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REPORTS

A. Applied Ecology & Conservation

ACADEMIC AFFAIRS

Reptile Diversity on Roads Surrounding the Selman Living Laboratory, Woodward County, Oklahoma

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Abstract: As human impacts on the environment accumulate, comprehensive species lists are needed as baselines to measure future change. During 2015–2022, we conducted 226 circuits of an 80.5 km road network surrounding the Selman Living Laboratory, Woodward County, Oklahoma. Sampling occurred during April-October and totaled 12000 road km. Roads were unpaved and not regularly maintained, resulting in slow speeds and light traffic. We documented 88% of the expected snake species in the study area. We observed 310 snakes representing 22 species, 81 turtles from three species, and 62 Slender Glass Lizards (*Ophisaurus attenuatus*). Western Ribbonsnakes (*Thamnophis proximus*, n = 63) were the most frequently encountered species, followed by Slender Glass Lizards, and Ornate Box Turtles (*Terrapene ornata*, n = 45). Neonates were well-represented, including 15.7% of all observations and 92.3% of observed species. We found 45 snakes (15.8% of observed snakes), 10 Slender Glass Lizards (16.1%), and zero turtles dead on the road. Road mortality was lower than rates reported in other studies. The study area harbors an intact reptile assemblage, even though 99% is private land, where ranching and fossil fuel exploration has been ongoing for over a century.

Introduction

As human impacts on the environment accumulate, documenting existing biodiversity is increasingly important. Long-term monitoring and comprehensive species lists are needed to document changes in assemblages and serve as baselines to measure future change (Green et al., 2009). Even though reptiles are important components of terrestrial ecosystems, species lists for reptiles are often incomplete due to the time and expense of data collection (Thompson et al., 2003; van Rooijen, 2009). Thorough surveys that document species occurrence and relative abundance in reptile assemblages are needed (Busby and Parmalee, 1996; Cagle, 2008).

Biological field stations are uniquely positioned for long-term monitoring of reptile assemblages (Michener et al., 2009). In rapidly changing environments, field stations provide stable habitats to monitor and a steady stream of researchers and students over time, allowing monitoring programs to exceed the career of any one researcher. As a local example, Watters et al. (2021) recently published an annotated list of reptiles and amphibians encountered during a 65-year period at the University of Oklahoma Biological Station at Lake Texoma.

In 1998, The Selman Living Laboratory (SLL) was acquired by the University of Central Oklahoma and soon after established as a member of the Organization of Biological Field Stations (McNulty et al., 2017). Located in the Cimarron

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Gypsum Hills of Woodward County, Oklahoma (36.685278, -99.277222, WGS84), the SLL consists of two tracts totaling 1.35 km^2, including several entrances to an extensive gypsum cave system. The SLL is the site of longitudinal studies on cave-dwelling bats, rodent Leishmaniasis, seasonal vegetation dynamics, and population genetics of small mammals. For the past eight years, the senior author (HLC) has conducted surveys for reptiles on the road network surrounding the SLL. Here, we summarize these surveys, presenting data on species richness, relative abundance, and road mortality of reptiles encountered on roads near the SLL during 2015–2022.

Materials and Methods

Study Area.—The study area was an 80.5 km network of farm roads within a 132 km^2 area in Woodward and Harper Counties, Oklahoma (Fig. 1). Climate and topography were typical of the Cimarron Gypsum Hills, a semi-arid region of rolling hills and plains, caves, canyons, and gypsum outcrops. Three creeks flowed intermittently northeast through the study area and drained into the Cimarron River less than 3 km north. Habitats surrounding roads were utilized for oil and gas wells, iodine production, cattle grazing, and wheat farming, with remnant mixed-grass prairie habitat interspersed, and numerous farm ponds constructed for watering livestock. Using aerial photographs taken on 21 June 2017 (Google Earth Pro, 2020), we counted 18 ranch houses, an iodine plant, and at least 45 pads for oil and gas wells in the study area. Two natural areas, Alabaster Caverns State Park (0.81 km^2) and Cimarron Bluff Wildlife Management Area (13.88 km^2), were on the eastern and northern boundaries of the study area, respectively. The study area was 1–3 km west of Oklahoma State Highway 50, and 5–6 km east of Oklahoma State Highway 34, the only paved roads in the vicinity. Study area roads were dirt or gravel and not regularly maintained, becoming impassable to two-wheel drive vehicles after even moderate rainfall. Because of the unevenness of the roads, speed limits were not posted, and traffic volume was low and comprised of local traffic. Often, no other vehicles were encountered during 2–3 h surveys. Roads were unevenly distributed, with two large parcels (64.7 km^2 and 18.1 km^2) near the center of the study area that were not bisected by roads. Within the largest of these parcels was the 1.35 km^2 Selman Living Laboratory.

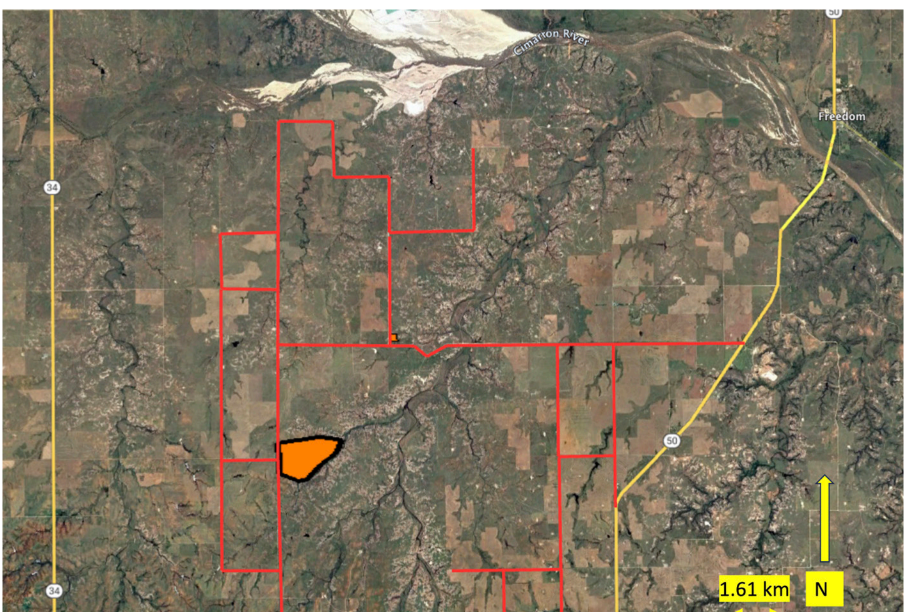

Fig. 1. Annotated map of the study area derived from Google Earth Pro (2020). The red lines show the network of dirt roads surveyed during the study. The orange polygons show the Selman Living Laboratory.

Road Surveys.—During 2015–2022, HLC made 48 trips to the study area (Table 1). Trips occurred opportunistically during April–October (Table 1). Trips lasted 1-4 days (mode = 2 days), with the SLL used for overnight stays. During each trip, HLC completed 1-12 circuits (mean ± SD = 4.7 ± 2.95 circuits/trip) of the road network around SLL. A total of 226 circuits (*ca*. 12000 road km) were completed (Table 1). The most common circuits were 48 km (n = 110) and 58 km (n = 78), which took 2–3 hours to survey, depending on how many sightings occurred. After heavy rains some roads were impassable, and abbreviated circuits were taken. Most circuits were early morning, late afternoon, or night, but depending on weather conditions, some circuits were conducted late mornings and early afternoons.

During sampling, HLC drove 16–24 km/h, scanning roads for reptiles. All snakes and turtles encountered were documented. For lizards, relatively small-bodied species that occupied roadside habitats, such as collared lizards, horned lizards, and whiptails, were not recorded, but occurrences of Slender Glass Lizards (*Ophisaurus attenuatus*) were recorded. For each sighting, HLC plotted location on a study area map and recorded GPS coordinates. Species, condition (alive or dead), and age class (adult or juvenile) were recorded, but specimens were not measured nor marked. HLC photographed some specimens and deposited photos in the University of Central Oklahoma Natural History Museum. If captured, specimens were released at the point of capture.

Table 1. Distance (km) surveyed by month and year for reptile surveys on roads surrounding the Selman Living Laboratory.

Year	April	May	June	July	August	September	October	Total	Circuits
2015	0	0	0	0	164	0	0	164	3
2016	174	290	232	174	348	348	338	1902	34
2017	266	193	0	579	48	193	0	1279	27
2018	0	0	483	451	0	193	0	1127	20
2019	97	0	354	145	48	193	0	837	16
2020	0	475	97	97	209	145	48	1070	24
2021	0	1081	356	436	728	819	0	3420	65
2022	0	431	241	1030	0	453	0	2155	37
Total	536	2470	1762	2912	1546	2343	386	11955	
Circuits	11	51	34	51	28	43	8		226

Data Analysis.— Our analyses focused on species richness, relative abundance, and rates of road mortality. Species richness was visualized using a species accumulation curve, a plot of number of species encountered vs. time. Our estimate of species richness was compared to the total species pool, estimated by examining range maps in the most recent edition of "A Field Guide to Oklahoma's Amphibians and Reptiles (Sievert and Sievert, 2021). We used Spearman's Rho to explore the correlation between the number of dead individuals and live individuals of each species. A significant correlation (alpha = 0.05) would be expected if natural history differences among species were absent, with outliers suggesting natural history differences that made species more or less susceptible to road mortality.

