PROCEEDINGS
of the
OKLAHOMA ACADEMY OF SCIENCE
Volume 101
2021

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The Official Organ of
the

OKLAHOMA ACADEMY OF SCIENCE

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Affiliated with the American Association for the Advancement of Science.

Publication Date: January 2022

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PROCEEDINGS OF THE OKLAHOMA ACADEMY OF SCIENCE

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REPORTS

A. Applied Ecology & Conservation

Human Impacts on the Prevalence of the Amphibian Infectious Diseases, *Batrachochytrium dendrobatidis* and Ranavirus, in Oklahoma, USA

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Abstract: *Batrachochytrium dendrobatidis* (*Bd*) and ranavirus (RV) are pathogens contributing to the global decline of amphibian populations. Both pathogens can be spread through direct contact between amphibians, through water carrying the infection, the accidental movement of disease particles between waterbodies by cattle, boats, or aquatic recreational equipment, or the intentional movement of infected amphibians used as fishing bait. Amphibians can also experience indirect human-caused effects due to environmental pollutants, including increased stress levels and reduced immunity. We conducted a meta-analysis regarding the effects of human impact on *Bd* and RV pathogen prevalence and infection loads in Oklahoma amphibians, based on field research conducted 2015–2017. Research sites were identified as having minimal, moderate, or high human impact with regard to the degree of land usage for aquatic recreation, grazing, and oil/natural gas. Samples were screened for both *Bd* (gene ITS1) and RV (gene MCP) via published qPCR methodologies; results are reported for both prevalence and infection load (calculated based on qPCR output of mean gene copies multiplied by the dilution factor and extraction volume). We found an average prevalence of 47% for *Bd* and 19.2% for RV infection in amphibians (sample sites pooled), with a trend of increasing prevalence for *Bd* and RV with increasing human interaction. For both pathogens, specimens collected from "moderate" sites had the highest infection loads. We advise land managers overseeing the public use of Oklahoma lands to share educational material regarding amphibian infectious disease, to prevent future spread.

Introduction

Global amphibian declines have been at the forefront of herpetological research for over three decades, yet no single threat has been pinpointed as the primary cause. Instead, amphibian declines have been linked to synergistic effects between several threats, including habitat loss and modification, environmental pollutants, over-exploitation for food and the pet trade, invasive species, climate change, and infectious diseases (McMenamin et al., 2008; Grant et al., 2020; Ford et al., 2020). The interplay of all of these largely anthropogenic factors has been difficult to tease apart, despite extensive research (Green et al., 2020) and making substantial

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*Current address: Hudson College of Public Health, University of Oklahoma, Oklahoma City, OK headway to decrease amphibian decline will require changes to policy and society as well (Beebee and Griffiths, 2005; Ford et al., 2020). Many researchers consider the modern spread of infectious diseases to be one of the most alarming threats to amphibians, especially as it is exacerbated by climate change and introductions to native amphibian communities (Pounds et al., 2006; Bienentreau and Lesbarreres, 2020). Two diseases now recognized to be major contributors to amphibian decline are chytridiomycosis, often referred to as chytrid and caused by the fungus *Batrachochytrium dendrobatidis* (*Bd*), and a systemic infection caused by strains of ranavirus (RV). Chytridiomycosis is a skin infection that clogs and unravels keratinized amphibian tissue, decreasing the host's ability to control osmotic balance and undergo cutaneous respiration, often leading to higher mortality in amphibian populations (Voyles et al., 2009). Less severe symptoms include lethargy, loss of appetite, and skin sloughing (O'Hanlon et al., 2018); however, these too have negative impacts on populations as they often lead to increased susceptibility to predation (Berger et al., 1998; Han et al., 2011). Keratinized structures in amphibians are reduced in pre-metamorphic individuals, often associated with oral regions only, therefore, they are often less susceptible to widespread *Bd* infection (Berger et al., 1998), though starvation has been known to occur (Venesky et al., 2010).

Like *Bd,* RV-infected individuals experience symptoms of emaciation and lethargy that increase their susceptibility to predation (Harp and Petranka, 2006). Furthermore, RV-infected organisms can experience organ necrosis and hemorrhaging, ultimately leading to death (Gray et al., 2009; Gray and Chinchar, 2015). Tadpoles are particularly vulnerable to RV infection, with mortality rates now shown to increase exponentially with each stage of larval development (Warne et al., 2011). Furthermore, because ranavirus is able to switch hosts easily among several major vertebrate groups, including amphibians, some reptiles, and fish, it raises great concern for those studying infectious diseases on these potentially vulnerable populations (Jancovich et al., 2005; Currylow et al., 2014; Gray and Chinchar, 2015). Amphibians with *Bd* and RV comorbidities are at greatest risk, with cases of coinfections documented previously in wild populations in the tropical Andes, Costa Rica, and Oklahoma, USA (Whitfield et al., 2013; Warne et al., 2016; Watters et al., 2018). For both *Bd* and RV, pathogens may only cause deleterious effects in some species, whereas tolerant species act as carriers that spread the disease to more vulnerable species (Schloegel et al., 2009; Hoverman et al., 2011; Currylow et al., 2014).

Research indicates that both *Bd* and RV have been spread worldwide through the global commercial amphibian trade (Schloegel et al., 2009; O'Hanlon et al., 2018), mostly during a period of time when traders did not use handling methods that would prevent cross-infections (Weldon et al., 2004; Fisher and Garner, 2007; Price et al., 2016). Humans continue to spread both diseases through direct effects and can contribute to disease susceptibility though indirect effects in a number of ways (Gray et al., 2017). First, some interactions such as human aquatic recreational activities and cattle grazing can spread both *Bd* and RV directly (Jancovich et al., 2005; Gray et al., 2007; Greer and Collins 2008; Gray et al., 2017). For example, humans engaging in recreational activities like fishing, hunting, and boating can spread *Bd* and RV by moving from pond to pond without disinfecting equipment (i.e. boats, waders, nets, etc.) that comes into contact with water or mud (Cunningham et al., 2003; Gray et al., 2017; Casais et al., 2019), or even moving infected individuals between locations by using them as fishing bait (Jancovich et al., 2005; Picco and Collins, 2008). Indirect human-mediated stressors have also been shown to increase amphibian susceptibility to disease through immune suppression, such as the use of pesticides, human road traffic, modification of habitats, and oil and natural gas extraction (Kerby and Storfer, 2009; Kerby et al., 2011; Brittingham et al., 2014; Guo et al., 2018; Robert et al., 2019; Bienentreau and Lesbarreres, 2020). Furthermore, significant habitat modification can result in increased amphibian densities in remaining, fragmented habitats, leading to higher rates of pathogen transmission (Bienentreau and

Lesbarreres, 2020). Additionally, the presence of cattle grazing in the vicinity of waterbodies also provides both direct and indirect effects through transfer microbes contained in sediments between locations (on hooves), degradation of water quality, and decreases in vegetation in an area (Harp and Petranka, 2006; Gray et al., 2007; Greer and Collins, 2008; Miller et al., 2011). For a state like Oklahoma, where pasture and rangeland make up approximately 50% of the land use in the state, oil and natural gas extraction are prevalent, and wildlife-related recreational activities are common (U.S. Department of the Interior, 2001; U.S. Department of Agriculture, 2017; U.S. Energy Information Administration, 2020), these anthropogenic factors are likely contributing collectively to the distribution and prevalence of both *Bd* and RV.

Amphibians in Oklahoma have been exposed to *Bd* for several decades (Watters et al., 2016), although the extent of disease prevalence across the state has become more well understood only recently through regional and statewide surveys (Marhanka et al., 2017; Davis et al., 2018; Watters et al., 2018, 2019; Smith et al., 2019). However, no study to date has assessed patterns of disease prevalence and distribution at a statewide level, nor has the impact of human-mediated habitat disturbance on pathogen threats of amphibian populations been evaluated. Oklahoma is home to many national wildlife refuges, state-managed wildlife management areas and state parks, and other conservation lands, all exposed to varying levels of anthropogenic impacts that may be contributing to amphibian disease spread and regionalized population susceptibility. In this study, we assess whether disease prevalence and pathogen load across Oklahoma has a positive correlation with the degree of human-mediated environmental impact. Such a correlation would indicate that amphibian populations in environments increasingly impacted by human-mediated stressors will be at a greater danger for mass mortality events and regional extirpation in the future (Leung et al., 2017).

Methods

Field Data Collection

Fieldwork was conducted in March–June and September–October in 2015, March–June in 2016, and March–June and October in 2017. Surveys were conducted around ponds, lakes, streams, and wetlands in Oklahoma Department of Wildlife (ODWC) Wildlife Management Areas (WMAs), National Wildlife Refuges (NWR), Oklahoma State Parks (SP), and The Nature Conservancy (TNC) preserves (Figure 1). This study is a meta-analysis and field study, combining reported data for central, northeastern, and southeastern Oklahoma (Marhanka et al., 2017; Davis et al., 2018;

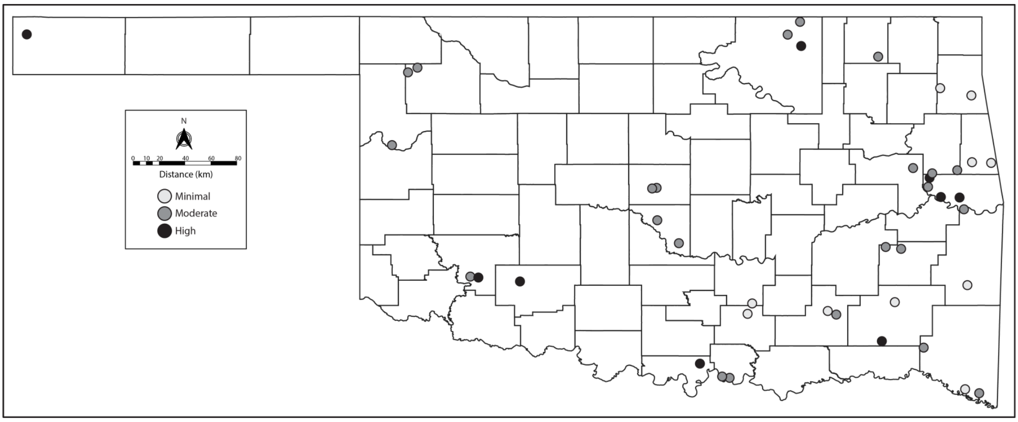

Figure 1. Map of Oklahoma, USA showing sampled sites for the study, with different colored circles representing minimal (light gray), moderate (dark gray), or high (black) human impact for each site. If multiple waterbodies were sampled within a site, the placement of each circle for a sampled area corresponds to the most sampled pond or lake at that site.

Watters et al., 2018, 2019; Smith et al., 2019), along with unpublished disease survey data for amphibian populations in western Oklahoma and TNC preserves. Overall, 43 distinct sites were sampled (Figure 1), with 3–179 individuals sampled per site (total *N* = 1,514 individuals for *Bd*; total *N* = 1,526 individuals for RV).

Sites were classified as having minimal, moderate, or high human impact based on the type and volume of human activity in that area (Table 2). Minimal human impact environments were defined as sites often forbidding public access, though some locations may allow minimal hunting or fishing (*N* = 11 sites). Moderate human impact environments were defined as sites having some amount of grazing, recreation, boating, fishing, hunting, and oil and/or natural gas exploitation, with waterbodies located near roadways and recreational parks (*N* = 23 sites). The volume of traffic at these sites was higher than at sites of minimal impact, but recreational use was not heavy on a frequent basis. High human impact environments were defined as sites subject to heavy mining and oil and/or natural gas extraction, grazing, fishing, recreational vehicle usage, hunting, and/or boating (*N* = 9 sites). All Oklahoma State Parks were designated as high human impact sites. Human impact status decisions were based on communications with land managers, public website descriptions of sampled locations, and our direct field observations (ODWC, 2020). There were no available statistics for numbers of visitors for most sites included in this study.

Each site was surveyed for a total of 12–48 h, with amphibians captured by hand, aquatic trap, dip net, or seine, and then kept in individual plastic bags until swabbing and euthanization or release. All field collecting equipment (i.e. waders, traps, nets) was sterilized between locations using 10% bleach to avoid any potential contamination of sites (Gray et al., 2017). In most cases, amphibians were transported back to the Sam Noble Oklahoma Museum of Natural History (SNOMNH) prior to disease sample collection. Rayon-tipped swabs (Medical Wire, MWE 113) were rubbed over the surface of the live amphibians before euthanization, for the collection of potential *Bd* spores (Lannoo et al., 2011). Swab heads were placed into sterile, individually labeled 1.5mL vials. In most cases, animals were then immediately euthanized via an aqueous solution of chloretone (hydrous chlorobutanol; 1, 1, 1, Tri-Chlora 2-methyl, 2-propanol), containing 1 teaspoon of crystals per 500mL of DI water, for a length of time appropriate to their body size and skin thickness, usually 3–5 minutes (Simmons, 2015). For those amphibians caught as part of a repeat sampling project in central Oklahoma, many individuals were released on-site after swabbing (Smith et al., 2019; Watters et al., 2019). In order to screen for ranavirus, a tissue sample was collected, either from the liver of euthanized animals or the tail or toe of released animals (St-Amour and Lesbarreres, 2007). Tissue samples were flash frozen in liquid nitrogen or preserved in 95% ethanol, then stored in 2mL cryovials. DNA from swabs were extracted using PrepMan Ultra (Applied Biosystems; Cheng et al., 2011) and tissue samples were extracted using a high salt extraction method (Esselstyn et al., 2008). All reusable equipment (i.e. scissors, forceps) was sterilized between sample collections using 10%

Table 1. Nucleotide sequence for forward primers, reverse primers, and probes used in *Batrachochytrium dendrobatidis* (*Bd*) and ranavirus (RV) screening in this study (Boyle et al., 2004; Forson and Storfer, 2006).

	Bd	**RV**
Forward primer	CCTTGATATAATACAGTGTGCCARARGTC	ACACCACCGCCCAAAAGTAC
Reverse Primer	AGCCAAGAGATCCGTTGTCAAA	CCGTTCATGATGCGGATAATG
Probe	CGAGTCGAACAAAAT	CCTCATCGTTCTGGCCATCAACCAC

bleach or Eliminase (DeconLabs); gloves were also changed between each sample collection (Gray et al., 2017).

Genetic Analysis

Quantitative PCR (qPCR) methodologies were employed to determine presence of *Bd* and RV genetic signatures from swab or tissue extractions and to estimate the number of gene copies per sample (infection load) using protocols from Kerby et al. (2013). DNA extracts were diluted 1:10 for *Bd* and 1:1 for RV with 0.25x TE Buffer to remove potential inhibitors. Samples from 2015 were analyzed at the Disease Testing and Sequencing Facility at the University of South Dakota (StepOnePlus Real-Time PCR, software v2.3); 2016–2017 samples were analyzed at the SNOMNH Genomics Core Facility (QuantStudio 3.0 Design and Analysis Software). For each qPCR run, samples for *Bd* and RV were run in triplicate, along with positive controls containing known gene copy numbers for both pathogens (gBlock DNA quantities $1e^1$–$1e^4$), and a single negative control (ddH_2O). For *Bd*, primers targeted the ITS-1 rRNA gene (Boyle et al., 2004), and for RV, primers targeted the major capsid protein (MCP; Forson and Storfer, 2006) (Table 1). Samples were considered positive for *Bd* (*Bd*+) or RV (RV+) if amplification occurred in at least two of the three wells and if the mean gene copy number per well (from qPCR output) was greater than 1.0. Any samples that tested positive in only one of three qPCR wells was re-run on a new qPCR plate, to determine whether it was a true negative or whether the pathogen DNA was simply present in very small quantities; if the rerun resulted in at least one positive well, the sample was considered positive. Infection load was calculated by obtaining the mean gene number copy/sample from all wells indicating positive results (from the qPCR analysis software), then multiplying this value by the original extract volume, and the appropriate dilution factor. Disease prevalence data was analyzed by human impact level using non-parametric Kruskal-Wallis tests for each pathogen. Additional Kruskal-Wallis tests were performed on infection load (mean gene copies/sample) by human impact level, with pairwise Wilcoxon-Rank Sum analyses performed as needed.

Results

The results of our metanalysis show an average pathogen prevalence of 47% and 19.2% for *Bd* and RV, respectively, across the state of Oklahoma (Table 2). Prevalence patterns for *Bd* in Oklahoma were not correlated significantly with environmental human impact level, despite a visible stair-step trend of increased prevalence when moving from minimal to high human impact ($H = 1.65$, $P = 0.438$; Figure 2). Although RV prevalence data also showed an increasing trend from minimal to moderate levels, high human impact environments possessed similar viral prevalence to moderate human impact sites (Figure 2); however, these trends were not statistically significant ($H = 2.86$, $P = 0.239$). With regard to infection load, there was no observed significant correlation to environmental human impact level for *Bd* ($H = 5.63$, $P = 0.0702$; Figure 3), although sites of moderate human impact tended to have individuals with higher infection loads (Table 2). In contrast, we do observe a statistically significant correlation between RV infection load and human impact level ($H = 28.9$, $P < 0.001$; Table 2; Figure 3), with post-hoc comparisons of RV infection load supporting a significant difference between sites of minimal and moderate human impact only ($z = 2.21$, $P = 0.0135$).

Discussion

Sampling efforts aimed at monitoring *Bd* and RV in Oklahoma have shown that the two diseases affect herpetofauna in communities across the state, with *Bd* found at higher prevalence levels than RV (Marhanka et al., 2017; Davis et al., 2018; Watters et al., 2018, 2019; Smith et al., 2019). Our meta-analysis found a non-significant trend of increasing prevalence in both *Bd* and RV as the degree of human impact on surveyed environments increased (Figure 2). Although we could not fully reject our null hypothesis, additional surveys across a greater temporal sampling of communities for disease may show more significant associations with direct and indirect human-mediated disturbances (Brittingham et al., 2014; Gray et al., 2017). Still, the observed trend of increased prevalence with increased human impact remains relevant to local land managers. Direct spread by human recreational activities could be mitigated through increased education regarding disinfection for people visiting the various locations and/or purchasing hunting and fishing permits for use in the state (Casais et al., 2019; Bienentreau and

Table 2. List of all Oklahoma sites sampled for *Batrachochytrium dendrobatidis* (*Bd*) and ranavirus (RV) prevalence from 2015–2017, sorted by human impact level. Total sample size, number of positive individuals (+), prevalence (%) ± standard deviation (SD), and mean ($\bar{X}$) infection load ± SD of infection load are listed for *Bd* and RV for each site. Site abbreviations are as follows: Oklahoma Department of Wildlife (ODWC) Wildlife Management Areas (WMAs), National Wildlife Refuges (NWR), Oklahoma State Parks (SP), and The Nature Conservancy (TNC) preserves.

					Bd				RV
Site name	**County**	**N**	**+**	**%**	**$\bar{X}$ infection load (± SD)**	**N**	**+**	**%**	**$\bar{X}$ infection load (± SD)**
MINIMAL		545	166	30.46	659,181.95 (± 2,897,358.30)	546	46	8.42	37,137.53 (± 143,442.36)
Grassy Slough WMA (33.78324, -94.76353)	McCurtain	27	20	74.07	275,147.29 (± 449,154.80)	27	2	7.41	301.69 (± 267.07)
Oka'yanahli TNC (34.43442, -96.64560)	Johnston	177	23	12.99	10,095.09 (± 32,364.02)	179	0	0	N/A (± N/A)
Ouachita WMA (34.68206, -94.74407)	Le Flore	54	25	46.3	899,866.83 (± 2,086,748.90)	14	1	7.14	586.10 (± 692.33)
Ozark Plateau NWR, Hamby Unit (36.31092, -94.70803)	Delaware	40	14	35	7,778.27 (± 136,431.57)	38	4	10.53	337,939.87 (± 150,252.20)
Ozark Plateau NWR, vicinity of Night Train Farm (35.74396, -94.70409)	Delaware	14	7	50	278,477.52 (± 632,016.70)	13	1	7.69	10,062.53 (± N/A)
Ozark Plateau NWR, Looney Unit (36.32050, -94.70953)	Adair	47	23	48.94	43,902.14 (± 84,784.26)	47	0	0	N/A (± N/A)
Ozark Plateau NWR, Sallybull Unit (35.73804, -94.53851)	Adair	20	6	30	1,897.25 (± 2,473.99)	19	0	0	N/A (± N/A)
Pontotoc Ridge TNC (34.52409, -96.60590)	Pontotoc	38	2	5.26	2,577.39 (± 3,361.56)	39	4	10.26	1,952.54 (± 2,164.88)
Pushmataha WMA (34.53523, -95.37177)	Pushmataha	47	24	51.06	2,505,419.96 (± 6,128,539.50)	49	14	28.57	1,305.41 (± 1,788.50)
Spavinaw WMA (36.38328, -94.97942)	Delaware	69	17	24.64	28,310.38 (± 65,379.88)	70	3	4.29	3,109.07 (± 1,459.82)
Stringtown WMA (34.45893, -95.95204)	Atoka	12	5	41.67	505,698.71 (± 815,319.18)	12	5	7.41	413.28 (± 367.44)
MODERATE		788	417	52.92	1,013,172.80 (± 7,347,673.10)	821	207	25.21	523,690.96 (± 4,657,370.82)
50th St. & Bartell Ave., Oklahoma City (35.52229, -97.43267)	Oklahoma	37	23	62.16	5,568,914.14 (± 23,237,913)	34	4	11.76	74,749.52 (± 147,447.02)
Arkansas River at Robert S. Kerr Lock and Dam 15 (35.52229, -97.43267)	Le Flore	14	9	64.29	843,889.22 (± 1,599,005.30)	14	1	7.14	202.96 (± N/A)
Camp Gruber WMA (35.69351, -95.21388)	Muskogee	28	21	75	165,010.91 (± 555,831.77)	28	21	75	38,619.80 (± N/A)
Cherokee WMA (35.64775, -95.04848)	Cherokee	14	1	7.14	76.64 (± N/A)	14	0	0	N/A (± N/A)
Cookson WMA (35.67467, -94.83175)	Adair/ Cherokee	142	89	62.68	N/A (± N/A)	151	54	35.76	N/A (± N/A)
Cooper WMA (36.56062, -99.50166)	Woodward	10	1	10	1,984.38 (± 995.25)	10	0	0	N/A (± N/A)

Table 2. Continued

Fobb Bottom WMA (33.89288, -96.86565)	Marshall	3	1	33.33	277,499.40 (± N/A)	3	1	33.3	496.68 (± N/A)
Fort Supply WMA (36.51990, -99.58335)	Woodward	4	1	25	1,968.00 (± N/A)	4	1	25	8,649.65 (± N/A)
Hulah WMA (36.95639, -96.19199)	Osage	11	4	36.36	N/A (± N/A)	11	4	36.36	N/A (± N/A)
James Collins WMA (35.01187, -95.45086)	Latimer	26	14	53.85	93,967.58 (± 162,633.49)	33	6	18.18	666.18 (± 370.12)
Lexington WMA (35.04437, -97.24004)	Cleveland	47	18	39.3	19,439.89 (± 27,500.96)	46	9	19.57	847.76 (± 1,219.16)
McClellan-Kerr WMA (35.53111, -95.08433)	Sequoyah	30	8	26.67	47,133.66 (± 73,122.65)	30	8	26.67	7,432.80 (± 6,688.91)
McGee Creek WMA (34.42796, -95.87825)	Atoka	29	19	65.52	255,365.08 (± 456,852.75)	30	4	13.33	459.22 (± 318.31)
Mountain Park WMA (34.75610, -99.04420)	Kiowa	18	1	5.56	24,856.20 (± 35,002.75)	18	0	0	N/A (± N/A)
Oklahoma City Zoo (35.51705, -97.47129)	Oklahoma	59	34	57.63	1,477,820.27 (± 6,219,816.90)	60	17	28.33	337.68 (± 455.31)
Oologah WMA (36.65560, -95.51593)	Nowata	62	26	41.94	191,331.69 (± 643,974.46)	62	26	41.94	5,588.7 (± N/A)
Osage Hills WMA (36.74756, -96.18187)	Osage	14	14	100	337,368.63 (± 558,724.25)	20	0	0	N/A (± N/A)
Packsaddle WMA (35.89135, -99.72193)	Ellis	14	13	92.86	283,679.73 (± 706,615.74)	14	2	14.29	245.35 (± 159.75)
Pine Creek WMA (34.14252, -95.12228)	McCurtain	16	5	31.25	46,234.47 (± 65,356.31)	21	16	24.53	2,455,477.09 (± 10,979,101.38)
Red Slough WMA (33.74901, -94.64159)	McCurtain	58	48	82.76	2,302,191.52 (± 8,603,747.50)	2	2	76.19	413.70 (± 363.21)
Robbers Cave WMA (34.99551, -95.31754)	Latimer	22	13	59.1	169,048.50 (± 245,605.34)	29	9	31.03	1,305,693.70 (± 3,904,431.08)
Sutton Urban Wilderness, (35.24266, -97.42689)	Cleveland	103	28	27.18	122,211.16 (± 326,647.18)	105	15	14.29	481.62 (± 558.68)
University of Oklahoma Biological Station (33.88150, -96.80122)	Marshall	27	26	96.3	5,890.84 (± 12,564.51)	26	0	0	N/A (± N/A)
HIGH		181	129	71.27	268,968.75 (± 955,328.46)	159	41	25.79	40,249.25 (± 102,739.85)
Black Mesa SP (36.84771, -102.88154)	Cimarron	9	4	44.44	91,993.60 (± 106,275.25)	10	5	50	7,523.29 (± 2,164.88)
Great Plains SP (34.74799, -98.97459)	Kiowa	5	0	0	N/A (± N/A)	5	1	20	2,507.95 (± N/A)
Hickory Creek WMA (34.00422, -97.05785)	Love	35	35	100	11,508.19 (± 41,099.02)	33	2	6.06	180.80 (± 88.63)
Hugo WMA (34.19843, -95.48391)	Choctaw/ Pushmataha	3	1	33.33	598.24 (± N/A)	2	2	100	459.89 (± 443.54)
KOA Group Campground, Sallisaw (35.43885, -94.81166)	Sequoyah	6	4	66.67	N/A (± N/A)	6	0	0	N/A (± N/A)
Osage Hills SP (36.74756, -96.18187)	Osage	35	26	74.29	24,048.69 (± N/A)	20	12	60	N/A (± N/A)
Sequoyah NWR (35.44331, -94.97335)	Sequoyah	28	10	35.71	10,837.95 (± 2,688.18)	28	12	42.86	93,658.03 (± 150,252.20)
Tenkiller WMA & SP (35.60904, -95.07096)	Sequoyah	4	1	25	281,947.92 (± N/A)	4	0	0	N/A (± N/A)
Wichita Mountains NWR (34.71400, -98.61472)	Comanche	56	48	85.71	524,981.54 (± 1,321,403.6)	51	7	13.73	5,271.92 (± 7,834.56)
TOTAL		**1,514**	**712**	**47.03**	**782,940.66** **(± 5,615,695.70)**	**1,526**	**294**	**19.27**	**331,055.59** **(± 3,619,434.80)**

Lesbarreres, 2020).

Interestingly, infection load data for both diseases was highest at moderate human impact sites (Figure 3). This effect was more pronounced for RV data, with significant differences between RV gene copies when comparing minimal and moderate environmental disturbances (Figure 3). Studies have proposed that stress to amphibian immune systems from human traffic, grazing, and land use can lead to infection load data mirroring disease prevalence data as human interaction with the environment increases (Brittingham et al., 2014; Gray et al., 2017; Bienentreau and Lesbarreres, 2020). One possible explanation for our observed results is that many individuals with higher infection loads may have already succumbed to the disease but not have been observed in mortality events. More research needs to be done to further elucidate the relationship between infection load and pathogen prevalence for both *Bd* and RV, and the interplay with direct and indirect human impacts (Warne et al., 2016; Bienentreau and Lesbarreres, 2020). Unfortunately, host responses to disease infection involve a myriad of environmental and host-specific factors, all of which together result in high variability of disease outcomes observed in field settings, making predictive assumptions difficult (Zamudio et al., 2020).

Future research to assess anthropogenic impacts on *Bd* and RV prevalence and infection load could be improved with improved assessment and temporal monitoring of human impacts on environmental health across the state, as well as more even sampling of amphibian communities and sites within disturbance categories. For example, Robbers Cave WMA exhibited a higher-than-average prevalence for both pathogens (Table 2), and human impacts on-site include natural gas pipelines, hunting, and fishing (Watters, personal observation; ODWC, 2020). However, our study did not address the impacts of each occurrence individually, merely as a whole. Assessing water quality, quantifying environmental contaminants, measuring distance from a pond to a pipeline, or counting numbers of fisherman and hunters per year would improve our understanding of individual disturbance activity impacts. Additionally, should more detailed information about daily traffic and levels of recreational use across the state become available, future studies may be able to re-evaluate the large, moderate impact category with more precision. Unfortunately, at present, the vast majority of sites have no metric by which to measure human visitation as they are broadly open to the public (no locked gates, etc.). However, as of June 2020, Oklahoma State Parks require a daily parking pass, which may at least provide relative data for comparison across parks.

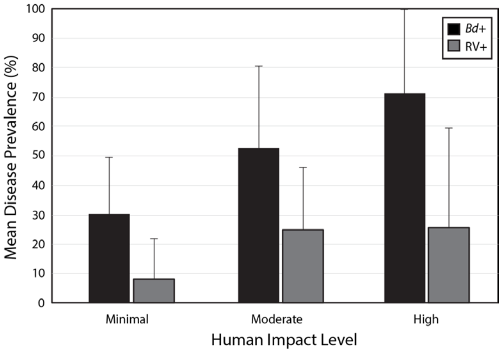

Figure 2. Comparisons of *Batrachochytrium dendrobatidis* (*Bd*+) and ranavirus (RV+) mean prevalence (%) with standard error bars among sites of minimal, moderate, and high human impact.

Although the observed trends along the human impact gradient lacked statistical significance, this could be the result of smaller sample sizes per site or category. For example, some sites in the moderate and high impact categories had fewer than 10 individual disease samples collected (Table 2). Additionally, there were comparatively fewer locations of minimal and high human impact (*N* = 11 and 9, respectively) compared to the number of locations classified as having moderate human impact (*N* = 23). The addition of increased species-specific and community-level sampling across sites, as well as additional surveys at minimal and high human impact environments, would allow for more robust tests for correlated patterns. In general, more objective classifications of human impact based on environmental measurements

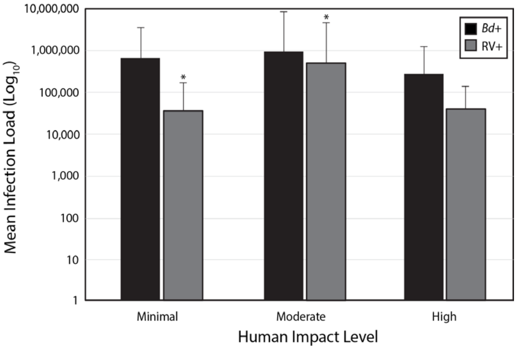

Figure 3. Comparisons of *Batrachochytrium dendrobatidis* (*Bd*+) and ranavirus (RV+) mean infection loads (calculated from mean gene copies) with standard error bars among sites of minimal, moderate, and high human impact. Statistical analysis resulted in a significant difference of RV infection loads between sites of minimal and moderate human impact only, as indicated by an asterisk (*).

and recreational and land usage metrics would increase statistical power in future studies. With identification of the most influential behaviors on human-mediated spread of *Bd* and RV, we can direct education efforts to land managers and the public accordingly.

In conclusion, our findings will help conservation efforts within Oklahoma by identifying specific areas in need of further preventive measures against the spread of amphibian infectious disease. Disease prevalence at several moderate and high areas of human impact are above 50% for *Bd* and around 25% for RV (Table 2). Additionally, since RV is not specific to amphibians, it has the potential to infect sympatric reptiles (particularly turtles) and fish in their ecosystems (Jancovich et al., 2005; Currylow et al., 2014), therefore, continued monitoring is recommended for all taxa potentially impacted. Additionally, while preliminary results on amphibian infectious diseases have been shared through an ODWC blog in 2018 (https://www.wildlifedepartment.com/ooj/health-checkup-oklahomas-frogs-and-salamanders), an official educational campaign should be developed for Oklahoma. This could include instructions for fisherman or boaters on properly sterilizing equipment when moving between bodies of water (Cunningham et al., 2003; Gray et al., 2017) and on the threats of using amphibians as bait that may carry infections as has been documented in other areas of the United States (Jancovich et al., 2005; Picco and Collins, 2008). Research indicates that the only way to mitigate amphibian population declines is to improve communication and collaboration among all possible stakeholders—researchers, land owners and managers, and the general public alike (Canessa et al., 2019).

Acknowledgments

We thank Siler Lab members for assistance in field sampling, DNA extractions, and qPCR screening necessary for completion of this project. We also thank the University of Oklahoma Honors College and the Sam Noble Oklahoma Museum of Natural History for providing SEH the opportunity to work on this project with the Siler Lab. Specimens were collected under applicable Oklahoma Department of Wildlife Scientific Collecting Permits to JLW and CDS, and the methodologies were approved by the University of Oklahoma IACUC Committee (#R14-026). Funding was provided to CDS and JLW by the Oklahoma Department of Wildlife Conservation (grant number F14F01225 [T-80-1]) and an Oklahoma City Zoo and Botanical Gardens Conservation Action Now grant. Lastly, we would like to thank two anonymous reviewers for their edits on this manuscript.

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Submitted December 16, 2020 Accepted July 29, 2021

Fish Communities, Species of Greatest Conservation Need, and Potential Protected Areas in Southeastern Oklahoma, 2014-2016

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Abstract: Conservation of native fish species relies on contemporary knowledge about their distributions and abundances. The Muddy Boggy, Kiamichi, and Little River drainages in southeast Oklahoma have diverse native fish communities, including numerous species with limited ranges within the state (i.e., “Species of Greatest Conservation Need” or SGCN). Comprehensive surveys of fish diversity had not been conducted within these drainages since the 1960s or ‘70s, meaning the current distribution and status of SGCN was not known. Therefore, we surveyed fish diversity in these drainages by making 167 collections by seine net in 2014-2016. We collected 35,236 individuals, 83 total species, and 11 of the 19 SGCN expected to occur in this region. Native fish communities throughout the region were similar relative to historical information from the 1920s through the 1970s. We suggest surveys of fish in streams of this region should be continued on a regular basis and priority should be given to locations with known populations of SGCN or high diversity of native species. We suggest that priority sites with 15 or more native species, high priority sites with 19 or more native species, or sites with multiple SGCN, should be candidates for special protection. We also provide suggestions about the status of several SGCN.

Introduction

The Muddy Boggy, Kiamichi, and Little River drainages in southeast Oklahoma (Figure 1) have diverse native fish communities, including numerous species with small, or very limited ranges within the state (Miller and Robison, 2004). The first scientific fish collections in the region were made in the Kiamichi drainage in 1894 by S. Meek (Meek 1896), and in the Muddy Boggy drainage by H. Pilsbry in 1903 (Fowler 1904). Fishes in Little River, and its Oklahoma tributaries (Glover River and Mountain Fork) were first collected for science in 1925 by A. I. Ortenburger from the University of Oklahoma and the newly formed Oklahoma Biological Survey (Ortenburger and Hubbs, 1926). Subsequent Little River collections were made in 1927 (Hubbs and Ortenburger 1929a, b), and during subsequent summers until 1934. Following the Ortenburger surveys, sampling within these rivers was sporadic until surveys of the Mountain Fork drainage in the late 1940s (Reeves 1950), the Little River drainage in the 1950s (Finnell *et al*. 1956), the Kiamichi River system by J. Pigg in 1972-1973 (Pigg and Hill, 1974), the Muddy Boggy drainage by J. Pigg in 1974-1975 (Pigg 1977), the Little River drainage by D. A. Rutherford, A. A. Echelle, and O. E. Maughan in 1981 and 1982 (Rutherford *et al*. 1987, 1992), and the Mountain Fork drainage in the 1970s (Eley *et al*. 1981). These included thorough descriptions of the river drainages, of previous fish sampling, and an annotated list of localities where fish occurred. Subsequently, there was annual sampling of about a dozen sites on larger streams in the region by J. Pigg or R. Parham until 2004 (Parham 2009), but no more drainage-wide surveys were conducted that included mainstems and tributaries of all sizes.

More recent fish sampling efforts were limited, but include: eight sites in the Kiamichi, Glover, and Little River drainages (Vaughn *et al.* 2021); targeted sampling for minnows in the three drainages (Wagner *et al*. 1987); sampling of numerous upland sites across the drainages (Dauwalter *et al*. 2008); and five sites in the Boggy drainage (Schenck and Smith 1973). Additionally, Matthews *et al*. (1988), Pyron *et al*. (1998), Porter and Patton (2015), and Sansom *et al*. (2017) sampled sites throughout the Kiamichi River mainstem; Pyron and Taylor (1993) surveyed the lower Little River drainage, and Taylor and Lienesch (1995) sampled minnows in the Little River drainage. These surveys, however, were all spatially limited and/or they focused on a subset of species. Therefore, a current survey was warranted to document contemporary fish species distributions and composition of fish communities throughout the region. From our survey, three papers have been published to date including a study addressing Beta diversity of fish communities in the Muddy Boggy system (Zbinden and Matthews 2017), and two papers focused on comparing recent collections to those of Pigg in the 1970s (Zbinden 2020, 2021). Here we summarize our fish collections from 2014-2016 across three Red River tributary drainages of southeast Oklahoma.

The Oklahoma Department of Wildlife Conservation (ODWC) periodically updates lists of fish (and other taxa) that are considered “Species of Greatest Conservation Need” (SGCN). SGCN designation is based on input from knowledgeable experts from ODWC, other agencies, non-governmental organizations, and universities. The SGCN are ones which experts have concern about their precarious existence in Oklahoma or a general lack of knowledge about their status. We were funded by a State Wildlife Grant from ODWC to conduct comprehensive fish community surveys throughout the Muddy Boggy, Kiamichi, and Little River drainages, with emphasis on detection of fish species listed as SGCN.

The ranks of SGCN include Tier I species (with the greatest need for information), followed by Tier II, and Tier III. The ODWC has identified 52 fish SGCN (Oklahoma Comprehensive Wildlife Conservation Strategy, Appendix E, ODWC 2013), of which 19 SGCN are Tier I (N = 11) or Tier II (N = 8) documented to occur in the drainages we surveyed (Miller and Robison 2004, W. J. Matthews collections from 1976 to present). The goals of this study were (1) determining the current distributions and community composition of native fishes in the Muddy and Clear Boggy, Kiamichi and Little River (including Glover River and Mountain Fork) drainages; and (2) assessing the status and distribution of Tier I and II SGCN in the region. For some SGCN we added information based on earlier collections we (WJM, EMM) made in southeast Oklahoma with university classes or during other research projects (e.g., Matthews *et al*. 1988, Cashner *et al*. 2010, Matthews and Marsh-Matthews 2015). We also evaluated the spatial overlap between sites with high numbers of species and/or SGCN and areas in which fish or streams have potential protection from state or federal land ownership or agreements.

Methods

Study Area

The rivers in the study area are direct tributaries of the Red River. Each tributary flows southward from high gradient lands in or at the western edge of the Ouachita Mountains into low gradient reaches near Red River. The Muddy Boggy and Kiamichi rivers flow directly into the Red River in south Oklahoma (Figure 1). The Little River flows eastward from Oklahoma into Arkansas, where it receives additional tributaries (Rolling Fork, Cossatot, and Saline) then joins the Red River near Fulton, Arkansas. The physical and geologic characteristics of each drainage are well documented (Muddy Boggy – Pigg 1977, Zbinden and Matthews 2017; Kiamichi – Pigg and Hill 1974, Matthews and Marsh-Matthews 2017; Little – Rutherford *et al*. 1987) and not repeated here. Upper reaches of the mainstems and upland tributaries are mostly clear water, over rocky substrate with distinct and repeating riffle-pool habitats. Mainstems and many tributaries lower in the drainages are mud or sand-bottomed, with moderate to high turbidity. Most river mainstem sites are wadeable in upper and middle reaches, but lower reaches are too deep to be sampled by wading, except at stream edges.

The Muddy Boggy drainage consists of two main branches (Clear Boggy Creek and Muddy Boggy Creek), and although the branches join just prior to connection with the Red River, the two branches are considered separately in results. The Kiamichi drainage has one mainstem that flows between two high ridges of the Ouachita Mountains and is therefore treated as a singular system in our results. The Little River drainage has two major sub-drainages in Oklahoma, the Glover River and Mountain Fork, also considered separately in some parts of results.

Study design

To aid selection of field sites we reviewed fish collection records from the Sam Noble Oklahoma Museum of Natural History, which included many samples by A. I. Ortenburger, C. Riggs, J. Pigg and R. Parham, and our own collections from much of the region (Matthews and Marsh-Matthews 2017). Site selection also depended on access, often on private land, so before collecting any fishes, WJM, EMM, and ZDZ traveled to many potential sites to determine stream accessibility and request landowner permission in person. During subsequent fish sampling, other sites were added by simply driving county roads to look for other accessible stream locations. The result was a semi-planned, haphazard sampling pattern, scattered throughout all drainages, to provide samples in all sizes of wadeable streams from river mainstems to small creeks or spring runs.

Sampling

A total of 167 fish collections was made during summers of 2014 and 2015 via seining. Collections included 66 sites in the Muddy Boggy River drainage (with 16 sites sampled a second time in 2016), 40 sites in the Kiamichi River drainage, and 45 sites in the Little River drainage, including 8 sites in the Glover River, 13 sites in the Mountain Fork, and 24 sites in Little River proper or its direct tributaries (Figure 1).

Fish communities were collected by seining all identifiable habitats, as described in detail in Matthews and Marsh-Matthews (2017, pages 5-10), within a target of 100 m of wadeable stream reach (sometimes shorter in small creeks if access was limited) using nets, 4.57 m × 1.22 m × 4.88 mm mesh and/or 2.44 m × 1.22 m × 4.88 mm mesh, depending on the width of the stream. Channel and pool habitats were sampled by pulling seines downstream; riffle and edge habitat including undercut banks were sampled by kick-seining. Specimens were preserved in 10% formalin, with large-bodied adults such as adult gars or buffalos identified and released. All other fishes were identified in our laboratory at the University of Oklahoma, then archived and cataloged in the Sam Noble Museum of Natural History.

At each seining site physical environmental variables were recorded by instream measurement (reach size, water quality, estimates of substrates and habitat types), riparian observation (land use, vegetation, and bank stability). The protocol used for gathering this data is described elsewhere (Zbinden and Matthews 2017), and these data are not analyzed directly herein but are provided for future reference. Exact geographic locations and environmental conditions for each site are provided in the Supplementary Material (Table S1).

We made no boat-mounted samples, so

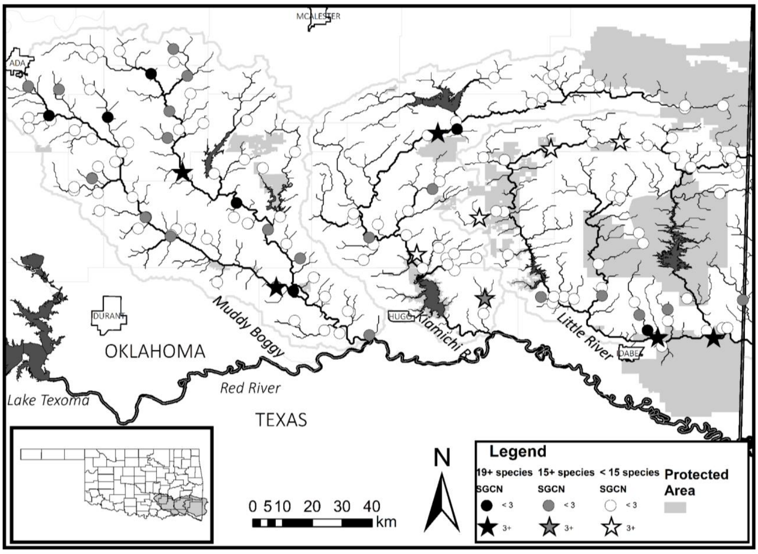

Figure 1. Sampled locations across the study region. Sites are color coded by the number of species collected (white ≤ 14, gray =15 to 18, and black ≥ 19). Sites with fewer than 3 SGCN are denoted by a circle and those with 3+ SGCN are denoted with a star. Protected areas from the Protected Areas Database (United States Geological Survey 2020).

large-bodied riverine species (e.g., skipjack herring, carpsuckers, buffalos, blue sucker) are under-represented in our collections. However, in June 2016, we set 15.2 m gill nets with 48 mm mesh in wadeable areas at two sites in the Muddy Boggy mainstem, one on the Kiamichi mainstem, one on the Glover River (Little River drainage) mainstem (overnight), and one on the lower Little River mainstem to seek larger species. Abundances of species collected at each site are provided in the Supplementary Material (Table S2).

Results and Discussion

Survey summary and community structure

Table 1 lists all species collected by seining, with their total abundances per drainage. We omitted individuals that were too young to identify (in order to prevent inflation of species numbers) and did not include them in diversity or similarity calculations. We also omitted a total of 172 putative hybrids of red shiner x blacktail shiner that we found in the Clear Boggy drainage, to avoid inflating numbers of species (not included in Table 1). We included in Table 1 one larval lamprey ammocoete that we collected in the Kiamichi drainage, as it was a putative SGCN (see below).

For taxonomy, we followed nomenclature in Robison and Buchanan (2020), which is the most recent authoritative work that includes detailed taxonomic accounts related to eastern Oklahoma species. Thus, *Phoxinus erythrogaster* is now *Chrosomus erythrogaster*, *Erimyzon oblongus* is now *Erimyzon claviformis*, and the orangethroat darter (formerly *Etheostoma spectabile*) in southeast Oklahoma is now *Etheostoma pulchellum*. In addition, we referred to all logperch captured in any of the drainages as *Percina caprodes*, although there is uncertainty as to the correct identity of some logperches in the Boggy or Kiamichi drainages (D. Lynch, pers. comm), and more research is needed on their systematics.

Across all seine collections, with 16 sites sampled twice, we netted a total of 35,236 fishes, including individuals that were released. Gill netting in 2016 added one species not included in Table 1, as two adult shortnose gar (*Lepisosteus platostomus*) were captured in the Muddy Boggy mainstem near Lane, Oklahoma.

Table 1: Fish collection summary. Number of fish collections made and species richness across each major drainage basins are shown. Each unique species collected, and the number of individuals collected in each of the major drainages are listed.

		CLEAR	MUDDY	KIAMICHI	LITTLE	GLOVER	MTN FK	TOTAL
	Number of Collections	40	42	40	24	8	13	167
	Number of species	54	54	51	54	25	32	83

Common Name	**Scientific Name**	CLEAR	MUDDY	KIAMICHI	LITTLE	GLOVER	MTN FK	TOTAL
Lamprey ammocoete	*Ichthyomyzon sp.*			1				1
Spotted gar	*Lepisosteus oculatus*	3	28	5	2			38
Longnose gar	*Lepisosteus osseus*	2	2	1	1		7	13
Gizzard shad	*Dorosoma cepedianum*	18	2	17	4			41
Threadfin shad	*Dorosoma petenense*	2		6				8
Central stoneroller	*Campostoma anomalum*	596						596
Highland stoneroller	*Campostoma spadiceum*	1	496	488	133	88	28	1234
Southern redbelly dace	*Chrosomus erythrogaster*	19						19
Red shiner	*Cyprinella lutrensis*	1747	792					2539
Blacktail shiner	*Cyprinella venusta*	1331	26	11	108			1476
Steelcolor shiner	*Cyprinella whipplei*		460	391	111	259	146	1367
Common carp	*Cyprinus carpio*	2						2
Pallid shiner	*Hybopsis amnis*		1					1
Striped shiner	*Luxilus chrysocephalus*				152		74	226
Ouachita Mtn. shiner	*Lythrurus snelsoni*				125	279	822	1226
Redfin shiner	*Lythrurus umbratilis*	339	498	2400	521		170	3928
Golden shiner	*Notemigonus crysoleucas*	175	151	39	16		2	383
Emerald shiner	*Notropis atherinoides*	1		30	51			82
Blackspot shiner	*Notropis atrocaudalis*	3	193	33	43			272
Bigeye shiner	*Notropis boops*	597	795	966	1550	1332	1138	6378
Ghost shiner	*Notropis buchanani*	14	187					201
Ironcolor shiner	*Notropis chalybaeus*				1			1
Kiamichi shiner	*Notropis ortenburgeri*			195	46			241
Sand shiner	*Notropis stramineus*	54						54
Rocky shiner	*Notropis suttkusi*	566	1307	680	128	24	19	2724
Mimic shiner	*Notropis volucellus*		1	19				20
Suckermouth minnow	*Phenacobius mirabilis*	75	68					143
Bluntnose minnow	*Pimephales notatus*	131	61	50	20	13	5	280
Fathead minnow	*Pimephales promelas*	379						379
Bullhead minnow	*Pimephales vigilax*	208	129	3				340
Creek chub	*Semotilus atromaculatus*					14	4	18
River carpsucker	*Carpiodes carpio*		3					3
Blue sucker	*Cycleptus elongatus*	1						1
Creek chubsucker	*Erimyzon claviformis*			72	17	3	6	98
Smallmouth buffalo	*Ictiobus bubalus*	6		2				8
Spotted sucker	*Minytrema melanops*	23	10	6	1	1		41
Black redhorse	*Moxostoma duquesnei*	1	1	7	4			13
Golden redhorse	*Moxostoma erythrurum*	10	5	24	1	1		41
Black bullhead	*Ameiurus melas*	12	23	4	1			40
Yellow bullhead	*Ameiurus natalis*	4	3	3	5	2		17
Blue catfish	*Ictalurus furcatus*		2					2

Table 1. Continued

Channel catfish	*Ictalurus punctatus*	15	73					88
Slender madtom	*Noturus exilis*						7	7
Tadpole madtom	*Noturus gyrinus*	1	4		1			6
Freckled madtom	*Noturus nocturnus*	101	45		1	1		148
Flathead catfish	*Pylodictis olivaris*	1	2			1		4
Redfin pickerel	*Esox americanus*		4	26	30	9	5	74
Starhead topminnow	*Fundulus blairae*				8			8
Blackstripe topminnow	*Fundulus notatus*	2	23	74	29	5	11	144
Blackspotted topminnow	*Fundulus olivaceous*			75	57		1	133
Pirate perch	*Aphredoderus sayanus*		18	5	3		1	27
Western mosquitofish	*Gambusia affinis*	936	1301	499	82	5	4	2827
Brook silverside	*Labidesthes sicculus*	67	289	394	90	24	107	971
Banded pygmy sunfish	*Elassoma zonatum*			1	9			10
Flier	*Centrarchus macropterus*				6			6
Green sunfish	*Lepomis cyanellus*	87	52	92	69	17	46	363
Warmouth	*Lepomis gulosus*	11	13	6	6			36
Orangespotted sunfish	*Lepomis humilis*	43	99	9				151
Bluegill	*Lepomis macrochirus*	382	363	367	145	5	99	1361
Longear sunfish	*Lepomis megalotis*	376	564	300	292	257	117	1906
Redear sunfish	*Lepomis microlophus*	54	39	47	24		16	180
Redspotted sunfish	*Lepomis miniatus*				1			1
Smallmouth bass	*Micropterus dolomieu*						4	4
Spotted bass	*Micropterus punctulatus*	13	7	1	6	18	6	51
Largemouth bass	*Micropterus salmoides*	80	87	179	106		37	489
White crappie	*Pomoxis annularis*	30	36	10	5			81
Black crappie	*Pomoxis nigromaculatus*	2	1	1	3		17	24
Scaly sand darter	*Ammocrypta vivax*				1			1
Bluntnose darter	*Etheostoma chlorosoma*		46	5				51
Swamp darter	*Etheostoma fusiforme*		8					8
Slough darter	*Etheostoma gracile*	14	144	28	2			188
Harlequin darter	*Etheostoma histrio*				34			34
Johnny darter	*Etheostoma nigrum*			12	1			13
Goldstripe darter	*Etheostoma parvipinne*	1	18					19
Cypress darter	*Etheostoma proeliare*			1				1
Plains darter	*Etheostoma pulchellum*	53	22		65	1		141
Orangebelly darter	*Etheostoma radiosum*	260	197	109	59	17	25	667
Logperch	*Percina caprodes*		1	3	3	1	5	13
Channel darter	*Percina copelandi*	1	4	4	1		8	18
Leopard darter	*Percina pantherina*						1	1
Slenderhead darter	*Percina phoxocephala*	31	27		5			63
Dusky darter	*Percina sciera*	70	29	3	18	1	3	124
Freshwater drum	*Aplodinotus grunniens*	2		1				3

For all fishes captured by seining, we averaged 211 individuals per sample, and number of species ranged from 1 to 28, with an average of 11.5 species per collection (Figure 2).

