 (98)	48 (100)	51 (100)	47 (90)	45 (94)
1000	51	56	46	51 (100)	53 (95)	42 (91)	48 (94)	52 (98)	42 (100)	49 (96)	53 (95)	42 (91)
3000	43	59	52	40 (93)	56 (95)	50 (96)	39 (98)	55 (98)	50 (100)	40 (93)	54 (92)	47 (90)

Table 3. Mean larval total length in mm (range, 2nd day post hatch and final) for fish A, B, and C treated at various levels of total water hardness.

	Mean total length (range)					
	2nd day post hatch			Final		
Treatment Level	A	B	C	A	B	C
Control	11 (10-12)	11.4 (10-12)	11.6 (11-12)	13.5 (13-14)	13.6 (13-14)	14.3 (14-15)
100	11.6 (11-13)	11.3 (11-13)	11.4 (11-12)	13.9 (13-15)	13.4 (13-14)	13.6 (13-14)
200	11.5 (11-12)	10.9 (10-12)	11 (10-12)	14.1 (13-15)	13.6 (13-14)	13.6 (13-14)
300	11.1 (10-12)	10.9 (10-12)	10.9 (10-12)	13.6 (13-14)	13.4 (13-14)	13.6 (13-14)
500	11.1 (10-13)	11.4 (11-12)	11 (10-12)	13.8 (13-15)	13.7 (13-15)	13.4 (13-14)
1000	11.7 (11-13)	11.6 (11-12)	11.2 (10-12)	13.6 (13-14)	13.8 (13-15)	13.8 (13-15)
3000	11.4 (10-12)	11.4 (11-12)	10.7 (10-12)	13.3 (13-14)	13.9 (13-15)	13.4 (13-15)

Discussion

Our results suggest water hardness did not influence channel catfish survival or growth early in life (i.e., egg to yolk-sac absorption). These findings appear to contradict those of Griffin et al. (2022) *prima facie*, as their results suggested water hardness influenced channel catfish recruitment in Thunderbird Reservoir, OK. However, these contrasting results may be the result of the life interval observed. Our study measured the effects of water hardness on survival up to yolk-sac absorption, whereas Griffin et al. (2022) investigated recruitment variation based on hardness exposure over the first year of life. Increased water hardness may increase mortality post yolk-sac absorption as external feeding requires greater energy expenditure and increased water hardness has been known to have adverse physiological effects on channel catfish (Buentello and Gatlin 2001). Future studies should investigate the effects of water hardness on channel catfish survival post yolk-sac abortion to determine if mortality increases after the ontogenetic shift to exogenous consumption.

Channel catfish eggs used in this study come from hatchery brood stock, not wild populations. Prior research has noted that there are distinct genetic differences between wild and domestic stocks (Simmons et al. 2006). Furthermore,

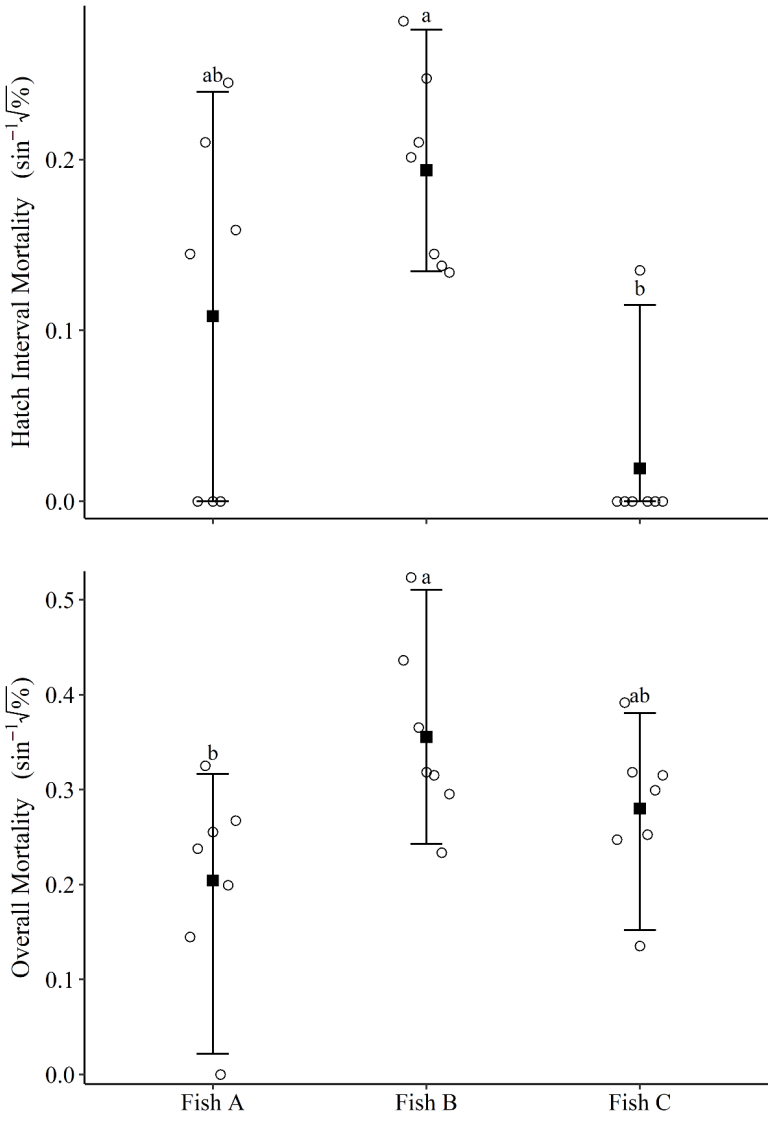

Figure 5. Observed hatch interval and overall percent mortality (sin-1√% transformed) for Fish A, B, and C across all hardness levels (circles). Mean mortality (squares) and 95% confidence intervals are included for each fish. Lowercase letters indicate statistical differences in hatch interval or overall mortality between fish.

domestic channel catfish exhibit different growth and reproductive characteristics than their wild counterparts (Broussard and Stickney 1981, Bondari 1984). Future work should determine if the results of water chemistry (e.g., hardness, salinity, temperature) studies conducted using domesticated stocks can be applied to wild populations. This is especially important given our results disagree with prior work on a wild channel catfish population (see Griffin et al. 2022). The methodology outlined within this study can be used to determine if wild and domestic stocks exhibit different responses (e.g., reduced survival, lower growth) to water chemistry variation.

This study highlights the importance of monitoring mortality over various life stages (e.g., egg, hatch, larva). No mortality differences were observed between fish at the egg stage

while fish C progeny exhibited the lowest mortality during the hatch interval, and fish B progeny exhibited the lowest mortality by the end of the study. To the best of our knowledge, this is the first study to document a significant difference between interval specific mortality between the same species of catfish. However, variation in interval specific mortality due to water chemistry (specifically salinity) has been noted between species of catfish (Abass et al. 2017). The variation in interval specific mortality between conspecifics, the consistently higher mortality of fish B from the hatching interval on, and the relatively stable density of individuals within treatments suggest differences may be due to genetic or epigenetic variation between individuals. However, further study would be required to determine if genetic or epigenetic variations are the source of differential mortality between channel catfish.

Genetic variation in channel catfish stocks is poorly understood within Oklahoma. Genetic information from Mexico (Lara-Rivera et al. 2019) and Alabama (Simmons et al. 2006) show that distinct genetic stocks can exist. Furthermore, genetic differentiation between channel catfish generally increases with spatial distance and is influenced by site-specific effects (Sotola et al. 2017). This suggests that there is potential for genetic variation within channel catfish populations in Oklahoma. If genetic differences do exist, there is potential for variation in water quality optima (e.g., salinity, temperature, hardness) between populations. At broader spatial extents (i.e., northern United States vs southern United States), differences in critical thermal maxima have been documented between channel catfish strains (Stewart and Allen 2014). Future work should determine if water quality optima vary between catfish populations at smaller spatial extents (e.g., Oklahoma) and if there is a genetic or epigenetic basis for such regulation.

Water chemistry can exhibit varying relationships on fish vital rates (e.g., mortality, growth) due to interactions between variable causing confounding effects. Prior studies have documented that lower water hardness increases copper-induced morality (Perschbacher and Wurts 1999) and mitigates sub-lethal effects (e.g., reduced growth) of chronic ammonia exposure (Sinha et al. 2022) in channel catfish. Additionally, our observation that the spawning matrix deteriorated in higher hardness concentrations might be a clue. Male channel catfish remain with the egg mass after fertilization to guard and fan water over the eggs (Tucker and Hargreaves 2004). In higher concentrations of water hardness, the breakdown of the egg matrix would likely allow the eggs to be either covered in silt, or fanned out of the spawning cavity, where they would be susceptible to predation. Future work should determine the impact of accelerated breakdown of the spawning matrix on eggs (i.e., eggs falling into silt/sediment, vulnerability to predation).

The results of this study help to contextualize the findings of Tucker and Steeby (1993), who recommended that water supply for hatchery rearing have a minimum hardness of 10 mg/L. These findings may be exclusive to channel catfish considering that other species, such as the silver catfish (*Rhamdia quelen*), had lower post hatch survival in higher water hardness trials (Silva et al. 2003). Our study did not find any difference in hatching or larval success based on hardness level contrasting with the findings of Griffin et al. (2022), where recruitment success was negatively correlated with higher water hardness values. Following this study, we recommend that fishery managers continue to follow past guidelines, such as those produced by Tucker and Steeby (1993), for channel catfish rearing. However, if supply water has high levels of hardness (≥ 500 mg/L) managers should use caution when handling, transporting, or incubating egg masses. Future studies on how water hardness impacts the uptake of potential toxins should be considered for channel catfish. Additionally, future work examining the effects of varying water chemistry levels on egg/larval fish survival can replicate our methods for experimental design.

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Ecology of the Harris Mud Crab (*Rhithropanopeus harrisii*) in Lake Texoma

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Abstract: Harris mud crab, *Rhithropanopeus harrisii,* was first reported in Lake Texoma in 2008. Since that time, very little research regarding this population has been conducted. Goals of this study included determining reproductive periods, noting microhabitat preferences, and documenting the distribution of crabs in Lake Texoma. Six sampling sites were established on the Oklahoma side of Lake Texoma along a transect from the OU Biological Station to near the Denison Dam. Sites were sampled from August 2019 to August 2021. A total of 1,396 crabs were observed with 1,326 collected for analyses. Results indicated a significantly male-dominated sex ratio in the population. Abundance and distribution of crabs varied across the lake, likely due to salinity values and microhabitat availability of each site. Population densities in Lake Texoma were higher than those observed in other locations. Seasonal trends noted the difference in crab frequency during the warm and cool seasons. A rapid drop in lake level during late winter of 2020 provided evidence these crabs migrate to deeper water to take refuge from cold temperatures. The presence of larvae and gravid females suggests a reproductive period from June to October and confirms the successful establishment of Harris mud crab in Lake Texoma.

Introduction

Rhithropanopeus harrisii is a small decapod crustacean commonly known as the Harris mud crab. *R. harrisii* is native to the Atlantic coast, ranging from Miramichi Estuary, Canada to Veracruz, Mexico (Boyle Jr. et al. 2010). Today, *R. harrisii* is one of the most widely distributed brachyuran species worldwide (Grosholz & Ruiz 1996, Roche & Torchin 2007). *R. harrisii* has been introduced in over 22 countries, two oceans, ten seas, and several US freshwater reservoirs in Texas (Boyle Jr. et al. 2010).

In 2008, Patton et al. (2010) confirmed the furthest inland report of Harris mud crab to date from Lake Texoma. Lake Texoma is a 360.27 km^2 freshwater reservoir located on the Oklahoma-Texas border. Boating and fishing are popular sports on Lake Texoma and are thought to be the source of invasive species introduction. Historically, Harris mud crabs have been introduced by anglers through live-wells and bait buckets, transported through ship ballast water, and released during marine fish stocking of striped bass (*Morone saxatilis*) and red drum (*Sciaenops oscellatus*) (Boyle et al. 2010). According to genetic analyses, the founding individuals likely originated from Texas estuaries (Huebner et al. 2021) and could have been introduced to Lake Texoma through any of these human-initiated methods (Boyle et al. 2010).

The Oklahoma Department of Wildlife Conservation (ODWC) recognizes the Harris mud crab as a potentially destructive species due to its documented impacts including pipe fouling, economic loss, displacement of native species (i.e. crayfish and midges), and spread of disease (Payen & Bonami 1979 cited in Roche & Torchin 2007). Currently, only two studies have been conducted on the Lake Texoma population. This study describes several ecological aspects of the Lake Texoma population of Harris mud crab for the first time.

Methods

Based on preliminary studies, six collection sites were established along the Oklahoma side of Lake Texoma. Locations of these sites listed from upstream to downstream are 1) OU Biological Station, 2) Lark Sandy Beach, 3) Caney Creek Yacht Club, 4) Texoma State Park, 5) Willow Springs Marina, and 6) West Burns Run Campground (Fig. 1). Sites were sampled monthly during the warm season and every other month during the cool season, with the exception of site #6 which was not accessible from October 2019 to April 2020. Sampling occurred from August 2019 to August 2021.

At each site, a 13.5 cm x 18.0 cm scoop was used to dredge two 1m^2 plots of submerged shoreline sediments. The entire benthic sample of the plots was transferred into 0.5 mm sieve buckets and sorted. Harris mud crabs were then separated from the sample and counted. Searching for crabs outside the plots was conducted if fewer than 10 crabs were found in the plots. Specimens were preserved in 70% ethanol and returned to the laboratory at the University of Central Oklahoma. Water temperature, salinity, specimen counts, and general observations from each site were recorded. A dissection microscope was used to determine the sex of each crab. A two-proportion z-test was used to analyze the data and calculate sex ratio.

A plankton net was used to sample Harris mud crab larvae during darkness. Ten tows of approximately 10 m in length were performed two to three hours after sunset. The samples were preserved in 70% ethanol and returned to the laboratory. Examination with a dissection microscope confirmed the presence or absence of zoea larvae during each sampling period. Larval presence-absence data and the collection of gravid females were used to determine reproductive period of Harris mud crab in Lake Texoma.

Results and Discussion

Sex ratio

A total of 1,396 crabs were collected during 18 sampling trips. Of the 1,326 crabs analyzed, 420 (31.7% were female, 524 (39.5%) were male,

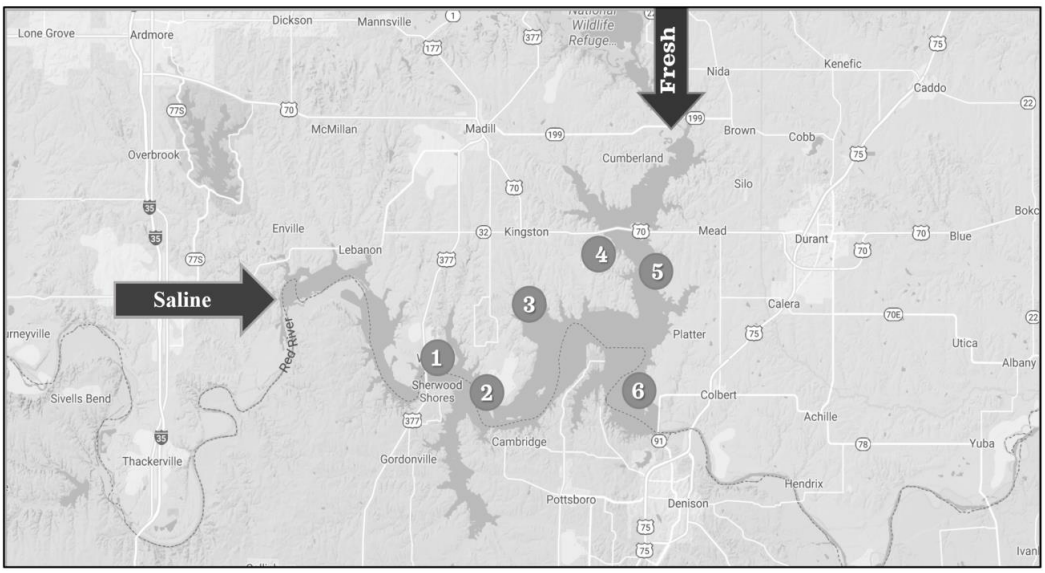

Figure 1. Map showing the Red River, Oklahoma-Texas border, and Lake Texoma. Study sites are 1) OU Biological Station, 2) Lark Sandy Beach, 3) Caney Creek Yacht Club, 4) Texoma State Park, 5) Willow Springs Marina, and 6) West Burns Run Campground. Arrows indicate the direction of flow and input of salts by the Red River and freshwater by the Washita River.

341 (25.7%) were immature, and 41 (3.1%) were too damaged to determine sex. Findings from a two-proportion z-test indicated that the number of males and females differed from an expected 1 male:1 female ratio, favoring males ($p<0.001$) and resulted in a 1.26:1 (male:female) sex ratio in this Lake Texoma population. Male-dominated sex ratios have been observed in other populations of Harris mud crab, including a 1.3:1 in the Dead Vistula River (Normant et al. 2004) and 2.4:1 in Vistula Lagoon (Rychter 1999 as cited in Normant et al. 2004).

One explanation for a male-dominated population is competition for limited space or resources. For instance, the crab's preferred microhabitat of rock/gravel was a limited resource at several of the sampling sites in Lake Texoma. The abundance of this microhabitat also fluctuated depending on lake level and the presence of inhabitable debris on the shoreline (i.e. burlap, trash, recreational equipment). It is possible that with limited space, the larger males outcompeted smaller females. Additionally, males are more motile and better able to obtain food, which is advantageous when resources are scarce (Czerniejewski 2009). However, based on the crab's opportunistic omnivorous nature, food is perhaps the least probable reason for a male-dominated population in Lake Texoma.

Another explanation for unequal sex ratio is sampling methods. Normant et al. (2004) states that sex ratios are largely dependent on the technique used to capture the crabs. For example, Normant et al. (2004) and Janta (1996) reported opposite sex ratios from the same area, and determined the results were likely due to a difference in sampling methodology. In similar studies, Krzywosz et al. (1995) concluded that the number of males is a characteristic feature of the collection method when sampling other decapod crustaceans, such as crayfish (cited in Normant et al. 2004). Method-dependent sex ratios are expected because males are more mobile, especially in warmer months. The sampling methods used in this study were most similar to those employed by Janta (1996), which yielded a sex ratio skewed towards female. Therefore, it is reasonable to assume this study was equally effective at capturing males and females because our results actually yielded a greater number of males.

Lastly, Tesch (1913) found that the sex ratio of *R. harrisii* favored males as the salinity of the water decreased (as cited in Turoboyski 1973). This concept seems to apply with the low salinity conditions of Lake Texoma, so it may partly explain the higher proportion of males.

Site Trends

The number of crabs varied greatly between sites. Figure 2. illustrates the relative abundance of crabs at each site and the frequency of male, female, and immature crabs in each collection. Sites #2 and #3 accounted for more than half of the total crabs collected (95% CI, 391.3, 473.7) and (95% CI, 414.1, 498.8), respectively (Fig. 2). Site #5 had the fewest number of crabs during the sampling period (2.9%) (Fig. 2e). One explanation for this distribution is the range in salinity. The Red River flows through 250-million-year-old salt beds in western Oklahoma and Texas, leaching up to 3,450 tons of sodium chloride per day (Malewitz 2013). Salinity in Lake Texoma generally decreases in the downstream direction, with a specific conductance of 3,740 µS/cm above Lake Texoma, closest to site #1, and 1,795 µS/cm at the outflow of Denison Dam near site #6 (USGS 2003) (Fig. 1). Additionally, there are inputs of freshwater from the Washita River between sites #4 and #5 in the north-east arm of the reservoir. Because these crabs are a marine species, it is possible that their distribution is influenced by the salinity gradient, resulting in a higher number of crabs in the west and fewer in the east (Fig. 2).

Another explanation could be habitat availability. Results from an unpublished independent study using a limited number of crabs, indicate that 94% of Harris mud crabs from Lake Texoma select gravel/cobble as a microhabitat over vegetation and sediments. This was reflected by results from the primary study. For example, site #4 contained primarily clay substrate, while site #5 was mostly sand. Neither site offered the cover or protection

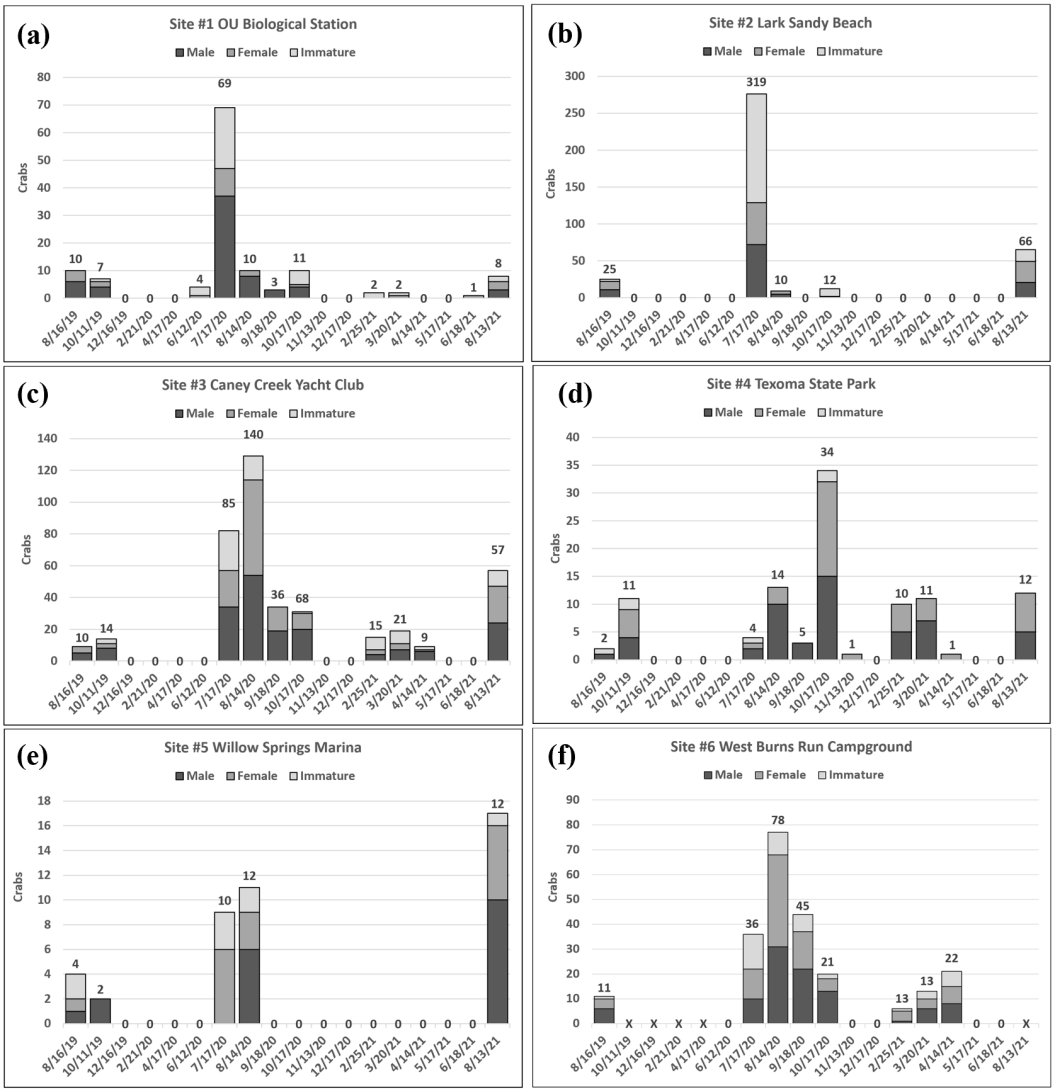

Figure 2. Number of male, female, and immature crabs collected at six collecting sites from August 2019 to August 2021. The y-axes are individually scaled. Data labels are based on the number of crabs observed, including those collected. The "X" indicates samples that did not occur due to site inaccessibility.

these crabs require (Nurkse et al. 2015), possibly explaining the lower numbers usually occurring at those sites. Sites #3 and #6 had rocky shorelines that made suitable habitats for Harris mud crabs resulting in higher population densities. Rocks and gravel at sites #1 and #2 were submerged only during periods of higher lake levels. Low lake levels left the gravel/rock exposed, and the submerged portion of the sampling areas were mostly sand and mud. Figure 2b illustrates the majority of the crabs collected at site #2 were from one sample when the lake level was elevated. The same site had considerably fewer crabs during the rest of the sampling period when lake water levels receded. This resulted in site #2 appearing to have a higher population than actually survived (Fig. 2b). Overall, the data suggests a combination of salinity and microhabitat availability influence the distribution pattern of Harris mud crabs in Lake Texoma.

Seasonal trends

The greatest number of crabs were observed on July 17, 2020. Crabs were most abundant throughout the study period when water temperatures were above 30°C (Fig. 3). This is likely due to the crab's increased locomotor activity during the warm season. Because they are ectotherms, crabs experience an increased demand for food as their metabolism responds to warming temperatures (Mat et al. 2017). Additionally, elevated water temperature induces breeding activity in Harris mud crabs. This not only means the crabs were more likely to be captured in samples because they are active and searching for mates, but the population totals also included the addition of new juveniles. During this time, large numbers of juveniles may be present in the population, but only a small percentage survive long-term (Gothland et al. 2014). The July 17, 2020 a peak in the number of crabs collected (488) included 212 (43%) juveniles. The following months showed a sharp decrease in the population as many crabs were likely consumed by predators or outcompeted for limited resources and/or space, a pattern typical of crabs and other r-selected species (Gothland et al. 2014).

Alternatively, very few crabs were observed during periods of cool water temperature and no crabs were collected in 6 of the 18 sampling periods when water temperatures were below 23°C (Fig. 3). The disappearance of crabs from the sampling plots may be explained by seasonal migration. Turoboyski (1973) suggests that Harris mud crabs migrate to deeper water during the cool months to take refuge from the cold intertidal conditions. To date, there are no studies describing this behavior in reservoir populations. However, seasonal migration was suspected during February 2021 when crabs were found despite the water temperatures being below 23°C (Fig. 3). Leading up to the February collection, the lake level dropped to 614 ft, which shifted the sampling area further into the lakebed (Fig. 4). It appeared crabs had remained in place from their earlier seasonal migration and 40 individuals were found. Crabs continued to be collected in samples until the lake level rose in April 2021 (Fig. 4). This indicates Harris mud crabs migrate to deeper water in Lake Texoma during the cool months, presumably seeking

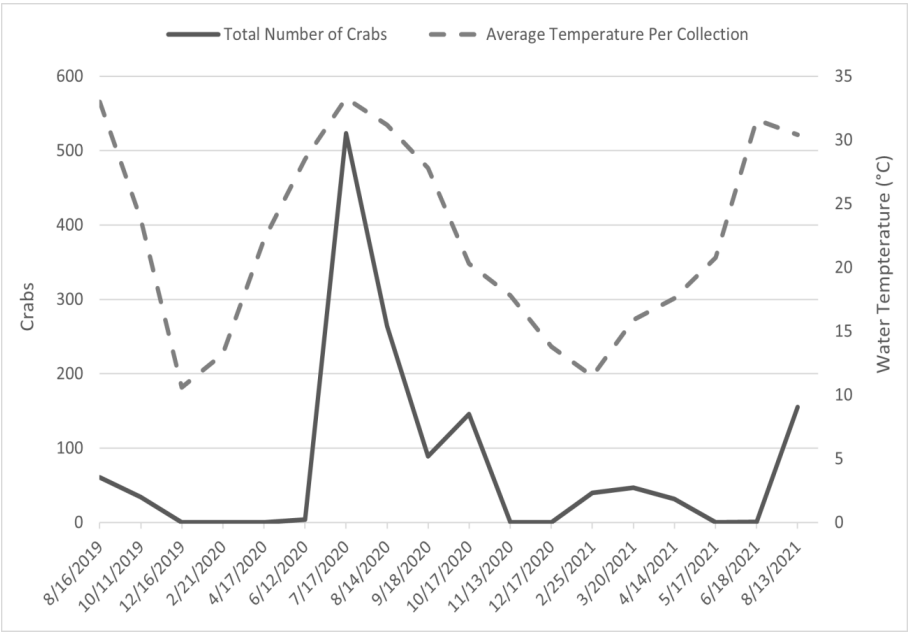

Figure 3. Line graph illustrating the total number of crabs observed and the average water temperature (°C) per collection from August 2019 to August 2021.

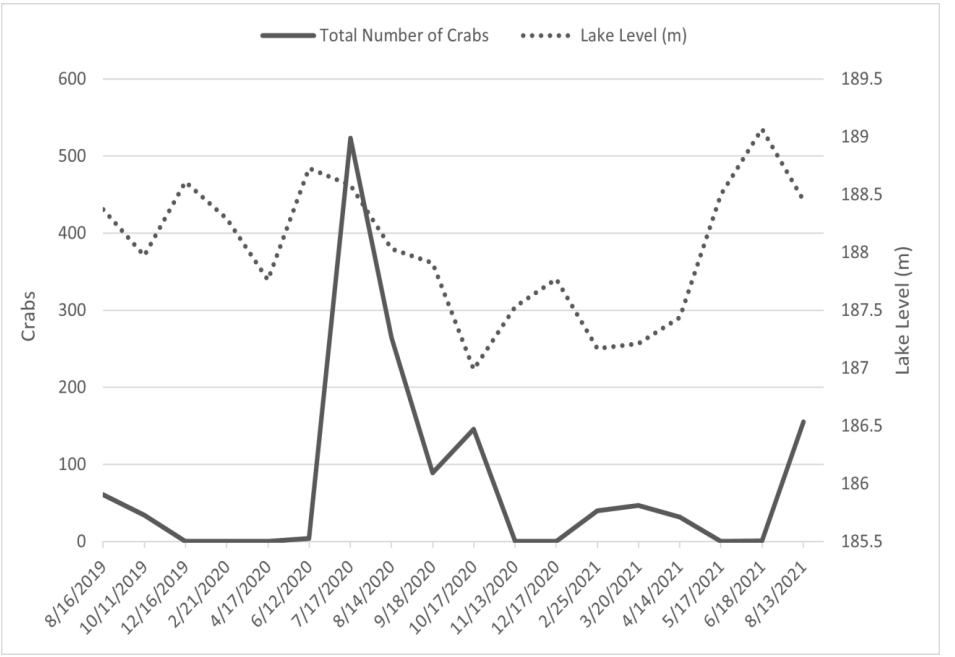

Figure 4. Line graph illustrating the total number of crabs observed and the lake level (m) at each collection from August 2019 to August 2021.

greater temperature stability.

Population trends for Harris mud crabs in Lake Texoma are best described as seasonal. The number of crabs present in the shoreline habitat is largely dependent on the water temperature and lake level. Additional studies are needed to accurately assess the frequency and distribution of crabs in the deeper water during the cool months.

Population density

The overall densities of Harris mud crab ranged from 0 individuals/m^2 to 186 individuals/m^2. When crabs were present, the lowest average population density was observed at site #5 (2.00 individuals/m^2 ± 1.00, $\overline{x}$ ± s.d.) while the highest was at site #2 (87.5 individuals/m^2 ± 85.92). The average density observed from all sites was 23.4 individuals/m^2 (± 38.62) when crabs were present. Population densities of crabs in Lake Texoma surpass densities reported from other locations. Hegele-Drywa et al. (2014) reported 19 individuals/100m^2 in the Gulf of Gdansk. Despite high crab densities in several of the quadrats in this Lake Texoma population, crabs were present in only 57 (26.4%) of the 216 total quadrats sampled during the collections, indicating an extremely patchy distribution. To avoid overestimating the population densities, quadrats were randomly selected rather than intentionally sampled where crabs would most likely occur. This often meant that few or no crabs were found in the plots, even if crabs were present elsewhere. Also, it should be noted that the population densities during the cooler months are most likely underestimated when crabs moved to deeper waters as described earlier.

Reproductive Periods

Harris mud crab larvae were found in plankton tows from July through October 2020 and in June and July 2021. Larvae were encountered only when water temperature was above 20°C. This larvae data is consistent with the spike in juvenile crabs found in July 2020. Furthermore, twelve gravid females were collected from quadrats in samples from October 2019, August 2020, September 2020, and August 2021. Based on these results, the reproductive period for Harris mud crabs in Lake Texoma is likely June

through October.

The presence of larvae and gravid females confirms Harris mud crabs are established and reproducing in Lake Texoma. However, prior studies found that the larvae could not hatch in salinities less than 5.0‰ (Costlow et al. 1966) and physiological problems occurred in adults exposed to freshwater for extended time (Turoboyski 1973). The salinity of Lake Texoma consistently ranged from <1‰ to 1‰ during the study, suggesting this population of crabs is adapted to freshwater conditions. This is noteworthy because Harris mud crabs from Lake Texoma exhibit potential to spread and colonize a greater range of habitats across North America.

Summary and Conclusion

The abundance of Harris mud crabs in Lake Texoma was considerably higher than the previous observations in 2008 by Patton et al. (2010). Since that time, Harris mud crabs have multiplied and spread to many available areas of the reservoir and now exist at densities of up to 186 individuals/m^2. Salinity and microhabitat availability appear to be the predominant factors determining the distribution and abundance of crabs at each site. Seasonal trends were observed, most notably a large increase in the population as water temperature rises at the beginning of breeding season and a disappearance of crabs during the cool months. A rapid drop in lake level provided evidence the crabs migrate to deeper water to take refuge from cold temperatures. The presence of Harris mud crab larvae and gravid females indicate a reproductive season from June to October in Lake Texoma. All life stages of the Harris mud crab are thriving despite Lake Texoma's low salinity and this oligohalene tolerance should raise concern for other areas susceptible to invasion. Future studies are needed to access the ecological consequences, if any, of Harris mud crabs in Lake Texoma. The crabs are known to cause economic loss (Zaitsev & Ozturk 2001), decrease in biodiversity (Jormalainen et al. 2016), and displacement of native species like crayfish in other areas of introduction (Richey 2004). Additional studies would benefit the current and potential areas of invasion, as well as provide a better understanding of Harris mud crabs.

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Population Characteristics of Black Bullhead *Ameiurus melas* in Two Small Oklahoma Close to Home Fishing Ponds.

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Abstract: Black Bullheads have both native and introduced populations throughout North America and introduced populations in Europe. Research of Black Bullhead populations has increased in recent years due to their native and introduced ranges having potential detrimental effects on fish communities. Black Bullheads are common throughout Oklahoma and are found in many Close to Home Fishing Ponds (CTHFP), however research in these small impoundments is limited. Therefore, our objective was to estimate abundance and describe population characteristics for two Black Bullhead populations in two Oklahoma CTHFP. We sampled two CTHFP in El Reno, Oklahoma within Canadian County, Southern Hills North (SHN) and Southern Hills South (SHS) with tandem baited hoop nets in June of 2022. To estimate population size, we used the K-pass depletion method. Lapilli otoliths were extracted from fish from each pond, but age and growth statistical analysis were only completed for SHS due to insufficient numbers of fish prohibiting analysis. Black Bullheads from SHS had a fish density estimate of 810 fish/ha, slow growth, ages ranging from 2-7, relatively low mortality (Z = 0.39), and was dominated by stock-sized individuals. The SHN pond had a lower fish density estimate (110 fish/ha) than SHS, fish ages ranging from 1-7, and was dominated by quality-sized individuals. Additional research should focus on understanding angler dynamics, variables that attribute to overabundant populations, and variability of Black Bullhead growth in CTHFP to allow for more effective fisheries management of native and invasive populations.

Introduction

Black Bullheads (*Ameiurus melas*) have native and invasive populations throughout North America and introduced populations throughout Europe (Rutkayová et al. 2013, Copp et al. 2016). They can tolerate poor water quality (i.e., high turbidity, low oxygen quality, high nutrient concentrations, high temperatures) which has aided in their expansion (Copp et al. 2016, Sikora et al. 2022). Black Bullheads are omnivorous and can outcompete native fish assemblages through direct competition, predation, or by negatively impacting water quality (Copp et al. 2016, Snow et al. 2017, Sikora et al. 2021, Montague et al. 2021). Their wide niche-breadth combined with a flexible maturity schedule allows them to attain high population densities, dominate the biomass where they reside, and establish populations outside of their native range (Stuber 1982, Copp et al. 2016, Sikora et al. 2022). Due to Black Bullhead's extensive native range, invasive populations, and robust population characteristics, interest has increased from managers to research their life history and population characteristics (Novomeská and Kovác. 2009, Copp et al. 2016, Sikora et al. 2021, Montague et al. 2021, Montague et al. *in review*). However, research on life history and population characteristics is still lacking compared to other ictalurid species (Channel Catfish [*Ictalurus punctatus*], Blue Catfish [*Ictalurus furcatus*], and Flathead Catfish [*Pylodictis olivaris*]) despite their potential negative effects on fish assemblages and water quality. Therefore, additional research on Black Bullhead populations is critical for managers to better understand how to manage them in both native and introduced ranges.

The Oklahoma Department of Wildlife Conservation (ODWC) manages Close to Home Fishing Ponds (CTHFP; small impoundments < 8.1 ha in size) to provide local-and-accessible fishing opportunities for urban anglers and "R^3" purposes (i.e., recruitment, retention, reactivation; Hinrichs et al. 2020). While most anglers utilizing Oklahoma's CTHFP target Channel Catfish, Crappie (*Poxomis spp*), Largemouth Bass (*Micropterus salmoides*) and sunfish (*Lepomis*) species (Balsman and Shoup 2008, York 2019), Black Bullhead are also commonly found in these ponds throughout the state (OFAA 2022). Black Bullheads inhabiting small impoundments can have a negative effect on sportfish assemblages if not managed, by altering biomass due to their low age-at-maturity, relatively high fecundity, parental care, and ability to alter water quality (Mork et al. 2009, Sikora et al. 2021). Therefore, it is imperative that biologists monitor and manage their populations in CTHFP to prevent their potential negative effects. Furthermore, population dynamics of Black Bullheads in small impoundments are poorly understood, limiting the ability of managers to manipulate populations for the benefit of other sportfish. Researchers must first determine Black Bullhead population characteristics (i.e., demographics and vital rates) to determine the applicability of potential management approaches in small impoundments.

During an electrofishing sampling assessment of sportfish populations on Southern Hills South (SHS) CTHFP in El Reno, Oklahoma, ODWC biologists observed high densities of Black Bullheads. Preliminary age and growth analysis of this population suggested that Black Bullheads in SHS consisted of primarily old fish (up to age-7) at small lengths (≤ 230 mm), which is characteristic of a stunted population (unpublished data, ODWC). This prompted further sampling efforts to better understand the Black Bullhead population in SHS. Biologists also sampled the Southern Hills North (SHN) Black Bullhead population. SHN is a CTHFP adjacently located (< 100 yards away), to compare population characteristics between ponds. Therefore, our objectives were to 1. estimate abundance and fish density (fish/ha) and 2. describe population characteristics (proportional size distribution, growth trajectories, length-weight relationships, length at maturity, mortality of two Black Bullhead populations in two Oklahoma CTHFP (Southern Hills North and South).

Methods

Study Area

Southern Hills South is a 0.3 ha impoundment with 0.32 km of shoreline located in El Reno, Oklahoma in Canadian County (latitude: 35.506520, longitude: -97.960830). Southern Hills North is a 0.81 ha impoundment with 0.4 km of shoreline located in El Reno, Oklahoma in Canadian County (latitude: 35.506520, longitude: -97.960830). Both ponds serve as CTHFP that are managed by the ODWC and support popular sportfish species such as Channel Catfish, Largemouth Bass, and Sunfish.

Study Design

Black Bullheads were collected using tandemly baited hoop nets (25-mm mesh) from both SHN and SHS in June 2022 (typically correlates with Black Bullhead spawning season in Oklahoma, [Montague et al. 2021]). Tandem, baited (Sportsman's Choice Trophy Fish Feed, Multi-species Formula, Cargill Animal Nutrition, Minneapolis, MN) hoop nets were rigged following ODWC standardized Channel Catfish sampling protocols. Specifically, three tandem hoop net sets were fished parallel to shore at depths of 1–3 m for a total of 72 hours, checked, and reset every 24 hours. Each net set consisted of three 3.4-m-long hoop nets (25-mm bar mesh; Miller Net Company, Inc., Memphis, Tennessee) containing seven fiberglass hoops, with the first hoop being roughly 0.8 m in diameter and each following hoop slightly decreasing in diameter toward the cod end. Every net included a throat on the second hoop and a restricted throat on the fourth hoop. Temperature (°C) and dissolved oxygen concentration (mg/L) were recorded with a YSI meter (model Pro 2030, Yellow Springs Instruments, Yellow Springs, OH) just above the bottom at each net set to ensure that dissolved oxygen was ≥ 4 mg/L. Due to the proximity of SHN and SHS being so close, water temperatures (range from 26.9 - 28.4), dissolved oxygen (ranging from 6.3 – 7.4), and secchi depth (2.1 – 2.7 m) measurements overlapped showing no difference between the two ponds.