Results

We made 453 observations of 26 species, including 310 snakes (0.026 snakes/km) representing 22 species (Table 2), 81 turtles from three species (0.0068 turtles/km), and 62 Slender Glass Lizards (*Ophisaurus attenuatus*, Table 2). Neonates were well-represented in the sample, including 15.7% of total observations and 92.3% of observed species. Western Ribbonsnakes (*Thamnophis proximus*, n = 63) were the most frequently encountered species, followed by Slender Glass Lizards and Ornate Box Turtles (*Terrapene ornata*, n = 45). We could not positively identify 28 snakes to species, including 26 racer/coachwhips (*Coluber* spp.) that crossed the road too fast for identification, and two dead gartersnakes (*Thamnophis* spp.) that were too badly damaged to identify. Among snakes, eight species were represented by more than 10 individuals, whereas three species were only observed once (Table 2). New snake species were added to the list for five years, but no new species have been observed for the last three years (Fig. 2).

We found 55 individuals (12.9% of total observations) of 17 species (65.3% of observed species) dead on the road, including 45 snakes (15.8% of observed snakes) and 10 Slender Glass Lizards (16.1%). No dead turtles were encountered. Among snake species, the relative abundance of live and dead individuals was significantly correlated ($r_s = 0.742$, $P = 0.00008$, Fig. 3).

Table 2. Total number of snakes, turtles, and lizards (dead + alive) observed by species each year on roads surrounding the Selman Living Laboratory. The last column is the number of individuals found dead in each species.

Taxon*	2015	2016	2017	2018	2019	2020	2021	2022	Total	# dead
Western Ribbonsnake (*Thamnophis proximus*)	0	16	14	6	3	8	12	4	63	8
Common Gartersnake (*Thamnophis sirtalis*)	0	0	1	2	1	0	1	2	7	1
Plains Gartersnake (*Thamnophis radix*)	0	2	0	0	1	1	0	0	4	1
Checkered Gartersnake (*Thamnophis marcianus*)	0	0	0	1	0	2	1	1	5	1
Unidentified Gartersnake (*Thamnophis* spp.)	0	0	0	1	1	0	0	0	2	2
Coachwhip (*Coluber flagellum*)	0	7	3	4	2	6	1	1	24	3
North American Racer (*Coluber constrictor*)	0	5	1	1	2	5	5	4	23	5
Unidentified Racer/Coachwhip (*Coluber* spp.)	1	8	7	1	2	1	5	1	26	0
Great Plains Ratsnake (*Pantherophis emoryi*)	1	0	0	1	0	3	1	1	7	1
Western Ratsnake (*Pantherophis obsoletus*)	0	0	0	1	0	3	1	2	7	0
Gophersnake (*Pituophis catenifer*)	0	1	4	2	2	9	4	2	24	6
Prairie Kingsnake (*Lampropeltis calligaster*)	2	0	4	0	2	0	1	0	9	2
Speckled Kingsnake (*Lampropeltis holbrooki*)	1	3	0	0	0	0	0	1	5	3
Glossy Snake (*Arizona elegans*)	0	1	0	0	2	0	0	0	3	0
Diamond-backed Watersnake (*Nerodia rhombifer*)	0	0	1	0	0	2	1	0	4	0
Plain-bellied Watersnake (*Nerodia erythrogaster*)	1	0	1	0	0	1	2	1	6	2
Eastern Hog-nosed Snake (*Heterodon platirhinos*)	1	1	3	0	1	5	1	0	12	2
Plains Hog-nosed Snake (*Heterodon nasicus*)	0	0	1	0	0	0	0	0	1	0
Dekay's Brownsnake (*Storeria dekayi*)	0	0	2	1	0	0	0	1	4	0
Ring-necked Snake (*Diadophis punctatus*)	0	0	1	0	0	0	0	0	1	0
Long-nosed Snake (*Rhinocheilus lecontei*)	0	0	0	0	1	0	0	0	1	0
Prairie Rattlesnake (*Crotalus viridis*)	5	7	1	5	3	2	4	1	28	4
Western Diamond-backed Rattlesnake (*Crotalus atrox*)	1	8	2	0	1	2	5	7	26	3
Western Massasauga (*Sistrurus tergeminus*)	0	5	5	3	4	1	0	0	18	1
Slender Glass Lizard (*Ophisaurus attenuatus*)	0	22	9	0	12	5	8	6	62	10
Ornate Box Turtle (*Terrapene ornata*)	2	8	8	5	6	9	5	2	45	0
Pond Slider (*Trachemys scripta*)	0	1	4	3	4	6	5	5	28	0
Yellow Mud Turtle (*Kinosternon flavescens*)	0	1	1	2	2	0	2	0	8	0
Individuals	15	96	73	39	52	71	65	40	453	55
Species**	9	16	20	16	19	18	19	17	26	17
Circuits	3	34	27	20	16	24	65	37	226	

* Taxonomy according to Crother 2017

**unidentified *Coluber* were not included in species totals, except in 2015, when *Coluber* was not otherwise observed

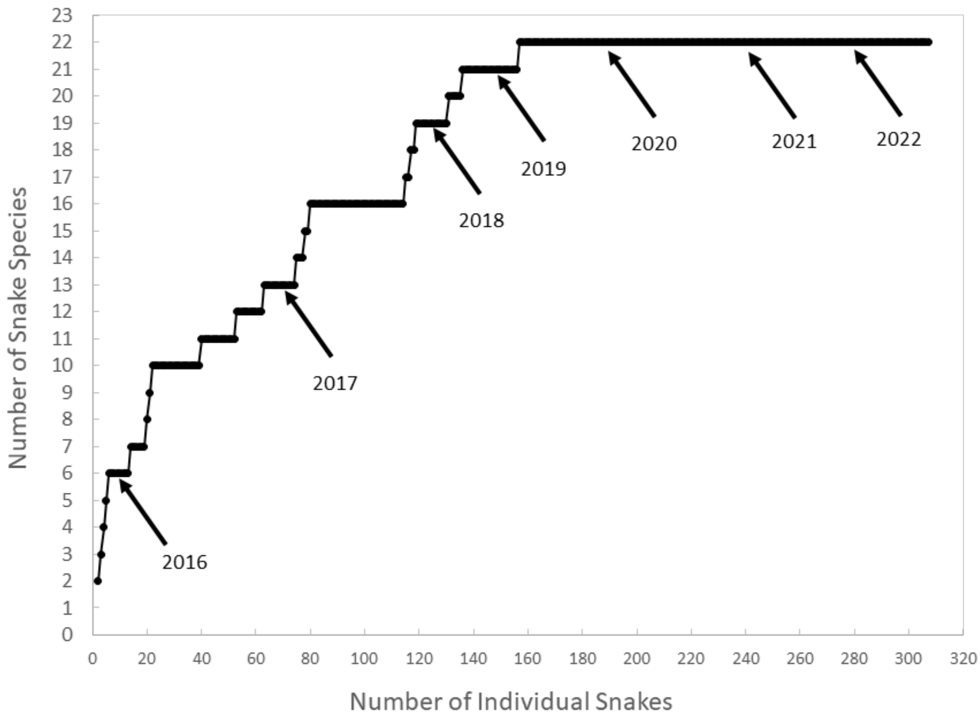

Fig. 2. Species accumulation curve for snakes on roads surrounding the Selman Living Laboratory. X-axis values increased each time a snake was encountered, whereas Y-axis values increased each time a new snake species was encountered.