We found a total of 83 distinct species (by major drainage): Muddy (including Clear) Boggy, 64; Kiamichi, 51; and Little, 59 species. Thirteen species were found in all major and minor drainages. Conversely, some species were found in only one of the minor drainages, including (number of species in parentheses), from west to east: Clear Boggy (5), Muddy Boggy (5), Kiamichi (2), Little

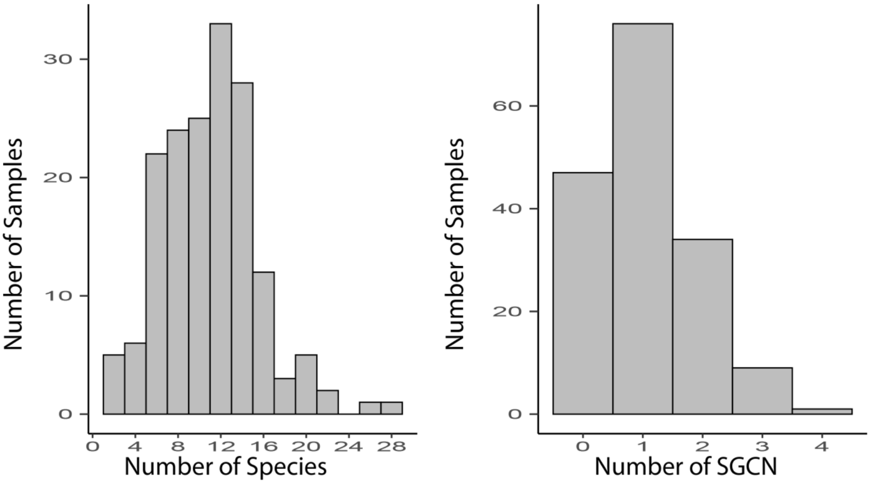

Figure 2: These histograms illustrate the frequency distribution of species richness (left) and total number of species of greatest conservation need collected at a site.

proper (6), Glover (none), and Mountain Fork (3). Localization, discussed more fully below for individual species, was increased by several western species not being found east of the Boggy drainages, and by some Coastal Plain species being found only in swampy habitats in the far southeast corner of the state in the lower parts of Little River drainage.

The site where we found the greatest number of species (N = 28) was at the "Cow Crossing" site east of Idabel on the Little River mainstem within the Little River Wildlife Refuge (McCurtain County). This was a complex site about 25 m wide with a mix of swift riffles and runs, channels, and backwaters, and substrates of sand, gravel, and cobble, offering a wide range of fish microhabitat. The site yielding only one species was a very small tributary of Cloudy Creek (Pushmataha County) where seining was difficult, and we caught only one golden shiner (*Notemigonus crysoleucas*). [We also saw several western mosquitofish (*Gambusia affinis*) and a green sunfish (*Lepomis cyanellus*), but they were not captured]. Several other sites on small tributaries yielded only 3 to 5 species each. But many of the local fish communities ranged from 8 to 16 species (Figure 2), with a few sites having more than 20 species. Sites with 15 or more species exceeded the 75th percentile of species per collection, and sites with 19 or more exceeded the 90th percentile. The values of 15 and 19 species per collection were used (below) to consider sites appropriate for protection because of high species richness.

Similarities among drainages

Qualitative (species presence) similarities between contiguous major drainages (Muddy Boggy, Kiamichi, Little) were calculated by Jaccard's Index, which ignores negative matches, and ranges from zero (no shared species) to one (if all species are shared). There were moderate, and nearly identical, similarities in species identities between contiguous drainages, with Jaccard's similarity of 0.6197 between Boggy and Kiamichi drainages, and 0.6176 between Kiamichi and Little drainages. Across the Boggy and Kiamichi drainages, combined, we found a total of 71 species, of which 44 were common to both drainages and 27 species were found in one of the drainages but not both. Comparing the Kiamichi to Little drainages, we found a combined total of 68 species, of which 42 were common to both drainages and 26 that were found in one drainage but not both. Thus, regarding species identity each major drainage was roughly 60% similar to the next, and, conversely, about 40% different. This level of similarity between adjacent drainages closely approximates similarities between adjacent river drainages in southwest Arkansas (Matthews and Robison 1998, their Table 1). All evidence within the region at large suggests that management plans for native fishes may need to be tailored to individual drainages, instead of expecting one broader plan to fit all.

Quantitative similarities between contiguous major drainages (Muddy Boggy, Kiamichi, Little) were calculated as Percent Similarity Index (PSI), based on the lesser proportion each species made up across any two drainages being compared. The PSI value can range from zero (no species in common) to 1.00 (perfect match in abundances of all species). In a comparison of the major drainages, from west to east, the Muddy Boggy and Kiamichi had a PSI = 0.50, and Kiamichi and Little had PSI = 0.47. Thus, there is not only substantial turnover in species presence (as above) but also in species abundances from one major drainage to the next in southeast Oklahoma, further suggesting that approaches to conservation and management of native fishes may need to be planned on a local basis.

Individual species abundances

Thirteen species each made up more than 1% of all individuals sampled (Table 2) across all drainages. Bigeye shiner (*Notropis boops*) was the most abundant, comprising 18% of all individuals, and abundant in all three major drainages (Table 1). The redfin shiner (*Lythrurus umbratilis*) was second in abundance, with 11% of all captures and common in all drainages. Other species comprising more than 5% of all individuals were: western mosquitofish, rocky shiner (*Notropis suttkusi*), red shiner (*Cyprinella lutrensis*), and longear sunfish (*Lepomis megalotis*) (Table 2).

Collectively, these species made up approximately 58% of all individuals in our samples. Conversely, we found 10 or fewer individuals for 22 species (Table 3). Of these, seven were “big water” species like blue catfish (*Ictalurus furcatus*), flathead catfish (*Pylodictis olivaris*), blue sucker (*Cycleptus elongatus*), or smallmouth buffalo (*Ictiobus bubalus*) that were under-represented by seining in wadeable streams. The other 15 were species amenable to collection by seining, and probably are truly scarce in the region [lamprey (*Ichthyomyzon* sp.), ironcolor shiner (*Notropis chalybaeus*)] or are restricted to specialized habitats [e.g., banded pygmy sunfish (*Elassoma zonatum*) flier (*Centrarchus micropterus*) or western starhead

Table 2: The most abundant species in southeastern Oklahoma ranked by total individuals collected and their percent contribution to the total of all individuals.

Common Name	Scientific Name	Total	Percent
Bigeye shiner	*Notropis boops*	6378	18.10%
Redfin shiner	*Lythrurus umbratilis*	3928	11.15%
Western mosquitofish	*Gambusia affinis*	2827	8.02%
Rocky shiner	*Notropis suttkusi*	2724	7.73%
Red shiner	*Cyprinella lutrensis*	2539	7.21%
Longear sunfish	*Lepomis megalotis*	1906	5.41%
Blacktail shiner	*Cyprinella venusta*	1476	4.19%
Steelcolor shiner	*Cyprinella whipplei*	1367	3.88%
Bluegill	*Lepomis macrochirus*	1361	3.86%
Highland stoneroller	*Campostoma spadiceum*	1234	3.50%
Kiamichi shiner	*Lythrurus snelsoni*	1226	3.48%
Brook silverside	*Labidesthes sicculus*	971	2.76%
Orangebelly darter	*Etheostoma radiosum*	667	1.89%
Central stoneroller	*Campostoma anomalum*	596	1.69%
Largemouth bass	*Micropterus salmoides*	489	1.39%
Golden shiner	*Notemigonus crysoleucas*	383	1.09%
Fathead minnow	*Pimephales promelas*	379	1.08%
Green sunfish	*Lepomis cyanellus*	363	1.03%

Table 3: Scarce species in our samples: asterisk indicates a "big water" species with lower probability of capture by seining.

Common name	Scientific name	Number	Percent
Banded Pygmy sunfish	*Elassoma zonatum*	10	0.03%
Threadfin shad	*Dorosoma petenense*	8	0.02%
Smallmouth buffalo	*Ictiobus bubalus**	8	0.02%
Western starhead topminnow	*Fundulus blairae*	8	0.02%
Swamp darter	*Etheostoma fusiforme*	8	0.02%
Slender madtom	*Noturus exilis*	7	0.02%
Tadpole madtom	*Noturus gyrinus*	6	0.02%
Flier	*Centrarchus macropterus*	6	0.02%
Flathead catfish	*Pylodictis olivaris**	4	0.01%
Smallmouth bass	*Micropterus dolomieu*	4	0.01%
River carpsucker	*Carpiodes carpio**	3	0.01%
Freshwater drum	*Aplodinotus grunniens**	3	0.01%
Common carp	*Cyprinus carpio**	2	0.01%
Blue catfish	*Ictalurus furcatus **	2	0.01%
Lamprey species	*Ichthyomyzon sp.*	1	0.00%
Pallid shiner	*Hybopsis amnis*	1	0.00%
Ironcolor shiner	*Notropis chalybaeus*	1	0.00%
Blue sucker	*Cycleptus elongatus**	1	0.00%
Redspotted sunfish	*Lepomis miniatus*	1	0.00%
Scaly sand darter	*Ammocrypta vivax*	1	0.00%
Cypress darter	*Etheostoma proeliare*	1	0.00%
Leopard darter	*Percina pantherina*	1	0.00%

topminnow (*Fundulus blairae*) that are found only in swamp-like habitats in far southeast Oklahoma]. The ten species that occurred most frequently (in 77 to 133 samples, Table 4) were from several families, including three minnows (Leuciscidae), one livebearer (Poeciliidae), one silverside (Atherinopsidae), four sunfish (Centrarchidae), and one darter (Percidae). Most widely occurring, overall, were longear sunfish, western mosquitofish, and bluegill (*Lepomis macrochirus*), occurring in 79.6%, 69.5%, and 66.5% of all samples, respectively. No other species occurred in more than 47 (28.1%) of our samples.

Species of greatest conservation need (SGCN)

We found 11 of the 19 SGCN that historically were known from the region (Table 5). Several in Tier I [blackspot shiner (*Notropis atrocaudalis*), Kiamichi shiner (*Notropis ortenburgeri*), Ouachita Mountain shiner (*Lythrurus snelsoni*), rocky shiner] and one in Tier II, the orangebelly darter (*Etheostoma radiosum*) were abundant at numerous sites in their historical ranges and are likely secure barring any widespread land use or water quality changes. We failed to find eight of the regional Tier I or II SGCN, some of which, like Alabama shad (*Alosa alabamae*) or alligator gar (*Atractosteus spatula*), likely were due to lack of sampling in their habitats. Failure to detect others, like bluehead shiner (*Pternotropis hubbsi*), crystal darter (*Crystallaria asprella*), peppered shiner (*Notropis perpallidus*), Creole darter (*Etheostoma collettei*), and mountain madtom (*Noturus eleutherus*), could have been lack of sampling in their preferred microhabitats, or because they actually are rare in southeast Oklahoma.

Tier I SGCN

Pallid shiner (*Hybopsis amnis*) was found only once, in extreme headwaters of the Muddy Boggy River drainage. One individual was taken in Caney Boggy Creek, Hughes County, at a stream site 4 m wide, including a water willow covered riffle feeding into a muddy channel. This species is rare in Oklahoma, and Matthews found it in the region only in 1990, as a single individual in each of two collections on the lower Little River within the Little River Wildlife Refuge (McCurtain County).

Ouachita Mountain shiner (*Lythrurus*

Table 4: The ten most frequently encountered species, and numbers of samples in which detected.

Common name	Scientific name	Occurrences
Longear sunfish	*Lepomis megalotis*	133
Western mosquitofish	*Gambusia affinis*	116
Bluegill	*Lepomis macrochirus*	111
Largemouth bass	*Micropterus salmoides*	94
Orangebelly darter	*Etheostoma radiosum*	91
Redfin shiner	*Lythrurus umbratilis*	89
Highland stoneroller	*Campostoma spadiceum*	85
Green sunfish	*Lepomis cyanellus*	80
Brook silverside	*Labidesthes sicculus*	79
Bigeye shiner	*Notropis boops*	77

snelsoni) was widespread in the upland parts of the Little, Glover, and Mountain Fork, occurring at 17 sites. There were more than 100 individuals in several collections, including 390 in Little Eagle Creek in the upper Mountain Fork drainage in LeFlore County. Being limited to and widespread in the upper Little River system is consistent with its historical distribution above the Fall Line (Taylor and Lienesch 1995, 1996). The species is probably secure, barring widespread changes in land use practices, or reservoir construction (Taylor and Lienesch 1995).

Blackspot shiner (*Notropis atrocaudalis*) was in all three major drainages, in the middle to lower Clear and Muddy Boggy drainage, in tributary creeks in the lower Kiamichi drainage and one creek at midstream near Clayton, in two tributaries to Little River downstream from Pine Creek Lake, and at two sites on the lower Little River mainstem. Blackspot shiner was mostly in small streams, and typically scarce when found (e.g., 1-5 individuals). However, we took 120 and 38 blackspot shiners, respectively, in Crooked and Lick creeks in Choctaw County, and 39 in Little River (McCurtain County) at the mouth of Yashau Creek within the Little River Wildlife Refuge.

Kiamichi shiner (*Notropis ortenburgeri*) was collected at seven sites, with a spotty distribution in the eastern part of our study region. It was at three sites in the upper Little River drainage and in Rock Creek near the Arkansas border, at two sites in the far upstream Kiamachi drainage, and one eastern tributary to the lower Kiamichi. The Kiamichi shiner was mostly in creeks 7-10 m wide, but also in streams up to 25 m wide with rocky riffles. The species can be locally abundant, and in this survey, we collected 25 at the Little River headwaters, 34 in Bohanan Creek and 157 in Little Pigeon Creek, in the upper Kiamichi drainage, all in Leflore County. Porter and Patton (2015) found a total of 126 Kiamichi shiners at seven sites on the mainstem Kiamichi River in 2012-2013.

Rocky shiner (*Notropis suttkusi*) was found at 11, 6, and 6 sites, respectively, in the Muddy Boggy, Kiamichi, and Little drainages. It was most common in river mainstems or large tributaries, not typically occurring in small creeks. It was locally abundant at several sites, with hundreds of individuals per collection. Our largest collection of 553 individuals was on the Kiamichi River mainstem between the towns of Antlers and Clayton. There, the river had a braided channel with riffles, rapids and runs, over large gravel and cobble. Channels were up to 20 m wide, with pool depths to 2 m. Most individuals, especially males in breeding colors of bright orange, were taken by seining in swift runs in knee deep water over bottoms of large gravel. Although the species is somewhat localized in abundance, it appears secure throughout the study region, and is also abundant in upper Blue River, to the west (Matthews and Marsh-Matthews collections in 2018). This species was called *Notropis rubellus* before it was elevated to species by Humphries and Cashner (1994), who designated the holotype and paratypes from a collection by Matthews *et al.* (1990) in the lower Little River east of Idabel.

Table 5. For SGCN in the study region: total individuals found, total number of sites where they occurred, and river drainages where they occurred.

Tier I Species

Common Name	Scientific Name	Total Found	Total Sites	River Drainage
Alabama shad	*Alosa alabamae*	0	0	n/a
Pallid shiner	*Hybopsis amnis*	1	1	Muddy Boggy
Ouachita Mountain shiner	*Lythrurus snelsoni*	1226	17	Little River
Blackspot shiner	*Notropis atrocaudalis*	272	20	All three drainages
Kiamichi shiner	*Notropis ortenburgeri*	241	7	Kiamichi & Little
Peppered shiner	*Notropis perpallidus*	0	0	n/a
Rocky shiner	*Notropis suttkusi*	2253	25	All three drainages
Bluehead shiner	*Pteronotropis hubbsi*	0	0	n/a
Western sand darter	*Ammocrypta clara*	0	0	n/a
Crystal darter	*Crystallaria asprella*	0	0	n/a
Leopard darter	*Percina pantherina*	1	1	Little River

Tier II Species

Common Name	Scientific Name	Total Found	Total Sites	River Drainage
Southern brook lamprey*	*Ichthyomyzon gagei**	1	1	Kiamichi
Alligator gar	*Atractosteus spatula*	0	0	n/a
Ironcolor shiner	*Notropis chalybaeus*	1	1	Little
Blue sucker	*Cycleptus elongatus*	1	1	Muddy Boggy
Mountain madtom	*Noturus eleutherus*	0	0	n/a
Creole darter	*Etheostoma collettei*	0	0	n/a
Goldstripe darter	*Etheostoma parvipinne*	19	2	Muddy Boggy
Orangebelly darter	*Etheostoma radiosum*	552	80	All three drainages

*Larval ammocoete, could not identify to species. It also could be *Ichthyomyzon castaneus*.

One leopard darter (*Percina pantherina*) was netted and released unharmed in Big Eagle Creek northwest of Smithville, McCurtain County. Because of its protected status as a Federally Threatened Species, we avoided its optimal habitat where it was known to occur (larger clear streams with flowing water over large boulders) and made no targeted search for this species. The US Fish and Wildlife Service, Tulsa, surveys leopard darters annually throughout its range. Working with USFWS, we found it common in the midreach of the Glover River in 1999-2000 (Schaefer *et al*. 2003). Matthews' only other collection of the species was in West Fork Glover River at Battiest, in 1982.

In addition, one ammocete (larval lamprey) was collected in the Kiamichi drainage, over a mud-sand bottom in Tuttle Branch of Bull Creek, in Choctaw County. This specimen was too small to identify to species and could be either the SGCN southern brook lamprey (*Ichthyomyzon gagei*) [Tier II] or chestnut lamprey (*I. castaneus*). Porter and Patten (2015) found one *I. castaneus* in the Kiamichi mainstem, but reported no *I. gagei*. Pigg (1974) found one specimens of *I. gagei*, but no *I. castaneus*. The ammocoete collected in our study could be either species.

Tier II SGCN

Ironcolor shiner (*Notropis chalybaeus*) was found only once, a single individual in Wildhorse Creek of the upper Little River, just west of Fewell, in Pushmataha County. The stream at this site was about 25 m wide, with boulders and bedrock, and fast flow in rapids and riffles. The species has been considered quite rare in Oklahoma (Williams and Echelle 1998).

Blue sucker (*Cycleptus elongatus*) was found at only one site, as a single individual netted in the lower mainstem of Clear Boggy Creek. At this site, maximum width was 7 m and maximum depth of 1.8 m, with a mix of pools and riffles. Finding only one blue sucker in our survey reflects the difficulty of capturing this big-water species using a seine, and not that it is rare. Using boat-mounted electrofishing, Dyer and Brewer (2020) found the species abundant in lower river reaches in southeast Oklahoma.

Goldstripe darter (*Etheostoma parvipinne*) was found only at two sites in the Muddy Boggy drainage. A single individual was taken in Davis Creek (Atoka County) of the Clear Boggy system, and 18 were found in Tanyard Creek (Choctaw County), a small tributary to the Muddy Boggy, dominated by riffles over a mostly sand bed. WJM had previously taken goldstripe darter only in Parker Creek, a small, sand-bed stream in southeastern McCurtain County, in 1993, within the Tiak District of the Ouachita National Forest.

Orangebelly darter (*Etheostoma radiosum*) was widespread throughout the study area, occurring at 36, 18, and 26 sites, respectively, in the Muddy Boggy, Kiamichi, and Little River drainages. It was essentially in streams of all sizes, but especially in medium to large upland streams wherever rocky riffle or rapids habitat was present, and often was the most abundant darter. It was not usually found in small headwater creeks. In numerous collections we found 20 to 40 individuals and took 56 in Clear Boggy Creek northeast of Boswell, Choctaw County. In this survey, and historically, this has been one of the most widespread and abundant darter species in the region. Note that designation of orangebelly darter as an SGCN included populations in Blue River, which have recently been elevated to full species status as the Blue River orangebelly darter (*Etheostoma cyanorum*) by Matthews and Turner (2019). This unique species, found only in the Blue River system, will likely deserve consideration for separate SGCN status in the future, or for other forms of legal protection. However, because of the widespread and abundant distribution of orangebelly darter (not including Blue River *E. cyanorum*) we suspect that it no longer needs to be a SGCN.

SGCN not found

We failed to find several small-bodied SGCN captured previously in southeast Oklahoma including bluehead shiner (Tier I), mountain madtom (Tier II), crystal darter (Tier I), peppered shiner (Tier I), and Creole darter (Tier II). Previous occurrences noted below are from collections by W. Matthews (WJM).

Bluehead shiner (*Pternotropis hubbsi*) – One adult male in breeding color was collected in a slough tributary to Crooked Creek in the Little River Wildlife Refuge in July 1990, the only capture of the species in WJM records, despite his dozens of samples in swampy, low-gradient habitats in the area in 1990 or 1993. Lemmons *et al*. (1997) found 16 bluehead shiners in borrow pits along Hwy 70 (Little River drainage), north of Idabel in 1996.

Mountain madtom (*Noturus eleutherus*) – Also collected only once in southeast Oklahoma by WJM, a single specimen was found in Little River near the mouth of Yashau Creek (McCurtain County) in 1990. We found none in the present survey, although many of our samples were in its appropriate habitat (i.e., flowing riffle-pool habitats in clear rocky streams). This indicates rarity in the state. K. Gido and G. Hopper (pers. comm.) found one individual in Little Yashau Creek (Little River drainage) in August 2015.

Crystal darter (*Crystallaria asprella*) – Found several times 1976 to 1990 (WJM) in waist-deep water flowing over a gravel bed in the main channel of the Little River mainstem west of US Hwy 70, McCurtain County, but in this survey and a collection at that site in 2018 they were not found. K. Gido and G. Hopper (pers. comm.) found one individual in August 2015 in Little Yashau Creek.

Peppered shiner (*Notropis perpallidus*) – Found in small numbers by WJM from 1976 to 1986 at several sites in the Glover, Little, and Kiamichi River mainstems, including 35 specimens in one seine haul below a fast rapid in the Glover River south of Hwy 3 (McCurtain County) in July 1982, but not seen by us since 1986. Wagner *et al*. (1987) found 172 individuals in 19 collections in the Kiamichi and Little River drainages in surveys in 1982. Robison (2006) searched 81 sites in Arkansas and Oklahoma for peppered shiners, including sites in the Kiamichi, Glover, and Mountain Fork Rivers, finding 17 individuals in Arkansas but none in Oklahoma. K. Gido and G. Hopper found none in their 2015 collections (pers. comm.). An exhaustive search was made for peppered shiners in 2018-2019 throughout their historical range in Oklahoma by Allen (2020) and his students, making 163 seining collections but finding no confirmed specimens (one juvenile in Little River downstream from Pine Creek reservoir was identified as "possible" peppered shiner but was too small to positively identify). The last known collections of peppered shiner in museum records at OU or OSU includes a single individual each in Kiamichi River near Clayton and in Glover River near Golden, in 1990 and 1991. The species must be extremely rare in Oklahoma if it still exists in the state.

Creole darter (*Etheostoma collettei*) – The species was initially determined to exist in Oklahoma by examination of old museum specimens (Matthews and Robison 1982). WJM took a single Creole darter at each of two sites on the lower Little River mainstem in October 1990, at "Cow Crossing" east of Idabel, and west of US Hwy 70, both within the Little River Wildlife Refuge. We took none in our recent survey, but Dr. Nick Lang (pers. comm.) found 2 or 3 individuals in Yashau Creek northwest of Broken Bow in 2018.

Other noteworthy local species distributions

Numerous other species were very localized or found in only one of the minor drainages (Table 1). Among minnows (Leuciscidae), the central stoneroller (*Campostoma anomalum*) was abundant in the Clear Boggy drainage but replaced to the east by highland stoneroller (*Campostoma spadiceum*). Southern redbelly dace (*Chrosomus erythrogaster*), characteristic of small, spring-fed streams, was found only once, in a clear, spring-fed creek in the Clear Boggy drainage. Red shiner was abundant in the Clear and Muddy Boggy drainage, but we took none in drainages to the east. Striped shiner (*Luxilus chrysocephalus*) was abundant in our survey but found only in the Little River and its minor drainages. Sand shiner (*Notropis stramineus*), and fathead minnow (*Pimephales promelas*) are two of the most widespread minnows in Oklahoma, but we found them only in the Clear Boggy drainage. The creek chub (*Semotilus atromaculatus*) was found only in the Glover River and Mountain Fork tributaries in the Little River drainage.

Among other families, we only found the slender madtom (*Noturus exilis*; Family Ictaluridae) in the Mountain Fork drainage. This species is more typical of Ozark streams in northeast Oklahoma and occurs in small numbers in upper Mountain Fork riffles or rapids. Western starhead topminnow (*Fundulus blairae*; Family Fundulidae) is endemic to and was found only in lower reaches of the Little River system. Banded pygmy sunfish (*Elassoma zonatum*; Family Elassomatidae) were in the lower Little River system, with one individual found in a small eastern tributary of the Kiamichi drainage. Banded pygmy sunfish were abundant in a marsh just north of Fort Towson in the 1970s and 1980s (WJM collections), but that marsh has since been highly modified or drained and our recent efforts to find the species there were unsuccessful. Echelle and Echelle (2005) reported taking the species in "the city spring at Fort Towson" in 1994. Two species in the family Centrarchidae, flier (*Centrarchus macropterus*) and redspotted sunfish (*Lepomis miniatus*), were found only in sluggish, coastal plain habitats of the Little River drainage in far southeastern Oklahoma. Several darters (Family Percidae) were limited to a single major or minor drainage. Scaly sand darter (*Ammocrypta vivax*) and harlequin darter (*Etheostoma histrio*) were found only in the Little River drainage. Cypress darter (*Etheostoma proeliare*) was found only in one eastern tributary of the Kiamichi drainage, and we only found swamp darter (*Etheostoma fusiforme*) in the Muddy Boggy drainage. This appears to be a westward range extension for the swamp darter, per Pigg (1977) and Miller and Robison (2004).

Species richness, SGCN and protected areas

An important question for native fish conservation is whether localities with high species richness or SGCN are in areas that offer some protection from change or degradation, by virtue of being owned by state or federal governments or non-governmental conservation organizations. Figure 1 shows locations with high species richness and/or three or more SGCN, versus protected areas in southeast Oklahoma. Sites are shown where we found 15-18 species (15 (> 75th percentile of species richness) and 19 or more (>90th percentile of species richness) species, which could be considered "priority" or "high priority" sites for conservation of native fishes. Sites are also shown that had 3 or 4 designated SGCN. The maps provide outlines (dark gray) of areas where streams and stream fishes could be protected by virtue of ownership or agreements that provide federal (e.g., national forest) or state control of the lands (e.g., wildlife management areas - WMA). The maps show protected areas compiled nationally (United States Geological Survey 2020). For each specific area, detailed maps or regulations by the controlling agency should be consulted.

In the Muddy Boggy drainage, there were 11 sites with 15-18 species, and 6 sites with 19 or more species (Figure 1), with priority sites occurring from lower mainstems to headwaters. The Muddy Boggy branch of the drainage had more of the highest priority sites with 19 or more species than the Clear Boggy, and more of the priority sites with 15 to 18 species. However, some sites on the Clear Boggy branch also were of high quality and had complex native fish communities. One site on the Muddy Boggy mainstem and one on the Clear Boggy mainstem had three SGCN, the latter being the only site where we seined a blue sucker. In the Muddy Boggy drainage (Figure 1), the protected areas are east of Atoka Reservoir to upper McGee Creek, including the Atoka WMA, Stringtown WMA, and McGee Creek WMA. In the drainage there were 17 sites with 15 or more species, scattered widely throughout the basin, but none specifically within the protected areas. There were two sites in the drainage where we found three or more SGCN, but not within the protected areas.

The upper reaches of the Kiamichi River drainage (Figure 1) have substantial protection with headwaters of the river and some tributary creeks in the Ouachita National Forest or the Leflore Unit of the Ouachita WMA. Downstream, the Pushmataha WMA provides some protection for southern tributaries of the Kiamichi River, and the small Hugo WMA can provide some protection for the river downstream from Hugo Reservoir. However, much of the lower and middle part of the Kiamichi River drainage is in private ownership, so protection of streams and native fishes would depend on landowner cooperation. In the Kiamichi River basin, only five of our samples had 15 or more species, but the most speciose sites, near Clayton, Oklahoma, were near but not within the Pushmataha WMA. One of those sites also had three SGCN.

In the Little River drainage, including Glover River and Mountain Fork, a combination of WMAs, the Three Rivers Area, wilderness areas, and the USFWS Little River Wildlife Refuge collectively protect large areas in the upper Little River and near Pine Creek Reservoir, the upper Glover River, much of the Mountain Fork River above and below Broken Bow Lake, and much of the lower Little River downstream from Idabel to the Arkansas state line (Figure 1). The Little River basin has numerous downstream sites, particularly in the lower Little River where we found 15 or more native species. Several of these sites are protected within the Little River Wildlife Refuge, and others are in or near the Three Rivers WMA. The Little River Wildlife Refuge provides protection for two sites where we detected three SGCN, but three other sites upstream in Little River where we detected three SGCN, are not within government protected areas. In general, much of the midreach of the three named rivers, and the lower Little River mainstem in Oklahoma has protection by government ownership or agreements. There is little or no protection of lands by government ownership or agreements in the headwaters of any of the Little River drainages, and 8 of the 11 sites with high native fish species richness or numbers of SGCN (Figure 1) are in areas with private ownership. Landowner cooperation is essential for protection of diverse native fishes throughout much of the Little River drainage.

Conclusions and recommendations

Documenting contemporary abundances and distributions of fishes is foundational for identifying appropriate conservation and management efforts. Our survey in 2014-2016 in the Muddy Boggy, Kiamichi, and Little River drainages of southeast Oklahoma provides recent information for SGCN, other native species, and the composition of local communities or the fish faunas of these river basins. This paper (and data provided in the Supplementary Material) provides a baseline for future assessment of fishes in these three major drainages.

Native fish communities throughout the region generally remained similar to historical information from the 1920s through the 1970s, with numbers of species per site in this survey typical of the diversity we found in our samples in the region from the 1970s to now (Matthews, unpublished data) or in other streams in Oklahoma (Matthews *et al*. 2013, Matthews and Marsh-Matthews 2015, 2017). The average of 11.1 species per site in this survey is extremely similar to that for many streams throughout North America. Matthews (1998, pp 39-40) found a modal number of 11 to 12 species per sample for 815 different whole-community samples streams throughout North America, and (Matthews 1998, pp 33-34) a mean of 12.9 species per collection for 182 collections in the Ouachita uplift in Oklahoma and Arkansas. Thus, streams in the three drainages we surveyed had species richness matching expectations for streams in this part of the United States. None of the sites we visited exhibited visible evidence of pollution, harmful exotic species, or any other obvious factors that would have impacted species richness. The low numbers of species at a few sites were probably natural, as can be expected at smaller stream sizes (Matthews 1998; Matthews and Marsh-Matthews 2017).

From sampling streams and observing environmental conditions across southeast Oklahoma, we offer suggestions for current actions and for future studies. First, surveys of fishes in streams of this region should be continued on a regular basis, more frequently than in the past, and should incorporate standardized sampling and metadata acquisition to allow accurate comparative temporal analysis. The ongoing efforts of the ODWC "Stream Team" to sample fishes in all regions of the state (T. Rodger, personal communication) are an excellent way to meet this need. In southeast Oklahoma, future surveys should include streams of all sizes because both headwaters and lower mainstems have fish communities that would benefit from protection. Additionally, local fish communities in these drainages can differ sharply in species composition from place to place (Zbinden and Matthews 2017), so broad coverage within drainages is essential. Our surveys in 2014-2015 were the first comprehensive fish surveys in the region since the 1960s or 1970s. More frequent assessment of status of SGCN and of entire native fish communities at least once per decade would be advisable, and more frequently at targeted locations with known populations of SGCN or high diversity of native species. The survey we report here provides one comprehensive snapshot in time, as a baseline against which managers can assess future changes in these communities, or in abundance and distribution of individual species. Long-term, repeated sampling will allow identification of trends in local and regional fish species abundances or community dynamics (Zbinden 2020, 2021). Long-term data are necessary to assess changes to community structure which may vary "loosely" around an equilibrium state (i.e., normal variance) or may undergo directional change (Matthews and Marsh-Matthews 2016, 2017).

We suggest that priority sites with 15 or more native species, high priority sites with 19 or more native species, or sites with multiple SGCN, should be identified for ODWC regional biologists and game wardens for special protection, and landowners or river regulation authorities should be so advised. For such sites on private property special efforts should be made to work cooperatively with landowners to assist in protection of streams and the surrounding land. Although there are stream reaches in all three drainages that can protect fish communities by virtue of state or federal ownership or cooperative agreements, managers should seek opportunities to expand areas protecting streams and complex fish communities in the region. Further efforts should be made to secure protection for stream reaches with multiple SGCN in headwaters of the Little River drainage.

We also have suggestions about the SGCN status of several species. The orangebelly darter (*Etheostoma radiosum*) (SGCN Tier II) was extremely widespread in the Muddy-Clear Boggy, Kiamichi, and Little River drainages, occurring in 80 of our sampling sites, often in large numbers in rocky riffle habitats. It is sufficiently secure in the region of study that might be considered for removal from SGCN status. However, the form of orangebelly darter in Blue River, now considered a distinct species (*Etheostoma cyanorum*) separate from *E. radiosum*, should continue to be (or be added to) the list of SGCN. The rocky shiner (*Notropis suttkusi*) occurred in 25 of our sampling sites across all three major drainages and was extremely abundant in some locations. Based on its wide distribution and abundance it is probably secure in Oklahoma and might be considered for removal from the SGCN list or in being lowered from Tier I to Tier II. The Ouachita Mountain shiner (*Lythrurus snelsoni*) (SGCN Tier I) is limited in Oklahoma to the upper portions of the Little, Glover, and Mountain Fork drainages, but where it occurred it often was in large numbers. Because of its limited range in Oklahoma, it should be retained on the SGCN list to encourage continued monitoring, but perhaps lowered from Tier I to Tier II priority. We envision no outright threats to this species so long as an abundance of high-quality water continues to flow in upland streams throughout the Little River drainage.

We recommend all other Tier I and Tier II fish species in southeastern Oklahoma remain as SGCN because their distributions or abundances remain poorly known or their existence in the state may be tenuous. Effort should be directed toward sampling more locations for crystal darter (*Crystallaria asprella*), because we occasionally found these in the Little River west of US Hwy 70 (Matthews, unpublished data) but they were not detected at their primary historic site, or elsewhere, in this survey. And in a subsequent effort at their historic site in 2018 (Matthews unpublished data) we also failed to find any crystal darters. Continued efforts should be made to determine if peppered shiner (*Notropis perpallidus*) still exists in Oklahoma. None were found in our survey or in a widespread survey in 2018-2019 by Dan Allen and students targeted specifically for peppered shiners (Allen 2020).

Other than as noted for some individual species, fish communities in southeastern Oklahoma streams seemed to be in good condition, relative to expectations from historical surveys or our own collections in the region over more than 40 years. This is consistent with the finding by Matthews and Marsh-Matthews (2015) that contemporary fish communities in much of Oklahoma were relatively similar to those found by Ortenburger almost a century ago. Emphasis should be placed on conservation actions within these river basins that assure continued availability of sufficient flow of high-quality water for fish or stream macroinvertebrates. Reduction of water volumes or quality in river mainstems or in their tributaries should be vigorously avoided. Any proposed removal of water by transfers out of basin, or by within-basin withdrawals, should be reviewed critically to assure that habitat needs of all the diverse native species in these streams are met. Dam operations that limit availability of downstream waters, especially in summer or during droughts, should be modified to assure adequate flow to maintain high quality habitats for all fish species. Likewise, timber harvest operations should be carried out with minimal disturbance of water quality, particularly as related to road construction, bridging of streams, or any activity that increases input of silt to these streams. The cooperation of agencies and environmental or political stakeholders in southeast Oklahoma to assure stream protection will have a tremendous impact on the future of the diverse native fishes in the region.

Acknowledgments

Financial support was provided by the Oklahoma Department of Wildlife Conservation through State Wildlife Grant FI 3AF01213 (T-74-1) and by the University of Oklahoma. We thank Barry Bolton, Chief of Fisheries, ODWC for providing collecting permits, Richard Standage and Elaine Sharp of the USDA Forest Service for approval to collect on the Ouachita National Forest, and David Weaver, Refuge Manager, for approval to collect on the Little River National Wildlife Refuge. We thank J. Cureton, D. Curtis, R. Lehrter, T. Shadid, and B. Tweedy for assistance in the field. We thank S. Cartwright and the Sam Noble Oklahoma Museum of Natural History for archiving fish collections and providing museum data, and D. Lynch and J. Agan for data from the Oklahoma State University collection of fishes. We thank S. Brewer, A. Echelle, D. Fenner, K. Gido, J. Schaefer, and J. Tucker for information on fish distributions in the study area. K. Carter, L. Cooley, and G. Martin of the OU Biology Department provided critical logistical support. All collections were made under approval by the IACUC of the University of Oklahoma, Protocol Number R14-002.

Supplementary material

Supplementary table S1 and S2 are available online at https://ojs.library.okstate.edu/osu/index.php/OAS)

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Submitted April 13, 2021 Accepted July 14, 2021

Updated Checklist of Amphibians and Reptiles at the University of Oklahoma Biological Station at Lake Texoma, Oklahoma

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Abstract: The University of Oklahoma Biological Station (UOBS) was established in 1950 in Marshall Co., Oklahoma along the newly created reservoir, Lake Texoma. Generations of biology students and independent researchers have documented the flora and fauna on the station grounds and surrounding areas. Herein we compare herpetological records for the area published in the 1950s to herpetology course survey events from 1978–1986 and 2008–2019. Overall, species richness has declined precipitously in the last 65+ years, likely due to several local anthropogenic factors such as habitat modification, environmental pollutants, and the spread of amphibian infectious diseases. We also highlight two species that are additions to the UOBS herpetofauna since its inception; one an introduced species and one a range expansion.

Introduction

The University of Oklahoma (OU) established a biological station on the shore of the newly created Lake Texoma (formed from the dammed Red and Washita Rivers) in the vicinity of Willis, OK (Marshall Co.) in 1950 (Riggs, 1955). The original purpose of the University of Oklahoma Biological Station (UOBS) was to provide infrastructure and protect several habitat types to be utilized by both biological researchers and OU students (Riggs, 1955). UOBS has continued that objective until the present day, with hundreds of research papers published from data collected on UOBS grounds and thousands of students taking courses on-site (UOBS, 2015). The station is currently comprised of 162 ha with a centralized dorm and dining hall building, additional student dorms and apartments, laboratory classrooms, a library, and several research laboratories and affiliated research spaces (greenhouse, mesocosm tanks, etc.), with the primary buildings surrounded by manicured lawns ~12 ha. The station also includes a small grassland, extensive sandy shoreline habitat, a small intermittent marsh (which changes in depth based on lake levels), and a strip of forested area between the shoreline and the access road (G. Wellborn, personal communication). The forest contains oaks of several species, cottonwood, elm, willow, cedar, pecan, and hackberry (G. Wellborn, personal communication).

Previous to dam construction, this region of Oklahoma was dominated by crop and pasture land, intermixed with natural grasslands and post oak/black hickory forests (Corbett et al., 2002, 2013). Little was known about local herpetofaunal species prior to impoundment, because research at that time prioritized fish and aquatic invertebrates only (White and White, 1977), so there are no historical data for comparison. Today, the primary forest vegetation within 15 m of the shoreline near UOBS is typical for riparian bottom-land forests, with minor invasions by Eastern Red Cedar (*Juniperus virginiana*) and Albizia (*Albezia julibrissin*) (Corbett et al., 2013). The watershed of Lake Texoma encompasses land used primarily for agriculture, ranching, and forest, with few human permanent residents (Eggleton et al., 2004). Due to its nature as a human-constructed reservoir, Lake Texoma water levels fluctuate rapidly within and between years, which has the potential to lead to decreased species richness as compared to a natural lake (Corbett et al., 2013; Roeder et al., 2018). However, these same changes in water levels and tributary flows also have the potential to increase species dispersal in the vicinity (Taylor and Laughlin, 1964). Additionally, unlike natural lakes, reservoirs tend to be highly dynamic in changes within the aquatic community structure, especially as they relate to abiotic (e.g. inshore wave turbulence) and biotic (e.g. dispersal between tributaries and the main reservoir) factors (Lienesch and Mathews, 2000; Matthews et al., 2004; Matthews and Marsh-Matthews, 2007).

The immediate area around Lake Texoma, and even adjacent to UOBS, is accessed heavily for tourism and aquatic recreation, such as boating, fishing, and swimming (An et al., 2002; An and Kampbell, 2003; Gonsoulin et al., 2003; Eggleton et al., 2004). The water in Lake Texoma exhibits higher than average conductivity and turbidity (Eggleton et al., 2004), and areas near marinas show evidence of high heavy metals and other environmental contaminants (An et al., 2002; An and Kampbell, 2003). A 1999–2000 study was conducted of Lake Texoma's littoral zone community responses to anthropogenic stressors, such as pollutants, nutrient enrichment from agricultural or septic run-off, and habitat modification (Eggleton et al., 2004). Researchers found that on a large scale, fish communities in the impacted sites were similar to non-impacted sites. Fish community differences between sites was linked more to habitat heterogeneity than point-source pollutants (Eggleton et al., 2004). In contrast, benthic invertebrate communities exhibited increased species richness at impacted sites, likely associated with degraded environmental conditions and increased eutrophication (Eggleton et al., 2004). A similar study of shoreline communities along the Oklahoma side of Lake Texoma quantified bird and plant community diversity in disturbed sites (i.e. in proximity to marinas, campgrounds, and boat launches) and undisturbed sites. Researchers found that both communities decreased in diversity in disturbed sites, with plants also showing reduced vegetation volume and percent canopy (Francl and Schnell, 2002). Forest communities along the Lake Texoma shoreline are dissimilar to others in Oklahoma, which has also been linked to human disturbance (Corbett et al., 2002).

Many of these anthropogenic factors could also impact local reptile and amphibian communities. The distributions of reptiles and amphibians have been well-studied at UOBS and in other nearby Lake Texoma habitats, with the earliest checklists dating back to the 1950s (Bonn and McCarley, 1953; Carpenter, 1955). Herein, we compare those earliest checklists to unpublished datasets obtained from intensive on-site Field Herpetology courses (1978–1986 and 2008–2019), and discuss potential changes in species composition due to anthropogenic factors in the area.

Methods

Presence/absence data for herpetological species expected to occur onsite at UOBS were collated from Marshall Co. (only) records from Carpenter (1955) and common/widespread Lake Texoma area species from Bonn and McCarley (1953) that one would expect to occur on-site (Table 1). Unfortunately, neither publication detailed the methods by which their species lists were obtained, so that information cannot be provided for direct comparison; however, both publications covered a larger land area than the UOBS grounds alone. Additional unpublished presence/absence data were collected from C. Carpenter's 8-week UOBS Herpetology course in June–July 1978, 1980, 1982, 1984, and 1986 (Table 1). A series of 1–2 drift fence arrays were placed on UOBS grounds and monitored daily by course participants. Drift fences were set up with 100 ft lengths, including six funnel traps (two on each side, at each end, and two in the middle) and eight pitfall traps (1 gal. metal cans). A renewed effort to survey the herpetofauna of UOBS as part of intensive undergraduate biology courses at OU began in 2008, with annual activities in the course Field Herpetology taking place from 2008–2019 (Table 1). Annual class survey efforts for the course lasted for 12 days and occurred in late May–early June (2009, 2011, 2012, 2015), mid-June (2013), mid-July (2018–2019), or late July–early August (2008, 2010, 2014, 2016–2017). Students spend up to eight hours per day sampling UOBS-controlled land for reptiles and amphibians through a combination of visual searches (including flipping rocks and logs), dipnetting, seining, various sizes/styles of aquatic traps, drift fence arrays with pitfalls and/or funnel traps, stand-alone funnel traps, cover boards, and frog call recognition (Willson and Gibbons, 2010; Graeter et al., 2013).

Details regarding the exact combination of sampling methods varied by year and were often determined by the students involved. By completing a wide variety of trapping and visual survey methods, which covered both active and passive sampling, we hoped to ensure that students caught or observed the maximum number of local herpetological species and reduced sampling bias (Crosswhite et al., 1999; Jenkins et al., 2003; Graeter et al., 2013). Results from the recent Field Herpetology courses are presented in two categories, based on differences in teaching/sampling styles: 2008–2015, as taught by C. Painter and G. Carpenter (or in the later years, by G. Carpenter alone) vs. 2016–2019, as taught by C. Siler and J. Watters (Figure 1). In addition to sampling during completed during Field Herpetology, we have also incorporated pooled citizen science data recently collected from within UOBS boundaries and exported from either iNaturalist (2014–present) or taxa lists from the 24-hr Oklahoma BioBlitz! in 2016 (Oklahoma Biological Survey, 2016; iNaturalist, 2020; Figure 1). Taxonomy follows

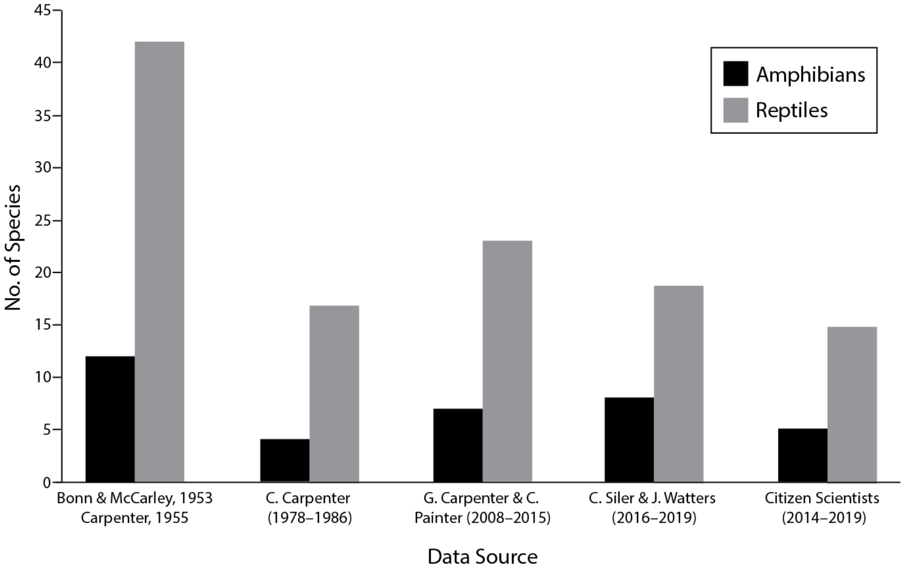

Figure 1. Summary of amphibian and reptile species numbers at the University of Oklahoma Biological Station as documented over time by publications (Bonn & McCarleyy, 1953; Carpenter, 1955), several on-site herpetology courses, and citizen science observations. Categories are representative of data source and in the case of herpetology courses, were divided by instructors and teaching style, in addition to dates.

Table 1. Checklist of University of Oklahoma Biological Station herpetological species comparing historical records from (A) the 1950s: pooled from the publications by Bonn and McCarley (1953) and Carpenter (1955), with unpublished course datasets from the following dates and professors (B) 1978–1986: C. Carpenter, (C) 2008–2015: C. Painter and G. Carpenter, (D) 2016–2019: C. Siler and J. Watters, in addition to (E) citizen science observations from 2014–2019 (Oklahoma Biological Survey, 2016; iNaturalist, 2020). The number in each column represents the total number of years the species was documented, or in the case of column A, the number of publications.