All fish caught were measured for total length (TL; mm) and weighed (g). Our goal was to collect 20 fish per 10-mm TL grouping for age estimation and sex determination. Every fish was removed from each net 24, 48, and 72 hours after setting to generate a population estimate using the depletion method. Fish kept for age estimation and sex determination were euthanized with a 1:1 ice water slurry (Blessing et al. 2010), placed on ice, and transported to the Oklahoma Fishery Research Laboratory in Norman to be processed. Fish were then re-measured, weighed, sex was determined, and the lapilli otoliths were removed for age estimation. Fish were assigned a maturity status (immature or mature) following methods of Davis and Posey (1958) and Perry and Carver (1972). Mature females were classified if they had developed ovaries that contained eggs (yellow or white in color) or their ovaries were spent (the eggs deposited). Mature males were classified if their testes were enlarged and white in color. Immature Black Bullhead showed no signs of gonadal development and their ovaries and/or testes were barely distinguishable or are readily distinguishable but not developed.

After extraction, both pairs of lapilli otoliths are placed into a uniquely numbered envelope (Montague et al. 2021, Montague et al. *in review*). The otoliths were dried for ≥ 24 hours prior to processing (Secor et al. 1992) and processed similarly to methods of Buckmeier et al. (2002) and Montague et al. (2021). After processing, otoliths were viewed using a stereo microscope (capable of 130x magnification) with a fiber optic filament attached to an external light source to illuminate annuli (Buckmeier et al. 2002, Montague et al. 2021). Two readers estimated the age of the otolith in concert read, however, if the readers disagreed on the estimated age, that otolith was reevaluated at a different time (Hoff et al. 1997). The second otolith's age was estimated if the first otolith's age was unreadable. If both otoliths were unreadable, the fish was removed from the age analysis. Each otolith was read randomly with no reference to length, weight, or sex (Hoff et al. 1997).

Analysis

Black Bullhead population estimates and

probability of capture (*p*) were determined for both ponds with the K-pass depletion method using the removal() function in the FSA package in R (Ogle 2022). Black Bullhead population size structure in both ponds were described with a length frequency histogram (10-mm length bins) of all fish captured. A Fisher's exact test (fisher.test() function, R Core Team 2022, version 1.4.1103) was used to determine if length frequencies from each pond differed ($P \leq 0.05$). Additionally, proportional size distribution (PSD; PSD-stock ≥ 150 mm, PSD-quality ≥ 230 mm, PSD-preferred ≥ 300 mm; Gabelhouse 1984) was calculated using the psdcalc() function in Ogle's Fisheries Stock analysis (FSA) package (2022) in R (R Core Team 2022) to describe each pond's size structure. A simple linear regression was used to describe the relationship between $\log_{10}$(weight):$\log_{10}$(length). The relationship of Black Bullhead length to weight was also used to evaluate fish condition by calculating relative weight (Wr) using the wrAdd() function in the FSA package (Ogle 2022) in R (R Core Team 2022).

Age and growth statistical analysis was only completed for SHS because the SHN had insufficient numbers of fish caught, prohibiting age and growth analysis. Therefore, the remaining analysis was completed for only SHS. A logistic regression model was used to determine the relationship between maturity at age for male and female Black Bullhead using binary variables (0 = immature, 1 = mature). Mean length at age was calculated for male and female Black Bullhead. These data were then log transformed to linearize the relationship, and differences in growth between sexes were tested using analysis of covariance (ANCOVA) with the aov() function in R (R Core Team 2022). Because prior analysis of growth between sexes was similar ($F_{1,108} = 1.29$, $P = 0.26$), all fish were combined to estimate growth rates using a von Bertalanffy growth model.

Growth trajectories and instantaneous mortality rates (Z) for Black Bullheads were estimated using a von Bertalanffy growth model fit to total length and age estimates using the Fisheries Stock Analysis R package (Ogle 2022, R Core Team 2022). Instantaneous mortality rates and annual survival (S) were estimated using the Chapman-Robson method with the chapmanRobson() function in the FSA package (Ogle 2022) in R (R Core Team 2022, version 1.4.1103). Black Bullhead < age-2 were not fully recruited to the sampling gear, so they were removed from mortality analysis.

Results

Southern Hills South

A total of 237 Black Bullhead were collected from SHS, with a high fish density estimate of 810 fish/ha. (Table 1). Of the fish collected, 165 fish were kept for age estimation and population assessment. Fish used for age analysis ranged from age-2 to age-7 and 149 - 247 mm TL. More male (55.9%) than female (44.1%) fish were represented in the sample. Both female and male Black Bullhead reached 100% maturity by age-7 (Figure 1). The earliest that

Table 1. Population estimates (95% CI) using the K-Pass depletion method for Black Bullheads caught with tandem, baited hoop nets in Southern Hills South and Southern Hills North Ponds in El Reno, Oklahoma. Fish density (fish/ha) estimates (95% CI) and capture probability (p; 95% CI) for tandem, baited hoop nets fished for 72 hours (checked and fish removed every 24 hours) are also shown.

System	Size (ha)	Population Estimate	Density (fish/ ha)	Capture probability
Southern Hills South	0.3	243 (236 - 250)	810 (787 - 833)	0.7 (0.64 - 0.77)
Southern Hills North	0.81	89 (74 - 104)	110 (91 - 128)	0.48 (0.32 - 0.64)

a Black Bullhead reached maturity was age-2, and 50% of all fish reached maturity by age-5. By age-7, 100% of all male fish have reached maturity. The earliest that a female reached maturity was age-2, 50% of all female fish reached maturity by age-4, and 100% by age-7.

Length frequencies derived from the catch rates of SHS and SHN differed significantly (Fisher's exact P value ≤ 0.01, Figure 2). The SHS Black Bullhead population was dominated by stock sized ($\geq$ 150 mm) fish (Table 2; Figure 2). As a result, PSD was low (PSD = 4), and no fish reached PSD-Q size (< 300 mm). Stock length fish had a mean age of 3.83, and quality length fish had a mean age of 5 (Table 1). The weight-length relationship ($\log_{10}(W) = 2.73(\log_{10}(TL)) - 4.26$) was significant ($r^2 = 0.84$, $P < 0.01$) resulting in a mean Wr of 84.3 (Figure 3, Table 2).

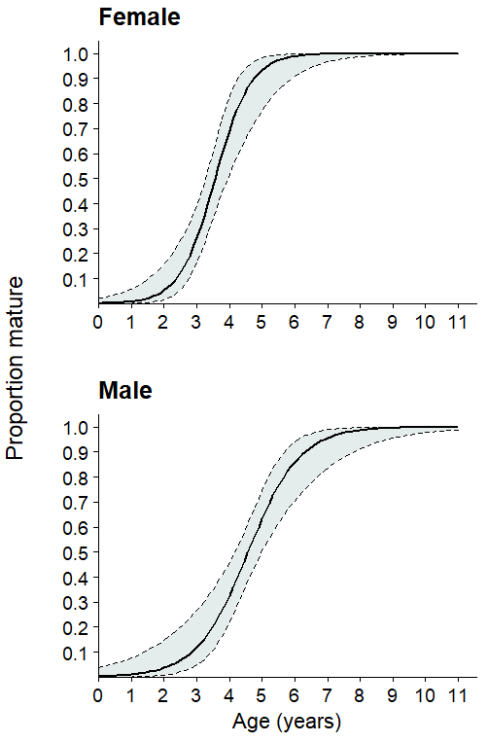

Figure 1. Results of logistic regression analysis displaying the proportion of mature female (top) and male (bottom) Black Bullheads by age caught from Southern Hills South Pond, El Reno, Oklahoma using tandem baited hoop nets. Grey dashed lines represent 95% confidence intervals.

The Von Bertalanffy growth model indicated that Black Bullhead approach L_∞ ($L_\infty = 224.5$ mm TL; predicted maximum total length) slowly (k = 0.377; Figure 4). The estimated instantaneous mortality was 0.39 (0.32 - 0.47) and the annual survival rate was 67.5%.

Southern Hills North

A total of 78 Black Bullheads were collected from SHN with a lower fish density estimate (110 fish/ha) than SHS (Table 1). Of the fish collected, 35 were kept for age estimation and population assessment. Fish used for age analysis ranged from age-1 to age-7 and 130 - 311 mm TL. More male (54.2%) than female (45.8%) fish were represented in the sample.

This population was dominated by quality sized ($\geq$ 230 mm) Black Bullhead (Table 2; Figure 2). As a result, PSD was higher than SHS (PSD = 72), and some fish reached PSD-P size (8; $\geq$ 300 mm). Stock length fish had a mean age of 3.83, and quality length fish had a mean age of 5 (Table 2). The weight-length relationship ($\log_{10}(W) = 2.69(\log_{10}(TL)) - 4.12$) was significant ($r^2 = 0.96$, $P < 0.01$;) resulting in a mean W_r of 85 (Figure 3, Table 2).

Discussion

Southern Hills South had a high abundance of Black Bullheads (810 fish/ha) with a fish density estimate nearly 8 times the estimate at SHN (110 fish/ha). Our results from the SHS population reflect similarly to other studies showing that Black Bullheads can have high abundances in small impoundments (Sikora et al. 2021, Sikora et al. 2022). The high densities in SHN may be the result of density-dependent factors such as interspecific competition over resources, lack of predators, or various environmental factors such as poor water quality (Shelley and Modde 1982, Anderson et al. 2016, Copp et al. 2016). Identifying and understanding the environmental and biological

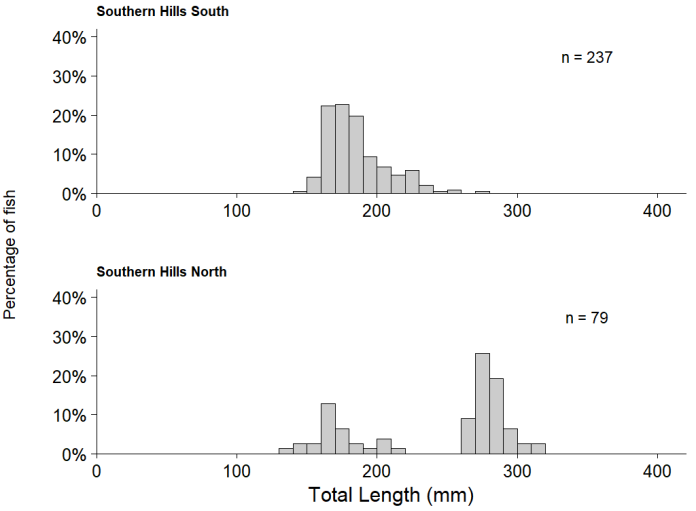

Figure 2. Length Frequency histogram (10-mm bins) of Black Bullheads caught using tandem, baited hoop nets from Southern Hills South and Southern Hills North Ponds, El Reno, Oklahoma.

factors that lead to overabundant Black Bullhead populations in small impoundments, specifically CTHFP, is warranted and will allow biologists to better manage this species and the specific system. Future research should examine the effects of Black Bullhead removal to aid in the management of overabundant populations in CTHFP as Black Bullhead removal has been shown to benefit native fish assemblages. For example, Barabe (2021) found that removal efforts of Black Bullhead in a California stream improved the abundance and recruitment of Coastal Rainbow Trout *Oncorhynchus mykiss irideus*. Sikora et al. (2021) found that Black Bullhead removal in Wisconsin lakes resulted in an increased abundance of Walleye *Sander vitreus* and Yellow Perch *Perca flavescens* and increased the diversity of the fish community. Additional research should also examine appropriate removal target rates to have the desired positive effect on the fish community. Further understanding of how Black Bullhead removal impacts the ecosystem in small impoundments will be essential to managing their overabundant populations.

Black Bullheads in SHS had slow growth, stunted population characteristics, and a different size structure compared to Black Bullheads in SHN. Due to insufficient sample

Table 2. Proportional size distribution (PSD, 95% CI), mean age (range), and mean relative weight (W_r; 95% CI) of Black Bullheads by size class from Southern Hills South and Southern Hills North Ponds in El Reno, Oklahoma.

System	Size Category	*n*	PSD Value	Mean Age	Wr
Southern Hills South	Sub-Stock (< 150 mm)	1	N/A	1.88 (1 - 3)	78.2
	Stock (≥ 150 mm)	227	96 (93- 98)	3.83 (2 -7)	84.5 (82.9 - 86.1)
	Quality (≥ 230 mm)	9	4 (2 – 7)	5 (5 - 5)	80.1 (75.3 - 84.9)
	Overall	237	-	3.59 (1 -7)	84.3 (82.8 - 85.8)
Southern Hills North	Sub-Stock (< 150 mm)	3	N/A	1 (1 - 1)	131 (88.6 - 173.4)
	Stock (≥ 150 mm)	24	28 (18 - 40)	1.75 (1 - 3)	91.4 (86.8 - 96)
	Quality (≥ 230 mm)	47	72 (60 - 82)	5.1 (4 - 6)	80 (77.1 - 82.9)
	Preferred (≥ 300 mm)	6	8 (3- 17)	6. 25 (6 - 7)	79.5 (64.9 - 94.1)
	Overall	78	-	4.55 (1 - 7)	85 (81.6 - 88.4)

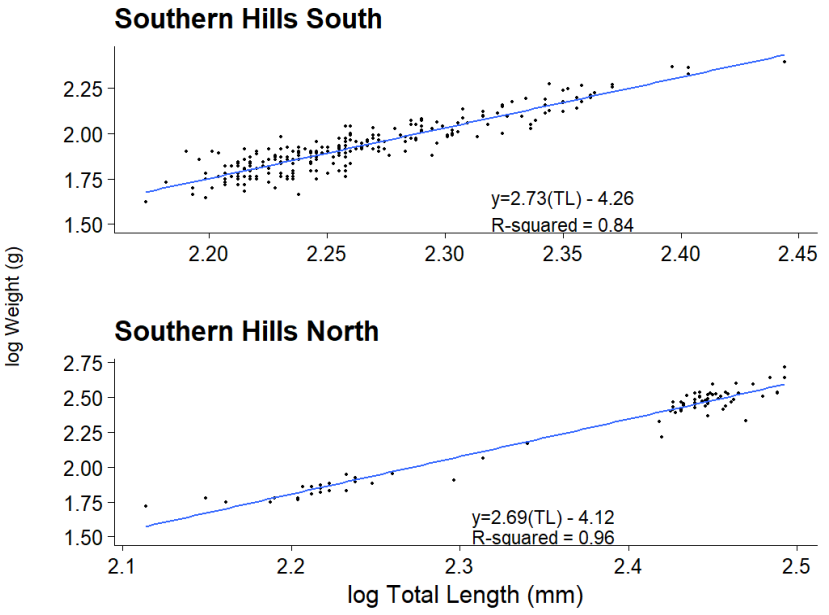

Figure 3. Weight-length relationship for Black Bullheads collected from Southern Hills South and Southern Hills North Ponds in El Reno, Oklahoma. The logarithmically transformed weight-length equations are shown for each pond.

size for age and growth analysis, we were unable to quantify age and growth (i.e., Von Bertalanffy growth curve) for SHN, however, the length frequencies were significantly different between ponds. Furthermore, the mean length at various PSD size categories at SHS suggests they had dissimilar growth rates. Our results depict the variability of Black Bullhead growth amongst populations and are consistent with what has been documented in the literature. For example, Mork (2009) found that Black Bullhead populations in Iowa varied and hypothesized that fish in poorer water quality (high nutrient concentrations and low water clarity) had higher growth rates than those with better water quality. In South Dakota Lakes, Hanchin (2002a) found that Black Bullhead growth was highly variable, and fish were more likely to overpopulate in shallow, productive lakes, resulting in slow growth. Hanchin (2002a) also found that growth was inversely related to size structure of predators suggesting a predator effect on Black Bullhead growth. Similarly, Sikora et al. (2022) found in Howell Lake, Wisconsin Black Bullheads exhibited fast growth rates due to the size structure of predators. Additionally, their initial analyses do not support the common ideology that black bullheads are suppressing sport fish populations through direct predation, but possibly diet overlap (Sikora et al. 2022). Black Bullhead typically grow faster where they are introduced in European countries compared to their native populations in North America (Copp et al. 2016). Future research should examine the statewide growth of Black Bullhead populations and how environmental and biological characteristics (e.g., lake size, productivity, predators) may impact growth rates.

Tandem baited hoop nets efficiently sampled and provided population density information for managing Black Bullhead in both CTHFP in our study. However, studies that examined the use of tandem, baited hoop nets with 25-mm mesh while sampling Channel Catfish suggest this gear may underrepresent smaller size classes of catfish, suggesting that juvenile Channel Catfish may not be fully recruited to the gear. (Montague et al. *in-press*, Tyszko 2021). If we assume this bias is true for Black Bullheads, then tandem baited hoop nets with 25-mm mesh may

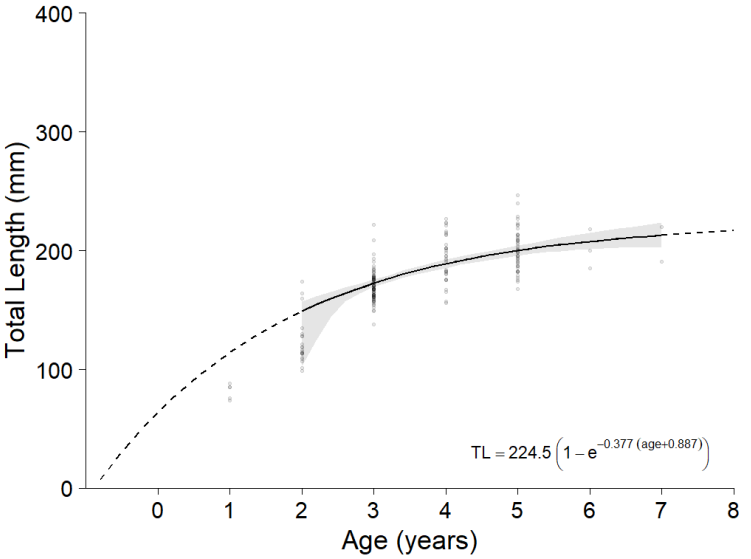

Figure 4. Von Bertalaffy growth curve calculated from 165 lapilli otolith age estimates for Black Bullheads collected using tandem, baited hoop nets from Southern Hills South Pond, El Reno, Oklahoma.

not effectively sample juvenile fish. Sampling juvenile Black Bullheads would be important for gaining information on recruitment and aiding in their overall management. Additionally, past research has sampled Black Bullhead populations with a variety of gears including fyke nets, trap nets, gill nets, hoop nets, and electrofishing (Hacnhin et al. 2002, Cucherousset et al. 2006, Mork et al. 2009, Snow et al. 2017, Sikora et al. 2021). Hacnhin et al. (2002) found that trap nets and experimental gill nets provided similar population size structure indices, however, trap nets may provide a better index of relative abundance for population monitoring. Cucherousset et al. (2006) found significant differences in length frequencies between trap nets and electrofishing for Black Bullheads, indicating a potential size bias between gears. Due to the discrepancies in which gear to use to sample Black Bullheads accurately, precisely, and efficiently, future research should evaluate the various gear types. This will allow managers to develop a standardized sampling protocol that most effectively and accurately evaluates these populations and allow for the use of catch-per-unit effort (CPUE) as a density index in reservoirs, increasing our understanding of population variation between reservoirs.

Close to Home Fishing Ponds are important for anglers in Oklahoma, providing fishing opportunities to urban areas and facilitating angler recruitment and reactivation in the face of declining license sales (Balsman and Shoup 2008). However, Black Bullhead populations can have detrimental effects on small impoundments and our results depict that the overcrowded and stunted population in SHS could potentially have a negative effect on sportfish populations and water quality in ODWC CTHFP. Therefore, management of CTHFP, especially in urban areas is needed. This study provides information on the population characteristics of two contrasting Black Bullhead populations and provides insights into future Black Bullhead research in CTHFP. Additional work should focus on understanding angler dynamics in CTHFP as this will be beneficial to managers trying to develop strategies that match angler preference, in turn providing quality fishing experiences to Oklahoman anglers. Lastly, identifying and understanding variables that attribute to overabundance and growth variability within and between Black Bullhead populations will allow for more effective fisheries management of both native and invasive populations in small impoundments.

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Morphometric Analysis of the Harris Mud Crab (*Rhithropanopeus harrisii*) in Lake Texoma

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Abstract: Harris mud crab, *Rhithropanopeus harrisii,* is a small crab native to the brackish waters of the Atlantic coast of North America. Today, *R. harrisii* exists well beyond its native range and in a wide variety of habitats, including freshwater reservoirs. *R. harrisii* was discovered in Lake Texoma in 2008 and is thought to have originated from Texas estuaries. The objectives of this study were to gather and describe the morphometrics of *R. harrisii* from Lake Texoma and document sex ratio, carapace size distributions, and handedness. Individuals were collected from August 2019 to August 2021 from six sampling sites located along the Oklahoma side of Lake Texoma. Various morphometrics were recorded from 1,326 crabs. Most crabs in the study were right-handed and they were smaller than crabs reported from other locations. Small size could be result from stressful conditions or early fatality. While there was no significant difference in average carapace size between males and females, males made up the highest percentage of large size classes. Males were also more numerous in the population and often attained larger claw sizes than the females. This is thought to be beneficial in intraspecific competition for females.

Introduction

Harris mud crab, *Rhithropanopeus harrisi*, is a small decapod crustacean belonging to the family Panopeidae (formally Xanthidae) (Marco-Herrero et al. 2014). Harris mud crabs possess a short abdomen hidden under the thorax and four pairs of walking legs (Ryan 1956). The chelipeds are often curved and unequal (Hegele-Drywa et al. 2014). *R. harrisii* are distinguished by a subquadrate carapace and four pairs of anterolateral teeth (Ryan 1956, Turoboyski 1973).

Harris mud crabs originate from the Atlantic coast of North America, ranging from Miramichi Estuary, Canada, to Veracruz, Mexico (Boyle Jr. et al. 2010). Today, *R. harrisii* occurs far beyond its native range, and is described as one of the most widely distributed brachyuran invaders (Grosholz & Ruiz 1996, Roche & Torchin 2007). The crabs are found in 82 locations worldwide (Fowler et al. 2013), including several US reservoirs throughout Texas and Oklahoma (Boyle Jr. et al. 2010, Patton et al. 2010).

In 2008, Patton et al. (2010) collected 24 crabs from Lake Texoma and documented the furthest inland occurrence of Harris mud crabs to date. Virtually no research was conducted on the Texoma population until Huebner et al. (2021) performed genetic analyses and determined the founding individuals were most likely from Texas estuaries. The purpose of the following project is to describe the morphometrics of this Harris mud crab population in Lake Texoma.

Methods

R. harrisii specimens were collected monthly during the warm seasons and every other month during the cool seasons from August 2019 to August 2021. Six sites were established on the Oklahoma side of Lake Texoma. At each site, two 1m^2 plots were randomly selected within a submerged transect along the shoreline. A 13.5 cm by 18.0 cm scoop was used to dredge the entire benthic area within the plots and the

substrate was washed through sieve buckets having a 0.5 mm mesh bottom. Visual surveys were conducted in addition to dredging if fewer than 10 crabs were found in the plots. Specimens were preserved in 70% ethanol and returned to the laboratory at the University of Central Oklahoma. Water temperature, salinity, and specimen count from each site were recorded.

A stereo microscope was used to determine sex and perform morphometric analyses. The following measurements to the nearest 0.01 mm were taken using a micrometer: carapace length (CL), from between eyes to the posterior margin of the carapace; carapace width (CW), greatest distance across the carapace; claw lengths (LCL, RCL), including the dactyl and propodus; and claw widths (LCW, RCW), left and right respectively. Crabs with unequally sized claws were labeled as "left-handed" or "right-handed," as commonly used in decapod research (Abby-Kalio 2008, Czerniejewski 2009). The data obtained was tested for normality using the Shapiro-Wilk test and then subjected to either the appropriate chi-square or Mann-Whitney tests.

Results and Discussion

A total of 1,326 crabs were collected in 18 sampling trips. Of the crabs analyzed, 420 (31.7%) were female, 524 (39.5%) were male, 341 (25.7%) were immature, and 41 (3.1%) were too damaged to determine sex. Results from a two-proportion z-test indicated that the number of males and females differed from an expected 1:1 (male:female) ratio ($p<0.001$). Male-dominated sex ratios have been observed in other populations of Harris mud crab, including a 1.3:1 in the Dead Vistula (Normant et al. 2004) and 2.4:1 in Vistula Lagoon (Rychter 1999 as cited in Normant et al. 2004).

The range of carapace width was larger in males than in females. The largest male measured 17.85 mm wide, while the largest female was 15.6 mm (Table 1). Although *R. harrisii* are sexually dimorphic and males usually attain larger sizes (Czerniejewski & Rybczyk 2008, Hegele-Drywa et al. 2014), there was no significant difference between the mean size of males and females according to a Mann-Whitney U Test (U = 111,722, p = 0.4455). Average carapace widths were also similar between the sexes when confidence intervals were compared (Fig. 1). To analyze size distribution of males and females, the individual crabs were assigned to 1 mm-wide carapace size classes and compared using chi-square tests. The test results showed multiple size classes with an unequal sex ratio of males and females. The following carapace width (CW) size classes differed significantly from expected (1:1): 6.00-6.99mm CW ($\chi2 = 6.20$, df = 1, p = 0.0128), 10.00-10.99mm CW ($\chi2 = 5.26$, df = 1, p = 0.0218), 11.00-11.99mm CW ($\chi2 = 6.53$, df=1, p = 0.0106), 12.00-12.99mm CW ($\chi2 = 9.00$, df = 1, p = 0.0028), and 13.00-13.99mm CW ($\chi 2= 7.14$, df = 1, p = 0.70546) (Fig. 2). All size classes with disproportionate sex ratios

Table 1. Mean carapace width, carapace length, and major chela length of the 524 male and 420 female *Rhithropanopeus harrisii* adults from Lake Texoma, Oklahoma.

	Carapace width (mm)		Carapace length (mm)		Major chela length (mm)	
	Range	Mean ± std	Range	Mean ± std	Range	Mean ± std
Males (524)	4.00 - 17.85	7.51 ± 2.60	3.00 - 12.6	5.71 ± 1.90	1.65 - 14.70	5.49 ± 2.44
Females (420)	4.00 - 15.60	7.24 ± 2.16	3.15 - 11.65	5.48 ± 1.53	1.65 - 10.15	4.61 ± 1.47

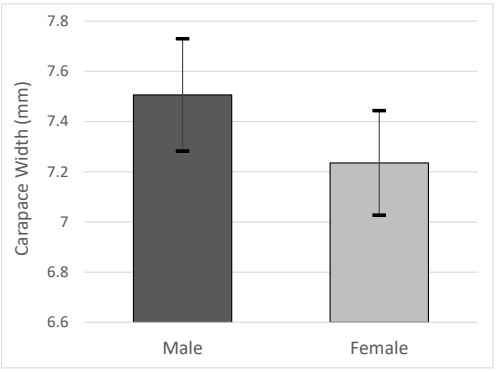

Figure 1. Carapace width (mm) averages for adult male and female *R. harrisii* crabs collected throughout the entire sampling period. Error bars display 95% confidence intervals.

were skewed towards male. Males also made up the majority of several large size classes (>10.00mm) (Fig. 2). As stated earlier, it is common in brachyuran crabs for males to reach larger sizes, and the trend has been documented in *R. harrisii* from Poland (Czerniejewski & Rybczyk 2008). However, there was a notable difference in overall carapace size between the crabs from Lake Texoma and the European crabs. The Lake Texoma crabs were smaller, averaging 12mm narrower than the Polish crabs. Additionally, *R. harrisii* in other locations are often larger than the maximum size (17.85 mm) observed in Lake Texoma (Turoboyski 1973, Normant et al. 2004, Czerniejewski & Rybczyk 2008). For example, Ryan (1956) reported a crab as large as 26.1mm, and Turoboyski (1973) determined the maximum size for the species is 27 mm.

A possible explanation for the reduced body size is the stressful conditions in Lake Texoma. Lake Texoma is a freshwater reservoir with low salinity (<1 PPT) and wide-ranging seasonal changes in water temperature (9-35 °C). Because Harris mud crab is native to brackish coastal waters, these conditions in

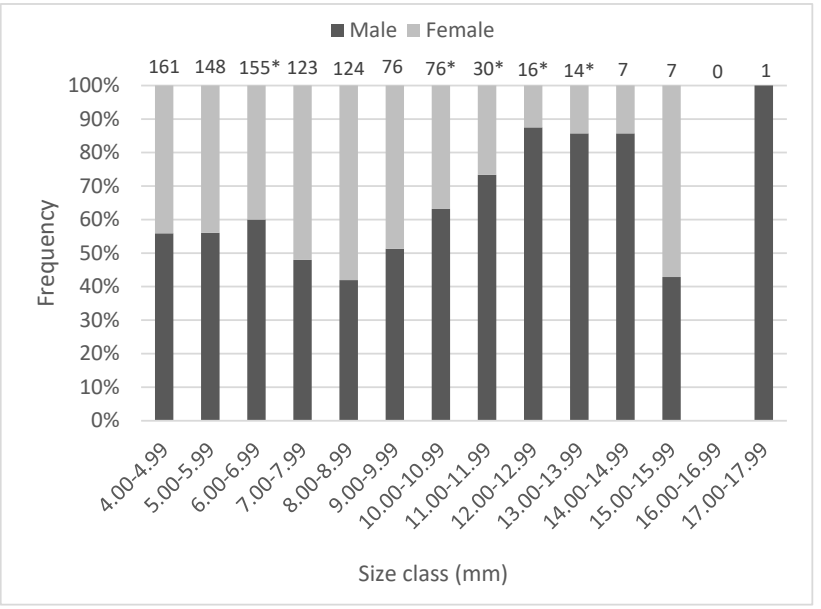

Figure 2. Sex ratio of *R. harrisii* adult male and females by carapace width (mm) size class, sampled from August 2019 to August 2021 from Lake Texoma, Oklahoma. Crabs larger than 4.0mm are considered adults. The sample size is denoted at the top of each class and an asterisk (*) indicates statistical difference from the expected 1:1 (male:female) sex ratio in each size class. Size classes with fewer than 2 observations were omitted from statistical analyses due to small sample sizes.

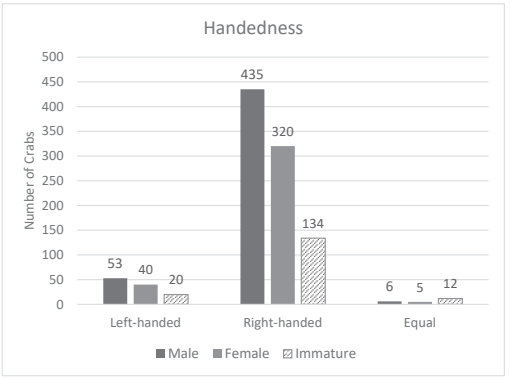

Figure 3. Bar graph showing the number of male, female, and immature *R. harrisii* crabs displaying dominant-right, dominant-left, or equally-sized claws

Lake Texoma likely hinder the growth and development of Harris mud crab. Another factor to consider is fatality. Without knowing the age of the crabs, it is difficult to determine whether the crabs are growing slower than expected or dying before a larger body size is reached. In addition, a constantly fluctuating water level in the reservoir can affect the survival of Harris mud crabs. Two massive fatality events were witnessed as a result of rapidly dropping lake levels leading to hundreds of crabs being stranded and desiccating on the shoreline at the sampling sites. Fatality events likely occur more often than observed, impacting the number of crabs surviving multiple years and attaining larger sizes.

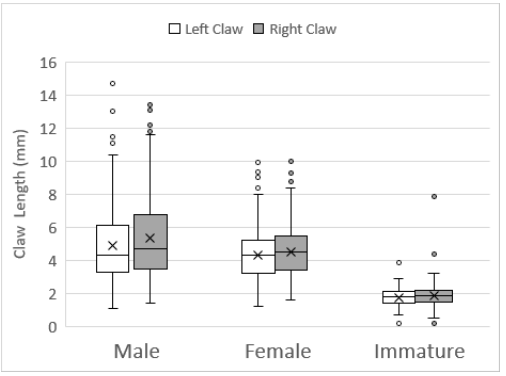

Figure 4. Left and Right claw length (mm) ranges for *R. harrisii* male, female, and immature crabs collected from Lake Texoma, Oklahoma throughout the 2019-2021 sampling period. Bars indicate the five-number summary of the data including the minimum, first quartile, median, third quartile, and maximum. Means are represented with an "x."

Results from a chi-square test show an unequal distribution of claw length ($\chi 2$ = 1329.65, df = 2, $p<0.001$). Heterochely was observed in male, female, and immature crabs (Fig. 3). Approximately 86.73% of crabs were right-handed, 11.02% were left-handed, and 2.24% of crabs were homochelous. These results are consistent with the literature stating Harris mud crabs are right-handed (Hegele-Drywa et al. 2014). When a Mann-Whitney U test was used to compare the dominant claws of adult male and female crabs (>4.0mm), claw lengths differed significantly ($U=61605$, $p<0.001$). Despite majority of crabs having a dominant right claw, the largest claw was a male's left claw measuring 14.7mm long (Table 1). However, this measurement was an outlier far beyond the upper quartile range (Fig. 4). Lastly, a positive linear relationship between major chela length (mm) and carapace width (mm) was observed in males, females, and immature crabs (Fig. 5). Males displayed a higher rate of change as major chela length (mm) and carapace width (mm) increased (Fig. 5). This could be because larger claws are advantageous for males in intraspecific competition for females (Jesse 2001, Czerniejewski & Rybczyk 2008).

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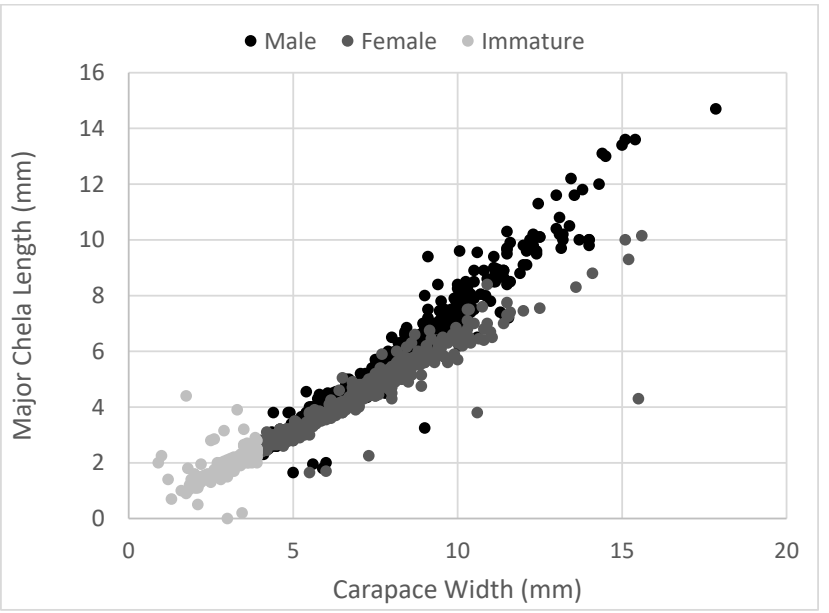

Figure 5. Carapace width (mm) versus major chela length (mm) for male, female, and immature *R. harrisii* crabs collected from Lake Texoma, Oklahoma during the 2019-2021 sampling period.

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First Record of *Aethycteron nigrofasciatus* (Harrises, 1962) (Monogenea: Dactylogyridae) from the Dusky Darter, *Percina sciera* (Swain) (Perciformes: Percidae), in Oklahoma

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Abstract: Virtually nothing is known regarding parasites of the Dusky Darter, *Percina sciera*. The only parasite reported to date from this host is a piscicolid leech. Here, we report *Aethycteron nigrofasciatus* on the gills of this host from the Little River Drainage of the Red River System of southeastern Oklahoma. Morphometric characters of *A. nigrofasciatus* from *P. sciera* from the state conform closely to those previously reported from its only other known host, the Blackbanded Darter, *Percina nigrofasciata* in Mississippi. Both possess a unique curved male copulatory organ with a spine on the distal half of the shaft and a flared distal end. *Aethycteron nigrofasciatus* on *P. sciera* represents a new host record and the first geographic distributional record of this monogenean from west of the Mississippi River.

Introduction

One of the most plentiful and ever-present groups of non-game fishes occurring in Oklahoma are the darters (Perciformes: Percidae: Etheostomatini), numbering about 32 species (Miller and Robison 2004). These active and multicolored fishes inhabit nearly all of the state's streams and rivers as well as many other watersheds. They are important ecologically in playing a role in the trophic structure of stream ecosystems (Cummins 1980). Yet, we know very little about the parasites of many darters in the state.

One species, the Dusky Darter, *Percina sciera* (Swain), is widely distributed in the Mississippi River basin from Ohio and West Virginia to eastern Illinois and south to Louisiana, and Gulf drainages from the Alabamba River in Alabama west to the Colorado River in Texas (Page and Burr 2011). In Oklahoma, *P. sciera* is found in the Red River and Poteau river systems and west to Rainy Mountain Creek, Kiowa County (Miller and Robison 2004). It primarily inhabits fast gravel riffles and runs of small to medium rivers, often associated with boulders, emergent vegetation, or fallen trees and brush. This darter is an invertivore that feeds mainly on small aquatic insects and crustaceans in riffles (Page and Smith 1970; Kuehne and Barbour 1983; Miller and Robison 2004; Robison and Buchanan 2020).

Although information is available on the natural history of *P. sciera* (Page and Smith 1970), very little is known about its parasites. Aside from a leech, *Myzobdella reducta* (Meyer,

1940), reported from *P. sciera* from Minnesota (Erickson 1978), nothing else is known of the parasitofauna of *P. sciera* (Hoffman 1999). This paper reports the presence of a monogenean on the gills of *P. sciera* in southeastern Oklahoma.

Methods

During stream surveys conducted between June 2014 and October 2017, three individual *P. sciera* (77, 77, and 90 mm total length [TL]) were collected from Yashau Creek, McCurtain County. The fish were collected by seine or backpack electrofisher and placed in containers with aerated creek water. We followed accepted guidelines for the use of fish in research per the American Fisheries Society (AFS 2014); specimens were overdosed by immersion in a concentrated chloretone (chlorobutanol) solution and preserved in 10% (v/v) neutral-buffered formalin. Gills were removed from the fish and examined for parasites under a stereomicroscope at 20–30×. Parasites (n = 2) were picked from the gills of a single host with minute needles. One was placed in glycerin as a temporary mount, and the other was mounted as a permanent slide in Grey and Wess medium stained with Gomori's trichrome (Kritsky et al. 1978). Observations were made with an Accuscope® 3000LED series phase contrast microscope (Accu-Scope Inc., Commack, New York). Measurements were made from digital images taken with a camera mounted on the microscope of sclerites, in micrometers (µm), as presented by Beverley-Burton and Suriano (1980) and Suriano and Beverley-Burton (1982). Prevalence and intensity were calculated according to Bush et al. (1997).

A voucher parasite specimen was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Voucher specimens of hosts were re-deposited in the Eastern Oklahoma State College Vertebrate Collection, Idabel, Oklahoma.

Results

A single *P. sciera* was found to harbor a monogenean that is listed below in annotated style as follows:

Monogenea Carus, 1863

Dactylogyridae Bychowsky, 1933

***Aethycteron* Suriano and Beverley-Burton, 1982**

Type-host and type locality: Mueller (1938) described *A. malleus* as *Cleidodiscus malleus* from Logperch, *Percina caprodes* (Rafinesque), and Blackside Darter, *Percina maculata* (Girard), from Chautauqua Lake, Chautauqua County, New York. Although Mueller (1938) did not explicitly establish a type host, he listed *P. caprodes* first (page priority) and provided line drawings only from that host. Thus, we recognize *P. caprodes* as the type host of *A. malleus* in spite of Suriano and Beverley-Burton (1982) providing a comprehensive redescription based on specimens from *P. maculata* from Ontario, Canada. This monogenean is also known from specimens from Manitoba, Canada (see Lubinsky and Loch 1979).