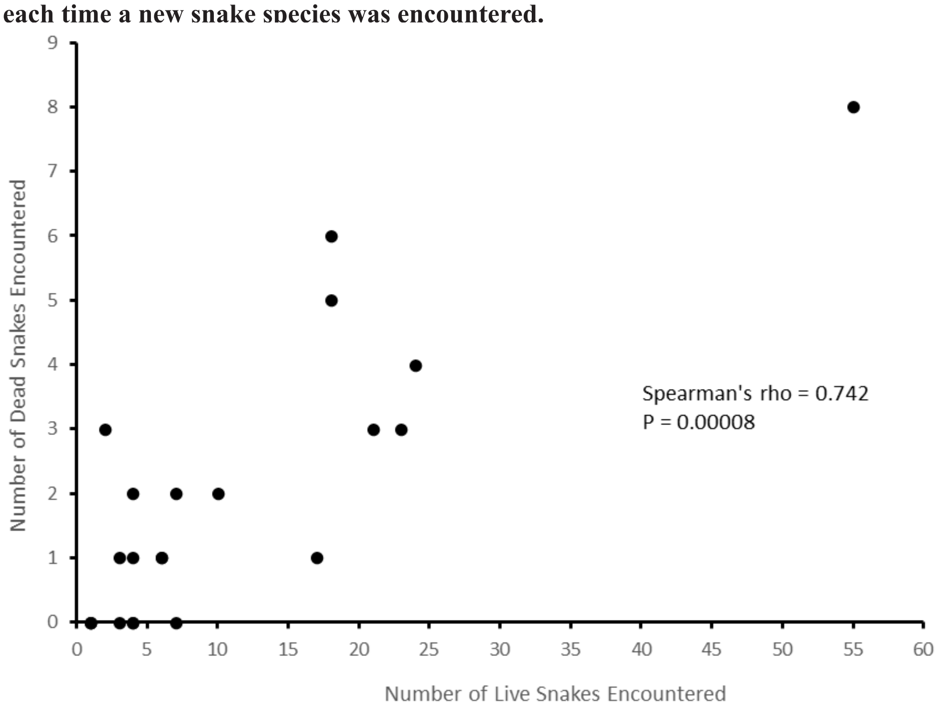

Fig. 3. Scatterplot of number of live individuals vs. number of dead individuals for each snake species encountered on roads surrounding the Selman Living Laboratory. Some points represent multiple species.

Discussion

We documented an apparently healthy and intact reptile assemblage: we observed 22 of 25 snake species (88%) and three of five turtle species expected to occur in the study area (Sievert and Sievert, 2021), with neonates representing 92.3% of observed species and 15.7% of all observations. We did not observe *Rena dissecta*, *Tantilla nigriceps*, and *Hypsiglena jani*, relatively small, nocturnal snakes, nor the highly aquatic turtles *Chelydra serpentina* and *Apalone spinifera*. It is likely that further road sampling, or the addition of different sampling methods, such as turning over objects in terrestrial habitats, using terrestrial drift fences with pitfall and funnel traps, and aquatic trapping with hoop nets, would eventually reveal the species not encountered.

Ours is the third study in the last decade to present a comprehensive species list for snakes in Oklahoma (McKnight et al., 2015; Watters et al., 2021). The three studies suggest Oklahoma snake assemblages are diverse and remain relatively intact despite 150 years of anthropogenic change. Between 22-24 species were observed in each study, accounting for 76-88% of local species pools (Table 3; species pools estimated from Sievert and Sievert, 2021). Despite the similarity in the results, the three studies used radically different methodologies (Table 3) but had one thing in common; sampling with high intensity (McKnight et al., 2015; Watters et al., 2021).

Table 3. A comparison of snake diversity documented during three recent Oklahoma studies.

	Selman Living Lab (This study)	Lake Texoma (Watters et al. 2021)	Boehler Seeps and Sandhills Preserve (McKnight et al. 2015)
Species Observed	22	24	22
Species Pool	25	30	29
% of Pool Observed	88%	80%	76%
Methods	8 years road surveys	65 years class field trips	2 years multiple methods

Road surveys of snakes produce biased samples because of interspecific variation in home range area, movement rates, habitat preference, and body size (Dodd et al., 1989; Jochimsen et al., 2014). As a result, in many systems multiple sampling methods are required for accurate estimates of species diversity and relative abundance, because small-bodied species with low movement rates remain undetected or underrepresented in road surveys (Seigel et al., 2002; Sullivan et al., 2017). However, snakes are difficult to trap, and several studies report incidental captures, on roads or otherwise, as the most effective way of sampling snakes (Busby and Parmalee, 1996; McKnight et al., 2015; Sullivan et al., 2017). Persistent surveys of the same network of roads can yield accurate estimates of species diversity in snake assemblages (Sullivan et al., 2017), but biases associated with relative abundance data likely remain inherent.

There are diminishing returns on adding new species to species lists by repeated sampling (van Rooijen, 2009). We repeatedly sampled the same area for eight years and eventually captured most of the expected species, observing one new species during the fifth year of sampling. Similarly, a study involving pitfall and funnel trapping of snakes in New Mexico added a new species after seven years of trapping the same sites (Bateman et al., 2009), and a study involving pitfall trapping of small reptiles in Western Australia added a new species after 16,500 individuals were captured (Thompson et al., 2003).

Observed mortality rates for snakes in our study were lower than rates reported in other studies (Smith and Dodd, 2003; Jochimsen et al., 2014; Lutterschmidt et al., 2019). Moreover, the absence of dead turtles in our sample is encouraging in light of numerous studies where road mortality had negative consequences on population structure and viability in turtles (Steen and Gibbs, 2004; Aresco, 2005; Piczak et al., 2019). None of the roads in our study area were paved, and speed limits were not posted because speeds in excess of 40 km/h were not sustainable due to road conditions. Moreover, after heavy rains when reptiles often move, road mortality was likely reduced because roads were impassable to vehicles. In contrast, studies reporting high rates of road mortality in snakes and turtles typically involved paved divided highways that were always passable, with multiple lanes and speed limits of 88 km/h or greater (Smith and Dodd, 2003; Aresco, 2005; Lutterschmidt et al., 2019). Our study area has been ranched and explored for oil and gas for over a century, and only about 1% of the study area is protected land. Despite these conditions, the reptile assemblage is diverse.

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Assessment of Beaver (*Castor canadensis*) Herbivory on a Bottomland Forest in Central Oklahoma

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Abstract: North American beaver (*Castor canadensis*) play a key role in engineering ecosystems by altering hydrology, forest structure, and plant species composition. We investigated beaver foraging in a bottomland forest at a human-made lake in central Oklahoma to understand if beaver were preferentially feeding on certain tree species. Beaver fed on four of the nine tree species present in the forest. Approximately 60% of trees had signs of beaver herbivory. We found a significant relationship between the degree of beaver herbivory and tree species. A significant difference was found in diameter between tree species, and a significant difference in diameter between beaver herbivory categories. We conclude that beaver were feeding more frequently on green ash due to their high density and smaller mean diameter to maximize their optimal foraging strategy.

Introduction

North American beaver (*Castor canadensis*) are recognized as ecosystem engineers that increase landscape heterogeneity and species richness (Wright et al. 2002), modify lotic functioning (Smith et al. 1991), and alter riparian forest stand density and basal area (Johnston and Naiman 1990). Beaver forage on aquatic vegetation and woody plant species in adjacent riparian and bottomland habitats (Jenkins and Busher 1979; Rosell et al. 2005).

Beaver foraging behavior affects riparian and bottomland forest succession. Previous research has shown that beaver can alter forest understory structure and species richness (Guillermo et al. 2006) and reduce the density and basal area of preferred tree species (Johnston and Naiman 1990; Barnes and Dibble 1988). Beaver selection for preferred tree species has the potential of shifting forest structure and species composition (Rosell et al. 2005). Changes in forest communities can also be driven by a combination of ungulate and beaver herbivory (Hood and Bayley 2009).

Several studies have indicated that beaver are selective foragers of woody plants. Jenkins (1979) indicated a temporal shift in foraging as beaver altered between feeding on pine (*Pinus*) to birch (*Betula*), oak (*Quercus*), and witch hazel (*Hamamelis*) over the course of two years. In one of the earliest studies of beaver herbivory, Shadle et al. (1943) found that members of the Genus *Populus* accounted for 33.8% of all tree cuttings in New York. Pinkowski (1983) showed that beaver used approximately 21% of *Populus* and 21% of *Fraxinus* that were available at the study site in North Dakota. Crisler and Russell (2010) reported that beaver girdled a greater proportion

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of *Celtis occidentalis*, *Morus rubra*, and *Salix* sp. than other species across multiple sites in south-central Kansas. In Nevada, Harper (2001) found that beaver preferentially fed on *Populus*, *Salix*, and *Fraxinus* along a 38 km stretch of riparian habitat. At sites in Georgia and Louisiana in the southeastern United States, beaver showed a preference for sweetgum (*Liquidambar styraciflua*) relative to species composition but had little overall effect on altering forest stand density (Chabreck 1958; Brzyski and Schulte 2009).

Most components of a tree (leaves, twigs, bark) are used as a food source (Allen 1983; Jenkins and Busher 1979) and for dam and lodge construction (Allen 1983). Tree diameters that had signs of beaver herbivory vary tremendously, ranging from 1.1 cm to 31 cm (Crisler and Russell 2010; Jenkins 1980; Brzyski and Schulte 2009; Pinkowski 1983; Barnes and Dibble 1983). While there is a wide range of tree diameters that beaver feed on, the frequency of feeding is often skewed towards trees < 10 cm diameter. Often the greatest amount of foraging occurs within 25-30 m from shoreline (Barnes and Dibble 1983; Jenkins 1980).