Classification	Common Name	A	B	C	D	E
	AMPHIBIANS					
Anura (frogs)						
Bufonidae						
Anaxyrus americanus	American Toad	1		2	2	
Anaxyrus woodhousi	Woodhouse's Toad	2	3	5	3	1
Hylidae						
Acris blanchardi	Blanchard's Cricket Frog	2		2	4	1
Hyla chrysoscelis/versicolor	Gray Treefrog Complex	2		1	4	1
Hyla cinerea	Green Treefrog			1	4	
Pseudacris clarkii	Spotted Chorus Frog	2				
Pseudacris streckeri	Strecker's Chorus Frog	2	1			
Microhylidae						
Gastrophryne olivacea	Western Narrow-mouthed Toad	1	2	2		2
Ranidae						
Lithobates blairi	Plains Leopard Frog				1	
Lithobates catesbeianus	American Bullfrog	2			2	
Lithobates sphenocephalus	Southern Leopard Frog	2	4	3	4	2
Scaphiopodidae						
Scaphiopus hurterii	Hurter's Spadefoot	1				
Spea bombifrons	Plains Spadefoot	1				
Caudata (salamanders)						
Ambystomatidae						
Ambystoma texanum	Small-mouthed Salamander	1				
	REPTILES					
Serpentes (snakes)						
Colubridae						
Coluber constrictor	North American Racer	2	4	3	2	2
Lampropeltis calligaster	Prairie Kingsnake	1	4	1		
Lampropeltis holbrooki	Coachwhip	1	2		1	
Masticophis flagellum	Speckled Kingsnake	1	2			
Opheodrys aestivus	Rough Greensnake	2	2	1	1	1
Pantherophis obsoletus	Western Ratsnake	2	4	5		2
Pituophis catenifer	Gophersnake	2				
Sonora semiannulata	Western Groundsnake	1				1
Tantilla gracilis	Flat-headed Snake	1				
Dipsadidae						
Diadophis punctatus	Ring-necked Snake	2				1
Heterodon platirhinos	Eastern Hog-nosed Snake	2				
Leptotyphlopidae						
Rena dulcis	Texas Threadsnake	1	1			
Natricidae						
Haldea striatula	Rough Earthsnake	2	1	2		1
Nerodia erythrogaster	Plain-bellied Watersnake	2	2	5	4	1
Nerodia rhombifer	Diamond-backed Watersnake	2		3	4	1
Storeria dekayi	Texas Brownsnake	1	4			
Thamnophis proximus	Western Ribbonsnake	2	4	1	1	1
Thamnophis sirtalis	Common Gartersnake	1				
Tropidoclonion lineatum	Lined Snake	1				

Table 1. Continued.

Viperidae						
Agkistrodon laticinctus	Broad-banded Copperhead	2	3	6	2	2
Agkistrodon piscivorus	Northern Cottonmouth	1			2	
Crotalus atrox	Western Diamondback Rattlesnake			1		
Crotalus horridus	Timber Rattlesnake	2		4	2	1
Sistrurus miliarius	Pigmy Rattlesnake	2			1	
Squamata (lizards)						
Anguidae						
Ophisaurus attenuatus	Slender Glass Lizard	1				
Gekkonidae						
Hemidactylus turcicus	Mediterranean Gecko			2	4	
Phrynosomatidae						
Phrynosoma cornutum	Texas Horned Lizard	2				
Sceloporus consobrinus	Prairie Lizard	2				
Scincidae						
Plestiodon fasciatus	Common Five-lined Skink	1	1	5	4	1
Plestiodon septentrionalis	Prairie Skink	1				
Scincella lateralis	Little Brown Skink	2	4	6	3	1
Teiidae						
Aspidoscelis sexlineata	Six-lined Racerunner	2	4	2		
Testudines (turtles)						
Chelydridae						
Chelydra serpentina	Snapping Turtle	1				1
Macrochelys temminckii	Alligator Snapping Turtle	1				
Emydidae						
Deirochelys reticularia	Chicken Turtle	1				
Graptemys ouachitensis	Ouachita Map Turtle	2		4	3	
Pseudemys concinna	River Cooter	1		2		
Terrapene carolina	Eastern Box Turtle	2	2	4	1	
Terrapene ornata	Ornate Box Turtle	2	2	3	1	1
Trachemys scripta	Pond Slider	2		7	4	1
Kinosternidae						
Kinosternon subrubrum	Eastern Mud Turtle	2	1	1		
Trionychidae						
Apalone mutica	Smooth Softshell	1		1	2	
Apalone spinifera	Spiny Softshell	2		1	1	

Frost (2020) for amphibians and Uetz (2020) for reptiles; common names follow SSAR (2017).

Results and Discussion

During the past nearly 70 years, many reptile and amphibian species have remained consistently present at UOBS (e.g. Woodhouse's Toad, Western Ratnsake, Plain-bellied Watersnake, Five-lined Skink, and Pond Slider), whereas several other species have not been seen since early collections (Table 1). Overall, four frog, one salamander, six snake, four lizard, and three turtle species have not been observed in the vicinity of UOBS since the 1950s, and an additional frog and snake species have not been observed during sampling in the 2000s (Table 1). While the earliest publications regarding Lake Texoma herpetofauna covered several counties, we have reduced our listing to those only expected to occur on UOBS grounds at that time, since in most cases, these details were not provided. However, all 1978–1986 and 2008–2019 data reflect the station grounds specifically, and it is possible to make direct comparisons between these latter groupings, within the larger context of the expected species from the 1950s.

Of particular note among the long-term observations is the loss of the following three reptile species, all of which are experiencing population declines throughout Oklahoma (and often throughout their range): Texas Horned Lizards, Alligator Snapping Turtles, and Chicken Turtles (Riedle et al., 2005, 2009; McKnight et al., 2012; Vesy et al., 2021). The habitat specialist Texas Horned Lizard has been declining in Oklahoma since at least the 1950s (Carpenter et al., 1993), with the primary causes linked to increased urbanization, habitat modification, exploitation for the pet trade, and most recently, the introduction of the red imported fire ant (Vesy et al., 2021), the last of which are known to be present on-site at UOBS (Helms and Tweedy, 2017). Alligator Snapping Turtles, were distributed historically throughout 15 Oklahoma counties, but recent research has located populations in five of these counties only, with populations found only in isolated or protected habitats (Riedle et al., 2005, 2009). Alligator Snapping Turtle declines are associated with overharvesting and habitat modification, particularly river damming, and as such, they would no longer be expected to occur in Lake Texoma (Riedle et al., 2005, 2009). Active conservation efforts to breed the species in captivity at the Tishomingo Fish Hatchery have continued from 2000–present, as well as reintroductions of individuals in appropriate river habitats in their original range and active monitoring post-release (Ligon and Voves, 2019). Chicken Turtles primarily inhabit vernal pools surrounded by pristine habitats during the spring, before going into estivation during dry summer months (McKnight et al., 2012). Although they were documented just west of UOBS in Mayfield Cove in the mid-1970s (G. Carpenter, personal observation), the species is known currently from only a handful of sites in Oklahoma (none in Marshall Co.) and, as such, requires conservation protection (McKnight et al., 2012). It is important to note that available habitat for all three species is lacking on the immediate UOBS grounds, so their loss was to be expected.

In addition to these highlighted declining reptile species, all UOBS amphibians are at risk from high levels of infectious disease on-site (Marhanka et al., 2017) and susceptibility to Lake Texoma environmental contaminants through their porous skin (An et al., 2002; An and Kampbell, 2003). Amphibian infectious disease sampling conducted on-site in 2015 resulted in a 96% prevalence rate for the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*), but a 0% prevalence rate for ranavirus (RV) (Marhanka et al., 2017; Davis et al., 2019); disease research is still ongoing at UOBS in order to understand changes of these two diseases over time. The observed environmental contaminants can also lead to numerous problems at various life stages, including sex reversal, difficulties in metamorphosis, changes in predator avoidance behavior, and an inability to fight off infectious disease (Polo-Cavia et al., 2016; McCoy and Peralta, 2018; Davis et al., 2020; Slaby et al., 2019).

According to the new Oklahoma field guide to reptiles and amphibians, there are an additional 13 species (2 amphibians and 11 reptiles) that are described as occurring in Marshall Co., but have yet to be documented at UOBS specifically (Sievert & Sievert, 2021). While some species of reptile and amphibian have declined in the last nearly 70 years, other species have moved into the Lake Texoma area. Two species of note have also been added to the more recent UOBS herpetofauna collections: Green Treefrog and Mediterranean Gecko (Table 1). The first recorded observation of Green Treefrogs at UOBS was in 2010 (Table 1), although no voucher specimen was collected. In 2011, an individual was captured nearby at Fobb Bottom Wildlife Management Area (WMA) and vouchered to obtain county record documentation for Marshall Co. (Butler and Juarez, 2011). The species has been seen consistently at UOBS ever since (Table 1). Mediterranean Geckos are unique case in that they are a non-native species that has been introduced throughout the southern United States through human-mediated dispersal events, both intentional and unintentional, and is found primarily in and around human habitation (White et al., 2019). The species was introduced to the Norman campus of the University of Oklahoma by Teague Self or C. Carpenter and/or their students in the 1950s–1960s, although there is some debate as to whether this was an intentional or accidental release (White et al., 2019; V. Hutchison, personal communication). Presumably the geckos were introduced to UOBS soon thereafter by C. Carpenter's student Dale Marcellini (G. Carpenter, personal observation), and have been seen fairly consistently to the present day (Table 1).

It is worth noting that the length of survey time (12 days maximum) and variation in the time of year (late Spring/early Summer vs. late Summer) for our most recent survey events may contribute to some biases in amphibian detection, especially for those species whose breeding cycles are linked to early Spring (e.g. many Hylidae) or only to intense rainfall events (e.g. all Scaphiopodidae) (Sievert and Sievert, 2021). Sampling events from 1978–1986 also occurred in mid-summer only, but lasted for 8 weeks. Similarly, the same combination of trapping and survey methods were not employed every year or in the same microhabitats, which may contribute to some annual variation in species-specific discovery. For example, turtle captures are highly dependent on bait type and trap style, and it is likely that large hoop traps and crab traps, baited with sardines, may have disproportionally caught turtle species that are more inclined to open water and basking, such as Pond Slider, River Cooter, and Ouachita Map Turtle (Riedle et al., 2009). However, despite each trapping and survey method having individual, associated biases (Willson and Gibbons, 2010; Graeter et al., 2013), by utilizing several methods simultaneously and repeatedly over this long-term monitoring program, including observations by citizen scientists, we feel confident in the inferred trends and patterns, for at least the collections from 2008–2019. Herpetological sampling from 1978–1986 involved land trapping only, further resulting in a potential loss of presence/absence data for turtles.

Long-term monitoring of species' natural history and habitats are necessary for understanding changes in species distribution through time as it relates to many human-mediated factors, such as urbanization and climate change (Bartholomew, 1986; Able, 2016). Additionally, accurate conservation assessments and mitigation cannot be completed without long-term monitoring (Able, 2016), yet these types of studies are in decline in herpetology (McCallum and McCallum, 2006) and many other biological disciplines (Tewksbury et al., 2014). At UOBS, we have a unique situation in that herpetological species presence/absence has been documented for nearly 70 years, albeit intermittently. Natural history-based courses like Field Herpetology, whether they occur in a classroom, field, or museum setting, provide students with an increased awareness of large-scale natural phenomena and allow them to make educated science-based decisions about the world they live in (King and Achiam, 2017). The data provided herein give both an important foundation for course-based research, but also a unique in-depth glimpse into one vertebrate group and locality, an area that is increasingly lacking in biology (McCallum and McCallum, 2006; Tewksbury et al., 2014).

Acknowledgments

The authors would like to thank all the herpetology mentors and collaborators they have had throughout their lives. We thank directors Dr. Larry Weider and Dr. Gary Wellborn and other University of Oklahoma (OU) Biological Station staff throughout the years for their invaluable assistance (Deanna Cathey, Donna Cobb, Richard Page, and Malon Ward), as well as the OU Biology Department for consistently supporting the Field Herpetology course. We would also like to acknowledge the Oklahoma Department of Wildlife Conservation (ODWC) for assistance in funding, as it relates to field sampling and infectious disease supplies (grant #: F14F01225 [T-80-1]). Lastly, we thank the Biology students from OU (and elsewhere) who commit whole-heartedly every year to a 12-day intensive field course full of water, mud, mosquitos, poison ivy, biting snakes, and more.

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Submitted April 29, 2021 Accepted September 17, 2021

Response of a Soil Invertebrate Community to a Brief Flood Event

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Abstract: Transient flooding affects both above and below ground ecosystems. Soil invertebrates may be especially susceptible because of their small size and, in some cases, lack of a cuticle. A degraded grassland area on Lake Texoma flooded in early 2019; I examined soil invertebrate communities following flooding. Transects were established in the flooded area, and compared to an adjacent non-flooded area about 3.5 meters higher. I calculated Shannon diversity indexes for each sampling period and compared abundance and number of orders present. I also examined rainfall data for the sampling periods. Soil invertebrate communities varied widely across sampling times, with a general trend of the diversity being higher in unflooded area in 2019, but both areas converging in 2020. In general, invertebrate communities recovered rapidly following flooding, suggesting some taxa may have used behavioral mechanisms to avoid the flooded area, or else survivors were able to rapidly reproduce.

Introduction

Soil invertebrates recycle leaf and plant litter and return nutrients to the soil. Their biomass varies widely between ecosystems; one estimate suggests a range from 83 to 786 kg/ha in temperate deciduous forest (Landsberg and Gower 1997). However, they are not widely studied because they are small and inconspicuous (Corbett 2015, Coyle 2017) and because identification to species requires a high degree of specialization and is often based on examination of mouthparts (Seastedt 2000). To examine the community of soil invertebrates as a whole, and especially to track changes in it over time, identification to species may not be necessary and a more-general taxonomic level could be used, especially for community-scale monitoring studies. In this study I examined abundance of individuals by order, and diversity based on order. I used the same method as in Corbett (2015), of identifying soil invertebrates to order to monitor community changes over time in response to environmental conditions.

Environmental conditions can impact soil invertebrate abundance and diversity. Temperature fluctuations (Dowdy 1944), fire (Seastedt et al 1988), grazing (Seastedt and Reddy 1991), and drought (Corbett 2015) all have the ability to affect soil invertebrate communities. Barnett and Facey (2016) suggested that terrestrial arthropods are generally sensitive to moisture changes in their habitats, because they have a high surface-to-volume ratio and some soil arthropods lack the waxy, moisture-barrier cuticle that other arthropods have. However, soil invertebrates are also known to have population resilience (Wall et al 2008), and seem to rebound fairly quickly following disturbance.

Flooding has the potential to alter soil invertebrate communities. It can cause direct mortality (Vetz et al 1979), induce dormancy, may alter soil chemistry in ways that may be harmful (e.g., anaerobic decomposition) (Ausden et al 2001), or cause them to migrate out of the area (Plum 2005). The pattern of community recovery may vary, as different groups experience different effects, or escape flooding in different ways.

Plum (2005) catalogued a variety of negative physiological effects that could happen to soil invertebrates in a flooded area, ranging from physically being carried off by flowing water, to swelling of the invertebrate's body from extreme water uptake, to poisoning by pollutants in the water or by compounds produced during anaerobic decomposition. Additionally, flooding can affect the soil habitat (through compaction, loss of soil structure, or siltation filling soil macropores) in ways that will make it less habitable by invertebrates in the future.

Vetz et al (1979) noted that an increased frequency of flooding in an area that had experienced infrequent flooding in the past would reduce species diversity and abundance, especially as compared to areas with a regular flooding regime. Over time, changes in soil invertebrate diversity and abundance could affect soil chemistry and plant growth by altering the rate of litter breakdown, nutrient turnover, and nutrient availability.

Mites, springtails, earthworms, and other small invertebrates are important parts of the detritivore web in the soil, but many of these groups are little-studied and there is not much known about community patterns over time in response to natural disturbance cycles. Vetz et al (1979) noted that "little [was] known" about the effect of disturbance on detritivore food webs and Plum et al (2005) emphasized the lack of knowledge about the effect of flooding on smaller species such as mites. There is likely a difference in effects of regular, periodic flooding versus flooding as an infrequent disturbance event, even though Plum (2005) notes that "there are no typical 'wetland' soil megafauna" and only species more-tolerant of wet conditions. Ausden et all (2001) suggest some earthworm species are more flood-adapted than others. There is evidence that isopods, millipedes, and centipedes are particularly sensitive to the effects of flooding (Plum 2005).

Lake Texoma, found in Southeastern Oklahoma, periodically floods. The lake was originally constructed, in part, for river flood control, but in the past 20 years has experienced an increased rate of flooding (USACE History of Lake Texoma). This is not an intentional management tool but rather the result of an unusually high period of rainfall. Many of the published studies on the effects of flooding (e.g., Vetz et all 1979, Ausden et al 2001) examine flooding as a regular (annual or seasonal) event, rather than an uncommon disturbance, and as a result, the soil invertebrate communities there may respond differently to a community suffering a rare flood event.

In the location of the current study, Lake Texoma near the border between Bryan and Johnston Counties, Oklahoma, flooding is infrequent and not used as a management tool. However, flooding frequency seems to be increasing in the past 20 years – after ~30 years without a flood event, the lake flooded in 2007, 2015, 2017, and again briefly (and less severely) in 2019. It is possible that climate change and increasingly-unpredictable patterns of rainfall are contributing to an increased frequency of flooding. As a result, this may increasingly be a factor in soil chemistry, soil moisture levels, compaction, and other factors that could affect soil invertebrates.

Materials and Methods

The research was conducted on a plot of US Army Corps of Engineers-managed (hereafter: USACE) land adjacent to Lake Texoma (33.99 N, 96.58 W). This land is shared between SE Oklahoma State University and USACE and is used for research and class field trips. The land is mostly used for recreation and lake access. Baseline lake level (called the conservation pool) is 619 feet above sea level (USACE Lake Texoma Data). Often in the summer the lake level is below this level. This site has occasionally flooded; when the lake is high enough to crest the spillway most access to the site is cut off, which happened in 1957, 2007, and twice in 2015 (USACE History of Lake Texoma). Parts of the site also flooded in 2017, and, most recently, in 2019. The most prolonged and extreme flood event was the 2007 event, where elevations exceeding 620 feet lasted from mid-May to mid-September, and the water crested the emergency spillway. In 2015, the flood event lasted from mid-May to Mid-August and there were two points where it was over 640 feet and crested the emergency spillway (USACE History of Lake Texoma).

The 2019 flooding was more limited in scope; maximum lake elevation was 630' feet above sea level, which flooded part of the research area. The flood lasted from early May through early July 2019. A low-lying area just north of Highway 70 was flooded during that time; an adjacent area that was about 3.5 meters higher remained dry. It is also unclear how deep the flooding penetrated: whether it was merely standing water inundating the top dozen centimeters of soil, or if it went deeper. The depth of wetting was not measured.

Having previously examined changes in soil invertebrate communities over time (Corbett 2015) and noting that the drought of 2011 affected their abundance and diversity, I wondered what effect flooding would have and how rapidly communities would rebound. After flooding receded (July 2019), I established two transects at the site; one in the recently flooded area and the other in an adjacent upland area that had not flooded. The two transects were separated by about six meters, and the unflooded area was 3-4 meters higher than the flooded area. Because soil invertebrate communities may have initially differed between the two locations due to elevation and vegetation differences, the primary objective was to compare the changes in the two communities. The flooded area was dominated by black willow saplings (*Salix nigra*), gaura (*Gaura biennis*), and grasses including Scribner's panic grass (*Dichanthelium oligosanthes var. scribnerianum*). The upland area was dominated by sericea lespedeza (*Lespedeza cuneata*) and Scribner's panic grass (Corbett, unpublished data). Both transects appear (from the USDA soil map: USDA 1978) to be a mixture of soil series; the area was disturbed and the soil replaced after disturbance – the code in the soils manual describes it as "pits." I did not do a laboratory test on the soil texture, but a quick field test suggested it was closest to a sandy clay loam in texture.

I located seven sampling points along each transect, separated by approximately 12 meters. On the first sample date (19 July 2019), surveyor's flags were placed so sample points could be relocated in the future. In total, there were five sampling events: July 2019, September 2019, October 2019, and July 2020 and October 2020. The author would have liked to have collected more samples in 2020, but campus closure in early 2020 due to the pandemic prevented samples being collected before July 2020.

Soil samples were collected using the same technique as in Corbett (2015): a 6.5 cm by 5 cm deep bulb planter was used to collect five haphazardly-spaced cores from a 2 m radius around the sampling point. Each set of cores was placed in a labeled zip-top bag and transported back to the biology department at Southeastern Oklahoma State University for extraction.

Samples were extracted by being placed in a large "funnel" with a plastic grid on the bottom (see Corbett 2015 for details) and were set over beakers containing 70% isopropyl alcohol. An incandescent light (40 watts, to avoid overheating during times when the room was unoccupied) shone on the soil for 48 hours to drive as many invertebrates as possible into the preservative. After the 48 hours, the preservative and any invertebrates captured was stored in a 100 mL plastic specimen cup with a lid. After that, a "float method" was applied (small subsamples of the soil mixed heavily with water and explored with a dissecting needle to find remaining invertebrates). Any additional invertebrates found were added to the appropriate specimen cup.

Following extraction, each sample was evaluated. The liquid and sediment in each specimen cup was dispensed into petri dish halves and examined under a dissecting microscope at 20x magnification. Organisms found were identified to order with the assistance of the "Kwik-Key to Soil Invertebrates" (Meyer, 1994). Each sample, thus, yielded both data on what orders were present in a sample as well as how many individuals of each order were present. This allowed for calculations of diversity and abundance.

To analyze the data, I first prepared tables showing the abundance of each order at each sampling date. The sample size was small (five dates with two transects on each date) so statistical comparisons were complicated by that fact. I used nonparametric testing (the Mann-Whitney U test: IBM SPSS 20, 2011) to compare total abundance and number of orders represented for the flooded vs. unflooded areas.

I also calculated Shannon indexes (H') for each transect for each sampling time. I used a base-10 logarithm with this calculation and also calculated evenness (J) as (H'/H' max)*100, where H' max was the base-10 logarithm of the number of orders represented in the sample (Magurran, 1988). Because of the nature of how Shannon indexes are calculated, the standard error is calculated differently from a typical t test. I followed the method given in Zar (2010) to perform a two-sample t test on Shannon index data, which is somewhat similar to the Behrens-Fisher t test. The standard error is calculated from a variance that is calculated based on a modification of the calculations used for the Shannon index, and the degrees of freedom is calculated similarly to that for the Behrens-Fisher test.

Five comparisons were made, flooded vs. unflooded at each sampling time.

Results and Discussion:

Table 1 lists the abundances by order for each of the sampling events; Table 2 lists the Shannon diversity and evenness values for each sampling event. Figure 1 shows a graph of the Shannon index values across the five sampling times. Notably, the flooded area starts out lower than the unflooded area, but the values converge in the second year of the study, when the unflooded area's diversity declines and the flooded area's diversity increases slightly. In fact, the main pattern seems to be one of higher but decreasing values in the unflooded area and low but increasing values in the flooded area. It is not clear why the unflooded area's diversity would decline in 2020.

The t-test comparisons of the Shannon indexes failed to achieve significance at the 0.05 value for all sampling periods. However, for the September 2019 period, the comparison was close to there being a significant difference: t = 1.93, critical value = 1.97 d.f. = 322. In this case the flooded area had a nearly-significantly lower value of diversity (table 2). None of the other pairs approached significance. This may be a result of small sample size (seven soil samples per transect) or that flooding genuinely does not affect species diversity of soil organisms. Because of the nature of how "standard error" is computed for statistical analysis, these values are not shown on the graph – they are typically only used for statistical analysis and as a result, they are not shown on Figure 1. Also, as Shannon index values are typically reported to three or four significant figures, I retained four decimal places in the Y axis of the graph.

There was no significant difference between flooded and unflooded areas over the course of the entire study for number of organisms (p=.690; SPSS does not post U values for Mann-Whitney tests). However, order number differed, with a p value of .032. The number of orders in the unflooded area was significantly higher over the span of the study (Table 1). This suggests that the main effect of flooding on the sites was in breadth of the community, rather than overall diversity.

Number of total organisms is extremely variable and can be influenced by small-scale or transient-in-time site factors. For example, if an active anthill is near where a sample was collected at a particular sampling time, large numbers of foraging ants may be collected in the sample, but nowhere else along the transect. Collembolans (springtails) also seemed to vary widely in population size between sampling periods. Russell et al (1992) suggest that collembola "react very flexibly to disturbance" and that they tend to have rapid population rebound after a disturbance. It seems likely different taxonomic groups will be affected to

Table 1. Abundance of different invertebrate orders (or higher taxonomic group, in some cases) by sampling date and site condition. Note that larvae are included in the count for their respective orders. Only orders with at least one individual present are noted for a time period.

July 2019	Unflooded area	Flooded area
Acarina	41	15
Aranae	2	0
Aschelminthes	2	0
Chilopoda	1	0
Coleoptera	31	21
Collembola	62	15
Diplura	4	4
Diptera	3	1
Gastropoda	1	0
Homoptera	2	0
Hymenoptera	13	0
Isopoda	1	0
Pauropoda	2	0
Thysanura	2	1
Total number	167	57
Orders represented	14	6
September 2019		
Acarina	34	24
Annelida	6	0
Aranae	1	3
Aschelminthes	1	0
Coleoptera	30	42
Collembola	41	118
Diplura	4	0
Diptera	4	3
Homoptera	2	1
Hymenoptera	5	3
Isoptera	0	1
Total number	94	195
Orders represented	10	8

Table 1. Continued

October 2019		
Acarina	23	79
Annelida	2	0
Aranae	1	2
Aschelminthes	1	0
Chilopoda	1	1
Coleoptera	13	20
Collembola	9	10
Diptera	1	1
Homoptera	4	4
Hymenoptera	8	8
Isopoda	3	3
Total number	145	128
Orders represented	10	9
July 2020		
Acarina	88	83
Annelida	5	2
Aranae	0	1
Coleoptera	38	53
Collembola	61	17
Diplura	10	5
Hymenoptera	11	69
Isopoda	0	1
Isoptera	0	3
Thysanura	1	0
Thysanoptera	1	0
Total number	215	238
Orders represented	9	10
October 2020		
Acarina	130	57
Annelida	3	5
Aranae	1	0

Table 1. Continued

Aschelminthes	2	1
Coleoptera	28	28
Collembola	53	15
Diplura	2	0
Homoptera	1	0
Hymenoptera	6	0
Isopoda	0	2
Pauropoda	4	0
Thysanoptera	3	0
Total number	233	108
Orders represented	11	6

different degrees by flooding; that difference might or might not show up in changes in overall community diversity. Some species of invertebrates (some Coleopteran larvae) have adaptations that allow them to withstand being in flooded areas; for example, water-repellant hair that will maintain a "bubble" of air around the larva (Barnett and Facey 2016). Ausden et al (2001) noted that recolonization following flooding could reconstitute soil-invertebrate communities (though possibly different taxonomic groups differ in their recolonization rate). In previous studies of grassland soil invertebrates (Corbett 2015), collembolans and mites (especially oribatid mites) were among the highest-abundance groups; this was also true in the current study. Beetles were the third-most-abundant group in the current study; beetles tend to be more mobile than many of the more hypogeic groups like proturans, and could recolonize the area following flooding or other disturbance.

There is a trend (Table 1) that the total numbers were higher in 2020 than in 2019; this could be a result of differences in rainfall, or recovery after a wetter year. Additionally, there may be some patterns within orders: Collembolans showed a large increase in the September 2019 sample, and there is some evidence (Coyle et al 2017) that their populations can rebound rapidly after flooding. It is also possible some organisms had migrated either laterally or to deeper regions of the soil (I did not test how deep the standing water penetrated) and migrated back as the site recovered. Presumably, different taxa will have different dispersal abilities, and that could affect community recovery over time, just as differences in reproduction rate between taxa could affect community recovery.

Rainfall amounts varied during the period of sampling although the two years did not differ greatly in total rainfall (Oklahoma Mesonet, last accessed 4/22/21). Total rainfall for 2019 was 127 cm and for 2020, it was 125.5 cm. However, in 2019, April, May, and June were high-rainfall months (49.9 cm combined) and for 2020, the April, May, and June combined rainfall were 35.1 cm). The months sampled in 2019 (July,

Table 2: Shannon diversity index (H', calculated using $\log_{10}$) and evenness (j) by sampling date and site condition.

Date	Unflooded		Flooded	
	H'	J	H'	J
July 2019	0.7568	29.18	0.6075	33.92
September 2019	0.8135	35.34	0.4948	23.80
October 2019	0.5698	24.75	0.5634	25.64
July 2020	0.6348	28.89	0.6690	29.05
October 2020	0.5740	23.94	0.5302	29.59

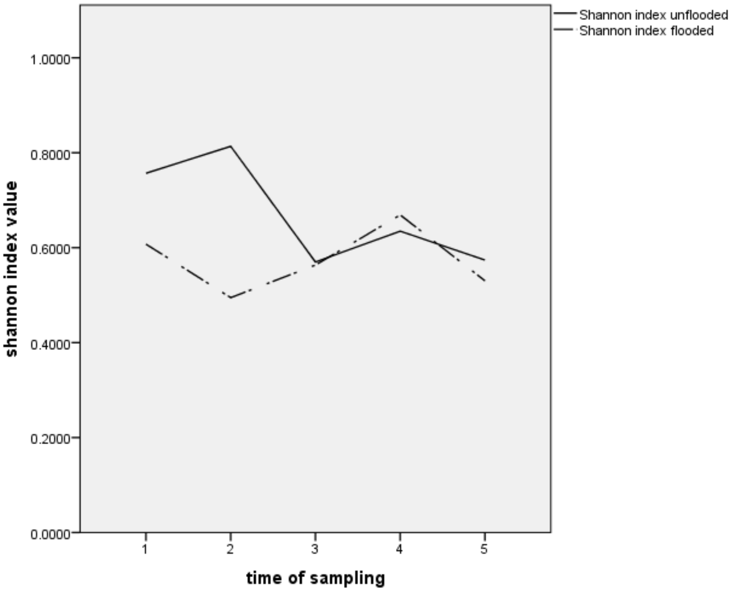

Figure 1: Shannon index values across the five sampling times. Sampling times 1, 2, and 3 were in 2019; sampling time 1 was shortly after flooding receded. Sampling times 4 and 5 were in 2020. The Shannon index values for the unflooded portion of the site are shown as a solid line, and for the flooded portion of the site, a dashed line. The Y axis is shown with four significant figures because typically the Shannon index is reported to three or four significant figures.

September, and October) had 7.8, 6.5, and 15.6 cm of rainfall, respectively, whereas July 2020 had 5.4 cm and October 2020 had 5.1 cm. Thus, the sampling times in 2020 were generally during dryer months which could affect the diversity levels of the sample; when it is drier, many soil invertebrates move to deeper levels of the soil (Barnett and Facey 2016, Dowdy 1944). The samples taken in this study were only about 5-7 cm deep in the soil.

Following the initial low level of invertebrates in the flooded area, populations seem to have rebounded quickly and there were few clear long-term affects on abundance and diversity. However, this was a short-term study (two years). Vetz et al. (1979) suggest that over longer term, with repeated flooding, there may be changes in nutrient cycling resulting from changes in the composition of the community. It is possible that climate change will cause long-term effects on both the invertebrate and plant communities throughout the temperate grassland that will alter nutrient cycling, community dynamics, and interspecific interactions (Barnett and Facey 2016), because of increased variability in rainfall regime and (possibly) increased frequency of flooding. Although these communities recovered quickly over the short term, it is possible increasing instability of rainfall regime with climate change could have long term effects for nutrient cycling and other soil alterations provided by soil invertebrates. Possible future studies could involve controlled flooding of areas and assessment of soil invertebrate communities before and after that process, focusing on individual taxonomic groups and their different responses.

Acknowledgments

The author thanks Laurinda Weisse for a critical read and comments on the pre-submission manuscript and two anonymous reviewers for their comments. Equipment and space for conducting this research were provided by Southeastern Oklahoma State University.

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Submitted June 9, 2021 Accepted August 18, 2021

Hooking Mortality Rates and Factors Influencing Mortality of Alligator Gar Caught Using Two Hook-and-Line Methods

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Abstract: The Alligator Gar (*Atractosteus spatula*) has become a recreationally popular species due to its trophy potential. Hook-and-line angling is one of the most popular methods for catching Alligator Gar and most anglers practice catch-and-release, however delayed mortality rates associated with these fishing methods are unknown. Therefore, we evaluated hooking mortality and factors affecting mortality for Alligator Gar caught using two hook-and-line fishing methods, juglines and rod and reel. Juglines were used to catch Alligator Gar from Texoma Reservoir, Oklahoma. Once caught, fish were placed into pools and hooking mortality was evaluated over a 120-h observation period. For the rod and reel component of the study, 32 Alligator Gar were captured from Texoma Reservoir using gillnets and transported to ponds at the Tishomingo National Fish Hatchery. Rod and reel angling was conducted in the ponds until 16 gar were caught and those fish were monitored for 268-d post-release. Following capture using either method, the tag number, water temperature, total length (mm), hooking depth, and anatomical hooking location for each Alligator Gar was recorded. A total of 74 Alligator Gar were caught using juglines, of which 60 died (mortality rate = 81.1%) during the observation period. Of the 16 Alligator Gar caught using rod and reel, one fish died (hooking mortality = 6.3%) during the observation period. No control fish died during either experiment. Mean hooking depth of Alligator Gar caught using juglines was 247 mm, which was significantly deeper than those caught using rod and reel angling (101 mm). A multiple logistic regression model indicated no significant relationship between gear type (juglines vs. rod and reel) and the fate (mortality or survival) of Alligator Gar, suggesting that most jugline mortality could be explained by deeper hooking depths. Additionally, temperature, total length, and hooking depth affected survival of Alligator Gar. Results from this study can be used to inform fisheries biologists of mortality associated with juglines and hook and line when managing recreational fisheries that permit these fishing methods or if they are considering implementation of these techniques for sampling Alligator Gar.

Introduction

Mortality rates and variables contributing to delayed hooking mortality have been described for numerous fish species (Muoneke and Childress 1994, Tomcko 1997, Bartholomew and Bohnsack 2005, Cooke and Suski 2005, Coggins et al. 2007, Hühn and Arlinghaus 2011, Schmitt and Shoup 2013). The majority of this research has focused on fish species that are economically and recreationally important, or have received considerable outdoor media attention (Muoneke and Childress 1994). Reported mortality rates associated with catch-and-release angling have ranged from 0 to 95% across freshwater and marine species (Muoneke and Childress 1994, Bartholomew and Bohnsack 2005). The wide range of reported mortality rates is attributed to a number of factors, including fish species, body size, bait type (natural vs. artificial), hook design (single, treble, barbed vs. barbless), gear types, hooking location, fish handing practices, water depth where fish was caught (barotrauma), and water quality where fish were caught and released (Muoneke and Childress 1994, Tomcko 1997, Bartholomew and Bohnsack 2005, Hühn and Arlinghaus 2011, Schmitt and Shoup 2013). Although delayed hooking mortality has been evaluated for many fish species, it is unknown how Alligator Gar (*Atractosteus spatula*) are affected by catch-and-release angling.

Historical negative perceptions towards Alligator Gar are rapidly changing, as this species has become recreationally popular and valuable due to its trophy potential (i.e., growth to >1,828 mm TL; Buckmeier et al. 2016, Adams et al. 2019). Hook-and-line angling is one of the most popular recreational methods for catching Alligator Gar and most anglers practice catch-and-release after documenting their experience with photographs. Catch-and-release angling operates under the assumption that released fish survive and continue to reproduce or are allowed to grow to a more desirable size before harvest thus limiting population effects (Coggins et al. 2007, Schmitt and Shoup 2013). If released fish do not survive, population size structure and abundance can be altered when sufficient proportions of the population are captured (Daugherty and Bennett 2019), which ultimately affects the sustainability of a fishery (Wydoski 1977, Coggins et al. 2007). Additionally, since management biologists use these methods to sample Alligator Gar (Buckmeier et al. 2016), high delayed fishing mortality rates could affect population structure or bias the sampling data.

Alligator Gar is considered vulnerable across its current range by the American Fisheries Society (Jelks et al. 2008), so it is critical to understand threats to this species, including the potential impacts of recreational angling. Alligator Gar exhibit a periodic life-history strategy (high longevity, delayed maturation, and specific spawning habitat requirements, which regulates recruitment and population densities; Buckmeier et al. 2017). These characteristics make this species particularly vulnerable to anthropogenic impacts on the environment and overfishing, which have historically resulted in significant population declines (Buckmeier et al. 2017, Smith et al. 2018). A small increase in angling mortality could have a negative impact on Alligator Gar populations as a result of its periodic life history characteristics (Coggins et al. 2007, Smith et al. 2018, Daugherty and Bennett 2019). Across the current range of Alligator Gar, unlimited catch-and-release angling (using hook-and-line methods) is permitted, however information regarding delayed hooking mortality associated with these methods is unknown.

The Oklahoma Department of Wildlife Conservation (ODWC) began using hook-and-line (juglines and rod and reel; Buckmeier et al. 2016) sampling in addition to gill netting (Bodine et al. 2015; Schlechte et al. 2016) to increase the number of Alligator Gar collected during mark-recapture efforts to attain a population estimate in Texoma Reservoir. However, soon after initiating these sampling methods, we realized numerous difficulties (jug design, hook type, bait type, sampling location, and rod and reel setup) that prohibited success of these sampling approaches. The first attempts to collect fish via hook-and-line methods relied on the use of J-style and circle hooks, but no Alligator Gar were landed using these hook types. To improve our methods we contacted professional fishing guides in Oklahoma and Texas that specialize in catching Alligator Gar. All of the guides suggested the use of size 3/0 or 4/0 treble hooks and recommended increasing treble hook size if fish were still being missed. However, these recommendations came with the warning that increased treble hook size would increase hooking mortality rates. Upon switching to 4/0 treble hooks, our ability to hook and land Alligator Gar improved, but all of the fish that were caught (7 with juglines and 5 with rod and reel) died from hooking-related injuries. Consequently, hook size was decreased to a size 3/0 treble hook and observed hooking mortality decreased but continued to occur. Use of hook-and-line techniques to mark and recapture Alligator Gar for estimation of population size was abandoned due to associated hooking mortality. However, these preliminary results identified the need to evaluate delayed hooking mortality of Alligator Gar using hook-and-line methods. Therefore, we sought to evaluate hooking mortality rates associated with two commonly used hook-and-line fishing methods (juglines and rod and reel) and to describe factors influencing the mortality of caught and released Alligator Gar.

Methods

Juglines

Jugline fishing for Alligator Gar occurred from June through September 2018 in the upper third of both river arms (Red and Washita rivers) of Texoma Reservoir. Side scan sonar was used to locate concentrations of Alligator Gar (Fleming et al. 2018), and once found juglines were set in these areas. Juglines were set just prior to sunset and lifted at sunrise the following morning (< 12 h set time). Juglines were similar in design to those used by Snow and Porta (2020), which consisted of inserting a PVC pipe through a swimming pool noodle and caps were added to each end of the pipe. Holes were drilled through each cap and 33.5 meters of 113 kg test jug line was run through the PVC pipe. Once the line was fed through pipe, a snap swivel was tied to one end for attaching a 0.45 kg weight and a 91.4 cm steel leader (84 kg test) with a snap swivel was added to the opposite end. This design allowed Alligator Gar to surface to breathe, without interference from the weight. Juglines were baited with cut or whole fish of various species (Snow and Porta 2020). The juglines were set to allow the bait to rest on the bottom of the lake.

Alligator Gar caught on jugs were played by hand. Once brought boat-side, the fish was noosed behind the pectoral fins with a rope, lifted onto the boat, and placed into a holding tank, which is commonly practiced by anglers. Once onboard, Alligator Gar were measured for total length (TL, mm) and implanted with a PIT tag (Biomark, Inc., Boise, Idaho) and stainless steel dart tag (FH-69W; Floy Tag and Manufacturing, Inc., Seattle, WA) for identification purposes. In cases when the baits were swallowed (fish not caught in a location where hook could be removed), the leader was cut at the corner of the mouth where the upper and lower jaws meet. The remaining section of leader was measured to determine the hook depth and anatomical hooking location (i.e. external [outside of mouth], mouth, esophagus, stomach) was recorded. Anatomical hooking location estimated using leader measurements was predicted with 86.7% accuracy, which was verified through dissection of mortalities.

Alligator Gars were then transported to one of two holding pools (4.6 m diameter x 1.2 m deep; Intex Recreation Corp., Long Beach, California) located on the shoreline of Texoma Reservoir at the University of Oklahoma Biological Station near Kingston, Oklahoma. Pools were filled using water from Texoma Reservoir, and water quality (temperature, dissolved oxygen, and pH) in each pool was monitored daily over the course of the study to ensure conditions were similar to those in the reservoir. An equal number of control fish (captured using multifilament gillnets; Schlechte et al. 2016) and treatment fish (caught using juglines) were placed into each pool. Densities of Alligator Gar did not exceed 6 individuals (3 control and 3 treatment) per pool. Fish were monitored daily for 5 d. When mortality occurred, that fish was removed from the pool and processed for TL and weight, and a necropsy was performed to determine the hooking location and document any internal injuries caused by hooking.

Rod and reel

In February 2018, Alligator Gar (n=32) were collected during winter using multifilament gill nets (Bodine et al. 2015, Schlechte et al. 2016). Side scan sonar was used to locate concentrations of Alligator Gar in the Washita River arm of Texoma Reservoir, and once found, gill nets were deployed in these areas (Fleming et al. 2018). Once captured, Alligator Gar were measured, weighed, implanted with a PIT tag and dart tag, and transported to the boat ramp where they were transferred into one of three fish hauling tanks. Water in the hauling tanks contained a 1% NaCl solution and a mild concentration (10 mg/l) of MS-222 to reduce handling and hauling stress while in transport to the Tishomingo National Fish Hatchery (TNFH; Porta et al. 2019). Alligator Gar were distributed evenly (n=8) among four 0.4 ha earthen ponds at the TNFH and ample live fish forage was added to each pond. Alligator Gars were allowed to acclimate to the ponds for 6 months prior to initiating the experiment. The experiment began in July 2018, but was quickly paused due to overabundant vegetation in the hatchery ponds that made fishing impossible. The fishing trials were successfully completed in May 2019 prior to extensive growth of vegetation in the ponds.

To mimic recreational Alligator Gar angling methods, a medium-heavy action rod with a spinning reel spooled with 36.3 kg test braided line was used. A 91.4 cm steel leader (84 kg test) was added to the braided line and a snap swivel was used to attach a size 3/0 treble hook. Hooks were baited with cut or whole fish (Common Carp [*Cyprinus carpio*], Gizzard Shad [*Dorosoma cepedianum*], River Carpsucker [*Carpiodes carpio*], or Smallmouth Buffalo [*Ictiobus bubalus*]) and fished on the bottom of the ponds with no weight. When an Alligator Gar picked up the bait, it was allowed to swim, stop to orient the bait in its mouth, and begin swimming again before setting the hook (time recorded from initial bait pickup to hook set averaged 6.43 min). Once an Alligator Gar was hooked, the fish was reeled to the shoreline, noosed behind the pectoral fins with a rope and brought on to the shore where the tag number was recorded, and the hook was removed (if caught in the mouth). When fish swallowed the bait (hooked in a location where hook could not be removed), the leader was cut at the corner of the mouth where the upper and lower jaws meet and the remaining section of leader was measured to determine the hook depth and anatomical hooking location. Alligator Gar were released into the same pond where they were captured. Fishing ceased once 4 Alligator Gar were caught from each pond. After all fish were caught, TNFH staff monitored the ponds daily for mortalities. When mortality of an Alligator Gar occurred, the date and time was recorded to determine the number of days following a fishing event and the fish was removed from the pond. All Alligator Gar mortalities were processed for TL and weight, tag number was recorded, and a necropsy was performed to determine the hooking location and internal injuries caused by hooking. In March 2020, 268-d following capture of the last fish, all four 0.4 ha earthen ponds at the TNFH were drained and tag numbers of the remaining Alligator Gar were recorded to determine if fish were caught and released (treatment fish) or never captured (control fish). All live Alligator Gar were transported back to Texoma Reservoir and released.

Statistical analysis

A length frequency histogram was used to describe the size distribution of Alligator Gar captured during this study for each method. A two-sample t-test was performed to determine if mean hooking depth differed between catch-and-release mortalities and survivors for juglines. Differences in the proportion of Alligator Gar mortalities between treatment (caught and released) and control (not caught) groups were analyzed using a chi-square test for rod and reel caught fish. Anatomical hooking location, fate (mortality or survival), and hooking depth was documented for each Alligator Gar for both methods. A logistic regression was performed to determine differences in mortality among anatomical hooking locations. A one-way ANOVA was performed to determine differences in hooking depth between anatomical hooking locations. If a significant difference was detected a Tukey Post-hoc test was performed.

A Pearson's correlation test was performed to ensure variables (temperature, total length, hooking depth, and ratio of hooking depth to Alligator Gar TL) were not correlated (Table 1). Variables were considered to be correlated at a level ≥0.60. Two variables: hooking depth and ration of hooking depth to alligator gar total length (% of TL) were correlated, so hooking depth was removed from further analyses. A logistic regression model was used to understand the relationship between juglines and rod and reel, while fitting temperature, total length, and

Table 1. Pearson correlation matrix of variables used in the multiple logistic regression model. Variables with bold values are strongly correlated (≥ 0.60). Hooking depth and hooking depth (% of TL) were correlated, therefore only hooking depth (% of TL) was used in the logistic model.

Variables	Temperature (°C)	Total Length (mm)	Hook Depth (mm)	Hooking Depth (% of TL)
Temperature (°C)		-0.34	0.46	0.34
Total Length (mm)	-0.34		-0.23	0.16
Hooking Depth (mm)	0.46	-0.23		**0.89**
Hooking Depth (% of TL)	0.34	0.16	**0.89**	

hooking depth (% of TL) to a binary variable (0 = survived, 1 = died) to understand if these variables affected the fate (mortality or survival) of Alligator Gar following capture. All analyses were performed using XLSTST 2020 (Addinsoft Inc., New York City, NY). All significance tests were evaluated at $P \leq 0.05$.

Results

Juglines

A total of 74 Alligator Gar were caught on juglines during summer 2018. Surface water temperatures ranged from 22.2ºC to 32.2 ºC during jugline fishing efforts. Alligator Gar ranged from 678 to 2,274 mm TL (Figure 1a). Of the 74 fish caught on juglines, 60 died from

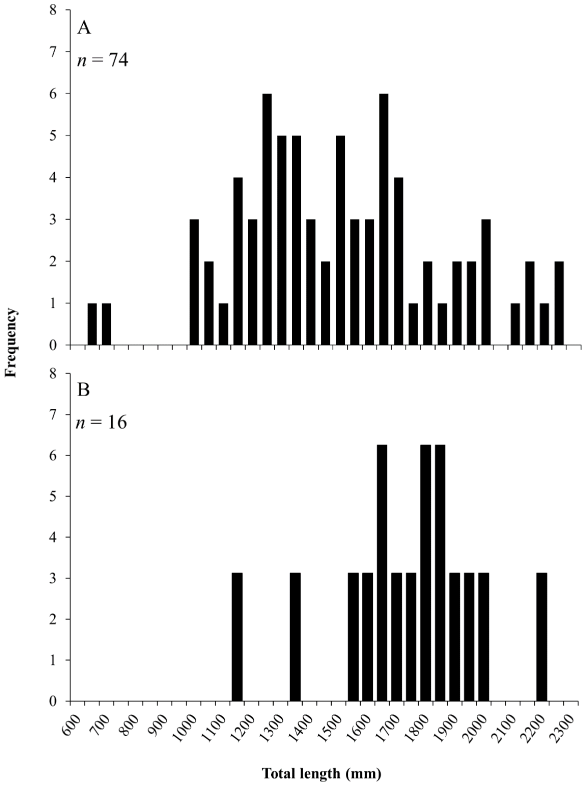

Figure 1. Length frequency histograms of Alligator Gar caught using (A) juglines and (B) rod and reel fishing methods. Alligator Gar caught using juglines were captured from Texoma Reservoir, Oklahoma. Alligator Gar caught using rod and reel were captured during fishing trials conducted in ponds at the Tishomingo National Fish Hatchery.

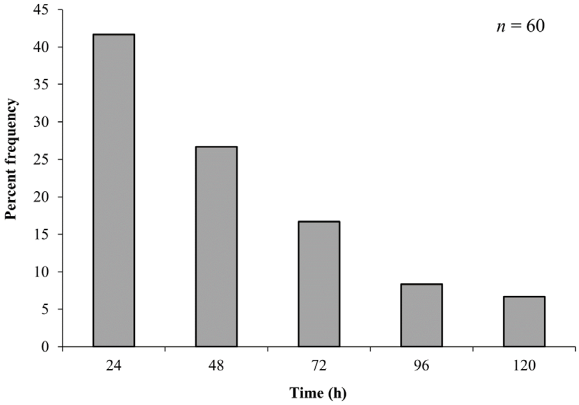

Figure 2. Daily (24 h period) mortality rates of Alligator Gar caught using juglines from Texoma Reservoir, Oklahoma during a post-capture 120 h observation period.