***Aethycteron nigrofasciatus* (Harrises, 1962)**

Type-host and type locality: Percina nigrofasciata Agassiz; Mississippi: Marion County, Foxworth Creek, 4 mi (6.4 km) N of Foxworth (Harrises 1962).

Other reported host and locality: P. nigrofasciata; Mississippi: Perry County, Thompson's Creek, 5 mi (8.0 km) S of Richton (Harrises and Vickery 1970).

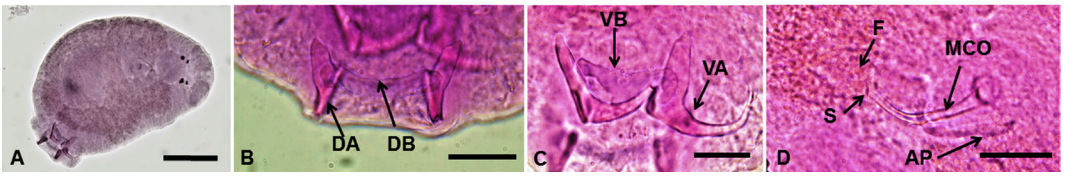
Host and locality (*present study*)*: Percina sciera*; Oklahoma: McCurtain County, Yashau Creek just S of Broken Bow off US 70, Little River Drainage (34°01'8.0004"N, 94°45'24.6996"W).

Deposited material: HWML 216914; 1 slide voucher.

Prevalence and intensity: 1 of 3 (33%), 2 worms.

Site of infection: Gills.

Comparative Description (Figs. 1A–D)



Figures 1A–D. ***Aethycteron nigrofasciatus*** **(HWML 216914). A. Whole mount (ventral). Scale bar = 50 µm. B. Haptor showing dorsal anchor (DA) and dorsal bar (DB). Scale bar = 20 µm. C. Haptor showing ventral anchor (VA) and ventral bar (VB). Scale bar = 20 µm. D. Male copulatory organ (MCO) and accessory piece (AP); MCO has small spine (S) in distal portion and flared distal end (F). Scale bar = 20 µm.**

With characters of the genus *Aethycteron* as diagnosed by Suriano and Beverley-Burton (1982) and Beverley-Burton (1984). Body (n = 2, based on formalin-fixed contracted specimens) 264–283 long × greatest width 188–210. No peduncle observed due to contracted body. Haptor 40–51 long × 101–107 wide. Cephalic lobes poorly developed, cephalic glands on lateral margins of pharyngeal region. Two pairs of pigmented light receptors, anterior pair smaller and closer apart than posterior pair. Pharynx ovate, 47–48 long × 36–38 wide. Gut smooth and confluent posteriorly. Two pairs of anchors; composed of solid base with short deep root, elongate superficial root, solid elongate, blade-like shaft curving to a sharp point; similar in shape, dorsal pair slightly smaller than ventral pair. Dorsal anchor 33–36 long (measurement "a" of Suriano and Beverley-Burton (1982) - distance from tip of superficial root to curve of blade); greatest width of base 20–22. Ventral anchor 35–36 long (measurement "a" of Suriano and Beverley-Burton (1982)); greatest width of base 27–30. Dorsal bar broadly curved with knobs on each end; 37–39 long. Ventral bar broadly yoke-shaped with wide medial posterior prong and large knobs on each end**;** 31–33 long. Fourteen hooks (7 pairs), similar in size and shape. Each hook composed of solid base (not observed in many of the hooks), solid slender shaft, sickle-shaped termination provided with opposable piece. Hook length 11–13. Copulatory complex composed of male copulatory organ (MCO) and sclerotized accessory piece. Male copulatory organ with lightly sclerotized base bearing slender, curved tubular shaft with a short spine on distal half of shaft and greatly flared distally, total length 62–70. Accessory piece solid, slender, sclerotized ribbon, 25–32 long. Testis post ovarian. Vagina not observed. Vitellaria distributed from pharynx to haptor.

Remarks: The morphological characteristics and measurements of the sclerites of *A. nigrofasciatus* parasitizing *P. sciera* from Oklahoma conform with the descriptions from *P. nigrofasciata* in Mississippi by Harrises (1962) and Harrises and Vickery (1970). The small hooked spine located in the distal half and the large flared distal end of a curved MCO of *A. nigrofasciatus* (Fig. 1D) are unique among species of *Aethycteron*. This is the first record of *A. nigrofasciatus* from *P. sciera* and from Oklahoma.

Discussion

The two known hosts of *A. nigrofasciatus* (*P. nigrofasciata* and *P. sciera*) are fairly closely related members of a clade traditionally recognized as the subgenus *Hadropterus* Agassiz (Near 2002; Near et al. 2011). Nine of the 13 (69%) described species of *Aethycteron* have been reported from only a single host, three (23%) from two hosts, and one (8%) from three hosts (Suriano and Beverley-Burton 1982; Beverley-Burton 1984; Hoffman 1999; McAllister et al. 2016, 2017; Cloutman and McAllister 2017; Million and Stallsmith 2019), indicating a fairly high degree of host specificity. This seemingly high host specificity, coupled with the apparent trend of a species or closely related species of *Aethycteron* parasitizing closely related hosts, may indicate considerable coevolution or host switching limited mainly to closely related hosts (Cloutman and McAllister 2017). However,

research concerning *Aethycteron* is in its infancy, and any phylogenetic inferences should be viewed with caution at this time.

As only 13 species of darters, representing 5% of the approximately 250 (including only 4 [10%] of 40 *Percina*) presently described (Near et al. 2011), have been reported as hosts for species of *Aethycteron*, much more descriptive work is necessary before diversity, host specificity, and definitive host-parasite phylogenies can be determined.

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First Report of *Haemogregarina* sp. (Apicomplexa: Haemogregarinidae) from Razor-Backed Musk Turtle, *Sternotherus carinatus* (Testudines: Kinosternidae), from Oklahoma, with a Summary of Hematozoans from the Family Kinosternidae

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Abstract: Generally, little is known regarding the hematozoan parasites of turtles in Oklahoma. Here, we report a *Haemogregarina* sp. from one of two razor-backed musk turtles, *Sternotherus carinatus*, collected from McCurtain County. The host possessed a single infected erythrocyte containing a bean to ovoidal-shaped gamont. Although *S. carinatus* has been previously reported, albeit with very limited information with a *Haemogregarina* sp. from Texas, this is the first time a *S. carinatus* from Oklahoma has been reported with a hematozoan to include a photomicrograph and information about the infection. We also provide a summary of the hematozoans known from the family Kinosternidae, to date.

Introduction

The razorback musk turtle, *Sternotherus carinatus* (Gray) is a medium-sized kinosternid with a prominent, sharp mid-dorsal carapace keel, that ranges from the Pascagoula River System of southeastern Mississippi and Alabama west to the Brazos River Basin of eastern Texas and north to the southern Ouachita uplift, the West Gulf Coastal Plain, and Delta of Arkansas and southeastern Oklahoma (Iverson 1979; Powell et al. 2016). In Oklahoma, *S. carinatus* had been reported from eight counties of the southeastern part of the state (Sievert and Sievert 2021). It occurs in slow-moving rivers and streams, oxbows, and swamps, including habitat with abundant aquatic vegetation and soft substrate (Ernst and Barbour 1972). This turtle feeds almost exclusively on mollusks but will also opportunistically take insects, carrion, and aquatic vegetation (Trauth et al. 2004).

Haemogregarines are apicomplexan blood-inhabiting parasites with an obligatory heteroxenous life cycle. In the life cycle, asexual multiplication occurs in a vertebrate host whereas sexual reproduction occurs in a hematophagous invertebrate vector (Telford 2009). They are omnipresent in all types of vertebrates, from fishes to mammals. Species of *Haemogregarina* infects lower vertebrates as intermediate hosts and various leeches serve as definitive hosts. When data on their life cycle stages is unknown, taxonomic classification of haemogregarines can be challenging. Therefore, the basis in classification of taxa of haemogregarines is related to the sporogonic cycle (Telford 2009). In addition, when sufficient sample is available, the inclusion of a molecular approach is an

excellent tool that aids in the identification of these parasites by elucidating their evolutionary relationships.

A good deal of information is known about the ecology of *S. carinatus* (Iverson 1979; Iverson and Iverson 1980) but data on its parasites, particularly intraerythrocytic hematozoans is mostly lacking. Herban and Yaeger (1969) examined five *S. carinatus* from Louisiana but none were infected, and McAllister et al. (2016) did not find hematozoans in a single *S. carinatus* from Arkansas. Wang and Hopkins (1965) reported a *Haemogregarina* sp. from a *S. carinatus* from Texas but it lacked detailed information, specifically by not providing a photomicrograph or any mensural or morphological data of the infection. Here, we report a hematozoan from a razor-backed musk turtle from southeastern Oklahoma, including the first photomicrograph of the infection. In addition, we provide a summary of the hematozoans from North American kinosternid turtles.

Methods

On 13 September 2022, two adult male *S. carinatus* (133 and 143 mm carapace length) were collected alive with a dipnet from two locales in McCurtain County, one site at Lukfata Creek (34°03'08.3916"W,-94°48'11.9154"N) and another at Yanubbee Creek (34°02'45.6426"N, -94°43'19.761"W). They were overdosed with a concentrated solution of tricaine methanesulfonate (TMS-222) via an intraperitoneal injection. The plastron was removed with a bone saw, a mid-ventral incision was made to expose the viscera, and the internal organs were visualized. Blood was obtained from their exposed heart by obtaining a sample using ammonium heparinized (75 mm long) capillary tubes and thin films were air-dried, fixed for 1 min in absolute methanol, stained for 20–30 min with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Two slides were scanned at 100× or 400× and if any infected erythrocytes were observed after counting 5,000 cells, photographs (digital images) were taken using a Swift model M10 light microscope (Microscope Central, Feasterville, Pennsylvania) under a 1,000× oil immersion lens. A host voucher is deposited in the Eastern Oklahoma State Vertebrate Collection (EOSC), Idabel, Oklahoma. A photovoucher of the parasite is deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results

One of two *S. carinatus* (133 mm CL) was found to be infected with an intraerythrocytic hematozoan. Information on the infection is presented below.

Apicomplexa Levine, 1970

Adeleorina Léger, 1911

Haemogregarinidae Léger, 1911

***Haemogregarina* sp. Danilewsky, 1885** – A single red blood cell (rbc) containing a gamont of a *Haemogregarina* sp. (Fig. 1, HWML 216885) was found infecting the host from Lukfata Creek. The gamont was ovoidal to bean-shaped but the infected erythrocyte was abnormally shaped and not the typical nucleated biconcave disc (Fig. 1); therefore, it was not possible to get an accurate length × width measurement. However, the morphology and size (Fig. 1) of this infected rbc had obviously undergone considerable aberrant changes. Hypertrophy of the cell clearly resulted in added intraerythrocytic volume from the gamont or may represent an erythrocyte adaptation to the presence of the parasite (Al-Quraishy et al. 2021).

Wang and Hopkins (1965) noted that in 24 of 33 (73%) infected turtles they examined from Brazos County, Texas (species not specified), less than 1% of the host erythrocytes possessed haemogregarines, including one of 142,000 erythrocytes (0.0007%) in one turtle, and one of 205,000 erythrocytes (0.0005%) in another. Here, we found an almost nearly undetectable intensity in *S. carinatus*. Interestingly, despite high prevalence of many turtles reported with haemogregarines, less than 1% of erythrocytes are infected on average (Davis and Sterrett 2011; Rossow et al. 2013; Nordmeyer et al. 2020) and

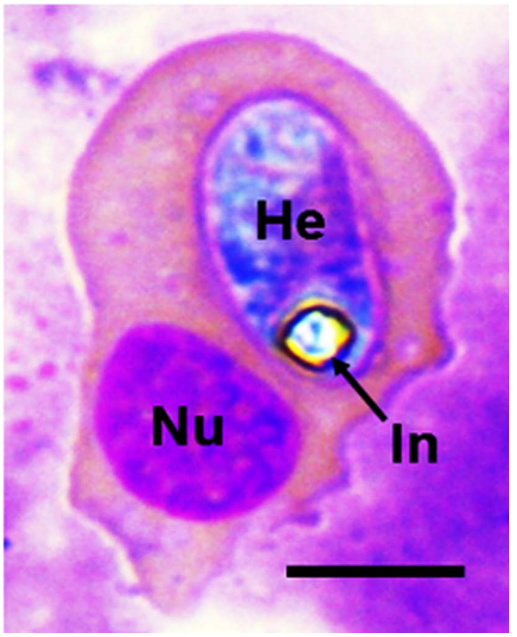

Figure 1. Photomicrograph of *Haemogregarina sp.* from *Sternotherus carinatus* from Oklahoma. Abbreviation: He (haemogregarine); In (inclusion); Nu (nucleus of host rbc). Scale bar = 5 µm.

sometimes only a single gamont is observed (Marquardt 1966; this study).

The mud turtle family Kinosternidae Agassiz includes 36 species/subspecies and four genera (van Dijk et al. 2011). In the Americas, the semiaquatic mud turtle genus *Kinosternon* Spix containing 31 species/subspecies ranges from New England to northern Argentina and the aquatic musk turtle genus *Sternotherus* Bell *in* Gray with five species/subspecies ranges from southern Ontario south to the Gulf states (van Dijk et al. 2011; Powell et al. 2016). Hematozoans have been previously reported from only nine of 36 (25%) species/subspecies of kinosternids (Table 1) with the eastern musk turtle or stinkpot, *Sternotherus odoratus* (Latreille, *in* Sonnini and Latreille) being reported most often as host, including specimens collected from Arkansas, Georgia, Illinois, Kentucky, Massachusetts, North Carolina, and Tennessee. This is not too unexpected as *S. odoratus* is the most widely ranging and perhaps most often collected kinosternid and is very common being found in 32 of 50 (64%) US states as well as Ontario and Québec, Canada (van Dijk et al. 2011).

González et al. (2019) examined three scorpion mud turtles, *Kinosternon scorpioides* (L.) from Colombia for hematozoans, but none were infected. We are also unaware of any reports of hematozoans from the remaining two genera (and three species) of kinosternids, namely *Claudius* (Cope) of Belize, Guatemala, and México, and *Staurotypus* Wagler from El Salvador, Guatemala, and México (van Dijk et al. 2011). For example, Nordmeyer et al. (2020) examined six captive northern giant musk turtles, *Staurotypus triporcatus* Wiegmann from Chiapas, México, but no hematozoans were observed.

There are 18 species and subspecies of turtles that inhabit Oklahoma (Sievert and Sievert 2021). Since turtles are hosts of numerous described and potentially novel hematozoans (Ernst and Ernst 1979), additional surveys on larger samples of turtles from the state need to be carried out as several species should be examined for hematozoans. Moreover, the inclusion of molecular characterization (DNA sequences) would be particularly helpful to identify some hematozoans which have limited morphological traits. As such, new host and geographic distributional records could be found, including the possibility of discovering new species.

Finally, Wang and Hopkins (1965) reported that one of two *S. carinatus* harbored *Haemogregarina* sp. Unfortunately, no detailed information was provided on this infection as well as lacking a photomicrograph. Therefore we document novel information herein and the first photomicrograph of a *Haemogregarina* from *S. carinatus*, as well as the first time this host has been reported with an infection from Oklahoma.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued Scientific Collecting

Table 1. Hematozoans (*Haemogregarina* sp.) reported from turtles of the family Kinosternidae.

Host	Prevalence*	Locality	Reference
	***Kinosternon* spp.**		
K. leucostomum	8/8 (100%)	Costa Rica	Rossow et al. (2013)
K. scorpioides albogulare	1/1 (100%)	Costa Rica	Rossow et al. (2013)
K. s. cruentatum	1/1 (100%)	Central America†	Plimmer (1913)
		North America†	Plimmer (1916)
K. sonoriense	2/3 (67%)	Alabama‡	Nordmeyer et al. (2020)
K. subrubrum hippocrepis	1/2 (50%)	Texas	Wang and Hopkins (1965)
	3/5 (60%)	Louisiana	Herban and Yaeger (1969)
	7/7 (100%)	Louisiana	Acholonu (1974)§
	1/4 (25%)	Arkansas	McAllister et al. (2016)
K. s. subrubrum	2/2 (100%)	North Carolina	Hahn (1909)
	***Sternotherus* spp.**		
S. carinatus	1/2 (50%)	Texas	Wang and Hopkins (1965)
	1/2 (50%)	Oklahoma	This study
S. minor	1/2 (50%)	Tennessee	Edney (1949)‖
S. odoratus	3/5 (60%)	Massachusetts, North Carolina	Hahn (1909)
	1/1 (100%)	Tennessee	Edney (1949)‖
	1/1 (100%)	Illinois	Marquardt (1966)
	26/27 (96%)	Kentucky	Strohlein and Christensen (1984)
	8/9 (89%)	Georgia	Davis and Sterrett (2011)
	3/7 (43%)	Arkansas	McAllister et al. (2016)
	1/3 (33%)	Texas	Nordmeyer et al. (2020)

*Prevalence = infected/examined (%).
†Exact locality not specified.
‡Captive specimen from Guthrie Turtle Farm, Birmingham, Alabama; host range includes Chihuahua and Sonora, México, and Arizona and New Mexico (Powell et al. 2016).
§Identified as *Haemogregarina pseudemydis* Acholonu, 1974, based solely on gamont stage (see Acholonu 1974).
‖Identified as *Haemogregarina stepanowi* Danilewsky, 1885, based solely on gamont stage (see Edney 1949).

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Noteworthy Parasites (Apicomplexa, Cestoda, Nematoda, Acari) from Plains Leopard Frog, *Rana blairi* (Anura: Ranidae), from Southern Oklahoma

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Abstract: A single adult plains leopard frog, *Rana blairi*, was collected from Marshall County, Oklahoma, and examined for ecto- and endoparasites. It was found to be infected with an unknown species of *Hepatozoon* and with trypanosomes of three distinct morphologies. This host also harbored a metacestode (*Mesocestoides* sp.) in the liver and mesenteries, a nematode (*Oswaldocruzia pipiens*) in the small intestine, and engorged larval *Hannemania dunni* and *Hannemania hegeneri* chiggers in skin tissue. Here, we provide the first report of hemoparasites from *R. blairi* in Oklahoma, including photomicrographs of the infection. In addition, the chigger *H. hegeneri* is reported from *R. blairi* and Oklahoma for the first time.

Introduction

The plains leopard frog, *Rana blairi* Mecham, Littlejohn, Oldham, Brown, and Brown occurs from western Indiana to southeastern North Dakota and eastern Colorado and south to central and west Texas and further westward to Arizona (Brown 1992; Powell et al. 2016). In Oklahoma, *R. blairi* is primarily found in the central and western parts of the state, including the Panhandle (Sievert and Sievert 2021). It inhabits plains and prairies and generally feeds on various insects, earthworms, and aquatic snails (Hartman 1906).

Although there is a great deal of ecological and natural history information on *R. blairi* (Smith and Keinath 1985; Brown 1992; Parris 1998; Parris and Semlitsch 1998; Bolek and Janovy 2004), only a moderate amount of studies have documented parasites of this frog. Walton (1929) originally described a nematode, *Raillietnema longicaudata* as *Aplectana longicauda* from hosts identified as northern leopard frogs, *Rana pipiens* (Schreber) from Illinois; however, in his redescription, Baker (1985) doubted the host identification as three species of this complex

with ranges that overlap (including *R. blairi*) occur in Illinois. Therefore, the host species cannot be verified. Brooks (1976) and Brooks and Welch (1976) reported trematodes from *R. blairi* in Nebraska. Bolek and Janovy (2005) found leeches, *Placobdella* (syn. *Desserobdella*) *picta* (Verrell, 1872) on *R. blairi* in Nebraska. Bolek et al. (2009) listed *R. blairi* as a host of the bladder fluke, *Gorgoderina attenuata* (Stafford, 1902) Stafford, 1905. The largest study, to date, on parasites of *R. blairi* was by Goldberg et al. (2000) who examined 73 specimens from Colorado, Kansas, Iowa, and Nebraska. Bolek and Janovy (2007) and Langford et al. (2013) reported *Haematoloechus coloradensis* (Cort, 1915) Ingles, 1932, and two species of lung parasites (*Haematoloechus* and *Rhabdias*) from *R. blairi* from Nebraska, respectively. In another study, Langford and Janovy (2013) reported two species of *Rhabdias* in *R. blairi* from Nebraska. McAllister et al. (2017) reported a chigger mite, *Hannemania dunni* Sambon, 1928, from *R. blairi* from Texas. However, none of these surveys included examination of the blood. Interestingly, the online Aquatic Parasite Observatory at the University of Colorado lists several parasites from *R. blairi* without detailed information or cited literature (http://www.aquaticparasites.org/hosts/amphibia/anura/ranidae/rana/blairi.php). We also are unaware of any previous studies of parasites of *R. blairi* from Oklahoma.

Haemogregarines are apicomplexan blood-inhabiting parasites with an obligatory heteroxenous life cycle. They are ubiquitous in all types of vertebrates, including fishes, amphibians, lizards, snakes, turtles, crocodilians, birds, and mammals. However, classification of haemogregarines is problematic when information about the life cycle stages is lacking. Therefore, the basis for taxonomic classification of genera/species of haemogregarines is related to the sporogonic part of the life cycle where sexual reproduction occurs in the hematophagous invertebrate vector (Telford 2009). The inclusion of a molecular approach is an excellent tool that can aid in the identification of these parasites by elucidating their evolutionary relationships.

Here, we document some new host and distributional records of some parasites of a plains leopard frog from the state, including histological information on the infections/infestations.

Methods

On 22 April 2022, a single adult gravid female *R. blairi* (85 mm snout-vent length) was collected alive by hand from just SE of Willis on the OU road, vicinity of the University of Oklahoma Biological Station (UOBS), Marshall County (33°53'03.732"W, -96°48'58.1472"N). It was anesthesized by immersion in a dilute solution of tricaine methanesulfonate (TMS-222). Orange to red-colored encapsulated chiggers were noted, excised from the skin (abdomen and legs), and placed in 70% (v/v) DNA grade ethanol or with attached tissue in 10% (v/v) neutral buffered formalin (NBF) for histosectioning. They were sectioned at 8 µm, stained with Pollak stain, and processed per the methods of Presnell and Schreibman (1997). For photomicroscopy, a Nikon Eclipse 600 epifluorescent light microscope with a Nikon DXM 1200C digital camera (Nikon Instruments Inc., Melville, NY) was used. Chiggers in ethanol were cleared in lactophenol, slide-mounted in Hoyer's medium (Walters and Krantz 2009), and identified using Brennan and Goff (1977).

Blood was drawn from the facial musculocutaneous vein of the anesthesized specimen following the methods of Forzán et al. (2012) and collected in an ammonium heparinized (75 mm long) capillary tube. Two separate blood smears were made and fixed briefly in absolute methanol, stained with Wright's-Giemsa stain for 30 min, and rinsed in buffer (pH = 7.0). They were screened initially at 400× magnification for extracellular hemoparasites, such as trypanosomes, and at 1000× for intraerythrocytic parasites, using a Swift Model M10 light microscope with an in-built digital camera (Commack, New York). Hematozoan parasites were identified to genus based on previous reports of hematozoa infecting other ranid frogs in North America (see McAllister et al. 2020).

The frog was euthanized with a concentrated solution of TMS-222 and a midventral incision was made from the cloaca to throat to expose the viscera. All major organs were examined under a stereomicroscope for parasites, including the entire gastrointestinal tract which was placed in a Petri dish containing 0.9% saline and split lengthwise. When suspected encapsulated tapeworms were observed, they were excised with a small portion of tissue and preserved in 10% NBF and processed for light microscopy (Presnell and Schreibman 1997). For photomicroscopy, a Nikon Eclipse light microscope as described above was used. Two nematodes were found in the small intestine, transferred to a vial containing 70% ethanol, and examined in a drop of glycerol under a coverslip.

We follow Yuan et al. (2016) in the adoption of the genus *Rana* instead of *Lithobates* for North American ranid frogs. Voucher ectoparasites (chiggers) are deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, GA (accession numbers L-3859A, L-3859B). Slide material, specimens in ethanol, and/or photovouchers were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, NE. The voucher host was deposited in the Eastern Oklahoma State College Vertebrate Collection, Idabel, OK.

Results and Discussion

A variety of different protozoan and metazoan parasites, including two blood-inhabiting parasites, a cestode, a nematode, and two species of ectoparasites were harbored by this single frog. Data are provided on each in an annotated format below.

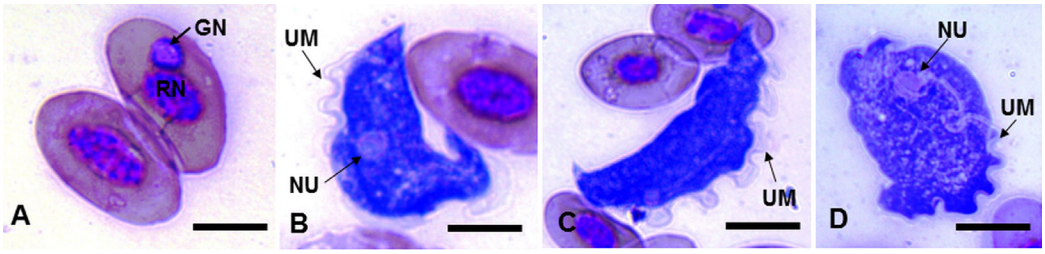
Apicomplexa: Adeleorina: Hepatozoidae

***Hepatozoon* sp. Miller, 1980 –** a single red blood cell (rbc) containing a gamont of an intraerythrocytic hematozoan belonging to an unknown species of the genus *Hepatozoon* (HWML 216905, Fig. 1A) was found. The gamont was spheroidal and the rbc nucleus was non-fragmented. No other erythrocytes were observed with gamonts.

In terms of those species within the family Ranidae Rafinesque, both the American bullfrog, *Rana catesbeiana* (Shaw) and green frog, *Rana clamitans* (Latreille) have been previously reported to harbor *Hepatozoon* spp. from New York and Ontario, Canada, including the latter frog species from Arkansas (see McAllister et al. 2020). To our knowledge, there have been no previous reports of hematozoans from amphibians of Oklahoma. We document the first report of a *Hepatozoon* sp. from *R. blairi* from the state.

Euglenozoa: Kinetoplastea: Trypanosomatida: Trypanosomatidae

***Trypanosoma* sp. Gruby, 1843 –** three distinct morphologies of trypanosomes ("a, b, and c") were found (HWML 216906, Figs. 1B–D) representing a combined intensity of



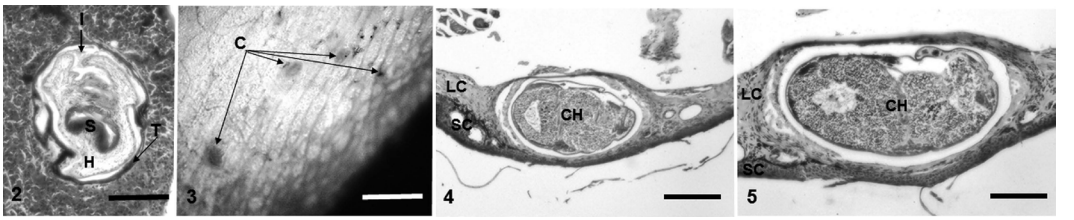
Figures 1A–D. Photomicrographs of hematozoan parasites infecting *Rana blairi*. (A) Gamont nucleus (GN) of a *Hepatozoon* sp.and host red blood cell nucleus (RN); scale bar = 10 µm. (B) *Trypanosoma* sp. ("form a") showing undulating membrane (UM) and nucleus (NU); scale bar = 10 µm. (C) *Trypanosoma* sp. ("form b") showing UM; scale bar = 20 µm. (D) *Trypanosoma* sp. ("form c") showing NU and UM; scale bar = 20 µm. All Wright's-Giemsa stain.

about 3%. McAllister et al. (2020) reported similar morphologies of three trypanosomes from *R. clamitans* from Arkansas. To our knowledge, trypanosomes have not been previously reported from *R. blairi*, but in addition to Arkansas, noted above for green frogs, they have been reported from *R. clamitans* from Louisiana (Southworth et al. 1968), and Ontario, Canada (Barta and Desser 1984). Trypanosomes have the remarkable ability to change their morphology throughout their life cycle, and as such, are considered pleomorphic (Desser 2001). Individual anurans are often infected with multiple morphologies and it is unknown whether these forms represent different taxa or a single pleomorphic species. Therefore, based on morphology alone, species of trypanosomes cannot be identified; however, careful isolation, culturing, and experimental infections of anurans are required to describe and to determine a specific identity (Desser 2001). We document the first report of *Trypanosoma* from *R. blairi* as well as the first amphibian from the state with this hemoparasite.

Cestoda: Cyclophyllidea: Mesocestoididae

***Mesocestoides* sp. Vaillant, 1863 –** numerous metacestodes (tetrathyridia) belonging to the genus *Mesocestoides* were found in the mesenteries and encapsulated in the liver (HWML 216904, Fig. 2). McAllister et al. (1990) examined seven *R. blairi* from Texas for *Mesocestoides* but none harbored the tapeworm. However, Goldberg et al. (2000) reported this cestode from *R. blairi*, including one of 18 (6%) individuals from Kansas, four of 16 (25%) in Iowa, and five of 55 (9%) from Nebraska, but no photomicrographs were provided. *Mesocestoides* sp. has been reported from an additional six species of North American ranid frogs from Arkansas, Michigan, New York, Oklahoma, South Dakota, Texas, and Wisconsin (see summary by McAllister et al. 2014). Interestingly, McAllister et al. (2005) reported *Mesocestoides* sp. from two species of spadefoot toads from the same general locality (UOBS) reported herein. In addition, other amphibians from Oklahoma have been reported to harbor *Mesocestoides* sp. (see summary by McAllister et al. 2021).

In the present infection, tetrathyridia possessed characteristic individual features of a single invaginated tetra-acetabulate scolex, a large and deep invagination canal, and a solid hindbody (Fig. 2). None possessed a divided scolex, somatic bud, or any excretory or tegumental anomalies, infrequently reported from tetrathyridia in some aberrant acephalic forms from other hosts (Conn et al. 2011). We document *Mesocestoides* sp. from *R. blairi* in Oklahoma for the first time.



Figures 2–5. Photomicrographs of metazoan parasites of *Rana blairi*. (2) Pollak stained histologic section of *Mesocestoides* sp. tetrathyridia in host-derived fibrotic capsule in liver showing characteristic solid cellular hindbody (H), deep invagination canal (I), tetra-acetabulate scolex (S), and syncytial tegument (T). Note the absence of buds, multiple scoleces, or any other evidence of asexual proliferation. Also note the thin host-derived capsule, normal appearance of hepatic parenchyma, and pigment deposition; scale bar = 200 µm. (3) Stereoscopic view of four *Hannemania hegeneri* chiggers (C, arrows) encapsulated in host tissue; scale bar = 5 mm. (4) Histosection of single encapsulated *H. hegeneri* (CH) in host tissue, LC (loose connective tissue), stratum corneum (SC); scale bar = 500 µm. Pollak stain. (5) Higher magnification of a different host-derived capsule showing CH, LC, and SC; scale bar = 250 µm. Pollak stain.

Nematoda: Strongylida: Molineidae

***Oswaldocruzia pipiens* Walton, 1929 –** Two adult specimens (one male, one female) of *O. pipiens* (HWML 118079) were found in the intestinal tract. Goldberg et al. (2000) reported that seven of 18 (22%) *R. blairi* from Kansas harbored this nematode. This roundworm has been previously reported from dwarf American toad, *Anaxyrus americanus charlesmithi* (Bragg), Woodhouse's toad, *Anaxyrus woodhousii* (Girard), *R. catesbeianus*, southern leopard frog, *Rana sphenocephalus utricularius* (Cope) and Hurter's spadefoot, *Scaphiopus hurterii* Strecker from Oklahoma (Trowbridge and Hefley 1934; Kuntz 1941; Kuntz and Self 1944; McAllister et al. 2005, 2014). We document *O. pipiens* from an Oklahoma *R. blairi* for the first time.

Arthropoda: Acari: Leeuwenhoekiidae

***Hannemania dunni* Sambon, 1928 –** a single engorged larval chigger was found that matched the description of *H. dunni*. McAllister et al. (2017) previously reported *H. dunni* from *R. blairi* from Texas. This chigger is a common ectoparasite of a variety of North American salamanders and frogs, including *Rana* spp. (see Watermolen [2021] for a summary of hosts). Previous reports of *H. dunni* from the state include specimens from *A. a. charlesmithi* (McAllister and Durden 2014), Woodhouse's toad, *A. woodhousii* (Loomis 1956), Blanchard's cricket frog, *Acris blanchardi* (as *A. gryllus*) (Loomis 1956), and *R. s. utricularis* (as *R. pipiens*) (Loomis 1956). This chigger has been previously reported from amphibian hosts from Alabama, Arkansas, Georgia, Kansas, North Carolina, Oklahoma, Texas, Virginia, and West Virginia (Watermolen 2021).

***Hannemania hegeneri* Hyland, 1956** – eight engorged larval specimens of *H. hegeneri* were taken from *R. blairi*. The type host and locality of *H. hegeneri* is *R. s. utricularius* from Sarasota, Sarasota County, Florida (Hyland 1956). It has also been previously reported from various anuran hosts (*Acris, Anaxyrus, Hyla* [*Dryophytes*]*, Rana* spp.) from Florida, Georgia, and Utah (Watermolen 2021). However, this is the first time *H. hegeneri* has been reported from Oklahoma as well as what we believe to be the first mixed parasitism of two species of *Hannemania* from a single host.

Chiggers were found encapsulated in a host-derived capsule of *R. blairi* and macroscopically (HWML 216903) appeared as conspicuous red to orange raised bumps, ca. 1 mm in diameter (Fig. 3). Similar to what Hyland (1961) reported for *R. s. utricularius* and the pickerel frog, *R. palustris* (LeConte), a local host immune reaction was produced and the surrounding connective tissue became restructured and an envelope of connective tissue (Figs. 4–5) formed around the chigger. This is the first time histopathology has been reported from an infestation of *H. hegeneri* in *R. blairi*.

In summary, although only a single *R. blairi* was examined for parasites, this study shows that a variety of parasites belonging to widely-different taxonomic groups can be found in one specimen collected from Oklahoma that resulted in novel information. However, for a more meaningful distribution of these and other parasites in *R. blairi*, larger sample sizes are recommended, as well as future surveys that take into account ecological differences in various geographic locales, and especially the abundance and identification of capable vectors for its hematozoan parasites.

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New Host and Distributional Records for Helminth and Arthropod Parasites of Birds (Aves: Strigiformes; Accipitriformes; Piciformes; Passeriformes) from Southeastern Oklahoma

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Abstract: Between November 2020 and March 2022, 14 individual salvaged birds (11 species) within four orders and nine families from McCurtain County, Oklahoma, were examined for ecto- and endoparasites. Avian orders (families) and host species included: Strigiformes (Strigidae): barred owl (*Strix varia*) and two great-horned owls (*Bubo virginianus*); Accipitriformes (Accipitridae): red-tailed hawk, (*Buteo jamaicensis*); Piciformes (Picidae): red-headed woodpecker (*Melanerpes erythrocephalus*) and downy woodpecker (*Dryobates pubescens*); Passeriformes (Turdidae, Troglodytidae, Mimidae, Parulidae, Paridae, Icteridae): two American robins (*Turdus migratorius*), Carolina wren (*Thryothurus ludovicianus*), brown thrasher (*Toxotoma rufum*), two yellow warblers (*Setophaga petechia*), tufted titmouse (*Baeolophus bicolor*), and common grackle (*Quiscalus quiscula*). Eighteen parasite taxa were found in the birds examined, including four digenean trematodes, four cestodes, three nematodes, two acanthocephalans, and five lice. We document five new host and 14 new geographic distributional records for the parasites from select birds of the state.

Introduction

Recently, our research collaborative provided novel information on parasites of raptors of Oklahoma (McAllister et al. 2017, 2018, 2019a, 2019b, 2019c; McAllister and Robison, 2020; Woodyard et al. 2021). However, Oklahoma supports 488 species of birds (Oklahoma Birds Records Committee 2022) and obviously many species remain to be surveyed for parasites. Indeed, there has been a general void of information on their parasites, especially those from songbirds (Passeriformes). To that end, we report new geographic and host records for several parasites of birds, including six species of passeriforms, from southeastern Oklahoma.

Methods

Between November 2020 and June 2022, 14 individual birds, including those within the orders **Strigiformes:** barred owl, *Strix varia* Barton, two great-horned owls, *Bubo virginianus* (Gemlin); **Accipitriformes:** red-tailed hawk, *Buteo jamaicensis* (Gmelin); **Piciformes:** red-headed woodpecker, *Melanerpes erythrocephalus* (L.) and downy woodpecker, *Dryobates pubescens* (L.); and **Passeriformes:** two American robins, *Turdus migratorius* L., Carolina wren, *Thryothorus ludovicianus* (Latham), two yellow warblers, *Setophaga*

petechia (L.), brown thrasher (*Toxotoma rufum*), tufted titmouse, *Baeolophus bicolor* (L.), and common grackle, *Quiscalus quiscula* (L.) from various sites in McCurtain County were examined for ecto- and endoparasites. Birds were either found freshly dead on the road (DOR), found dead in the field (for unknown reasons), killed by feral cats (*Felis catus*), or died after hitting glass windows at private residences. Specimens were placed in collection bags on ice and taken to the laboratory within 24 hr for necropsy. Their feathers were vigorously brushed over a white enamel tray to collect ectoparasites. Those found were placed in a vial of 70% (v/v) DNA grade ethanol; specimens were cleared in 10% (v/v) potassium hydroxide, dehydrated through an ethanol series, further cleared in methyl salicylate or xylene, and slide-mounted in Canada balsam (Price et al. 2003).

A mid-ventral incision was made from the cloaca to throat of each bird to expose the trachea, lungs, air sacs, esophagus, proventriculus, gizzard, gallbladder, liver, kidneys, and intestines. Organs were placed in individual Petri dishes containing 0.9% (v/v) saline, opened, and their contents washed. Several 100 mm sections of the tissues were cut, split lengthwise, and examined under a stereomicroscope at 20 to 30× to aid in locating endoparasites. Trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, transferred to 70% DNA grade ethanol, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate or xylene, and mounted in Canada balsam. Nematodes were fixed in hot tap water, transferred to ethanol (as above), and studied as temporary mounts on a microscopic slide in a drop of glycerol. Acanthocephalans were placed in tap water overnight in a refrigerator, transferred to 70% ethanol, and cleared in 80% phenol.

Ectoparasites were deposited in the General Ectoparasite Collection (L-series) in Department of Biology, Georgia Southern University Collection, Statesboro, Georgia or the Price Institute of Parasite Research (PIPR), School of Biological Sciences, University of Utah, Salt Lake City, Utah. Actual specimens of helminth parasites (or photovouchers) were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska-Lincoln; specimens without HWML numbers at this time are being retained for further study. Host voucher specimens (in ethanol) are deposited in the Eastern Oklahoma State College Collection (EOSC), Idabel, Oklahoma.

Results and Discussion

Eighteen parasite taxa were found in the avians examined, including four digenean trematodes, four cestodes, three nematodes, two acanthocephalans, and five lice (Table 1). Their data is presented below in annotated format.

Trematoda: Plagiorchiida: Dicrocoeliidae

***Lutztrema monenteron* (Price and McIntosh, 1935) Travassos, 1941** – a single American robin, collected DOR on 10 January 2021 from just N of Broken Bow off US 259 (34°04’22.479”N, -94°44’21.4764”W), harbored four *L. monenteron* (Fig. 1, HWML 216476, 216908) in its gall bladder. Specimens possessed a single cecum with a sinuous curve in the middle of the body, a defining taxonomic characteristic of the genus.