We report the results of a study that assessed characteristics of trees that had signs of beaver herbivory at a man-made lake in central Oklahoma. Previous research has described the structure of the bottomland forest at the lake (King and Buck 2018). The bottomland forest floods frequently following periods of precipitation. Observations at the bottomland forest since 2016 suggest increased beaver herbivory during periods of flooding. The bottomland forest overstory is dominated by *Salix nigra* (black willow) and *Fraxinus pennsylvanica* (green ash). However, black willow dominates basal area while green ash dominates density (King and Buck 2018). Given this difference between these two tree species, we tested whether beaver at the man-made lake have a selection preference. Green ash are, on average, smaller diameter and higher density relative to black willow. We expect that beaver would maximize their foraging by preferentially feeding on and collecting the smaller diameter and higher density green ash. The objectives of this study were: 1) categorize the degree of beaver herbivory on black willow and green ash; and 2) assess beaver preference for tree species.

Methods

Study Area

This study occurred in a section of bottomland forest at Arcadia Lake, Oklahoma County, Oklahoma (35˚38'54"N, 97˚24'03"W). The lake is a man-made water source that covers 736.5 ha (1820 ac) when the lake is at its standard pool elevation (306.6 m; 1006 ft). Construction of the lake began in the early 1980s, and the lake reach conservation pool status in 1987 (U.S. Army Corps of Engineers 2020, https://www.swt.usace.army.mil/Locations/Tulsa-District-Lakes/Oklahoma/Arcadia-Lake/).

Regional climate (Oklahoma Climate Division 5) is warm-temperate with mean annual temperature of 15.7˚C (60.2˚F). The warmest months include July and August and the coldest months are December and January. Mean annual precipitation is 87.4 cm (34.41 in) (NOAA National Centers for Environmental Information 2020, https://www.ncdc.noaa.gov/cag/).

Our study site is 4.7 ha and is located in the northwest section of Arcadia Lake. The site was previously defined as a black willow-green ash (*Salix nigra-Fraxinus pennsylvanica)* bottomland forest (King and Buck 2018). Cottonwood (*Populus deltoides*), silver maple (*Acer saccharinum*), Osage orange (*Maclura pomifera*), and honey locust (*Gleditsia triacanthos*) are minor components of the forest overstory.

Data Collection

We established three belt transects that were 150 m in length and 10 m wide. Transects were oriented southeast-northwest based on the shape of the study site. Distance between belt transects was exactly 50 m. All trees (diameter at breast height (DBH) > 8 cm) and saplings (height > 1.3 m; DBH < 8 cm) were identified to species. Diameter of trees and saplings were measured at breast height (1.3 m above ground level) using a DBH tape measure and assessed for beaver herbivory. All data collection occurred during September-October 2019.

Beaver herbivory was categorized in the field during sampling. We classified the degree of beaver herbivory into five categories based on visual observations at the study site: 0 = no evidence of beaver herbivory; 1 = bark removed, < 50% circumference of tree; 2 = bark removed, > 50% circumference of tree; 3 = gnawing, < 50% through tree, tree standing; 4 = gnawing, > 50% through tree, tree standing; 5 = felled tree. For statistical analysis, we pooled data into two categories based on the presence or absence of beaver herbivory (1 = no herbivory, 2 = herbivory).

Data Analysis

We analyzed the effect of beaver herbivory on the two most common tree species in our sampling, green ash and black willow, due to sample sizes. We tested for effects of tree species and beaver herbivory category on tree diameter using a two-factor analysis of variance (ANOVA). We used a chi-square (χ^2) contingency test to determine if there was a relationship between the category of tree damage and the species of tree. All analyses were conducted at $\alpha = 0.05$.

Results

Table 1. Descriptive statistics of trees sampled and tree characteristics that exhibited beaver herbivory at Arcadia Lake, Oklahoma.

Species	Number of Stems	Overall Mean Diameter (cm) (±SD)	Beaver Herbivory Tree Diameter (cm) (±SD)	Percentage of Trees with Beaver Herbivory (# stems)
Black willow	83	25.4 (7.55)	26.5 (6.82)	28% (23)
Green ash	170	12.5 (5.23)	13.2 (4.69)	78% (132)
Silver maple	6	17.9 (8.23)	[a]7.30	17% (1)
Boxelder	3	5.97 (6.70)	8.05 (7.99)	67% (2)
Total	262			

[a]The value represents the diameter at breast height (cm) of a single silver maple.

We collected data on 262 trees across four species. Green ash was the most common tree species encountered within the belt transects (n = 170) followed by black willow (n = 83), silver maple (n = 6), and boxelder (n = 3). The tree species with the largest diameter (cm) was black willow (¯ = 25.4 cm ± 7.55 SD). Green ash had the highest proportion of trees that exhibited signs of beaver herbivory (78%, n = 132) (Table 1).

Beaver herbivory was greater on the two most common tree species, green ash and black willow. The largest diameter black willow and green ash that had beaver herbivory were 37.9 cm and 26.2 cm, respectively. Black willow had a greater number of trees that did not have herbivory compared to green ash (Table 2). Smaller diameter size classes (< 20 cm DBH) of green ash accounted for approximately 70.6% of beaver herbivory (Figure 1). This is in contrast to black willow in which approximately 21.7% of trees in the larger size classes (> 20 cm DBH) exhibited evidence of beaver herbivory. There was also a difference in total proportion of trees that exhibited beaver herbivory (green ash = 77.7%; black willow = 27.7%).

Table 2. Categories of beaver herbivory and number of green ash and black willow trees that exhibited the degree of beaver herbivory. 0 = no sign of beaver herbivory; 1 to 2 = bark removed; 3 to 4 = bark removed and evidence of gnawing; 5 = a completely felled tree.

	0	1 to 2	3 to 4	5
Green ash	38	48	48	36
Black willow	60	17	6	0
Total	98	65	54	36

There was a significant relationship between the degree of beaver herbivory and tree species based on chi-square contingency test results ($\chi^2_{3, 253} = 66.315$, $P < 0.0001$). Approximately 72.2% of black willow sampled at the study site did not have any sign of beaver herbivory while only 22.4% of green ash did not have beaver herbivory (Figure 1). There was a greater number of green ash that had been felled by beaver compared to black willow. Statistical analysis indicated a significant difference in diameter between tree species ($F_{1, 249} = 241.233$, $p < 0.0001$) and a significant difference in diameter between herbivory categories ($F_{1, 249} = 7.254$, $P = 0.008$). There was no significant interaction between tree species and herbivory category ($F_{1, 249} = 1.268$, $P = 0.261$). Approximately 61.3% (n = 155) of trees among green ash and black willow had evidence of beaver herbivory (Figure 1).

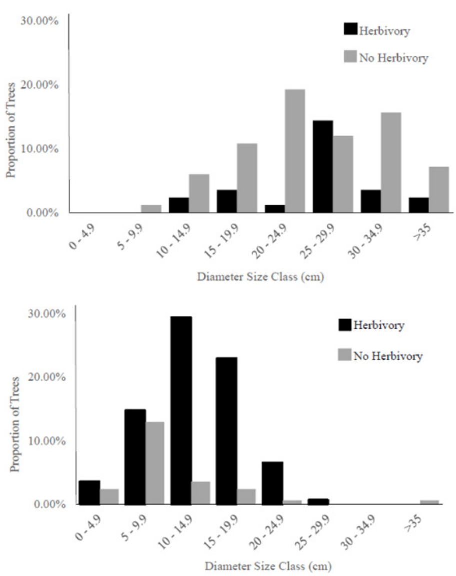

Figure 1. Proportion of black willow (top) and green ash (bottom) that had evidence of beaver herbivory or no herbivory based on tree size classes (cm).

Discussion

Our study at Arcadia Lake in Oklahoma County, Oklahoma indicates that beaver herbivory occurred on four tree species. The most common tree species that were fed on at the study site were black willow and green ash. Analysis of beaver herbivory on the two most common tree species indicates a significant relationship between beaver herbivory category and tree species.

We found that black willow and green ash accounted for 98% of trees with evidence of beaver herbivory (Table 1). Shadle et al. (1943) found that seven tree genera across six beaver colonies accounted for 93% of beaver cuttings with *Populus* and *Carpinus* accounting for 56.9% of total beaver cuttings. Crisler and Russell (2010) in Kansas found that the most abundant tree species, northern hackberry and red mulberry, were also the most frequently girdled at their study sites. Gerwing et al. (2013) suggest that beaver selection of certain plant species is a function of the site, distance of plant species from water, and the plant species, themselves.

Previous research at our study site indicated that black willow and green ash are the two most important overstory tree species (King and Buck 2018). This suggests that beavers at Arcadia Lake are utilizing more often what is available to them. The previous literature (Shadle et al. 1943, Crisler and Russell 2010) and others at sites across North America (Brenner 1962, Pinkowski 1983, Johnston and Naiman 1990, Harper 2001) all indicate the most common tree species at their study sites and beaver selection for those common species. This suggests a flexibility in the beaver's generalist herbivory.