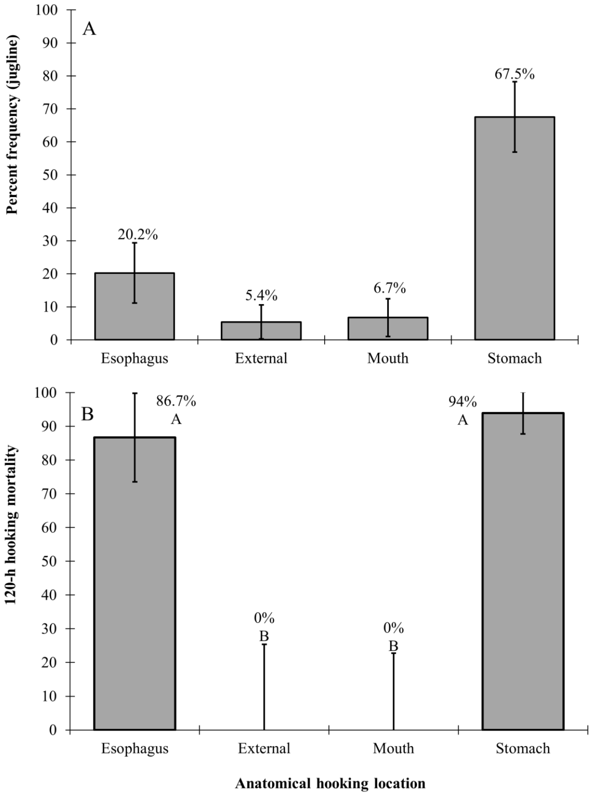

Figure 3. Percent of total fish (A) and mortality rate (B) by anatomical hooking location for Alligator Gar caught using juglines at Texoma Reservoir, Oklahoma during June-September 2018. Error bars represent 95% confidence intervals. A difference in letters above bars reflects statistical significance at P > 0.05.

hooking injuries (mortality rate = 81.1%). Delayed morality occurred within the first 24-h for 42% of the Alligator Gar. Although the mortality rate decreased with increasing time, mortality events occurred over the entire 120-h observation period (Figure 2), suggesting mortalities could have occurred beyond 120-h. No control fish died during the 120-h observation

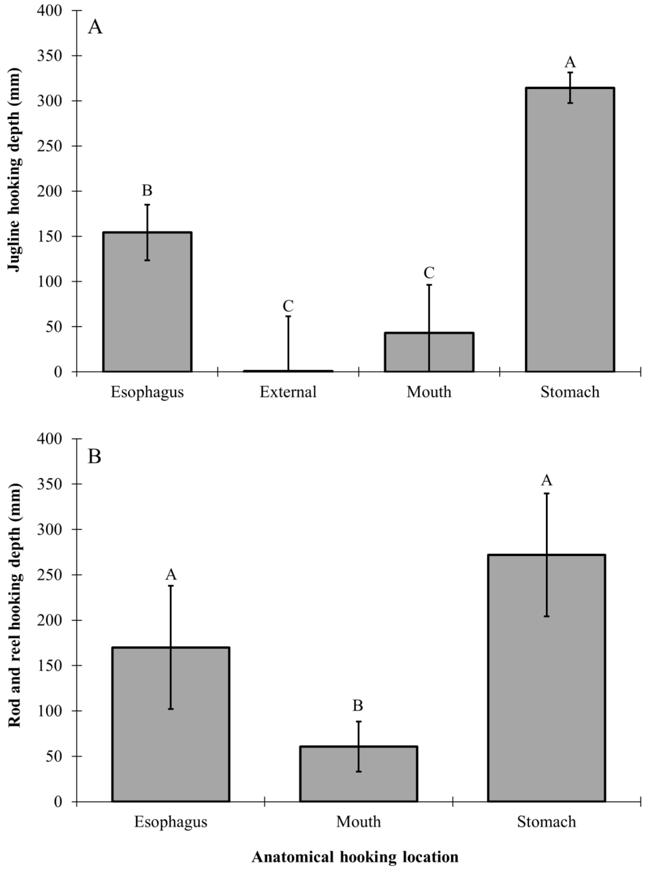

Figure 4. Mean hooking depth of Alligator Gar caught using (A) juglines and (B) rod and reel by anatomical hooking locations. Error bars represent 95% confidence intervals. A difference in letters above bars reflects statistical significance at P > 0.05.

period.

The majority of Alligator Gar caught with juglines were hooked in the stomach (67.5%). The remaining fish were hooked in the esophagus (20.2%), mouth (6.7%), or externally (5.4%; Figure 3a). Alligator Gar hooked in the esophagus or stomach experienced a mortality rate exceeding 86%. There was no significant difference in mortality rate between esophagus- and stomach-hooked fish. However, Alligator Gar hooked in the esophagus and stomach had a significantly higher mortality rate ($X^2 = 14.516$, df = 3, $P < 0.01$) than both external- and mouth-hooked Alligator Gar (Figure 3b). No mortality was observed for Alligator Gar hooked in the mouth or externally.

Table 2. Results of multiple logistic regression model examining hooking mortality of Alligator Gar in relation to temperature (°C), TL (mm), hooking depth (% of TL), and gear type (juglines and rod and reel). Variables with bold P-values were significant at an alpha level = 0.05.

Variable	df	X^2	*P*-value
Temperature (°C)	1	3.741	**0.05**
Total length (mm)	1	7.946	**<0.01**
Hooking depth (% of TL)	1	14.198	**<0.01**
Gear	1	0.327	0.58

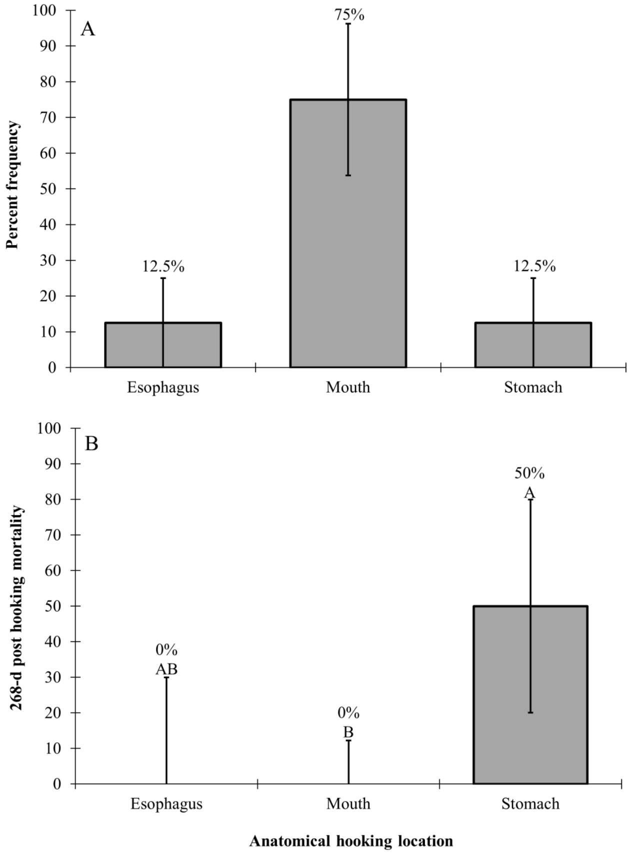

Figure 5. Percent of total fish (A) and mortality rate (B) by anatomical hooking location for Alligator Gar caught using hook and line angling in ponds at Tishomingo National Fish Hatchery during May 2019. Error bars represent 95% confidence intervals. A difference in letters above bars reflects statistical significance at P > 0.05.

Mean hooking depth of Alligator Gar caught using juglines was 247 mm (range = 218.1 – 275.9 mm). The mean hooking depth resulting in Alligator Gar mortality (270 mm, range = 244.5 – 295.5 mm) differed significantly ($t = 4.3$, df = 72, $P < 0.01$) from the mean hooking depth of Alligator Gar that survived (125 mm, range = 29.5 – 220.5 mm). Additionally, a significant difference was detected between hooking depths among anatomical hooking locations ($F_{3,\ 70} = 75.647$, $P < 0.01$; Figure 4a), particularly for Alligator Gar hooked in the stomach. No difference in hooking depth was detected for Alligator Gar hooked externally or in the mouth.

Rod and reel

A total of 16 Alligator Gar were caught using rod and reel during May 2019. Water temperature of the TNFH ponds ranged from 19.7ºC to 22.3ºC. Alligator Gar ranged from 1,168 to 2,215 mm TL (Figure 1b). Of the 16 Alligator Gar caught using rod and reel angling, only one died (mean mortality rate = 6.3%) over the 268-d post-release observation period. The mean hooking depth of Alligator Gar that survived the catch-and-release process was 101 mm (range = 55.2 -146.8 mm). No control fish died during the 268-d post-release observation period. The proportion of Alligator Gar that survived catch-and-release angling did not significantly differ from the proportion of control fish remaining at the end of the study period ($X^2 = 0.065$, df = 1, $P = 0.79$).

The majority of rod-and-reel-caught Alligator Gar were hooked in the mouth (75%), followed by esophagus (12.5%) and stomach (12.5%; Figure 5a). No Alligator Gar were hooked

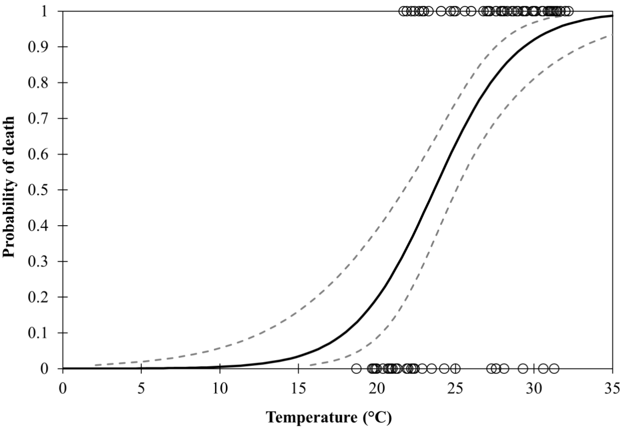

Figure 6. Logistic regression curve displaying relationship between temperature (°C) and fate (mortality or survival) of Alligator Gar following catch-and-release angling. Grey dashed lines represent the 95% confidence intervals.

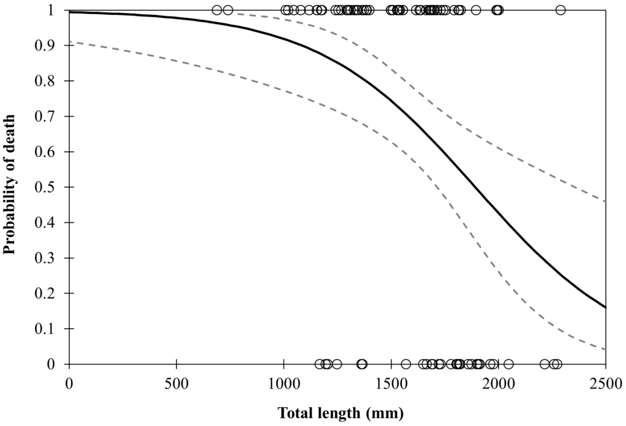

Figure 7. Logistic regression curve displaying relationship between total length (mm) and fate (mortality or survival) of Alligator Gar following catch-and-release angling. Grey dashed lines represent the 95% confidence intervals.

externally. Mortality associated with hooking location varied and was significantly different ($X^2 = 6.971$, df = 2, $P = 0.03$). Mortality rate of stomach-hooked Alligator Gar was significantly different than the morality rate of fish hooked in the esophagus and mouth (Figure 5b). The one Alligator Gar that died from hooking-related injuries was caught in the stomach at a depth of 267 mm. Additionally, a significant difference was detected between hooking depth and anatomical hooking location ($F_{2, 13} = 22.189$, $P < 0.01$; Figure 4b). Alligator Gar hooking depth for stomach- and esophagus-hooked fish were not significantly different, but differed from fish hooked in the mouth.

Comparison between gears

The logistic regression model indicated that temperature, total length and hooking depth (% of TL) significantly affected survival of Alligator Gar, but gear type (rod-and-reel or juglines) did not (Table 2). Mortality occurred more frequently at higher temperatures (Figure 6), with shorter Alligator Gar (mm TL; Figure 7), and greater hooking depths (% of TL; Figure 8). For every 1°C increase in water temperature, mortality was 1.23 times more likely (odds ratio 1.227). For every 1 mm increase in total length, Alligator Gar mortality decreased by one percent (odds ratio .0994). Hooking depth (% of TL) had the greatest effect on fate of

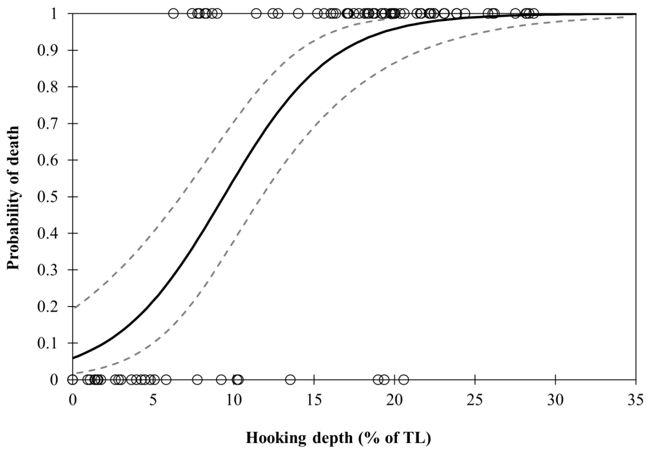

Figure 8. Logistic regression curve displaying relationship between hooking depth and fate (mortality or survival) of Alligator Gar following catch-and-release angling. Grey dashed lines represent the 95% confidence intervals.

caught-and-released Allgator Gar, which were 1.57 times more likely to die for every one percent increase in hooking depth (odds ratio 1.570). For example, the probability of death increases to ≥50% when hooking depth is ≥10% of Alligator Gar TL. When hooking depth is ≥30% of Alligator Gar TL mortality is 100%. The logistic regression line never reaches a probability of death equaling 0% for total length and hooking depth (% of TL), indicating that low rates of fish mortality are possible regardless of total length or hooking depth.

Discussion

Our results suggest that catch-and-release mortality rates of Alligator Gar can be very high, but depends upon hooking depth (% of TL), hooking location, total length of fish and water temperature. We found that hooking mortality associated with juglines (81%) was substantially higher than with rod and reel (6%), and appeared to be a function of hooking depth (hooking depth = 16.4% of TL for juglines vs. 5.5% of TL for rod and reel). Although both are passive angling approaches, there are differences between these methods that may explain the disparities in observed mortality rates. Juglines were set overnight, whereas rod and reel angling occurred over a shorter duration and employed a more immediate hook set. Bartholomew and Bohnsack (2005) found that passive fishing approaches, such as juglines, results in higher morality than active fishing methods (rod and reel with lure). While fishing with rod and reel, we allowed Alligator Gar to pick up the bait, swim until they stopped to orient the bait in their mouth, and the hook was set when the fish began swimming again. The longer duration that juglines are fished compared to rod and reel may explain why the majority (86%) of Alligator Gar caught with this gear were hooked in the esophagus or stomach (more time to consume and digest the bait). However, the majority of Alligator Gar caught using rod and reel were hooked in the mouth (75%). Differences in hooking locations, and associated hooking depths, have been observed to influence mortality rates.

The anatomical location of a hooking wound is often determined by bait type and the manner in which the bait is ingested (Muoneke and Childress 1994, Tomcko 1997, Bartholomew and Bohnsack 2005). Fishing with natural baits increases the risk of deep hooking, because fish voluntarily ingest the bait (Daugherty and Bennett 2019). Alligator Gar anglers use natural baits (cut or whole fish) when fishing with juglines or rod and reel, which we found can result in deep hooking and increased mortality rates. Similarly, Wilde et al. (2000) found

Striped Bass (*Morone saxatilis*) caught with live bait were more likely to be deep hooked, which resulted in increased mortality. Payer et al. (1989) compared mortality rates of Walleyes (*Sander vitreus*) caught using different bait types and found artificial lures tended to catch fish in the mouth and resulted in low mortality, whereas fish caught with natural baits were hooked deeper (esophagus or stomach) and had higher mortality rates. In Northern Pike (*Esox lucius*), Tomcko (1997) found when fish consumed natural baits they were generally hooked deeply, and that these wounds inflicted internal damage that resulted in greater hooking mortality. In this study, Alligator Gar mortality was high (particularly for jugline-caught fish) when hooked in the esophagus or stomach, and the proximity of these hooking injuries to vital organs likely resulted in the high observed mortality rates.

During necropsies of deceased Alligator Gar, we observed that fish hooked in the esophagus or stomach often had lacerations or punctures to adjacent organs. Reeves and Bruesewitz (2007) documented high mortality rates of Walleye when deep hooking wounds resulted in damage to major internal organs. Similarly, Loftus et al (1988) reported high mortality rates (71.4%) of Lake Trout (*Salvelinus namaycush*) when hooking wounds affected vital organs. Alligator Gar hooked in the esophagus often experienced injuries to the heart or surrounding vasculature and those hooked in the stomach often sustained injuries to the swim bladder and liver. In cases where the liver was affected, the hepatic vein and other vasculature was typically lacerated. Injuries to the heart or liver vasculature resulted in rapid mortality, often occurring within 24 h, which has been documented for numerous fish species when suffering similar injuries (Muoneke and Childress 1994).

Mortality of Alligator Gar was considerably slower when they suffered lacerations to the swim bladder. Difference in time to death between these injuries is likely due to the mechanism causing death (hemorrhaging vs. drowning). We observed Alligator Gar with lacerated swim bladders struggling to maintain buoyancy and equilibrium, and these fish were usually unable to rise to the water surface to breathe. The effects of swim bladder injuries may be intensified at higher water temperatures, as Alligator Gar become obligate air breathers with increasing water temperature (Rahn et al. 1971). When water temperatures warm (> 22°C), Alligator Gar transition to almost exclusively breathing atmospheric oxygen by consuming air at the water surface, which is processed by a highly vascularized swim bladder (Rahn et al. 1971). When fish were able to surface for air, consumed air appeared to enter the peritoneal cavity and was released through the vent or mouth without being processed. Upon dissection, we also determined that the peritoneal cavity of these fish was filled with water. The combination of these factors likely resulted in the fish drowning, which occurred more often when higher water temperatures increased the need for Alligator Gar to surface breathe. This likely explains why mortality rates increased with increasing water temperature in this study.

The relationship between water temperature and hooking mortality rates has been well described for other fish species (Muoneke and Childress, 1994; Bartholomew and Bohnsack 2005). For example, Titus and Vanicek (1988) reported that hooking mortality of Cutthroat Trout (*Oncorhynchus clarkia*) was low (2%) when water temperatures were cool (< 17°C), but increased dramatically (49%) when water temperatures were warmer (~21°C). Similarly, hooking mortality of cool water species (tiger muskellunge [*Esox lucius* × *E. masquinongy*] and Walleye) increases with warmer water temperatures (Newman and Storck 1986, Fielder and Johnson 1994, Reeves and Bruesewitz 2007). Further, seasonal effects on hooking morality (higher mortality rates in summer than winter because of higher water temperature) has been described for several warm water species (Striped Bass; Hysmith et al. 1994, Bettoli and Osborne 1998 and Blue Catfish and Flathead Catfish [*Pylodictis olivaris*]; Muoneke 1993, Schmitt and Shoup 2013). Our finding that warmer water temperatures resulted in higher mortality rates is concerning, because these angling techniques are typically conducted during summer months when water temperatures are the highest.

We found that mortality decreased with increasing size of Alligator Gar in this study. Previous studies evaluating hooking mortality of large-bodied fish have determined that handling times increase with increasing fish size, which results in higher angling mortality rates (Weithman and Anderson 1976, Muoneke and Childress 1994, Bartholomew and Bohnsack 2005). Because of their large size, we secured a rope around the body of Alligator Gar (usually behind the pectoral fins) following capture to aid in lifting the fish onto the boat (similar to methods used by anglers). Once on the deck of the boat, the weight of the fish may press on the hook or hooking wound, causing additional injuries. The combination of the rope constriction and body weight of the fish applying pressure on the hook inside of the fish may have contributed to the high mortality rates we observed for Alligator Gar caught on juglines. Additionally, Alligator Gar caught on juglines could have been hooked for up to 12-h depending when the bait was taken, and these fish required longer handling times (compared to those caught on rod and reel). Both stressors could have affected hooking mortality rates. Although not tested, it appears that handling stress could be reduced if Alligator Gar were not removed from the water, which could reduce mortality rates.

This study is the first to provide information regarding hooking mortality rates and associated factors affecting mortality of Alligator Gar, resulting in important management implications for this species. The mortality rates of jugline-caught Alligator Gar that we observed is concerning. Therefore, biologists tasked with managing recreationally exploited Alligator Gar populations should evaluate the potential population-level effects of high fishing mortality rates associated with juglines. Additionally, fisheries managers using juglines to sample Alligator Gar populations should consider alternate sampling methods, because mortality associated with this technique may be unacceptably high and could bias study results. For example, if estimating population size of Alligator Gar with juglines and tagged fish die due to high catch and release mortality rates, the population estimate could be artificially high since fewer tagged fish can be recaptured. It is difficult for us to make decisions about rod and reel angling based on the limited results of this study. While this study is an important first step to understand mortality associated with rod and reel angling, it was conducted in ponds using a small number of fish. We limited the number of fish used in this study due to the high mortality rates observed using juglines and because Alligator Gar are a species of concern in Oklahoma as a result of their limited range. Future rod and reel studies should be applied to wild Alligator Gar populations and consider the many variables that may affect catch-and-release mortality (bait type, fight time, fish size, handling methods and time, terminal tackle, and water temperature). Based on our results, fisheries managers should evaluate the potential effects of catch-and-release angling (juglines and rod and reel) on Alligator Gar populations in other states, as this species is sensitive in many areas of the native range.

Acknowledgments

We thank J. Bartinicki, M. Lyons, M. Waters and S. Jeter for assistance with field work. We thank K. Graves and staff at Tishomingo National Fish Hatchery for assistance with transporting and holding Alligator Gar. We thank Dr. Gary Wellborn and the OU Biological Station staff for allowing the use of their grounds and assistance with setup of holding pools. We thank K. Kuklinski (ODWC) and three anonymous reviewers from Texas Parks and Wildlife Department for reviewing an earlier draft of this manuscript. We thank Dr. D. Shoup (Oklahoma State University) and Dr. J. Long (Oklahoma State University) for statistical guidance and reviewing an earlier draft of this manuscript. Financial support was provided by U.S. Fish and Wildlife Service, Sportfish Restoration Grant F-86-D-1, F-50-R-25, and F-112-D-1 to the Oklahoma Department of Wildlife Conservation.

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Submitted September 30, 2021 Accepted November 2, 2021

Examination of the Current State Record River Carpsucker in Oklahoma

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Abstract: River Carpsucker (*Carpiodes carpio*) are found throughout Oklahoma and are recognized as a non-game species by the Oklahoma Department of Wildlife Conservation, which allows the species to be pursued by anglers using any method and without limits. The current state record River Carpsucker was harvested with bowfishing on May 30, 2021 from Fort Cobb Reservoir, Oklahoma, which we examined to estimate age, back calculate length-at-age (growth rates), and back calculate spawning year. Based on those analyses, we estimated this fish to be 14 years old with rapid growth (52.8% of total length) within the first 4 years, and then growth slowed with increasing age. Based on the estimated age of this fish, the state record River Carpsucker was spawned in 2007. This hatch year corresponds with flooding events that occurred across the state, and signifies the importance of river flow for successful spawning of this species. Even though this study is limited to a single specimen, it provides insight and adds knowledge to this understudied species in Oklahoma.

Introduction

The River Carpsucker (*Carpiodes carpio*) is one of three species of carpsuckers, including Quillback (*Carpiodes Cyprinus*) and Highfin Carpsucker (*Carpiodes velifer*), that are found in Oklahoma (Miller and Robison 2004). This species is widely distributed and abundant throughout Oklahoma's reservoirs, rivers, and streams. The native range of River Carpsucker includes the Great Plains from Texas to Montana, throughout the Mississippi drainage, and extends into northeastern Ohio (Miller and Robison 2004).

The River Carpsucker has received comparatively little attention by fisheries managers throughout its range compared to other fish species. This is driven by a negative connotation from anglers, and perceived as having little economic importance (Rypel et al. 2021). For example, in the 2019 Oklahoma angler survey, "carp" ranked 15 out of 18 for species most often targeted when fishing (York 2019). Further complicating the management of River Carpsucker is the limited and outdated literature for the species. For example, age and growth characteristics of this species is based on scales and fin rays (Buchholz 1957; Purkett 1958; Morris 1965; Stucky and Klaassen 1971; Hesse et al. 1978), which generally underestimate age when compared to otoliths (Quist et al. 2007, Muir et al. 2008, Grabowski et al. 2012). Authors who have studied River Carpsuckers have considered the species to be long-lived (Sandheinrich and Atchison 1986; Braaten and Berry 1997).

On May 30, 2021, a new Oklahoma state record (612 mm TL, 4.3 kg) River Carpsucker was harvested by an angler at Fort Cobb Reservoir, Oklahoma. This fish exceeded the weight of the previous River Carpsucker record by 272 g. The Oklahoma Department of Wildlife

Conservation (ODWC) requested to obtain this fish for examination because of the increase in popularity from anglers taking non-game fish coupled with previous literature claims that this species is long-lived. Our objective was to examine the new state record River Carpsucker to estimate age, evaluate growth rate through back calculated length-at-age, and estimate hatch year (i.e., back calculated) so that we can better understand the environmental conditions that contributed to production of this fish. We realize that this assessment is limited to a single individual but considered the opportunity to examine a large specimen would benefit future elucidation of this species' life-history.

Methods

On May 30, 2021, ODWC Oklahoma City region fisheries staff weighed (using a certified scale, A&D SK-20KZ, A&D Store, Inc., Wood Dale, IL) and measured (TL and girth; mm) the potential state record River Carpsucker. Meristic counts of lateral line scales, dorsal fin rays, anal rays, pectoral rays, pelvic rays, and gill rakers were taken to verify the species' identification (Pflieger 1997, Miller and Robison 2004). Following inspection, this fish was confirmed as the new state record River Carpsucker for Oklahoma.

Once certified, the fish was donated to the Oklahoma Fishery Research Laboratory (OFRL) in Norman, Oklahoma. The fish was dissected to determine sex, and to examine stomach contents. The ovaries were removed to determine number of eggs, and lapilli otoliths were removed for age estimation. We estimated egg abundance in both ovaries by taking 10 subsamples of 50 eggs (500 total), and then weighed and averaged the samples (0.80 g total weight; 0.08 g per sample). Then, using those metrics, we took the total weight of the egg mass (g), dividing by the mean subsampled weight (0.08 g), and multiplying by 50 eggs to get total eggs.

After removal, lapilli otoliths were cleaned of organic material and placed into an envelope to dry for a period > 24 hrs. After drying, lapilli otoliths were embedded in epoxy resin and cut in the sagittal plane (Secor et al. 1992). The otolith was then sanded wet with 2000 grit sandpaper and viewed in cross section under a dissecting microscope capable of 130X magnification. We estimated age by placing the otolith, polished side up, in a dish containing modeling clay and immersed the otolith in water (to reduce glare). We then illuminated the otolith by using a fiber-optic filament attached to an external light source, which enables the reader to manipulate the light angle to better enhance annuli marks. The annuli appeared as dark rings on a light background and were counted to assign an age estimate. Two independent readers evaluated both otoliths. If the estimate was not agreed on, then a concert reading was conducted to finalize an age estimate (Hoff et al. 1997). After age estimates were finalized, we used the Dahl-Lea method to back-calculate length-at-age to describe growth (mm; Quist et al. 2012). We then subtracted the final age estimate from the capture year to estimate hatch year.

Results and Discussion

The new state record River Carpsucker was a female that measured 612 mm TL, had a girth of 515 mm, and weighed 4.3 kg. The two independent readers estimated the age of this fish to be 13 and 14 years-old, respectively. The agreed consensus age of this fish was 14 years old (Figure 1), which is older than prior studies age estimates ranging from 8-9 years old. However age estimates for the previous studies were based on dorsal fin rays, age 9 (Braaten et al. 1999), and scales, age 8 (Stucky and Klaassen 1971).

During the necropsy, we found that the stomach was empty, and that the specimen likely had not spawned. The egg mass from both ovaries weighed 750.5 g and accounted for 17.5% of the total weight of the fish. We estimated the total number of eggs contained by this River Carpsucker to be 469,063. Based on an equation developed by Behmer (1969), our estimate was 161,642 eggs higher than expected. However, Behmer (1969) suggested that any estimates derived from this equation would be conservative because River Carpsucker have

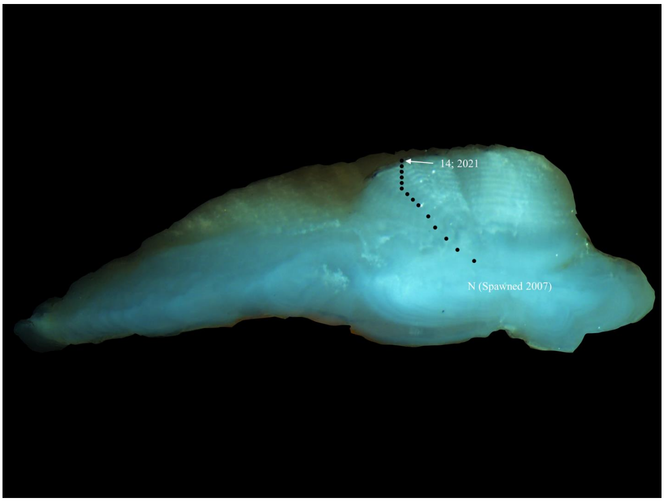

Figure 1. Photograph of a sectioned lapilli otolith from the current state record River Carpsucker (age 14) caught on 30 May 2021. ● = indicate annuli that reflect the age and year (age; year) on photograph.

multiple spawning events in a season (Behmer 1967).

Our evaluation is one of few to present growth information for River Carpsucker using otoliths. River Carpsucker have been found to grow rapidly during their first 3-4 years of life based on scale derived age estimates (Stucky and Klaassen 1971; Hesse et al. 1978). Similarly, back-calculated lengths-at-age in this study suggest that this River Carpsucker grew 52.8% of its TL during the first 4 years of life and 81.6% of TL by age 9. Even though growth slowed with age it never plateaued (Figure 2), suggesting this species can live longer than 14 years, as has been reported for other species in the family Catostomidae. For example, Bigmouth Buffalo (*Ictiobus cyprinellus*), have been observed to reach 112 years old (Lackmann et al. 2019), Blue Sucker *Cycleptus elongates*, to 42 (Radford et al. 2021), Quillback (*Carpiodes Cyprinus*) to 52 years; (Lackmann et al. 2019), Razorback Sucker (*Xyrauchen texanus*) to 44 (McCarthy and Minckley 1987), and Smallmouth Buffalo (*Ictiobus bubalus*) to 62 years of age (Snow et al. 2020).

We were able to back-calculate the hatch year to 2007 (Figure 1). In that year, heavy precipitation and severe flooding impacted Oklahoma during late spring and again in the summer when the remnants of Tropical Storm Erin affected the state (Dong et al. 2011). A total of 87.12 cm of rain fell at the Ft. Cobb Mesonet station from May through August, 8.2 cm above the average amount that the region typically receives over an entire year (Mesonet.org). Also, the top of conservation pool elevation of Ft. Cobb lake is 409 m and during the period of June-August the lake maintained an average elevation of 411.1 m meaning the lake was 2.03 m higher than normal (US Army Corps of Engineers 2007). River Carpsuckers journey up rivers and streams in large schools to spawn over soft substrata (Etnier and Starnes, 1993) during flooding events in late spring/early summer (May through June; Miller and Robison 2004, Catalano and Bozek 2015). Presumably, the extreme floods during the summer months of 2007, allowed young-of-year to utilize inundated floodplain habitat for nursery cover, resulting in high juvenile survival and successful recruitment.

Although this study is limited to one specimen, it adds to the growing knowledge of this species based on the largest individuals available for study. Additionally, it provides a need to better understand the basic ecology of this species throughout Oklahoma's reservoirs, rivers, and streams. Currently ODWC allows unlimited take and any method on River

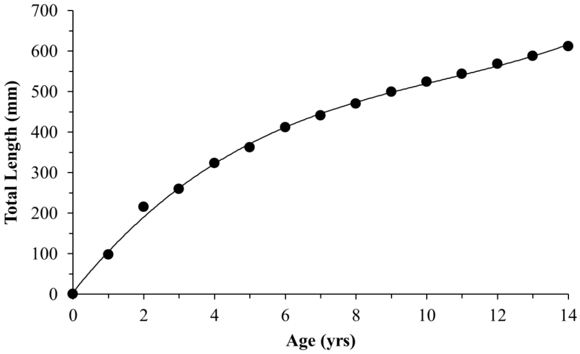

Figure 2. Back-calculated total length-at-age (mm) for the state record Smallmouth Buffalo captured at Fort Cobb Reservoir in Oklahoma.

Carpsucker, however future changes may be needed to further protect this species. We hope this information will support a better understanding of River Carpsuckers natural history and assist with future conservation and management of this species.

Acknowledgments

The authors thank Jaci Williams for her willingness to donate the state record River Carpsucker to ODWC. A special thanks to the Oklahoma City Regional office for collection of information and storage of the specimen. We thank Kurt Kuklinski (ODWC) and Dr. James Long (OSU) for reviewing an earlier draft of this manuscript and providing comments that greatly improved this manuscript. We also thank Dr. Mostafa Elshahed and anonymous reviewers for providing edits that improved this manuscript. Financial support was provided by the U.S. Fish and Wildlife Service Sportfish Restoration Grant F-86-D-1and F-66-D-8 to the Oklahoma Department of Wildlife Conservation.

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Submitted September 30, 2021 Accepted October 11, 2021

Validation and Timing of Annulus Formation in Sagittal Otoliths of Alligator Gar

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Abstract: Successful management of fish populations relies on accurate age estimates for calculation of age-based population parameters. Otoliths are considered the most accurate and reliable structure for estimating age of most fish species. Validation studies should be conducted for populations or species that have not been evaluated to ensure otoliths can be aged reliably. Marginal increment analysis is a technique that can be used to validate annulus formation in otoliths. Our objective was to validate that a single annulus was formed yearly and to determine the timing of annulus formation in the sagittal otoliths of Alligator Gar. A total of 152 Alligator Gar were collected from Texoma Reservoir, Oklahoma for marginal increment analysis. We found that a single opaque band (annulus) formed yearly in otoliths of Alligator Gar. Annulus formation occurred during May and the completed band was evident in June. Findings from this study suggest that sagittal otoliths can be used reliably for aging Alligator Gar, since a single annulus forms yearly in this structure. Furthermore, collection of Alligator Gar for age estimation should occur after May to minimize age estimation errors caused by annulus formation timing.

Introduction

Characterizing growth, mortality, and recruitment of fish populations relies on accurate age estimates (Campana 2001). An assumption of fish age estimation is that counted annuli correspond with the number of years since hatch (Buckmeier et al. 2017). Otoliths have been validated for many fish species, and therefore, are typically considered the most reliable and accurate aging structure (Spurgeon et al. 2015). Sagittal otoliths of Alligator Gar (*Atractosteus spatula*) have been validated by injecting fish with oxytetracycline to create a distinct time stamp on the otolith and then counting the number of rings formed post-injection (Buckmeier et al. 2012). However, identification of the annulus was sometimes difficult due to false annuli (growth checks) formation in otoliths of young fish and the constriction of annuli (reduced growth) in otoliths of older fish (Buckmeier et al. 2012). Misinterpretation of annuli can result in inaccurate age estimates, which can produce erroneous age-based population parameters and lead fisheries biologists to make incorrect management decisions (Lai and Gunderson 1987, Beamish and McFarlane 1995, Porta et al. 2018).

Marginal increment analysis is an alternate technique for validating annulus formation in otoliths and has been used to validate that aging structures produce a single annulus yearly and to determine the time of year to sample fish for collection of aging structures (Buckmeier et al. 2017, Porta and Snow 2018, Snow et al. 2018). This method relies on measurements of the translucent zone of the otolith, that when plotted against the month that fish were collected, exhibits an annual sinusoidal pattern where the smallest point (slowest growth) on the curve represents the month that the annulus is formed (Okamura et al. 2013). Our objective was to validate that a single annulus forms yearly in Alligator Gar otoliths collected from Texoma Reservoir, Oklahoma. Additionally, because the timing of annulus formation can differ by fish age, we sought to determine the timing of annulus formation for young (ages 1-15) and older (ages 16-49) Alligator Gar.

Methods

Alligator Gar used for marginal increment analysis were collected via angler donations and direct sampling from Texoma Reservoir during 2017 - 2020. Fish were processed at the Oklahoma Fishery Research Laboratory in Norman, Oklahoma where they were measured for total length (TL, mm) and sagittal otoliths were removed. Following removal, otoliths were cleaned, placed into a small envelope, and allowed to dry for at least 2 weeks prior to processing.

Sagittal otoliths were processed following the methods of Buckmeier et al. (2012). In short, otoliths were ground on a plane perpendicular to the anterior–posterior axis using a rotary tool fitted with a grinding bit (Dremel, Racine WI). The rotary tool was secured to a laboratory bench using a vice. Forceps coated in a rubberized tool dip (Plasti Dip International, Blaine MN) were used to securely hold the posterior portion of the otolith during processing. Otoliths were polished using wetted 2000 grit sandpaper to smooth the surface of the otolith. Age estimation was accomplished by standing the otolith polished side up in a glass dish containing black modeling and the otolith was submerged in water to improve clarity. Otoliths were viewed with a variable-power Olympus SZX16 stereomicroscope capable of 130× magnification (Olympus Corporation, Lake Success, New York), and a single strand fiber-optic filament attached to an external light source was shined through the otolith to illuminate the annuli. An age estimate was assigned to each Alligator Gar by two independent readers. In cases where readers disagreed on an age estimate, a concert read was conducted by both readers and a final age was assigned.

Marginal increment analysis was performed by measuring the width of the hyaline zone following the most recently deposited opaque band (annulus) on the ventral edge of the otolith (Clayton and Maceina 1999, Blackwell and Kaufman 2012, Porta and Snow 2017, Snow et al. 2018). Three measurements were taken at similar points along the ventral edge of each otolith and the average of the three measurements was used as the marginal increment distance for each fish. Otoliths were measured (mm) using an Olympus DP74 digital camera attached to the stereomicroscope and cellSens entry imaging software (Olympus Corporation, Lake Success, New York). Marginal increment measurements were separated into two age groups, young fish (ages 1-15) and older fish (ages 16-43), and the mean increment distance was graphed by month. Marginal increment data were evaluated by age classes to determine if timing of annulus formation varied with age.

Results and Discussion

A total of 152 Alligator Gar ranging 590 - 2,310 mm TL were collected for marginal increment analysis (Figure 1). Alligator Gar age estimates ranged from 1 to 43 years old, which resulted in 107 fish in the young age group and 45 fish in the older age group. The marginal increment measurements indicated that a single annulus was formed yearly during May in the sagittal otoliths of Alligator Gar from Texoma Reservoir for both age classes (Figure 2). An annulus was fully completed in June, as indicated by deposited translucent material beyond the annulus. The marginal increment method has also been used to validate annulus formation in otoliths of Bluegill (*Lepomis macrochirus*; Hales and Belk 1992), Gizzard Shad (*Dorosoma cepedianum*; Clayton and

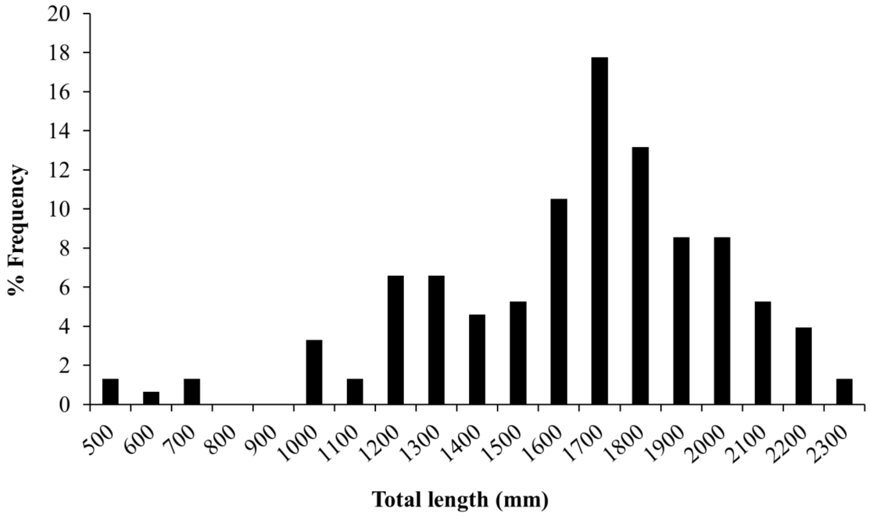

Figure 1. Length frequency distribution of Alligator Gar collected from Texoma Reservoir, Oklahoma for marginal increment analysis

Maceina 1999), Redband Trout (*Oncorhynchus mykiss gairdneri*; Schill et al. 2010), Saugeye (*Sander vitreus and S. Canadensis*; Snow et al. 2018), Spotted Suckers (*Minytrema melanops*; Strickland and Middaugh 2015), White Perch (*Morone Americana*; Porta and Snow 2017), and Yellow Perch (*Perca flavescens*; Blackwell and Kaufman 2012).

A single annulus formed in otoliths of young and old Alligator Gar. Formation of a single annual mark in the sagittal otoliths of Alligator Gar validates this structure for aging this species. Other methods, including oxytetracycline marking (Buckmeier et al. 2012, Buckmeier and Smith 2020) and bomb radiocarbon dating (Daugherty et al. 2020) have been used to validate sagittal otoliths of Alligator Gar to age-31 and > age-60, respectively. Buckmeier et al. (2012) found that false annuli are sometimes formed in Alligator Gar sagittal otoliths, therefore we utilized the marginal increment measurement technique because this method can identify the presence and timing of false annuli formation (Snow et al. 2018). False annuli were not observed in the sagittal otoliths of Alligator gar from Texoma Reservoir, suggesting that this structure can be used reliably to assign age estimates to fish in this population since only one opaque band (annulus) forms yearly.

Annulus formation occurred in the sagittal otoliths of Alligator Gar during May for both age classes, and annual marks were apparent by June. Buckmeier et al. (2012) observed annulus formation in sagittal otoliths of Alligator Gar in May. Buckmeier and Smith (2020) determined that annulus formation in sagittal otoliths of juvenile Alligator Gar was completed in June, but could not be observed until additional material was added to the otolith beyond the newly formed annulus. Similarly, we could not discern the annulus from the edge of the otolith until translucent material associated with new growth had been deposited beyond the annulus, which occurred after May. Therefore, we recommend that Alligator Gar should be collected for aging purposes during summer following formation of the annulus. Collection during this time will reduce age estimation errors caused by the timing of annulus formation. This study adds to the body of literature confirming annulus formation in the sagittal otoliths of Alligator Gar. Additionally, this study provides fisheries managers with information regarding the timing of annulus formation, which is important when collecting Alligator Gar to understand their population dynamics.

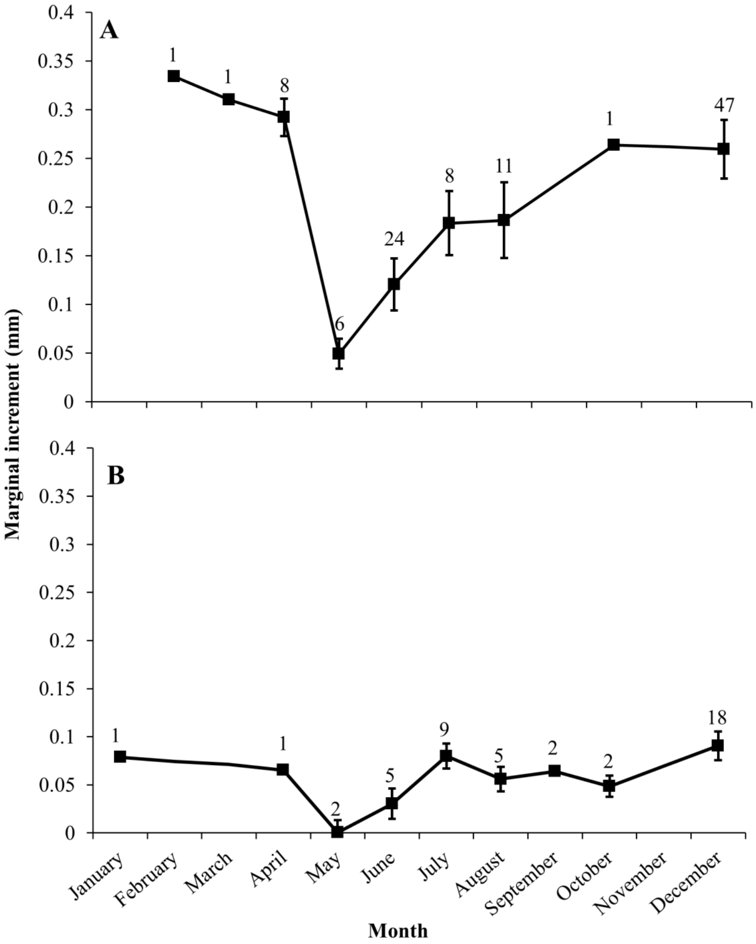

Figure 2. Mean monthly marginal increment measurements for (A) young Alligator Gar (ages 1-15) and (B) older Alligator Gar (ages 16-43) using sagittal otoliths. Error bars represent the standard error of the mean. Numbers above each point represent the monthly sample size.

Acknowledgments

We thank M. Lyons, S. Jeter, S. O'Donnell, M. Waters and SCR staff for assisting in field sampling and lab work for this project. We thank Kurt Kuklinski (ODWC) and Dr. Daniel Shoup (Oklahoma State University) for reviewing an earlier draft of this manuscript. We thank Dr. Mostafa Elshahed and anonymous reviewers for providing edits that improved this manuscript. Financial support for this publication was provided by the Sport Fish Restoration Program grant [F-50-R-25] and [F-86-D-1] to the Oklahoma Department of Wildlife Conservation.

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Submitted October 6, 2021 Accepted November 2, 2021

Scanning Electron Microscopy of the Gonopods of the Milliped, *Thrinaxoria lampra* (Diplopoda: Polydesmida: Xystodesmidae)

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Abstract: Although the gonopods of the xystodesmid milliped, *Thrinaxoria lampra*, have been described via light microscopy and line drawings provided, nothing is known concerning their ultrastructural detail. The gonopods and gonopodal aperture of two male specimens collected in January 2005 from Bowie County, Texas, were examined using scanning electron microscopy (SEM). Compared to previously published line drawings of *T. lampra*, finer detail was observed on the solenomeres and tibial processes using SEM. As such, we present new ultrastructural data on the gonopods of *T. lampra*.

Introduction

The xystodesmid flat-backed milliped, *Thrinaxoria lampra*, was described as *Fontaria lampra* by Chamberlin (1918) from Creston, Natchitoches Parish, Louisiana. The range of *T. lampra* comprises two allopatric populations; one has widely separated localities extending from west-central Tennessee to southwestern Alabama, and the other, which is continuous, covers an area of approximately 176 km N/S and 440 km E/W extending from the vicinity of Texarkana, Arkansas/Texas, to central Sabine Parish, Louisiana, and from Longview, Gregg County, Texas, to northern Natchitoches Parish, Louisiana (Shelley 1984; Shelley and McAllister 2006).

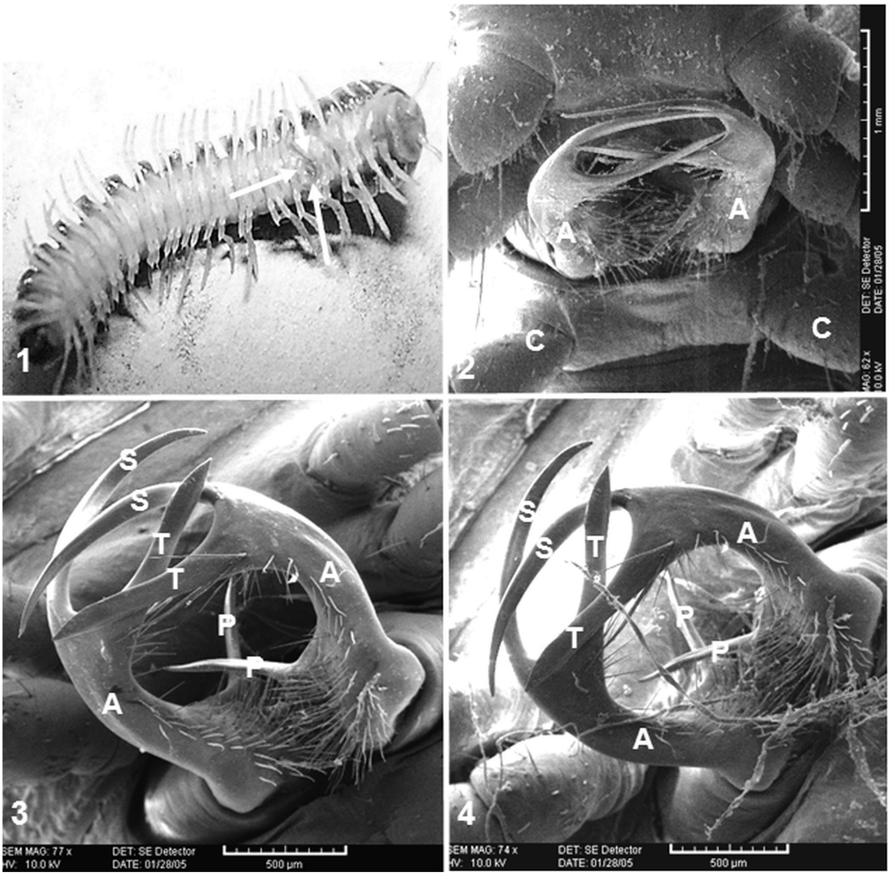
Detailed information on the ecology and geographic distribution of *T. lampra* is available (Chamberlin 1918; Chamberlin and Hoffman 1950; Shelley 1984, 1990; McAllister et al. 2002; Marek and Bond 2006; Shelley and McAllister 2006). Its modified male sexual organs or gonopods are specialized for copulation and are very important taxonomically by aiding in specific identification of milliped taxa. The gonopods of *T. lampra* occur around the seventh segment (body ring). In his original description, Chamberlin (1918) provided a description of the gonopods of *T. lampra* (as *F. lampra*) without providing any figures. Chamberlin (1942, his fig. 7 as *Zinaria aberrans*), Shelley (1984, his figs. 24–26), and Shelley and McAllister (2006, their figs. 7–8) included line drawings of the gonopods of specimens from Caddo County, Louisiana, and Henderson and Harrison counties, Texas. However, nothing is available of the ultrastructure of gonopods of *T. lampra*. Here, we provide, for the first time, information on the gonopods of *T. lampra* using scanning electron microscopy (SEM).