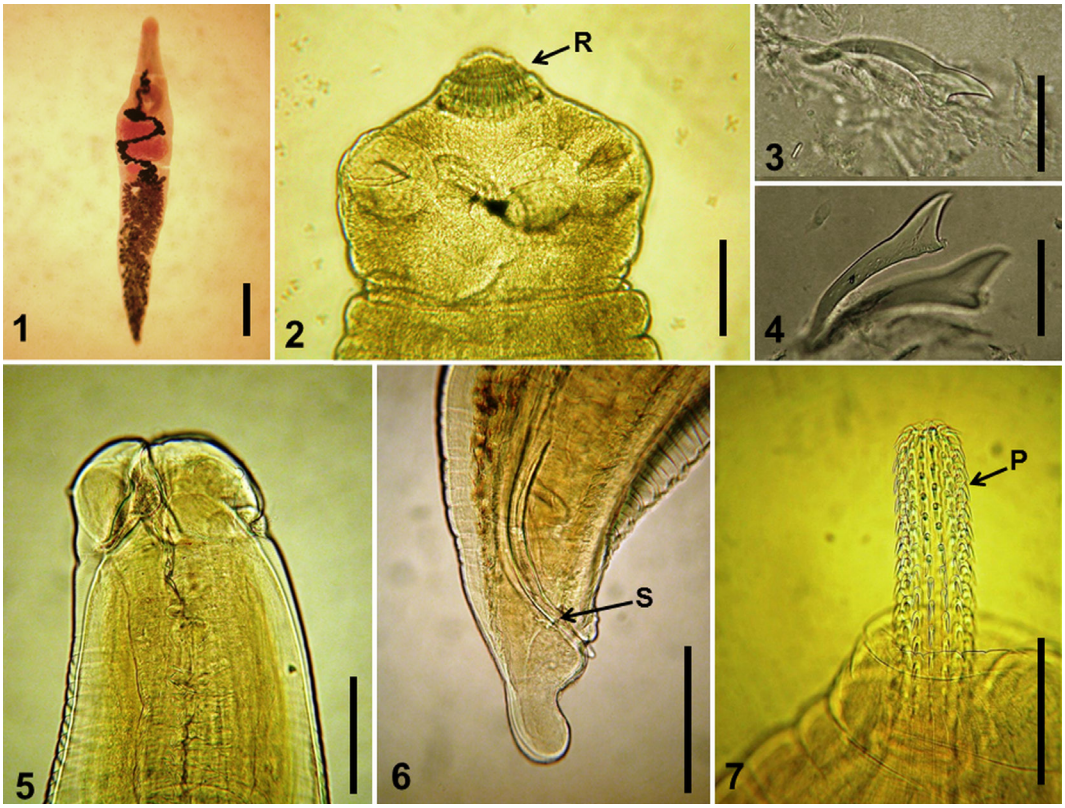
Interestingly, no type host or type locality was specifically designated for the trematode in the original description by Price and McIntosh (1935). They listed *T. migratorius* from Charlottesville, Albermarle County, Virginia, and Washington, D.C., and the eastern bluebird, *Sialia sialis* L., from Falls Church, Fairfax County, Virginia as co-hosts of *L. monenteron*. This worm is a commonly reported trematode as it has also been documented from this host from Colorado, Georgia, Illinois, Ohio, North Carolina, New York, Tennessee, and Texas, and Canada (Webster 1943; Denton and Byrd 1951; Slater 1967; Cooper and Crites 1974; Hamer and Muzzall 2013). In addition, the species has little host specificity as members of six families of passerine birds have been reported as natural definitive hosts of *L. monenteron*, including *Catharus guttata* (Pallas), *Cyanocitta cristata* (L.), *Cyanocorax chrysops* (L.), *Corvus corona* L., *Corvus frugilegus* (L.), *Ixoreus*

Table 1. Parasites of selected birds from McCurtain County, Oklahoma.

Phylum/Parasite Species	Host	New Host Record	New Locality Record
Platyhelminthes			
Lutztrema monenteron	*Turdus migratorius*	No	Yes
Strigea elegans	*Bubo virginianus**	No	Yes
S. macroconophora	*Buteo jamaicensis**	No	Yes
Neodiplostomum reflexum	*Strix varia*	No	Yes
Raillietina sp.	*Dryobates pubescens*	Yes	No
Dilepis undula	*T. migratorius* *	No	Yes
Anomotaenia sp.	*Thryothorus ludovicianus*	Yes	Yes
Anonchotaenia sp.	*Setophaga petechia*	Yes	Yes
	Baeolophus bicolor	Yes	No
Nematoda			
Capillaria exilis	*T. migratorius* *	No	Yes
Porrocaecum angusticolle	*B. jamaicensis* *	No	Yes
P. depressum	*B. virginianus**	No	No
Acanthocephala			
Plagiorhynchus cylindraceus	*Toxotoma rufum*	Yes	Yes
Mediorhynchus robustus	*T. migratorius* *	No	No
Arthropoda			
Strigiphilus oculatus	*B. virginianus**	No	Yes
Philopterus quiscali	*Quiscalus quiscula**	No	Yes
Menacanthus quiscali	*Q. quiscula**	No	Yes
Brueelia straminea	*Melanerpes erythrocephalus*	No	No
Picicola snodgrassi	*M. erythrocephalus*	No	Yes

*Type-host.

naevius (Gmelin), *Mimus polyglottos* (L.), *Pipilo erythrophthalmus* (L.), *Sialia sialis* (L.), *Sturnella magna* (L.), *Sturnus vulgaris* L., *T. rufum*, *Turdus pilaris* (L.), *Turdus merula* (L.), *Turdus philomelos* (L.), and *Turdus viscivorus* (L.). This species has one of the widest geographical distributions known for a member of the genus, including North America, Europe (Czech Republic, England, France, Germany), and South America (Brazil) (Dollfus 1957; Mettrick 1958; Rysavý 1960; Groschaft 1969; Odening 1970; Binder 1971; Rietschell 1971). In North America alone, this trematode is widespread in the eastern and southeastern states with a few reports from the northwestern states; reports of this fluke are mostly lacking



Figures 1–7. Helminth parasites of Oklahoma birds. (1) *Lutztrema monenteron* from *Turdus migratorius*; scale bar = 1 mm. (2) *Dilepis undula* from *T. migratorius*; scale bar = 200 µm. (3) Small rostellar hook of *D. undula*; scale bar = 100 µm. (4) Large rostellar hook of *D. undula*; scale bar = 100 µm. (5) *Porrocaecum depressum* from *Bubo virginianus*; scale bar = 100 µm. (6) Spicule of *P. depressum*; scale bar = 200 µm. (7) *Plagiorhynchus cylindraceus* from *Toxotoma rufum*; scale bar = 1mm. Abbreviations: P (proboscis); R (rostellum); S (spicule).

from the southwestern states, there is only one report from the middle west, including Colorado, Connecticut, Georgia, Idaho, Illinois, Iowa, Michigan, Montana, New York, North Carolina, Ohio, Oklahoma (this report), Tennessee, Texas, Virginia, Washington, Washington, D.C., and Alberta and Quebéc, Canada (Webster 1943; Krissinger 1975).

The American robin has been reported by numerous investigators as a natural definitive host of this digenean in North America. It appears from these reports that *T. migratorius* is the primary definitive host for *L. monenteron* with the other passerine species serving as secondary or accidental hosts. We document *L. monenteron* from an Oklahoma host for the first time.

Diplostomida: Strigeidae

***Strigea elegans* (Chandler and Rausch, 1947) Dubois and Rausch, 1950 –** a DOR *B. virginianus* collected on 10 January 2021 from off US 70, east of Broken Bow (34°02'30.7968"N, -94°37'36.9696"W), harbored this trematode (HWML 118080) in its small intestine. The type host and type locality of *S. elegans* is *B. virginianus* from Poynette, Columbia County, Wisconsin (Chandler and Rausch 1947). Other reported hosts include *S. varia* from Florida (Kinsella et al. 2001) and *B. virginianus* from Arkansas (McAllister et al. 2019a). The life

cycle involves snails as first intermediate hosts, bufonid and ranid anuran tadpoles as second intermediate hosts, watersnakes and ducks with tetracotyles as third intermediate hosts, and owls as definitive hosts (Pearson 1959; Miller et al. 1965). We document *S. elegans* from an Oklahoma *B. virginianus* for the first time.

Strigea macroconophora **(Chandler and Rausch, 1947) Dubois and Rausch, 1950 –** this trematode was taken from the small intestine of a DOR *B. jamaicensis* (Gmelin) collected on 24 June 2022 from off St. Hwy 93, just S of Wright City (34°02'30.7862"N, -95°01'2.874"W). Dubois and Rausch (1950) originally described *S. macroconophora* from *B. jamaicensis* from Poynette, Wisconsin. This is the first report of this strigeid from an Oklahoma host.

Diplostomidae

Neodiplostomum reflexum **(syn. *delicatum*) Chandler and Rausch, 1947 –** a single *S. varia* collected DOR on 23 May 2021 from off US 259, 16.1 km N of Hochatown (34°13'35.7162"N, -94°46'47.0424"W) harbored this trematode in its small intestine. The type host and type locality of *N. reflexum* is *B. virginianus* from Michigan (Chandler and Rausch 1947). It has also been reported from *S. varia* from Texas (Little and Hopkins 1975). We document *N. reflexum* from Oklahoma for the first time.

Cestoda: Cyclophyllidea: Davaineidae

Raillietina **sp. Fuhrmann, 1920 –** two specimens representative of this tapeworm genus (HWML 216912) were found in the small intestine of a *D. pubescens* found dead on 24 March 2022 in Hochatown (34°10'17.0286"N, -94°45'05.7414"W). It is cosmopolitan as well as being the most common genus among davaineids with about 295 species reported from a wide variety of both domestic and wild avians as well as mammalian hosts, including humans (Schmidt 1986). Rigney (1943) reported *R. centuri* from a red-bellied woodpecker, *Melanerpes carolinus* (L.) from near Stillwater, Payne County, Oklahoma. Movsesyan (2003), in Osnovy Tsestodologii, did not include *D. pubescens* as a host of *Raillietina*. We therefore document a new host record for a *Raillietina* sp. in a downy woodpecker.

Dilepididae

Dilepis undula **(Schrank, 1788) Fuhrmann, 1908 –** the same *T. migratorius* reported above had a single *D. undula* (Figs. 2–4, HWML 216909) in its small intestine. Other reported hosts of *D. undula* include American robins from Colorado, Illinois, Indiana, New York, Ohio, Washington, D. C., and Newfoundland, Canada (Baker and Hamon 1967; Slater 1967; Cooper and Crites 1974, 1976) and various passerine birds, primarily different species of the genus *Turdus*, as well as mammals (Mettrick 1958; Haukisalmi 2015). The species is cosmopolitan in distribution as there are records from other hosts in Brazil, Bulgaria, Canada, Chile, China, Czech Republic, Finland, Germany, Israel, Nicaragua, New Zealand, Poland, Russia, Spain, Ukraine, the United Kingdom, and the USA (Ohio, Oklahoma) (see Llanos-Soto et al. 2019).

Members of this family are characterized by an armed rostellum possessing a double row of rostellar hooks (Figs. 3–4), a post-ovarian position of the compact vitellarium, a sacciform or lobulated ovary, a single set of reproductive organs per proglottid, lack of seminal vesicles, numerous testes, and a ventral position of the persistent uterus (Mariaux et al. 2017). The life cycle includes larval *D. undula* developing in earthworm intermediate hosts, which are the most important food items of birds of the subfamily Turdinae (Rysavý 1973). We document *D. undula* in an Oklahoma host for the first time.

Anomotaenia **sp. Cohn, 1900** – a single *T. ludovicianus* found dead on 4 July 2021 from off US 259 in Hochatown (34°10'17.0286"N, -94°45'05.7414"W) had three tapeworms in its small intestine belonging to the genus *Anomotaenia* (HWML 216907). These specimens possessed an armed rostellum with two rows of small hooks (~15 µm) with irregularly alternating genital pores. Generic identification of this specimen was confirmed with molecular analysis (VV Tkach *pers. comm.*); however, there are no comparative sequence data on North American *Anomotaenia*

spp. in GenBank.

Anomotaenia tapeworms are parasites of birds (Bona 1994) with the majority (27 species) being parasites of waders (suborder Charadrii) (Matevosyan 1963; Spassky 1968; Spasskaya and Spassky 1978). In the life cycle of one species in this genus, ova of *A. brevis* (Clerc, 1902) are transmitted from a definitive host (woodpecker) to the intermediate host (ant, *Leptothorax nylanderi* Foerster) by ingestion of infected bird feces during the ants' larval stage (Plateaux 1972). We document the genus for the first time in this host as well as in Oklahoma. This is also the initial report any helminth parasite, to our knowledge, having been documented in a Carolina wren.

***Anonchotaenia* sp. Cohn, 1900** – a tufted titmouse collected on 2 April 2022 from Hochatown harbored two individual tapeworms (HWML 216911). In addition, two individual yellow warblers found dead on 3 and 13 March 2022 from Hochatown harbored tapeworms belonging to this genus. Specimens possessed an unarmed rostellum as well as a parauterine organ. The genus has been previously reported many other North American passeriform birds as well as other New World warblers (Parulidae), including blue-winged warbler, *Vermivora cyanoptera* Olson and Reveal and yellow-rumped (or myrtle) warbler, *Setophaga coronata* (L.) (Rausch and Morgan 1947). In addition, it appears that members of the family Fringillidae (true finches) in North and South America are the most common hosts of this genus (Voge and Davis 1953).

Our generic identification of these specimens was confirmed with molecular analyses (28S and NADH dehydrogenase subunit 1 [nad1] mitochondrial gene, VV Tkach *pers. comm.*); unfortunately, these data would only be useful when there is a comparison from other species or specimens identified to species. However, there are no data on *Anonchotaenia* from North America in GenBank. Nonetheless, this is the first time the genus has been reported from these two hosts and/or from Oklahoma.

Nematoda: Enoplida: Capillariidae

***Capillaria exilis* (Dujardin, 1845) Travassos, 1915** – the same American robin reported previously had one gravid female and a single male *C. exilis* in its small intestine. Three species of *Capillaria* have been reported from *T. migratorius*, including *C. exilis*, *C. ovopunctatum* (von Linstow, 1873) and *C. caudinflata* = *Aonchotheca caudinflata* (Molin, 1858) from Illinois, and Ohio, and Québec, Canada (Cooper and Crites 1974, 1976). In the Osnovy Nematodologii (Skrjabin et al. 1957), both *C. exilis* and *C. caudinflata* are reported to possess caudal alae (see also Boyd, 1951; host = starlings); however, Lopez-Neyra (1947) does not show caudal alae in *C. exilis*. As the current specimens clearly do not possess caudal alae, we report them as *C. exilis* and also document the species for the first time from Oklahoma.

Ascaridida: Ascaridae

***Porrocaecum angusticolle* (Molin, 1860) Baylis and Daubney, 1922 –** a single specimen was taken from the same *B. jamaicensis* (Gmelin) noted above. This nematode species primarily infects birds of the orders Accipitriformes and Strigiformes. It has been previously reported from *B. jamaicensis* as well as other raptors (Canavan 1929; Morgan and Schiller 1950). More recently, McAllister et al. (2019a) reported *P. angusticolle* from a red-shouldered hawk, *Buteo lineatus* (Gmelin) from Arkansas, and also noted this nematode has been reported from six species of hawks from the Nearctic Realm. We document this ascarid from Oklahoma for the first time.

***Porrocaecum depressum* (Zeder, 1800) –** the same *B. virginianus* collected herein harbored this nematode (Figs. 5–6, HWML 216910) in its small intestine. Specimens were identified as *P. depressum* based on the length of the esophagus and length of the spicules (425 µm) (Fig. 6). Other previously reported hosts include *S. varia* (Nadler and Hudspeth 1998; Kinsella et al. 2001), *A. otus*, Eurasian eagle-owl, *Bubo bubo* L. (Sitko 1994), *M. asio* (McAllister et al. 2019a), and spotted owl, *Strix occidentalis* Xantus de Vesey (Hoberg et al. 1989). Its North American range includes Florida (Kinsella et al.

2001), Louisiana (Nadler and Hudspeth 1998), Oklahoma (McAllister et al. 2019c, this report), Oregon (Hoberg et al. 1989), and Alberta and Manitoba, Canada (Wong et al. 1990). Kinsella et al. (2001) previously reported *P. depressum* from *B. virginianus*. Here, we document the species in *B. virginianus* from Oklahoma for the first time.

Acanthocephala: Polymorphida: Plagiorhynchidae

***Plagiorhynchus* (*Prosthorhynchus*) *cylindraceus* (Goeze, 1782) Schmidt and Kuntz, 1966** – A single specimen (Fig. 7, HWML 118082) was found in the small intestine of a *T. rufum* found dead on 31 March 2022 from the Hochatown site. This acanthocephalan is considered to have a cosmopolitan distribution and has usually been reported from passerine birds (Smales 1988; Hamer and Muzzall 2013). It has been reported from various Eurasian, North American, Australian, and South African avian definitive hosts, including sandpipers, ducks (rarely), and birds of prey (Listsnya 2010); shore and aquatic arthropods (crustaceans and insects) serve as intermediate hosts (Amin et al. 1999). In the US, this parasite has been reported from Colorado, Kansas, Nebraska, New Hampshire, New York, and Oregon (Amin et al. 1999). We document a new host and distributional record for *P.* (*P.*) *cylindraceus*.

***Mediorhynchus robustus* Van Cleave, 1916** – a single immature specimen (HWML 118081) was taken from the small intestine of a *T. migratorius* found dead on 3 March 2022 from Hochatown. This acanthocephalan was previously reported from *T. migratorius* from Ohio (Cooper and Crites 1976). Also, Riggins (1953) previously reported *M. robustus* from killdeer, *Charadrius vociferous* (L.) from Lake Texoma, Oklahoma. Other avian hosts of this parasite include *T. rufum*, northern flicker, *Colaptes auratus* (L.), Florida scrub jay, *Aphelocoma coerulescens* (Bosc), eastern meadowlark, *Sturnella magna* (L.), *Q. quiscala*, red-winged blackbird, *Agelaius phoeniceus* (L.), and eastern towhee, *Pipilo erythrophthalmus* (L.) (Van Cleave 1947; Kinsella 1974). We document the first time *M. robustus* has been reported from a *T. migratorius* in Oklahoma.

Arthropoda: Phthiraptera: Ischnocera: Philopteridae

***Strigiphilus oculatus* (Rudow, 1870) –** Two *B. virginianus*, one of them is the same specimen reported above as well as another individual collected DOR on 10 February 2022 from just NW of Idabel off US 70 (33°56'02.07"N, -94°53'31.07"W) was found to be infested with three females and two nymphs (L-3855, L-3856) and the latter with one male, three females, and eight nymphs (L-3866) of *S. oculatus*. The type host of *S. oculatus* is *B. virginianus* (Rudow 1870; Emerson 1961) and, as far as we know, the only other reported host is the snowy owl, *Bubo scandiacus* (L). This louse has also reported from *B. virginianus* from Alaska, California, Florida, Indiana, Massachusetts, Michigan, Minnesota, Oregon, Nebraska, New York, Pennsylvania, Tennessee, Washington, and Wyoming, and British Columbia and Saskatchewan, Canada (Peters 1936; Carriker 1966; Clayton and Price 1984; Forrester et al. 1995). As Emerson (1940) did not list the species from the state, we report it here from an Oklahoma host for the first time.

***Philopterus quiscali* (Osborn, 1896) –** a single male *P. quiscali* (L-3864) was taken from a DOR adult *Q. quiscula* collected on 7 November 2021 in Idabel (33°52'24.8"N, -94°47'35.2"W). Peters (1936) reported *P. quiscali f*rom *Q. quiscula* but no locality or date was given. In addition, Emerson (1940) did not list this louse from the state so the finding here represents the first report of *P. quiscali* from Oklahoma. *Quiscalus quiscula* is the only host listed for *P. quiscali* by Price et al. (2003).

***Menacanthus quiscali* (Price, 1977) –** the same *Q. quiscula* reported above harbored two male (Fig. 8, L-3863, L-3865) and one female *M. quiscali* (L-3866). Emerson (1940) did not list the species from Oklahoma. Interestingly, there are three specimens of *M. quiscali* collected on 2 July 1957 by G. M. Sutton in the PIPR collection (#001-967) from a great-tailed grackle, *Quiscalis mexicanus* (Gemlin) from Willis, Marshall County, Oklahoma (see https://scan-bugs.org/imglib/scan/misc/202003/

Figures 8–9. Lice from Oklahoma birds. (8) *Menacanthus quiscali*; scale bar = 500 µm. (9) *Picicola snodgrassi* from *Melanerpes erythrocephalus*; scale bar = 500 µm.

PIPR001967.jpg). However, we document this louse for the first time from the state in a refereed publication. *Quiscalus quiscula* is the type host for *M. quiscali* but Price et al. (2003) list two additional species of *Quiscalus* as hosts as well as rusty blackbird, *Euphagus carolinus* (Muller) and brown-headed cowbird, *Molothrus ater* (Boddaert). However, the latter two avian species are probably accidental hosts.

***Brueelia straminea* Denny, 1842 –** a red-headed woodpecker found dead in Hochatown on 2 November 2021 harbored this louse (PIPR collection). Emerson (1940) listed this louse from Oklahoma without providing host information; later he (Emerson 1972) provided a host list without localities. Dalgleish (1971) reported the species from *M. erythrocephalus* from Minnesota and Nebraska as well as other piciform birds from various US states. The genus is one of the largest taxa within Ischnocera, containing about 426 described species of which roughly 90% are host specific (Gustaffson and Bush 2017). We document the first report of *B. straminea* from a specified Oklahoma avian host.

***Picicola snodgrassi* Kellogg, 1896 –** the same *M. erythrocephalus* listed above also was infested with a male and female *P. snodgrassi* (L-3858, Fig. 9). It was originally described as *Lipeurus snodgrassi* from the rufous hummingbird, *Selasphorus rufus* (Gmelin) from California (Kellogg 1896). Emerson (1972) lists *M. erythrocephalus* as well as other North American piciform birds as hosts but without localities. In addition, Dalgleish (1969) provides records of *P. snodgrassi* from other woodpecker species from California, Oregon and British Columbia, Canada. Since then, it has also been reported from various piciform birds (https://phthiraptera.myspecies.info/). However, Emerson (1940) did not include this louse in his list of bird lice from Oklahoma; therefore, to our knowledge, this is the first time *P. snodgrassi* has been reported from the state.

In conclusion, we document five new host and 14 new distributional records for various parasites of birds collected in Oklahoma. Although this survey included only a few species examined for parasites, it continues to illustrate the significance of salvaging DOR raptors and other birds. Indeed, this data can yield knowledge, all in the spirit of conservation on bird parasites, that could not be obtained otherwise because of state and federal restrictions on collecting and euthanizing live migratory birds,. As many avian species remain to be surveyed in the state, additional records for their parasites should be expected, including the possibility of discovery of novel species with the use of molecular techniques.

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An Analysis of COVID-19 Infection Rates among Native American Tribal Nations in Oklahoma

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Abstract: COVID-19, the infectious disease caused by the variant of coronavirus SARS-CoV-2, has had a significant impact in the United States. However, recent research indicates that among demographic groups, Native Americans are one of the most severely affected. This study utilized the Supreme Court case *McGirt v. Oklahoma* to analyze COVID-19 cases and deaths among areas in seven tribal nations in Oklahoma to determine how they have been affected by COVID-19 compared to the general population of Oklahoma. For the analysis, descriptive statistics and incidence and case-fatality rates were evaluated. Time series plots were created to illustrate the rates of new cases. Finally, multiple linear regression models were used to predict COVID-19 deaths from cases, population, and tribal status. The analysis showed that, in general, areas within tribal nations do not have significantly different COVID-19 case and death rates from the state of Oklahoma. Since these results contradict previous findings, they indicate both the need for and importance of research on COVID-19 among Native American populations.

Introduction

In December 2019, the world was introduced to a mysterious new strain of coronavirus that would significantly change the course of events in the months to come (Tirupathi et al. 2020). Coronavirus 19, referred to as COVID-19, is an infectious disease caused by the coronavirus strain severe acute respiratory syndrome coronavirus 2, or SARS-CoV-2 (CDC 2021). It is the seventh discovered strain of coronavirus known to infect humans, but only one of three strains that can cause severe illness or even death (Andersen et al. 2020; He et al. 2020). In March 2020, after assessing the rapid spread of the virus to more than 100 countries in three months' time, the World Health Organization (WHO) officially declared COVID-19 a global pandemic (Puspitasari et al. 2020; Cucinotta and Vanelli 2020).

In the United States, the impact of COVID-19 has been felt by every demographic group, but this impact has been disproportionately distributed among minority groups (Tai et al. 2021; Tirupathi et al. 2020). Native Americans, Latinos, Pacific Islanders, and Black Americans have significantly higher rates of death from COVID-19 compared to Caucasian and Asian Americans. However, the group with the highest mortality rates is Native Americans, who have a death rate at least twice as high as that of Caucasian Americans and 2.8 times higher than that of Asian Americans (Gawthrop 2021; Gawthrop 2022). Other studies have supported these findings, reporting that tribal areas and reservations have significantly greater numbers of COVID-19 cases and deaths compared to other demographic areas (Kakol et al. 2021; Rodriguez-Lonebear et al. 2020; Wang 2021).

Although research is suggesting significantly higher mortality rates for Native Americans, the actual amount of research on COVID-19 among Indigenous Americans is lacking, especially when compared to other demographic groups (Yellow Horse and Huyser 2021). Abigail Echo-

Hawk, the director of the Urban Indian Health Institute, called the available COVID-19 data for Native Americans "a national disgrace," referencing the insufficient data on Native Americans in ethnic minority studies (Wade 2020). A significant portion of this data is aggregated and does not accurately portray the situation in individual native populations, reservations, or nations (Carroll et al. 2021). Without complete and accurate data analyses, the impact of COVID-19 on these communities will not be fully known. This will affect decisions about funding, treatment, and other measures that can help mitigate the effects of COVID-19 in these communities (Curtice and Choo 2020; Yellow Horse and Huyser 2021).

This study analyzed COVID-19 case and death rates among areas in seven tribal nations in Oklahoma to determine how they have been affected by COVID-19 compared to the state of Oklahoma. We hypothesized that, overall, areas within tribal nations experienced higher COVID-19 case and death rates compared to the state of Oklahoma. Also, Oklahoma counties that lie within the borders of tribal nations will have significantly higher numbers of cases and deaths compared to counties that do not lie within tribal nations.

Methods

Data Collection

This study relied primarily on data collection and analysis. The dataset used for this study was retrieved online from the independent nonprofit COVID Act Now (COVID Act Now 2020). As addressed earlier, there has been little data collected and analyzed for unaggregated demographic groups, especially Native Americans. This study analyzed daily COVID-19 data in Oklahoma by county rather than ethnicity. Only the data columns reporting the collective numbers of cases and deaths per day, as well as the rates of new cases and deaths per day were used. The populations of the counties in Oklahoma were retrieved from the U.S. Census Bureau (2021). The time frame of this study is from March 11, 2020, to May 1, 2021. March 11, 2020, is the date on which the WHO declared COVID-19 a global pandemic (Cucinotta and Vanelli 2020).

Organization of Data

Since this data analyzed COVID-19 data by Oklahoman county populations rather than tribal nation populations, the tribal nations analyzed in this study are defined by the counties that lie within their borders. Thus, 'tribal nations' as addressed in this paper refers to the geographic jurisdiction of the tribal nations, not the actual populations. The tribal nations were chosen in accordance with the Supreme Court decision in *McGirt vs. Oklahoma* (McGirt v. Oklahoma 2020). The Court ruled that half of Oklahoma's land lies within Native American reservation borders; therefore, their decision maintained the sovereignty and territorial borders of Native American nations in eastern Oklahoma (Fig. 1). Although this case applied specifically to the Muscogee Nation, the decision has since affected all the Five Tribes in Oklahoma and is likely to impact other tribal nations in the future (Healy and Liptak 2020; Wamsley 2020; Schwartz 2020).

The seven geographically largest tribal nations in eastern Oklahoma were examined in this analysis: The Cherokee Nation, Choctaw Nation, Chickasaw Nation, Muscogee (Creek) Nation, Seminole Nation, Citizen Potawatomi Nation, and Osage Nation. For each tribal nation, the Oklahoman counties that lie within the borders were grouped together to collectively define the tribal nation (Table 1). It is important to note that the borders of the counties and the borders of the tribal nations do not perfectly align with each other. Consequently, some counties lie only partially within a tribal nation, while others lie within two different tribal nations. To compensate for this, a county that lies partially within a tribal nation was only included in the tribal nation for this study if more than half of its land lies within the nation's border. For a county that lies within the borders of two tribal nations, the county was included in the tribal nation that contained more of its land. The only two counties for which this system was not applied were Tulsa and Muskogee counties. These counties lie within the Cherokee Nation and the

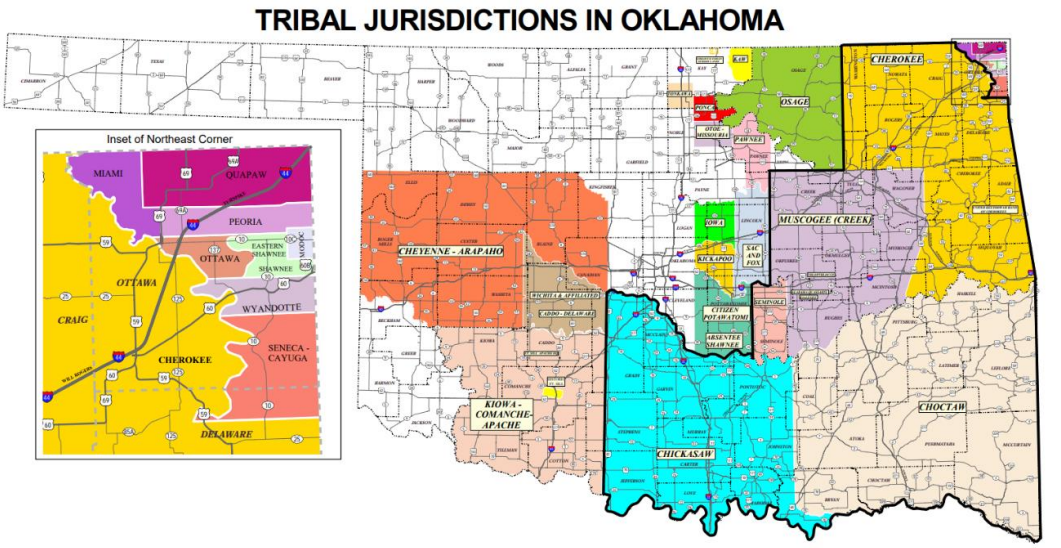

Figure 1. Map of *McGirt vs. Oklahoma* boundaries.

Note. Reprinted from "Supreme Court upholds American Indian treaty promises, orders Oklahoma to follow federal law", by Murphy S & Gresko J. 2020, July 10. The inset on the left of the map is an enlarged picture of the tribal nations in the upper right corner of the state. Due to the nature of this study, these nations were not included.

Muscogee Nation, with both nations containing about half of each county. Since the Cherokee Nation is larger in size and population, we have included Tulsa County in the Cherokee Nation, and Muskogee County in the Muscogee Nation.

To justify the assignment of the counties for our study, we also retrieved the tribal population percentages of each county in Oklahoma from the U.S. Census Bureau (2020) and determined whether these percentages were significantly different. We found that the tribal population percentages in our tribal-assigned counties are significantly higher than those in nontribal-assigned counties ($t = 7.3786$, $p < 0.001$). The average tribal population percentage in the tribal-assigned counties is 16.29, while the average trial population percentage in nontribal-assigned counties is 5.806 (Fig. 2). Therefore, these results support our choice of counties assigned to the tribal nations in this study.

Table 1. List of counties in each tribal nation.

Tribal Nations	Counties
Seminole	Seminole
Osage	Osage
Cherokee	Adair, Cherokee, Craig, Delaware, Mayes, Nowata, Ottawa, Rogers, Sequoyah, Tulsa, Washington
Chickasaw	Carter, Garvin, Grady, Jefferson, Johnston, Love, McClain, Marshall, Murray, Pontotoc, Stephens
Choctaw	Atoka, Bryan, Choctaw, Coal, Haskell, Latimer, LeFlore, McCurtain, Pittsburg, Pushmataha
Muscogee	Creek, Hughes, McIntosh, Muskogee, Okfuskee, Okmulgee, Wagoner
Citizen Potawatomi	Pottawatomie, Cleveland

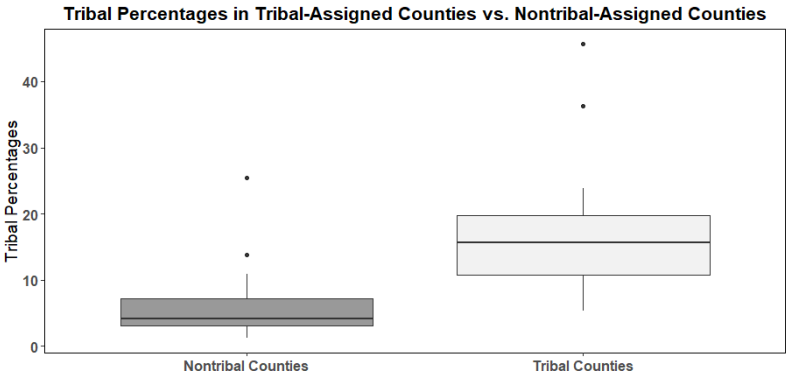

Figure 2. Boxplot of tribal percentages in tribal-assigned counties versus nontribal-assigned counties.

Data Analysis

All analyses in this study were completed using RStudio software (Version 1.4.1717). Descriptive statistics for new COVID-19 cases and deaths per day were computed for each tribal nation-assigned area. Incidence and case fatality rates were also calculated for each tribal nation-assigned area and compared to the state of Oklahoma's incidence and case-fatality rates. Proportion tests were used to compare these rates by means of the prop.test function in R. Time series graphs of the rates of new COVID-19 cases per day were created for each tribal nation. These plots were then compared to the time series graph for Oklahoma reported by COVID Act Now to determine whether the tribal nation-assigned areas experienced similar or greater rates and peaks of COVID-19 cases. All graphs were created using the ggplot package in R (Wickham 2016).

Multiple linear regression models were created to estimate the association between a county's tribal status, population, COVID-19 case total, and COVID-19 death total. The tribal status of a county was a binary categorical independent variable represented by either "0" (county is not in a tribal nation) or "1" (county is in a tribal nation). For the first regression model, the independent variables were the county's population, tribal status, and the interaction between population and tribal status, while the dependent variable was the county's COVID-19 case total. For the second regression model, the independent variables were the county's COVID-19 case total, tribal status, and the interaction between COVID-19 case total and tribal status, and the dependent variable was the county's COVID-19 death total.

After creating the first two regression models, two outlier counties were evident: Tulsa County and Oklahoma County. These two counties are the most populous in Oklahoma by far, with a difference of around 365,000 between Tulsa County and the third most populous county. To determine whether these counties were significantly affecting the results of the regressions, they were removed from the data sets, and the two regression models were re-created. All tests in this analysis were two-tailed and conducted using a p-value of 0.05.

Results

Analysis of Tribal Nation Rates

The average number of new COVID-19 cases per day and the average number of new COVID-19 deaths per day were calculated over the entire period of this study. The number of COVID-19 cases and deaths per day were both highest on average for the area within Citizen Potawatomi Nation ($\bar{x}$ = 46.73 and $\bar{x}$ = 0.63, respectively) and lowest on average for the area within the Choctaw Nation ($\bar{x}$ = 6.54 and $\bar{x}$ = 0.10, respectively). The area within Cherokee Nation had the greatest range of both new COVID-19 cases and deaths per day (*range* =

1224 and *range* = 220, respectively).

Incidence and case-fatality rates were calculated over the time period of this study for all seven tribal nations and compared to the rates for Oklahoma. The incidence rates for the tribal nation-assigned areas ranged from about 9.97 to 12.33. The area within Chickasaw Nation saw the highest incidence rate of COVID-19, while the area within Osage Nation saw the lowest incidence rate. The incidence rates for all the tribal nations were significantly different from the incidence rate for Oklahoma, which was approximately 11.30 (Table 2).

The case-fatality rates of the tribal nation-assigned areas ranged from 1.34 to 2.41. The areas within Seminole Nation had the highest case-fatality rate, while the area within Citizen Potawatomi Nation had the lowest case-fatality rate. The case-fatality rate for Oklahoma was approximately 1.52. The case-fatality rates for the areas within Osage, Cherokee, and Choctaw nations were not significantly different from the rate for Oklahoma, while the other four tribal nation-assigned areas were significantly different (Table 3).

Time series graphs of the daily number of new COVID-19 cases in the areas of each tribal nation were created (Fig. 3). For all tribal nation-assigned areas except the area within Osage Nation, the number of daily new COVID-19 cases began to steadily increase in late October 2020, peaking in January 2021. This is consistent with the time series graph of new COVID-19 cases per 100K for Oklahoma (COVID Act Now). The area within Osage Nation experienced an additional peak of new cases in early October 2020 that was not present in the other tribal nations.

Analysis of Counties Within Tribal Nation-Assigned Areas

Multiple linear regression models were created to estimate the association between the county's tribal status, population, case total, and death total (Fig. 4 and 5). The results of the first regression show that there is a significant effect on the total number of COVID-19 cases, with about 99.8% of the variation predicted by county population and tribal status ($F(3, 73) = 14750$, $p < 0.001$, $r^2 = .998$). The individual predictors indicate that county population is a significant predictor of total cases ($t = 157.133$, $p < 0.001$). However, the tribal status of a county is not a significant predictor of total cases ($t = -1.635$, $p = 0.106$). The interaction between county population and tribal status, though, is a significant predictor ($t = 4.512$, $p < 0.001$).

The results of the second regression indicate that there is a significant effect on the total number of COVID-19 deaths, with about 98.8% of the variation predicted by the total number of COVID-19 cases and county tribal status ($F(3, 73) = 2139$, $p < 0.001$, $r^2 = 0.988$). The individual predictors indicate that the total number of cases is a significant predictor of total deaths ($t = 59.65$, $p < 0.001$). However, the tribal status of a county is not a significant predictor of total deaths ($t = 1.269$, $p = 0.209$). The interaction between total cases and tribal status is also not significant ($t = 0.211$, $p = 0.833$).

Table 2. Incidence rates for each tribal nation-assigned area and results of proportion test between these areas' rates and Oklahoma's incidence rate. The X^2 values come from the proportion test. Note that Oklahoma's incidence rate is 11.30.

Tribal Nation	Incidence Rates	X^2 values	P-values
Seminole	11.794047	5.78	0.0162
Osage	9.967421	82.612	< 0.001
Cherokee	11.760782	166.03	< 0.001
Chickasaw	12.325385	299.26	< 0.001
Choctaw	11.838850	62.429	< 0.001
Muscogee	10.836421	61.63	< 0.001
Citizen Potawatomi	10.974857	35.33	< 0.001

Table 3. Case-fatality rates for each tribal nation-assigned area and results of proportion tests between these areas' rates and Oklahoma's case-fatality rates. The X^2 values come from the proportion test. Note that Oklahoma's case-fatality rate is 1.52.

Tribal Nation	Case-Fatality Rates	X^2 values	P-values
Seminole	2.411744	14.56	< 0.001
Osage	1.559496	0.054	0.816
Cherokee	1.469208	1.48	0.224
Chickasaw	1.761049	13.86	< 0.001
Choctaw	1.460093	0.57	0.449
Muscogee	1.896353	28.72	< 0.001
Citizen Potawatomi	1.343997	7.33	0.0068

The final two regression models were created by removing the outliers from the first two models. The results of the third regression indicate that there is a significant effect on the total number of COVID-19 cases, with about 98.7% of the variation predicted by county population and tribal status ($F(3, 73) = 10.51$, $p < 0.001$, $r^2 = 0.987$). The individual predictors indicate that county population is a significant predictor of total cases ($t = 42.920$, $p < 0.001$). However, the tribal status of a county is not a significant predictor of total cases ($t = 0.473$, $p = 0.637$). The interaction between county population and tribal status for this model is not significant unlike the model with the outliers ($t = -1.570$, $p = 0.121$).