We found a discrepancy in beaver herbivory when assessing tree diameter. Black willow were the largest diameter trees while green ash were smaller diameter (Table 1). Beaver did feed on larger diameter black willow and smaller diameter green ash (Figure 1). King and Buck (2018) have previously demonstrated that black willow dominated basal area while green ash dominated tree density at our study site. Allen (1983) based on a review of early 20th century beaver research reported that beaver selected trees less than 11 cm DBH. However, Jenkins (1980), and Raffel et al. (2009), report that beaver selected a range of tree size classes and that the selection of trees changed with an increasing distance from water. While we did not assess changes in beaver selection with distance from lake edge, we do note that most herbivory on black willow occurred along the edge of the lake while herbivory on green ash occurred at a greater distance from the lake edge. The feeding on large diameter black willow and small diameter green ash may be an artifact of the study site given the current size classes of trees that are available to the beaver.

There was evidence of different feeding behavior by beaver at the study site. This is highlighted by the beaver herbivory categories (Table 2). We show a significant relationship between beaver herbivory category and tree species (black willow and green ash). Collectively, 38.7% of black willow and green ash had no signs of beaver herbivory (Table 2). Based on our categories of beaver herbivory, green ash had similar number of trees that had bark removed only (categories 1-2), gnawing (categories 3-4), and completely felled (category 5). There were fewer black willow with signs of herbivory, but bark removal was the most common sign of beaver herbivory (categories 1-2). Bark is an important woody food source for beaver, and Svendsen (1980) reported that beaver in southeast Ohio fed on bark during early spring and fall months often when terrestrial and aquatic herbaceous vegetation was limiting. We found that bark removal was the most common type of damage to black willow and green ash, accounting for 41.9% of trees that were damaged. Similar to our results from Oklahoma, King et al. (1998) found that bark removal was the most common type of beaver damage to trees at Caddo Lake, Texas.

All felled (beaver herbivory category 5) trees were green ash (Table 2). Green ash at the study site have the highest tree density but a lower basal area relative to black willow (King and Buck 2018). While we did not directly assess the number of ash trees removed by beaver at the site, most ash trees remained lying next to the stump. This likely indicates the felling of ash trees was to access canopy leaves and branches either for food or for caching (Jenkins and Busher 1979). Additionally, Busher (1996) found that beaver in western Massachusetts cached or immediately consumed branches of the most common tree species. We found a significant difference between tree diameter and beaver herbivory category which may indicate that beaver are maximizing their energy consumption (Gallant et al. 2016) by selecting for a resource that is a higher density but smaller in size. Selecting for a smaller size resource requires less time and energy to fell but maximize the energy intake by felling multiple ash trees relative to larger black willow at the study site. This would also minimize predation risk (Pinkowski 1983). Nolet et al. (1994) also found that Eurasian beaver (*Castor fiber*) selected for shrub willows, which are smaller in diameter, than tree willows.

While black willow and green ash were most commonly fed on by beaver, other tree species are present at the study site. We documented beaver herbivory on silver maple and boxelder, but that was rare. King and Buck (2018) documented nine tree species that were in the forest overstory or understory. Cottonwood is the third most important overstory species at the study site (King and Buck 2018), but we did not document beaver herbivory on cottonwood within the belt transects. One possible explanation for the lack of herbivory on cottonwood is that the majority of cottonwood are furthest from the lake edge that would require beaver traveling overland. This is also the case for the other tree species at the study site. Most black willow and green ash are at or near the lake edge.

Another possible explanation for higher beaver herbivory on black willow and green ash are higher nutrient availability and lower secondary compounds in the wood and bark of these tree species. Nolet et al. (1994) argue that selection for willow may be an avoidance of plant defenses like resins, which are generally lower in willow species. Multiple ash species are known to have secondary defense compounds that are associated with plant-herbivore interactions (Eyles et al. 2007). Our data on beaver herbivory suggests that secondary compounds likely have a minimal influence on tree selection by beaver given that a greater proportion of green ash exhibited evidence of beaver herbivory compared to black willow. Nutritional value of bark and wood may be an explanation of beaver selection for black willow and green ash at our study site. Hill et al. (2012) assessed the concentrations of multiple nutrients in four ash species and found that green ash was intermediate in nutrition relative to other ash species. Five and ten-year-old cultivar willows in New Zealand were found to have similar protein levels (Kemp et al. 2001) compared to eight-year-old green ash in Hill et al. (2012). In a cafeteria-style study, Doucet and Fryxell (1993) found that while beaver had a preference for trembling aspen (*Populus tremuloides*), they consumed all five species that were presented. They found that each plant species had different nutrient concentrations and concentrations changed throughout the study period. Jenkins (1979) argued that seasonal and annual shifts in beaver herbivory may be related to changes in nutrient concentrations in bark and wood. While we did not assess temporal patterns in tree selection, it is possible that beaver at Arcadia Lake are foraging black willow and green ash at particular times during the year that is associated with changing nutrient availability.

Our study indicates that beavers at Arcadia Lake, Oklahoma County, Oklahoma are using the most common tree species available to them. Evidence indicates that the beavers are foraging green ash more frequently than black willow, the two most common tree species at the study site. Foraging on green ash is likely due to their smaller diameter and higher density. As beaver densities continue to increase, we encourage further studies of beaver herbivory in southern states where beaver have the ability to be active year-round due to less long-term freezing temperatures during the winter.

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Seasonal Activity Patterns of Mesocarnivores in Southcentral Oklahoma

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Abstract:- The primary goal of this study was to identify temporal activity patterns of mesocarnivores, and to identify activity overlap and seasonal variations in activity overlap at Oka' Yanahli Preserve (OYP), located in southcentral Oklahoma. We used camera traps to collect photographs of mesocarnivores in the preserve during winter (November 2016 – February 2017) and summer (May 2017 – August 2017). We deployed six remotely-triggered infra-red cameras, moving cameras to different, random locations every 4 weeks. Twenty-five camera locations in winter resulted in 1531 mesocarnivore pictures, and 18 camera locations during the summer resulted in 1455 mesocarnivore pictures. We identified coyote (*Canis latrans),* raccoon *(Procyon lotor),* bobcat (*Lynx rufus)*, Virginia opossum (*Didelphis virginiana)*, and striped skunk (*Mephitis mephitis)* during both seasons. All species were more active in winter than in summer, as they were detected more frequently in winter (Kernel Density Estimates). Temporal activities, measured by the coefficient of overlap (Δ), showed substantial overlap among all species in winter ($\Delta > 0.7$). The summer did not yield sufficient detections of bobcats, opossums, and skunks to assess their activity patterns. Moderate activity overlaps between coyotes and raccoons ($0.4<\Delta<0.7$) were recorded in summer. The data show that mesocarnivore species do not necessarily avoid each other, rather they co-exist through resource partitioning, as supported by large temporal overlap between the species, specifically in the winter season.

Introduction

Animal activity encompasses the time spent on essential processes crucial for survival (Clapham 2017; Cid et al., 2020; Caetano et al., 2020). This includes foraging, mating, resting, and other behaviors vital for energy acquisition, reproduction, and avoiding predators (Zhang et al., 2017). Studies suggest that the level of activity plays a significant role in determining an animal's resilience to environmental stressors. For instance, more active marine invertebrates were found to be more likely to survive during global change extinctions (Clapham 2017). Additionally, wildlife activity patterns are influenced by internal states (e.g., pregnancy) and external factors (e.g., seasonal resource availability and weather conditions) (Cid et al., 2020).

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Engaging in any activity requires energy. To survive, animals must undertake various activities to manage their energy budgets while navigating their habitats (Bu et al. 2016). The activities they perform could expose them to elevated predation risk or thermal stress, thus they need to perform these activities in a way that is as energetically beneficial as possible (Rowcliffe et al. 2014). However, due to environmental pressures such as habitat fragmentation and other human influences, the realized niches of numerous species worldwide have been altered. To survive, animals have to utilize the resources available by niche partitioning, and most importantly, by co-existing with minimal conflicts (Monterroso et al. 2013). Therefore, time has become a niche dimension that animals could use to segregate from each other to prevent agonistic encounters (Carothers and Jaksić 1984). Daily routines of animals within the time structure are inherited through evolution but adapted according to the environment in which they live (Carothers and Jaksić 1984, Monterroso et al. 2013,). That is, the adaptive significance of diel activity and circadian rhythms are intrinsic and their plasticity for local environmental adaptations is rather restricted (Kavanau and Ramos 1975). For example, nocturnal animals have physical and physiological adaptations that could maximize their energetical expenditures when they behave nocturnally whereas diurnal animals have different types of adaptations. Using all of these adaptations, animals utilize niche dimensions with low energetic demand that avoid mortality (Brown et al. 1999; Monterroso et al. 2013).