Methods

Two adult male *T. lampra* (total length = 35–36 mm) were collected by hand in January 2005 from decaying hardwood logs in deciduous forest habitat along county road 1370 in Bowie County, Texas (33°22’30.1512”N, -94°04’44.8206”W). Specimens were preserved in 10% neutral-buffered formalin and processed for SEM. They were dehydrated in a graded series of ethanols (70–100% [v/v]), transferred to amyl acetate transition solvent, critically point dried with a Autosamdri®–815 critical point drier (Tousimis Research Corporation, Rockville, MD; 31◦C, 1072 psi, ventilation rate ~100 psi/min), coated with a gold-palladium with a Cressington sputter coater (Cressington Scientific Instruments Ltd, Watford, UK), and viewed with a Vega TS 5136XM digital scanning SEM (Tescan USA Inc., Cranberry Township, PA) at an accelerating voltage of 20 kV. Voucher specimens are deposited in the North Carolina State Museum (NCSM), Raleigh, North Carolina.

Results and Discussion

In *T. lampra*, the modified legs on the seventh segment (body ring) are withdrawn into a pouch on the body (Fig. 1) and as such, the specimen appears to be lacking legs on the seventh ring. Ultrastructural characters of the gonopods and gonopodal aperture (Figs. 2–4) are as



Figures 1-4. *Thrinaxoria lampra*. 1. Stereoscopic view of venter showing location of gonopods in situ on body ring seven (arrows). 2. Ventral view of SEM of gonopods in situ; scale bar = 1 mm. 3. Higher magnification SEM of left and right gonopods; scale bar = 500 µm. 4. Another SEM view of left and right gonopods; scale bar = 500 µm. Abbreviations: A (acropodite), C (coxa), P (prefemoral process), S (solenomere), T (tibial process).

follows: gonopodal aperture ovoidal without anteriolateral depressions; the acropodites project anteriad and bending mediad overlapping in mid-length; the tips project just beyond the anterior margin of the aperture; solenomore and tibial process arising from acropodite, directed and curving keenly caudad; solenomere pointed and acutely sharp; tibial process pointed but not as sharp, and the prefemoral process is short but thin and spikey; scattered hairs distributed from base of acropodites to near the base of the telopodite.

In comparison, Shelley and McAllister (2006) provided information via light microscopy on the morphology of the left gonopod of *T. lampra* as follows: the solenomere and tibial process arise from the acropodite, directed or curving caudad; the acropodite is deeply cleft apically; and hairs extend from the base of the acropodite to the beginning of the tibial process. In our SEMs (figs. 3–4), finer detail is observed on the solenomeres and tibial processes. The line drawings of Shelley and McAllister (2006) reveal solenomeres and tibial processes that come to more of a broader blunt, rather unsharpened appearing point.

In summary, SEM micrographs of *T. lampra* are provided for the first time. Comparison with descriptions from light microscopy and line drawings show some important differences *vs.* the current SEMs. We suggest using caution in taxonomic descriptions and especially use of gonopod line drawings if SEM showing their ultrastructure is not readily available.

Acknowledgments

The Texas Parks and Wildlife Department issued a Scientific Collecting Permit to CTM.

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Submitted October 11, 2021 Accepted November 28, 2021

An Annotated Checklist of the Millipeds (Arthropoda: Diplopoda) of Oklahoma

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Abstract: Although a great deal of information has been reported over the last two decades on millipeds (Diplopoda) of Oklahoma, no comprehensive surveys have been published, and records of those from the state, particularly those of older records, are scattered throughout the myriapod literature. Here, we provide an annotated checklist of millipeds of Oklahoma reporting 33 species, representing 16 families within eight orders. This contribution is meant to be an initial effort and additional fieldwork will certainly provide new geographic records of additional species in the state.

Introduction

Worldwide, the number of described milliped species has exceeded 12,000 from a global fauna that is estimated, based on known degrees of endemism, to contain around 80,000 species, making the Diplopoda the fourth largest class in the phylum Arthropoda and the most speciose class in the subphylum Myriapoda (Sierwald and Bond 2007). They represent the major component of terrestrial ecosystems throughout the temperate and tropical zones where they constitute a major component of the soil-litter macrofauna. Therefore, knowledge of which species occur geographically is fundamental to conservation and ecological efforts, and documentation of their overall range in various U.S. states.

Early on, studies concerning diplopods were mostly neglected in Oklahoma, and the earliest treatment, primarily restricted to the northeastern part of the state, was documented in the early 1930s through the late 1950s by Ralph Vary Chamberlin (Chamberlin 1931, 1940, 1943) and Nell Bevel Causey (Causey 1950, 1951, 1952, 1954, 1959). One report appeared in the 1960s when Branson (1961) provided records of four species of millipeds from the University of Oklahoma Biological Station (Marshall County). Since then, taxonomy of millipeds has undergone major revisions. For example, main contributions and revisions by William Shear (Shear 1972, 2003, 2010) of members of the Chordeumatida, as well as Rowland Shelley's comprehensive studies and monographs of several major North American milliped groups, including *Pleuroloma* (Shelley 1980), *Auturus* (Shelley 1982), Abacionidae (Shelley 1984), Eurymerodesmidae (Shelley 1990), Polyzoniidae (Shelley 1998), Desmonidae (Shelley 2000b), Parajulidae (Shelley 2000a), *Oriulus* (Shelley 2002), *Brachycybe* (Shelley et al. 2005b), *Scytonotus* (Shelley 2005a), and *Narceus* (Shelley et al. 2006) provided additional records for Oklahoma millipeds.

Interestingly, a significant number of Oklahoma milliped species are known either as troglophiles or troglobites from caves (Shear 1972, 2003, 2010; Graening et al. 2011). For example, Harrel (1960, 1963) reported three milliped taxa from Wild Woman Cave, Murray County, Black (1971) reported records of several millipeds from additional caves of the state, and Lewis (2002) reported on *Chaetaspis* spp. from

Oklahoma caves.

Since the turn of the century, a great deal has been published on the state's millipeds (Lewis 2002; McAllister et al. 2002, 2004, 2005, 2013; McAllister and Shelley 2003, 2005, 2008, 2010; Shear 2003; Shelley et al. 2003, 2005a, 2005b, 2006; McAllister and Robison 2011, 2018; Lewis and Slay 2012; Robison and McAllister 2012; Shelley and Snyder 2012; McAllister and Connior 2016). Although a consolidated listing has never been prepared, we provide an initial list of the millipeds of the state, including a compilation of the milliped literature that cites specific county/state records of specimens from Oklahoma. In addition, this checklist specifically includes records of rarer millipeds from fragile, energy-limited cave environments of the state.

Methods

A review of the milliped literature was undertaken to provide a list of species known to inhabit Oklahoma. The late Richard Lawrence Hoffman, recognized as the world's leading authority of millipeds and his catalogue of North and Middle American Diplopoda (Hoffman 1999), served as a helpful resource although it is now more than two decades old. Another supportive resource was Millibase (http://www.millibase.org), an on-line database which covers the global milliped fauna. In addition, previous collection records by the authors of Oklahoma millipeds as well as continuing collections were used to develop this checklist. Collections were made in preferred shelters of deciduous hardwood forest using a potato rake by turning over decaying logs, moist leaves, rocks, and debris, as well as peeling off bark from trees.

Identification of millipeds were made initially by CTM and confirmed by the late RM Shelley (North Carolina State Museum [NCSM], Raleigh). All recent voucher specimens collected and reported for this project are deposited in the NCSM collection, the Sam Noble Oklahoma Museum of Natural History (SNOMNH), Norman, or the Florida State Collection of Arthropods (FSCA), Gainesville.

The checklist is arranged hierarchically for each taxon starting with the Order Polyxenida, then alphabetically by family and currently accepted scientific name. For each species, county records are provided as well as supplemental notes given as "remarks". Each entry is followed by synonyms and/or new combinations in chronological order. Taxonomy follows Hoffman (1999), Shelley (2003), and Sierwald and Speida (2021).

Results and Discussion

To our knowledge, all known published Oklahoma records are included in this checklist and it documents 33 species, representing 16 families in eight orders as follows:

CLASS DIPLOPODA DE BLAINVILLE IN GERVAIS, 1844
SUBCLASS CHILOGNATHA LATREILLE, 1802-1803
ORDER POLYXENIDA VERHOEFF, 1934
FAMILY POLYXENIDAE LUCAS, 1840

***Polyxenus fasciculatus* (Say, 1821) (syn. *Pollyxenus fasciculatus* Say, 1821; *Polyxenus fasciculatus* var. *pallidus* Ryder, 1878).**

Remarks: Say's (1821) original description simply states…"inhabits the southern states." However, according to Bollman (1893)…"its habitat is from Massachusetts to Indian Territory, and it does not seem to be found in the North Central States." Hoffman (1999) noted that this species occurs chiefly on the Coastal Plain from Maryland to Texas, north to Illinois; it is also recorded from Bermuda and the Canary Islands. We therefore tentatively include it herein for Oklahoma although there are apparently no specific locales.

ORDER PLATYDESMIDA COOK, 1895
FAMILY ANDROGNATHIDAE COPE, 1869

***Brachycybe lecontii* Wood, 1864 (syn. *Platydesmus lecontei* Bollman, 1888; *Brachycybe lecontei* Cook and Loomis, 1928; *Brachycybe lecontii* Gardner, 1975).**

Delaware, Le Flore, McCurtain (Black 1971; McAllister et al. 2002a; Shelley et al. 2005b; Brewer et al. 2012; McAllister and Connior 2016).

Remarks: Black (1971) reported *B. lecontii* from Bear Den Cave (Le Flore County). In their molecular analysis of the genus, Brewer et al. (2012) examined specimens of *B. lecontii* from Le Flore and McCurtain counties; their results had individuals more closely aligned with specimens in branch "LC4" from Arkansas and Missouri, and that they… "represent collectively a genetically divergent lineage." This species is a fungivorous social milliped known for paternal care of eggs and forming multi-generational aggregations (Wong et al. 2020).

ORDER JULIDA BRANDT, 1833

FAMILY BLANIULIDAE C. L. KOCH, 1847

***Brachyiulus lusitanus* Verhoeff, 1898 (syn. *Brachyiulus* (*Microbrachyiulus*) *pusillus lusitanus* Verhoeff, 1898; *Microbrachyiulus lusitanus* Verhoeff, 1910; *Microbrachyiulus calcivagus* Verhoeff, 1910; *Brachyiulus pusillus* Shelley, 1978).**

Cleveland (McAllister and Robison 2018).

Remarks: This is an introduced species whose native range includes the Caucasus and Mediterranean regions, the Azores, and Canary Islands; it is also introduced in North Carolina and California (Hoffman 1999).

***Nopoiulus kochii* (Gervais, 1847) (syn. *Julus pulchellus* C. L. Koch 1838; *Blaniulus venustus* Meinert, 1868; *Iulus kochii* Gervais, 1847; *Nopoiulus* (*Nopoiulus*) *kochii* Enghoff, 1984).**

Latimer (McAllister and Robison 2018).

Remarks: This is another introduced species widely distributed in both Old (Asia and Europe) and New World localities, including northeastern North America (Nova Scotia, Canada, south to Virginia) and in Washington state (Hoffman 1999; Enghoff and Kime 2005).

***Virgoiulus minutus* (Brandt, 1841) (syn. *Julus pusillus* Say, 1821; *Julus minutus* Brandt, 1841; *Nopoiulus minutus* Brandt, 1841; *Julus sayi* Newport, 1844; *Julus lineatus* McNeill, 1887; *Nopoiulus minutus* Chamberlin, 1922; *Virgoiulus minutus* Enghoff, 1984).**

McCurtain (McAllister et al. 2005).

Remarks: This milliped was found by CTM underneath peeled decaying pine bark in Beaver's Bend State Park (McAllister et al. 2005), which is typical microhabitat of *V. minutus*.

FAMILY PARAJULIDAE BOLLMAN, 1893

***Aliulus caddoensis* Causey, 1950**

Caddo (type locality), Latimer, Le Flore, Murray (new record) (Causey 1950; McAllister and Shelley 2003).

Remarks: Causey (1950) did not provide a specific type locale in Caddo County for the holotype but two additional males were collected from Wilburton, Latimer County. In addition, CTM collected a male, a female, and 11 juveniles as well as two more males of *A. caddoensis* in Caddo County on 8 November 2003 from Cobb State Park and Red Rock Canyon State Park, respectively. Two males collected by CTM on 6 November 2005 from Turner Falls, Murray County, represent a new county record.

***Aniulus (Hakiulus) diversifrons diversifrons* (Wood, 1865) (syn. *Iulus sp.* Wood, 1864; *Iulus diversifrons* Wood, 1867; *Julus diversifrons* Preudhomme de Borre, 1884; *Parajulus castaneus* Bollman, 1887; *Parajulus diversifrons* Bollman, 1893; *Ethoiulus diversifrons* Chamberlin, 1931; *Hakiulus diversifrons* Chamberlin, 1940; *Hakiulus parallelus* Chamberlin, 1940).**

Caddo (new record), Canadian, Cleveland, Coal, Delaware, Hughes, Logan, McCurtain, Murray, Pittsburg (Chamberlin 1940; Causey 1953; Shelley 2000a; McAllister et al. 2013).

Remarks: Chamberlin (1940) described *Hakiulus parallelus* (now a synonym) from at an unspecified location in Cleveland County (Causey 1953; Shelley 2000a). The Caddo County specimen is a new county record collected on 8 November 2003 from Ft. Cobb State Park by CTM. The southeasternmost record for this milliped in the state is McCurtain County (McAllister et al. 2013).

***Okliulus carpenteri* Causey, 1950**

Latimer (type locality).

Remarks: This parajulid was originally described from Wilburton, Latimer County (Causey 1950); there are apparently no other records from the state.

***Oriulus venustus* (Wood, 1864) (syn. *Iulus venustus* Wood, 1864; *Parajulus venustus* Bollman, 1889; *Oriulus grayi* Causey, 1950; *Oriulus venustus* Chamberlin and Hoffman, 1958).**

Marshall, Muskogee, Pittsburg, Pottawatomie (Branson 1961; Shelley 2002).

Remarks: This is the most widespread native milliped species in North America (Shelley and Snyder 2012). This species is found in at least 34 states of the United States, stretching from Massachusetts to Montana, blanketing most of the continental United States in its range.

ORDER SPIROBOLIDA COOK, 1895
FAMILY SPIROBOLIDAE BOLLMAN, 1893

***Narceus americanus/annularis* complex (Palisot de Beauvois, 1817) (syn. see Keeton [1960] and Hoffman [1999] for extensive list of synonyms).**

Caddo, Choctaw, Comanche, Craig, Hughes, Le Flore, Marshall, McCurtain, Murray, Okmulgee, Osage, Pittsburg, Pushmataha, Wichita (Chamberlin 1931; Branson 1961; Keeton 1960; McAllister et al. 2002a, 2013; McAllister and Shelley 2003; Shelley et al. 2006).

Remarks: The synonym *Spirobolus oklahomae* Chamberlin, 1931, was synonymized by Keeton (1960) and described by Chamberlin (1931) from Murray County with additional samples from Pushmataha County. It was also reported by Black (1971) from the entrance of Bear Den Cave (Le Flore County). This commonly encountered milliped is found in every U.S. state east of the Mississippi River and nine states to the west (Shelley et al. 2006). The usage of "*Narceus americanus/annularis* complex" to represent species in the genus was recommended by Shelley et al (2006), and the complex is urgently in need of a molecular-based study to help organize the systematics of the group. This widely ranging species-complex contributes about two tons of frass/acre yearly to deciduous forests (Coville 1913).

ORDER SPIROSTREPTIDA BRANDT, 1833
FAMILY CAMBALIDAE BOLLMAN, 1893

***Cambala minor* Bollman, 1888 (syn. *Cambala annulata* (nec Say, 1821); *Cambala annulata* subsp. *minor* Bollman, 1888; *Cambala minor* Loomis, 1938; *Cambala minor* Loomis, 1943; *Cambala arkansana* Chamberlin, 1942; *Cambala cara* Causey, 1953).**

Adair, Sequoyah (Black 1971; Shelley 1979).

Remarks: This troglophilic milliped has been reported from Three Forks and Cottonwood caves, Adair and Sequoyah counties, respectively (Black 1971; Shelley 1979).

FAMILY SPIROSTREPTIDAE BRANDT, 1833

Orthoporus ornatus* (Girard, 1853) (syn. *Julus ornatus* Girard, 1853; *Spirostreptus montezumae* (nec DeSaussure) Bollman, 1888; *Orthoporus punctilliger* Chamberlin, 1923; *Orthoporus wichitanus* Chamberlin, 1931; *Orthoporus entomacis* Chamberlin and Muliak, 1941; *Orthoporus vallicolens* Chamberlin, 1943; *Orthoporus torreonus* Chamberlin, 1947; *Scaphiostreptus caperanus* Attems, 1950; *Orthoporus crotonus* Chamberlin, 1952; *Orthoporus arizonicus

Loomis, 1953).

Comanche (Chamberlin 1931).

Remarks: The synonym *Orthoporus wichitanus* was described by Chamberlin (1931) from Elk Mountain, Comanche County.

ORDER CALLIPODIDA POCOCK, 1894
FAMILY ABACIONIDAE SHELLEY, 1979

***Abacion tesselatum* Rafinesque, 1820 (syn. *Reasia spinosa* Sager, 1856; *Lisiopetalum eudasym* McNeill, 1887; *Lisiopetalum eudasum* McNeill, 1887; *Lisiopetalum eudasum* McNeill, 1888; *Callipus lactarius* Bollman, 1888; *Lysiopetalium rugulosum* Pocock, 1893; *Lysiopetalum lactarium* Packard, 1883; *Spirostrephon creolum* Chamberlin, 1942; *Platops rugulosa* Newport, 1944; *Spirostrephon lactarium* Johnson, 1954; *Abacion tesselatum tesselatum* Chamberlin and Hoffman, 1958; *Abacion tesselatum creolum* Chamberlin and Hoffman, 1958).**

Craig, Le Flore, McCurtain (Shelley 1984; McAllister et al. 2002a).

Remarks: *Abacion tesselatum* can be differentiated from *A. texense* by possessing a tibiotarsus with only a minute flexure *vs.* a tibiotarsus strongly reflected mediad apically in the latter (Shelley 1984).

***Abacion texense* (Loomis, 1937) (syn. *Lysiopetalum lactarium* Kenyon, 1893; *Spirostrephon texensis* Loomis, 1937; *Spirostrephon texense* Chamberlin, 1942; *Spirostrephon jonesi* Chamberlin, 1942; *Tynomma messicanum* Chamberlin, 1943; *Abacion texense* Chamberlin and Hoffman, 1958).**

Adair, Cherokee, Choctaw, Comanche, Ellis, Garfield, Kiowa, Latimer, Le Flore, Major, Mayes, McCurtain, Okmulgee (new record), Pittsburg, Pottawatomie, Sequoyah, Stephens (Chamberlin 1931; Chamberlin and Hoffman 1958, Loomis 1968; McAllister and Shelley 2010; McAllister et al. 2013).

Remarks: Black (1971) reported *A. texense* from Gittin' Down Mountain Cave (Adair County), Dressler Cave (Cherokee County), and Bear Den Cave (Le Flore County). The specimen collected by CTM on 11 September 2004 from Dripping Springs State Park, Okmulgee County, is a new county record.

ORDER CHORDEUMATIDA POCOCK, 1894
FAMILY CLEIDOGONIDAE COOK, 1896

***Tiganogona brownae* Chamberlin, 1928**

Murray (McAllister and Shelley 2005)

Remarks: This was only the second report of *T. brownae* since its description and the Murray County specimen collected by CTM is from the Arbuckle Mountains of southcentral Oklahoma, more than 765 km southwest of the type locality at St. Charles, St. Louis County, Missouri (Chamberlin 1928).

FAMILY TRICHOPETALIDAE VERHOEFF, 1914

***Trichopetalum uncum* Cook and Collins, 1895**

Muskogee (Shear 2010).

Remarks: The single record is a male from Dresser Cave, 8.0 km N of Ft. Gibson, Muskogee County (Shear 2010).

***Trigenotyla blacki* Shear, 2003**

Adair, Delaware (type locality) (Black 1971; Shear 2003; Robison and McAllister 2012).

Remarks: All specimens of the endemic *T. blacki* are from either Delaware County caves, including Bell's Bluff, Jail House, Stansbury-January, and Twin caves, or Cave #AD-14 (Adair County) (Shear 2003). It is considered the only true troglobitic milliped known from Oklahoma.

***Trigenotyla seminole* Shear, 2003**

Seminole (Black 1971; Shear 2003; Robison and McAllister 2012).

Remarks: The holotype was collected from Whiskey Cave, Seminole County; others were from Doolin and Cold Springs caves in same county (Shear 2003). It is an endemic species in the state (Robison and McAllister 2012).

***Trigenotyla vaga* Causey, 1959**

Johnston, Latimer (type locality), Le Flore, Murray (Causey 1959; Black 1971; Shear 2003; McAllister and Shelley 2003; Robison and McAllister 2012).

Remarks: The type specimen was described from a "river ravine" in Latimer County (Causey 1959), so it is impossible to know the exact type locality. Additional specimens have been reported from Wild Women Cave, Murray County (Shear 2003). It is another Oklahoma endemic milliped species (Robison and McAllister 2012).

ORDER POLYDESMIDA POCOCK, 1887
FAMILY SPHAERIODESMIDAE

***Desmonus pudicus* (Bollman, 1888) (syn. *Sphaeriodesmus pudicus* Bollman, 1888; *Desmonus pudicus* Cook, 1898; *Ethocyclus atophus* Chamberlin and Mulaik, 1941; *Desmoniella curta* Loomis, 1943; *Desmonus inordinatus* Causey, 1958; *Desmonus austrus* Causey, 1958: *Desmonus conjunctus* Loomis, 1959; *Desmonus crassus* Loomis, 1959; *Desmonus distinctus* Loomis, 1959; *Desmonus atophus* Loomis, 1959; *Stilbopagus acclivus* Loomis, 1966; *Tetraporosoma seriata* Loomis, 1966).**

Pontotoc (Shelley 2000b).

Remarks: Six females and two juveniles were collected in the Arbuckle Mountains, 3.7 km S Pittstown, Pontotoc County; these were initially described by Loomis (1943, his fig. 3) as *Desmoniella curta* (see Shelley 2000b). Although there are several records from the surrounding states of Arkansas, Louisiana, Missouri, and Texas (Shelley 2000b, his fig. 17), none, to our knowledge, have been collected since that time in Oklahoma.

FAMILY TRICHOPOLYDESMIDAE VERHOEFF, 1910

Remarks: The *Chaetaspis* spp. below was formally included in the family Macrosternodesmidae Brölemann, 1916, which was recently classified by Golovatch (2013) as a synonym of the Trichopolydesmidae. However, Shear and Reddell (2017) do not agree, and as they say…"since this family, almost completely endemic to North America, seems to us to be clearly diagnosable and distinct." For the time being, we follow Golovatch (2013) until more conclusive evidence is provided to the contrary.

***Chaetaspis* sp. (undescribed)**

Murray (Harrell 1960; Black 1971; Lewis 2002)

Remarks: This undescribed troglobitic species was originally collected by R.C. Harrell. He (Harrell 1960) reported it a new species of *Chaetaspis* (as *Antriadesmus*) from Wild Woman Cave that was being studied by N. Causey. Lewis (2002) reported this milliped as *Chaetaspis* undescribed species 2 from Murray County, 427 m into the dark zone of an unspecified cave.

***Chaetaspis* sp. (undescribed)**

Cherokee (Lewis and Slay 2012).

Remarks: Lewis and Slay (2012, their fig. 6) reported this milliped as *Chaetaspis* n. sp. 2. It is yet to be named and formally described and there are surely others in the genus that inhabit subterranean habitat in the state (see Graening et al. 2011).

FAMILY XYSTODESMIDAE COOK, 1895

Remarks: A recent study (Shelley and Smith 2018) subsumed the families Eurymerodesmidae and Euryuridae under Xystodesmidae; this higher-level change was justified based solely on similarity of male genitalic morphology.

***Apheloria virginiensis reducta* Chamberlin, 1931 (syn. *Apheloria reducta* Chamberlin, 1939)**

McCurtain (Causey 1954; McAllister et al. 2003a; Shelley and McAllister 2007).

Remarks: Causey (1954) was the first to report *A. reducta* from Oklahoma from an unspecified locality in McCurtain County; it is known from Beaver's Bend State Park, McCurtain County (McAllister et al. 2002). This xystodesmid is known to possess the aroma of benzaldehyde (similar to maraschino cherries) and squirt mandelonitrile (a cyanogen) and hydrogen cyanide from pores lining the sides of its body as a chemical defense, so care should be taken when collecting.

***Auturus louisianus louisianus* (Chamberlin, 1918) (syn. *Euryurus louisiana* Chamberlin, 1918; *Auturus louisiana* Causey, 1955; *Auturus louisianus louisianus* Shelley, 1982).**

Latimer, Le Flore, McCurtain (Shelley 1982; McAllister et al (2002, 2003).

Remarks: Hoffman (1999), in error, stated the range included southwestern Oklahoma but it is actually southeastern Oklahoma.

***Auturus evides* (Bollman, 1887) (syn. *Paradesmus evides* Bollman, 1887; *Auturus mimetes* Chamberlin, 1942; *Auturus florus* Causey, 1950; *Auturus evides* Shelley, 1982).**

Adair, Cherokee, Mayes, Sequoyah, Wagoner (Black 1971; Shelley 1982).

Remarks: Black (1971) reported *A. evides* from Ft. Gibson Cave no. 4 (Wagoner County).

***Eurymerodesmus birdi birdi* Chamberlin, 1931 (syn. *Leptodesmus hispidipes* Gunthorp, 1913; *Eurymerodesmus birdi* Chamberlin, 1931; *Eurymerodesmus creolus* Chamberlin, 1942; *Eurymerodesmus schmidti* Chamberlin, 1943; *Eurymerodesmus plishneri* Causey, 1950).**

Cherokee, Cleveland (new record), Garvin, Hughes, Le Flore, McCurtain, Murray (type locality), Payne, Pittsburg, Pottawatomie, Seminole, Wagoner (Chamberlin 1931; Causey 1952; Chamberlin and Hoffman 1958; Branson 1961; Black 1971; Shelley 1990; McAllister et al. 2002, 2003).

Remarks: Shelley (1990) examined several specimens from Oklahoma and noted that habitat data was given on vial labels for those individuals from counties as follows: Cherokee (Dressler Cave), Garvin (on patio), Le Flore (Bear Den Cave), Payne (on driveway), Seminole (Whiskey Cave), and Wagoner (under rocks in woods). The Cleveland County specimen is a new county record collected by CTM on 7 November 2003 at Lake Thunderbird State Park.

***Eurymerodesmus digitatus* Loomis, 1976**

Comanche (Shelley 1990)

Remarks: Thus far only known from a single site in the state, three male specimens collected at Mt. Scott in October 1974 (see Shelley 1990).

***Eurymerodesmus dubuis* Chamberlin, 1943 (syn. *Paresmus columbus* Causey, 1950).**

Choctaw, McCurtain (McAllister et al. 2002)

Remarks: To date, only reported from two counties in the southeastern part of the state.

***Eurymerodesmus mundus* Chamberlin, 1931 (syn. *Leptodesmus floridus* Kenyon, 1893; *Leptodesmus hispidipes* Gunthorp, 1913; *Eurymerodesmus mundus* Chamberlin, 1931).**

Caddo, Canadian, Cleveland (type locality), Comanche, Craig, Grady, Latimer, Le Flore, Logan, McClain, McCurtain, Latimer, Noble, Osage, Pittsburg, Pushmataha, Tulsa, Washita, Woodward (Chamberlin 1931; Chamberlin and Hoffman 1958; Shelley 1990; McAllister et al. 2004, 2013; McAllister and Shelley 2008; McAllister and Robison 2011.

Remarks: Shelley (1990) reported that size varies greatly between specimens of *E. mundus* and those from Oklahoma are among the largest. In addition, the holotype was collected by RD Bird from blackjack oak (*Quercus marilandica*) litter at or very near the University of Oklahoma campus at Norman (Chamberlin 1931). This milliped is known from at least 19 Oklahoma counties, with Washita County being the westernmost distribution in the state.

***Pleuroloma flavipes* Rafinesque, 1820 (syn. see Shelley [1980], and Marek et al. [2012] who lists no less than 28 synonyms).**

Adair, Cherokee, Latimer, Mayes, McIntosh, Noble, Nowata, Payne, Pittsburg, Rogers, Sequoyah, Tulsa, Wagoner (Causey 1951; Shelley 1980; Shelley et al. 2003).

Remarks: Causey (1951) reported the synonym *Zinaria warreni* from Latimer County. In addition, Black (1971) reported this species as *Pleuroloma brunnea*, another synonym, from Christian School Study, Three Forks, and Gittin' Down caves, Adair County. The species has the most extensive distribution of any known xystodesmid (Shelley 1980).

FAMILY PARADOXOSOMATIDAE DADAY, 1889

***Oxidus gracilis* (Koch, 1847) (syn. *Fontaria gracilis* C. L. Koch, 1847; *Paradesmus dasys* Bollman, 1887; *Orthomorpha gracilis* Bollman, 1893; *Orthomorpha dasys* Bollman, 1893).**

Bryan, Caddo (new record), Comanche, Marshall, Tulsa (McAllister and Robison 2018).

Remarks: The introduced "hothouse" milliped is ubiquitous in the lower 48 states and could be expected in any Oklahoma county. Specimens were collected by CTM from Red Rock Canyon State Park, Caddo County on 8 November 2003.

FAMILY POLYDESMIDAE LEACH, 1815

***Pseudopolydesmus pinetorum* (Bollman, 1888) (syn. *Polydesmus pinetorum* Bollman, 1888; *Polydesmus americanus* Carl, 1902; *Polydesmus pinetorum* Chamberlin, 1943; *Polydesmus modocus* Chamberlin, 1943; *Pseudopolydesmus hubrichti* [Chamberlin, 1943])**

Adair, Atoka, Caddo, Choctaw, Cleveland, Delaware, Johnston, Latimer, Le Flore, Logan, Marshall, McCurtain, McIntosh, Murray, Osage, Payne, Pittsburg, Pontotoc, Woodward (Chamberlin 1931; Causey 1953; Harrel 1960, 1963; Black 1971; Withrow 1988; McAllister et al. 2002, 2013).

Remarks: Chamberlin (1943) described *Polydesmus hubrichti*, a synonym of *P. pinetorum* from Latimer and Murray counties. Black (1971) reported *P. pinetorum* from Stansberry-January Cave (Delaware County), HorseThief Cave no. 2 (Johnston County), and Wild Woman Cave (Murray County). Throughout its range, this milliped is most commonly collected west of the Mississippi River (Sierwald et al. 2019).

FAMILY SCYTONOTIDAE COOK AND LOOMIS, 1924

***Scytonotus granulatus* (Say, 1821) (syn. *Scytonotus laevicollis* C. L. Koch, 1847; *Scytonotus scabricollis* C. L. Koch, 1847; *Stenonia hispida* Sager, 1856; *Polydesmus setiger* Wood, 1865; *Scytonotus cavernarum* Bollman, 1887; *Scytonotus granulatus* Bollman, 1887).**

Latimer, Le Flore (Shelley et al. 2005a)

Remarks: The exact locale of *S. granulatus* in Latimer County is unknown (Shelley et al. 2005a). The specimen from the Choctaw Nation State Historic site in Le Flore County represents a westward range expansion of at least 405 km from sites in Arkansas (Shelley et al. 2005a).

The following millipeds have not yet been reported from Oklahoma but are found in adjacent states and are possible in the state according to the following authors:

ORDER JULIDA

FAMILY PARAJULIDAE

Gosiulus conformatus Chamberlin, 1940

Remarks: Shelley and Smith (2018) reported it was plausible that *G. conformatus* occurs north of the Red River in southern Oklahoma and conceivably even in western Kansas.

ORDER CHORDEUMATIDA
FAMILY CLEIDOGONIDAE

Causeyella sp.

Remarks: Shear (2003) erected the genus *Causeyella* based on *Scoterpes dendropus* Loomis, 1939 from Missouri and described two additional species, C. *youngsteadtorum* and *C. causeyae* in adjacent Arkansas**.** He (Shear 2003) does not provide any county records for this genus in the state. However, Graening et al. (2011, their Table 4.3) list this genus as occurring in Oklahoma without providing any records and/or voucher specimens. It is possible this cave adapted species may eventually be found in caves of northeastern Oklahoma.

FAMILY TRICHOPETALIDAE

Trigenotyla parca Causey, 1951

Remarks: Shear (2003) mentions that "*T. parca*….probably also occur in adjoining Oklahoma."

ORDER CALLIPODIDA
FAMILY ABACIONIDAE

Abacion wilhelminae Shelley, McAllister, and Hollis, 2003

Remarks: This Critically Imperiled (S1) species is so far only known from a single site on Rich Mountain, Polk County, Arkansas (Shelley et al. 2003; NatureServe 2021). Additional collecting efforts by CTM and colleagues failed to find *A. wilhelminae* at other similar locales on the mountain. However, as the same Ouachita uplift extends further westward into Oklahoma, it may eventually be found there with future collections.

ORDER POLYDESMIDA
FAMILY POLYDESMIDAE

Pseudopolydesmus serratus (Say, 1821)

Remarks: Shelley and Snyder (2012, their fig. 4) show its peripheral range includes most of central and eastern Oklahoma without giving any specific records; the closest record is Barber County, Kansas, just over the border on the northern edge of the state. In addition, Sierwald et al. (2019) does not mention any Oklahoma records in their taxonomic synthesis of the genus. Therefore, it is conceivable this species occurs in the state where little collecting has been done in counties south of the Kansas line.

Our checklist includes a total of 33 milliped species within 24 genera, 16 families, and eight orders to inhabit 51 (66%) of Oklahoma's 77 counties. The three most speciose milliped families from the state are the Xystodesmidae, which represents 24% (eight species) of the described Oklahoma fauna, followed by Trichopeltidae (12%) and Parajulidae (12%), each with four species. The largest represented genera are *Eurymerodesmus* which represents (12%) of the state's fauna with four species, *Trigenotyla* (9%) with three species, and *Auturus* and *Abacion* (6%) with two species, each. Only 6% of the described Oklahoma fauna is non-native.

To date, the majority of Oklahoma millipeds have been reported from counties of the oak-hickory-pine Ouachita Highlands of the southeastern corner (Fig. 1), including McCurtain (12 species) and Latimer and Le Flore, each with 11 species. The central and eastern part of Oklahoma supports the majority of milliped biodiversity in the state; the only counties in far eastern Oklahoma without any records are Haskell and Ottawa (Fig. 1). Comparatively speaking, the western and southwestern regions have fewer records of millipeds. Furthermore, there are no current records of millipeds in any of the three counties (Beaver, Cimarron, and Texas) making up the Panhandle of Oklahoma. This might be explained possibly by a combination of two factors: (1) fewer milliped surveys have been conducted in those regions, and (2)

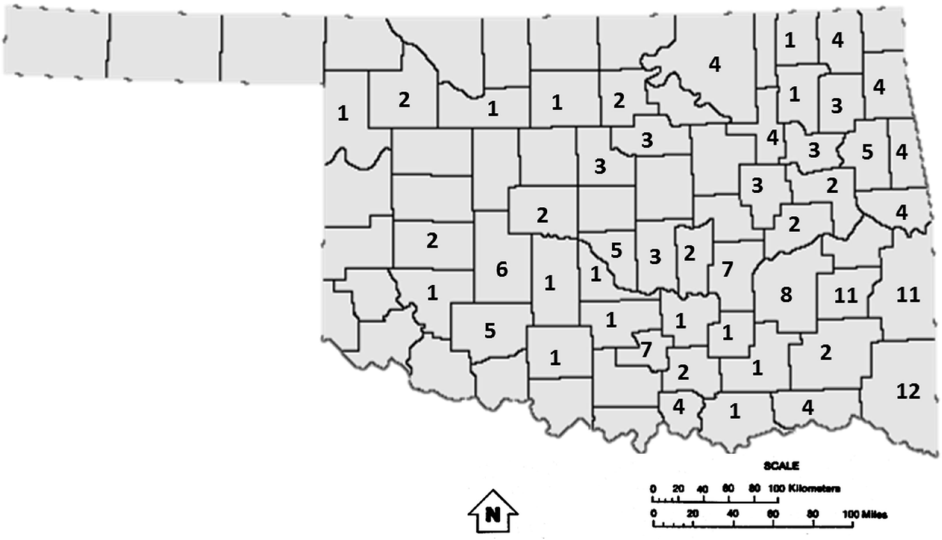

Figure 1. Number of milliped species within each Oklahoma county.

compared to eastern Oklahoma, the region has a harsher climate, less precipitation, and sparser vegetation in the Pinon-Juniper Mesas and Shortgrass Plains physiognomic regions (see Caire et al. 1989).

Given the paucity of information about the taxonomy/systematics, distribution, ecology, and natural history of millipeds in Oklahoma, there are sufficient opportunities to add to this growing body of knowledge by intensive collecting and further biological studies of these invertebrates. In addition to routine collecting techniques noted herein, sifting dead leaves, use of Berlese funnel extraction, and using pitfall traps is recommended. Deposition of voucher specimens is a must and collectors should make every effort to preserve specimens in DNA grade ethanol, 10% neutral-buffered formalin (for scanning electron microscopy of gonopods), and accession them into a publically accessible museum collection. We also suggest that future surveys should target sites with accessible trails in the western part of the state and Panhandle, where virtually nothing is known to date about millipeds and their distributional patterns in those regions.

Acknowledgments

A Scientific Collecting Permit was issued to CTM by the Oklahoma Department of Wildlife Conservation. We dedicate this paper to the memory of Richard Lawrence Hoffman (1927-2012) who was the leading authority of the world's milliped fauna and who also hosted and tutored both authors on millipeds at his laboratory at the Virginia Museum of Natural History in Martinsville.

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Submitted October 18, 2021 Accepted November 18, 2021

Scanning Electron Microscopy of the Gonopods of the Milliped, *Eurymerodesmus dubius* (Diplopoda: Polydesmida: Xystodesmidae)

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Abstract: Information on the gonopods of the polydesmid milliped, *Eurymerodesmus dubius*, have been described previously using light microscopy; however, nothing is known concerning their ultrastructural detail. The gonopods and gonopodal aperture of an adult male *E. dubius* collected in April 2011 from Beaver's Bend State Park, McCurtain County, Oklahoma, was examined using scanning electron microscopy (SEM). Compared to previous information from descriptions and line drawings, the morphology of the gonopods and gonopodal aperture of *E. dubius* via SEM is quite similar but the finer detail observed herein adds to earlier works. Here, for the first time, we provide new ultrastructural data on the gonopods of *E. dubius*.

Introduction

The former milliped family Eurymerodesmidae Causey, 1951, was the dominant representative of the order Polydesmida that occupied various habitats in the central, southcentral, and southeastern United States. However, a recent study by Shelley and Smith (2018) subsumed the families Eurymerodesmidae and Euryuridae Pocock, 1909, under Xystodesmidae Cook, 1895; this higher-level change was justified based solely on similarity of the male genitalic morphology. The genus *Eurymerodesmus* Brölemann, 1900, is a relatively speciose taxon with about 37 species (Shelley 1990; Hoffman 1999; Sierwald and Spelda 2021). One species of flat-backed milliped, *Eurymerodesmus dubius* Chamberlin, 1943 (syn. *Paresmus columbus* Causey, 1950), was described from Delight, Pike County, Arkansas (Chamberlin 1943). Since then, it has been reported from additional Arkansas counties as well as two southeastern counties in Oklahoma (Shelley 1990; McAllister et al. 2002a, 2002b, 2003, 2004, 2013; McAllister and Shelley 2003, 2008). An excellent color photograph of *E. dubius* from Arkansas is provided by Means et al. (2021, their fig. 1E).

In xystodesmids, the male copulatory organs or gonopods and the gonopodal apertures that occur around the seventh segment (body ring) hold taxonomic utility (Shelley 1990); previous information on gonopods from *E. dubius* includes a description and line drawings (Chamberlin 1943, his fig. 8; Shelley 1990, his figs. 46–50). Nothing, however, is available on the ultrastructure of gonopods of *E. dubius*. Here, we provide, for the first time, information using scanning electron microscopy (SEM) of the gonopods of *E. dubius*.

Methods

On 1 April 2011, a single adult (31 mm total length) *E. dubius* was collected by hand from under a rock in deciduous forest off the David Boren trail at Beaver's Bend State Park, McCurtain County, Oklahoma (34°07'43.7694"N, -94°41'11.3928"W). For SEM, it was preserved in 10% neutral-buffered formalin, dehydrated in a graded series of ethanols (70–100% v/v), transferred to amyl acetate transition solvent, critically point dried with a Autosamdri®–815 critical point drier (Tousimis Research Corporation, Rockville, MD; 31◦C, 1072 psi, ventilation rate ~100 psi/min), coated with a gold-palladium with a Cressington sputter coater (Cressington Scientific Instruments Ltd, Watford, UK), and viewed with a Vega TS 5136XM digital scanning SEM (Tescan USA Inc., Cranberry Township, PA) at an accelerating voltage of 20 kV. A voucher specimen is deposited in the North Carolina State Museum (NCSM), Raleigh, North Carolina.

Results and Discussion

The gonopodal aperture of *E. dubius* (Fig. 1) is mostly spheroidal with entire sides but without lobes and caudolateral pouches; a slight depression is found on the anterior margin of the aperture. The gonopods (Figs. 1A–B) slant inward toward the midline slightly curving with the apices virtually coming into contact; the telopodites are located completely over the aperture and are relatively short and vertical, terminating below the level of distal limits of hairs. The prefemur is relatively long. The gonopod acropodite is very short, broadly terminal and covered by prefemoral hairs, principally just short of a diminutive spur on the distal extremity of the prefemur, but continuous with and poorly delineated from the latter, curving gently dorsad with sides narrowing to acuminate tip.

Compared to information from descriptions and line drawings provided by Chamberlin (1943) and Shelley (1990), the morphology of the gonopods and gonopodal aperture of *E. dubius* provided by us via SEM is quite similar

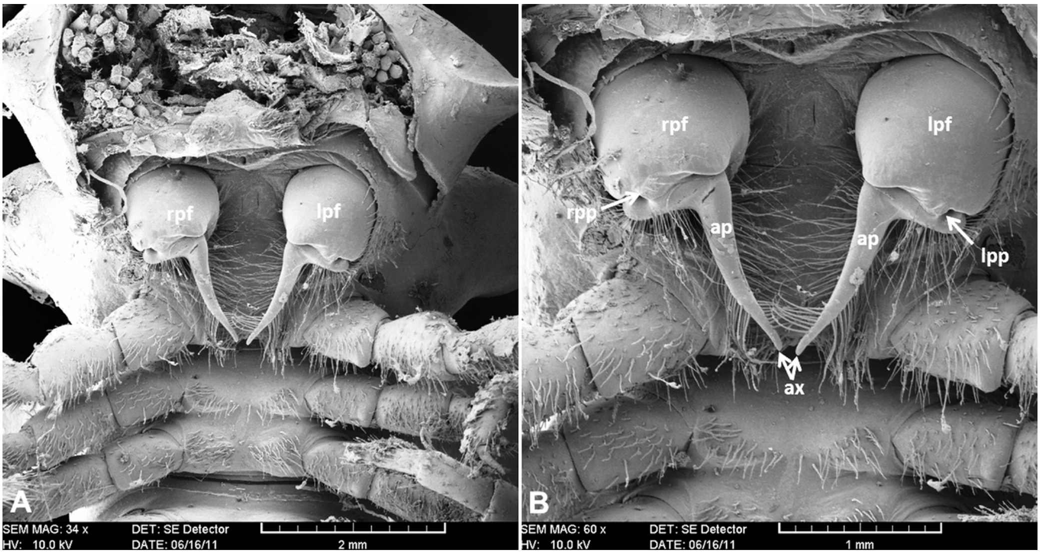

Figure 1. Scanning electron micrographs showing the ventral views of the gonopodal aperture and gonopods in situ on its first pair of legs on the seventh segment (8th leg pair) of a male *Eurymerodesmus dubius*. (A) View showing right and left gonopods. (B) Higher magnification showing right and left gonopods. Abbreviations: ap (acropodite); ax (acropodite apices); lpf (left prefemur); rpf (right prefemur); lpp (left prefemoral process); rpp (right prefemoral process).

but the finer detail observed herein adds to those earlier works. We suggest SEM of additional eurymerodesmids to help reveal details of male copulatory organs not observed in their classical line drawings and figures.

Acknowledgments

A Scientific Collecting Permit was issued to CTM by the Oklahoma Department of Wildlife Conservation. The late Dr. RM Shelley (NCSM) verified the identity of the voucher specimen of *E. dubius*.

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Submitted October 27, 2021 Accepted November 28, 2021

Tetrathyridia of *Mesocestoides* sp. (Cestoda: Cyclophyllidea: Mesocestoididae) from Pickerel Frog, *Rana palustris* (Anura: Ranidae), with a Summary of Hosts from Oklahoma

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Abstract: We collected four pickerel frogs (*Rana palustris*) from McCurtain County, Oklahoma, and examined each for the cyclophyllidean cestode, *Mesocestoides* sp. A single (25%) *R. palustris* harbored tetrathyridia of this tapeworm. We document additional information on *Mesocestoides* sp. and report it for the first time from *R. palustris* in Oklahoma. In addition, we provide a summation of the amphibians and reptiles of Oklahoma reported to harbor this enigmatic tapeworm.

Introduction

The tetrathyridial or metacestode stage of the cyclophyllidean cestode, *Mesocestoides* Vaillant, 1863, occurs free in the body cavity and as encapsulations in tissues of a broad range of vertebrate second intermediate hosts such as amphibians, reptiles, and small rodents; the adult worm is found infecting definitive hosts including birds of prey, placental mammals (canids, felids, mustelids, and hyaenids), non-human primates, and rarely humans (Fuentes et al. 2003; Padgett et al. 2012; Montalbano Di Filippo et al. 2018). Although the classical literature on parasites from anuran amphibians of Oklahoma reported several hosts infected with cestodes (Trowbridge and Hefley 1934; Kuntz 1941; Kuntz and Self 1944), none reported any hosts with *Mesocestoides* sp. However, for more than a decade, our research consortium has reported *Mesocestoides* sp. tetrathyridia from several amphibians and reptiles of Oklahoma (see McAllister and Bursey 2004; McAllister et al. 2005, 2017, 2018a, 2018b, 2020). The purpose of this report is to document additional information on *Mesocestoides* sp. in a common anuran of the state. We also provide a summation of the amphibians and reptiles of Oklahoma reported to harbor this enigmatic tapeworm.

Methods

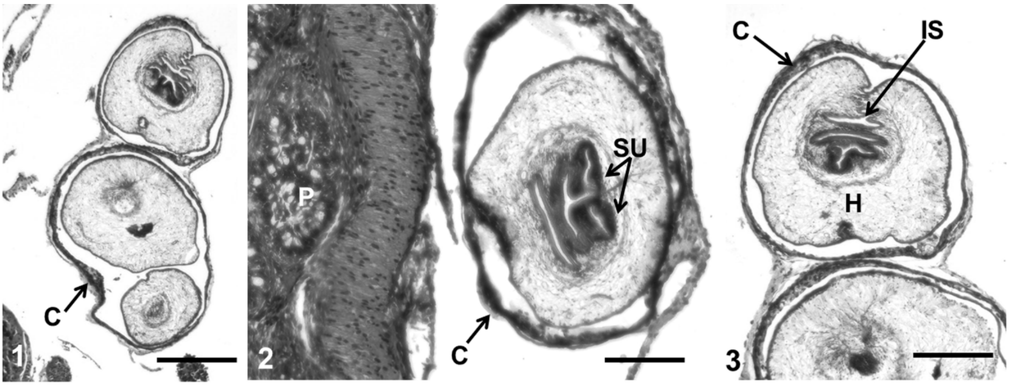
Between March 2018 and September 2021, four adult (1 male, 3 female, mean ± 1SD snout-vent length [SVL] = 65.8 ± 8.1, range 52–72) pickerel frogs, *Rana palustris* (LeConte, 1825), were collected by hand from Hochatown, McCurtain County, Oklahoma (34°10’17.0286”N, -94°45’5.7414”W), and examined for *Mesocestoides* sp. Specimens were euthanized with a concentrated tricaine methanesulfonate solution following accepted guidelines (SIH 2004) and a mid-ventral incision was made and the coelomic cavity and various organs (placed in Petri dishes with saline) was examined with a stereomicroscope. When suspected encapsulated tapeworms were observed, they were excised with a portion of tissue and preserved in 10% (v/v) neutral buffered formalin. Tissues were prepared for light microscopy following Presnell and Schreibman (1997). The histological steps included the following: dehydrating tissue through a graded ethanol series, clearing in 100% xylene, infiltrating in paraffin wax overnight in a paraffin oven (56C), embedding in paraffin using plastic molds, sectioning on a rotary microtome into 10-µm strips (affixed onto glass slides with Haupt's adhesive prior to floating strips in 2% NBF on a slide warmer) and staining to reveal general cytology with Pollak trichrome stain for the enhancement of tissue. Coverslips were adhered to the microscopic slides using Permount. For photomicroscopy, specimens were examined and photographed with a Leica DM 2000 LED microscope (Leica Microsystems, Inc., Buffalo Grove, Illinois). A subset of sample was saved in 70% (v/v) DNA grade ethanol for future molecular analysis (VV Tkach, *pers. comm.*).

A voucher host specimen was deposited in the Eastern Oklahoma State College Vertebrate Collection, Idabel, Oklahoma. Voucher specimens of *Mesocestoides* sp. were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. We generally adopt the amphibian and reptile taxonomy of Crother et al. (2017), except we follow Yuan et al. (2016) for North American *Rana* spp., rather than *Lithobates*.

Results and Discussion

A single gravid female *R. palustris* (73 mm SVL) harbored tetrathyridia (HWML 216710) in its mesenteries (Figs. 1, 3) and near the pancreas (Fig. 2). Tetrathyridia possessed the characteristic features of a single invaginated scolex (Fig. 2), a generally deep invagination canal (Fig. 3), a prominent single excretory pore at the end opposite the scolex, and a solid hindbody (Fig. 3). None of the tetrathyridia possessed any anomalies such as those reported in some aberrant acephalic tetrathyridia from other hosts, including a divided scolex, somatic bud, or any tegumental or excretory irregularity (see review by Conn et al. 2011).