The results of the fourth regression indicate that there is a significant effect on total number of COVID-19 deaths, with about 90.6% of the variation predicted by total number of COVID-19 cases and county tribal status ($F(3, 71) = 237.8$, $p < 0.001$, $r^2 = .906$). The individual predictors indicate that total number of cases is a significant predictor of total deaths ($t = 13.26$, $p < 0.001$). However, the tribal status of a county is not a significant predictor of total deaths ($t = 0.193$, $p = 0.847$). The interaction between total cases and tribal status of a county is also not significant ($t = 1.971$, $p = 0.0526$).

Discussion

Key Findings

The results of this study indicate that areas within tribal nations in Oklahoma do not have significantly higher COVID-19 case and death rates when compared to Oklahoma's rates, contradicting our hypothesis. The incidence rates of COVID-19 for tribal nation-assigned areas were significantly different from the incidence rate for Oklahoma. Four of the tribal nation-assigned areas had higher incidence rates than Oklahoma, while the other three areas had lower rates. The highest incidence rate for the tribal nation-assigned areas was approximately 1.09 times greater than Oklahoma's rate. According to the CDC, the incidence rate for Native Americans is approximately 1.6 times higher than the rate for Caucasian Americans (2022). While the incidence rates for these tribal nation-assigned areas were significantly greater, they were not as high as the incidence rates for Native Americans found in previous studies. This suggests that Native Americans in Oklahoma have not been infected by COVID-19 to the degree of Native Americans across the United States.

The case-fatality rates for four of the tribal nation-assigned areas were significantly different from the case-fatality rate for Oklahoma, while the case-fatality rates for the other three areas were not significantly different. The rates for the areas within Seminole, Chickasaw, and Muscogee nations were significantly higher than Oklahoma's rate. The areas within Seminole and Muscogee nations, which had the two largest case-fatality rates, were approximately 1.59 times and 1.25 times higher than Oklahoma's case-fatality rate respectively. Previous studies

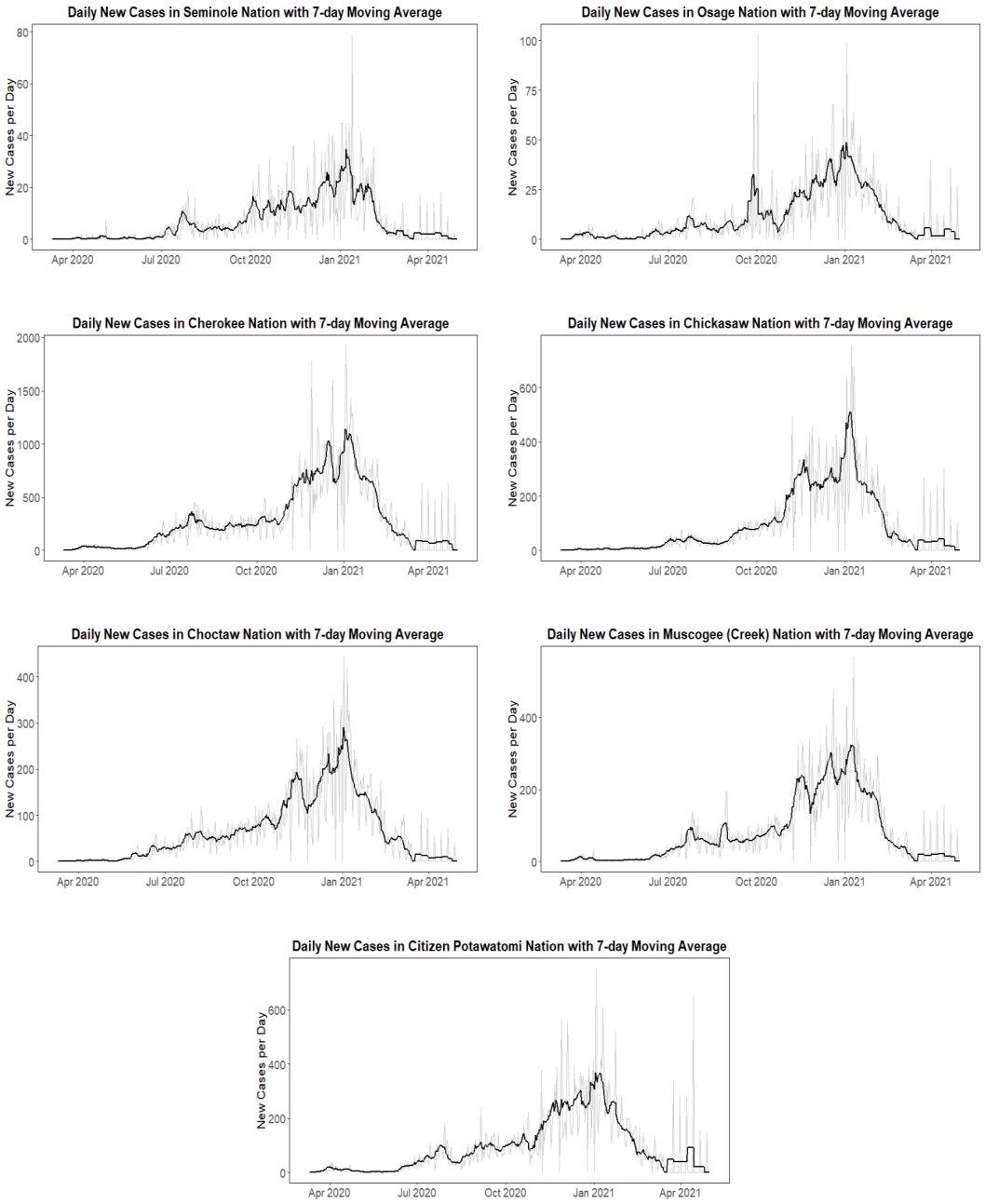

Figure 3. Time series graphs of new COVID-19 cases per day for each tribal nation-assigned area from March 11, 2020, to May 1, 2021. The trend in each tribal nation-assigned area is consistent with the trend of daily new COVID-19 cases in Oklahoma provided by COVID Act Now, https://covidactnow.org/us/oklahoma-ok/chart/5?s=39732721.

have shown that the case-fatality rate for Native Americans is at least double that of Caucasian Americans (CDC 2022; Gawthrop 2021). Thus, the case-fatality rates for tribal nations in Oklahoma were not as high as the case-fatality rates for Native Americans found

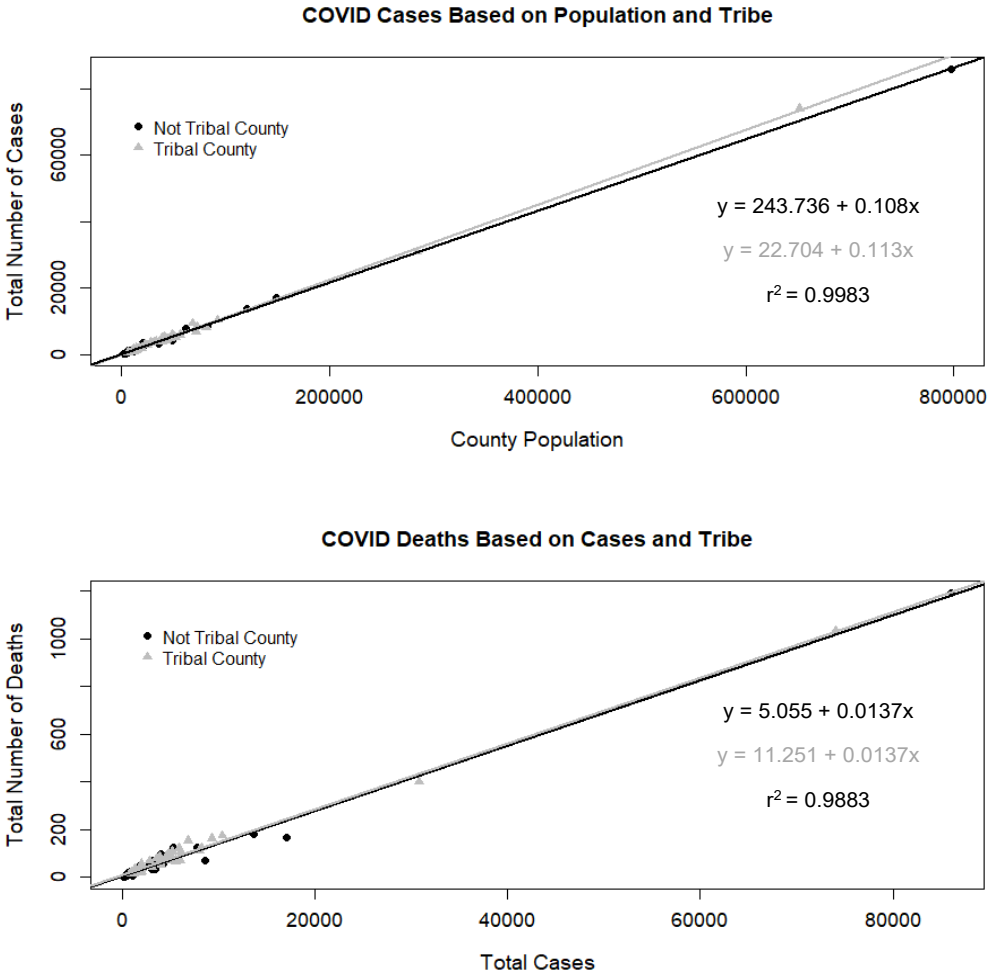

Figure 4. Multiple linear regression models for total numbers of COVID-19 cases and deaths in Oklahoman counties with outliers.

in these previous studies. This indicates that Native Americans in Oklahoma are not dying from COVID-19 at the rates of other Native Americans across the United States.

The time series plots of new COVID-19 cases in each tribal nation-assigned area revealed that, during the period of this study, the trends of new cases were consistent with the trend of new cases for the general population of Oklahoma. New COVID-19 cases began to increase in Oklahoma around early November 2020 and peaked in January 2021 (COVID Act Now). Similarly, new cases for all seven tribal nation-assigned areas increased around November 2020 and peaked in January 2021. This suggests that the tribal nations did not experience any additional surges of COVID-19 not seen in the rest of the state.

The only tribal nation area in this study that experienced an additional peak of COVID-19 cases was Osage Nation during early October 2020. According to the Osage News (2020), there were 62.1 cases for every 100,000 persons. While Osage Nation experienced this additional surge of cases, the incidence rate for the area within Osage Nation was significantly lower than Oklahoma's rate, and the case-fatality rate was not significantly different from Oklahoma's.

This indicates that Osage Nation did not have higher COVID-19 activity compared to the state.

One important thing to note is that around March 17, 2021, OSDH switched from reporting daily COVID-19 numbers to weekly numbers. The sudden decrease in new daily cases around March 2021 and the fluctuating peaks in April 2021 in the time series plots are thus due to the OSDH's change in reporting COVID-19 updates (OSDH 2021a). On April 7, 2021, the OSDH added around 1300 previously unreported cases and 1800 unreported deaths to their COVID-19 dashboard (OSDH 2021b). This addition of new cases created an unusually high report for that day, which may also explain the unusual peaks of cases in April 2021.

When analyzing the counties within the tribal nations compared to the other counties in Oklahoma, the regression models found that county population size was an independent predictor of a county's COVID-19 case total. The number of COVID-19 cases in a county was also an independent predictor of the county's COVID-19 death total. The tribal status of a county was not an independent predictor of the number of COVID-19 cases or deaths in that county.

When examining the interaction between a county's population and tribal status, the first regression model found this interaction to be significant. This means that the effect of a county's population on its COVID-19 case total depends on the tribal status of the county, which suggests that the tribal status of a county may influence total COVID-19 cases of the county. When the two outliers were removed from the model, however, the interaction was no longer significant, and the variables were independent of each other. This indicates that the outlier counties were influencing the model, and the tribal status of a county does not likely influence the number of COVID-19 cases in a county.

The results of the second regression model found that the interaction between a county's COVID-19 case total and tribal status was not significant, indicating that the variables were independent and did not influence each other. When the two outlier counties were removed from this regression, the interaction was still not significant. Both models suggest that the tribal status of a county does not likely influence the number of deaths from COVID-19 in a county. Therefore, the results of the regressions indicate that counties within tribal nation borders do not have significantly different numbers of COVID-19 cases or deaths compared to other counties in Oklahoma.

Implications

One of the most important implications of this study is the need for more reliable and accurate data for Native American populations. This is demonstrated through both the lack and inadequacy of data for indigenous populations at the state level (Yellow Horse and Huyser 2021). Due to the lack of disaggregated COVID-19 data on indigenous populations, this study relied on Oklahoma state data collected by county. However, this data does not report cases and deaths for the specific demographic groups in each county. While COVID-19 data by demographic group is available for the state of Oklahoma, this data either combines all indigenous people into an "American Indians and Alaskan natives" group or into an aggregate "other" group (APIAHF 2021; Huyser et al. 2021). The aggregation of COVID-19 data for Native Americans not only indicates the inadequacy of indigenous representation in public health data, but also presents challenges in mitigating the effects of COVID-19 on individual tribal nations and populations (Curtice and Choo 2020; Wade 2020; Yellow Horse and Huyser 2021).

Limitations

This study has potential weaknesses. Due to the lack of available COVID-19 data for Native Americans as well as the challenges of collecting data from the tribal nations, this study relied on data that includes all demographic groups in Oklahoma, not just Indigenous data. The dataset used for this study consisted of Oklahoma COVID-19 rates by county. This data includes all demographic groups in Oklahoma. Since white Oklahomans are the majority in all

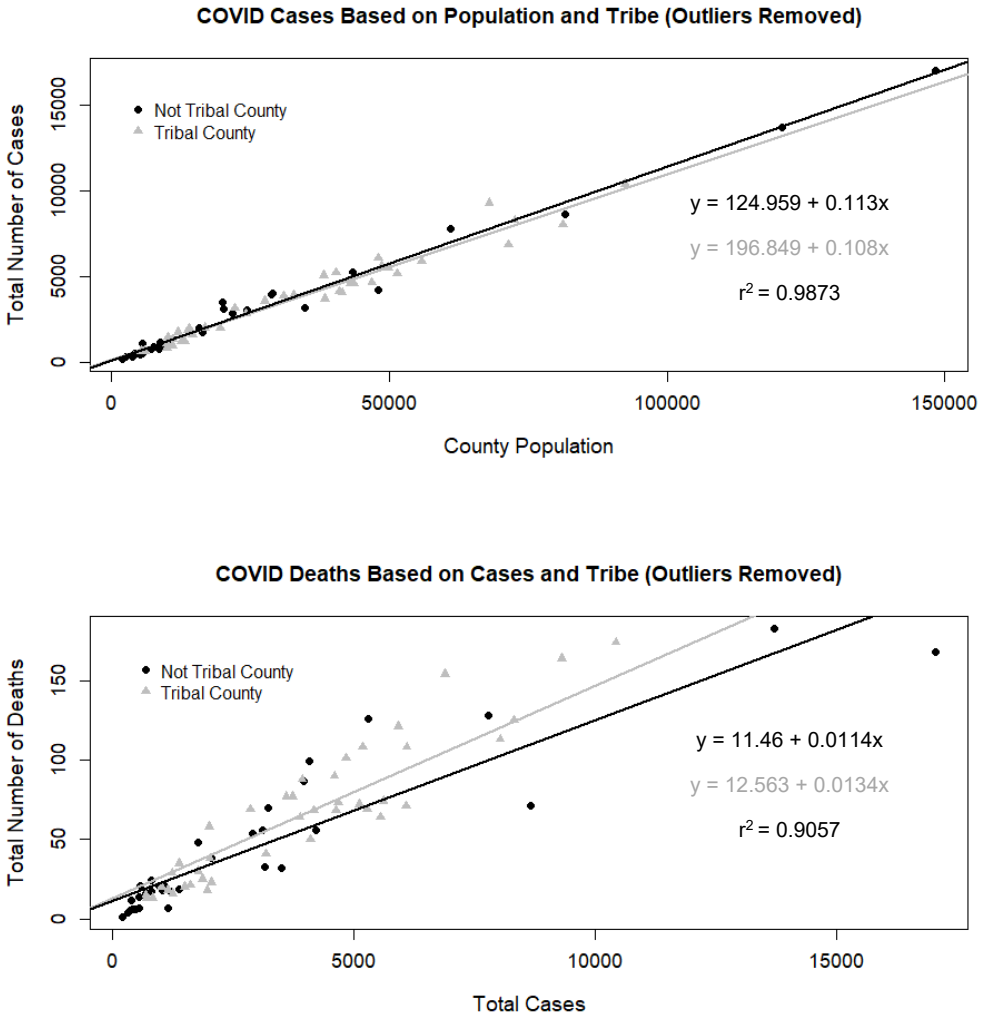

Figure 5. Multiple linear regression models for total numbers of COVID-19 cases and deaths in Oklahoman counties without outliers

counties, and white Americans have the greatest raw numbers of COVID-19 cases and deaths, the data is skewed toward the rates among white Oklahomans (Gawthrop 2021). This means that it does not accurately reflect COVID-19 rates among Native Americans in Oklahoma.

Another limitation of this study is the division of the tribal nations by county. Some counties do not completely lie within one tribal nation's borders, while others lie within two different tribal nations. Since this study relied predominately on geographic distribution, we attempted to reduce as much error as possible by only including a county in a tribal nation if more than half of its land is within the nation's borders. However, this method involves error, as some counties are either included in or excluded from tribal nations. This created overestimation and underestimation of population sizes and, thus, COVID-19 cases and deaths for the tribal nations. An analysis using data from the actual populations of the tribal areas would reduce this error and ensure more accurate counts of COVID-19 rates for the tribal nations.

Conclusion

This study aimed to determine how tribal nations in Oklahoma have been affected by COVID-19 compared to the state of Oklahoma. In general, areas within tribal nations do not have significantly different COVID-19 case and death rates from the general population of Oklahoma. These areas did not have significantly higher incidence and case-fatality rates from Oklahoma, nor did they experience additional surges of COVID-19 cases throughout the time period of this study. Finally, Oklahoma counties that lie within the borders of tribal nations did not have significantly different numbers of cases and deaths compared to counties that do not lie within tribal nations.

Many people and organizations continue to advocate for greater representation of Native Americans in COVID-19 studies to get more accurate results about the effects of COVID-19 on these people. This study not only demonstrates the importance of collecting both more and accurate data, but also disaggregating this data to analyze individual indigenous populations and tribal nations. In this way, we can determine the populations most affected by COVID-19 and provide assistance to those who need it most.

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Comparing Nucleophilic Substitutions Versus Elimination Reactions in Comprehensive Introductory Organic Chemistry Textbooks

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Abstract: In order to facilitate learning nucleophilic substitution and elimination, 17 comprehensive introductory organic chemistry textbooks are compared with respect to topic ordering, term abbreviation selection, including important topics, and content accuracy. Differences among texts are noted, and detailed comparisons are discussed. Pedagogically useful, consistent, and concise descriptions are given to understand the differences.

Introduction

Nucleophilic substitutions versus elimination reactions constitute an integral part of introductory organic chemistry, which is required for many graduate and professional programs in science.[1] The underlying chemistry principles governing these reactions are critical for comprehending material in other scientific disciplines. The importance of introductory organic chemistry is evidenced by its being a prerequisite for nursing, medicine, engineering, dentistry, pharmacy, biochemistry, and many sciences. If students in these majors do not understand material in the text adopted for their organic chemistry courses, then this can influence them to consult other textbooks, for additional and/or alternative explanations. Complex or weak points in course-designated texts can similarly compel students to supplement the course-designated textbook with other organic texts, which could present different definitions, terminology, acronyms, and abbreviations. While multiple explanations can provide the supplement or clarification which students seek, these explanations must be unequivocal, and multiple sources should at least agree on basic facts, terminology, acronyms, and abbreviations.

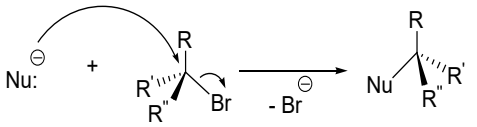
Many introductory organic chemistry students identify nucleophilic substitution versus elimination reactions as the most confusing section in the first semester introductory organic chemistry course.[2] This is due to the concurrent nature of these reactions and their similarities in reagents and reaction conditions, which hinder predicting the predominant reaction. Such confusion in students is further intensified when these texts disagree on basic facts, terminology, acronyms, and abbreviations. Variations and inconsistencies, in facts and terminology of some topics across introductory organic chemistry texts have previously been identified.[1,3] These variations and inconsistencies can originate from personal preferences of the authors and/or instructors.[4] Therefore, an analysis differentiating the two could (a) limit confusion originating from personal preferences, (b) prevent the dissemination of such discrepancies in subsequent texts, and (c) spawn general agreement on the presentation of facts, terminology, and abbreviations. The benefits of limiting such discrepancies due to personal preferences of authors and instructors have been highlighted.[1,3-5] Therefore, the treatment of these reactions in current texts is analyzed herein in order to (1) reveal discrepancies identified in nucleophilic substitutions versus elimination reactions across current organic chemistry texts and (2) spawn a general consensus among authors and instructors.

Background

In nucleophilic substitution, a molecule or atom with an electron pair replaces a leaving group on a substrate. The attacking molecule or atom is referred to as a nucleophile, and the molecule containing the positive or partially positive leaving group is called an electrophile. An example of nucleophilic substitution is hydrolysis of alkyl bromide R-Br, under alkaline conditions (eq 1), where the nucleophile is OH^- and the leaving group is Br^-.

$$\text{R-Br} + \text{OH}^- \rightarrow \text{R-OH} + \text{Br}^- \quad (1)$$

Nucleophilic substitution can involve two molecules (S_N2) or one molecule (S_N1) in the rate-determining transition state (eqs. 2 and 3 respectively). For S_N2, the departure of the leaving group from the electrophile is simultaneous with a backside attack by the nucleophile, producing stereochemical inversion in the product. In S_N1, the leaving group departs to produce a positively charged planar carbocation intermediate. Because the nucleophile can attack the resulting carbocation with equal probability from either side, S_N1 substitution is associated with racemization.



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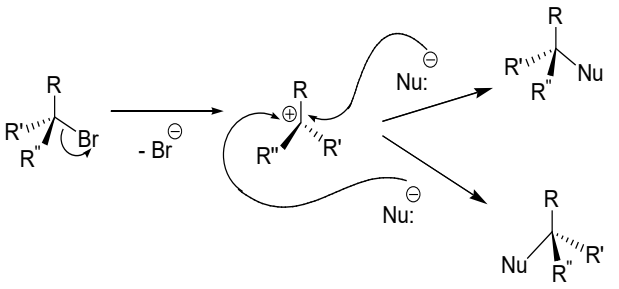


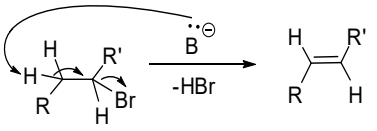
(3)

Elimination is a reaction in which two substituents are removed from a molecule, usually to form a π bond. Either the unsaturation of the molecule increases by one (to form a π bond) or the valence of an atom in the molecule decreases by two; the latter process is also known as reductive elimination.

$$\text{PhCH}_2\text{CH}_2\text{Br} + \text{MeO}^- \rightarrow \text{PhCH=CH}_2 + \text{MeOH} + \text{Br}^- \quad (4)$$

The one- and two-step mechanisms are known as E1 and E2, respectively. Often an alkane bearing a good leaving group reacts with a base to form an alkene, such as an alkyl bromide reacting with methoxide to yield an alkene (eq 4). When leaving group departure is simultaneous with proton abstraction by base, a concerted bimolecular elimination (E2)

mechanism is favored, in which two molecules participate in the rate-determining transition state (eq 5). When the leaving group departs first in order to form a carbocation, followed by rapid proton abstraction by base, a unimolecular elimination (E1) mechanism is effected (eq 6). If the proton is removed first in order to form a carbanion, followed by slow loss of the leaving group (eq 7), this mechanism (eq 7) is known as unimolecular elimination conjugate base (E1cb). The E1cb mechanism is common when there is at least one good electron-withdrawing group (EWG) adjacent to the alkane proton to be abstracted. The EWG serves to increase the Bronsted acidity of the adjacent alkane proton by stabilizing the resultant conjugate base. However, the weak acidity of alkane protons dictates that most organic molecules will not undergo E1cb.



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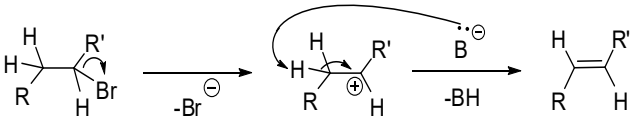

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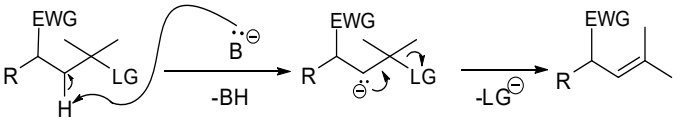

(7)

EWG = Electron Withdrawing Group

Methodology

There were selected 15 commonly used current comprehensive introductory organic chemistry textbooks (Solomons,[6] Klein,[7] J. G. Smith,[8] Hornback,[9] McMurry,[10] Jones,[11] Loudon,[12] M. B. Smith,[13] Clayden,[14] Bruice,[15] Wade,[16] Carey,[17] Brown,[18] Vollhardt,[21] and Sorrell)[22] and two additional recently used ones (Eğe[19]and Fox),[20] both of which were very popular while they were available. These textbooks were published before COVID came and posed problems for education generally, including publishing textbooks. The textbooks selected for comparison were provided by publishers; textbook descriptors (senior author, publisher, edition, and year) are listed in the extreme left four columns of Table 1.

A group of undergraduate students, who recently had completed Organic Chemistry I and Organic Chemistry II, each enrolled in an independent study course in order to assist with the project and help formulate the recommendations discussed herein. Over two semesters, about 20 students participated, joining and leaving at different times, with flexibility in their participation. Each student brought a fresh eye to the project, so each brought the valuable perspective of a student who was learning the material, rather than the perspective of a professor who had taught the course for years. Each came in at regular intervals, reviewed pertinent sections of each textbook, and offered his/her individual evaluation of each text. The results from the students for each book are compiled and compared in Tables 1-4.

Results and Discussion

There were 17 comprehensive introductory organic chemistry textbooks[6-22] selected for comparison perused, and characteristics critical to nucleophilic substitution versus elimination reactions were identified. Results of the comparisons are listed in Table 1. Many variations and inconsistencies were identified in the chapter(s) covering the reactions (Tables 1-4). For clarity, results have been grouped into subsections, and corresponding recommendations are given in each subsection, based on the patterns observed across the texts.

I. Presentation of Nucleophilic Substitution versus Elimination Reactions.

The comparison herein of Nucleophilic Substitution versus Elimination Reactions is extremely important because it is a topic which undergraduates enrolled in organic chemistry consider to be difficult and confusing. All texts canvassed[6-22] covered nucleophilic substitution and elimination reactions, but they differed in the number and/or order of chapters in which they were presented (Table 1). While six texts[12,13,17,20-22] cover nucleophilic substitution and elimination reactions in more than two chapters, six other texts[7-9,11,14,15] limit discussions of the reactions to only two chapters. The material is condensed into a single chapter by the remaining five texts.[6,10,16,18,19]

The preferred paradigm for ordering substitution and elimination mechanisms begins with the simplest reaction mechanism and trends with increasing mechanistic complexity. The S_N2 mechanism is relatively straightforward and therefore should be presented before the S_N1, because the S_N1 mechanism typically leads to a mixture of product stereoisomers and may involve a carbocation rearrangement. Similarly, nucleophilic substitution should be discussed before elimination, because the concepts learned for the former assist learning the latter. E1cb is the most complicated of the elimination reaction mechanisms, so it should be discussed last. Therefore, the preferred order of presentation of these reactions (Table 1) is bimolecular nucleophilic substitution (S_N2), unimolecular nucleophilic substitution (S_N1), bimolecular elimination (E2), unimolecular elimination (E1), and elimination unimolecular conjugate base (E1cb).

This ordering is consistent with three texts,[7-9] and four other texts[6,12,13,15] use the same order except omitting E1cb. All texts except one cover the S_N2 reaction first; one text,[22] presents the S_N1 reaction first followed by S_N2, E1, E2, then E1cb. Three texts[10,11,20] use the recommended order, except they reverse the order of the E1 and E2 reactions; three additional texts[16,17,21] do the same except they omit the E1cb reaction also. One text[14] discusses S_N2 and S_N1 together and then E2, E1, and E1cb in the recommended order. Two texts[18,19] present the S_N2 and S_N1 reactions together and the E2 and E1 reactions together also.

II. Nucleophilicity versus Basicity.

Some students find the concept of nucleophilicity versus basicity confusing. Given that nucleophilicity and basicity are kinetic and thermodynamic phenomena respectively, a clear discussion of the competition between substitution and elimination is crucial, especially in borderline situations where both are possible. To this end, it is imperative to compare nucleophilicity versus basicity in nucleophilic substitution and elimination reactions. Fifteen texts[6-18,21,22] detail such comparisons between nucleophilicity and basicity in these reactions, but two texts[19,20] omit them (Table 1).

III. Abbreviations for Leaving Group and Nucleophile.

There is great variation in abbreviations used for leaving group and nucleophile (Table 2). Eight texts[6,8-11,14,18,21] use Nu to represent nucleophile, while four texts[7,12,16,20] use Nuc. Some[13,15,17,22] opt for Y, while one[19] uses B as an abbreviation for nucleophile. We favor using Nu with a lone pair of electrons (Nu:). Using only Nu: for nucleophile avoids the ambiguity that could emerge with Nuc, B, or Y, because these could be construed to mean nucleus, base, and yield, respectively. Because nucleophiles can be neutral or negatively charged, Nu can be used for the neutral nucleophile and Nu^- for the anion.

Table 1. Substitution vs. Elimination Presentation in Comprehensive Introductory Organic Chemistry Textbooks.

	Textbooks				Substitution and Elimination Presentation								Nucleophilicity vs Basicity Comparision	
	First Author	Publisher	Current Edition	Pub. Year	# of Chapters 1	2	2+	Presentation Ordering S_N2	S_N1	E2	E1	Incl. E1cb		
Preferred Entries →					x			1	2	3	4	y	y	Ref
1	Solomons	Wiley	11	2013	x			1	2	3	4	n	y	6
2	Klein	Wiley	1	2012		x		1	2	3	4	y	y	7
3	Smith, JG	McGraw Hill	4	2014		x		1	2	3	4	y	y	8
4	Hornback	Cengage	2	2006		x		1	2	3	4	y	y	9
5	McMurry	Cengage	8	2012	x			1	2	4	3	y	y	10
6	Jones	Norton	5	2014		x		1	2	4	3	y	y	11
7	Loudon	Roberts	5	2009			x	1	2	3	4	n	y	12
8	Smith, MB	CRC Press	1	2010			x	1	2	3	4	n	y	13
9	Clayden	Oxford Univ.	2	2012		x		1	1	3	4	y	y	14
10	Bruice	Pearson	7	2013		x		1	2	3	4	n	y	15
11	Wade	Pearson	8	2013	x			1	2	4	3	n	y	16
12	Carey	McGraw Hill	9	2014			x	1	2	4	3	n	y	17
13	Brown	Cengage	7	2014	x			1	1	3	3	n	y	18
14	Ege	Houghton Mifflin	5	2004	x			1	1	3	3	n	n	19
15	Fox	Jones/Barlett	3	2004			x	1	2	4	3	y	n	20
16	Vollhardt	Macmillan	7	2014			x	1	2	4	3	n	y	21
17	Sorrell	Univ. Science	2	2006			x	2	1	4	3	y	y	22

For leaving group abbreviation, the letter X is used by ten texts,[8,10,12-17,21,22] LG is preferred in five,[6,7,11,19,20] Lv in one,[18] and L in one.[9] We recommend that LG represent leaving group, because it is most self-explanatory. It is not surprising that the letter X might be used to represent leaving group, since X is often used to represent halide, and halide could serve as a leaving group in alkyl halides during nucleophilic substitution and elimination reactions. However, halides are not the only leaving groups on alkanes, which undergo nucleophilic substitution and elimination reactions. This supports LG being used to represent leaving group instead of the letter X, because it is self-explanatory and encompasses leaving groups other than halide, such as tosylate and mesylate. The use of Lv should be avoided, because it is unclear.

IV. Comparing Pairs of Competing Mechanisms

A. S_N2 versus S_N1

The majority of texts[6-8,10,11,16,17,19,22] examined herein compare the reactivity of substrates toward S_N2 versus S_N1 reactions by listing the substrates as a series. In these nine texts, substrates most reactive toward S_N2 and least reactive towards S_N1 are listed first, then the trend is to substrates least reactive toward S_N2 and most reactive towards S_N1 (Table 2). Six texts[9,12,14,15,20,21] compile similar substrates in the form of a table, while maintaining the trend. In both series and table treatments, borderline cases can be found mid-way through the trend where either S_N1 or S_N2 is plausible, depending upon reaction conditions and reagents involved. However, one text[18] implements both series and table representations for S_N2 versus S_N1 reactions, and one[13] omits the comparison totally.

Most texts present S_N2 reactivity as a series, two in charts,[14,21] one in a table,[12] and two[6,13] in both series and table formats. While one text[6] employs only a series presentation for S_N2 versus S_N1 reactivity, it utilizes both series and table presentations for correlating S_N2 reactivity. Similarly, one text[13] omits the S_N2 versus S_N1 comparison, but it presents reactivity just for S_N2 using both series and table presentations. When discussing S_N2 reactivity, only eight texts[6,11,15-17,19,20,22] include the bulky neopentyl substrate.

We recommend that the reactivities of substrates toward S_N2 versus S_N1 be presented together, and the data be outlined both in series and in a table. This will help students to contrast

Table 2. Reactivity Toward S_N2/S_N1 and Abbreviations Used in Comprehensive Introductory Organic Chemistry Textbooks.

	S_N2 Reactivity		S_N2/S_N1 Reactivity	Abbreviation		
	Include Neopentyl	Presentation		Leaving Group	Nucleophile	
Preferred Entries →	y	Series or Both		LG	Nu:	Ref
1	y	both[a]	series	LG	Nu	6
2	n	series	series	LG	Nuc	7
3	n	series	series	X	Nu	8
4	n	series	table	L	Nu	9
5	n	series	series	X	Nu	10
6	y	series	series	LG	Nu	11
7	n	table	table	X	Nuc	12
8	n	both[a]	none	X	Y	13
9	n	chart	table	X	Nu	14
10	y	series	table	X	Y	15
11	y	series	series	X	Nuc	16
12	y	series	series	X	Y	17
13	n	series	both[a]	Lv	Nu	18
14	y	series	series	LG	B	19
15	y	series	table	LG	Nuc	20
16	n	chart	table	X	Nu	21
17	y	series	series	X	Y	22

[a]Both = series and table

the contributing role of steric and electronic requirements in S_N2 versus S_N1 reactions. Both formats should be used throughout, in analyzing S_N2 and S_N1 reactions individually as well. This is because using both tables and series for comparisons or one reaction, and then switching to only a series presentation for a different reaction within the same text, can hinder grasping and assimilating information. Also, using only a series presentation for S_N2 and S_N1 reactions separately, introduces unnecessary separation between the concepts. Therefore, we suggest that reactivities of S_N2 and S_N1, as well as their comparisons, should be treated simultaneously and should use both series and table presentations.

B. S_N1 vs E1, and S_N2 vs E2

1. Presentation.

When competing nucleophilic substitutions and elimination reactions are plausible, it can be difficult to select the favored reaction pathway. It can be especially difficult to choose between S_N2 versus E2 reactions, and between S_N1 versus E1 reactions, because of similarities in each pair. S_N2 versus E2 reactions arising from a common reactant have similarities such as concerted mechanisms and an identical leaving group, while S_N1 and E1 reactions proceed through a common first step and cationic intermediate. These similarities in each reaction pair create similarities in the contributing roles of base strength, temperature, steric requirements, and nucleophile strength. Therefore, an effective discussion must compare not only nucleophilic substitution vs elimination pathways generally, but also the four sets of mechanism pairs which frequently compete: S_N1 versus S_N2, S_N1 versus E1, S_N2 versus E2, and E1 versus E2. Also, in each of these pairs, the factors which favor one selection over the other must be discussed.

However, there is little agreement among the textbooks about the comparisons which should be included. One point of agreement is that all textbooks examined do compare S_N1 versus S_N2 mechanisms and the factors which favor each.

Some texts do not compare these pairs of mechanisms or even nucleophilic substitution versus elimination generally. Only two[17,18] include both a general comparison between nucleophilic substitution versus elimination, as well as specific comparisons between S_N2 versus E2 mechanisms and between S_N1 versus E1 mechanisms.

While eleven texts[7,8,10,12-14,17-19,21,22] present general comparisons between nucleophilic substitution and elimination reactions, six texts[6,9,11,15,16,20] omit such comparisons, but effectively contrast S_N2 versus E2 and S_N1 versus E1.

However, we recommend thorough and comprehensive comparisons, not just between nucleophilic substitutions (S_N1 versus S_N2) and between elimination reactions (E1 versus E2), but for S_N2 versus E2, and for S_N1 versus E1 as well. Such information gives students criteria needed to predict the major product in each reaction, and the mechanism by which it is formed.

2. Factors Affecting the Reactions.

Predicting whether nucleophilic substitution or elimination predominates in these reactions eludes many organic chemistry students, due to similar reaction conditions and reagents involved. Factors affecting nucleophilic substitutions versus elimination reactions are crucial in predicting which mechanism predominates. Therefore, it is logical to include all factors and to discuss them thoroughly both for S_N1 versus E1, and for S_N2 versus E2 comparisons. Knowing the contributing roles of these factors will help students readily identify the dominant mechanism when competing mechanism are plausible. Competing reactions (S_N1 versus E1, and S_N2 versus E2) and side reactions (carbocation rearrangement in S_N1 and E1 reactions) warrant that factors (such as base strength, temperature, steric effects, and nucleophilicity) affecting these reactions be considered carefully.

Table 3. Substitution vs. Elimination Characteristics in Comprehensive Introductory Organic Chemistry Textbooks.

	Comparison			Factors Compared								
				S_N1/E1				S_N2/E2				
	S vs. E	S_N1 vs. E1	S_N2 vs. E2	Base Strength	Temp-erature	Steric Effects	Nucleo-philicity	Base Strength	Temp-erature	Steric Effects	Nucleo-philicity	
Preferred Entries →	x	x	x	x	x	x	x	x	x	x	x	Ref
1		x	x		x			x	x	x	x	6
2	x			x	x	x	x	x	x	x	x	7
3	x	x		x		x	x	x	x	x	x	8
4		x	x	x			x	x	x	x	x	9
5	x		x	x				x	x		x	10
6		x	x				x	x	x	x	x	11
7	x			x			x	x			x	12
8	x			x		x	x	x		x	x	13
9	x			x	x	x	x	x	x	x	x	14
10		x	x	x				x	x	x	x	15
11		x	x	x	x		x	x		x	x	16
12	x	x	x	x	x	x		x	x	x	x	17
13	x	x	x	x				x	x	x	x	18
14	x			x	x	x		x	x	x		19
15		x	x		x		x	x			x	20
16	x			x		x	x	x	x		x	21
17	x		x		x	x	x		x	x	x	22

In spite of the pivotal role of these factors in predicting whether substitution or elimination predominates, there seems little agreement among these texts, in presentation method, technique, or order for comparing S_N2 versus E2, or S_N1 versus E1. In general, the texts emphasize comparisons between S_N2 versus E2 more than those between S_N1 versus E1. This is demonstrated by fewer entries in the S_N1 versus E1 comparison column versus the S_N2 versus E2 column (Table 3).

In comparing S_N2 versus E2, nine texts[6-9,11,14,15,17,18] consider all four reaction mechanism determinants: base strength, temperature, steric effects, and nucleophilicity. Six texts[10,13,16,19,21,22] elaborate upon three of the four characteristics; one[22] excludes basicity, two[13,16] omits temperature, two[10,21] exclude steric requirements, and one[19] omits nucleophilicity.

In comparing S_N1 versus E1, only two texts[7,9] use all four factors. Most texts[8,13,16,17,19,21,22] use three of the four above factors, one[22] of these omits base strength, three[8,13,21] omit temperature, one[16] omits steric requirements, and two texts[17,19] omit nucleophile strength. One text[12] relies on base strength and nucleophile strength, and one[20] uses reaction temperature and nucleophile strength. Surprisingly, in some texts, only one factor is used to compare S_N1 versus E1; three[10,15,18] discuss only base strength, while two[6,11] consider reaction temperature and nucleophile strength only, respectively.