Ecological niche partitioning in spatial and temporal scales is important when studying predator-prey relationships and intraguild predation. According to optimal foraging theory, predators have to utilize energy budgets in a way to maximize the energy gain during foraging that could ultimately increase fitness (Porfirio et al. 2016). Alternatively, prey species should avoid potentially risky areas by either using their behavioral adaptations or physical characteristics to avoid predators. Prey species try to minimize activity overlap with predators while predators try to maximize and synchronize their temporal overlap with prey species. Therefore, animals are in an arms race when they partition their niche (Brown et al. 1999; Lima 2002; Monterroso et al. 2013).

In ecosystems without large carnivores, mesocarnivores assume the role of the apex predator (Roemer et al. 2009). Like other species, mesocarnivores divide their time performing several behaviors such as resting, hunting, defending their territories, and protecting themselves. Mesocarnivores can feed on a variety of prey species (Porfirio et al. 2016; Rowcliffe et al. 2014). Simultaneously, they are a very diverse group of mammals, that could experience intraguild predation (Prugh and Sivy 2020; Thompson and Gese 2007). Mesocarnivores occur across a large geographic distribution and how they utilize their resources, their activity patterns, and diet will vary across that distribution. It is therefore important to understand mesocarnivore ecology throughout their range.

Mesocarnivores are a diverse group of mammals ranging from elusive, nocturnal behaviors to dietary specialists, or sometimes generalists (Roemer et al. 2009). Hence, monitoring their temporal activity patterns can be challenging. The use of camera traps is convenient in this situation because they can provide a large set of data that could be analyzed by using modern robust methods like habitat use modeling, Bayesian modeling, and even in machine learning and computer vision. The degree of spatial overlap between sympatric species would help to understand intraguild predation or avoidance.

We used detection data of mesocarnivores at Oka' Yanahli Preserve (OYP) in south-central Oklahoma. We fitted them in circular density estimates to identify temporal activity patterns of individual mesocarnivore species in the winter and summer seasons. We then analyzed temporal activity overlap between sympatric mesocarnivore species to identify any activity avoidance among mesocarnivore guild. Circular density estimates are designed for data with a cyclical nature, such as time-of-day activity patterns, where there is no true beginning or end (e.g., midnight connects seamlessly to the next midnight). Kernel density estimation (KDE), on the other hand, is a more general method for estimating the probability density function of data. When applied to circular data, KDE must be adapted to handle the continuous nature of the circle, ensuring that density estimates wrap around smoothly. In the context of animal activity studies, KDE for circular data allows researchers to understand and visualize patterns of activity throughout a 24-hour cycle, providing insights that account for the biological rhythms influenced by sunrise and sunset.

We expected that coyotes (*Canis latrans)* and bobcats (*Lynx rufus)* should avoid each other temporally, hence they should have minimal activity overlap because bobcats are more elusive than coyotes and bobcat kittens could be eaten by coyotes (Knick 1990; Koehler and Hornocker 1991; Palomares and Caro 1999). We also expect that Virginia opossum (*Didelphis virginiana)*, raccoons (*Procyon lotor)*, and skunks (*Mephitis mephitis)* should also avoid coyotes because of their larger body size and the potential for conflict (Gehring and Swihart 2003; Prange and Gehrt 2007). Finally, we expected a significant difference in the coefficient of activity overlap among mesocarnivores between winter and summer seasons, due to more abundant resources during summer months that can affect the activity levels.

Methods

Field-Site Description.

We conducted our study during winter (November 2016-February 2017) and summer (May-August 2017) at Oka' Yanahli Nature Preserve (OYP) located in Johnston County, southcentral Oklahoma (34°26'14.7"N 96°38'09.9" W) and managed by The Nature Conservancy. It is located about 40 km south of Ada and about 24 km north of Tishomingo, on the Arbuckle Mountain Plains (Fig. 1). The preserve consists of 1457 hectares along 3.2 km of the Blue River. OYP is at the intersection of the Cross Timbers Forest and mixed grass prairies of the Great Plains (Woods et al. 2005). Apart from limestone prairie grassland, oak/hickory bottomland forests occurred on the eastern side of OYP and by the Blue River (Diamond and Elliott 2015).

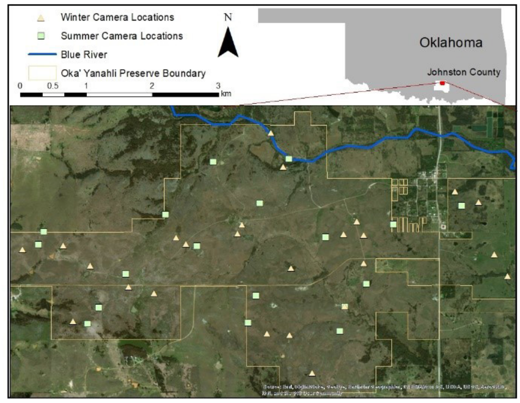


Figure 1. Location of study area, Oka' Yanahli Preserve in Arbuckle Mountain Plains, Johnston County, Oklahoma. Camera survey locations at Oka' Yanahli Preserve are triangles for winter (November 2016-Februry 2017) and squares for summer (May-August 2017).

The prairie is dominated by silver bluestem (*Andropogon saccharoides*), little bluestem (*Andropogon scoparius*), broomsedge bluestem (*Andropogon virginicus*), oldfield threeawn (*Aristida oligantha*), and buffalograss (*Bouteloua dactyloides*). Some other grasses and forbs include prairie dropseed (*Sporobolus hetrolepis*), sideoats grama (*Bouteloua curtipendula*), compass plant (*Silphium laciniatum*), leadplant (*Amorpha canescens*), wild alfalfa/scurf pea (*Psoralea tenuifolia*), Illinois bundleflower (*Desmanthus illinoensis*), blazing star (*Liatris sp*.), goldenrod (*Solidago sp*.), Indian paintbrush (*Castillega coccinea*), and Maximillian sunflower (*Helianthus maximilliani*) (Diamond and Elliott 2015).

Before purchase by The Nature Conservancy, OYP was used primarily as rangeland. We identified the following habitats: hardwood tree patches, aquatic habitats consisting of abandoned ponds and spring water accumulations, riparian corridors, bottomland forests, and prairie. There are some man-made and natural trails and roads that run through the preserve. The Nature Conservancy limits human use of the property; however, they allow limited deer hunting during winter and cattle grazing during summer as part of their land management plan.

The average annual precipitation of the study area ranged from 99–120 cm, with most precipitation occurring from midsummer to fall (Oklahoma Climatological Survey 2018). Snowfall during the winter months averaged 2 cm. In summer the temperature was as high as 35 ^{0}C, in winter it was as low as -1.6 ^{0}C, and the annual average temperature was 17 ^{0}C. Average frost-free days ranged between 224 –231 and the average growing season was 212 days. Wind speed was on average 12 km/hr. and relative humidity was 42 % –96 %. The highest humidity was in May and the lowest in August. On average there are 45 thunderstorms per year in the area (Oklahoma Climatological Survey 2018).

Camera trapping

We conducted camera trap sampling during the winter (November 2016 – February 2017) and summer (May – August 2017) seasons. We used Reconyx HC 500 Hyper Fire Semi-Covert Cameras, which use an infra-red, motion trigger function (Reconyx Inc., 3828 Creekside Ln, Site 2, Holmen, WI 54636). We programmed cameras to take 3 pictures every time the camera was triggered, record the date, time, and temperature, and rest for 5 minutes between bursts. The delay between each photo in the 3-photo burst was 1 second.

We used the ArcGIS 10.4 computer program (Environmental Systems Research Institute, Redlands, CA) to generate 100 random camera trap locations with 1.5 km between each camera location. Locations were selected from random locations in areas that were accessible and maintained a distance of at least 1.5 km between each camera in winter and 1.4 km in summer. In November 2016, 8 camera traps were used. Afterward, 6 camera traps were set each month in new, random locations. Cameras were set at random locations and moved every 3-4 weeks, to different random locations based on accessibility (Cove et al. 2013). Cameras were placed about 0.5 –1 m above the ground, angled downwards. Usually, cameras were attached to trees, bushes, or fence posts, but when not available wooden posts with stable stands (Christmas tree stands) were used. Winter season had 25 camera locations and summer season had 18 camera locations.

Data analysis

For each mesocarnivore detection, we recorded the species, number of animals per picture, location, camera ID, date the trap was set, date detected, time of the first picture, time of the last picture, number of pictures recorded during that detection, and temperature. Consecutive pictures of the same species and multiple animals in pictures were considered as single detection.