The complete life cycle of *Mesocestoides* remains an enigma. Several authors have suggested that terrestrial (perhaps coprophagic) arthropods, including coleopterans (dung beetles), hymenopterans (ants), blattodeans (roaches), and mites could serve as potential



Figures 1–3. *Mesocestoides* sp. tetrathyridia from *Rana palustris*. (1) Three tetrathyridia from mesenteries; two in single capsule (arrow). (2) Single tetrathyridium from near pancreas; note capsule and suckers (arrows). (3) Two tetrathyridia from mesenteries; note capsule and invaginated scolex (arrows). Abbreviations: capsule (C); solid hindbody (H); IS (invaginated scolex); pancreas (P); suckers (SU). Scale bars (1) 1 mm; (2-3) 250 µm.

Table 1. Amphibians and reptiles of Oklahoma* reported as hosts of *Mesocestoides* sp.

Host (Class, Order, Family, Species)	Prevalence†	Reference(s)
Amphibia		
Caudata		
Plethodontidae		
Plethodon sequoyah	3/14 (21%)	McAllister and Bursey (2004)
Anura		
Bufonidae		
Anaxyrus fowleri	1/1 (100%)	McAllister et al. (2020)
Scaphiopodidae		
Scaphiopus hurterii	3/14 (21%)	McAllister et al. (2005)
Spea bombifrons	2/3 (67%)	McAllister et al. (2005)
Ranidae		
Rana catesbeiana	1/18 (6%)	McAllister et al. (2017)
Rana palustris	1/5 (20%)	This report
Rana sphenocephalus utricularius	21/74 (28%)	Vhora and Bolek (2015)
	1/1 (100%)	McAllister et al. (2020)
Reptilia		
Squamata		
Scincidae		
Scincella lateralis‡	1/1 (100%)	McAllister et al. (2018b)‖
Colubridae		
Heterodon platirhinos	1/1 (100%)	McAllister et al. (2020)
Thamnophis sirtalis sirtalis	2/2 (100%)	McAllister et al. (2018a)

*All hosts were collected in McCurtain County except spadefoot toads came from the University of Oklahoma Biological Station (Marshall County) and *R. s. utricularius* were collected from Stillwater, Payne County (Vhora and Bolek 2015).

†Prevalence = number infected/number examined (%).

‡Harbored pre-tetrathyridia with evaginated scolex and neck region.

‖McAllister et al. (2014b) also examined 20 *S. lateralis* from McCurtain County and none were infected with *Mesocestoides*.

first intermediate hosts (see Sapp and Bradbury 2020). However, it has also been proposed that *Mesocestoides* could perhaps develop through a simple two-host (diheteroxenous) life cycle rather than an obligate three-host cycle (triheteroxenous) by utilizing only vertebrates as the intermediate host (McAllister et al. 2018b).

McAllister et al. (1995) provided a survey of the parasites of *R. palustris* from the southern part of its range. In that study, two specimens were collected in Le Flore County, Oklahoma, but they only harbored nematodes, *Abbreviata* sp. However, a single specimen of 24 (4%) collected in the same survey from Arkansas had encapsulated *Mesocestoides* sp. tetrathyridia in its mesenteries. Numerous other surveys of *R. palustris* collected in other parts of it range, including Illinois, Indiana, Maine, Massachusetts, Missouri, North Carolina, Texas, and Wisconsin, and Ontario, Canada, did not report the presence of this tapeworm in any hosts (see McAllister et al. 1995, their Table 2). Therefore, *Mesocestoides* appears to be a rarely encountered helminth of pickerel frogs and generally of other North American anurans as well (McAllister and Conn 1990; McAllister et al. 2014b, 2017).

In Oklahoma, to date, 10 species of amphibians and reptiles, including a single salamander, six species of anurans, a skink, and two colubrid snakes have been reported to harbor *Mesocestoides* sp. (Table 1). As Oklahoma supports 157 species/subspecies of amphibians and reptiles (Sievert and Sievert 2011), only 6% have been reported in the state infected with *Mesocestoides*, so there is plenty of opportunity to discover this unusual parasite by surveying additional herpetofauna.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued Scientific Collecting Permit No. 1551646 to CTM.

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Submitted October 27, 2021 Accepted November 28, 2021

Noteworthy Records of Helminth Parasites (Monogenea, Trematoda, Cestoda, Nematoda, Acanthocephala) from Select Herpetofauna (Anura, Testudines, Ophidia) from McCurtain County, Oklahoma

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Abstract: As part of our continual pursuit to report on the geographic distribution and host records of the helminth parasites of southeastern Oklahoma herpetofauna, we recently had the opportunity to examine select amphibians (two anuran species) and reptiles (two turtle and eight snake species) from the southeastern part of the state. Found were: two monogeneans, two digeneans, three tapeworms, five nematodes, and an acanthocephalan. We document several new host and distributional records for these parasites.

Introduction

For the last several years, our research group has reported biological information in a series of reports on the parasites of amphibians and reptiles in Oklahoma (McAllister and Bursey 2012; McAllister et al. 2014, 2015, 2016, 2018, 2020, and references therein). The present work compliments our aforementioned surveys and provides some new records for helminth parasites of select Oklahoma herpetofauna.

Methods

Between April 2019 and September 2021, two species of amphibians and 10 species of reptiles, including (single specimens except where noted): Cajun chorus frog, *Pseudacris fouquettei* Lemmon, Lemmon, Collins, and Cannatella, three Coastal Plains leopard frogs, *Rana sphenocephala utricularius* (Cope), Mississippi mud turtle, *Kinosternon subrubrum hippocrepis* Gray, eastern river cooter, *Pseudemys concinna concinna* (LeConte), southern black racer, *Coluber constrictor priapus* Dunn and Wood, eastern hog-nosed snake, *Heterodon platirhinos* (Latreille), plain-bellied watersnake, *Nerodia erythrogaster* (Forster), broad-banded watersnake, *Nerodia fasciata confluens* (Blanchard), northern diamond-backed watersnake, *Nerodia rhombifer rhombifer* (Hallowell), flat-headed snake, *Tantilla gracilis* Baird and Girard, red-sided gartersnake, *Thamnophis sirtalis parietalis* (Say), and three northern cottonmouths, *Agkistrodon piscivorus* (Lacépède) were collected by hand, snake tong or dead off the road (DOR) from various sites in McCurtain County and examined for helminth parasites. Specimens were placed in cloth collection bags, placed in a refrigerator, and necropsied within 24 hr. Turtles were measured for straight-line carapace length (CL) and anurans and snakes for snout-vent length (SVL), killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004), and examined for helminth parasites. A bone saw was used to remove the plastron from turtles to expose the heart and a mid-ventral incision from mouth to cloaca was made to expose the same in other reptiles. For intravascular trematodes in turtles, we followed methods of Snyder and Clopton (2005). Visceral organs, particularly those of the GI tract of all specimens, were examined for helminths by removing and splitting them lengthwise, placing separate organs in a Petri dish with 0.9% saline, and their contents scanned at 20–30× using a stereomicroscope. Trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, transferred to 70% (v/v) ethanol, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were fixed in hot tap water and studied as temporary mounts on a microscopic slide in a drop of glycerol. Encapsulated acanthocephalans were teased from tissues and fixed in 70% (v/v) ethanol and examined as temporary mounts. Further examinations were made at 100 to 400× with an Olympus BX-51 upright research microscope configured for Brightfield (BF) and Differential Interference-Contrast (DIC) microscopy.

We followed the common and scientific names of North American herpetofauna of Crother (2017) except for adopting Yuan et al. (2016) in our usage of *Rana* rather than *Lithobates* for Oklahoma's ranid frogs. Host vouchers are deposited in the Eastern Oklahoma State Vertebrate Collection (EOSC), Idabel, Oklahoma. Genuine vouchers or photovouchers of parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Monogeneans, digeneans, and a nematode species are saved in DNA grade ethanol and being utilized in molecular studies at the University of North Dakota, Grand Forks (VV Tkach, *pers. comm.*) and as well as *Ophiotaenia* spp. at the Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Czech Republic (T Scholz, *pers. comm.*).

Results and Discussion

Fourteen taxa of endoparasites, including two monogeneans, three digeneans, three tapeworms, five nematodes, and an acanthocephalan were harbored by 12 host species; two hosts harbored multiple infections. An annotated list of the parasites found and the host data follows.

Platyhelminthes: Trematoda: Monogenea: Polystomatidea: Polystomatidae

***Neopolystoma* sp.** – A single specimen was found in the urinary bladder of an adult (115 mm CL) *K. s. hippocrepis* collected on 22 May 2021 from off US 259, north of Hochatown (34°10'25.23"N,-94°43'35.2878"W). This individual possessed a type III sucker characteristic of *Neopolystoma* (Du Preez and Theunissen 2021). At present, at least 54 turtle polystome species are known from 55 host species (DuPreez and Van Rooyen 2015). The family Polystomatidae (Platyhelminthes: Monogenea) comprises 25 genera of which three (*Polystomoides* Ward, 1917, *Polystomoidella* Price, 1939, and *Neopolystoma* Price, 1939) infect freshwater turtles (Morrison and DuPreez 2011). *Neopolystoma* is found in the oral, nasal, and ocular cavities, cloaca, and urinary bladder and has no hamuli. *Neopolystoma orbiculare* (Stunkard, 1916) Price 1939, has previously been reported from Oklahoma in red-eared slider, *Trachemys scripta elegans* (Wied) and western painted turtle, *Chrysemys picta bellii* (Gray) (Harwood 1931; Price 1939; Everhart 1957; McKnight 1959). A *Neopolystoma* sp. was reported from snapping turtle, *Chelydra serpentina* (L.) from Oklahoma by McAllister et al. (2015); however, specimens were found in the conjunctival sacs of this host. We document the first report of a *Neopolystoma* sp. from *K. s. hippocrepis* and the first time a member of the genus that inhabits the urinary bladder has been reported in any Oklahoma host.

***Polystomoidella oblongum* Wright, 1879** – Two individuals were found in the urinary bladder of a 270 mm CL *P. c. concinna* collected on 29 June 2021 from Broken Bow (34°02' 11.4648"N, -94°45'25.0662"W). *Polystomoidella* parasitizes the urinary bladder of turtles and possesses a single pair of hamuli. Two *Polystomoidella* species are known from North America and are *P. oblongum* Wright, 1879, and *Polystomoidella whartoni* Wright, 1879. The former was originally described from *S. odoratus* from Canada (Wright 1879) and has also been reported from *C. serpentina*, razor-backed musk turtle, *Sternotherus carinatus* (Gray), and southeastern mud turtle, *Kinosternon subrubrum subrubrum* (Lacépède) (Stafford 1900; Price 1939). McAllister and Bursey (2012) reported *P. whartoni* from the urinary bladder of a *K. s. hippocrepis* from Latimer County, Oklahoma. The eastern river cooter represents a new host for *P. oblongum*.

Digenea: Schistosomatoidea: Spirorchiidae

***Hapalorhynchus* sp.** – Several spirochiid flukes were found in the blood and body wash of the same *K. s. hippocrepis* above; it appears to not fit any described species. As currently defined (Platt 2002), the genus *Hapalorhynchus* Stunkard, 1922, contains 19 species (Smith 1997). Three of the six species of *Hapalorhynchus* previously reported from North America infect various kinosternids, including: *H. albertoi* Lamothe-Argumento, 1978, from white-lipped mud turtle, *Kinosternon leucostomum* Duméril and Bibron *in* Duméril and Duméril, from Tabasco, México, *H. reelfooti* Byrd, 1939, from eastern musk turtle, *Sternotherus odoratus* (Latreille, *in* Sonnini and Latreille) from Tennessee, and *H. stunkardi* Byrd, 1939, from *S. carinatus* from Georgia (Byrd 1939; Lamonthe-Argumento 1978; Platt 1988; Platt and Snyder 2002). More recently, however, several species were reported by Roberts et al. (2017) from Alabama and Florida musk turtles, including a new species, *Hapalorhynchus conecuhensis* Roberts and Bullard, 2017, from innominate musk turtle, *Sternotherus* cf. *minor* and loggerhead musk turtle, *Sternotherus minor* (Agassiz), as well as previously described *H. reelfooti* from *S. minor*, stripe-necked musk turtle, *Sternotherus peltifer* (Smith and Glass), *S. odoratus*, and *S.* cf. *minor*, and *Hapalorhynchus* cf. *stunkardi* from *S. minor* and *S. odoratus*. However, this is the first time a *Hapalorhynchus* sp. has been reported from *K. s. hippocrepis*.

Plagiorchiida: Plagiorchiidae

***Styphlodora magna* Byrd and Denton, 1938** – Eight *S. magna* (Fig. 1) was found in the gallbladder of an adult (480 mm SVL) *A. piscivorus* collected on 1 May 2021 from Hochatown (34°10'12.4926"N, -94°45'01.1442"W). McAllister et al. (2020) previously reported *S. magna* from western cottonmouths from the same locale. Byrd and Denton (1938) described *S. magna* from the gallbladder of northern watersnake, *Nerodia sipedon sipedon* (L.) from Georgia and Mississippi. It has been previously reported from *A. piscivorus* from North Carolina (Collins 1969). We document the second report of *S. magna* from *A. piscivorus* in Oklahoma.

Ochetosomatidae

***Renifer ancistrodontis* (MacCallum, 1921) Talbot, 1934 –** Six specimens were found in the oral cavity of the same *A. p. leucostoma* above. The type host and locality of *R. ancistrodontis* is a captive specimen of the copperhead, *Agkistrodon contortrix* (L) from the New York Zoological Gardens, New York (MacCallum 1921). Since then, this digenean has been previously reported from northern cottonmouths from Alabama (Detterline et al. 1984) and Texas (McAllister et al. 2008). However, this is the first time this parasite has been reported from Oklahoma. The genus is badly in need of revision which should embrace molecular analyses.

Cestoda: Eucestoda: Bothriocephalidea: Proteocephalidae

***Ophiotaenia grandis* La Rue, 1911** – Several specimens (Fig. 2) were taken from the intestine of two *A. piscivorus* (437 mm SVL) collected on 23 June 2019 and 17 August 2019 in Hochatown and from off US 259 at US 259A junction (34°07'10.3038"N, -94°44'23.3016"W), respectively. La Rue (1911) originally described

Figures 1–3. Macrophotographs of some of the helminth parasites reported herein. (1) Several *Syphlodora magna* from intestine of *Agkistrodon piscivorus*; scale bar = 1 mm. (2) Numerous *Ophiotaenia grandis* from intestine of *A. piscivorus*. (3) Two *Rhabdias* cf. *joaquinensis* from lungs of *Rana sphenocephala utricularius*; scale bar = 1 mm.

O. grandis from *A. piscivorus* from a captive specimen at the National Zoological Park, Washington, D.C. (exact collection locality unknown). It has been previously reported from *A. piscivorus*, Mississippi green watersnake, *Nerodia cyclopion* (Duméril, Bibron and Duméril), Florida green watersnake, *Nerodia floridana* (Goff), northwestern gartersnake, *Thamnophis ordinoides* (Baird and Girard), and *T. sirtalis* from Florida, Louisiana, Mississippi, Oklahoma, and Texas, and Sasketchewan, Canada (see Ernst and Ernst 2006).

***Ophiotaenia perspicua* La Rue, 1911** – Several individuals were collected from the intestine of an adult (755 mm SVL) *N. rhombifer* collected on 10 June 2019 from 4.8 km N of Idabel from an oxbow lake off the Little River at the Turner Ranch (33°55’58.3278”N, -94°43’42.6642”W). This tapeworm was originally described by La Rue (1911) from *N. rhombifer* from the Illinois River at Havana, Illinois; additional specimens came from an unknown locality in Oklahoma from the same host species. Detterline et al. (1984) reported it from *N. rhombifer*, *N. sipedon*, and *A. piscivorus* from Alabama, Fontenot and Font (1996) reported it from *N. cyclopion*, *N. f. confluens*, *N. rhombifer*, and *A. piscivorus* from Louisiana, and McAllister et al. (2012) reported *O. perspicua* from prairie ringneck snake, *Diadophis punctatus arnyi* Kennicott from Oklahoma. The geographic range of this cestode includes Alabama, Florida, Idaho, Illinois, Louisiana, Mississippi, North Carolina, Ohio, Oklahoma, Texas, and Québec, Canada, and Hidalgo and Veracruz, México (Brooks 1978; Detterline et al. 1984; Fontenot and Font 1996; Ernst and Ernst 2006; Goldberg et al. 2012; McAllister et al. 2012). These specimens are being retained for molecular analyses (T Scholz, *pers. comm*.).

***Ophiotaenia* cf. *perspicua* La Rue, 1911** – Several individuals that differ from typical *O. perspicua* were found in the intestinal tract of an adult (425 mm SVL) *N. f. confluens* collected on 29 June 2019 from Broken Bow (34°00’41.994”N, -94°44’58.9662”W). *Ophiotaenia perspicua* has been previously reported from *N. fasciata* from Louisiana (Fontenot and Font 1996). Additional material from two adult colubrid snakes from Hochatown, including *N. erythrogaster* (735 mm SVL) collected on 26 August 2020 and a *T. s. parietalis* (700 mm SVL) collected on 30 April 2021, were identified as *O.* cf. *perspicua*. *Ophiotaenia perspicua* has been previously reported from *T. sirtalis* (Ernst and Ernst 2006). However, no tapeworms, to our knowledge, have been previously reported from *N. erythrogaster* (Detterline et al. 1984; Ernst and Ernst 2006) so we here document the first report of an *Ophiotaenia* in this host.

Nematoda: Rhabditoidea: Rhadiasidae

***Rhabdias* cf. *joaquinensis* Ingles, 1935** – A total of nine (3.0 ± 1.4, range 2–5, Fig. 3) specimens that differed from typical *R. joaquinensis* were taken from the lungs of three (49, 75, 80 mm SVL) *R. s. utricularius* collected on 20 July 2020, 9 May 2021, and 25 July 2021 from the Hochatown site. The type host and locality of *R. joaquinensis* is the northern red-legged frog, *Rana aurora* Baird and Girard from California (Ingles 1935). Since then, the species has been reported in various ranid frogs, including *R. s. utricularius* and the American toad, *Anaxyrus americanus* (Holbrook) from Arkansas, Iowa, Kansas, Mississippi, Nebraska, Oklahoma, and Tennessee (Kuzmin et al. 2003; Vhora and Bolek 2015). We document the first report of *R.* cf. *joaquinensis* in an Oklahoma host.

Enoplida: Trichuroidea: Capillaridae

***Capillaria* sp. –** Ova of an unknown species of *Capillaria* (Fig. 4) was found in the feces of *A. piscivorus* collected on 1 May 2021 from Hochatown. *Capillaria heterodontis* Harwood, 1932, has been previously reported from *A. piscivorus* from Texas (Harwood 1932) and Louisiana (Fontenot and Font 1996); a *Capillaria* sp. and *Capillaria colubra* Pence, 1970, was reported from *A. piscivorus* from North Carolina (Collins 1969; Davis et al. 2016). In Oklahoma, McAllister et al. (2018) reported a *Capillaria* sp. egg from the feces of a timber rattlesnake, *Crotalus horridus* L. This nematode belongs to the only known trichuroid genus that infects reptiles and which possesses a direct life cycle. The presence of thick-shelled ova with polar plugs at both ends of the egg (Fig. 4) allows for generic diagnosis. Although we are unable to provide a specific identity, we document the genus from an *A. piscivorus* from Oklahoma for the first time.

Spirurida: Physalopteridae

***Physaloptera abjecta* Leidy, 1856** – Three specimens (2 males, 1 female) were found in the stomach of a *H. platirhinos* (600 mm SVL) collected from Hochatown on 15 April 2019. *Physaloptera abjecta* was originally described by Leidy (1856) from *Coluber* (=*Masticophis*) *flagellum* Shaw (host identification is questionable) from Pennsylvania. This nematode has been previously reported by Mawson (1956) as *Physaloptera variegata* Reiber, Byrd, and Parker, 1940, from *H. platirhinos* from Georgia. It has also been documented in various colubrid and viperid snakes from at least 10 U.S. states, and Québec and Saskatchewan, Canada (see McAllister et al. 2008). McAllister and Bursey (2012) previously reported *P. abjecta* from a *C. c. priapus* from Oklahoma. This is the first report of *P. abjecta* from an eastern hog-nosed snake in Oklahoma.

Figure 4. Light microscopy of an egg of a *Capillaria* sp. from feces of *Agkistrodon piscivorus* showing the characteristic bipolar plugs; scale bar = 30 µm.

Trichostrongyloidea: Molineidae

***Oswaldocruzia pipiens* Walton, 1929 –** A single male specimen was collected from an adult (31 mm SVL) *P. fouquettei* collected on 21 February 2019 from Hochatown. Trowbridge and Hefley (1934) were the first to report *O. pipiens* from Oklahoma in *Rana* spp., and Woodhouse's toad, *Anaxyrus woodhousii* and Kuntz and Self (1944) reported an *Oswaldocruzia* sp. from an unspecified anuran host. This nematode was earlier reported from *P. fouquettei* (as *P. nigrita triseriata*, *P. triseriata* or *P. feriarum*) in Oklahoma in an unpublished thesis by Kuntz (1940). Bouchard (1953), in an unpublished dissertation, reported *O. pipiens* from *P. fouquettei* (as *P. triseriata*) from Oklahoma. In addition, McAllister et al. (2015) previously reported *O. pipiens* from Fowler's toad, *Anaxyrus fowleri* (Hinckley) from Hochatown. Other hosts in Oklahoma include *A. a. americanus*, Rocky Mountain toad (*A. woodhousii woodhousii*), *L. s. utricularius*, and Hurter's spadefoot (*Scaphiopus hurterii*) (see McAllister et al. 2014a). This nematode has been reported from various amphibians and reptiles from at least 12 U.S. states and México (see McAllister et al. 2014a for summary). McAllister et al. (2013, 2015) previously reported the similar *Oswaldocruzia leidyi* Steiner, 1924 from *P. fouquettei* from Arkansas and Texas, and Oklahoma, respectively. This is the first definitive published host record of *O. pipiens* from *P. fouquettei*.

Ascaridida: Cosmocercidae

***Cosmocercoides* sp.** – A single female specimen was taken from the lower intestine of an adult male (130 mm SVL) *Tantilla gracilis* collected on 26 September 2021 from the Hochatown site. Unfortunately, without a male, it is not possible to determine the specific identity of this nematode. *Cosmocercoides variabilis* (Harwood, 1930) Travassos, 1931 was reported previously from Dekay's brownsnake, *Storeria dekayi* (Holbrook) from Oklahoma by McAllister et al. (2015). The life cycle involves terrestrial gastropods as intermediate hosts and amphibians and reptiles as definitive hosts (Anderson 2000). This is the first report of any helminth parasite from *T. gracilis*.

Acanthocephala: Giganthorhynchidea: Oligacanthorhynchidae

Oligacanthorhynchid cystacanths – Four cystacanths were encysted in subdermal tissues and muscle fascia of a gravid female (960 mm SVL) *C. c. priapus* collected DOR on 3 June 2021 from the vicinity of the Eastern Oklahoma State College Campus in Idabel (33°55'10.3584"N, -94°46'30.6336"W). Snakes serve as paratenic (transport) hosts of oligacanthorhynchids and, as adults, they are parasitic in terrestrial birds and mammals. The eventual development of a cystacanth to an adult occurs when a paratenic host is ingested by an appropriate definitive host. This is the first time acanthocephalan cystacanths have been reported in this host.

In summary, a number of new host and geographic distribution records are reported herein for some parasites of select herpetofauna from southeastern Oklahoma. Future surveys should include amphibians and reptiles from other parts of the state where they have been rarely examined for parasites in general. Therefore, the prospect of additional records as well as the likelihood of discovery of novel species is promising, especially with inclusion of molecular analyses.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit No. 1551646 to CTM. Drs. Scott L. Gardner and Gabor R. Racz (HWML) provided expert curatorial assistance, and Nikolas H. McAllister (Broken Bow High School, Broken Bow, OK) and Johnny Ray (EOSC-Idabel, OK) assisted with collections. We also thank Drs. Vasyl V. Tkach (University of North Dakota, Grand Forks, ND) for identifying several trematodes, and Alain de Chambrier (Natural History Museum, Geneva, Switzerland), Olena Kudlai (Institute of Ecology, Vilnius, Lithuania), and Tomáš Scholz (Biology Centre of the Czech Academy of Sciences, Czech Republic) for detailed information on *Ophiotaenia* spp.

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Submitted October 29, 2021 Accepted November 18, 2021

Evaluation of the Feasibility of Phosphorene for Electronic DNA Sequencing Using Density Functional Theory Calculations

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Abstract: Electronic DNA sequencing using two-dimensional (2D) materials such as graphene has recently emerged as the next-generation of DNA sequencing technology. Owing to its commercial availability and remarkable physical and conductive properties, graphene has been widely investigated for DNA sequencing by several theoretical and experimental groups. Despite this progress, sequencing using graphene remains a major challenge. This is due to the hydrophobic nature of graphene, which causes DNA bases to stick to its surface via strong $\pi - \pi$ interactions, reducing translocation speed and increasing error rates. To circumvent this challenge, the scientific community has turned its attention to other 2D materials beyond graphene. One such material is phosphorene. In this article, we performed first-principle computational studies using density functional theory (DFT) to evaluate the ability of phosphorene to distinguish individual DNA bases using two detection principles, namely, nanopore and nanoribbon modalities. We observe that binding energies of DNA bases are lower in phosphorene compared to graphene. The energy gap modulations due to interaction with DNA bases are very significant in phosphorene compared to graphene. Our studies show that phosphorene is superior to graphene, and hence a promising alternative for electronic DNA sequencing.

Introduction

The progress towards cheaper and faster sequencing has been very impressive since the Human Genome Project first sequenced the human genome using the classical Sanger method (Lander et al. 2001). Recently, Oxford Nanopore Technologies developed a sequencing device based on protein nanopores (Mikheyev and Tin 2014). Despite this progress, there are still several challenges (Jain et al. 2015, Dekker 2007, Heerema and Dekker 2016) with DNA sequencing using protein nanopores such as: high startup and consumables costs; sensitivity of pore to environmental conditions *e.g.,* temperature, pH, and applied voltage; and high error rate (~15%). Due to these challenges, the current focus is on electronic DNA sequencing using 2D materials. Electronic DNA sequencing has the capability to produce larger current signals (~ µA) compared to the low ionic-current signals (~ pA) used in protein nanopores (Heerema 2016). Electronic methods could also lead to label-free, single-nucleotide, long read length automated sequencing without requiring additional consumables (Patel et al. 2017), which could drive down cost and improve accuracy.

Owing to its commercial availability and remarkable physical and conductive properties, graphene has been widely investigated for DNA sequencing by several theoretical (Prasongkit et al. 2011 and Prasongkit et al. 2015) and experimental (Schneider et al. 2010, Merchant et al. 2010, and Garaj et al. 2010) groups. Despite this progress, sequencing using graphene remains a major challenge. This is due to the hydrophobic nature of graphene, which causes DNA bases to stick to its surface via $\pi - \pi$ interactions, reducing translocation speed and increasing error rates (Sathe et al. 2011 and Wells et al. 2012).

Recently, the scientific community has turned its attention to other 2D materials beyond graphene. For instance, molybdenum disulfide (MoS_2) has been shown to produce better signal-to-noise ratios, and non-stickiness of DNA bases to its surface (Farimani et al. 2014). Furthermore, the presence of a finite energy gap (energy difference between valence band maximum and conduction band minimum) makes MoS_2 to be suitable for advanced sequencing devices such as field-effect transistors (Graf et al. 2019).

Another potential 2D material for DNA sequencing is phosphorene (Novoselov et al. 2016). Phosphorene is an elemental 2D material similar to graphene with remarkable electronic properties including a finite energy gap. Furthermore, phosphorene is hydrophilic and biocompatible (not toxic to cells), making it suitable for biological applications (Cortes-Arriangada et al. 2018, Kumawat et al. 2019, and Kumawat and Pathak 2019).

In this article, we performed first-principle computational studies using density functional theory (DFT) to evaluate the ability of graphene and phosphorene to distinguish individual DNA bases using two detection principles, namely, nanopore and nanoribbon modalities.

Materials and Methods

We focus on two advanced detection principles, namely, the nanopore and nanoribbon methods (Heerema et al. 2016), as shown in Figure 1. For simplicity, we will refer to our nanodevice concepts using the following abbreviations: GNP (graphene nanopore), PNP (phosphorene nanopore), GNR (graphene nanoribbon), and PNR (phosphorene nanoribbon). The four DNA bases are guanine (G), adenine (A), cytosine (C), and thymine (T). For GNP, the active region has a dimension of 1.91 nm x 1.80 nm, with a pore diameter of 1.07 nm. For PNP, the active region measures 2.48 nm x 1.20 nm, with a pore diameter of 1.03 nm. For GNR, the dimension of the active region is 1.07 nm x 1.17 nm. For PNR, the dimensions are 1.33 nm x 1.32 nm. For both nanopore and nanoribbon systems, the size of the active region is comparable to the interbase distance ~0.7 nm (Lagerqvist et al. 2006), and hence suitable for single-base resolution. For the nanoribbon model, the

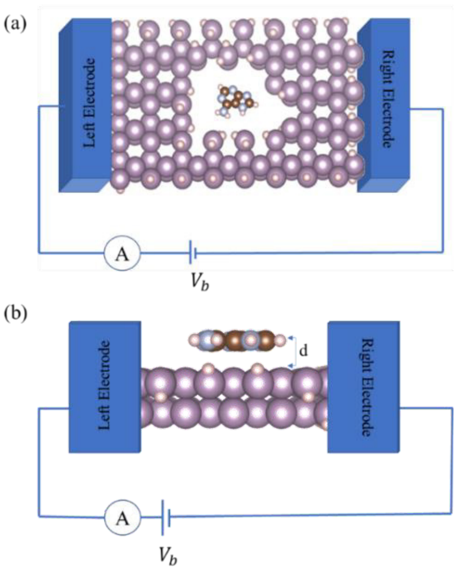

Figure 1. Schematic of electronic DNA sensing device concepts. (a) Translocation of DNA base through nanopore causes variations in in-plane current. (b) Changes in electronic current due to physisorption of DNA bases onto surface of 2D nanoribbon can be detected.

DNA bases were placed 3.0 Å above center of the nanoribbon prior to geometry optimization calculations. The structural relaxation calculations were performed at the B3LYP level of theory using the 6-31G (d, p) basis set, with a force convergence cutoff of 0.02 eV/Å (Kumawat et al. 2019). All calculations were performed using the GAUSSIAN 16 software package (Frisch et al. 2016). Computational resources were provided by the University of Central Oklahoma Buddy Supercomputing Center.

To evaluate the ability of graphene and phosphorene to distinguish individual DNA bases, two evaluation metrics were computed. The binding energy was calculated for both nanopore and nanoribbon methods as the difference in total energy, that is, $E_b = E_{system+base} - E_{system} - E_{base}$. The energy gap was calculated as the difference between the HOMO and LUMO energies: $E_{gap} = E_{HOMO} - E_{LUMO}$.

Results and Discussions

Table 1 shows the energy gaps and binding energies for the four systems considered. The binding energies for GNP (0.871 – 1.063 eV) are larger than those for PNP (0.207 – 0.405 eV). Similarly, the binding energies for GNR (0.423 – 0.592 eV) are larger than those for PNR (0.182 – 0.330 eV). For PNR, our calculated binding energy order (G > A > C > T) is consistent with reported results using nanoribbons from MoS_2 (Farimani et al. 2014) and phosphorene (Kumawat and Pathak, 2019). Figure 2 shows the binding energy comparisons for all four systems. It shows that graphene nanomaterials typically have a higher binding energy, and hence greater tendency for bases to stick their surfaces, compared to phosphorene nanomaterials.

To further compare the performance of each system, we computed the change in energy gap as follows: $\Delta E_{gap} = E_{gap}(system + base) - E_{gap}(system)$. Table 2 shows the magnitude of energy gap change for all systems. For GNP, the change in energy gap is small ($\Delta E_{gap} \sim 7 - 10$ meV), while for PNP, the change is very significant ($\Delta E_{gap} \sim 13 - 281$ meV). Similarly, for GNR, the change in energy gap is small ($\Delta E_{gap} \sim 1 - 3$ meV), while for PNR, the change is very significant ($\Delta E_{gap} \sim 16 - 358$ meV).

Our idealized calculations show that phosphorene is superior to graphene for electronic DNA sequencing. In our model calculations, the effect of ions and solvating water were not included. We would expect the effects of solution and orientation of bases to produce changes in the magnitude of the calculated quantities, but not the trends (Henry et al. 2021, Feliciano et al. 2018, and Lagerqvist et al. 2007).

Table 1. Energy Gap and Binding Energy (absolute value) for GNP, PNP, GNR, and PNR.

	Energy Gap (eV)				Binding Energy (eV)			
Base	**GNP**	**PNP**	**GNR**	**PNR**	**GNP**	**PNP**	**GNR**	**PNR**
Pristine	0.221	3.070	0.259	3.038	-	-	-	-
G	0.228	3.083	0.260	2.680	0.888	0.395	0.592	0.330
A	0.230	2.789	0.257	2.783	0.936	0.307	0.546	0.293
C	0.230	3.046	0.262	3.022	1.063	0.405	0.578	0.182
T	0.231	3.025	0.258	3.055	0.871	0.207	0.423	0.169

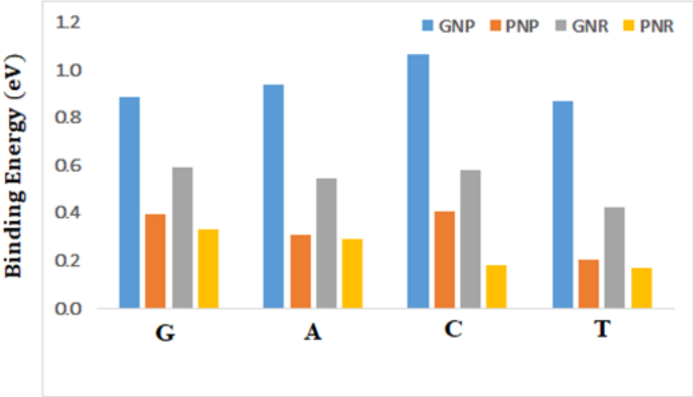

Figure 2. Binding energy for graphene and phosphorene device concepts.

Table 2. Change in energy gap for graphene and phosphorene models.

	ΔE_{gap} (eV)			
Base	**GNP**	**PNP**	**GNR**	**PNR**
G	+0.007	+0.013	+0.001	-0.358
A	+0.009	-0.281	-0.002	-0.255
C	+0.009	-0.024	+0.003	-0.016
T	+0.010	-0.045	-0.001	+0.017

In conclusion, using two metrics, namely the binding energy and energy gap, we calculated the modulation of electronic properties of nanomaterials from graphene and phosphorene due to interaction with DNA bases using two advanced detection principles, namely, nanoribbon and nanopore concepts. Our calculations show that the binding energies for phosphorene systems are generally lower compared to graphene. Also, the modulation in energy gaps are pretty significant for phosphorene nanomaterials compared to graphene. Our studies show that phosphorene is superior to graphene, and hence a promising alternative to graphene for electronic DNA sequencing applications.

Acknowledgments

Funding for this research was provided by the Office of Research and Sponsored Programs at the University of Central Oklahoma and the CURE-STEM grant from the College of Mathematics and Sciences.

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Submitted May 10, 2021 Accepted July 25, 2021

Modern Perspectives on Einstein's General Theoryof Relativity

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Abstract: This paper addresses the mathematics of Einstein's General Theory of Relativity with regard to the force of gravity. A unique approach is used, with some historical background, to show the progression of events that have led to our current understanding of how energy, mass, and light are interrelated. Our viewpoint includes the work of a physical chemist, an organic chemist, and a biologist, interested in how the universe works. The mathematics described for Einstein's General Theory of Relativity in this paper incorporates use of Tensor calculus, which involves a set of rules and methods for mathematical objects that have an arbitrary, but known, number of situations. This approach focuses on how mathematics can be applied, quantitatively, to explain Einstein's General Theory of Relativity for gravity; such as that used to calculate the slight procession of the elliptical orbit of the planet Mercury about the sun every hundred years; the slight bending of starlight by the sun; and the slight time dilation of Global Positioning System satellites. Gravity is the force one feels at the surface of the earth and matches that which one would observe if they are inside a rocket that is accelerating at a rate of 9.80 meters per second-squared (9.80 m/s^2) or 32.2 feet per second-squared (32.2 ft/s^2) in outer space. In this paper, we modified Newtonian theory by using the Schwarzschild Metric to derive kinetic and negative gravitational energy by guiding the reader through the mathematics with key references.

Introduction

We are professors, fascinated by Einstein's Theory, and the equation, $E = mc^2$, which for all intents and purposes, explains much of the how and why of the basic nature of light and gravity exists. As humanity continues to explore beyond our world, we are convinced that Einstein's Theory will hold in most cases, but anxious to see how discoveries may show deviations in what we currently understand. What has been interesting is that Einstein's General Theory of Relativity has applications in other areas of science, such as electromagnetism, global navigation satellite systems, nuclear power, and even the very existence of life itself.

Recently, a photograph of a black hole was generated (The Event Horizon Telescope Collaboration, 2019). Many do not appreciate that the initial conjecture of black holes was suggested by Karl Schwarzschild (Schwarzschild, 1916; see translation Schwarzschild, 2003), by solving the Einstein Field Equations a few months after Albert Einstein published his Theory on General Relativity (Einstein, 1915a; 1915b; 1915c; 1915d; see translations "Collected Papers of Albert Einstein" in 1987). There were other investigators, including Johannes Droste (1916) and David Hilbert (1917) who did much of the work to solve the Einstein Field Equations and the conjecture of black holes. It was Schwarzschild who attempted to solve the Field Equations for the static case (Schwarzschild, 1916; see translation Schwarzschild, 2003) a few months after Albert Einstein published his Theory on General Relativity (Einstein, 1915a; 1915b; 1915c; 1915d; see translations "Collected Papers of Albert Einstein" in 1987). Droste and

Hilbert were able to take Schwarzschild's work further and derived what some refer to as the "Schwarzschild Metric." Our tactic describes the Schwarzschild Metric derivation to solve the Einstein Field Equations in the vacuum solution of solving for the Ricci Tensor (Ricci and Levi-Civita, 1901; see translation, Ricci-Curbastro, 1975) and the Ricci Scalar (Ricci, 1904) by considering the mass density in the Einstein Field Equations to be equal to zero. The Cosmological term is also assumed to be zero. Schwarzschild and Hilbert considered a body to be stationary, which is a non-rotating body that can approximate real life cases such as the sun and earth. Hilbert first derived a solution that is called in modern parlance, an "Event Horizon" or what Hilbert called the "Schwarzschild Radius." If such a body has a Schwarzschild Radius that is larger than the radius of the object, the outcome is what is called, in modern language, a black hole. In the area around a black hole, a light-like vector that is stationary along the space axis may be produced. This is considered to be the Event Horizon of a black hole in which light itself cannot escape. It is possible to calculate the bending of light around a massive object in this scenario. Einstein calculated the bending of light and confirmation of this was provided in an expedition during a total solar eclipse. Hence, Einstein developed a new way of describing the nature of light that many believed was needed and a new theory of gravity emerged (Coles, 2019).

At the end of the 19th century, there was a need for new thinking on the nature of light and its propagation. Light was considered to be a wave and therefore needed some media to propagate in space. The concept of "luminiferous aether" (e.g. ether) was used as the sole propagating medium, and it should be possible to measure the speed of light as light passes from the sun with and against the movement of the ether. The motion of the ether should add or subtract as vector quantities from the velocity vector of the light. The speed of light experiment by Michelson and Morley (1887) shows no such change in the speed of light.

This lack of need for the ether as evidenced by this experiment, and by the Maxwell Equations of Electromagnetism (Maxwell, 1861a; 1861b; 1862a; 1862b; see Fleisch, 2010), which are independent of motion showing a constant velocity of light, also had a profound change on another paradigm. The Newtonian concept (Newton, 1687; see translation Newton, 1999) of absolute space in which a position can be determined with coordinates with the ether with a fixed position was overturned. Minkowski (1908; see translation Minkowski, 2012) developed the concept of space-time in which the geometry of space and time for any object exists with three dimensions of geometry and one dimension of time.

Although Einstein never personally refuted the presence of the ether, this lack of proof of its existence allowed Einstein to develop his General Theory of Relativity from the work of Minkowski. In this theory, relative space means all inertial frames can be considered to be equally valid in that all the laws of physics are the same within each frame. In having a fixed speed of light and all frames being equally valid, time and space might appear to be either compressed or time appears to run more slowly to an observer in another inertial frame of reference that would be very noticeable as one approaches the speed of light. An important consequence of this was a new insight concerning gravity. Because space-time sets up a manifold, one can think of it as a trampoline as space-time with a heavy object as a star represented by a bowling ball. One could try to roll the lighter object, such as a marble around the heavy object, but the depression in the trampoline would cause the marble to appear to swing around the heavier object. Gravity was the result of this disturbance of space-time.

Schwarzschild, Droste, and Hilbert used differential geometry to solve the Einstein Field Equations in the first non-trivial solution. Johannes Droste was the first to use the final Einstein paper to develop a non- trivial solution that shows repulsive gravity (Droste, 1916) Some of the mathematics used by Einstein and Schwarzschild to solve this equation was developed before Luigi Bianchi developed the geometry of topology (Bianchi, 1891) and fellow Italian, Gregorio Ricci (also referred to as Gregorio Ricci-Curbastro) developed the use of Tensor calculus that describes such a space. Tensors are geometric objects that map for the point of physics, two vectors to a new tensor. The calculus of these differential shapes can be treated by field equations that the first investigators of relativity (Schwarzschild, Droste, and Hilbert) used. In the following description, a set of vectors in such a geometric space is called a basis. Such a basis is described by a multidimensional array. In Tensor calculus, when a vector is invariant or whenever the direction and magnitude of the resulting vector is the same, its components of the field equation must transform to a contravariant rule to keep the direction and magnitude of the resulting vectors the same. In differential geometry, the Christoffel symbols (Γ) that are used in the field equations (Christoffel, 1869; see Eisenhart, 1940) are the array of numbers that describe a metric connection which is the topology, or surface geometry, that can be described by a vector bundle with a metric bundle.

Einstein's Special Theory of Relativity and Minkowski Space in Rectangular Cartesian Coordinates

Much of the focus on Einstein's work revolves around five papers published in 1905 and 1906 (Einstein, 1905a; 1905b; 1905c; 1905d; 1906). Einstein's Special Theory of Relativity states that the speed of light is a universal constant in an inertial reference frame, being that the sum of all forces is equal to zero for an object at rest or is in linear motion at a constant speed. From this result, Albert Einstein developed the theory that energy E equals the relative mass m times the speed of light squared c^2, where c represents the speed of light (Einstein, 1905d):

$$E = mc^2 = \left(\frac{m_0}{\sqrt{1 - v^2/c^2}}\right) c^2, \qquad (1)$$

and the relativistic mass m is the function of object's rest mass m_0, the object's speed v, and the speed of light c as shown in Equation 1.

Another interesting effect with Einstein's Special Theory of Relativity is time dilation. If one travels near the speed of light from our solar system to one of the nearby stars, their biological clock will move at a slower pace than those remaining on planet earth. This is referred to as Lorentz time dilation (Lorentz, 1899) whereby proper time change for one in the moving space craft is referred to as $\Delta\tau$ and time change for one on planet earth is Δt. The change in proper time $\Delta\tau$ for the fast-moving space craft will be equal to the following expression as a function of time Δt on planet earth, the speed v of the moving space craft, and the speed of light c:

$$\Delta\tau = \Delta t \sqrt{1 - v^2/c^2}. \qquad (2)$$

Thus, one has the following derivative function $dt/d\tau$, the derivative of earth time t with respect to time τ, for the individual flying away in the fast moving space craft:

$$\frac{dt}{d\tau} = \frac{\Delta t}{\Delta\tau} = \frac{1}{\sqrt{1 - v^2/c^2}}. \qquad (3)$$

In other words, a person moving near the speed of light will age at a slower rate than a person who is at rest with our solar system. If one squares Equation 3, takes the reciprocal, and then multiplies through with the speed of light squared c^2, the following important expression is derived:

$$c^2 - v^2 = c^2 \left(\frac{d\tau}{dt}\right)^2 = c^2 \frac{d\tau^2}{dt^2}. \tag{4}$$

Then we can multiply dt^2 through both sides of Equation 4 to obtain

$$c^2 dt^2 - v^2 dt^2 = c^2 d\tau^2. \tag{5}$$

Realizing that the quantity v^2 is the dot product of the velocity vector **v** with itself such that

$$\mathbf{v} \cdot \mathbf{v} = v_x^2 \hat{\mathbf{x}} \cdot \hat{\mathbf{x}} + v_y^2 \hat{\mathbf{y}} \cdot \hat{\mathbf{y}} + v_z^2 \hat{\mathbf{z}} \cdot \hat{\mathbf{z}} = v_x^2 + v_y^2 + v_z^2 = \left(\frac{dx}{dt}\right)^2 + \left(\frac{dy}{dt}\right)^2 + \left(\frac{dz}{dt}\right)^2. \tag{6}$$

Equation 5 becomes the following differential expression since dt^2 divides out to one for $v^2 dt^2$:

$$c^2 dt^2 - dx^2 - dy^2 - dz^2 = c^2 d\tau^2. \tag{7}$$

After multiplying Equation 7 by negative one, –1, the next expression is the linear function for what is referred to as Minkowski space or space-time in the four dimensions of x, y, z, and time t, with regards to proper time τ:

$$dx^2 + dy^2 + dz^2 - c^2 dt^2 = -c^2 d\tau^2. \tag{8}$$

Note that Equation 8 is simply the dot product of the any position vector in Minkowski space or space-time as referred to in Einstein's Special Theory of Relativity with the three unit vectors $\hat{\mathbf{x}}$, $\hat{\mathbf{y}}$, and $\hat{\mathbf{z}}$ as in Cartesian rectangular coordinates. Plus, there is an additional time unit vector $\hat{\mathbf{t}}$ for the fourth dimension of time t and unit vector $\hat{\boldsymbol{\tau}}$ for proper time τ concerning distance traveled in Minkowski space at the speed of light *c*:

$$ic\, d\tau\, \hat{\boldsymbol{\tau}} = dx\, \hat{\mathbf{x}} + dy\, \hat{\mathbf{y}} + dz\, \hat{\mathbf{z}} + ic\, dt\, \hat{\mathbf{t}}. \tag{9}$$

In Equation 9, $ic\, d\tau\, \hat{\boldsymbol{\tau}}$ represents velocity vector of an object moving through the four dimensions of Minkowski space, and the square of the distance traveled in Minkowski space is $-c^2 d\tau^2$. Thus, all objects move at the speed of light in the four-dimensional Minkowski space. Two objects appearing to be at rest with one another are moving at the speed of light along the t-axis relative to one another.

Concerning the derivation of Equation 1, the magnitude of the momentum vector is the relativistic mass m times the speed v of a moving object:

$$p = mv = \left(\frac{m_0}{\sqrt{1 - v^2/c^2}}\right) v. \tag{10}$$

By Newton's Second Law of Motion, force is equal to derivative of momentum with respect to time, so by using Equation 10 for the relativistic momentum, force is the following definition by Newton's Second Law in one-dimension for linear motion:

$$F = \frac{dp}{dt} = m\frac{dv}{dt} + \frac{dm}{dt}v = \frac{m_0}{(1 - v^2/c^2)^{3/2}}\frac{dv}{dt}. \tag{11}$$

Since by definition, work or change in kinetic energy is equal to the integral of force times distance, and when including relativistic momentum, work or kinetic energy is the following integral for an object accelerated by a constant force from rest to final velocity value v:

$$W = KE = \int_0^v \frac{m_0}{(1 - v^2/c^2)^{3/2}}\frac{dv}{dt}dx = \int_0^v \frac{m_0}{(1 - v^2/c^2)^{3/2}}\, v\, dv = mc^2 - m_0c^2. \tag{12}$$

It is important to note that the integral in Equation 12 is the derivation for Albert Einstein's famous equation relating mass and energy given in Equation 1, $E = mc^2 = KE + m_0c^2$. With regard to the expression in Equation 12, for speeds much less than the speed of light $(v \ll c)$, the kinetic energy for an object with rest mass m_0 is nearly equal to the classical kinetic energy value $KE = \frac{1}{2}m_0v^2$ using the binomial expansion for $1/\sqrt{1 - v^2/c^2} \approx 1 + \frac{1}{2}v^2/c^2$ for $v \ll c$. Note that Equation 12 applies to flat or Minkowski space that is not stretched. Stretched Minkowski space is how Einstein's General Theory of Relativity explains the force of gravity.