3. Zaitsev and Hofmann Elimination.

Zaitsev rules and Hofmann rules predict the regio- and stereochemistry of the new double bond resulting from E2 elimination. Both Zaitsev elimination and Hofmann elimination should be discussed as parts of E2 elimination, because they include concepts critical to predicting the elimination products. The Zaitsev elimination should be discussed first

Table 4. Zaitsev vs. Hoffman Elimination on Presentation in Comprehensive Introductory Organic Chemistry Textbooks.

	Zaitsev Elim.	Hofmann Elim.	Zaitsev vs. Hofmann Regiochemical Determinants				
	Rationale Included	Presented w/E2	Steric Effects	Carbanion Stability	Proton Acidity	LG Basicity	
Preferred Entries →	y	y	y	y	y	y	Ref
1	y	y	n	y	n	n	6
2	n	n	y	y	n	n	7
3	y	n	y	n	n	n	8
4	y	y	y	y	y	n	9
5	n	n	y	n	n	n	10
6	y	y	y	y	n	y	11
7	y	n	n	n	n	y	12
8	y	n	y	n	n	n	13
9	n	y	y	n	y	n	14
10	y	y	y	y	n	n	15
11	n	y	y	n	n	n	16
12	n	n	y	n	n	n	17
13	n	n	y	n	n	y	18
14	y	n	n	n	n	n	19
15	y	y	y	n	n	n	20
16	y	y	y	n	n	n	21
17	n	n	n	n	n	n	22

due to its straightforward general mechanism, followed by the Hofmann elimination, which applies in specific cases. The rationale for the Zaitsev elimination is included only in some texts.[6,8,9,11-13,15,19-21] Some textbooks[6,9,11,14-16,20,21] present the Hofmann elimination alongside the E2 elimination, while others[7,8,10,12,13,17,18] present it independently (Table 4). Two texts,[19,22] omit the Hofmann elimination entirely.

Comparing the Zaitsev and the Hofmann eliminations uses substrate steric requirements, carbanion stability, proton acidity, and leaving group basicity as regiochemical determinants (Table 4). However, no text uses all four criteria. Although one text[9] considers steric effects, carbanion stability, and proton acidity, it omits basicity; another text[11] omits proton acidity, but considers the other three factors. Carbanion stability, proton acidity, or leaving group basicity are discussed in a few texts; some[6,7,9,11,15] utilize carbanion stability, others concentrate on proton acidity[9,14] or basicity[11,12,18] only. Most texts[7-11,13-18,20] present steric hindrance as a regiochemical determinant. It is preferable that all four determinants indicated be used in comparing the Zaitsev elimination versus the Hofmann elimination, in order to help students better understand the underlying principles and how to apply them.

Conclusion

Pertinent sections of 17 comprehensive and recently used introductory organic chemistry textbooks dealing with nucleophilic substitution versus elimination reactions were canvassed. These textbooks have many variations and differences in content and presentation of nucleophilic substitution versus elimination reactions. Comparing the texts will reduce confusion in students, especially those who consult multiple texts for alternate or multiple explanations and guide modifications to later editions.

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Comparing Atomic versus Molecular Orbitals in Comprehensive Introductory Organic Chemistry Textbooks

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Abstract: Variations, factual inaccuracies, inconsistencies in presentation, and ordering of various topics related to atomic and molecular orbitals in comprehensive introductory organic chemistry textbooks prompted their comparison. This work analyzes atomic orbitals, molecular orbitals, and related concepts in introductory organic chemistry and provides student-identified remedies. The recommendations are fact-based, pedagogically useful, and designed to clarify discrepancies in comprehensive introductory organic chemistry textbooks and avoid the inadequacies from being carried to future editions.

Introduction

Understanding atomic and molecular orbital characteristics is important to learning molecular structure, bonding, and reactivity. Because these are basic concepts in introductory organic chemistry, their accurate presentation is critical in order for students to learn the subject. Inconsistencies in these concepts found in some current comprehensive introductory organic chemistry textbooks can impede student learning. Accordingly, we methodically compared currently used comprehensive organic chemistry textbooks in order to assess their accuracy and consistency in presenting atomic and molecular orbital characteristics.

The importance of topic sequencing in comprehensive introductory organic chemistry has been highlighted recently.[1] Therefore, we also evaluated selected additional aspects of atomic and molecular orbitals in these texts, including topic sequence.

I. Atomic Orbital and Molecular Orbital Characteristics

Discussing the variety of atomic and molecular orbitals found in chemistry is beyond the scope of this article. Therefore, the atomic and molecular orbitals considered herein are the orbitals and hybridized orbitals which are most often used in organic chemistry.

A. Atomic Orbitals

An atomic orbital (AO) is a theoretical region of space around an atomic nucleus where the probability of finding an electron is high. The AOs which are most used in organic chemistry reactions are s, p, d, and f orbitals.[2] The following descriptions and explanations seem to be the minimum needed for undergraduates to understand the role of orbitals in bonding and molecular structure in organic chemistry and should therefore be included in undergraduate organic chemistry texts.

B. Molecular Orbitals

Molecular orbitals, as the name implies, are orbitals representing the region of space occupied by electrons in molecules.[3a] A molecular orbital (MO) encompasses more than one nucleus and is obtained by combining AOs in the molecule.[3a] According to MO theory, the two ways for orbitals to interact and form a molecular bond are via an additive interaction or a subtractive interaction. The additive

interaction (constructive interference) leads to formation of a MO that is roughly egg-shaped, whereas the subtractive interaction (destructive interference) leads to formation of a MO than contains a node between the atoms.[3a] The overlap of two AOs with matching symmetry, similar energy, and close contact forms two MOs; these are one lower-energy bonding MO and one higher-energy antibonding MO, as shown in Figure 1.[3a] The symmetry properties and relative energies of AOs determine how the orbitals interact or mix to form MOs.[3a,b] AOs are less stable than the bonding MOs which they form, and more stable than the corresponding anti-bonding MOs.[3a]

Electrons populate MOs preferring lower-energy MOs.[3b] The result is that the overall energy of the electrons in the occupied MOs are lower in energy than the overall energy of the electrons in the original AOs, and the resulting molecule has a lower total energy than the separate atoms.[3b] When two AOs have quite different energies, their interaction is weak, and the resulting MOs have characteristics such as energy and shape, which are similar to those of the AOs (1s versus 2s, or 2s versus 2p).[3b] Hybridized orbitals can also combine to form MOs, provided they have matching symmetries, have similar energies, and can make close contact. AO hybridization involves mixing AOs on an atom to create hybrid AOs.[3b] These new hybrid AOs permit greater overlap when forming MOs, and the hybridization determines the shape of the molecule.[3]

1. Sigma Molecular Orbitals (σ and σ*)

The orbital resulting from end-on overlap and constructive interference of AOs is called a bonding MO, because electron(s) in this orbital spend most of the time in the region directly between the two nuclei.[3a,b] This orbital is called a sigma (σ) MO because it looks like an s orbital when viewed along the axis of the bond.[3a,b] Placing an electron in this lower-energy orbital (Figure 1) therefore stabilizes the molecule relative to the original AOs.[3a,b]

The orbital resulting from end-on overlap and destructive interference of AOs is called an antibonding MO or sigma star (σ*) MO,[3a,b] because electrons placed in one of these orbitals spend most of their time in regions away from the area between the two nuclei.[3a,b] Because the σ* antibonding MO forces the occupying electron(s) to spend most of the time away from the area between the nuclei, placing an electron in this higher-energy orbital makes the molecule less stable, relative to the original AOs (Figure 1).[3a,b]

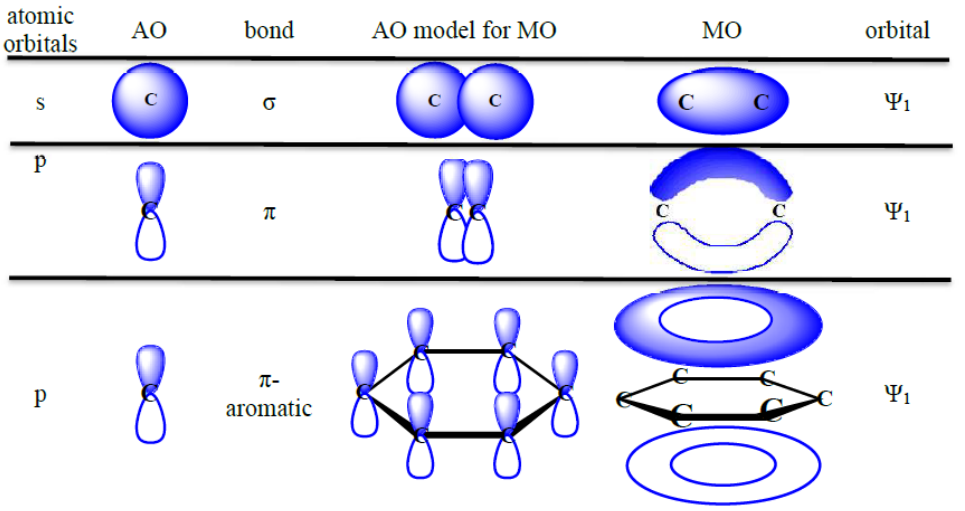

Figure 1. Schematic diagram of MOs formed from interactions between pairs of AOs.

2. Pi Molecular Orbitals (π and π^*)

The sidewise overlap of two AOs of appropriate shape and orientation results in formation of a bonding π orbital and an antibonding π^* orbital (Figure 1).[3c,d] Two atomic p orbitals constructively overlapping will result in a bonding π MO with one nodal plane in which are found the sp^2-hybridized carbons and all atoms directly bonded to them.[3c-e]

Destructive interference resulting from sideways overlap of two AOs of appropriate shape and orientation forms an antibonding π^* MO.[3c-e] The shapes of these orbitals are decided by the orbitals participating in their formation. The antibonding π^* MO should have two nodal planes; these are the nodal plane mentioned above plus an additional nodal plane resulting from destructive interference of the participating orbitals.[3c-e]

3. Nonbonding Molecular Orbitals (n)

The number of AOs which mix equals the number of resulting bonding and antibonding MOs.[3] If two AOs mix, then two MOs will result --- a bonding MO and an antibonding MO (Figure 1). However, if there are three AOs of the same symmetry and similar energy, then three MOs are formed (a low-energy bonding orbital, a high-energy antibonding orbital, and an intermediate energy nonbonding orbital).[3] AOs whose symmetries do not match, and therefore remain unchanged in the molecule, are also called nonbonding.[3] If more than three AOs are involved, even then the number of AOs will be equal to the number of MOs formed (i.e. bonding, antibonding, and nonbonding orbitals).[3]

Methodology

We compared the ordering and content of material pertaining to AOs and MOs across fourteen[4-11,13-16,18,19] currently used comprehensive introductory organic chemistry textbooks, and two additional recently used ones[12,17] (marked with asterisks (*)). In order to do this, we (A) evaluated the current edition of each textbook[4-18] and (B) compared their presentations and drawings of AOs and MOs, as well as the presentation and drawing orderings.

The same student participation as detailed in our previous publications[20] was used for textbook comparison. A group of undergraduate students, who recently had completed Organic Chemistry I and Organic Chemistry II, each enrolled in an independent study course in order to assist with the project and help formulate the recommendations discussed herein. Over two semesters, about 20 students participated, joining and leaving at different times, with flexibility in their participation. Each student brought "fresh eyes" to the project -- the valuable perspective of a student who was learning the material, rather than the perspective of a professor who had taught the course for years. Each came in at regular intervals, reviewed pertinent sections of each textbook, and offered his/her individual evaluation of each text. The results from the students for each book are compiled and compared in Tables 1-3.

Results and Discussion

Variations in the order, descriptions, and drawings of AOs and MOs are found across these textbooks.[4-19] Some texts had errors, which may be well-meant attempts to simplify the material, but which could nevertheless (1) confuse students who try to supplement their adopted text with a second text or (2) leave students ill-prepared for questions on AOs and MOs in standardized exams, such as the ACS Standardized Exam in Organic Chemistry.[21] Furthermore, recent terminology changes have not been adopted uniformly across these textbooks.

Describing AO versus MO

All textbooks explored herein[4-19] discuss and compare AOs and MOs, but some differ in the number of chapters devoted to the topics (Table 1). Textbooks also differ in how early the concepts are presented, as demonstrated by the chapter number in which AO and MO appear (Table 1, Column 5). Eight texts[4,6, 7,9,10,12,13,14] present these concepts in the first chapter, while three texts[5,16,19] discuss AO and MO in chapter three. Four texts[8,15,17,18] spread the topics across

two chapters, with the first being chapter one for AOs. One text[11] presents these concepts in chapter four.

These textbooks have appropriate figure captions except one text,[11] in which the AO and MO figure captions are missing. This textbook[11] does explain the figures (page 80-101) with in-text descriptions, but figure captions would unambiguously further explain the points (Table 1, Column 7).

Figures in the texts generally correlate well with their corresponding descriptions. Figures should help students visualize the concept, and therefore facilitate understanding it. However, figures in some texts[15,18] are less easily understood than in others (Table 1).

In another text,[10] the description of Figure 1-7 (page 26) mentions p-orbital diagrams, but the figure shows an s orbital. The text describes "the three rules for assigning electrons to atomic orbitals", on page 27 and notes Figure 1-8 as an example, but Figure 1-8 only shows an empty energy level AO diagram. Figure 1-9 in this text provides an example of filled energy diagrams, but the textbook does not provide enough examples of AO diagrams (Table 1, Column 8). This text also describes interaction of atomic p orbitals as MOs in Figures 14-2, 14-7, and 15-4 at pages 581, 590, and 648, respectively. Similarly, five other texts[11-14,16] also label atomic p orbitals as MOs (Figures at pages 147 and 159)[11]; Figures 13.16 and 14.10, pages 597 and 648[12]; Figure 20.2, page 834[13]; Figure 15.4, page 540[14]; Figures 23.5 and 24.1, pages 1206 and 1245[16].

One text[18] (Section 17.9b, page 652) has correct descriptions, but Figure 17.9 describes the interactions of atomic p orbitals of benzene as molecular orbitals, but it does not show MOs. This hinders understanding the types of orbitals represented there. Inconsistent depiction of AOs and MOs in this the section causes the "N" in the column "AO vs. MO differentiation clear" (Table 1, Column 11).

The descriptions of AOs and MOs in two texts[15,18] are not easy to understand. In one[18] (Section 17.9a, page 651), AOs are combined to form a single-colored drawing for the lower energy bonding MO and two separate antibonding MOs. The description in Section 17.9b states "Because each of the six carbon atoms of benzene has a p orbital, six atomic p orbitals combine to form six π molecular orbitals" in referring to Figure 17.9. This instance does not follow previous representations of AOs combining to form MOs. When the six p orbitals combine into the benzene model, the orbitals

Table 1. Atomic Orbital (AO) and Molecular Orbital (MO) Characteristics in Organic Chemistry Textbooks.

textbooks					figures			text			
first author	publisher	current edition	pub year	chapter	captions	placement in-text maximizes relevance	in-text description easy to understand	clear definitions of AO & of MO	AO vs MO differentiation included	AO vs MO differentiation clear	ref
Bruice	Pearson	8	2016	1							4
Hornback	Cengage	2	2006	3							5*
Loudon	Roberts	6	2016	1							6
Solomons	Wiley	12	2016	1							7
Wade	Pearson	9	2016	1,2							8
Klein	Wiley	3	2016	1							9
Vollhardt	Macmillan	8	2018	1		N					10
Clayden	Oxford Univ.	2	2012	4	N						11
Jones	Norton	5	2014	1				N			12
Brown	Cengage	8	2017	1						N	13
McMurry	Cengage	9	2015	1							14
Carey	McGraw Hill	10	2016	2			N	N			15
M Smith	CRC Press	2	2018	3							16
Sorrell	Univ. Science	2	2006	2				N			17*
J Smith	McGraw Hill	5	2015	1			N	N	N	N	18
Karty	W W Norton	2	2018	3			N	N	N	N	19

are still depicted as the author had previously referred to as AOs. The bonding MOs appear as individual AOs instead of MOs. They show no orbital overlap (Table 1, Column 9). One text[15] has difficulty in chapter 2 in describing AO and MO theories, and their respective definitions are not explicitly stated. Although in Section 2.4 an accurate description is referenced, atomic and molecular orbital theories are not restated in Chapter 10.1, which should be the appropriate location.[15] The sections should be revised, and information should be reformatted, in a way that clearly presents the main ideas in simpler labeling directly under the figures instead of only in the text (Table 1, Columns 9 & 10).[15]

The definitions of AO and MO are very clear in some texts[4-11,13,14,16] (Table 1, Column 9), while others[12,15,17,18,19] do not define them so clearly. Color can be more effectively used if AOs and MOs are represented in different colors or different shades of one color (Table 1, Column 10). Students opined that the texts[4-9,11-18] have relevant figures. Most of the textbooks discuss the differences between AO and MO,[4-17] except one[18] which omits the differentiation completely. Figure references in all texts are correct. However, in one,[13] the bottom "cartoon" representation of the "in-phase addition" in Figure 1.21 seems incorrect; the MOs should overlap if the atoms are in-phase as shown in the "computed" representation (Table 1, Column 12). In another text[17] (Section 2.4b, pages 49-50), a clear definition of AO is not given before the term is used to define valence bond theory. Furthermore, the definition of valence bond theory is related to the definition of MO theory, which is not mentioned until almost three hundred pages later (page 307).[17]

In one text[15] (pages 372-373), definitions of AO and MO are introduced in a complicated, alternative approach, which makes the descriptions unclear. While describing one situation, another is introduced in the middle of the sentence. Also, the figure description should be more theory based. Sentences with too many clauses are distracting and difficult for students to follow (Table 1, Column 10), such as, "Recalling from Section 2.4 that the number of orbitals is equal to the number of AOs that combine to form them, we combine the three 2p AOs, one from each of the three sp^2-hybridized carbons of allyl, into the system of three π MO's shown in Figure 10.2" (page 373).

Describing and Presenting the Concept of Node in AOs and MOs

Characteristics of the description and presentation of AO node and MO node are collated in Table 2. Texts differ in the chapter number in which AO node and MO node are presented. Ten texts[4,7-10,12-15,18] discuss them in the first chapter. Two texts[5,19] present them in chapter three and another text[11] presents them in chapter 4, while the description of node is spread across two chapters in three texts[6,16,17] (Table 2, Column 2). We recommend presenting the definition and discussion of node in one chapter along with the introduction of AO structure, because spreading the discussion across multiple chapters unnecessarily splits this closely related information. Overall, the concept of node is explained clearly in all the books,[4-6,8-18] except one.[15]

The texts also differ in the number of figures used to explain the concepts of AO and MO nodes. Six[4,7,8,10,11,19] use two figures to explain them, while two[12,13] use three figures, and another two[6,9] use four. Four texts[5,15,16,18] use one figure to discuss node, while two texts[14,17] use no figures at all to explain the concept of node (Table 2, Column 4).

In addition to figures, an appropriate description is also required in order to clearly present the concept of node. However, different textbooks use different amounts of text to explain node. It is difficult to judge how many paragraphs are needed to explain the concept of node, but the descriptive text should be enough to explain node characteristics, so that it is clear and does not overwhelm students. Four texts[9,10,11,12] have four paragraphs, while one text[4] uses one and one-half paragraphs. Three texts[5,7,17] explain all properties of a node in one paragraph, while four texts[8,14,15,18] devote less than one complete paragraph to this (Table 2,

Table 2. Descriptions and Presentation of Node in AOs and MOs in Texts.

first author	content				term			ref
	chapter	# of figures showing node	# of paragraphs discussing node	figure accuracy	node	nodal plane (planar node)	others	
Bruice	1	2	1.5				radial node	4
Hornback	3	1	1				spherical node / nodal sphere	5
Loudon	1	4	5				spherical node / nodal sphere	6
Solomons	1	2	1				nodal surface	7
Wade	1,2	4	2					8
Klein	1	4	4			N		9
Vollhardt	1	2	3.5					10
Clayden	4	2	4				spherical node / nodal sphere	11
Jones	1	3	4				spherical node / nodal sphere	12
Brown	1	3	1	N				13
McMurry	1	0	<1			N		14
Carey	1	3	<1		N	N	nodal surface	15
M Smith	3	1	2	N		N		16
Sorrell	2	0	1			N		17
J Smith	1	1	<1			N	node of electron density	18
Karty	3	2	2					19

Column 5).

In one text,[13] Figures 1.12a, 1.14a, and 1.16a (pages 34, 35, and 36) all depict the "cartoon" versions of AOs for sp, sp^2, and sp^3 to be identical, while the computerized versions of these images show differences and only slight similarities that exist among these AOs. The size of the smaller lobe on each "computed" representation decreases as the s character increases in these hybridized orbitals. One text[16] was found to be incomplete with respect to orbital images showing the contributions from different orbitals. Although Figure 3.1 correctly depicts the orbital images and their nodes from wave functions, the shapes of the AO nodes are not shown in a manner that is easy to understand. There are some 2-D versions showing possible shapes for these orbitals but a 3-D depiction would convey a much clearer message (Table 2, Column 6). In one text,[15] (Figure 2.5b) the node is mentioned in the figure, but not in the text (Table 2, Column 7).

The use of different names for node, such as "node of electron density," "nodal surface," "radial node," and "planar node" was found during the study. One text[18] used both terms "node" and "node of electron density" interchangeably, which could lead to confusion (Table 2, Column 9). We recommend using "node" consistently throughout, because "node of electron density" might lead a student to think that a node contains electron density, which is the exact opposite of the fact that the node actually has no electron density. Two texts[7,15] use "nodal surface" in addition to "node" when explaining the area of no electron density, but one text[4] separately defines "radial node." Most texts[4-8,10-13] explain "planar node" or "nodal plane," while six texts[9,14-18] completely overlook this (Table 2, Column 8). In addition to the above-mentioned terms, four texts[5,6,11,12] use the terms "spherical node" or "nodal sphere" (Table 2, Column 9). Explaining nodal plane is important to understanding the shapes of orbitals, so it is recommended that this be included in all textbooks.

Although nine texts[4-6,8-10,12,13,16] present accurate information about node by using merely figures alone, four texts[7,10,15,18] do not have such clarity in node explanation. Similarly, nine textbooks[4,5,7-10,12,13,16] present appropriate

terms for the explanations, while five[6,11,14,15,18] do not. Some books[6,8,11-13] use multiple terms as mentioned above and clearly define and differentiate each term, while others[4,7,10,16,18] use multiple terms, but do not define and differentiate them.

Hybridization

The concept of hybridization is key in understanding the shape and geometry of a molecule. Eight texts[4,6,7,9,10,13,14,18] discuss this concept in the same chapter with AOs, while four texts[8,12,15,17] present it in the chapter immediately following AOs (Table 3, Column 2). Three texts[5,11,16] place hybridization in chapter 3, and one of them[16] splits the discussion into chapters 3 and 5. We recommend that hybridization be discussed in the same chapter with AOs, in order to understand the concept better, at a time when AOs are still fresh in students' minds. All of the textbooks[4-10,12-16,18] used appropriate terms in illustrating hybridization, with the exception of two texts.[11,17] One text[16] states, "The hybridization process is illustrated by the 'blue lobes' for electrons in the 2p-orbitals 'donated' by carbon and the 'red lobe' the electrons in the 2s-orbital".

All textbooks used appropriate figures for explaining hybridization, with accurate captions and text descriptions. Except for one text,[16] all books present the concept in a way such that it is easy for students to understand (Table 3, Column 3). Surprisingly, three texts[11,12,14] do not refer to VSEPR (Valence Shell Electron Pair Repulsion) theory in discussing hybridization, while the others do (Table 3, Column 4). VSEPR theory is a model used to predict the geometry of individual molecules from the number of electron pairs surrounding their central atoms.

Aromaticity vs Pericyclic reactions

Both AOs and MOs play a central role in explaining aromaticity as well as stereochemistry in pericyclic reactions. MO theory also enables understanding aromaticity and predicting the stability of aromatic systems. Therefore, it is important to compare across textbooks for presentation of aromaticity and pericyclic reactions with AOs and MOs. Aromaticity is explained before pericyclic reactions in nine texts,[4-6,11-14,16,18] while the reverse is true in six texts.[7-10,15,17] Aromaticity should be discussed before pericyclic reactions, because it will help understand the transition states of pericyclic reactions. If the transition state has a continuous flow of electron density, a planar geometry, and a (4n+2) number of electrons, this will satisfy the requirements for the transition state to be aromatic and more stable than expected otherwise. It is important that students know what imparts stability to a cyclic conjugated system of continuous flowing electrons. Therefore, we recommend presenting aromaticity before pericyclic reactions and conjugated systems. One text[10] only discusses electrocyclic reactions in the pericyclic reactions chapter.

The texts range widely in the number of chapters separating aromaticity and pericyclic reaction discussions. One text[8] presents aromaticity immediately followed by pericyclic reactions (0 chapters apart), two texts[13,16] separate them by two chapters, one text[5] by five, one text[12] by six, one text[18] by nine, one text[6] by eleven, one text[14] by fourteen, one text[4] by nineteen, and one text[11] has a twenty-five-chapter separation. Furthermore, one text[16] chapter on pericyclic reactions focuses on sigmatropic rearrangements only.

Eleven texts[5,7-10,13-18] explain aromaticity in the benzene and aromatic compounds chapter. Eight texts[4-6,11,12,14,16,18] discuss pericyclic reactions separately from benzene and conjugated π systems, while six texts[7-10,15,17] discuss it with the conjugated pi-electron systems. Only one text[11] discusses pericyclic reactions in two chapters while the other books discuss the topic in one chapter either alone or with π-conjugated systems. One text[13] presents pericyclic reactions with other C-C bond formation reactions, which include charge-controlled reactions (electrophilic additions to conjugated dienes) in addition to orbital-controlled pericyclic reactions (electrocyclic, cycloaddition, and sigmatropic reactions).

Table 3. Hybridization Characteristics in Textbook.

first author	hybridization			
	chapter	easy to understand	reference to VSEPR theory	ref
Bruice	1			4
Hornback	3			5
Loudon	1			6
Solomons	1			7
Wade	2			8
Klein	1			9
Vollhardt	1			10
Clayden	4		N	11
Jones	2		N	12
Brown	1			13
McMurry	1		N	14
Carey	1,2			15
M Smith	3,5	N		16
Sorrell	2			17
J Smith	1			18
Karty	3			19

Conclusion

Comparing fifteen currently used comprehensive introductory organic chemistry textbooks reveals discrepancies, variations, and inconsistencies in their presentations, figures, and discussions of AO and MO concepts. Recommendations with appropriate and reasonable justifications have been provided in order to remedy these shortcomings. In order to facilitate students learning these concepts, the recommendations are pedagogically useful, consistent, descriptive, and in agreement with research literature.

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Women in Space: From Historical Trend to Future Forecasts

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Abstract: In the history of space exploration, the gender balance has tended to favor men. However, the STEM (science, technology, engineering, and mathematics) fields have increasingly had women represented. This trend is often encouraged nowadays as openness to gender diversity in technology has been realized to be beneficial to the overall level of expertise in the fields, to the economy, and to those wishing to be part of these fields. Astronautics, the occupation charged with the direct human exploration of outer space forms an interesting case study.

Our study shows that the percentage of women throughout the world in the astronautics field and thus involved in space exploration has increased over time. This article explores the contribution of women as astronauts in terms of the percentages of astronauts that are women, the historical trend of increase in these percentages, and the extrapolation of the historical trend to provide foresight into future percentages. We are able to project, assuming the historical trend continues, the possible future increases in percentages of female astronauts worldwide.

Introduction

Why should we be interested in whether women are counted among astronauts or not? One reason is the often-noted need for increasing numbers of women in science and technology. For astronauts in particular, if humankind is to expand into the cosmos without limits, self-perpetuating colonies will be necessary, making both male and female astronauts essential. This article explores the historical trend in women as astronauts and its extrapolation into the future.

Historical Notes on Women's Involvement in Astronautics

Perhaps the earliest effort to put women in space was the 1959 WISE (Women in Space Earliest) program, which was initially cancelled but soon reconstituted as the Woman in Space Program which lasted until 1962 (Ryan et al. 2009). This led to a method for selecting female astronauts that was subsequently proposed and designed (Betson and Secrest 1964) but ultimately not implemented.

Ryan et al. (2009) note that the trend nowadays of women's participation in space has been related to national pride. The National Aeronautics and Space Administration (2017) discusses how exploring space has long been of great interest to humankind. Women have not participated in this exploration as much as men for various reasons. Women and men can see things differently and therefore can complement each other, yet their presence and full participation in the process has often been impeded by the "glass orbit" (Beall 2019), despite the fact that simulated spaceflight stress experiments on small groups comparing men and women found complementary responses across genders that have the potential to strengthen community problem-solving efforts (Sýkora et al. (1996).

Various other articles have discussed different aspects of the topic of women as astronauts. Individual profiles of female astronauts appear in Williamson (2021), Betz (2020), Space.com (2022), and Gibson (2014). The status of women in the space industry in general, not just as astronauts, is discussed in United Nations (2021), while the US Bureau of Labor Statistics (BLS Reports 2021) explores the wider labor market, providing context. A proposal for sending women to Mars, instead of men, is explained in Landis (2000), an idea expanded in scope in Nadia (2019). Some of the authors' personal experiences in undergraduate classrooms indicates that, with Mars as a destination, there would be plenty of volunteers.

Motivation of the Study

Given the historical interest in women as astronauts, and the apparent growth in their involvement over time, we have conducted a data analysis to project the future levels of women in the astronautics profession.

Data Analysis & Results

Some initial figures provide context by showing basic tabulations. The data is from McDowell (2022). These figures collectively indicate the disparity of women's involvement in space exploration.

Figure 1 shows the overall number of astronauts by gender as of the end of 2021. Figure 2 shows each year's percentage of male astronauts taking their first flights, out of the 100% total for all years from 1961 to 2021. Figure 3 provides the percentages of female astronauts' first flights for the years from 1961 to 2021. A gap between 1964 and 1981 is prominent, illustrating that women were not very active in the field during the initial period of the space age.

Figure 4 presents the numbers of female astronauts that have traveled into space by country. From the graph, clearly the USA has had more female astronauts than any other country.

Figure 5 is a chart showing the % of male and female astronauts' first space flights by year. The percentage of women traveling into space appears to be erratic but generally increasing.

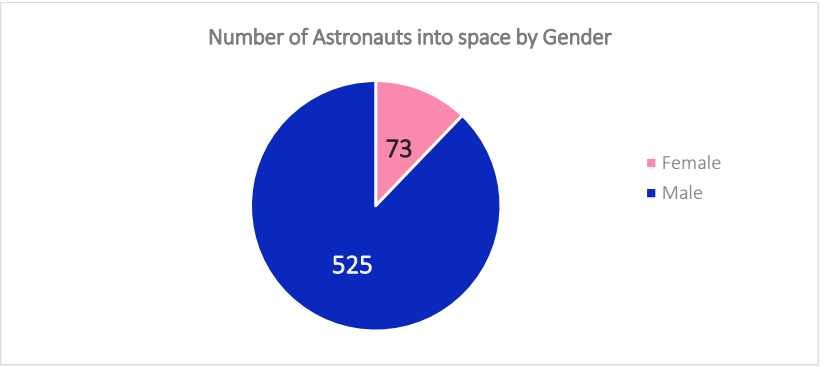

Figure 1. Cumulative number of astronauts' first trips into space by gender.

Similar to Figure 5, Figure 6 compares female and male astronauts by number from 1961 to 2021. From Figure 5 we can see that the % of women in space is relatively high for the year 2019 and 2006. However, the exact numbers were 4 and 6 respectively for years 2019 and 2006 (Figure 6), so the data is susceptible to random fluctuation due to the small totals, which likely played a significant role in the specifics for those and other years.

A New Analysis

Figure 7 fits trend curves to the historical data, then extrapolates them to show possible futures. This approach was applied to other aspects of space exploration (Hall et al. 2017, Tsai et al. 2021), and here we apply it to astronaut gender percentages. We believe this analysis to be a new contribution.

Notice that near the beginning of the spacefaring era only one female astronaut flew her first flight (1963), and after that, astronauts' first flights into space were exclusively male from 1964 to 1981. After that the percentages of female astronauts' first flights shows a fluctuating tendency of increase. Nonlinear regression was applied to fit logistic curves to the data. The

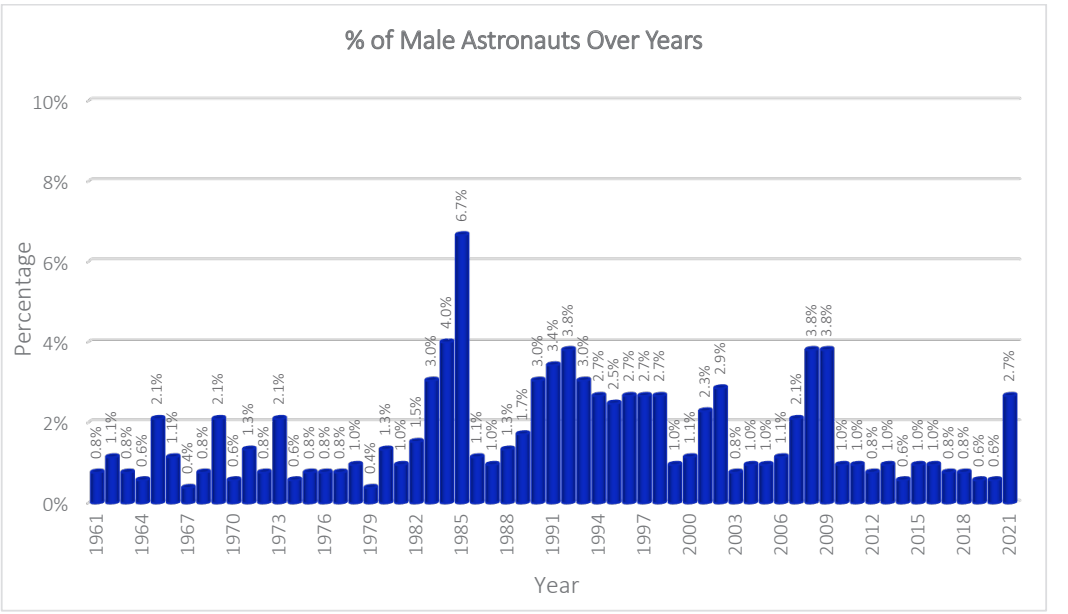

Figure 2. Percentage of male astronauts by year of first flight.

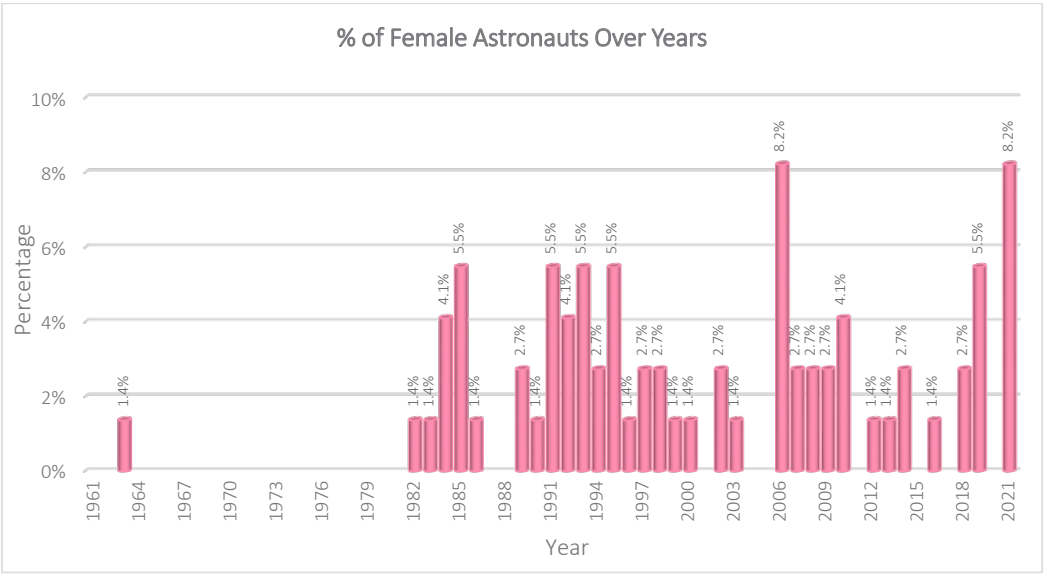

Figure 3. Percentage of female astronauts by year of first flight.

Legend: CA: Canada, CN: China, F: France, I: Italy, J: Japan, KR: South Korea, RU: Russia (including Soviet Union), UK: United Kingdom, US: United States (Source: Wikipedia, List of female astronauts).

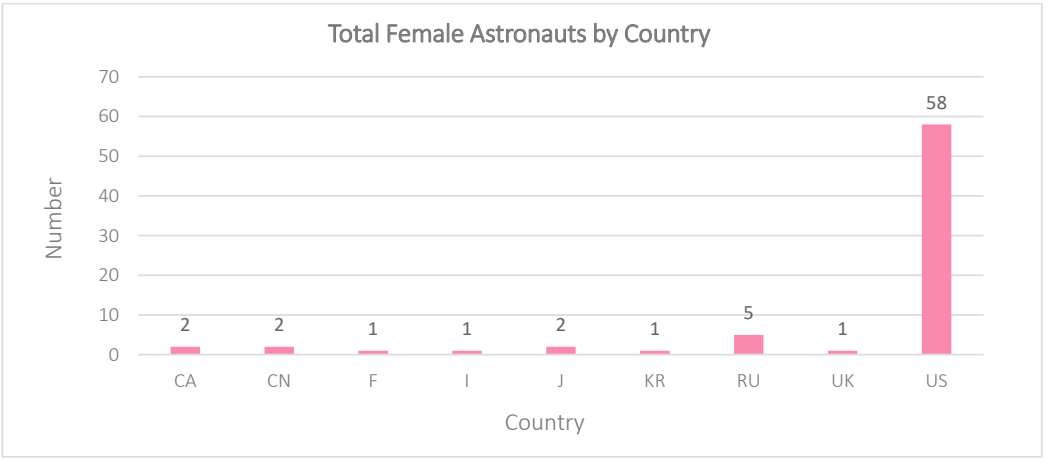

Figure 4. Numbers of female astronauts by country.

logistic curve, or S-curve, has the form:

$$f(t) = \frac{k}{1 + e^{-(t-t_0)/s}}$$

where $f(t)$ is the fraction of astronauts who are female, k is the asymptotic maximum the function reaches on the y-axis, s is the steepness (logistic growth rate), and t_0 is the inflection time point, which occurs at $k/2$ on the y-axis.

The lower curve in Figure 7 (red) was the best fit logistic, obtained by regression on all three logistic curve parameters k, s, and t_0 to find the values for them which minimize the summed squared residuals (SSR) between the data points and the curve. The value of k is 0.23, reflecting

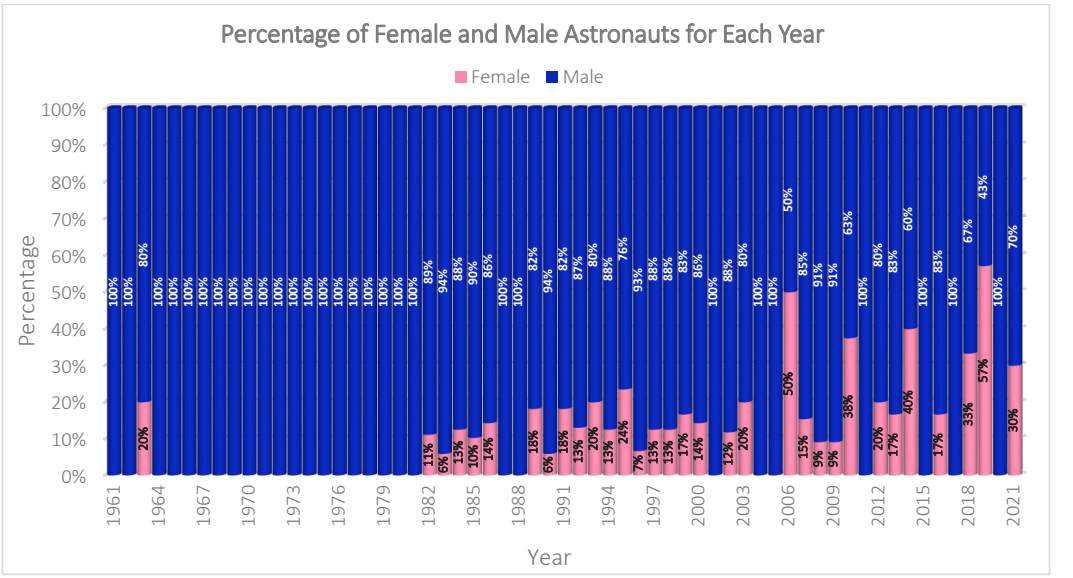

Figure 5. Percentages of female and male astronauts by year.