We analyzed mesocarnivore activity periods using nonparametric circular density functions (Frey et al. 2017; Linkie and Ridout 2011; Ridout and Linkie 2009). Since animal behavior patterns tend to change according to the daylight changes and the position of the sun: the time of sunrise, zenith, or sunset, analyzing the activity according to the clock time of sunrises and sunsets has no biological meaning (Azevedo et al. 2018; Caravaggi et al. 2018; Haswell et al. 2020; Noor et al. 2017; Nouvellet et al. 2012). The clock time of sunrises and sunsets changes according to the latitudes and the longitudes of the study location. Therefore, we adjusted each record to a specific day sunrise and sunset time. We standardized each clock time activity detection to sun time using "sunTime" and "overlap" packages in R version 4.0.3 (Meredith and Ridout, 2020). We estimated the activity pattern of each species during each season using Kernel Density Estimation (KDE) for circular data and activity overlap using the coefficient of overlap (Δ) (Porfirio et al.2016; Ridout and Linkie 2009). The coefficient of overlap varies from zero to one, where zero is no overlap and one is complete overlap (Penido et al. 2017). According to Ridout and Linkie (2009), KDE considers camera trap pictures as random samples with an underlying continuous distribution, therefore, they are not categorized into discrete time variables. We used the Δ_1 estimator which is recommended when the smallest sample size is below 50 records for all mesocarnivore pairs except for coyote-raccoon. With more than 50 records for coyote-raccoon, we used Δ_4 estimators. Confidence intervals of Δ_1

and Δ_4 were calculated as a percentile of intervals from 10,000 bootstraps (Frey et al. 2017; Linkie and Ridout 2011; Ridout and Linkie 2009). If $\Delta > 0.7$, it was considered a higher activity overlap, and $\Delta<0.4$ was considered a lower activity overlap (Bu et al. 2016). To minimize pseudo replication, we pooled detections of the same species at the same trap location that occurred within two hours. Also, if there were more than one animal in one picture, it was considered as a single detection (Bu et al. 2016). All statistical analyses were performed using R software (R Software Core team 2020 version 4.0.3) using packages Overlap, Circular, Boot, SunTime, Suncalc, and Maptool (Meredith and Ridout 2014). All figures were made using ggplot2 package.

Results

We had 844 camera nights in winter and 600 camera nights in summer for a total of 1444 camera nights (Table 1). More than 100,000 pictures were recorded; among them, 4233 pictures were obtained of mesocarnivores. The winter season had a higher number of pictures (2778) than the summer season (1455). Mesocarnivore species recorded were coyote, bobcat, Virginia opossum, raccoon, and striped skunk. Camera malfunctions were very rarely identified and did not affect the survey during either season.

Table 1. Camera days and numbers of independent detections of each mesocarnivore species; coyote, bobcat, raccoon, Virginia opossum, and striped skunk during the camera trap survey in winter 2016-2017 and summer 2017 at Oka' Yanahli Preserve, southcentral Oklahoma.

	Winter season	Summer season	Totals
Camera days	844	600	1444
Species			
coyote	80	24	104
bobcat	22	3	25
raccoon	138	31	169
opossum	60	1	61
skunk	19	4	22

KDE for single species during the winter season showed that all species were highly crepuscular and peak activities were seen at sunrise and sunset (Fig. 2). The graphs overall show that all mesocarnivore species recorded were highly nocturnal during the winter season. While coyotes had higher activity density during both sunrise and sunset; bobcats, opossums, and skunks had higher activity density during sunset and thus nocturnal. Raccoons had a higher activity peak during sunrise than sunset.

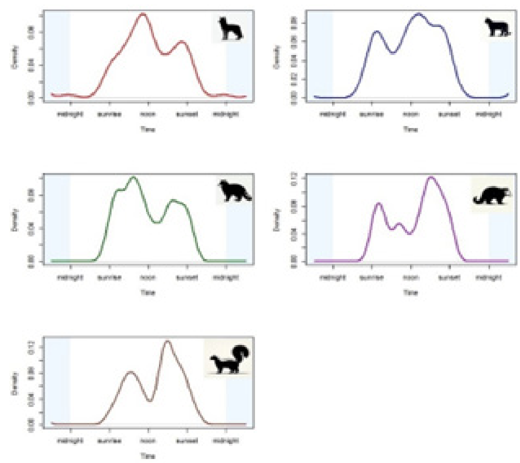


Figure 2. Winter Activity patterns of five species of mesocarnivores; coyote, bobcat, raccoon, Virginia opossum, and striped skunk at Oka' Yanahli Preserve, southcentral Oklahoma, as captured by camera trap records. Curves are fitted with circular kernel density distributions along with the time of the day during the winter season of 2016-2017.

In summer, only coyotes and raccoons had enough data to analyze activity patterns. bobcats, opossums, and skunks were removed from the analysis due to low detection rates. Raccoons were active slightly after sunset while coyotes were active throughout the day with a slightly higher peak between noon and sunset (Fig. 3).

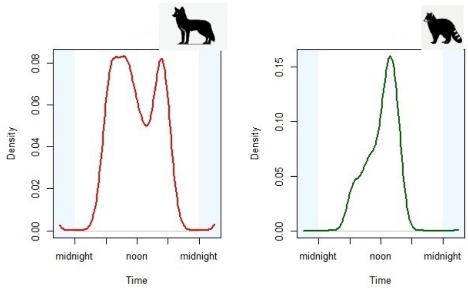
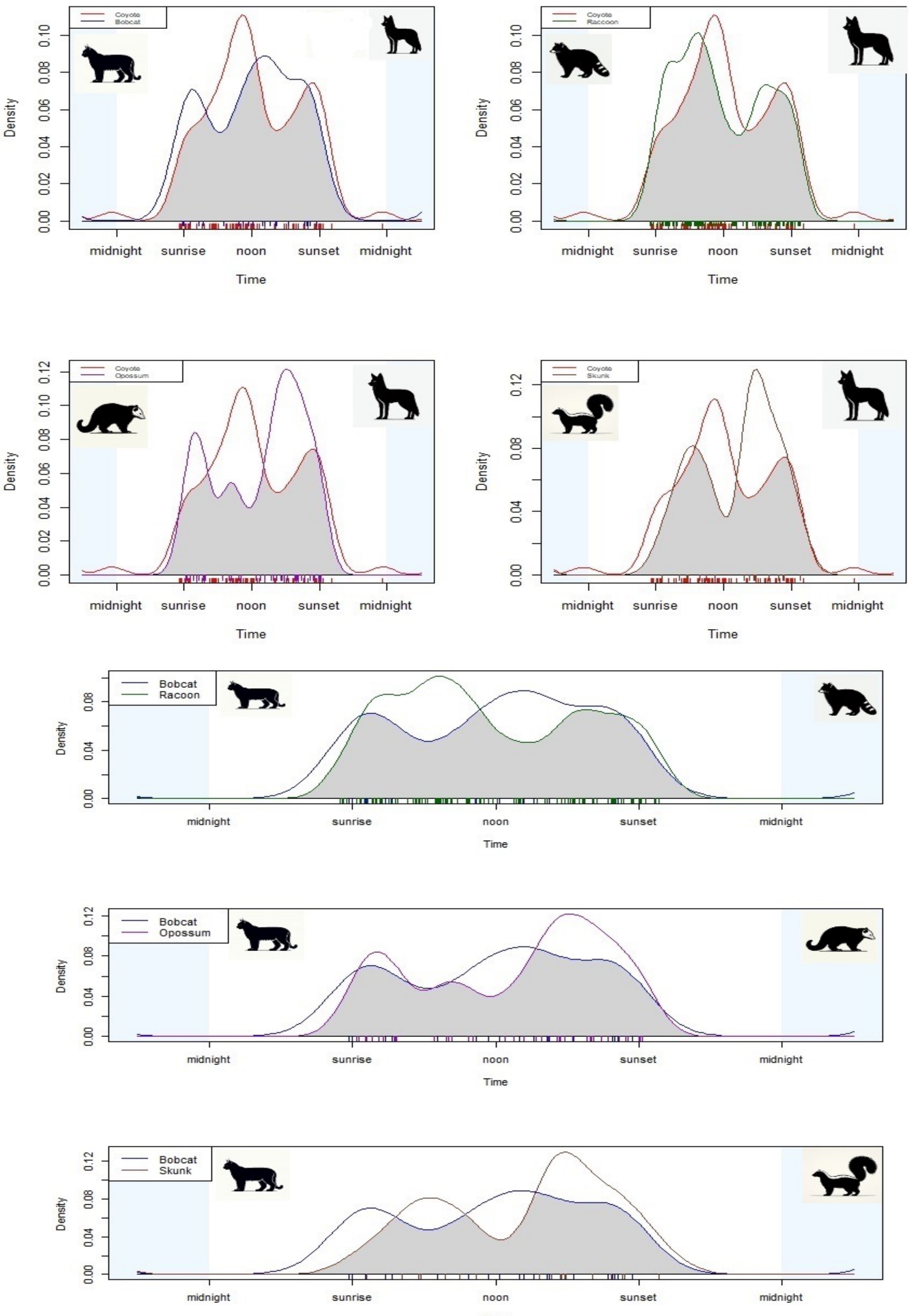


Figure 3. Summer activity patterns of four species of mesocarnivores; coyote and raccoon at Oka' Yanahli Preserve, southcentral Oklahoma, as captured by camera trap records. Curves are fitted with circular kernel density distributions along with the time of the day during the summer season of 2017.