Spherical Polar Coordinates

Usually one uses the Cartesian coordinate system for three-dimensions comprised of x-, y-, and z-axes. The Cartesian coordinate system is appropriate when one involves the calculation of linear motion. However, when one wants to mathematically describe curved motion, it is much more conveniently to apply what is referred to as spherical polar coordinates r, θ, and ϕ. In vector notation, any position vector **r** in three-dimensions is represented below using Cartesian rectangular coordinates with orthonormal unit vectors **x̂, ŷ**, and **ẑ**:

$$\mathbf{r} = r_x\hat{\mathbf{x}} + r_y\hat{\mathbf{y}} + r_z\hat{\mathbf{z}}. \tag{13}$$

In addition concerning Equation 13 above, r_x, r_y, and r_z represents the x-, y-, and z-components of the position vector **r**, such that:

$$r_x = r\,\cos\phi\,\sin\theta\,; \qquad r_y = r\,\sin\phi\sin\theta\,; \qquad r_z = r\,\cos\theta. \tag{14}$$

Also, in Equations 14, r represents the magnitude of the position vector **r**:

$$r = \sqrt{r_x^2 + r_y^2 + r_z^2}. \qquad (15)$$

Angle ϕ represents the direction of the position vector **r** about the x-axis in the xy-plane at $z = 0$, and angle θ represents the direction of the position vector **r** about the z-axis. Henceforth, the position vector **r** can be represented as the following function of the vector magnitude r and angles ϕ and θ as

$$\mathbf{r} = r\ \cos\phi\ \sin\theta\,\hat{\mathbf{x}} + r\sin\phi\sin\theta\ \hat{\mathbf{y}} + r\cos\theta\ \hat{\mathbf{z}} = r\hat{\mathbf{r}}. \qquad (16)$$

Normally in spherical polar coordinates, the unit vector $\hat{\mathbf{r}}$ is presented as the following function of angles ϕ and θ and orthonormal unit vectors $\hat{\mathbf{x}}$, $\hat{\mathbf{y}}$, and $\hat{\mathbf{z}}$:

$$\hat{\mathbf{r}} = \cos\phi\ \sin\theta\,\hat{\mathbf{x}} + \sin\phi\sin\theta\ \hat{\mathbf{y}} + \cos\theta\ \hat{\mathbf{z}}. \qquad (17)$$

In the Cartesian rectangular coordinate system, the orthonormal unit vectors $\hat{\mathbf{x}}$, $\hat{\mathbf{y}}$, and $\hat{\mathbf{z}}$ have fixed directions and are always parallel with their corresponding x-, y-, and z-axes. On the other hand, in spherical polar coordinate system, orthonormal unit vectors $\hat{\mathbf{r}}$, $\widehat{\boldsymbol{\theta}}$, and $\widehat{\boldsymbol{\phi}}$ do not have fixed directions and change their directions with a moving position vector **r** while remaining perpendicular or orthogonal to each other, with unit vector $\hat{\mathbf{r}}$ always being parallel with position vector **r**.

By definition for the tangent of any curved function $y = f(x)$, the slope of the tangent at x is equal to the derivative of the function $y = f(x)$ with respect to x, slope = $df(x)/dx$. Thus, unit vectors $\widehat{\boldsymbol{\theta}}$ and $\widehat{\boldsymbol{\phi}}$ are the following derivative functions of $\hat{\mathbf{r}}$ with respect to θ and ϕ:

$$\widehat{\boldsymbol{\theta}} = \frac{\partial\hat{\mathbf{r}}}{\partial\theta} = \cos\phi\ \cos\theta\ \hat{\mathbf{x}} + \sin\phi\ \cos\theta\ \hat{\mathbf{y}} - \sin\theta\ \hat{\mathbf{z}}; \qquad (18)$$

$$\widehat{\boldsymbol{\phi}} = \frac{1}{\sin\theta}\frac{\partial\hat{\mathbf{r}}}{\partial\phi} = \frac{1}{\sin\theta}\left(-\sin\phi\ \sin\theta\ \hat{\mathbf{x}} + \cos\phi\ \sin\theta\ \hat{\mathbf{y}}\right) = -\sin\phi\ \hat{\mathbf{x}} + \cos\phi\ \hat{\mathbf{y}}. \qquad (19)$$

For Equation 19 to be a unit vector such that the dot product of $\widehat{\boldsymbol{\phi}}$ with itself is equal to one ($\widehat{\boldsymbol{\phi}} \cdot \widehat{\boldsymbol{\phi}} = 1$), it is necessary to divide by the quantity $\sin\theta$.

Before discussing the mathematics of Einstein's General Theory of Relativity, it also necessary to give a discussion about classical physics of bodies in motion using spherical polar coordinates. The next section not only discusses the employment of spherical polar coordinates for classical physics, but it also includes some digression on Tensor calculus, the mathematics used in Einstein's General Theory of Relativity.

Velocity and Acceleration in Spherical Polar Coordinates using Tensor Calculus

With regard to the velocity vector **v** and the acceleration vector **a** in rectangular Cartesian coordinates, they are simply the first and second derivatives with respect to time t of the position vector **r** for linear motion:

$$\mathbf{v} = \frac{d\mathbf{r}}{dt} = \frac{dr_x}{dt}\hat{\mathbf{x}} + \frac{dr_y}{dt}\hat{\mathbf{y}} + \frac{dr_z}{dt}\hat{\mathbf{z}}; \qquad (20)$$

$$\mathbf{a} = \frac{d\mathbf{v}}{dt} = \frac{d^2 r_x}{dt^2}\hat{\mathbf{x}} + \frac{d^2 r_y}{dt^2}\hat{\mathbf{y}} + \frac{d^2 r_z}{dt^2}\hat{\mathbf{z}}. \tag{21}$$

However, for curved motion with change in values of angles ϕ and θ as well as for the vector magnitude r with time, the mathematics becomes more complex which can be handled mathematically using Tensor calculus.

In spherical polar coordinates, the velocity vector **v** becomes the following first derivative of position vector **r** with respect to time:

$$\mathbf{v} = \frac{\partial \mathbf{r}}{\partial t} = \frac{\partial \mathbf{r}}{\partial r}\frac{dr}{dt} + \frac{\partial \mathbf{r}}{\partial \theta}\frac{d\theta}{dt} + \frac{\partial \mathbf{r}}{\partial \phi}\frac{d\phi}{dt}, \tag{22}$$

and now one must use the chain-rule when taking the first derivative of **v** with respect to time t, since r, θ, and ϕ are all functions of time which includes the changing directions of the unit vectors $\hat{\mathbf{r}}$, $\widehat{\boldsymbol{\theta}}$, and $\widehat{\boldsymbol{\phi}}$ with curved motion. The first derivative of vector **r** with respect to r can be represented as $\mathbf{r}_\mathbf{r}$ in Tensor calculus, as shown below, which is equal to the unit vector $\hat{\mathbf{r}}$ when taking the derivative of Equation 16 with respect to r:

$$\mathbf{r}_\mathbf{r} = \frac{\partial \mathbf{r}}{\partial r} = \cos\phi \sin\theta\, \hat{\mathbf{x}} + \sin\phi \sin\theta\, \hat{\mathbf{y}} + \cos\theta\, \hat{\mathbf{z}} = \hat{\mathbf{r}}. \tag{23}$$

Likewise, taking the derivative of vector **r** with respect to angle θ in Equation 16 results in the following expression defined as vector $\mathbf{r}_{\boldsymbol{\theta}}$, which ends up being equal to the product of the magnitude of the position vector **r** times the unit vector $\widehat{\boldsymbol{\theta}}$:

$$\mathbf{r}_{\boldsymbol{\theta}} = \frac{\partial \mathbf{r}}{\partial \theta} = r\,[\cos\phi \cos\theta\, \hat{\mathbf{x}} + \sin\phi \cos\theta\, \hat{\mathbf{y}} - \sin\theta\, \hat{\mathbf{z}}] = r\widehat{\boldsymbol{\theta}}. \tag{24}$$

Then when taking the derivative of Equation 16 with respect to angle ϕ, this results in the next expression for vector $\mathbf{r}_{\boldsymbol{\phi}}$ which is equal to the quantity $r \sin\theta\, \widehat{\boldsymbol{\phi}}$:

$$\mathbf{r}_{\boldsymbol{\phi}} = \frac{\partial \mathbf{r}}{\partial \phi} = r\,[-\sin\phi \sin\theta\, \hat{\mathbf{x}} + \cos\phi \sin\theta\, \hat{\mathbf{y}}] = r \sin\theta\, \widehat{\boldsymbol{\phi}}. \tag{25}$$

So in Tensor calculus, the velocity vector **v** can be represented as the following function by substitution of Equations 23 to 25 into Equation 22:

$$\mathbf{v} = \frac{dr}{dt}\mathbf{r}_\mathbf{r} + \frac{d\theta}{dt}\mathbf{r}_{\boldsymbol{\theta}} + \frac{d\phi}{dt}\mathbf{r}_{\boldsymbol{\phi}}. \tag{26}$$

In terms of unit vectors $\hat{\mathbf{r}}$, $\widehat{\boldsymbol{\theta}}$, and $\widehat{\boldsymbol{\phi}}$, the velocity vector in Equation 26 becomes

$$\mathbf{v} = \frac{dr}{dt}\hat{\mathbf{r}} + r\frac{d\theta}{dt}\widehat{\boldsymbol{\theta}} + r \sin\theta \frac{d\phi}{dt}\widehat{\boldsymbol{\phi}}. \tag{27}$$

Thus, the square of the velocity vector, via vector dot-product, becomes

$$\mathbf{v}\cdot\mathbf{v}=\left(\frac{dr}{dt}\right)^2+r^2\left(\frac{d\theta}{dt}\right)^2+r^2\sin^2\theta\left(\frac{d\phi}{dt}\right)^2. \tag{28}$$

In Tensor calculus, the velocity vector in Equation 26 is defined as a covariant vector that has orthogonal contravariant basis vectors $\mathbf{r_r}$, $\mathbf{r_\theta}$, and $\mathbf{r_\phi}$, with radial velocity component defined as $v^r = dr/dt$ and angular velocity components defined as $v^\theta = d\theta/dt$ and $v^\phi = d\theta/dt$, such that the velocity vector is represented instead as

$$\mathbf{v^i}=v^r\mathbf{r_r}+v^\theta\mathbf{r_\theta}+v^\phi\mathbf{r_\phi}, \tag{29}$$

with vector components being the following when using unit vectors $\hat{\mathbf{r}}$, $\hat{\boldsymbol{\phi}}$, and $\hat{\boldsymbol{\theta}}$:

$$v^r\mathbf{r_r}=\frac{dr}{dt}\hat{\mathbf{r}} \qquad v^\theta\mathbf{r_\theta}=\frac{d\theta}{dt}r\hat{\boldsymbol{\theta}} \qquad v^\phi\mathbf{r_\phi}=\frac{d\phi}{dt}r\sin\theta\,\hat{\boldsymbol{\phi}}. \tag{30}$$

The superscript **i** used for velocity vector in Equation 29 represents that this velocity vector is a covariant vector in Tensor calculus with the contravariant basis vectors $\mathbf{r_r}$, $\mathbf{r_\theta}$, and $\mathbf{r_\phi}$. Because the magnitudes of these basis vectors are not equal one, these three basis vectors are orthogonal but not orthonormal. Since the basis vectors are not orthonormal, in Tensor calculus the square of the magnitude of the velocity vector v by definition is equal to the following vector dot-product instead:

$$v^2=\mathbf{v^i}\cdot\mathbf{v_i}=\mathbf{v_i}\cdot\mathbf{v^i}=\left(\frac{dr}{dt}\right)^2+r^2\left(\frac{d\theta}{dt}\right)^2+r^2\sin^2\theta\left(\frac{d\phi}{dt}\right)^2. \tag{31}$$

In Equation 31, $\mathbf{v_i}$ is the following contravariant vector with covariant basis vectors $\mathbf{r^r}$, $\mathbf{r^\theta}$, and $\mathbf{r^\phi}$:

$$\mathbf{v_i}=v_r\mathbf{r^r}+v_\theta\mathbf{r^\theta}+v_\phi\mathbf{r^\phi}. \tag{32}$$

From the stipulation in Equation 28, the following vector dot-product applies concerning the contravariant and covariant basis vectors due to the orthogonal condition of the basis vectors:

$$\mathbf{r_i}\cdot\mathbf{r^j}=\delta_i^j=\mathbf{r^j}\cdot\mathbf{r_i}=\delta_j^i=1\ (\text{If } i=j); \tag{33}$$

$$\mathbf{r_i}\cdot\mathbf{r^j}=\delta_i^j=\mathbf{r^j}\cdot\mathbf{r_i}=\delta_j^i=0\ (\text{If } i\neq j). \tag{34}$$

In Equations 33 and 34, i and j represent parameters r, θ, and ϕ and the values of i and j range in value from 1 to 3. In Tensor calculus, x^1 is vector magnitude r, x^2 is angle θ, and x^3 is angle ϕ. Note that i and j are not exponents but indices instead. From the orthogonal conditions given in Equations 33 and 34 and the result from the dot product in Equation 31, covariant basis vectors $\mathbf{r^r}$, $\mathbf{r^\theta}$, and $\mathbf{r^\phi}$ are, therefore, equal to the following functions:

$$\mathbf{r^r}=\cos\phi\sin\theta\,\hat{\mathbf{x}}+\sin\phi\sin\theta\,\hat{\mathbf{y}}+\cos\theta\,\hat{\mathbf{z}}=\mathbf{r_r}=\hat{\mathbf{r}}; \tag{35}$$

$$\mathbf{r}^{\boldsymbol{\theta}} = \frac{1}{r}\cos\phi\cos\theta\,\hat{\mathbf{x}} + \frac{1}{r}\sin\phi\cos\theta\,\hat{\mathbf{y}} - \frac{1}{r}\sin\theta\,\hat{\mathbf{z}} = \frac{1}{r}\hat{\boldsymbol{\theta}}; \tag{36}$$

$$\mathbf{r}^{\boldsymbol{\phi}} = -\frac{1}{r\sin\theta}\sin\phi\,\hat{\mathbf{x}} + \frac{1}{r\sin\theta}\cos\phi\,\hat{\mathbf{y}} = \frac{1}{r\sin\theta}\hat{\boldsymbol{\phi}}. \tag{37}$$

Therefore, vector magnitudes v_r, v_θ, and v_ϕ are defined as follows:

$$v_r = \frac{dr}{dt} \quad \left(v_r\mathbf{r}^{\mathbf{r}} = v^r\mathbf{r}_{\mathbf{r}} = \frac{dr}{dt}\hat{\mathbf{r}}\right); \tag{38}$$

$$v_\theta = r^2\frac{d\theta}{dt} \quad \left(v_\theta\mathbf{r}^{\boldsymbol{\theta}} = v^\theta\mathbf{r}_{\boldsymbol{\theta}} = r\frac{d\theta}{dt}\hat{\boldsymbol{\theta}}\right); \tag{39}$$

$$v_\phi = r^2\sin^2\theta\frac{d\phi}{dt} \quad \left(v_\phi\mathbf{r}^{\boldsymbol{\phi}} = v^\phi\mathbf{r}_{\boldsymbol{\phi}} = r\sin\theta\frac{d\phi}{dt}\hat{\boldsymbol{\phi}}\right). \tag{40}$$

Also, by Tensor calculus, we have the following conditions for the orthogonal basis vectors:

$$\mathbf{r}_{\mathbf{i}}\cdot\mathbf{r}_{\mathbf{j}} = g_{ij} = g_{ji} \neq 0 \;\; (\text{If } i = j) \text{ and } \mathbf{r}_{\mathbf{i}}\cdot\mathbf{r}_{\mathbf{j}} = g_{ij} = g_{ji} = 0 \;\; (\text{If } i \neq j); \tag{41}$$

$$\mathbf{r}^{\mathbf{i}}\cdot\mathbf{r}^{\mathbf{j}} = g^{ij} = g^{ji} \neq 0 \;\; (\text{If } i = j) \text{ and } \mathbf{r}^{\mathbf{i}}\cdot\mathbf{r}^{\mathbf{j}} = g^{ij} = g^{ji} = 0 \;\; (\text{If } i \neq j). \tag{42}$$

In spherical polar coordinates, therefore,

$$\mathbf{r}_{\mathbf{r}}\cdot\mathbf{r}_{\mathbf{r}} = g_{rr} = 1; \quad \mathbf{r}_{\boldsymbol{\theta}}\cdot\mathbf{r}_{\boldsymbol{\theta}} = g_{\theta\theta} = r^2; \quad \mathbf{r}_{\boldsymbol{\phi}}\cdot\mathbf{r}_{\boldsymbol{\phi}} = g_{\phi\phi} = r^2\sin^2\theta; \tag{43}$$

$$\mathbf{r}^{\mathbf{r}}\cdot\mathbf{r}^{\mathbf{r}} = g^{rr} = 1; \quad \mathbf{r}^{\boldsymbol{\theta}}\cdot\mathbf{r}^{\boldsymbol{\theta}} = g^{\theta\theta} = \frac{1}{r^2}; \quad \mathbf{r}^{\boldsymbol{\phi}}\cdot\mathbf{r}^{\boldsymbol{\phi}} = g^{\phi\phi} = \frac{1}{r^2\sin^2\theta}. \tag{44}$$

When taking the first derivative of the velocity vector **v** in Equation 26 with respect to time to derive the acceleration vector **a**, it is important to realize that one has to take the derivative of each vector $\mathbf{r}_{\mathbf{r}}$, $\mathbf{r}_{\boldsymbol{\theta}}$, and $\mathbf{r}_{\boldsymbol{\phi}}$ with respect to r, θ, and ϕ as well as with respect to time t using the chain rule Therefore, the acceleration vector **a,** the derivative of Equation 26 with respect to time, becomes the following expression:

$$\begin{aligned}\mathbf{a} = \frac{d\mathbf{v}}{dt} = \frac{d^2\mathbf{r}}{dt^2} = \mathbf{r}_{\mathbf{r}}\frac{d^2r}{dt^2} + \frac{\partial\mathbf{r}_{\mathbf{r}}}{\partial r}\left(\frac{dr}{dt}\right)^2 + \frac{\partial\mathbf{r}_{\mathbf{r}}}{\partial\theta}\frac{dr}{dt}\frac{d\theta}{dt} + \frac{\partial\mathbf{r}_{\mathbf{r}}}{d\phi}\frac{dr}{dt}\frac{d\phi}{dt} + \mathbf{r}_{\boldsymbol{\theta}}\frac{d^2\theta}{dt^2} + \frac{\partial\mathbf{r}_{\boldsymbol{\theta}}}{\partial r}\frac{dr}{dt}\frac{d\theta}{dt} + {}\\ \frac{\partial\mathbf{r}_{\boldsymbol{\theta}}}{\partial\theta}\left(\frac{d\theta}{dt}\right)^2 + \frac{\partial\mathbf{r}_{\boldsymbol{\theta}}}{\partial\phi}\frac{d\phi}{dt}\frac{d\theta}{dt} + \mathbf{r}_{\boldsymbol{\phi}}\frac{d^2\phi}{dt^2} + \frac{\partial\mathbf{r}_{\boldsymbol{\phi}}}{\partial r}\frac{dr}{dt}\frac{d\phi}{dt} + \frac{\partial\mathbf{r}_{\boldsymbol{\phi}}}{\partial\theta}\frac{d\theta}{dt}\frac{d\phi}{dt} + \frac{\partial\mathbf{r}_{\boldsymbol{\phi}}}{\partial\phi}\left(\frac{d\phi}{dt}\right)^2.\end{aligned} \tag{45}$$

In Tensor calculus, derivatives of each vector $\mathbf{r}_{\mathbf{r}}$, $\mathbf{r}_{\boldsymbol{\theta}}$, and $\mathbf{r}_{\boldsymbol{\phi}}$ with respect to r, θ, and ϕ are defined by the following two expressions:

$$\frac{\partial \mathbf{r_i}}{\partial x^j} = \Gamma_{i,ij}\mathbf{r^i} + \Gamma_{j,ij}\mathbf{r^j} + \Gamma_{k,ij}\mathbf{r^k}; \tag{46}$$

$$\frac{\partial \mathbf{r_i}}{\partial x^j} = \Gamma^i_{ij}\mathbf{r_i} + \Gamma^j_{ij}\mathbf{r_j} + \Gamma^k_{ij}\mathbf{r_k}. \tag{47}$$

In Equations 46 and 47, x^j represents parameters r, θ, and ϕ, and orthogonal contravariant-basis vector $\mathbf{r_i}$, $\mathbf{r_j}$, and $\mathbf{r_k}$ represent vectors $\mathbf{r_r}$, $\mathbf{r_\theta}$, and $\mathbf{r_\phi}$ and likewise the same for covariant-basis vectors $\mathbf{r^i}$, $\mathbf{r^j}$, and $\mathbf{r^k}$ represent vectors $\mathbf{r^r}$, $\mathbf{r^\theta}$, and $\mathbf{r^\phi}$. In Tensor calculus, as stated previously, x^1 is vector magnitude r, x^2 is angle θ, and x^3 is angle ϕ. In Equations 46 and 47, $\Gamma_{i,ij}$ is call the Christoffel symbol of the first-kind and Γ^i_{ij} is called the Christoffel symbol of the second-kind, where i, j, and k range from values 1 to 3. With reference to Equations 46 and 47, the Christoffel symbols of the first- and second-kind are the following dot-products due to the orthogonality of the basis vectors:

$$\Gamma_{k,ij} = \frac{\partial \mathbf{r_i}}{\partial x^j}\cdot \mathbf{r_k} \qquad \left[\Gamma_{j,ik} = \frac{1}{2}\left(\frac{\partial g_{ij}}{\partial x^k} + \frac{\partial g_{kj}}{\partial x^i} - \frac{\partial g_{ik}}{x^j}\right) = g_{jj}\Gamma^j_{ik} \text{ since } g_{ij} = 0 \text{ if } i \neq j\right]; \tag{48}$$

$$\Gamma^k_{ij} = \frac{\partial \mathbf{r_i}}{\partial x^j}\cdot \mathbf{r^k} \qquad \left(\Gamma^j_{ik} = \sum_{l=1}^{3} g^{jl}\Gamma_{l,ik} = g^{jj}\Gamma_{j,ik} \text{ since } g^{ij} = 0 \text{ if } i \neq j\right). \tag{49}$$

What is also shown in each parenthesis for both Equations 48 and 49 is another way to evaluate both the first- and second-kind of Christoffel symbols.

When using mathematics to quantitatively analyze gravity from Einstein's General Theory of Relativity, the Christoffel symbol of the second-kind is use instead of the first-kind. Since there are 3 dimensions in spherical polar coordinates, there are a total of $3^3 = 27$ different Christoffel symbols of the first- and second-kind each, but only 9 out of the 27 of the first- and second-kind turn out not to be equal to zero using Equations 48 and 49. The 9 Christoffel symbols of the first- and second-kind not equal to zero are shown in Table I, and 3 pairs of the 9 non-zero Christoffel symbols of the first- and second-kind being equal because of the condition $\Gamma_{k,ij} = \Gamma_{k,ji}$ and $\Gamma^k_{ij} = \Gamma^k_{ji}$.

If one substitutes the summations in Equation 46 for the derivatives $d\mathbf{r}_\mathrm{i}/x^j$ into Equation 45 for the acceleration vector **a,** collect all like terms for basis vectors $\mathbf{r_r}$, $\mathbf{r_\theta}$, and $\mathbf{r_\phi}$, one has for each component, $\mathbf{r_r}$, $\mathbf{r_\theta}$, and $\mathbf{r_\phi}$, the following expression of the acceleration vector **a**:

$$\mathbf{a}_\mathrm{i} = \left(\frac{d^2x^i}{dt^2} + \sum_{j=1}^{3}\sum_{k=1}^{3}\Gamma^i_{jk}\frac{dx^j}{dt}\frac{dx^k}{dt}\right)\mathbf{r_i}. \tag{50}$$

Table I. Christoffel Symbols for spherical polar coordinates.

Christoffel Symbols of the Second-Kind	Christoffel Symbols of the First-Kind
$\Gamma^r_{\theta\theta} = \mathbf{r}^{\mathrm{r}} \cdot \frac{\partial \mathbf{r}}{\partial \theta} = \Gamma^1_{22} = -r$	$\Gamma_{r,\theta\theta} = \mathbf{r}_{\mathrm{r}} \cdot \frac{\partial \mathbf{r}_{\boldsymbol{\theta}}}{\partial \theta} = -r$
$\Gamma^r_{\phi\phi} = \mathbf{r}^{\mathrm{r}} \cdot \frac{\partial \mathbf{r}_{\boldsymbol{\phi}}}{\partial \phi} = \Gamma^1_{33} = -r\sin^2\theta$	$\Gamma_{r,\phi\phi} = \mathbf{r}_{\mathrm{r}} \cdot \frac{\partial \mathbf{r}_{\boldsymbol{\phi}}}{\partial \phi} = -r\sin^2\theta$
$\Gamma^\theta_{r\theta} = \mathbf{r}^{\boldsymbol{\theta}} \cdot \frac{\partial \mathbf{r}_{\mathrm{r}}}{\partial \theta} = \Gamma^\theta_{\theta r} = \Gamma^2_{12} = \Gamma^2_{21} = \frac{1}{r}$	$\Gamma_{\theta,r\theta} = \mathbf{r}_{\boldsymbol{\theta}} \cdot \frac{\partial \mathbf{r}_{\mathrm{r}}}{\partial \theta} = \Gamma_{\theta,\theta r} = r$
$\Gamma^\theta_{\phi\phi} = \mathbf{r}^{\boldsymbol{\theta}} \cdot \frac{\partial \mathbf{r}_{\boldsymbol{\phi}}}{\partial \phi} = \Gamma^2_{33} = -\cos\theta\sin\theta$	$\Gamma_{\theta,\phi\phi} = \mathbf{r}_{\boldsymbol{\theta}} \cdot \frac{\partial \mathbf{r}_{\boldsymbol{\phi}}}{\partial \phi} = -r^2\cos\theta\sin\theta$
$\Gamma^\phi_{r\phi} = \mathbf{r}^{\boldsymbol{\phi}} \cdot \frac{\partial \mathbf{r}_{\mathrm{r}}}{\partial \phi} = \Gamma^\phi_{\phi r} = \Gamma^3_{13} = \Gamma^3_{31} = \frac{1}{r}$	$\Gamma_{\phi,r\phi} = \mathbf{r}_{\boldsymbol{\phi}} \cdot \frac{\partial \mathbf{r}_{\mathrm{r}}}{\partial \phi} = \Gamma_{\phi,\phi r} = r\sin^2\theta$
$\Gamma^\phi_{\theta\phi} = \mathbf{r}^{\boldsymbol{\phi}} \cdot \frac{\partial \mathbf{r}_{\boldsymbol{\phi}}}{\partial \theta} = \Gamma^\phi_{\phi\theta} = \Gamma^3_{23} = \Gamma^3_{32} = \cot\theta$	$\Gamma_{\phi,\phi\theta} = \mathbf{r}_{\boldsymbol{\phi}} \cdot \frac{\partial \mathbf{r}_{\boldsymbol{\phi}}}{\partial \theta} = \Gamma_{\phi,\theta\phi} = r^2\sin\theta\cos\theta$

Also, when one substitutes only those Christoffel symbols of the second-kind which are not equal to zero, Equation 45 becomes the following expression:

$$\frac{d^2\mathbf{r}}{dt^2} = \left[\frac{d^2r}{dt^2} - r\left(\frac{d\theta}{dt}\right)^2 - r\sin^2\theta\left(\frac{d\phi}{dt}\right)^2\right]\mathbf{r}_{\mathrm{r}} + \left[\frac{d^2\theta}{dt^2} + \frac{2}{r}\frac{dr}{dt}\frac{d\theta}{dt} - \cos\theta\sin\theta\left(\frac{d\phi}{dt}\right)^2\right]\mathbf{r}_{\boldsymbol{\theta}} + \left[\frac{d^2\phi}{dt^2} + \frac{2}{r}\frac{dr}{dt}\frac{d\phi}{dt} + 2\cot\theta\frac{d\theta}{dt}\frac{d\phi}{dt}\right]\mathbf{r}_{\boldsymbol{\phi}}. \tag{51}$$

With constant speed for tangential velocity along a curve of a surface, meaning no change in kinetic energy and shortest distance between two points on a curve, each of the magnitudes within the brackets, for all three components $\mathbf{r}_{\mathrm{r}}$, $\mathbf{r}_{\boldsymbol{\theta}}$, and $\mathbf{r}_{\boldsymbol{\phi}}$, will be equal to zero in Equation 51. This is a result for the traditional Geodesic equation being equal to zero concerning the minimum distance between two points on a curved path:

$$\frac{d^2x^i}{dt^2} + \sum_{j=1}^{3}\sum_{k=1}^{3}\Gamma^i_{jk}\frac{dx^j}{dt}\frac{dx^k}{dt} = 0. \tag{52}$$

With regard to each term in the expression of Equation 52, zero acceleration implies the following equations being equal to zero:

$$\frac{d^2r}{dt^2} - r\left(\frac{d\theta}{dt}\right)^2 - r\sin^2\theta\left(\frac{d\phi}{dt}\right)^2 = 0; \tag{53}$$

$$\frac{d^2\theta}{dt^2} + \frac{2}{r}\frac{dr}{dt}\frac{d\theta}{dt} - \cos\theta\sin\theta\left(\frac{d\phi}{dt}\right)^2 = 0; \tag{54}$$

$$\frac{d^2\phi}{dt^2} + \frac{2}{r}\frac{dr}{dt}\frac{d\phi}{dt} + 2\cot\theta\frac{d\theta}{dt}\frac{d\phi}{dt} = 0. \tag{55}$$

If the angle θ is equal to π/2 radians (or for a 90° angle) and is constant, Equations 53 to 55 become:

$$\frac{d^2r}{dt^2} - r\left(\frac{d\theta}{dt}\right)^2 = 0; \tag{56}$$

$$\frac{d^2\theta}{dt^2} = 0; \tag{57}$$

$$\frac{d^2\phi}{dt^2} + \frac{2}{r}\frac{dr}{dt}\frac{d\phi}{dt} = 0. \tag{58}$$

Equation 56 matches with circular motion where radial acceleration is zero, such as a perfect circular planetary orbit, and the $r(d\theta/dt)^2$accounts for centripetal acceleration towards the center. In addition, when multiplying r through the expression given in Equation 58, the second term in Equation 58 represents the Coriolis force $2(dr/dt)(d\phi/dt)$, which one feels as they walk outward or inward in the radial direction of a rotating Carousel or Merry-Go-Round.

The derivation of the Geodesic expression in Equation 52 being equal to zero is obtained from the following Lagrangian L for motion of constant speed v in three-dimensions first by multiplying ½ though Equation 28:

$$L = \frac{1}{2}v^2 = \frac{1}{2}\left(\frac{dr}{dt}\right)^2 + \frac{1}{2}r^2\left(\frac{d\theta}{dt}\right)^2 + \frac{1}{2}r^2\sin^2\theta\left(\frac{d\phi}{dt}\right)^2. \tag{59}$$

If one has constant speed moving between two points, and for minimum distance between two points on a curve, the following expression, known as the Euler-Lagrange equation (see Fox, 1963), is equal to zero for all three components of $\mathbf{r_r}$, $\mathbf{r_\theta}$, and $\mathbf{r_\phi}$:

$$\frac{d}{dt}\left(\frac{\partial L}{\partial(dx^i/dt)}\right) - \frac{\partial L}{\partial x^i} = 0. \tag{60}$$

Applying the condition in Equation 60 for terms in Equation 59 will also result in determining which Christoffel symbols of the second-kind are not equal to zero in spherical polar coordinates. When the expression in Equation 60 is set equal to zero, this yields the minimum distance between two points on a curved path which also matches up with constant speed for motion upon a curved surface.

For Minkowski space, Equation 8, represented in spherical polar coordinates becomes the following expression with reference to Equation 28:

$$-c^2 d\tau^2 = -c^2 dt^2 + dr^2 + r^2 d\theta^2 + r^2 \sin^2\theta d\phi^2. \tag{61}$$

Theoretically, gravitational force is due to the stretching of Minkowski space, or four-dimensional space-time. When Schwarzschild developed his metric using Einstein's General Theory of Relativity, he assumed the stretching was about time t and radial distance r for a spherically shaped planet, star, collapsed star, or black hole in space. His initial expression for stretched Minkowski space is the following function involving exponents:

$$-c^2 d\tau^2 = -e^a c^2 dt^2 + e^{-a} dr^2 + r^2 d\theta^2 + r^2 \sin^2\theta d\phi^2. \tag{62}$$

Karl Schwarzschild initially set both exponents as the same two unknown functions of radial distance r, $a = a(r)$, with one being positive in the first addition term in Equation 62 and negative in the second addition term. He made this choice in order that the Minkowski space could be stretched but not curved. This correlates with the theory that outside a planet, star, collapsed star, or black hole, Minkowski space should not be curved but only stretched. If we divide both sides of Equation 62 with $d\tau^2$ and multiply through by ½, one has the following Lagrangian L:

$$L = -\frac{1}{2}c^2 = -\frac{1}{2}e^a c^2 \left(\frac{dt}{d\tau}\right)^2 + \frac{1}{2}e^{-a}\left(\frac{dr}{d\tau}\right)^2 + \frac{1}{2}r^2\left(\frac{d\theta}{d\tau}\right)^2 + \frac{1}{2}r^2\sin^2\theta\left(\frac{d\phi}{d\tau}\right)^2. \tag{63}$$

Unknown function of r, $a = a(r)$, should be evaluated using the mandatory condition that the diagonal elements of the Ricci tensor $\mathcal{R}_{ii}$ are all equal to zero. This is true if there is no curvature in the four-dimensions of Minkowski space.

All the off-diagonal elements $\mathcal{R}_{ij}$ are, of course, equal to zero due to the orthogonality of unit vectors $\hat{\mathbf{r}}$, $\hat{\boldsymbol{\theta}}$, $\hat{\boldsymbol{\phi}}$, and $\hat{\mathbf{t}}$. There is a total of 16 elements (4×4) in the Ricci tensor which is a 4-by-4 matrix. When all of these 16 elements in the Ricci tensor are equal to zero, then there is no curvature in the four-dimensional space-time outside the planet, star, collapsed star, or black hole. But first, we must use the Euler-Lagrange equation to evaluate which Christoffel symbols of the second-kind in Minkowski space that are not equal to zero, by using the mathematical condition in Minkowski space that the speed is always equal to c, that of light, because there is no acceleration or deceleration. This is similar to the derivation of Equation 51 for curved motion in three-dimensions parameterized by time that all the components for basis vectors $\mathbf{r}_\mathbf{r}$, $\mathbf{r}_\boldsymbol{\theta}$, and $\mathbf{r}_\boldsymbol{\phi}$ are equal to zero. We first determine the Geodesic equation for time t coordinate x^0, such that for the four-dimensions indices 0 is used for the time axis, and as previously 1 for r, 2 for θ, and 3 for ϕ. We take the first derivative of Equation 63 with respect to proper time τ:

$$\frac{\partial L}{\partial \tau} = 0, \tag{64}$$

and then we take the first derivative of Equation 63 with respect to $dt/d\tau$:

$$\frac{\partial L}{\partial(dt/d\tau)} = -c^2 e^a \frac{dt}{d\tau}. \qquad (65)$$

The next step is to take the derivative of Equation 65 with respect to proper time τ:

$$\frac{d}{d\tau}\left[\frac{\partial L}{\partial(dt/d\tau)}\right] = -c^2 \frac{da}{dr} e^a \frac{dr}{d\tau}\frac{dt}{d\tau} - c^2 e^a \frac{d^2t}{d\tau^2}. \qquad (66)$$

Afterwards, one substitutes Equations 65 and 66 into Equation 60, the Euler-Lagrange equation becomes the following expression:

$$\frac{d}{d\tau}\left[\frac{\partial L}{\partial(dt/d\tau)}\right] - \frac{\partial L}{\partial t} = -c^2 \frac{da}{dr} e^a \frac{dr}{d\tau}\frac{dt}{d\tau} - c^2 e^a \frac{d^2t}{d\tau^2} - 0 = 0. \qquad (67)$$

To obtain the Geodesic equation, one simply multiplies Equation 67 with negative one and then divide by the product c^2e^a:

$$\frac{d^2t}{d\tau^2} + \frac{da}{dr}\frac{dr}{d\tau}\frac{dt}{d\tau} = 0. \qquad (68)$$

In comparison with the Geodesic equation in Equation 52, replacing time t with proper time τ, Equation 52 is the following expression:

$$\frac{d^2x^i}{d\tau^2} + \sum_{j=0}^{n}\sum_{k=0}^{n} \Gamma^i_{jk} \frac{dx^j}{d\tau}\frac{dx^k}{d\tau} = 0. \qquad (69)$$

When comparing Equation 69 with Equation 68, obviously there are only two Christoffel symbols of the second-kind that are not equal to zero and are also equal to one another. Because of the double summation in the Geodesic equation for coordinates t and r (x^0 and x^1 for $j = 0$, $k = 1$ and $j = 1$, $k = 0$), the Christoffel symbol is one-half of the first-derivative of function a with respect to r, da/dr. Table II lists all the Christoffel symbols of the second-kind derived using the Euler-Lagrange equation for Equation 63 involving the function $a(r)$ to be determined. There are a total of 14 Christoffel symbols of the second-kind that are not equal to zero when the other 50 are equal to zero. There are a total of 64 Christoffel symbols of the second-kind due to having four-dimensions, 4×4×4 = 64.

To evaluate the exponent a as some function of r, we will need to set the diagonal terms of the Ricci tensor to zero. We have up to 16 contravariant Riemann curvature tensor values $\mathcal{R}^i_{kik}$ to calculate, four for each Ricci tensor components $\mathcal{R}_{ii}$, with i and k equal 0 to 3 for all four dimensions. By definition (see Cheng, 2010), the covariant and contravariant Riemann tensors are the following two expressions for 4 dimensions of Minkowski space:

$$\mathcal{R}_{kmji} = \frac{\partial \Gamma_{k,mi}}{\partial x^j} - \frac{\partial \Gamma_{k,mj}}{\partial x^i} + \sum_{n=0}^{3} \Gamma_{n,mj}\Gamma^n_{ki} - \sum_{n=0}^{3} \Gamma_{n,mi}\Gamma^n_{kj}\,; \tag{70}$$

$$\mathcal{R}^k_{mji} = \frac{\partial \Gamma^k_{mi}}{\partial x^j} - \frac{\partial \Gamma^k_{mj}}{\partial x^i} + \sum_{n=0}^{3} \Gamma^k_{nj}\Gamma^n_{mi} - \sum_{n=0}^{3} \Gamma^k_{ni}\Gamma^n_{mj}. \tag{71}$$

Table II **Christoffel symbols of the second-kind before and after deriving the Schwarzschild metric with $\boldsymbol{a(r) = \log_e[1 - GM_0/(c^2 r)]}$**

$\Gamma^t_{rt} = \Gamma^t_{tr} = \Gamma^0_{10} = \Gamma^0_{01} = \frac{1}{2}\frac{da}{dr}$	$\Gamma^t_{rt} = \Gamma^t_{tr} = \Gamma^0_{10} = \Gamma^0_{01} = \frac{GM_0/(c^2 r^2)}{1 - 2GM_0/(c^2 r)}$
$\Gamma^r_{tt} = \Gamma^1_{00} = \frac{1}{2}\frac{da}{dr} e^{2a} c^2$	$\Gamma^r_{tt} = \Gamma^1_{00} = \frac{GM_0}{r^2}\left(1 - \frac{2GM_0}{c^2 r}\right)$
$\Gamma^r_{rr} = \Gamma^1_{11} = -\frac{1}{2}\frac{da}{dr}$	$\Gamma^r_{rr} = \Gamma^1_{11} = -\frac{GM_0/(c^2 r^2)}{1 - 2GM_0/(c^2 r)}$
$\Gamma^r_{\theta\theta} = \Gamma^1_{22} = -e^a r$	$\Gamma^r_{\theta\theta} = \Gamma^1_{22} = -\left(1 - \frac{2GM_0}{c^2 r}\right) r$
$\Gamma^r_{\phi\phi} = \Gamma^1_{33} = -e^a r \sin^2\theta$	$\Gamma^r_{\phi\phi} = \Gamma^1_{33} = -\left(1 - \frac{2GM_0}{c^2 r}\right) r \sin^2\theta$
$\Gamma^\theta_{r\theta} = \Gamma^\theta_{\theta r} = \Gamma^2_{12} = \Gamma^2_{21} = \frac{1}{r}$	
$\Gamma^\theta_{\phi\phi} = \Gamma^2_{33} = -\sin\theta\cos\theta$	
$\Gamma^\phi_{r\phi} = \Gamma^\phi_{\phi r} = \Gamma^3_{13} = \Gamma^3_{31} = \frac{1}{r}$	
$\Gamma^\phi_{\theta\phi} = \Gamma^\phi_{\phi\theta} = \Gamma^3_{23} = \Gamma^3_{32} = \cot\theta$	

In flat space, no matter the number of dimensions, the two expressions in Equations 70 and 71 will all be equal to zero, but not in curved or stretched space. The two expressions in Equations 70 and 71 are derived from the following double derivative when using Tensor calculus, which we are showing an example of for 3 dimensions in spherical polar coordinates:

$$D_r D_\theta \mathbf{R}(r,\theta,\phi) - D_\theta D_r \mathbf{R}(r,\theta,\phi) = f(r,\theta,\phi). \tag{72}$$

$D_r\mathbf{R}(r,\theta,\phi)$ is the following total derivative of a function:

$$D_r\mathbf{R}(r,\theta,\phi) = \frac{\partial}{\partial r}\left[R_r(r,\theta,\phi)\mathbf{r}_\mathrm{r} + R_\theta(r,\theta,\phi)\mathbf{r}_\theta + R_\phi(r,\theta,\phi)\mathbf{r}_\phi\right]. \tag{73}$$

Note that components of the **R** vector are all functions of the three spherical polar coordinates. Thus, just taking the first derivate with respect to r yields the following:

$$D_r\mathbf{R}(r,\theta,\phi) = \frac{\partial R_r}{\partial r}\mathbf{r_r} + R_r\frac{\partial \mathbf{r_r}}{\partial r} + \frac{\partial R_\theta}{\partial r}\mathbf{r_\theta} + R_\theta\frac{\partial \mathbf{r_\theta}}{\partial r} + \frac{\partial R_\phi}{\partial r}\mathbf{r_\phi} + R_\phi\frac{\partial \mathbf{r_\phi}}{\partial r}. \tag{74}$$

Note that this involves taking partial derivatives of the basis vectors which also result in further expansions using the Christoffel symbols of either the first- or second-kind. Using the first-kind gives the result in Equation 70, and use of the second-kind yields the result in Equation 71. This mathematics is very tedious and lengthy, and so it will not be shown in further detail. However, if one works out the math for the subtraction of the double derivative function $D_rD_\theta\mathbf{R}(r,\theta,\phi) - D_\theta D_r\mathbf{R}(r,\theta,\phi)$ and collects like terms, one has either of the two forms of the Riemann curvature tensor for basis vector $\mathbf{r^k}$ or $\mathbf{r_k}$:

$$\mathcal{R}_{kmji}\mathbf{r^k} = \left(\frac{\partial \Gamma_{k,mi}}{\partial x^j} - \frac{\partial \Gamma_{k,mj}}{\partial x^i} + \sum_{n=0}^{3}\Gamma_{n,mj}\Gamma^n_{ki} - \sum_{n=0}^{3}\Gamma_{n,mi}\Gamma^n_{kj}\right)\mathbf{r^k}; \tag{75}$$

$$\mathcal{R}^k_{mji}\mathbf{r_k} = \left(\frac{\partial \Gamma^k_{mi}}{\partial x^j} - \frac{\partial \Gamma^k_{mj}}{\partial x^i} + \sum_{n=0}^{3}\Gamma^k_{nj}\Gamma^n_{mi} - \sum_{n=0}^{3}\Gamma^k_{ni}\Gamma^n_{mj}\right)\mathbf{r_k}. \tag{76}$$

Note that if $D_rD_\theta\mathbf{R}(r,\theta,\phi) - D_\theta D_r\mathbf{R}(r,\theta,\phi) = f(r,\theta,\phi) = 0$, both terms in the parenthesis above will be equal to zero for all basis vectors $\mathbf{r^k}$ and $\mathbf{r_k}$ due to the space being flat and not stretched.

The Riemann tensor matrix has up to 256 curvature components due to the presence of four dimensions (4×4×4×4=256). By another definition (see Cheng, 2010), the Ricci curvature tensor components $\mathcal{R}_{ii}$ are equal to the following summation of the contravariant Riemann curvature tensor components for the four dimensions in Minkowski space:

$$\mathcal{R}_{ii} = \sum_{j=0}^{3}\mathcal{R}^j_{iji}. \tag{77}$$

To calculate $\mathcal{R}_{00}$ or $\mathcal{R}_{tt}$, we must first calculate the following Riemann curvature tensor components $\mathcal{R}^0_{000}$, $\mathcal{R}^1_{010}$, $\mathcal{R}^2_{020}$, and $\mathcal{R}^3_{030}$ as follows. The first Riemann curvature tensor component $\mathcal{R}^0_{000}$ or $\mathcal{R}^t_{ttt}$ is simply equal to zero since the terms being subtracted out are identical:

$$\mathcal{R}^0_{000} = \frac{\partial \Gamma^0_{00}}{\partial x^0} - \frac{\partial \Gamma^0_{00}}{\partial x^0} + \sum_{n=0}^{3}\Gamma^0_{n0}\Gamma^n_{00} - \sum_{n=0}^{3}\Gamma^0_{n0}\Gamma^n_{00} \quad (\mathcal{R}^t_{ttt} = 0). \tag{78}$$

However, we will need to evaluate $\mathcal{R}^1_{010}$ or $\mathcal{R}^r_{trt}$:

$$\mathcal{R}^1_{010} = \frac{\partial \Gamma^1_{00}}{\partial x^1} - \frac{\partial \Gamma^1_{01}}{\partial x^0} + \sum_{n=0}^{3} \Gamma^1_{n1}\Gamma^n_{00} - \sum_{n=0}^{3} \Gamma^1_{n0}\Gamma^n_{01}. \tag{79}$$

Because the three Christoffel symbols of the second-kind Γ^0_{01}, Γ^1_{00}, and Γ^1_{11} or Γ^t_{tr}, Γ^r_{tt}, and Γ^r_{rr} are not equal to zero:

$$\mathcal{R}^1_{010} = \frac{\partial \Gamma^1_{00}}{\partial x^1} + \Gamma^1_{11}\Gamma^1_{00} - \Gamma^1_{00}\Gamma^0_{01} \quad \left(\mathcal{R}^r_{trt} = \frac{\partial \Gamma^r_{tt}}{\partial r} + \Gamma^r_{rr}\Gamma^r_{tt} - \Gamma^r_{tt}\Gamma^t_{tr}\right). \tag{80}$$

After substituting in Γ^0_{01}, Γ^1_{00}, and Γ^1_{11} given in Table II before deriving the Schwarzschild metric, Equation 80 becomes the following expression:

$$\mathcal{R}^1_{010} = \frac{\partial}{\partial r}\left(\frac{1}{2}\frac{da}{dr}e^{2a}c^2\right) + \left(-\frac{1}{2}\frac{da}{dr}\right)\left(\frac{1}{2}\frac{da}{dr}e^{2a}c^2\right) - \left(\frac{1}{2}\frac{da}{dr}e^{2a}c^2\right)\left(\frac{1}{2}\frac{da}{dr}\right); \tag{81}$$

$$\mathcal{R}^1_{010} = \frac{1}{2}\frac{d^2a}{dr^2}e^{2a}c^2 + \frac{1}{2}\left(\frac{da}{dr}\right)^2 e^{2a}c^2 = \frac{1}{2}\left[\frac{d^2a}{dr^2} + \left(\frac{da}{dr}\right)^2\right]e^{2a}c^2. \tag{82}$$

Since two of the following Christoffel symbols of the second-kind Γ^1_{00} and Γ^2_{12} or Γ^r_{tt} and $\Gamma^\theta_{r\theta}$ are not equal to zero, likewise we need to evaluate Riemann curvature tensor component $\mathcal{R}^2_{020}$ or $\mathcal{R}^\theta_{t\theta t}$:

$$\mathcal{R}^2_{020} = \Gamma^2_{12}\Gamma^1_{00} \quad \left(\mathcal{R}^\theta_{t\theta t} = \Gamma^\theta_{r\theta}\Gamma^r_{tt}\right); \tag{83}$$

$$\mathcal{R}^2_{020} = \frac{1}{r}\left(\frac{1}{2}\frac{da}{dr}e^{2a}c^2\right). \tag{84}$$

Concerning the Riemann curvature tensor component $\mathcal{R}^3_{030}$ or $\mathcal{R}^\phi_{t\phi t}$, the Christoffel symbols of the second-kind Γ^1_{00} and Γ^3_{13} or Γ^r_{tt} and $\Gamma^\phi_{r\phi}$ are not equal to zero, so the computation of $\mathcal{R}^3_{030}$ is as follows:

$$\mathcal{R}^3_{030} = \Gamma^3_{13}\Gamma^1_{00} \quad \left(\mathcal{R}^\phi_{t\phi t} = \Gamma^\phi_{r\phi}\Gamma^r_{tt}\right); \tag{85}$$

$$\mathcal{R}^3_{030} = \frac{1}{r}\left(\frac{1}{2}\frac{da}{dr}e^{2a}c^2\right). \tag{86}$$

So, our first diagonal element of the Ricci curvature tensor $\mathcal{R}_{00}$ is the following summation:

$$\mathcal{R}_{00} = \mathcal{R}^0_{000} + \mathcal{R}^1_{010} + \mathcal{R}^2_{020} + \mathcal{R}^3_{030}; \tag{87}$$

$$\mathcal{R}_{00} = 0 + \frac{1}{2}\frac{d^2a}{dr^2}e^{2a}c^2 + \frac{1}{2}\left(\frac{da}{dr}\right)^2 e^{2a}c^2 + \frac{1}{r}\left(\frac{1}{2}\frac{da}{dr}e^{2a}c^2\right) + \frac{1}{r}\left(\frac{1}{2}\frac{da}{dr}e^{2a}c^2\right). \tag{88}$$

Factoring out $e^{2a}c^2$ and $1/2$ we obtain the following expression for Equation 88:

$$\mathcal{R}_{00} = \frac{1}{2}\left[\frac{d^2a}{dr^2} + \left(\frac{da}{dr}\right)^2 + \frac{2}{r}\frac{da}{dr}\right]e^{2a}c^2. \tag{89}$$

For $\mathcal{R}_{00}$ to be equal to zero, the expression, within the brackets, of Equation 89 must be equal to zero:

$$\frac{d^2a}{dr^2} + \left(\frac{da}{dr}\right)^2 + \frac{2}{r}\frac{da}{dr} = 0. \tag{90}$$

Table III displays all the diagonal elements of the Ricci curvature tensor calculated using the above discussion. In Table III, it is important to note that the Ricci curvature tensor $\mathcal{R}_{11}$ contains the expression of Equation 90 within brackets also. So if Equation 90 is equal to zero, $\mathcal{R}_{11}$ likewise is equal to zero.