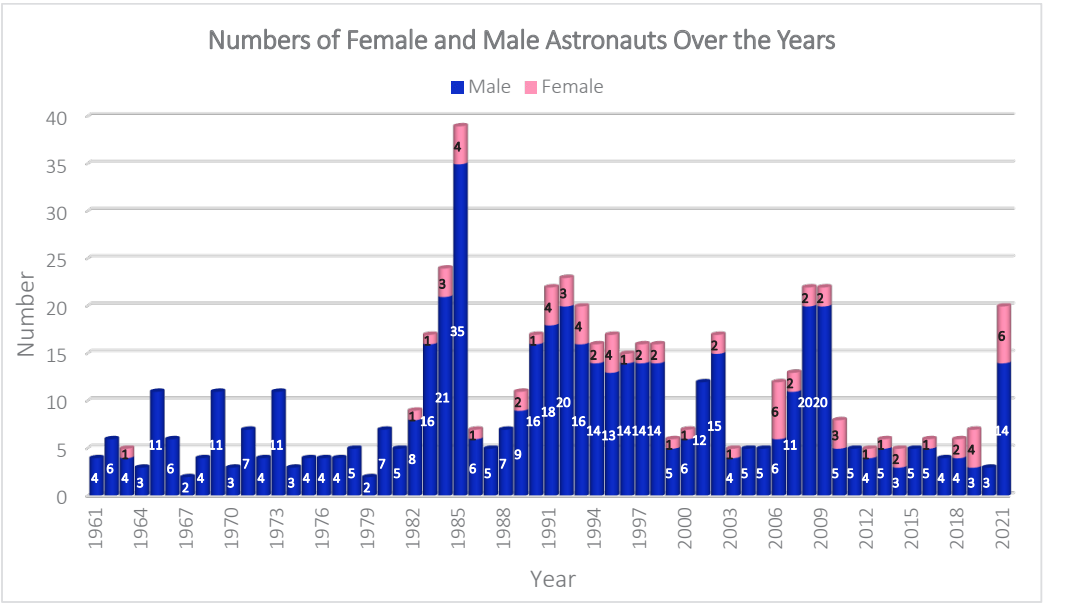

Figure 6. Number of female and male astronauts by year.

a future that ends with the percentage of female astronauts leveling off at 23%.

But what about the possibility of a future percentage that asymptotically levels off at 50%? The upper logistic curve (blue) is the result of forcing k to 50% and regressing the other two parameters to the values that reflect a logistic curve that best fits the data under the constraint $k = 0.5$.

Although the two logistic curves are, as Figure 7 illustrates, quite different overall, within the time period for which data exists they are fairly close together. In fact, the measure of fit, the SSR (summed squared residual), is

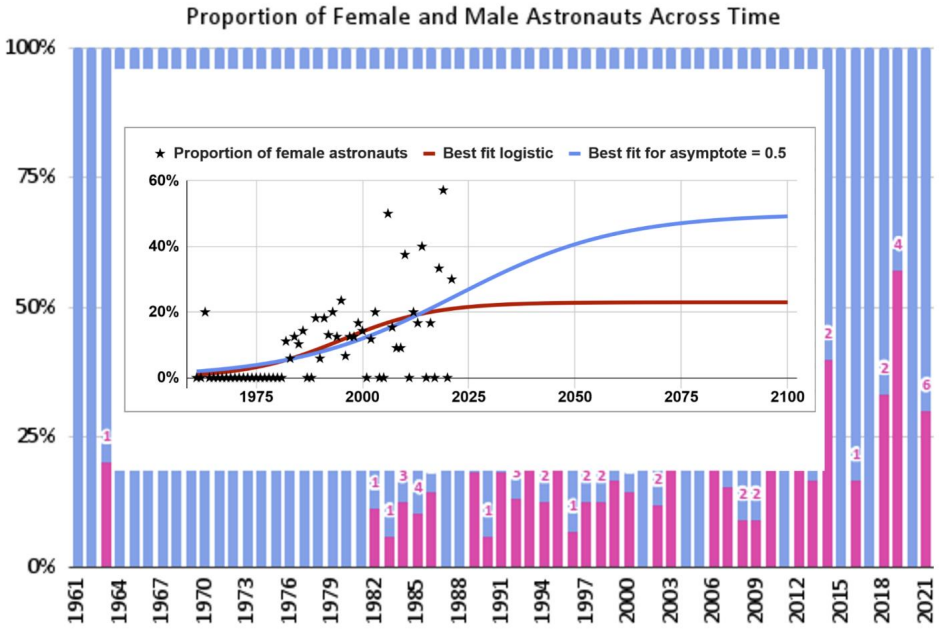

Figure 7. Logistic curves regressed to the yearly percentages of female astronauts. The black star data points in the inset correspond to the heights of the pink bars in the background histogram.

a mere 0.8% higher (worse) for the blue curve compared to the red curve. This indicates that the blue curve is almost as good a fit as the red curve, despite its much different asymptotic height, so although the blue curve is not the best fit it is well within the range of possibility (Berleant et al. 2003). Thus, the red curve is not destiny — the blue curve, projecting an eventual 50% female astronauts, is a plausible eventuality. The future values of the blue curve provide attainable yearly subgoals on the trajectory that according to the historical data is the most likely one to end at the 50% level. This is important to know since a long-term goal of self-perpetuating extraterrestrial colonies will require both male and female astronaut colonists, and the higher the percentage of female colonists the more robust the potential for population growth.

In Figure 8 the data is analyzed to show 95% confidence limits and 95% prediction intervals with an attempt to fit a second-order polynomial using statistical regression analysis with year as the independent variable to response F% over the forty-year time period between 1981 and 2021. All except two of the data points are contained with the 95% prediction interval but many of the data points lie outside the 95% confidence interval. The R-square value of 11.5% indicates that there is not significant regression between year and F% where F is defined as a ratio which compares the variation explained by the model (SSR) to unexplained variation (SSE). The result of this analysis of the data on the yearly proportion of male to female astronauts' first flights is that unexplained variation is a major factor in the overall participation of women in space over the forty-year period of 1981 to 2021.

Discussion and Conclusion

Challenges encountered during the 21st century have led to rethinking and restructuring of society in many ways. Women's contributions to success in science and technology in general, and in space exploration in particular, have increased dramatically during this period, and numerous women were pioneers of some of today's widely used inventions (White House 2022). Yet obstacles to further increases remain

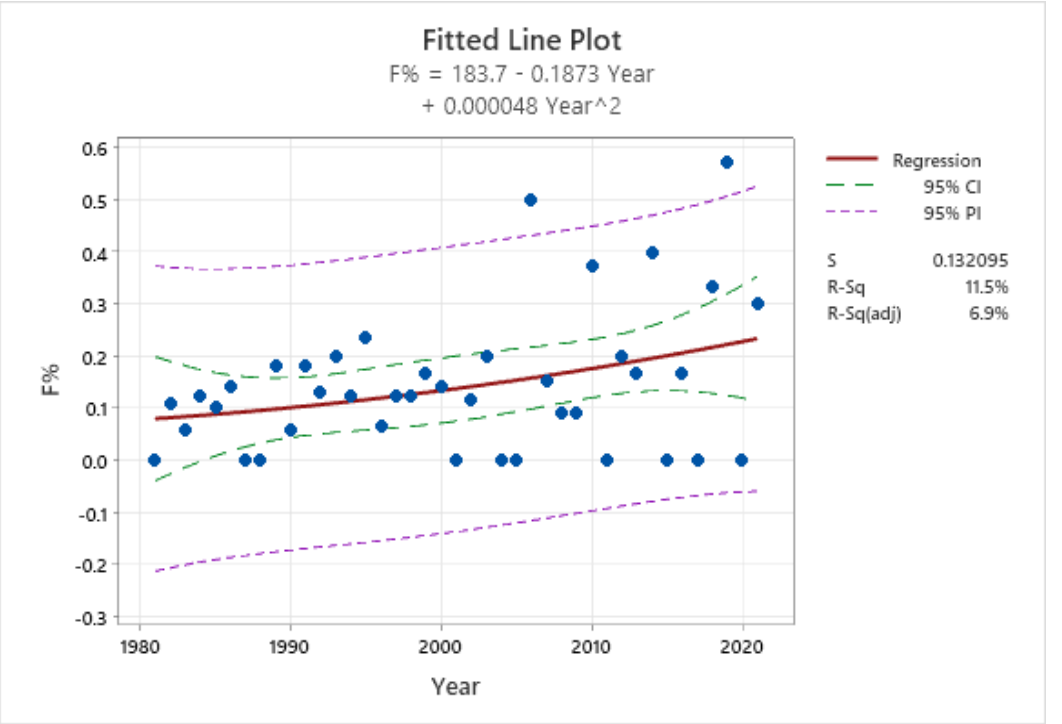

Figure 8. A study of the proportion of male versus female participation in space activities. This shows both the confidence and predictions intervals for a polynomial regression.

as challenges for society to alleviate, ranging from subcultural traits of particular fields to the need of society for child care. If society decides to commit to solving these problems, the benefits of increased contributions should follow. Such changes could enable faster and more effective progress in science and technology in general and space exploration in particular by ushering in further contributions by women participating and contributing to these fields.

The various graphs plotted have shown that women astronauts as a percentage of all astronauts was low early in the space age, but has been increasing in recent decades. We have contributed a new analysis in which the historical data record was regressed to a best-fit logistic curve which was extrapolated to identify two plausible trajectories for the future percentages of women astronauts. In the most likely scenario, the percentage of women as astronauts ultimately reaches 23%. Another likely scenario shows the most likely trajectory in which the level ultimately reaches 50%.

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Characterization of Mycobacteriophage Fulbright Isolated from Oklahoma Soil

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Abstract: Increasingly unsuccessful antibiotic remedies against emerging drug resistant bacteria make research on alternative treatments paramount. In this project, we characterized the mycobacteriophage Fulbright, evaluated the phage's efficacy against *M. smegmatis* and *M. abscessus*, and explored the possibility of using it for phage therapy applications. We found that Fulbright is stable at pH range 4-9 and temperatures ranging 20-60°C. We observed a 90-minute latent period and a lytic burst plateau 3 h after adsorption. We observed that Fulbright can infect *M. abscessus* at a concentration of $1x10^9$ PFU/mL and higher, but loses efficacy at concentrations lower than $1x10^9$ PFU/mL. In an effort to demonstrate the feasibility of using mycobacteriophage Fulbright in phage therapy applications, we electrospun Fulbright with polycaprolactone (PCL) nanofiber to serve as a model wound dressing and observed PCL_Fulbright successfully infecting *M. smegmatis.*

Introduction

The genus *Mycobacterium* is part of the order Actinomycetales and the phylum Actinobacteria and is composed of bacteria that are aerobic, acid-fast, rod-shaped, and non-spore forming. Some species have evolved into potential human pathogens, presumably due to genomic events such as genome reduction, critical gene acquisition, gene transfer, mutations, and recombination (Singh et al. 2011; Röltgen et al. 2012; Prasanna et al. 2013; Bottai et al. 2014; Franco-Paredes et al. 2019). Mycobacterial species are typically present in water and soil that humans encounter (Stinear et al. 2007; Falkinham 2009; Franco-Paredes et al. 2019), and mycobacterial infections predominantly occur by entering through open skin and mucosal barriers, leading to cutaneous or pulmonary infections, respectively (Adjemian et al. 2012; Wu & Holland 2015; Szymanski et al. 2015; Ryu et al. 2016).

Nontuberculosis Mycobacteria (NTM) are divided into two distinct groups: slow-growing mycobacteria (SGM) and rapid-growing mycobacteria (RGM) (Kim et al. 2013). *M. smegmatis* is a model organism that allows scientists to study the genus *Mycobacterium*. Using *M. smegmatis* is advantageous because it is not pathogenic and is an RGM (Gordon & Smith 1953; Beltan et al. 2000); additionally, it has been observed to be a part of the normal flora

in human sebaceous gland secretions (Brooks et al. 2010). The strain *M. smegmatis* mc^{2}155 is one of the most studied strains of *M. smegmatis* that was derived from the carbenicillin resistant parent strain, mc^{2}6 (Snapper et al. 1990).

Nontuberculosis Mycobacteria (NTM) cutaneous infections occur via direct inoculation through skin barrier damage (Griffith et al. 2007; Wang and Pancholi 2014; Forbes et al. 2018). Treatment of NTM *M. abscessus* infections typically include antibiotics such as azithromycin and imipenem (Marion et al. 2014); however, a recent case of *M. abscessus* infection on a 15-year-old patient with cystic fibrosis showed that the bacteria were not responsive to the antibiotic regimen given. Researchers genetically engineered a cocktail of phages that were collected by students in the Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program that were observed to infect *M. abscessus* in vitro. Intravenous administration of the cocktail was given over several months and patient sera did not show evidence of phage neutralization and weak cytokine responses (IFNγ, IL-6, IL-10, TNF α) were observed (Dedrick et al. 2019). Phage Fulbright discussed in this manuscript was also found under the SEA-PHAGES program and has the potential to be used in therapy against *M. abscessus* as described above.

As bacteria continue to develop resistance to antibiotics, there is a growing need for alternative treatments. Bacteriophage therapy presents as a promising alternative; however, the very nature of bacteriophages is still poorly understood. Mycobacteriophages are phages (viruses) that specifically infect mycobacteria. Because mycobacteriophages can act as bactericidal agents, they could be used directly to destroy bacterial hosts, or phage-encoded products such as lysins could be used against pathogens (Samaddar et al. 2016). Phages can also inhibit the metabolism of their hosts (host inactivation) (Miller et al. 2003; Samaddar et al. 2016), and deeper mechanistic understanding could lead to another potential therapeutic strategy.

To determine suitable phages for phage therapy applications, isolation, identification, and full characterization of the phage, while using reliable and reproducible methods, is necessary (Montso et al. 2019). Extreme temperatures can denature the protein capsid shell of phages; for example, experiments on a phage infecting *B. thailandensis* showed that the phage undergoes a lytic cycle at higher temperatures (37°C) and remains temperate at lower temperatures (25°C) (Shan et al. 2014). Extreme pH values cause hydrogen and hydroxyl ions to be highly concentrated in the water, resulting in viral inactivation (Feng et al 2003). Because highly reactive radicals in aqueous environments (such as hydroxyl and superoxyl ions) have a long lifespan, they oxidize materials in the environment and can affect phage capsid shell by removal, deformation, or denaturation of critical ligand sites and overall dissociation of the capsid. Protein shell degradation could cause RNA hydrolysis inside or outside of the phage particle, resulting in the loss of infectivity (Feng et al. 2003)

In this study, we characterized a previously isolated and sequenced mycobacteriophage Fulbright (Kotturi et al. 2021) by testing its stability in various pH and temperature conditions. We observed phage-host interaction using a one-step growth curve assay and evaluated its ability to infect *M. smegmatis* and *M. abscessus.* Lastly, we incorporated phage Fulbright into polycaprolactone (PCL_ Fulbright) to serve as a potential antibacterial wound dressing and tested its efficacy against *M. smegmatis* and *M. abscessus* bacterial lawns.

Materials and Methods

Culture medium and bacterial culture preparation

Mycobacterium smegmatis mc^{2}155 provided by the Hatfull lab at the University of Pittsburgh (Pittsburgh, PA, USA) was grown in the standard 7H9 liquid medium complete (7H9 broth base, 0.2% glycerol, albumin dextrose catalase (ADC) (10%V/V), 1 mM calcium chloride ($CaCl_2$)) and was incubated in a shaking incubator (Fisher Scientific # SHKE4450). On solid media, the

bacteria were grown on the standard 7H10 agar plates (0.5% glycerol, 0.2% dextrose, and 1 mM calcium chloride ($CaCl_2$)). Cultures grown were incubated at 37 °C. 50 µg/mL of cycloheximide and 50 µg/mL of carbenicillin were added to the liquid culture medium and 7H10 agar plates to reduce contamination. 2X Middlebrook top agar (7H9 broth base, 0.8% agar) was diluted at a 1:1 ratio with 7H9 liquid medium neat (7H9 broth base, 0.2% glycerol, 1 mM calcium chloride ($CaCl_2$)) to make 1X Middlebrook top agar, which was used to plate the bacterial lawn. The phage lysate was diluted in phage buffer (pH 7.2, 10 mM Tris, 10 mM magnesium sulfate ($MgSO_4$), 70 mM sodium chloride (NaCl), and 1 mM $CaCl_2$) to quantify the phage. For the agar-overlay method, 10 µL of each dilution was added to 250 µL of *M. smegmatis* mc^{2}155 bacteria and incubated for 10 min. After incubation, 4.5 mL of 1X Middlebrook top agar was added to the bacteria and phage mixture and transferred to a 7H10 agar plate. The plates were incubated at 37°C and assessed for plaques. *Mycobacterium abscessus* ATCC 19977 was grown in similar conditions as *M. smegmatis* but without cycloheximide and carbenicillin. Middlebrook 7H10 Oleic Albumin Dextrose Catalase (OADC) supplementation (cat #MBS652952) was used instead of calcium chloride, dextrose, and glycerol to improve bacterial growth.

Phage Fulbright stability in pH conditions

The stability of the mycobacteriophage was evaluated by incubating the phage (initial concentration of 1×10^{11} PFU/mL) in phage buffer adjusted to a range of pH values (2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12) at room temperature for 1 h following the protocol described in previous publications (Kotturi & Lopez-Davis et al. 2022; Patton 2019). The pH of the phage buffer was adjusted using HCl or NaOH and was 0.22 µm filter-sterilized. Phage was incubated at its respective pH value for 1 h at room temperature. Following the 1 h incubation, the phage solution was serially diluted, plated using the agar-overlay method, and incubated at 37 °C prior to assessment of plaques.

Phage Fulbright stability at various temperature conditions

The stability of phage Fulbright was evaluated at different temperatures (20°C, 30°C, 40°C, 50°C, 60°C, 70°C, 80°C), following the protocol described in previous works (Kotturi & Lopez-Davis et al. 2022; Patton 2019). 1 mL volume of the phage lysate (1×10^{11} PFU/mL) was dispensed onto a 1.5 mL tube and incubated at the temperature conditions for 1 h. Following thermal incubation, lysates were serially diluted in sterile phage buffer and plated using the standard agar-overlay method. After incubation, the number of PFU/mL was determined and plotted. The PFU/mL was measured at 60 minutes of incubation.

One-step growth curve of Fulbright

The one step growth curve was done at a multiplicity of infection (MOI) of 1.0 (one bacterial cell to one bacteriophage) following a protocol from previous work with mycobacteriophages (Patton 2019). The host bacterium and phage were incubated at 37ºC for 50 minutes to allow for complete phage adsorption. Following incubation, 0.4% sulfuric acid (H_2SO_4) was added to inactivate unattached phage particles, and the solution was incubated again for 5 minutes at room temperature. The H_2SO_4 was neutralized by adding 0.4% sodium hydroxide (NaOH). The bacteriophage suspension was diluted in 7H9 broth and incubated at 37 ºC. Every 30 minutes, for a duration of 8 hours, the sample solution was diluted and plated using the double agar layer method. Following incubation, the plaques were counted to determine PFU/mL and plotted.

Determining infectivity of Fulbright

Bacterial lawns were plated on standard 7H10 agar plates by mixing 250 uL of bacteria with 4.5 mL of standard 7H9 Middlebrook 1X top agar. *M. abscessus* ATCC 19977 was plated on standard 7H10 agar supplemented with OADC, while *M. smegmatis* mc^{2}155 was supplemented with 0.5% glycerol, 0.2% dextrose, and 1 mM calcium chloride ($CaCl_2$). 5 uL of serially diluted Fulbright lysate was spotted onto bacterial lawn. Plates were incubated right side up overnight to allow spots to absorb and were flipped upside-

down the next day. *M. smegmatis* plates were incubated for 2 days, while *M. abscessus* plates were incubated up to 6 days.

Comparing efficacy of PCL_Fulbright against host bacteria *M. smegmatis* and *M. abscessus*

Phage Fulbright was incorporated into polycaprolactone (PCL) fiber using methods described in our previous work (Kotturi & Lopez-Davis et al. 2022). After electrospinning, ~2cm^2 of the phage incorporated fiber was cut and plated on agar plates containing host bacterial lawn, *M. smegmatis* or *M. abscessus.* The plates were incubated at 37°C for 48 hours and assessed for clear borders surrounding the fiber.

Data analysis

All experiments were performed in triplicates unless otherwise noted. GraphPad prism was used to calculate one-way ANOVA and Tukey post hoc tests and establish multiple comparisons between samples. A p-value below 0.05 was considered statistically significant.

Results and Discussion

Our results showed that mycobacteriophage Fulbright is stable for up to 60 minutes when exposed to temperatures 20-60°C, and the phage particle is inactivated at 70°C and above (Figure 1). In contrast, other novel mycobacteriophages described in Stella et al. (2013) were isolated at 30°C and were unable to propagate at a higher temperature of 37°C. Phage Cepens was found to be unstable at temperatures 37°C and higher (Cantrell 2019). Our results are comparable to bacteriophages against *Vibrio parahaemolyticus*, which retained infectivity between 25-50°C (Yin et al. 2019).

pH stability assays showed that Fulbright is stable in pH 4-9, with a ten-fold decrease at pH 3. There was a significant difference in phage titer between pH 3 and 4 ($p<0.0001$). No viable plaques were observed at pH 2, 10, 11, and 12 (Figure 2). *Vibrio* phages studied by Yin and others (2019) were found to retain activity at a broad range of pH 2-12. In contrast, mycobacteriophage Cepens showed stability in a narrow pH range of 7-9 (Cantrell 2019).

Because Fulbright can maintain stability in a wide range of temperature and pH conditions, it serves as an excellent candidate for phage therapy. The human body at homeostasis is 37°C, and upon infection and/or sepsis, the body temperature typically climbs to 38°C and higher. Thus, Fulbright can endure these higher temperatures if used for therapy. The human saliva has a typical pH range of 6.2-7.6 (Baliga

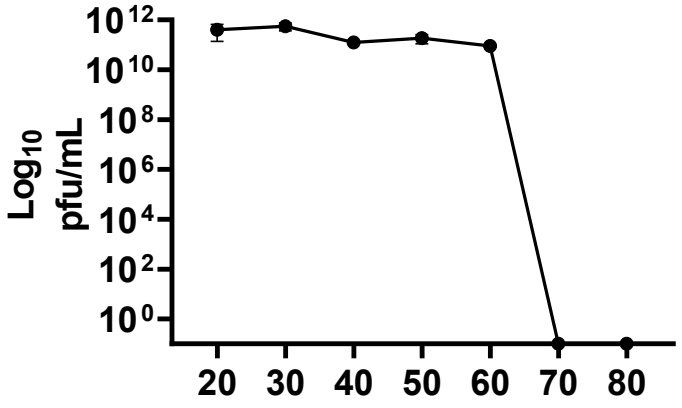

Figure 1. Temperature stability of phage Fulbright. Error bars represent standard error of the mean (SEM).

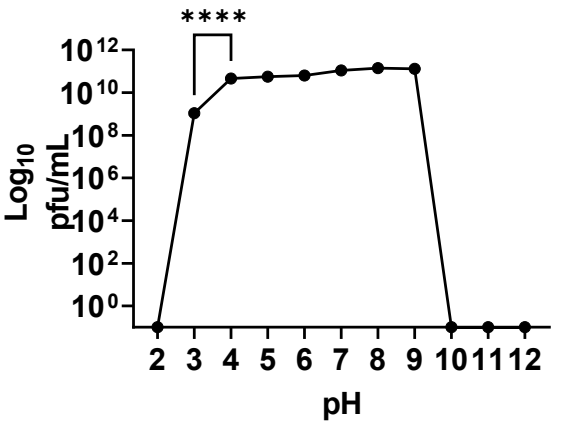

Figure 2. pH stability of phage Fulbright. ****p<0.0001.

et al. 2013). The human blood has a normal pH range of 7.35-7.45 (Hopkins et al. 2021), and values greater than 7.8 (alkalemia) or less than 6.8 (acidemia) often result in death. Lastly, open wounds are characterized to have a neutral to alkaline pH of around 6.5 to 8.5, while chronic wounds exist at a range of 7.2 to 8.932 (Bennison et al. 2017). Fulbright is stable from pH 4-9 and can thus be used for oral, intravenous and cutaneous applications. Ingestion of the phage would not be favorable as Fulbright begins to destabilize at a pH of 3 and lower which are optimal pH conditions for human stomach (Fujimori 2020).

Because a good understanding of a phage's latent period is needed to determine its potential use for phage therapy, we constructed a one-step growth curve for phage Fulbright. We found the latent period of phage Fulbright to be 90 min, followed by a rise period of 90 min (Figure 3). The PFUs plateaued after the rise period, approximately 3 h after adsorption. These results are similar to previous work with mycobacteriophages (Patton 2019). Kalapala et al. (2020) tested the infectivity of mycobacteriophages at various MOIs (10, 1, 0.1, and 0.01), and found that MOI of 10 and 1 both significantly reduced bacterial growth 3 h post treatment. Bavda and Jain (2020) observed a latent period of 60 minutes for mycobacteriophage D29; moreover, when they generated a D29 holin knockout phage, the latent period increased to 90 minutes. Studies by Fan et al. (2016) showed mycobacteriophage SWU1 to have a latent period of 30 minutes and a burst time of 270 minutes. BO1 and BO2a phages had latent periods of 150 min and 260 min, respectively (Kraiss et al. 1973). Samaddar used a different approach to observe mycobacteriophage-mycobacterial host interaction and used flow cytometry to measure bacterial cell viability and observed similar results, with a ~60 min latent period (Samaddar et al. 2016).

To determine whether Fulbright is a good candidate for phage therapy applications for treating human mycobacterial infections, we also tested the infectivity of Fulbright against human pathogen *M. abscessus.* Results showed that at high concentrations, Fulbright can effectively lyse *M. abscessus.* Figure 4b shows Fulbright is effective up to 10^{-2} dilution in lysing the host cell. These results are comparable to preliminary work done with Fulbright (Ali 2019). This limited infectivity against *M. abscessus* has also been observed by the Hatfull lab, where other phages (Muddy, ZoeJ, and BPs) were first isolated using host *M. smegmatis* (The Actinobacteriophage Database). The three phages were genetically modified and engineered to improve infectivity against *M. abscessus* subsp. *massiliense* and were then intravenously administered to a cystic fibrosis patient, making it the first therapeutic use of phages for a human mycobacterial infection. (Dedrick et al. 2019).

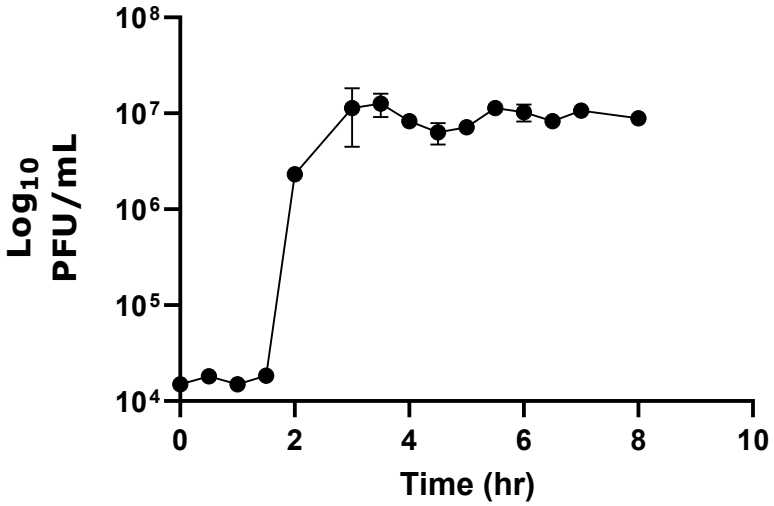

Figure 3. One-step growth curve of phage Fulbright. Error bars represent SEM.

In our previously published work with Fulbright (Kotturi and Lopez-Davis 2022) we successfully incorporated our phage into a PCL wound dressing. Here, we tested phage incorporated fiber against *M. smegmatis* and *M. abscessus.* Our results showed PCL_Fulbright to be effective against *M. smegmatis* (Figure 5a), but not *M. abscessus* (Figure 5b). A possible reason for this is the low-concentration and slow release of phage Fulbright from the nanofiber. We previously observed that ~2cm^2 of PCL_Fulbright released +/−2.1 × 10^5 PFU/mL infectious particles after 1 h incubation in 1 mL of phage buffer. Another reason that PCL_Fulbright was able to infect *M. smegmatis* but not *M. abscessus* could be due to the properties of mycolic acid present. Mycolic acids are a major component of mycobacterial cell walls (Sethiya et al. 2020; Kurosu 2019; Marrakchi et al. 2014; Takayama et al. 2005), and despite providing similar functions in all mycobacterial pathogens (cell protection, virulence, structural

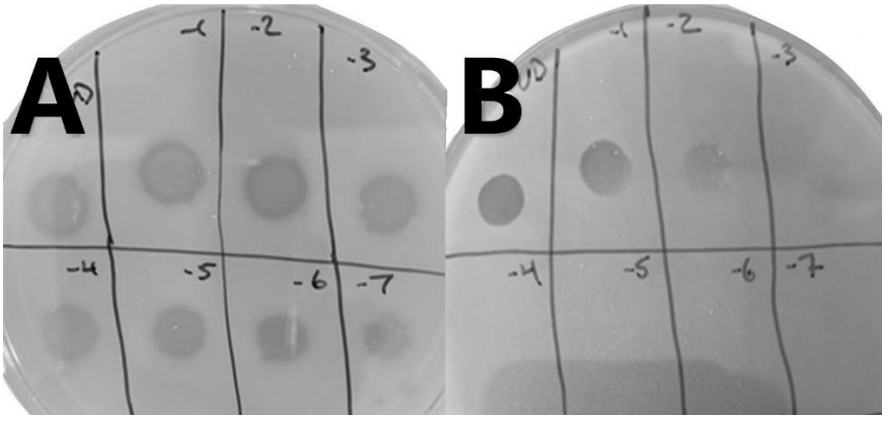

Figure 4. Testing infectivity of phage Fulbright. Fig 4a shows spot test results at ten-fold dilutions against *M. smegmatis;* Fig 4b shows that Fulbright can lyse *M. abscessus* at high titer.

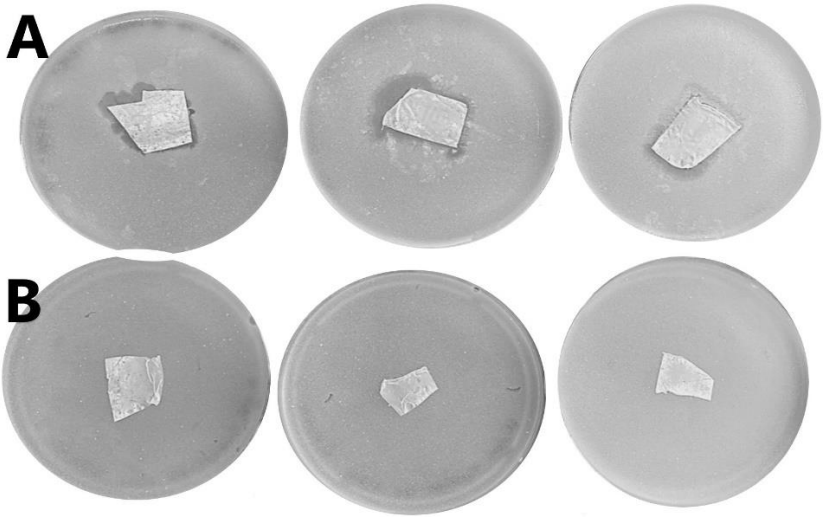

Figure 5. Testing the efficacy of phage Fulbright incorporated PCL against *M. smegmatis* bacterial lawn (Fig 5a) and against *M. abscessus* (Fig 5b). Clear lysis zones surrounding the edges of the fiber on Figure 5a shows that the fiber is effective in lysing *M. smegmatis*; No clear zone is observed in Fig 5b.

integrity), subtle differences in the carbon chain length are found that may contribute to their susceptibility to phage infections (Sethiya et al. 2020). Furthermore, we hope to optimize the efficacy of the phage fiber by improving our electrospinning techniques via increasing porosity and hydrophilicity, or by incorporating other materials such as collagen and hydrogel in order to increase the phage release and effectiveness while promoting optimal conditions for appropriate wound healing.

Bacteriophages have the potential for therapeutic and food safety uses due to their ability to lyse multi-drug resistant pathogens (Montso et al. 2019). They are easy to manufacture and have no documented negative side effects to date. Our research lays a good foundation for continuing research and possible application of Fulbright in the future.

Acknowledgments

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ABSTRACTS OF THE 111TH OKLAHOMA ACADEMY OF SCIENCE TECHNICAL MEETING

NOVEMBER 4, 2022

OKLAHOMA STATE UNIVERSITY CENTER FOR HEALTH SCIENCES, TULSA

THE ROLE OF RETINAL ENDOTHELIAL CELL CAVEOLIN-1 IN SMOOTH MUSCLE CELL LOSS IN RETINA MICRO-VESSELS

Jennifer Ballheim and Jami Gurley, University of Oklahoma Health Sciences Center

Loss of Smooth muscle cell (SMC) coverage in retina micro-vessels occurs with age and metabolic disease. At present, the mechanism behind the SMC loss is unclear. Previous data from our lab show that SMC loss in retina micro-vessels also occurs in mice with endothelial cell (EC) Caveolin-1 (Cav1) depletion. Here, we establish a method to silence the Cav1 gene in human retinal endothelial cells (HRECs) to explore the role of EC Cav1 in loss of SMC coverage in retina micro-vessels. HRECs were cultured and transduced with Adenovirus-5 (Ad5) Cav1- silencing shRNA. Increasing viral titers and incubation periods were performed to optimize the shRNA-mediated Cav1 transduction and CAV1 protein knockdown. Transduction was confirmed by the presence of a green fluorescent protein (GFP) reporter imaged via fluorescence microscopy 24 hours post viral transduction. The culture was incubated in viral-infected media for 48 hours before being replaced with new media. The cells were harvested 96 hours post viral transduction. Cav1 silencing was confirmed with Western blotting for CAV1 protein. The Western blot CAV1 protein was quantified via densitometry using LI-COR Image Studio Lite. Our preliminary data suggests that Cav1 protein is reduced by more than 80% with transduction by the Ad5 Cav1 silencing via shRNA compared to the un-transduced HREC samples. HREC Cav1 silencing was achieved with over 80% efficacy with shRNA viral transduction. Depletion of CAV1 in HRECs will allow exploration of retinal EC-CAV1's role in retinal microvascular SMC loss. Future studies include assessments of retinal EC-CAV1 expression on retinal EC metabolic functions.

A STATISTICAL ANALYSIS OF COVID-19 CASE AND DEATH RATES AMONG TRIBAL NATIONS IN OKLAHOMA

Bethany Bengs and Jessica Brumley, East Central University

Outstanding Undergraduate Paper in Mathematics, Computer Science, & Statistics

COVID-19, the infectious disease caused by the variant of coronavirus SARS-CoV-2, has had a significant impact in the United States. Recent research indicates that Native Americans are among the most severely affected groups. However, the data used in these studies are often aggregated and do not accurately reflect the situation in individual tribal nations or populations. This study utilized the Supreme Court case McGirt v. Oklahoma to analyze COVID-19 cases and deaths among areas in seven tribal nations in Oklahoma to determine how they have been affected by COVID-19 compared to the general population of Oklahoma, as well as what factors might have influenced these rates. Descriptive statistics and incidence and case-fatality rates were evaluated and correlated with population density, access to healthcare, and government funding allotments. Time series plots were created to illustrate the rates of new cases per day in each tribal nation. Finally, multiple linear regression models were created to predict COVID-19 deaths from cases, population, and tribal status of counties in Oklahoma. This analysis found that, in general, areas within tribal nations do not have significantly different COVID-19 case and death rates from Oklahoma. Higher population density correlates with higher case and death rates, while healthcare access and government funding have no correlation with incidence and case-fatality rates. Since these results contradict the findings of previous studies, they indicate both the need for more research among Native American populations and the importance of data analysis in research.

ANTIFUNGAL ACTIVITY OF NOVEL COMPOUND EIPE-1 AGAINST THE FUNGAL PATHOGEN *CRYPTOCOCCUS NEOFORMANS*

Priscilla Chatman, Brittney Conn, Emma Maritz, Toby L. Nelson, and Karen L. Wozniak, Oklahoma State University - Stillwater Campus

Outstanding Undergraduate Paper in Biochemistry & Molecular Biology

Cryptococcus neoformans is an opportunistic fungal pathogen that affects immunocompromised individuals. Antifungal drugs have been used to treat fungal infections for many decades; however, due to similarities between fungal and mammalian cells, these drugs are often toxic. In these last few decades, the fungi have also become resistant to the antifungal drugs. EIPE-1 was synthesized from vanillin, and was shown to have activity against methicillin resistant *S. aureus* (MRSA), and other gram-positive bacterial pathogens. We hypothesized that EIPE-1 could be used to kill fungal pathogens. For this study, we tested EIPE-1 against *C. neoformans* using a minimum inhibitory concentration (MIC) assay and an in vitro model of intracellular fungal growth using RAW macrophages. EIPE-1 has antifungal activity against *C. neoformans* in our MIC assay, with an MIC value of 1.749 μg/ml. In addition, following phagocytosis of *C. neoformans* by RAW macrophages, treatment with EIPE-1 had significant antifungal effects on *C. neoformans* compared to *C. neoformans* alone and compared to *C. neoformans* with RAW macrophages (without treatment). In further studies, we will perform RNA sequencing experiments and more comparison studies with other antifungal drugs.

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CHARACTERIZATION OF A UROPATHOGENIC STRAIN OF *KLEBSIELLA PNEUMONIAE*

Jonathan Crosse, Joseph Moberly, and Janaki K. Iyer, Northeastern State University

Outstanding Undergraduate Paper in Microbiology

Urinary tract infections (UTIs) are common bacterial infections that affect a wide variety of people including children. They account for 25% of the bacterial infections encountered by women and are associated with significant costs for treatment. Uropathogenic *Escherichia coli* (*E. coli*) is the most common etiologic agent followed by *Klebsiella pneumoniae* (*K. pneumoniae*). *E. coli*-mediated pathogenesis, in the context of UTIs, is widely studied but similar detailed information is not available on *K. pneumoniae*-mediated pathogenesis. UTIs caused by *K. pneumoniae* are harder to treat and cause more morbidity. There are also reports of increased antimicrobial resistance in different uropathogenic *K. pneumoniae* strains. In the current study, we have characterized a strain of *K. pneumoniae* (UCI-41) isolated from the urine of a patient diagnosed with a UTI. This strain is resistant to different bacteriostatic and bactericidal antibiotics and hence can serve as a good model to study pathogenesis mechanisms employed by antibiotic-resistant *K. pneumoniae* strains. Experiments involving ELISAs showed that *K. pneumoniae* UCI-41 induced the secretion of pro-inflammatory cytokines in a human epithelial bladder cancer cell line. This strain was able to internalize into bladder cells as determined by a gentamicin protection assay. This indicated that this strain was invasive and hence had the potential to cause recurrent UTIs. These findings support the use of *K. pneumoniae* UCI-41 as a model for future experiments that study invasive antibiotic-resistant uropathogenic bacteria and design novel strategies for treatment of infections caused by these strains.