The next step is by setting equal to zero the Ricci curvature tensor $\mathcal{R}_{22}$ (Table III) ensuring that Ricci curvature tensor $\mathcal{R}_{33}$ is also equal to zero:

$$-\left(r\frac{da}{dr} + 1\right)e^a + 1 = 0. \tag{91}$$

Table III **The Ricci curvature tensors $\mathcal{R}_{ii}$ for $i = 0$ to 3 (t, r, θ, ϕ) set equal to zero to derive the Schwarzschild metric determining that $a(r) = \log_e[1 - GM_0/(c^2r)]$**

$$\mathcal{R}_{00} = \frac{1}{2}\left[\frac{d^2a}{dr^2} + \left(\frac{da}{dr}\right)^2 + \frac{2}{r}\frac{da}{dr}\right]e^{2a}c^2$$

$$\mathcal{R}_{11} = -\frac{1}{2}\left[\frac{d^2a}{dr^2} + \left(\frac{da}{dr}\right)^2 + 2\frac{da}{dr}\frac{1}{r}\right]$$

$$\mathcal{R}_{22} = -\left(\frac{da}{dr}r + 1\right)e^a + 1$$

$$\mathcal{R}_{33} = \left[-\left(\frac{da}{dr}r + 1\right)e^a + 1\right]\sin^2\theta = \mathcal{R}_{22}\sin^2\theta$$

To evaluate the exponent a as a function of radius r, we will use the following static situation:

$$dr = 0, \qquad d\theta = 0, \qquad d\phi = 0. \tag{92}$$

Insert the Christoffel symbols of the second-kind into the Geodesic equation for coordinate r (Table II):

$$\frac{d^2r}{d\tau^2} + \frac{1}{2}\frac{da}{dr}e^{2a}c^2\left(\frac{dt}{d\tau}\right)^2 - \frac{1}{2}\frac{da}{dr}\left(\frac{dr}{d\tau}\right)^2 - e^a r\left(\frac{d\theta}{d\tau}\right)^2 - e^a r\sin^2\theta\left(\frac{d\phi}{d\tau}\right)^2 = 0, \tag{93}$$

and Equation 93 becomes the following expression for the static condition:

$$\frac{d^2r}{d\tau^2} + \frac{1}{2}\frac{da}{dr}e^{2a}c^2\left(\frac{dt}{d\tau}\right)^2 = 0. \tag{94}$$

In theory, Equation 94 should match Newtonian gravity because we are using the static condition:

$$\frac{d^2r}{d\tau^2} = -\frac{1}{2}\frac{da}{dr}e^{2a}c^2\left(\frac{dt}{d\tau}\right)^2 = -\frac{GM_0}{r^2}. \tag{95}$$

In Equation 95, M_0 is the rest mass of the sun and G is the constant for Newton's Universal Law of Gravitation. To determine the value of the squared differential expression $(dt/d\tau)^2$, we will use the same static case, dividing $d\tau^2$ through Equation 62 for the Schwarzschild metric, or stretched Minkowski space, in the following expression:

$$-c^2 = -c^2e^a\left(\frac{dt}{d\tau}\right)^2 + e^{-a}\left(\frac{dr}{d\tau}\right)^2 + r^2\left(\frac{d\theta}{d\tau}\right)^2 + r^2\sin^2\theta\left(\frac{d\phi}{d\tau}\right)^2, \tag{96}$$

and because of the static case in Equation 96, $(dt/d\tau)^2$ is equal to e^{-a} or $(dt/d\tau)^2 = e^{-a}$. Thus, we can now substitute e^{-a} for $(dt/d\tau)^2$ in Equation 95 to obtain:

$$\frac{1}{2}\frac{da}{dr}e^{2a}c^2\left(\frac{dt}{d\tau}\right)^2 = \frac{1}{2}\frac{da}{dr}e^{a}c^2 = \frac{GM_0}{r^2}. \tag{97}$$

Therefore,

$$\frac{da}{dr} = \left(\frac{2GM_0}{c^2r^2}\right)e^{-a}. \tag{98}$$

One can then substitute the expression of Equation 98 into Equation Ricci tensor component $\mathcal{R}_{22} = 0$ from Table III for da/dr to arrive at:

$$-\left[\left(\frac{2GM_0}{c^2r^2}\right)e^{-a}r + 1\right]e^a + 1 = 0, \tag{99}$$

and if one solves for unknown a as a function of radial distance r using Equation 99, the result is the following natural logarithmic function:

$$a = \log_e\left(1 - \frac{2GM_0}{c^2 r}\right). \tag{100}$$

Thus, the Schwarzschild metric becomes

$$-c^2 d\tau^2 = -\left(1 - \frac{2GM_0}{c^2 r}\right)c^2 dt^2 + \frac{dr^2}{\left(1 - \frac{2GM_0}{c^2 r}\right)} + r^2 d\theta^2 + r^2 \sin^2\theta d\phi^2. \tag{101}$$

From Equation 101, the radius r of a black hole in outer spaced produced by the collapse of a sufficient large star is equal to the ratio $2GM_0/c^2$.

The final step is to utilize Equation 100 in Equation 90 to see if $\mathcal{R}_{00} = 0$ and $\mathcal{R}_{11} = 0$ (See Table III). Initially, take the first- and second-derivatives of Equation 100 with respect to r, and then square the first-derivative. Substituting these into Equation 90, the term in the brackets of Ricci curvature tensors $\mathcal{R}_{00}$ and $\mathcal{R}_{11}$, one will note that Equation 90 is equal to zero matching with $\mathcal{R}_{11}$ and $\mathcal{R}_{00}$ being zero in value:

$$\frac{d^2a}{dr^2} + \left(\frac{da}{dr}\right)^2 + 2\frac{da}{dr}\frac{1}{r} = -\frac{\left(\frac{2GM_0}{c^2r^2}\right)^2}{\left(1 - \frac{2GM_0}{c^2r}\right)^2} - 2\left(\frac{\frac{2GM_0}{c^2r^2}}{1 - \frac{2GM_0}{c^2r}}\right)\frac{1}{r} + \frac{\left(\frac{2GM_0}{c^2r^2}\right)^2}{\left(1 - \frac{2GM_0}{c^2r}\right)^2} + 2\left(\frac{\frac{2GM_0}{c^2r^2}}{1 - \frac{2GM_0}{c^2r}}\right)\frac{1}{r} = 0. \tag{102}$$

The first and third terms of Equation 102 add up to zero, and the second and fourth terms likewise add up to zero also, because of the way Schwarzschild had assumed the exponents in Equation 62 would yield $\mathcal{R}_{00}$ and $\mathcal{R}_{11}$ both being equal to zero. Table II lists the Christoffel symbols of the second-kind that match up the Schwarzschild metric as shown in Equation 101.

Conclusion

If a collapsing star had sufficient mass to form a black hole instead of a white dwarf, it would take infinity for the collapsing star to reach the radius of a black hole, as calculated for a black hole using the Schwarzschild metric $2GM_0/c^2$, because of time dilation to a value of zero at this radius value. If one could ever vision an object falling into a black hole, initially they would see it accelerate in velocity, but due to time dilation in very strong gravitational fields, as the falling object approaches the surface of a black hole, or even a white dwarf, they would see the object begin to slow down in speed due to the time dilation effects of strong gravitational fields. This is one of the mysteries of our universe, and we humans may find it a challenge to observe experimentally, due to our short life-time expectancies and the vast distances of our universe.

Finally, if one performs the correct number of algebraic manipulations on Equation 101, the Schwarzschild metric, the following equation results:

$$\left[\left(1-\frac{2GM}{c^2r}\right)^2\left(\frac{dt}{d\tau}\right)^2-1\right]\frac{1}{2}m_0c^2=\frac{1}{2}m_0v^2-\frac{GM_0m_0}{r}-\frac{GM_0m_0}{c^2}r\left(\frac{d\phi}{d\tau}\right)^2. \qquad (103)$$

Note that the expression in Equation 103 matches the classical Newtonian gravity for planetary motion around the sun for the first two terms on the right-hand-side of the equation, the summation of the kinetic energy and the negative gravitational potential energy:

$$\frac{1}{2}m_0v^2-\frac{GM_0m_0}{r}=E \quad (E<0 \text{ for bound orbits}) \quad (E\geq 0 \text{ for unbound orbits}). \qquad (104)$$

The third term becomes important if for a stable orbit, the planetary speed begins to approach that of light in a very strong gravitational field such as that of a white dwarf or black hole. For orbital speeds much less than that of light, the third term in Equation 103 is nearly equal to zero:

$$\frac{GM_0m_0}{c^2}r\left(\frac{d\phi}{d\tau}\right)^2\approx 0 \text{ for speeds much less than that of light } (v\ll c).$$

Therefore, Equation 103 approaches the expression in Equation 104 for orbital speeds much less than that of light.

Karl Schwarzschild is a much-underappreciated physicist of the 20th century. He was an experimental and theoretical physicist. He developed tools and concepts that are still salient in astronomy. He came up with the concept of spectral type and the color of a star, and developed tools with course grating that measured the separation of stars. Schwarzschild also made significant contributions to quantum theory by explaining the Stark Effect or how light splits in an electric field. Schwarzschild died too early at the age of 43 in 1916 from complications of war injuries. He never had a chance to improve upon his 1915 paper, as Droste and Hilbert did. Schwarzschild, with his short life, made a huge impact on physics, and it is hard to name another 20th century physicist that impacted quantum theory and relativity and astronomy, with the theory and the experiments. It is equally important to remember other key investigators such as Droste and Hilbert who elaborated further on our understanding of Einstein's Theory of Relativity. For example, Droste's work on Repulsive Gravity is considered by some researchers to be a possible basis of Dark Energy (Droste, 1916).

In this paper, we have undertaken a unique approach to explain Einstein's Theory of Relativity for the educated mathematician, and provided some historical aspects on how these ideas evolved. We have demonstrated the effect on light and the origin of black holes by using the first non-trivial solution to the Einstein Field Equations. The further exploitation of Einstein's Field Equations led by other scientists such as Stephen Hawking has been more recent and much of it still trails back to these initial contributions on black holes. Much of modern physics owes a great debt to these early investigators.

As a society, we are fortunate that Einstein's General Theory of Relativity inspired many talented investigators such as Schwarzschild, Droste, and Hilbert. The practical applications from concepts of these investigators have been immense on society. For instance, global navigation satellite systems are more accurate with use of relativistic corrections, which has many implications in agriculture, communications, conservation, real estate, meteorology, military, travel, and other vocations where navigation precision is important. In a nutshell, Einstein's Theory of Relativity explains why different observers, traveling at different speeds can and will have different perspectives about their surroundings. We do not know where this concept will take humanity even in the next few decades. It is understood that a person or object that exceeds the speed of light may, indeed, travel into the future, but is that really possible? It is not much of a prognostication that we will call 21st century physics the *Age of Relativity.*

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Submitted June 8, 2021 Accepted October 11, 2021

ABSTRACTS OF THE
110TH OKLAHOMA ACADEMY OF SCIENCE TECHNICAL MEETING
NOVEMBER 5, 2021
EAST CENTRAL UNIVERSITY – ADA

SECONDARY SCHOOLING TYPE AND DIFFERENCES IN ACADEMIC SELF-EFFICACY AND SELF-REGULATION

LaDonna Autrey. East Central University

College students who received secondary education of homeschooling or traditional schooling were compared using self-efficacy for learning and academic self-regulation ability. A quantitative nonexperimental study was used with a convenience sample of 184 traditional undergraduate college students (44 homeschooled and 140 traditionally schooled) from 18 to 24 years of age. Participants completed the SELF, MSLQ, Parental Involvement-Student Section, and demographic questions. An ANCOVA found homeschoolers had significantly higher academic self-efficacy but found no significant differences in academic self-regulation when holding parental involvement and SES constant. No significant difference was found between participants' SES or parental involvement levels, so an ANOVA analysis was used and found homeschooled students had significantly higher academic self-efficacy but no significant difference from traditional students on academic self-regulation. Differences in academic self-efficacy could stem from differing pedagogical approaches and role students play in directing homeschool education not found in traditional schooling. Lack of difference found in academic self-regulation could stem from erratic answer patterns displayed on the MSLQ not found on the other surveys.

CLONING, SEQUENCING AND IDENTIFICATION OF TWO CLINICAL ENTEROVIRUS ISOLATES FROM OKLAHOMA

Earl Blewett, McKayla Muse, Brett Szymanski, and BJ Reddig, Oklahoma State University Center for Health Sciences

We obtained clinical enterovirus isolates from the Oklahoma State Department of Health. Two isolates have proven very useful and have been studied in two publications (Brett, et al. 2019a, 2019b). We are continuing research on anti-viral drugs that act on a wide spectrum of viruses, using these isolates. To confirm the identity of the isolates, which were typed at the Department of Health, we are cloning, sequencing and analyzing the viral genomic nucleic acid sequence using bioinformatics. The isolate CoxA9-01 has been positively identified as a Coxsackie A Virus. Isolate Echo2-01 has been confirmed as an Echovirus 2.

NURSE PLANT EFFECT INCREASES PLANT SIZE AND INSECT ABUNDANCE ON *SOLANUM CAMPYLACANTHUM* ON AN AFRICAN SAVANNAH

Sudikshya Budhathoki and H. George Wang, East Central University

Nurse plant effect is a type of plant–plant interaction that can facilitate the focal plant by microclimate modification or protection from herbivory. It also has the potential to affect the community pattern at other trophic levels. We conducted a controlled field experiment to examine the nurse plant effect of *Acacia etbaica* on a perennial shrub *Solanum campylacanthum* in an area frequented by large mammalian herbivores in Laikipia County, Kenya. Ninety *S. campylacanthum* saplings were randomly assigned to three treatments: Thorn, Non-thorn, and Control. The initial height, stem length, and number of leaves were recorded for each plant. Plants of the Thorn treatment were covered with branches of *A. etbaica*, plants of the Non-thorn treatment were covered with branches of *Croton dichogamous*, and plants of the Control treatment were not covered. The *Solanum* plants were allowed to grow for nine months. At the end of the experiment the plant sizes were measured again and the insects on the plants were sampled. We also collected five leaves from each plant and used ImageJ to quantify leaf damage from insect herbivory. We used generalized linear modeling to compare the plant growth and insect density between the treatments. We also used linear mixed-effects modeling to compare leaf damage between treatments. The nurse plant significantly increased the growth of the *Solanum* plants and the density of insects on them relative to the Control plants. *Solanum* plants in the Thorn treatment had more leaf damage than the Control plants. The nurse plants, especially the type with thorns (*A. etbaica*), appear to have reduced large mammalian herbivory on *S. campylacanthum*, which in turn facilitated insect herbivory on the focal plant.

ANTIBIOTIC RESISTANT AND GROWTH PATTERNS OF INVASIVE SPECIES IMPORTED ON MELONS FROM CENTRAL AMERICA

Katlyn Hampton, D. Melton, A. Gauchan, and C. Biles, East Central University

Invasive species of fungi are commonly imported into the United States on fruits and vegetables. Our goal with this project was to establish what the differences were between the species imported locally. The 2 genera that we studied were *Fusarium* and *Diaporthe*. We investigated a range of physiological traits in order to better understand them including antibiotic resistance and growth characteristics. We performed a growth study where we used different kinds of media to observe species' specific growth rates across a range of 5 agar plates. We also performed an antibiotic study where we tested the isolate cultures to analyze resistance and susceptibility to 10 different antibiotics. Finally, we performed a temperature study to determine what the optimum temperature was for each species to grow. Results indicate that Czapek-Dox (CZD) and Corn Meal Agar (CMA) provided the best nutrients for growth for most species. The most effective antibiotic we tested on all isolates was econazole 10 mcg, and the least effective was fluconazole 25 mcg. The optimum temperatures were 23° C and 25° C. Invasive species threaten immunocompromised people as well as the environment. By better understanding what these species prefer, we can work towards minimizing their impact.

BIOLOGICAL CHARACTERIZATION OF THE CYTOTOXIC EFFECTS OF NOVEL LACTATE DEHYDROGENASE INHIBITORS IN MIA PACA-2 CELLS

Hanna Hill, Horrick Sharma, and Pragya Sharma, Southwestern Oklahoma State University

Outstanding Undergraduate Paper in Biomedical Sciences

Pancreatic ductal adenocarcinoma cancer (PDAC) is one of the most lethal cancers, with a 5-year survival rate of ~8% and a median survival duration of fewer than six months. Therefore, identifying new biological drug targets is urgent. In the attempt to develop innovative anticancer treatments and selectively target cancer cells, growing interest has recently focused on the peculiar metabolic properties of cancer cells. One of the characteristic metabolic hallmarks of tumor metabolism is aerobic glycolysis. The Warburg effect in cancer cells is regulated by lactate dehydrogenase-A (LDH-A), which is shown to be overexpressed in many cancer types, including PDAC. Several studies have shown great potential in targeting this enzyme as a means of developing novel treatments. Our lab, in collaboration, has identified diverse novel lactate dehydrogenase inhibitors, which have shown promising anticancer activity against pancreatic cancer cells without having any cytotoxic effect in normal cells. We are currently investigating the mechanism of antiproliferative activity of lead LDHA inhibitors through Western blotting and Annexin V assays.

ANALYSIS OF THE SPATIAL DISTRIBUTION OF GEOREFERENCED HERBARIUM RECORDS IN OKLAHOMA AND TEXAS

Sierra Hubbard, Oklahoma State University

Outstanding Graduate Paper

Digitized and georeferenced herbarium collections are invaluable for research focused on spatial distributions of plants. However, herbarium data are often spatially biased; easily accessible locations are often overrepresented in herbaria, while remote locations are underrepresented. These spatial biases are compounded by the lack of digitization and georeferencing efforts in certain areas. The Texas Oklahoma Regional Consortium of Herbaria (TORCH) database currently houses 1.2 million vascular plant records, but only ~30% have been assigned geographic coordinates. Additional georeferencing improves data accessibility for researchers and can help address spatial bias, but it is necessary to develop methods for prioritizing locations for georeferencing. My objective is to analyze the spatial distributions of herbarium collections in Oklahoma and Texas to identify underrepresented locations that should be targeted for georeferencing efforts. My dataset is made up of ~300,000 georeferenced vascular plant records from Oklahoma and Texas. Examining the abundance of herbarium records from each county and the proportions of georeferenced records revealed that there are very few herbarium records from many counties in north and northwest Texas. The majority of these records do not have coordinates. Additionally, many counties of eastern and east-central Texas and one county in central Oklahoma have less than 15% of specimens georeferenced. Based on these findings, I recommend that TORCH georeferencing efforts should first focus on plant collections from the west-northwest and eastern-central regions of Texas. More specifically, locations in and near Haskell, Midland, Roberts, and Robertson counties appear to be in greatest need of attention.

IDENTIFYING CRITICAL HIGHER-ORDER INTERACTIONS IN COMPLEX NETWORKS

Sidra Jawaid, Mehmet Aktas, Thu Nguyen, Rakin Riza, and Esra Akbas ,University of Central Oklahoma

Outstanding Undergraduate Paper in Math, Computer Science & Statistics

Diffusion on networks is an important concept in network science observed in many situations such as information spreading and rumor controlling in social networks, disease contagion between individuals, and cascading failures in power grids. The critical interactions in networks play critical roles in diffusion and primarily affect network structure and functions. While interactions can occur between two nodes as pairwise interactions, i.e., edges, they can also occur between three or more nodes, which are described as higher-order interactions. In this talk, we present a novel method to identify critical higher-order interactions in complex networks. We propose two new Laplacians to generalize standard graph centrality measures for higher-order interactions. We then compare the performances of the generalized centrality measures using the size of giant component and the Susceptible-Infected-Recovered (SIR) simulation model to show the effectiveness of using higher-order interactions. We further compare them with the first-order interactions (i.e., edges). Experimental results suggest that higher-order interactions play more critical roles than edges based on both the size of giant component and SIR, and the proposed methods are promising in identifying critical higher-order interactions.

THE PALEOECOLOGY OF YAHUAI CAVE IN GUANGXI, CHINA AT 120 KYA: IMPLICATIONS FOR EARLY MODERN HUMAN DISPERSAL INTO EAST ASIA

Kathleen Kelley, University of Tulsa

One of the main questions in human evolution is the dispersal of modern humans across a range of ecological niches. Given that today South China is situated in a tropical environment, the question remains, if early modern humans dispersing into this region could penetrate the rainforest to forage for food and if the environment in that area was suitable for early hominins to seek out during their exodus out of Africa around 100kya. As a case study we present the small mammal remains from Yahuai Cave, Guangxi, China, dated to around 120,000 years ago as they dispersed into East Asia. The study aims to identify the paleoecology of the area by utilizing Middle Range and Niche Construction theories to make an analogous comparison between extant and the extinct micromammals around the region of the Yahuai cave. Reconstruction of the paleoecology will include a three-step process: taxonomic identification, quantification and a taphonomic study. The study will look at similar comparisons at other sites that were along the path of hominin's exit, to further solidify the paleoecological finding and the probability that early hominins would have utilized this novel ecosystem.

INTERACTION OF DNA NUCLEOBASES WITH ARMCHAIR GRAPHENE NANORIBBONS: A VAN DER WAALS DENSITY FUNCTIONAL THEORY INVESTIGATION

Pujan Khatri, Sagar Ghimire, and Sanjiv K. Jha, East Central University

Benjamin O. Tayo, University of Central Oklahoma

Outstanding Undergraduate Paper in Physical Science

Graphene is a suitable candidate for a wide spectrum of applications, including the biosensing and sequencing of DNA nucleobases. In this work, we computationally examined the interaction of four DNA bases [Adenine (A), Cytosine (C), Guanine (G), and Thymine (T)] with graphene nanoribbons (GNRs) using periodic density functional theory (DFT). Our calculations were performed using the van der Waals corrected DFT (vdW-DF2 and semi-empirical Grimme's-D2) methods, as implemented in Quantum Espresso simulation package. N-Armchair graphene nanoribbons (AGNRs) with three different widths corresponding to the indices N = 13, 14, and 15, passivated with the hydrogen atoms were considered in our study. The binding energies of nucleobases on GNRs were examined for GNRs containing no surface defects, containing Stone-Wales (SW) defects, and containing di-vacancy (DV) defects. Our results show that DNA nucleobases form stable complexes with GNRs. The DNA bases showed different interaction strengths on graphene nanoribbons, and their binding energies followed the order: G > A > T > C. It was found that the presence of structural defects on the GNRs has no significant effect on the computed binding energies of DNA bases on GNRs. The computing for this project was performed at the OU Supercomputing Center for Education & Research (OSCER) at the University of Oklahoma (OU).

CONSERVATION OF KINASE DOMAINS WITHIN THE INSULIN/TOR SIGNALING PATHWAY OF *DROSOPHILA*

Aidan Long, Ryan Dufur, and Jimmy O'Brien, Oklahoma Christian University

Outstanding Undergraduate Paper in Biochemistry & Molecular Biology

The insulin/TOR signaling pathway is a key pathway within organisms that contributes to maintaining homeostasis and allows the uptake of glucose into cells. This pathway has been found to be well conserved in *Drosophila* species when compared to humans. Sik3 and S6k are two proteins that both fall within the insulin/TOR pathway. Sik3 was found to be less connected within the pathway than S6k, so we hypothesized that S6k would be more highly conserved than Sik3 due to the number of interactions within the pathway. After confirming the higher conservation of S6k in comparison to Sik3, we shifted our focus to the conservation of kinase proteins. Kinases are proteins that catalyze the movement of a phosphate from ATP to another protein, these proteins have kinase domains that are the structural active site of the phosphorylation process. Due to these domains having more interactions when compared to the whole protein, we hypothesized that the kinase domains would show a higher level of conservation than the overall whole protein. It was shown that the kinase domains of Sik3 and S6k had a lower degree of percent change when compared to the whole protein sequence. The proteins Sik3 and S6k are more conserved in the kinase domain than the overall protein sequence due to the interactive active site that is key for the protein function.

DNA POLYMERASE EPSILON MUTANTS EXHIBIT DELAYED RECOVERY AFTER DNA DAMAGE

Ostmo Lydia and Michael Smith, Northeastern State University

DNA replication requires many proteins to interact together to keep copies of our DNA intact and free of errors. Recent work in our lab with budding yeast has shown that Mcm10 plays an integral role in DNA polymerase epsilon (Polε) functionality. Polε contains three structural subunits and one essential catalytic subunit known as POLE1 in mammals and Pol2 in budding yeast. The N-terminal half of Pol2 contains functionally characterized DNA polymerase and exonuclease domains but the C-terminal half contains no experimentally characterized domains aside from two putative Zn-finger modules that are conserved from yeast to humans. Previously, we have shown that the C-terminus of Pol2 interacts with Mcm10 in budding yeast. Expanding on this research, we constructed mutations in yeast Pol2 that interrupted interaction with Mcm10. The current project studied cell cycle progression in the yeast POL2 mutants after exposure to DNA damage. Our results suggest that the specific mutants of Pol2 take longer to complete chromosome replication when treated with hydroxyurea. Our second project investigated the interaction of MCM10 and POLE1 in human cells. Co-immunoprecipitation experiments confirmed the interaction in HEK293T cells. Current experiments are focused on exploring POLE1 C-terminus mutants and their interactions in human cells. Our work in yeast and human cells will shed light on Polymerase Epsilon and MCM10 interaction and the function of this interaction in the maintenance of genome stability.

RECOVERY STUDY OF SILVER NANOPARTICLES THROUGH LIMESTONE AND DOLOMITE PACKED COLUMNS

Randall Maples, Rachel L Bley, and Jarett A Williams, East Central University

Nanomaterials have found use in diverse applications such as materials coatings and pharmaceutics, among many others because of the unique properties they possess. Due to this increased usage in society, the fate of these engineered nanoparticles being released into the ecosystem is important as potential environmental contaminants when devices and materials containing these nanoparticles are disposed of. Studies of the behavior of various nanoparticles in the environment is not new and has been underway for some time. Still, however, the environmental toxicity of these materials has not been fully determined due to the sheer variety of engineered nanoparticles available and in use. It is important to be able to assess the short and long-term fate of these engineered materials and the distribution of various nanoparticles in groundwater. This preliminary study examines the percent recovery of water dispersible functionalized silver nanoparticles using columns packed with limestone or dolomite as a potential model for their behavior in the local groundwater environment.

LEAF WEIGHT AND SURFACE AREA OF THE THREE SISTERS IN MONOCULTURES AND POLYCULTURES

Jamian Maxwell and Leah S. Dudley, East Central University

It has been shown that plants grown in polycultures can have increased productivity when comparing them to monocultures. Leaf weight and surface area were taken when looking at the three sisters (bean, corn, and squash) in both monocultures and polycultures. Looking at these factors in relation to each other can give some insight on whether the three sisters grow more effectively in a polyculture or a monoculture. Four varieties of each sister were grown in a factorial design in two culture treatments: monoculture (3 varieties of the same sister) or polyculture (one variety each of corn, bean, and squash). Three seeds were sown into a mound randomly located within a garden space. They were then randomly assigned one of three watering treatments: control, medium (5mL/day on average of rainwater added) and high (10mL/day) Leaves were haphazardly collected from each living plant at the end of the flowering season, pressed and later weighed and scanned for leaf area The area was measured by using the program ImageJ. Surface area to leaf weight ratio has been linked to photosynthetic potential and yield in plants. We assess this ratio for the Three Sisters in monoculture compared to polyculture and hypothesize that plants in polyculture may benefit from the association compared to monoculture. If results support our hypothesis, then growing plants as a polyculture supports this long-held tradition.

MOLECULAR AND MORPHOLOGICAL CHARACTERISTICS OF PATHOGENIC FUNGAL SPECIES IMPORTED FROM CENTRAL AMERICA

Dylan Melton, Angeela Gauchan, Katlyn Hampton, Alisha Howard and Charles Biles, East Central University

Plant pathogenic fungi are the leading cause of plant diseases in the world. The increasing discovery of plant pathogenic fungal species in the United States is, in many cases, due to the introduction of infected host crops via international trade. The genus *Diaporthe* (Phomopsis) is distributed to several geographical locations and many host crops as an endophyte or a latent pathogen, causing disease at the host crop's maturity. The purpose of this study was to identify the presence of Diaporthe spp. on specifically melons (*Cucumis melo* L. var. *cantalupensis* Naudin) being imported from Central America to Oklahoma. We purchased a total of 61 melons from 3 different markets in Ada, Oklahoma. Melons were imported from Honduras, Mexico, and Costa Rica. The melons were washed in a 10% bleach solution and set out on a clean surface for 4 to 6 days, until lesions were visible. Infected tissue was isolated from the mesocarp tissue of the melon and put onto acidic potato dextrose agar (APDA) to facilitate fungal growth and prevent any bacterial contamination. Once in pure culture, the DNA was extracted using an OmniPrep Fungal Kit and amplified using polymerase chain reaction (PCR). Three different loci were targeted during this study: Beta-tubulin (TUB), Transcription Elongation Factor 1-alpha (TEF), and HIS. Prior to analysis, Koch's postulates was conducted with each *Diaporthe* isolate using healthy melons to determine virulence. We found variation in the population of *Diaporthe* spp. being imported as well as some isolates that were of a different genus. This includes *Stagonosporopsis* spp. The information gathered indicates that there is a multitude of fungi being imported from Central America, increasing the variety of pathogenic fungi in the United States.

A COMPARISON OF TICK ABUNDANCE ON DISC GOLF COURSES IN URBAN AND SUBURBAN CENTRAL OKLAHOMA PARKS

Jessie Merrifield and Heather R. Ketchum, University of Oklahoma

Outstanding Undergraduate Paper, Outstanding Undergraduate Paper in Biological Science

Tick surveillance is an important aspect of vector control because it provides necessary information about species abundance, the risk of a tick encounter, and the potential presence of pathogens. Risk assessment through flagging focuses on the possibility of a human coming into contact with a questing tick, one that is actively seeking a host, while carbon dioxide traps can be used to determine the presence and abundance of ticks in a defined area. For our project, we surveyed ticks on disc golf courses from two central Oklahoma parks, one urban and one suburban. Disc golf is an increasingly popular sport where players are exposed to a variety of environments where they could potentially encounter ticks. The fairways of disc golf courses are typically mowed and well maintained while the periphery of the fairway is relatively untouched. At each park, we flagged for ticks around the periphery and the fairway of disc golf courses to determine a player's risk of encountering a questing tick. Carbon dioxide traps were used around the park to determine tick abundance at each park. While ticks were present at both parks, the greatest abundance was at the suburban park. Generally, on the disc golf courses, there was a greater abundance of ticks around the periphery, which included unmanaged and wooded areas as well as tall grasses and shrubs. For less skilled players, chasing a disc into the periphery of the course could increase their risk of coming into contact with a tick.

G3MP2 COMPUTATIONAL STUDY OF GASEOUS BORON AND ALUMINUM HYDROXIDES

Dwight L. Myers, Brenna S. Hefley, and Uendi Pustina, East Central University

Refractory oxides and nitrides are important materials in high temperature applications. In environments containing water vapor, hydroxides and oxyhydroxides are important reaction products, particularly in combustion environments, which can contain 3 – 10 % water vapor. Formation of gaseous hydroxides and oxyhydroxides is an important mode of corrosion. This study is a computational study of the thermodynamic stability of two of the gaseous hydroxides of boron and aluminum, boron(III) hydroxide and aluminum(III) hydroxide. Calculations were performed using composite methods at the G3MP2 level of theory. The geometries, vibrational frequencies, thermodynamics, and steps toward computations at higher levels will be discussed.

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UNDERSTANDING EPIGENETIC MECHANISM: A NOVEL WAY TO APPROACH THERAPEUTIC TARGETS FOR THE TREATMENT OF COLITIS

Radhika Pande, Kenneth E. Miller, and Subhas Das, Oklahoma State University Center for Health Sciences

Inflammatory bowel disease (IBD) is a term used to characterize the conditions like Crohn's disease and ulcerative colitis, which involve inflammation of the digestive tract. The main symptoms include repeated abdominal pain, diarrhea, fatigue, reduced appetite, and weight loss. According to CDC, approximately 3 million Americans were reportedly diagnosed with IBD. Reasons underlying IBD are still unknown, but several factors such as environmental, genetics, diet, and microbiome composition might play an essential role in disease development and the pharmacological therapies are limited. Previous studies have linked the Nerve growth factor (NGF), which plays an important role in inflammation and immune response, expression with neurogenic inflammation in various inflammatory animal models. Numerous studies have shown that epigenetic regulation, especially DNA methylation, plays an important role in inflammatory modulation. Epigenetics is the study of changes in gene expression, which occurs without any changes in the DNA sequence. The epigenetic mechanism for NGF regulation during colitis is still unexplored. Aim: In this study, we evaluated the epigenetic mechanisms which regulate the gene expression of NGF during TNBS induced colitis in rats. Method: Colitis was induced in 8-10 weeks old female Sprague-Dawley rats by infusing TNBS into the colon. The colon was collected after 24 hours of inflammation. Azacitidine (Aza) was pre- and co-administered to/with TNBS in the colon. Bisulfite converted DNA was used for Methylation-specific PCR (MSP) to analyze the DNA methylation patterns in the NGF promoter's CpG islands. RNA and protein expression of NGF was determined by qualitative, quantitative PCR, and immunoblot techniques. Results & Conclusion: Our findings show altered NGF expression in the colon during TNBS induced colitis due to hypermethylation of CpG dinucleotides in the NGF promoter. Aza treatment mitigated this hypermethylation and reduced neurogenic inflammation in these animals suggesting NGF expression can be epigenetically regulated in colon inflammation.

ENVIRONMENTAL STEWARDSHIP IN CITIZEN SCIENCE PARTICIPANTS

Cheyanne Olson, Rogers State University

Outstanding Graduate Poster

In recent years, citizen science programs have gained momentum and involved members of the public in active and ongoing science projects. Citizen science is often defined as a form of research collaboration that engages members of the public in scientific research projects that involve data collection, analysis, and dissemination (Haklay, 2013; Dickinson et al., 2012; Conrad & Hilchey, 2011; Wiggins & Crowston, 2011). In Oklahoma, Blue Thumb is a state-sponsored citizen science program that focuses on water quality monitoring. Blue Thumb has over 300 active volunteers monitoring 80 streams across Oklahoma annually. Focus on citizen science has turned to evaluation of participant outcomes. Behavior and stewardship is defined as "measurable behaviors that result from engagement in citizen science projects but are external to protocol or skills of the specific citizen science project" (Phillips et al., 2018). Behavior change is considered to be the "most sought-after outcome" for environmental citizen science program, but initial pro-environmental behaviors are not always documented before participation (Phillips et al., 2017). There is a need in Blue Thumb to evaluate whether volunteers change environmental behaviors as a direct result of participation in citizen science activities or if environmental behaviors already exist in their participant demographics. This poster serves as a discussion of preliminary findings of pro-environmental behaviors of new BT volunteers. This study is part of an on-going multi-phase mixed methods evaluation of participant learning outcomes in water monitoring citizen science programs like Blue Thumb. New volunteer environmental stewardship scores will be compared to experienced volunteer scores to infer if their environmental behaviors have changed over time. Older, experienced volunteers will be asked to reflect on what specific actions they have undertaken as a result of something they learned or experienced within their Blue Thumb Participation. Results anticipated Spring 2022.

SEASONAL VARIATION OF ARTHROPODS COLONIZING DECOMPOSING MAMMALIAN CARCASSES

Sam Ray and George Wang, East Central University

Outstanding Undergraduate Paper in Applied Ecology & Conservation

Decaying mammalian carcasses attract a variety of insect detritivores and other colonizers. The succession of insect colonizers can be used to indicate the rate of decomposition in forensics. The type and abundance of insect colonizers can be affected by season. We examined insect colonization of medium-sized mammalian carcasses between spring and summer seasons at the Botanical Outdoor Learning & Outreach Space (BOLOS) of East Central University in Ada, Oklahoma. We collected meso-mammalian (*Didelphis virginiana*, *Procyon lotor*, *Castor canadensis*, and *Dasypus novemcinctus*) carcasses from highways and weighed them. We enclosed the specimens in metal-wire cages and placed them in a wooded area of BOLOS. We conducted the experiment twice, once in late April 2021, and then in early August 2021. During each experiment, we performed daily sampling of insects and other arthropods on all specimens for one week, and then sampled once every two days until the specimens fully decomposed. The species and densities of arthropods were recorded. The arthropod species richness is comparable for both seasons; however, the species composition differs. The densities of American carrion beetle (*Necrophila americana*) and margined carrion beetle (*Oiceoptoma noveboracense*) are higher in the spring whereas the densities of rove beetles (*Platydracus maxillosus* and *Creophilus maxillosus*) were higher in the summer. Fly larvae development period was shorter in the summer than in the spring. The mammalian carcasses decomposed faster in the summer. Our results suggest that the use of insect abundance to indicate the decomposition rate of bodies in forensics should consider the season and other environmental conditions.

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NOVEL MELANIN INSPIRED COMPOUND POSSESSES MEMBRANE-DIRECTED ANTIBACTERIAL MECHANISM FOR GRAM-POSITIVE BACTERIA

Daniel Reed, Toby L. Nelson, Gabriel A. Cook, and Erika I. Lutter, Oklahoma State University

Daniel Reed and Franklin R. Champlin, Oklahoma State University Center for Health Sciences

Outstanding Undergraduate Paper in Microbiology

Melanin-inspired compounds (EIPE) synthesized by our group possess a core that provides scaffolding for the attachment of various functional groups. The purpose of this study was to investigate the antibacterial potential of Melanin-inspired compounds EIPE-1 and EIPE-HCl which are hydrophobic and hydrophilic, respectively. Antibiotic resistance remains a threat as more pathogenic bacteria increasingly acquire resistance to clinically useful drugs. This creates a need for novel compounds to be developed to combat resilient pathogens like methicillin resistant *Staphylococcus aureus* (MRSA). A standardized disk agar diffusion bioassay was performed to qualitatively compare the susceptibility and resistance levels of 12 gram-positive and 13 gram-negative bacteria to EIPE-1 and EIPE-HCl. The hydrophobic derivative EIPE-1 exhibited a gram-positive spectrum that included two methicillin resistant *Staphylococcus aureus* (MRSA) strains, while the hydrophilic derivative EIPE-HCl possessed no antibacterial properties at the concentrations examined. Turbidimetric growth curves were constructed to investigate the EIPE-1 mechanism of action. Bacteriolysis occurred immediately upon treatment for Staphylococcus epidermidis SK01 and at the five-hour mark for B. subtilis ATCC 6633, likely resulting from dissolution of their cytoplasmic membranes. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) bioassays were employed to quantitatively determine EIPE-1 potency. All gram-positive bacteria tested were susceptible with MIC values ranging from 0.25 to 2.0 µg/mL, while gram-negative bacteria were resistant with MICs in excess of 128 µg/mL. MICs and MBCs for two MRSA strains and two strains of the obligate anaerobe Clostridioides difficile were less than 8.0 µg/mL. These data suggest that hydrophobic EIPE-1 is a novel compound that possesses a gram-positive antibacterial spectrum that involves disruption of the cytoplasmic membrane and does not involve the involvement of molecular oxygen. The intrinsic resistance of 13 disparate gram-negative bacteria is likely due to the typical impermeability properties of the gram-negative outer membrane for hydrophobic molecules.

BEE POLLINATION PATTERNS WITHIN A THREE SISTERS GARDEN

Hallie Reed, Bobby L. Cothren, and Leah S. Dudley, East Central University

Outstanding Undergraduate Poster

Bee pollination is an essential ecosystem service required for many plant species to set fruit. Mutualistic plant-pollinator interactions vary in efficiency, depending on the plant-pollinator species and frequency of flower visits. With the human demand for insect-pollinated crops increasing and the population of bee pollinators declining, understanding the plant-pollinator interactions of these insects are crucial. This study focused on three bee groups: the honey bee, the bumblebee, and the solitary bee and their behavior within a Three Sister (corn, bean, & squash) garden. With its open, zoophilous floral design, squash flowers may entice more visitors than the other two sisters. Here, we address the following three questions: 1) Will squash flowers receive more visitors than the other two sisters? 2) Will a squash plant receive more visitors as the number of open squash flowers increases? and 3) Do the different types of bees visit squash flowers equally? The study took place during June-July of 2021 in a Three Sister garden consisting of various Sister varieties grown in mounds in a random, full-factorial design. Plant species, number of open flowers, visitor morphospecies, visitor behavior, and number of visitors were recorded. Results show that squash flowers received the most insect visitation compared to the other two sisters. The number of open squash flowers on a single plant had no notable effect on the number of visits, and the most frequent visitor to the squash flowers were honey bees. Our results suggest that agricultural systems may benefit from planting squash to attract pollinators. By interspersing squash throughout an agricultural system, it may increase the overall pollination and yield of the entire system, not just that of squash. In addition, squash not only is used as a food source for the non-native honey bee but also is capable of supporting native bee populations.

VIRTUAL TWIN STUDY: EFFECTS ON PARENTING EFFICACY

Robin Roberson, East Central University

In this study, 235 developmental psychology students (n=169 female; n=66 male) were assigned the task of raising two virtual twins using Pearson's My Virtual Child© (n.d.). A parental locus of control pre/post survey was used to determine if students improved in their understanding of parental influence on child/adolescent development. Significant changes were seen overall, as well as when disaggregated by student classification, ethnicity, and course delivery mode. Results suggest using My Virtual Child© (n.d.) as a twin study improves student understanding of parental influence on human development.

SINGLE-MOLECULE ANALYSIS OF DNA NUCLEOBASES USING VAN DER WAALS HETEROSTRUCTURES: A COMPUTATIONAL STUDY

Benjamin O. Tayo, University of Central Oklahoma

Sanjiv K. Jha, East Central University

Electronic DNA sequencing using two-dimensional (2D) materials such as graphene has recently emerged as the fourth-generation of DNA sequencing technology. Owing to its commercial availability and remarkable physical and conductive properties, graphene remains the most widely investigated material for DNA sequencing by both theoretical and experimental groups. One of the major issues with graphene is its lack of a band gap. Furthermore, the hydrophobic nature of its surface causes DNA bases to stick to its surface, slowing down translocation speed and increasing sequencing error rates. As part of the materials research project, our goal is to extend these studies to several 2D materials beyond graphene, including van der Waals heterostructures. In this talk, we discuss recent accomplishments and future perspectives.

THE NEAR SIGNIFICANCE OF THE RELATIONSHIP AMONG CHILDHOOD TRAUMA, ATTACHMENT STYLE, AND RELATIONSHIP SATISFACTION

Kaylee Thoma, East Central University

This study was conducted to find any relationships among Adverse Childhood Experiences (ACEs), attachment style, and relationship satisfaction. It was anticipated that attachment style would act as a mediator between ACE score and relationship satisfaction, and that people who are more securely attached would have higher relationship satisfaction and lower ACE scores than those who are not securely attached. Participants were 130 and individuals within the Amazon MTurk panel who indicated being in a relationship for 3 months or longer within the past month. Three valid and reliable scales were used to measure Relationship Satisfaction (CSI), Adult Attachment Style (RSQ), and Childhood Trauma (ACE questionnaire). The sample of 130 individuals reached near significance, leading to further research of people with 3 or more ACEs, those results will not be discussed in this presentation.

THE POTENTIAL FOR USING CERVIDS AS PROXIES FOR PALEOECOLOGICAL RECONSTRUCTION THROUGH STABLE ISOTOPE ANALYSIS: IMPLICATIONS FOR OUT OF AFRICA I

Forrest Valkai, University of Tulsa

Approximately 1.8 million years ago, Homo erectus moved through the Levantine corridor to spread throughout Europe and Asia. ‘Ubeidiya, a 1.5-million-year-old archaeological site in Israel, provides some of the earliest evidence of this dispersal. The details of the environment of the region at the time would provide a great deal of insight into the lives of our ancestors but is largely unknown. The analysis of the differential fractionation of stable isotopes is a powerful tool for paleoecological and paleoclimate reconstruction. The usefulness of cervids as proxies for this kind of reconstruction, however, is debated due to their foraging habits as variable browsers and grazers. In this study, I first develop a modern model to test if stable isotopes can distinguish between deer populations. I sample the tooth enamel from the M3 of different modern cervid populations to test for the δ13C and δ18O content of the tooth enamel. These data, when combined with data from GIS, will allow me to build an isotopic map of different cervid environments which can then be compared to samples taken from fossil cervids excavated from ‘Ubeidiya. This preliminary study will provide support for using cervids as a robust paleoecological proxy and could be a large step along the way to reconstructing the environment in which our ancestors lived and gaining a better understanding of *Homo erectus’s* movement out of Africa.

CO-LOCALIZATION STUDIES REVEAL DISTINCT PATTERNS OF POLE: MCM10 ASSOCIATION DURING CELL CYCLE IN HUMAN CELLS

Sarah Woller, B. Okda, M. Anderson, and S. Das-Bradoo, Northeastern State University and Oklahoma State University Center for Health Sciences

In eukaryotic DNA replication, Polymerase epsilon (POLE) is responsible for synthesizing the leading strand of DNA. POLE is known to have roles in the activation of the DNA damage response pathway and is believed to have roles in cell cycle control. Unfortunately, the distinct roles of POLE are not well understood and heavily debated. However, mutations in POLE have been known for tumor predispositions in several types of cancer. Our laboratory has shown that Minichromosome maintenance protein 10 (MCM10) plays a vital role in the functionality of POLE through studies in budding yeast. MCM10 is essential for DNA replication and, its interactions are highly regulated by the cell cycle. Genetic amplification and or over expression of MCM10 has been observed in colon, breast and prostate cancer. Therefore it is imperative to determine if the POLE: MCM10 interaction is conserved in human cells and if the interaction is regulated by the cell cycle. To achieve our goal we cloned POLE and MCM10 into RFP and GFP vectors, respectively. The vectors were transfected into HEK293T cells and visualized by fluorescence microscopy as well as confocal microscopy. Interestingly we observed interactions between POLE and MCM10 in human cells using co-localization fluorescence studies. To understand the dynamics of POLE and MCM10 throughout the cell cycle, cells were synchronized in G0 phase by serum starvation and interaction was studied by fluorescence and confocal microscopy. We were able to identify localization patterns of MCM10 and POLE in the nucleus throughout the S phase. Interestingly, MCM10 and POLE interaction is only seen in the mid to late S phase even though both proteins are present throughout the S phase. Our data suggest that both POLE: MCM10 interact in human cells to carry out specific functions during DNA replication.

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STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID
FOR THE YEAR ENDED DECEMBER 31, 2020

REVENUES COLLECTED		
Membership Dues:	$1,469.16	$1,469.16
Investment Income:	$42.02	$42.02
Meetings:		
Registration – Fall Field Meeting	$0.00	$0.00
Registration – Technical Meeting	$2,752.83	$2,752.83
Donations:	$55.00	$55.00
Woody Plants:	$0.00	$0.00
POAS:	$5,687.25	$5,687.25
Other Income:	$0.00	$0.00
Total Revenue Collected		**$10,006.26**
EXPENSES PAID		
Stipends and other Compensation:		
Stipends	$6,141.24	
Social Security & Medicare	$1,017.44	$7,158.68
Professional Fees:		
Tax Preparation	$100.00	$100.00
Meeting Expenses:		
Fall Field Meeting	$25.00	
Technical Meeting	$481.27	$506.27
Dues/Donations to AAAS/AJAS:	$675.00	$675.00
POAS:	$3,874.32	$3,874.32
Woody Plants:	$178.00	$178.00
Other Expenditures:	$321.09	$321.09
Total Expenses Paid		**$12,813.27**
Revenues Collected Over Expenses Paid		**$-2,807.01**

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF ASSETS, LIABILITIES AND FUND BALANCE
ARISING FROM CASH TRANSCATIONS
DECEMBER 31, 2020

ASSETS

Cash:		
Checking Account	$21,055.41	
Savings Account	$3,277.95	
Endowment Savings Account	$2,736.31	$27,069.67
Investments:		
Certificate of Deposit	$60,000.00	$60,000.00
Total Assets:		**$87,069.67**

LIABILITIES AND FUND BALANCE

Liabilities:	$0.00	
Fund balance:		
Beginning operation fund balance	$89,874.86	
Excess revenues collected over expenses	$-2,805.19	
Total Funds:		**$87,069.67**

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID
FOR THE YEAR ENDED DECEMBER 31, 2019

REVENUES COLLECTED		
Membership Dues:	$2,940.09	$2,940.09
Investment Income:	$43.87	$ 43.87
Meetings:		
Registration - Fall Meeting	$3,713.01	
Registration - Technical Meeting	$8,600.38	$12,313.39
Donations:	$721.17	$721.17
Woody Plants:	$496.00	$496.00
POAS:	$4,190.52	$4,190.52
Transfer from OJAS	$1,172.48	$1,172.48
Other Income:	$3.50	$3.50
Total Revenue Collected		**$21,881.02**
EXPENSES PAID		
Stipends and other Compensation:		
Stipends	$6,141.24	
Social Security	$1,030.75	
Medicare	$241.05	$7,413.04
Professional Fees:		
Audit	$300.00	
Tax Preparation	$1,119.00	$1,419.00
Meeting Expenses:		
Fall Meeting	$3,825.00	
Technical Meeting	$3,128.97	$6,953.97
Dues:	$1238.17	$1238.17
POAS:	$3,528.58	$3,528.58
Woody Plants:	$487.13	$487.13
Other Expenditures:	$1,775.62	$1,775.62
Total Expenses Paid		**$22,815.51**
Revenues Collected Over Expenses Paid		**$-934.49**

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF ASSETS, LIABILITIES AND FUND BALANCE
ARISING FROM CASH TRANSCATIONS
DECEMBER 31, 2019

ASSETS

Cash:		
Checking Account	$23,862.50	
Savings Account	$3,277.44	
Endowment Savings Account	$2,734.92	$29,874.86
Investments:		
Certificate of Deposit	$60,000.00	$60,000.00
Total Assets:		**$89,874.86**

LIABILITIES AND FUND BALANCE

Liabilities:	$0.00	
Fund balance:		
Beginning operation fund balance	$90,809.35	
Excess revenues collected over expenses	$-934.49	
Total Funds:		**$89,874.86**

OKLAHOMA ACADEMY OF SCIENCE

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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.

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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.

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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.

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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:

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D. Review Process.

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