VOLE (*MICROTUS GUENTHERI*) STABLE CARBON ISOTOPES AS CLIMATE PROXIES FOR THE MIDDLE AND LATE PLEISTOCENE OF THE LEVANT AND CAUCASUS

Logan Guthrie, Abigale Rogers, and Miriam Belmaker, University of Tulsa

Amy Prendergast and Zuorui Liu, School of Geography, University of Melbourne

Orr Comay and Michal Zyztov, The Steinhardt Museum of Natural History, Tel Aviv, Israel

Yoav Motro, Ministry of Agriculture, Israel

The Mediterranean Levant and Caucasus are situated in mid-latitudes. Therefore, climate oscillations during the Last Glacial Period (LGP, c. 115,000–11,700 years ago) are not as pronounced as in northern latitudes such as Europe and North America. One of the main questions is whether the LGP was cold and dry or cold and humid. Stable carbon isotopes are often used to distinguish between C3 vs. C4 plants. However, in mid-latitudes most vegetation is dominated by C3 plants. Hence, δ13C varies according to precipitation. To develop a modern model of the Levant's paleoecology, stable carbon isotopes of modern social voles, *Microtus guentheri*, were sampled from sites across Israel and correlated with GIS-derived mean annual precipitation. For the fossil study, vole teeth were selected from two Israel sites and from one Georgian site. Rantis Cave, Israel, (160 - 120 Kya) is in the central region of Israel, Amud Cave (45 Kya) is in the North of Israel while Dzudzuana (ca. 40 Kya), is found in the Republic of Georgia. Results of the modern study indicate a positive correlation between δ13C and mean annual rainfall with an average of -15.47 ± 1.277 (n=39). This confirms observations of previous studies that more enriched δ13C are indicative of higher mean annual precipitation. Results indicate that the carbon values for all fossil sites were enriched compared to modern voles; Rantis (n=9, -9.8 ± 0.25), Amud (n=20, -7.96 ± 0.46), and Dzudzuana (n=19 -7.2 ± 0.82). This suggests that Middle and Late Pleistocene sites had an increase in mean annual precipitation compared to modern populations. These results support the hypothesis that glacial periods in the Levant were cold and humid rather than cold and dry and demonstrates how stable isotopes in voles can provide relevant palaeoecological information in mid-latitude regions such as the Levant and Caucasus.

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DANDELION EXTRACT ALTERS EXPRESSION OF GENES REGULATING ATP AND NUCLEOTIDE BINDING IN CERVICAL CANCER CELLS

Christina Hendrickson, Oklahoma City University

Melville Vaughan and Nikki Seagraves, University of Central Oklahoma

Cancer continues to be a major public health burden and one of the leading causes of death. Despite many forms of expensive existing cancer therapies, there continues to be a high mortality rate among cancer patients. Therefore, we based our research on plant derived products due to their anti-cancer effects that can help to produce an efficient and inexpensive pharmaceutical that is widely accessible. One such product is dandelion (*Taraxacum officinale*). It was hypothesized that anti-cancer properties of dandelion extract acts by disrupting key cellular processes in tumor cells which can result in growth inhibition, decreased invasiveness, and increased apoptosis of tumor cells. We performed our experiments by preparing dandelion whole extract (DWE), filtering, freeze-drying, and resuspending them in sterile PBS. Then cultured HeLa cells and Human Cervical Epithelial Cells (HCEC), under standard in vitro conditions, were treated with DWE concentrations between 0 to 8 mg/ml for 96 hours. The quantitative polymerase chain reaction (qPCR) was performed to further investigate the anti-cancer mechanism of DWE. The results showed that DWE inhibited proliferation and migration and promoted cell death in HeLa cells while leaving HCEC cells unaffected. The qPCR showed the analysis of 8 most significantly differential expressed genes ($p<0.05$) resulted in enrichment of three annotation clusters. The top annotation cluster included genes associated with UP keywords ATP-Binding, Nucleotide-binding, Kinase, and Transferase as well as GO Terms ATP binding (Enrichment score=1.68). The next annotation cluster included genes associated with GO Term focal adhesion (Enrichment score=1.28). These data can provide a foundation to further investigate the mechanism of DWE toxicity in HeLa cells which can pave the way for future research in finding new anticancer pharmaceuticals.

A SUSTAINABILITY ASSESSMENT OF OKLAHOMA CITY UNIVERSITY

Ellie Howell and Adam K. Ryburn, Oklahoma City University

Outstanding Undergraduate Paper in Environmental Sciences

Over the summer of 2022, a comprehensive sustainability assessment for Oklahoma City University (OCU) was completed. The need for this project presented itself as a response to a growing concern from the campus community about OCU's environmental sustainability, and a lack of transparency from the administration and facilities departments. Utilizing a self-assessment program from the American Association for Sustainability in Higher Education's (AASHE) called STARS (Sustainability Tracking Assessment and Rating System), each section of sustainability at the university was clearly evaluated and the results were compared with that of peer and benchmark institutions who have also enrolled in the STARS program. The main sections of the report detailed Academics, Engagement, Operations, Administration, and Leadership in relation to sustainability in higher education. The main areas for improvement discovered throughout this summer-long survey were primarily in Operations, given that there is currently not a complete recycling program, nor does any designated tracking of emissions, energy, or water usage take place at the university. A more positive part of the results came from the dining services subsection of Operations, in which it was found that our dining services provider actively makes strides to locally source produce and reduce food waste through consistent inventory and documentation. This report establishes a framework for other campuses to follow in order to evaluate and track sustainability in their institutions and suggests possible initiatives to implement in response to areas lacking in sustainability.

SYNTHESIS AND POWER GENERATION CHARACTERIZATION OF PEROVSKITE SOLAR CELLS

McClain Irby, Amanda Nichols, Will Clothier, and Kevin Plumlee, Oklahoma Christian University

Outstanding Undergraduate Paper in Physical Science

Perovskite solar cells are a type of solar cell that uses perovskite as one of the layers due to its semiconductor capabilities. The cell materials are layered to allow electron and hole transport. Patwardhan, et al showed that perovskite solar cells can be constructed using a deposition method by undergraduate students as a way to introduce solar energy and this unfamiliar type of solar cell. While perovskite solar cells that have different halides and divalent cations have been constructed, they have not been made using a simple deposition method. Two other halides were used instead of iodide (bromide and chloride ions), and different mixtures of the halides were used to fabricate the solar cells. Electrical power output of the cells was collected and compared to the commercial and literature values.

COULD ARCHAIC *HOMO SAPIENS* SURVIVE IN THE TROPICS OF SOUTHEAST ASIA? IDENTIFYING SMALL MAMMAL REMAINS FROM YAHUAI CAVE IN GUANGXI, CHINA AT 120 KYA TO DETERMINE THE PALEOECOLOGY

Kathleen Kelley, Guangmau Xie, Qiang Lin, and Miriam Belmaker University of Tulsa

It has been hypothesized that the lack of protein sources and technological skills prevented archaic *Homo sapiens* from penetrating the rainforest to forage for food prior to 40,000 years ago (kya). Accordingly, early modern humans dispersing from Africa to Asia ca. 120 kya would have preferred savanna over tropical environments. As a case study, we present an analysis of small mammal remains (Chiroptera, Rodentia, Eulipotyphla, Primates) from Yahuai Cave, Guangxi, China. The area in Yahuai cave focused on for this research excavated 53 stratigraphic layers, dated by OSL to 124.2 ±16 kya. Early modern human remains were found nearby at several contemporaneous sites, such as Tongtianyan and Mulan cave indicating the region was inhabited by ca. 120 - 100 kya. Species found include a wide range of murids such as *Niviventer andersoni* (Anderson's white-bellied rat), *Mus pahari* (Gairdner's shrewmouse) and other murid specimens identified only to the genus level, such as *Leopoldamys*, *Rattus* and additional species of *Niviventer*. Other species include several squirrel species such as *Hylopetes alboniger* (Particolored flying squirrel), and *Belomys personii* (hairy-footed flying squirrel). Ecological analogy as well as community structure methods are utilized in the paleoecological analysis. This analysis indicates a warm, humid, dense forested environment, probably more humid than the contemporaneous Indochinese peninsula. A diachronic comparison shows no appreciable differences in species composition across strata. This suggests the ecology of the area was similar in the lower strata, ca. 120 kya, to that in the upper levels, ca. 40 kya. This confirms the ability of early modern humans to utilize this novel ecosystem earlier than previously assumed.

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EFFECTS OF ER-SIGNALING ON ADIPOCYTE PROGENITOR EXPANSION: POTENTIAL ROLE FOR WISP2

Laci Liter, Nisha Susan Thomas, and Elizabeth Wellberg, Oklahoma City University

Dysfunctional adipose tissue (AT) occurs when progenitors fail to expand and form new adipocytes during a positive energy balance. In this context, mature adipocytes become hypertrophic and ectopic lipid deposition can increase the risk for type 2 diabetes. Disrupted estrogen receptor alpha (ERα) signaling has been shown to contribute to AT dysfunction. We found elevated expression of Wnt1-inducible signaling protein (WISP2/CCN5) in APCs from obese mice after estradiol (E2) treatment. WISP2 regulates APC proliferation in mice and is induced by estrogen in human breast cancer cells. Analysis of human AT revealed a correlation between WISP2 and serum insulin levels. We hypothesized that WISP2 influences APC renewal and differentiation in response to ERα and potentially insulin signaling in AT. We aimed to investigate the connections between WISP2 and the progenitor phenotype plus WISP2 regulation by ERα in an in vitro model of adipogenesis. Mouse APCs (mAPCs) were cultured for all experiments. Adipocyte differentiation was visualized with Oil Red O-staining at days 0, 2, 4, 6, 8, 10, and 15. Expression of ERα and WISP2 after treatment with TAM, fulvestrant (ICI), insulin, and/or E2 in mAPCs was analyzed by PCR and Western blotting. The effect of WISP2 and E2 on mAPC progenitor proportions was measured by flow cytometry analysis of CD24 and Sca1. ORO-staining confirmed mAPC adipocyte differentiation over time. WISP2 expression increased after insulin-, E2-, and insulin+E2 treatments and decreased in treatments containing ICI. All treatments decreased ESR1 expression except ICI+E2+insulin. Western-blotting confirmed gene expression analyses. Consistent with previous work, E2 and insulin induced WISP2 expression in mAPCs. Further classifying associated signaling cascades will aid in investigating AT expansion and the pathology of adipocyte progenitors in obesity and diabetes.

ATR-FTIR DETECTION OF CHLORINATED HYDROCARBONS IN GROUNDWATER

Randall Maples, East Central University

Chlorinated hydrocarbons including aliphatic and aromatic compounds (CHCs) are toxic contaminants commonly found in groundwater samples and efficient detection and monitoring of these contaminants is an important part of the evaluation of water quality. Analysis is often complicated due to the presence of many compounds as well as interfering molecules. In this preliminary study, with an overall end-goal of the development of a novel, time and cost-efficient procedure for the determination of complex mixtures of CHCs in groundwater employing digital signal processing techniques, a method was developed for the determination of various CHCs in aquifer groundwater using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR).

TURMERIC FOR CANCER PREVENTION

Madeline McTigue and William P. Ranahan II, Oral Roberts University

Outstanding Undergraduate Paper in Biological Sciences

Most chemotherapy, though advancing in its complexity, is killing healthy cells, and harming patients while generally providing low efficacy against tumor suppression. Given that many of the successful drugs on the market today are naturally occurring or designed from natural sources, a holistic cancer prevention model will be fundamental to fighting this disease, and plants are our best bet in preventing such an elusive illness. In 2011, scientists Douglas Hanahan and Robert Weinberg consolidated our understanding of cancer progression into ten "hallmarks of cancer". The ten hallmarks of cancer describe the ten steps required for a normal or healthy cell to become tumorigenic. It is in these ten areas that cancer must be addressed to properly treat and prevent the disease from occurring. Medicinal plants and herbs were identified that biochemically interact with each of these hallmarks to prevent cancer progression. Of the identified plants, turmeric root was found to interact with the most hallmarks via its polyphenolic pigment curcumin. As such, we began to deduce the process of sterilization and tissue culturing the turmeric root. Once this process was successful, a callus was induced, providing the future site for epigenetic modification using a biolistic particle delivery system. The aim of such epigenetic modification is to upregulate turmeric's expression of curcumin for a more potent anti-cancer product.

CELL SURFACE HYDROPHOBICITY PROPERTIES AND BIOFILM ADHESION IN OPPORTUNISTIC *SERRATIA* SPECIES HAVING DISPARATE SUSCEPTIVITY TO TRICLOSAN SENSITIZATION

Katherine Nehmzow, Abby S. Rigsbee, Christopher Godman, Sam Hudgeons, Sue Katz Amburn, and Franklin R. Champlin, Northeastern State University

We have shown all but one of 1 *Serratia* species capable of opportunistic pathogenicity to be intrinsically resistant to the hydrophobic biocide triclosan. However, they differed markedly regarding their susceptivity to triclosan sensitization by outer membrane permeabilization using the cationic detergent compound 48/80. Representative organisms exhibiting slight (*Serratia marcescens*), complete (*Serratia fonticola*), transitorily complete (*Serratia liquefaciens*), and intermediate (*Serratia rubidaea*) susceptivity were selected for further analysis. The purpose of the present study was to determine if cell surface hydrophobicity properties of these phenotypically disparate Serratia species are related to susceptivity to triclosan sensitization and the initial adhesion stage of biofilm formation. NPN fluorescent probe and hydrocarbon adherence assays were employed to quantitatively determine cell surface hydrophobicity properties, while an in vitro biofilm assay was used to assess adhesion of planktonic cells to a solid substrate. While S. rubidaea was seen to be extremely hydrophobic, *S. marcescens* and S. liquefaciens were only slightly to moderately hydrophobic, and *S. fonticola* was seen to be hydrophilic to slightly hydrophobic. These data do not appear to support the notion that the degree of susceptivity to triclosan sensitization by outer membrane permeabilization is directly related to cell surface hydrophobicity. However, the initial adhesion stage of biofilm formation appears to be influenced to some degree by cell surface hydrophobicity properties.

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INVESTIGATING ANTIMICROBIAL EFFECTS OF AQUEOUS DANDELION EXTRACT

Ashley Nguyen, Kayla Nguyen, Stephanie Rojas, Lindsey Morris, and Christina Hendrickson, Oklahoma City University

Certain plant-derived products have pharmaceutical uses due to their anti-inflammatory and anti-cancer effects. Dandelion (*Taraxacum officinale*) is one of them. It has long been consumed safely as part of Middle Eastern and Ancient Chinese Medicine. Anticancer effects of aqueous DWE (Dandelion Whole Extract) have been vastly studied on HeLa cells and other cancer cell lines. As some anticancer compounds are also used as antibiotics, this study aimed to further investigate the antibacterial effects of DWE. The disk diffusion method was utilized to test various concentrations (5 - 100 mg/mL) of DWE on bacterial growth. DWE was tested on six bacteria: *Escherichia coli*, *Citrobacter freundii, Morganella morganii, Salmonella typhi*, *Staphylococcus aureus*, and *Neisseria sicca*. All bacterial cultures were incubated at 37 ℃ for 24 hours. Isolated bacterial colonies were suspended in tryptic soy broth (TSB), compared with 0.5 McFarland Standard, and cultured on Mueller-Hinton Agar (MHA). Sterilized paper disks were impregnated with DWE and applied to bacterial plates. A Mueller-Hinton Agar plate devoid of bacteria was treated with DWE disks to serve as a control to ensure the DWE disks did not introduce contamination. After incubation, all plates were visualized for indication of DWE impact on bacterial growth. The results showed no zone of inhibition; indicating all six bacteria were resistant to aqueous DWE in this method. In the future, broth dilution antibiogram assays will be conducted utilizing additional bacterial species and different formulations of dandelion extracts.

MICROWAVE IMAGING SYSTEM (PASCO SYSTEM) MOTION DETECTION AND TRACKING OF MOBILE PHANTOM FOR HUMAN TISSUE

Moses Omeneki, Kwabena Boateng, and Nesreen Alsbou, University of Central Oklahoma

Imad Ali, University of Oklahoma Health Sciences Center

Outstanding Graduate Paper

Breast cancer is a disease that occurs mostly in female cancer patients and is the leading cause of cancer-related death among females worldwide. Breast screening and early detection are currently the most successful, most common method for the management, reduction, and treatment of this disease or mortality rate. Various imaging methods such as X-ray and MRI are currently utilized for detecting breast cancer. Microwave Imaging is gaining quite a lot of attention as a promising diagnostic tool for early breast cancer detection. MWI is inexpensive, fast, convenient, and a safe screening tool. The purpose of this research is to use a specially designed object that is utilized as a human tissue equivalent material and can be scanned/imaged to evaluate, analyze, and fine-tuned the performance of an imaging device. This is an effort to provide an update on the principles, developments, and current research status of MWI for breast cancer detection. The project is structured to provide an overview of MWI system techniques used for detecting the motion of fourteen different human tissue equivalents to provide reliable and quantitative data to determine how effective an imaging system is compared to imaging systems used in a real-world setting. For this project, a Pasco system consists of a transmitter, a receiver, a breadboard circuit, an Arduino, a stepper motor driver, a computer, and a DC power supply. The innovative technique of the MWI system is significant in that it has the potential to provide a safe and reliable method of enhancing the overall performance of imaging systems in a very safe, cost-effective, and non-invasive way before it can be applied in a clinical setting.

EFFECTS OF MULTIPLE POLYCYCLIC AROMATIC HYDROCARBONS ON CARDIAC DEVELOPMENT IN CHICK EMBRYOS

Yulianis Pagan, Hallum Ewbank, and Christopher Goodchild, University of Central Oklahoma

Outstanding Undergraduate Paper in Applied Ecology & Conservation

Following oil spills, avian embryos may be exposed to polycyclic aromatic hydrocarbons (PAHs) when crude oil is transferred from oiled nesting material or oiled feathers of brooding parents to the eggshell surface. While several studies have examined the effects of PAHs on adult birds, the developmental effects of embryonic exposure to PAHs remain unclear. In other taxa, like fish, embryonic exposure to PAHs causes cardiac impairments like bradycardia and a decline in cardiac output. Similar trends have been detected in avian embryos, specifically external application of crude oil to the eggshell reduces embryonic heart and metabolic rates. However, the mechanism and specific PAHs driving these effects in developing avian embryos are still poorly understood. This experiment investigated the effects of sublethal exposure of six PAHs (anthracene, phenanthrene, pyrene, chrysene, benzo[a]pyrene, and fluoranthene), at four concentrations (100, 200, 400, and 800 ng PAH / g egg mass), on avian embryonic heart rate, heart organ mass, morphology, and mRNA expression of phase I and phase II detoxification enzymes. We exposed chicken (*Gallus gallus*) embryos to PAHs on embryonic day (ED) 3 via egg-injection. We recorded heart rate on ED 10, 14, and 18, and collected heart organ mass, morphology, and transcriptional data on ED 18. Chick embryos exhibited a decrease in ED 18 heart rate at the highest concentrations for fluoranthene, phenanthrene, chrysene, and pyrene. Additionally, we found an increase in heart mass in chicks exposed to phenanthrene, pyrene, chrysene, and fluoranthene at intermediate concentrations. Preliminary results also indicate several transcriptional responses in chicks exposed to various PAHs. Collectively, these data indicate in ovo exposure to various PAHs interferes with avian embryonic development and may contribute to reduced hatchling survival, especially if these impaired cardiac functions continue post-hatch.

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EPIGENETIC MECHANISMS HOLD THE KEY TO DEVELOPING NOVEL THERAPEUTIC TREATMENTS FOR ULCERATIVE COLITIS

Radhika Pande and Subhas Das, Oklahoma State University Center for Health Sciences

Background: Inflammatory bowel disease (IBD) includes Crohn's disease (CD) and ulcerative colitis (UC) and is associated with symptoms like abdominal pain, diarrhea, fatigue, reduced appetite, and weight loss. According to CDC, approx. 3 million Americans are reportedly diagnosed with IBD. Compared to normal individuals, IBD patients are more prone to colorectal cancer and arthritis. The causes of IBD are unknown; however, environmental, nutritional, microbiological, and genetic factors have been suggested to play a role in disease development. Nerve Growth Factor (NGF), a neurotrophic factor, is significantly elevated during several inflammatory and autoimmune diseases, including IBD and is essential for a robust inflammatory response. Studies suggest the importance of epigenetic mechanisms in chronic gastrointestinal inflammation and colorectal cancer, offering important insights into IBD's molecular basis. Although epigenetic regulators are well-explored in IBD, the regulations controlling NGF gene expression are unknown. Epigenetic modifications, including DNA methylation and covalent histone modifications, influence gene expression at the transcription level without altering the DNA sequence. We found that colon inflammation causes hypermethylation of the NGF promoter, resulting in its activation. Hypermethylation recruits proteins containing methylated DNA binding domains (MBDs), such as MeCP2. The evidence suggests that MeCP2 links DNA methylation and histone modifications to control gene expression. Aim: To understand the involvement of MeCP2 and identify novel histone modifications associated with NGF transcription. Method: TNBS-induced colitis animal model was used for this study. After inflammation, colon tissue was collected to study the DNA-protein and protein-protein interactions by Chromatin-immunoprecipitation-assay and Immunoprecipitation-assay, respectively. Results and Conclusion: Our findings show that MeCP2 and tri-methylation of histone 3 lysine 4 (H3K4me3) are elevated during the TNBS-induced inflammation compared to control animals. ChIP and pull-down assays prove that MeCP2 interacts with H3K4me3, and both are associated with the hypermethylated NGF gene promoter for the active transcription during colon inflammation.

EXAMINING THERAPEUTIC EFFECTS OF EXT-4U ON NEOVASCULARIZATION AND FIBROSIS IN AMD

Melissa Testut, Oklahoma Christian University

Henry Shin, Excitant Therapeutics

Outstanding Undergraduate Paper in Biomedical Sciences

EXT-4U is a novel small molecule that selectively activates Peroxisome Proliferator-Activated Receptor Alpha (PPAR-α). Here we show its therapeutic effects on retinal fibrosis and neovascularization in a mouse model of Age-related Macular Degeneration (AMD). To test the selectivity and potency of EXT-4U for PPAR-α agonism, we first performed a PPAR-α reporter assay. We then conducted a Cellular Thermal Shift Assay (CETSA) in ARPE19 cells to analyze the binding affinity of EXT-4U to human PPAR-α. We performed western blotting to examine the anti-fibrotic effects of EXT-4U in ARPE19 cells treated by TGF-β2 and tested the anti-angiogenic effects by conducting an ex vivo choroidal sprout assay. Finally, we conducted in vivo efficacy tests in a mouse model of laser-induced Choroidal Neovascularization (CNV). The PPAR-α reporter assay confirmed that EXT-4U is a selective and potent PPAR-α agonist, and the CETSA assay further demonstrated PPAR-α binding affinity. In ARPE19 cells treated with TGF-β2, co-treatment with EXT-4U downregulated levels of connective tissue growth factor. EXT-4U treatment also inhibited choroidal endothelial cell sprouting. Lastly, EXT-4U treatment in CNV mice decreased CNV lesion size. When considered together, these results demonstrate that EXT-4U exhibits a therapeutic effect on fibrosis and neovascularization.

HTLV-1 VIRAL PROMOTER NUCLEOSOME DYNAMICS

Landen Underwood and Alisha Howard, East Central University

Outstanding Poster

Human T-Cell Leukemia Virus Type 1, or HTLV-1, is a retrovirus infecting primarily T-cells. This viral infection is known to be the causative agent in a subset of patients into Adult T-Cell Leukemia (ATL), or if it crosses the blood-brain barrier into HTLV-1 associated myelopathy, or tropical spastic paraparesis (HAM/TSP). The virally-expressed transcription factor Tax has been found to be pivotal in the malignant transformation of infected cells. Control of viral expression by Tax from the proviral promoter varies with stage of infection. The association of the HTLV-1 viral promoter and the associated viral and host activators/co-activators within the context of the chromatin environment could provide insights into target interactions and interfaces leading to dynamic promoter control. To investigate this, a plasmid, pHTLV208-8, containing the HTLV promoter surrounded by 5S nucleosome positioning sequences was prepared. Four biotinylated oligos corresponding to complimentary regions near the 5S or promoter sites along the plasmid were designed with various lengths and sent for synthesis with a 5' biotinylation modification included. Triplex strand invasions were performed to attach each specific biotinylated oligo and pHTLV208-8. Bead binding of the triplex strand mixtures were performed using streptavidin-bound magnetic beads. Restriction digests were then used to quantitatively analyze binding success and durability of the triplex-bound plasmids. Use of the stationary bound plasmid will allow analysis of Tax chromatin positioning effects as well as pulldowns from cell lysates in the absence of non-specific end-binding proteins.

BIOHYBRID MICROSWIMMER FABRICATION AND CHARACTERIZATION

Kathy Vo, Laurel Eze, Trung Le, and Christian Santizo, University of Central Oklahoma

Outstanding Undergraduate Paper in Engineering Sciences

The long-term objective of this project is to develop a novel drug delivery method in the form of biohybrid microswimmers with the purpose of improving and accelerating patient outcomes. Traditionally, drugs are delivered through the skin, mouth, veins, etc. in order to treat diseases, improve health, and extend lives. Usually, this treatment comes with a cost in the form of side effects due to the interactions between said drugs and healthy tissues. The background of this project stems from the need to develop a better and more precise way to deliver treatments and substances into the body exactly where they are needed. The biological part of the micro-swimmer is by establishing the green algae cells, which are created through a process called cell cultures that is done in labs. The algae cells will be tested with polystyrene beads that will ultimately contain drugs, and this will identify the most effective and efficient ratio of the beads to the cells. Subsequently, to ensure the beads are attached to the algae surface, the cells will be coated in an electropositive solution expected to attract the beads even more. The guidance of these cells will be placed in an experimental setup. There is possibly a light probe that will be emitting light with an ideal wavelength for the cell's reaction. As the cells are being guided by the light probe, another high intensity light operating in the 40mA range will be used for imaging, serving as a sort of PIV system to observe the movement on a more detailed level. The idea behind this project lies in the expansion of a safer, more effective drug delivery method that will utilize the motile flagella-powered-biohybrid microswimmers by transporting drugs to the targeted areas.

OKLAHOMA ACADEMY OF SCIENCE

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** Technical Meeting: A posthumous special thanks to Dr. Earl Blewett for bringing this meeting to OSU-CHS. A well-deserved special thanks to the OSU-CHS planning committee for realizing Dr. Blewett's vision for this meeting: Darlene DuBois; Subhas Das, George Huang, Gerwald Koehler, and Franklin Champlin.

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID
FOR THE YEAR ENDED DECEMBER 31, 2021

REVENUES COLLECTED:		
Membership Dues:		$1,670.30
Investment Income:		$29.97
Meetings:		$4,875.83
Registration – Fall Field Meeting	$0.00	
Registration – Technical Meeting	$4,875.83	
Donations		$100.00
POAS:		$5,471.04
Woody Plants:		$0.00
Other Income:		$83.94
Total Revenue Collected		**$12,231.08**
EXPENSES PAID		
Stipends and Other Compensation:		$7,158.68
Stipends	$6,141.24	
Social Security & Medicare	$1,017.44	
Meeting Expenses:		$1,334.20
Fall Field Meeting	$0.00	
Technical Meeting	$1,334.20	
Dues/Meetings of NAAS/AJAS/AAAS:		$559.41
POAS:		$4,036.49
POAS Editor	$2,500.00	
POAS Printing	$1,227.00	
POAS Mailing	$309.49	
Woody Plants:		$0.00
Other Expenditures:		$107.20
Total Expenses Paid:		**$13,195.98**
Revenues Collected Over Expenses Paid		**$-964.90**

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF ASSETS, LIABILITIES, AND FUND BALANCE
ARISING FROM CASH TRANSACTIONS
FOR THE YEAR ENDED DECEMBER 31, 2021

ASSETS		
Cash:		$26,168.47
Checking Account	$20,090.51	
Savings Account	$3,278.31	
Endowment Savings Account	$2,799.65	
Investments:		$60,000
Certificate of Deposit	$60,000	
Total Assets:		**$86,168.47**
LIABILITIES AND FUND BALANCE		
Liabilities:	$0.00	
Fund balance:		
Beginning operation fund balance	$87,069.67	
Excess revenues collected over expenses	$-901.20	
Total Funds:		**$86,168.47**

OKLAHOMA ACADEMY OF SCIENCE

Name_____Affiliation _____
Last First Middle

Professional Address (if applicable) _____
Dept., Bldg., Office, etc. (if necessary for campus mail delivery)

_____
City State Zip
OR (not both)

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Note all annual memberships expire December 31 if you do not prepay for the following year.

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_ Life $600 Professional $40 Family $35 Undergraduate/Graduate Student $20
$40 Library/Institute

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____A Biological Sciences_____E Science Education_____I Engineering Sciences ____M Environ. Sci.
_____ B Geology _____F Geography_____J Biochemistry/Biophysics_____N Biomedical Sci.
_____ C Physical Sciences_____G Fish and Wildlife_____K Microscopy_____Y Collegiate Acad.
_____ D Social Sciences_____H Microbiology_____L Mathematics/Computer Sci_____Z Junior Academy

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The *Proceedings of the Oklahoma Academy of Science* is published by the Oklahoma Academy of Science. Its editorial policies are established by the Editor and Associate Editors, under the general authority of the Publications Committee. The Editor is appointed by the Executive Committee of the Academy; Associate Editors are appointed by the Publications Committee in consultation with the Editor. The suitability for publication in the *Proceedings* of submitted manuscripts is judged by the Editor and the Associate Editors.

All manuscripts must be refereed critically. The *POAS* Editors have an obligation to the membership of the Academy and to the scientific community to insure, as far as possible, that the *Proceedings* is scientifically accurate. Expert refereeing is a tested, effective method by which the scientific community maintains a standard of excellence. In addition, expert refereeing frequently helps the author(s) to present the results in a clear, concise form that exceeds minimal standards.

The corresponding author is notified of the receipt of a manuscript, and the Editor sends the manuscript to at least two reviewers, anonymous to the author(s). After the initial review, the Editor either accepts the manuscript for publication, returns it to the author for clarification or revision, sends it to another referee for further review, or declines the manuscript.

A declined manuscript will have had at least two reviews, usually more. The Editors examine such manuscripts very carefully and take full responsibility. There are several grounds for declining a manuscript: the substance of the paper may not fall within the scope of the *Proceedings*; the work may not meet the standards that the *Proceedings* strives to maintain; the work may not be complete; the experimental evidence may not support the conclusion(s) that the author(s) would like to draw; the experimental approach may be equivocal; faulty design or technique may vitiate the results; or the manuscript may not make a sufficient contribution to the overall understanding of the system being studied, even though the quality of the experimental work is not in question.

A combination of these reasons is also possible grounds for declining to publish the MS. In most cases, the Editors rely on the judgment of the reviewers.

Reviewer's Responsibilities

We thank the reviewers who contribute so much to the quality of these *Proceedings*. They must remain anonymous to assure their freedom in making recommendations. The responsibilities or obligations of these reviewers are

- Because science depends on peer-reviewed publications, every scientist has an obligation to do a fair share of reviewing.
- A reviewer who has a conflict of interest or a schedule that will not allow rapid completion of the review will quickly return the manuscript; otherwise, the review will be completed and returned promptly.
- A reviewer shall respect the intellectual independence of the author(s). The review shall be objective, based on scientific merit alone, without regard to race, religion, nationality, sex, seniority, or institutional affiliation of the author(s). However, the reviewer may take into account the relationship of a manuscript under consideration to others previously or concurrently offered by the same author(s).
- A reviewer should not evaluate a manuscript by a person with whom the reviewer has a personal or professional connection if the relationship could reasonably be perceived as influencing judgment of the manuscript.
- The manuscript is a confidential document. If the reviewer seeks an opinion or discusses the manuscript with another, those consultations shall be revealed to the Editor.
- Reviewers must not use or disclose unpublished information, arguments, or interpretations contained in a manuscript under consideration, or in press, without the written consent of the author.
- Reviewers should explain and support their judgments and statements, so both the Editor and the author(s) may understand the basis of their comments.

Brief Instructions to Authors

The instructions to authors wishing to publish their research in the Proceedings of the Oklahoma Academy of Science are listed below. We ask the authors to recognize that the intent is not to establish a set of restrictive, arbitrary rules, but to provide a useful set of guidelines for authors, guidelines that, in most cases, are also binding on the Editors in their task of producing a sound and respected scientific journal.

A. Submission Process.

Manuscripts for the *Proceedings* should be submitted electronically via electronic mail (email) to:

poas@okstate.edu

Prospective authors should note carefully the policy statement "Policies of the *Proceedings*" on page ii. Complete instructions for manuscript formatting requirements, as well as a template for use may be found at:

https://ojs.library.okstate.edu/osu/index.php/OAS/submit

The Editors review the MS and carefully select other reviewers as described in "Editorial Policies and Practices" (see p. 158); all referee and editorial opinions are anonymous. Send a resubmitted and/ or revised manuscript and a point-by-point response to the reviewers'/Editor's comments.

All authors should approve all revisions (the corresponding author is responsible for insuring that all authors agree to the changes). A revised paper will retain its original date of receipt only if the revision is received by the Editor within two months after the date of the letter to the author(s).

B. Types of Manuscripts.

A manuscript may be a paper (report), review, note (communication), a technical comment, or a letter to the editor. All manuscripts should be submitted as a Microsoft Word document, 10-point Times New Roman font, single spaced, and include line numbers. Authors should carefully consider page size when producing manuscripts. The journal's page size is roughly 7 by 10 inches, portrait orientation, and does include margins.

Paper (a report; traditional research paper). A Paper may be of any length that is required to describe and to explain adequately the experimental observations.

Review. The Editor will usually solicit review articles, but will consider unsolicited ones. The prospective writer(s) of reviews should consult the Editor; in general, the Editor needs a synopsis of the area proposed for review and an outline of the paper before deciding. Reviews are typically peer- reviewed.

Note (Communication). The objective of a *Note* is to provide an effective form for communicating new results and ideas and/ or describing small but complete pieces of research. Thus, a *Note* is either a preliminary report or a complete account of a small investigation. *Notes* must not exceed four printed pages including text, figures, tables, and references. One journal page of standard text contains about 600 words; hence, there is space for presentation of considerable experimental detail. *Notes* are peer-reviewed.

Technical Comment. Technical comments (one journal page) may criticize material published in an earlier volume of *POAS* or may offer additional useful information. The author(s) of the original paper are asked for an opinion on the comment and, if the comment is published, are invited to reply in the same volume.

Letter to the Editor. Letters are selected for their pertinence to materials published in *POAS* or because they discuss problems of general interest to scientists and/or to Oklahomans. Letters pertaining to material published in *POAS* may correct errors, provide support or agreements, or offer different points of view, clarifications, or additional information.

Abstract. You may submit an abstract of your presentation at the OAS Technical Meeting. For specific instructions, contact the Editor. Even though abstracts are not peer-reviewed, they must align with the policies and scope of the *Proceedings*. The quality or relevance of work may not be in question, but the printed material is still subject to scientific accuracy.

The same guidelines that apply to manuscripts and notes submitted for peer-review, also apply to abstracts submitted for print. Just as manuscripts and notes are subject to thorough testing, so are comments written in abstracts (supported by data). The *Proceedings* understands that all disciplines are in a search for a deeper understanding of the world some of which are through creative expression and personal interpretation. Science is a system by which one discovers and records physical phenomena, dealing with hypotheses that are testable. The domain of "science" while working within nature is restricted to the observable world. There are many valid and important questions to be answered but lie outside the realm of science.

C. Manuscript Organization.

1. General organization.

For papers (reports), the subsections should typically include the following: Abstract, Introduction, Experimental Procedures (or Methods), Results, Discussion, Acknowledgments (if any), and References. In the case of notes or short papers, you may combine some headings, for example, "Results and Discussion":

I. The title should be short, clear, and informative; it should not exceed 150 characters and spaces (three lines in the journal), and include the name of the organism, compound, process, system, enzyme, etc., that is the major object of the study.

II. Provide a running title of fewer than 60 characters and spaces.

III. Spell out either the first or second given name of each author. For example, Otis C. Dermer, instead of O.C. Dermer, or H. Olin Spivey, instead of H.O. Spivey.

IV. Every manuscript (including Notes) must begin with a brief Abstract (up to 200 words) that presents clearly the plan, procedure, and significant results of the investigation. The Abstract should be understandable alone and should provide a comprehensive overview of the entire research effort.

V. The Introduction should state the purpose of the investigation and the relationship with other work in the same field. It should not be an extensive review of literature, but provide appropriate literature to demonstrate the context of the research.

VI. The Experimental Procedures (or Methods) section should be brief, but adequate for repetition of the work by a qualified experimenter. References to previously published procedures can reduce the length of this section. Refer to the original description of a procedure and describe any modifications.

VII. You may present the Results in tables or figures or both, but note that it is sometimes simpler and clearer to state the observations and the appropriate experimental values directly in the text. Present a given set of results *in only one form:* in a table, or figure, or the text.

VIII. The Discussion section should interpret the Results and how these observations fit with the results of others. Sometimes thecombination of Results and Discussion can give a clearer, more compact presentation.

IX. Acknowledgments of financial support and other aid are to be included.

X. References are discussed below.

2. References

POAS uses the name-year system for citing references. Citations in the text, tables and figure legends include the surname of the author or authors of the cited document and the year of publication. The references are listed alphabetically by authors' surnames in the reference list found at the end of the text of the article. Below are given several examples of correct formats for citing journal articles, books, theses and web resources. For Additional information regarding the name- year system, consult the CBE Manual [Scientific *Style and Format: The CBE Manual for Authors, Editors, and Publishers,* 6th edition]. Abbreviate journal names according to the *International List of Periodical Title Word Abbreviations.*

If it is necessary to refer to a manuscript that has been accepted for publication elsewhere but is not yet published, use the format shown below, with the volume and page numbers absent, the (estimated) publication year included and followed by the words *in press* for papers publications and *forthcoming* for all other forms (CBE 30.68). If the materials are published before the manuscript with that reference is published in *POAS,* notify the Editor of the appropriate volume and page numbers and make the changes as you revise.

Responsibility for the accuracy of bibliographic references rests entirely with the author(s); confirm all references through comparison of the final draft of the manuscript with the original publications. *We expect that the only changes in galley proof will be for typographical errors.* Any mention of *manuscript in preparation, unpublished experiments,* and *personal communication* should be in parenthesis. Use of *personal communication* should be with written permission of the communicator and should be entered only in the text, not in the Reference list.

Examples of References in CBE Style and Format

Journal Articles

Miller LF, Chance CJ. 1954. Fishing in the tail waters of TVS dams. Prog Fish-Cult 16:3-9.

Ortenburger AI, Hubbs CL. 1927. A report on the fishes of Oklahoma, with descriptions of new genera and species. Proc Okla Acad Sci 6:123-141.

Books

Book with Authors:

Miller RJ, Robison HW. 1980. The fishes of Oklahoma. Stillwater (OK): Oklahoma State University Press. 246 p.

Book with Editors:

Gilman AG, Rall TW, Nies AS, Taylor P, editors. 1990. The pharmacological basis of theraputics. 8th ed. New York: Pergamon. 1811 p.

Book with Organization as Author: International Union of Pure and Applied Chemistry, Physical Chemistry Division. 1993. Quantities, units, and symbols in physical chemistry. 3rd. Oxford (UK): Blackwell Science. 166 p.

Chapter in Book with Editors:

Hamilton K, Combs DL, Randolph JC. 1985. Sportfishing changes related to hydro- power generation and non-generation in the tailwater of Keystone Reservoir, Oklahoma. In: Olsen FW, White RG, Hamre RH, editors. Proceedings of the symposium on small

hydropower and fisheries. Bethesda (MD): American Fisheries Society. p 145-152.

Theses: Knapp MM. 1985. Effects of exploitation on crappie in a new reservoir [MSc thesis]. Stillwater (OK): Oklahoma State University. 84 p. Available from: OSU Library.

Internet: Oklahoma Climatological Survey. 2003. Climate of Oklahoma [online]. Available from: http://climate.ocs.ou.edu. (Accessed August 15, 2005).

D. Review Process.

The Editors review the MS and carefully select reviewers for all submitted manuscripts. All referee and editorial opinions are anonymous. A decision to accept, revise, or reject the manuscript is made by the editor after careful consideration of reviewers' comments and recommendations. If a "revise" decision is reached, the authors will be allowed to resubmit a revised version of the manuscript within a given time window. The authors are considered to address all reviewers' comments and concerns, or provide compelling reasons to explain why they chose not to do so. A point- by-point rebuttal letter is required with each revised manuscripts, which clearly indicates the nature and locations of corrections within the revised manuscript. All authors should approve all revisions, with the corresponding author being responsible for insuring that all authors agree to the changes.

E. Page Charges

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