During the winter season, almost all mesocarnivore pairs had a high coefficient of activity overlap ($\Delta > 0.7$) (Table 2, Fig. 4). The highest activity overlap was recorded between skunk-opossum Δ_1=0.87 (95% CI,0.52 to 0.89), while the lowest occurred between coyote and opossum Δ_1=0.74 (95% CI,0.67 to 0.98) (Table 2, Fig. 4). The coyote-opossum activity overlap plot shows higher activity peaks from opossum when coyote had low activity peaks, especially in sunset and sunrise (Fig. 4). Skunk and opossum both had higher activity peaks during sunset, where there were low activity peaks with bobcats and raccoons respectively (Fig. 4).

Table 2. Estimated activity level overlap of five mesocarnivore species, coyote, bobcat, raccoon Virginia opossum, and striped skunk at Oka' Yanahli Preserve southcentral Oklahoma, during winter 2016-2017 obtained from camera trap data. Values were obtained from circular density functions and the coefficient of Overlap (Δ) and 95% Confidence intervals Δ are shown.

Species	The coefficient of Overlap (Δ)			
	Type	Lowest Value	Overlap Estimate	95% CI
coyote-bobcat	Δ_1	22	0.822	0.997—0.676
coyote-raccoon	Δ_4	76	0.856	0.813—0.420
coyote-opossum	Δ_1	42	0.741	0.977—0.674
coyote- skunk	Δ_1	18	0.762	0.975—0.616
bobcat-raccoon	Δ_1	22	0.827	0.889—0.549
bobcat-skunk	Δ_1	18	0.765	0.935—0.594
bobcat-opossum	Δ_1	22	0.842	0.985—0.699
raccoon-opossum	Δ_1	42	0.804	0.842—0.542
raccoon-skunk	Δ_1	18	0.798	0.892—0.528
skunk- opossum	Δ_1	19	0.874	0.895—0.526


Coyote
Bobcat
Coyote
Raccoon
Coyote
Opossum
Coyote
Skunk
Bobcat
Racoon
Bobcat
Opossum
Bobcat
Skunk
Density
Time
midnight
sunrise
noon
sunset
midnight

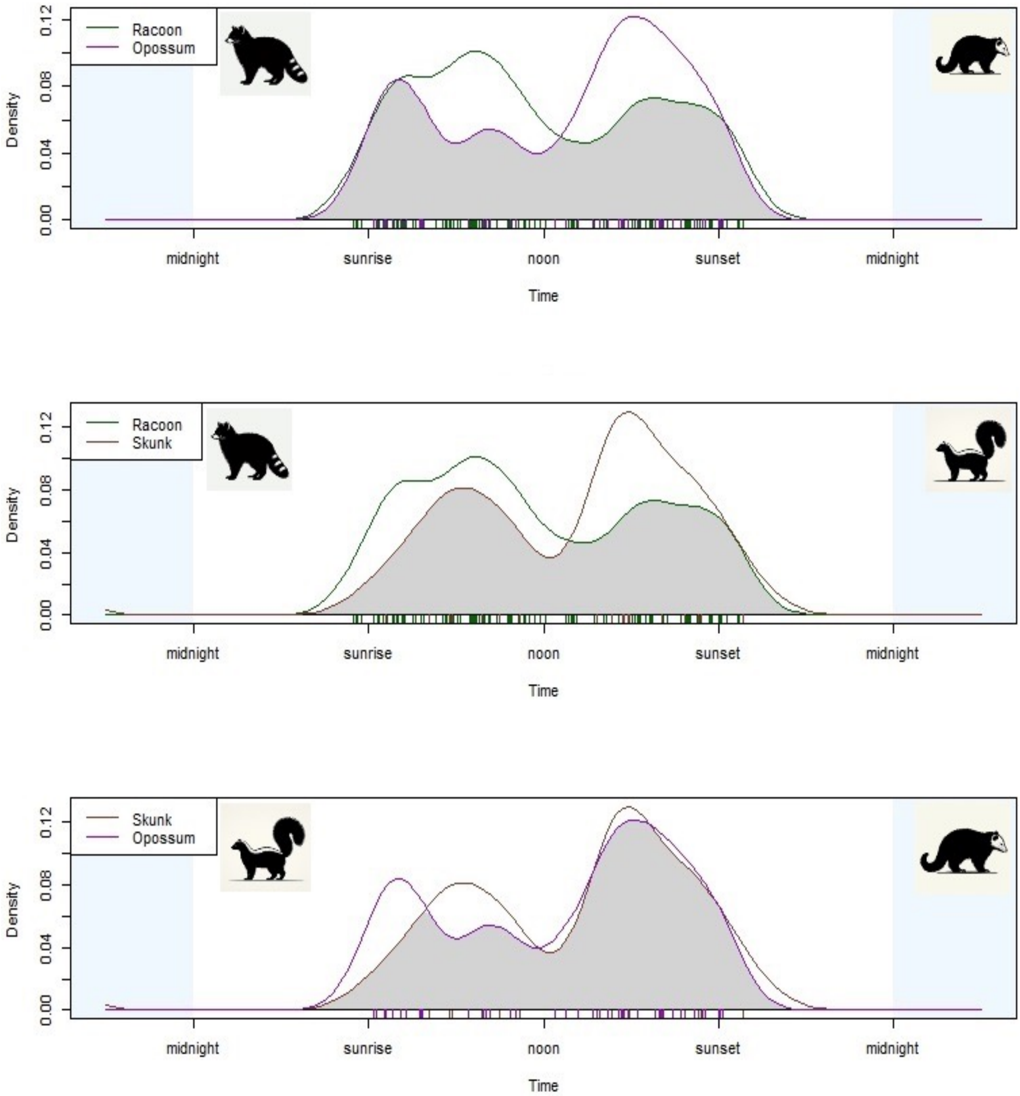


Figure 4. Temporal overlap of activity patterns of mesocarnivores; coyote, bobcat, raccoon, Virginia opossum, and striped skunk during the winter season (November to February 2016-2017) in Oka' Yanahli Preserve at southcentral Oklahoma. The y-axis represents the kernel density estimate. The overlap area was denoted in grey. Species names are given in the legend of each graph.

During summer, moderate activity overlap ($0.4 < \Delta < 0.7$) was recorded between coyote and raccoon Δ_1=0.63 (95% CI,0.45 to 0.81) (Table 3, Fig. 5). However, activity overlap patterns were dissimilar from winter activity patterns. In summer, both raccoons and coyotes had activity peaks during sunset, but raccoons were more active than coyotes during this time (Fig. 5).

Table 3. Estimated activity level overlap of Coyote and striped skunk at Oka' Yanahli Preserve south-central Oklahoma, during summer 2017 obtained from camera trap data. Values were obtained from circular density functions the coefficient of Overlap (Δ) and 95% Confidence intervals Δ are shown.

Species	The coefficient of Overlap (Δ)			
	Type	Lowest Value	Overlap Estimate	95% CI
coyote-raccoon	Δ_1	24	0.628	0.448—0.808

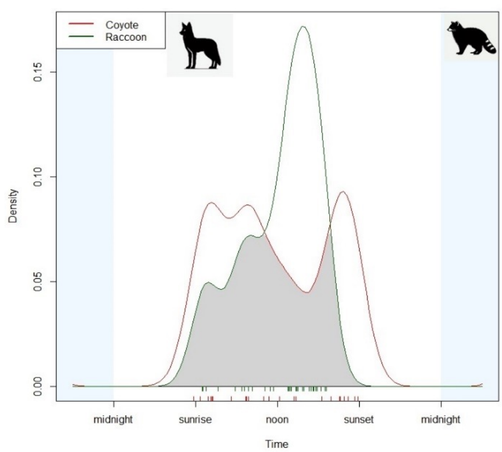


Figure 5. Temporal overlap of activity patterns of mesocarnivores; coyotes and raccoons during summer season (May to July 2017) in Oka' Yanahli Preserve southcentral Oklahoma. The y-axis represents the Kernel Density Estimates. The overlap area was denoted in grey. Species names are given in the legend of each graph.

Discussion

Our study revealed that during winter, there was a high degree of temporal activity overlap among all pairs of mesocarnivore species. The interplay of factors such as increased scavenging opportunities, reduced competition, behavioral adaptations, physiological needs, and changes in habitat use explains why mesocarnivores in North America tend to be more active in winter than in summer (Bell et al., 2023). This adaptability is crucial for their survival as they face seasonal challenges, and our study provides additional support for these findings. In contrast, during summer, we observed a moderate level of activity overlap between coyotes and raccoons, as anticipated. However, we could not identify seasonal variations in individual activity patterns or the activity overlap of bobcats, opossums, and skunks due to low detections in summer. Several similar studies have reported that there was a reduction in mesocarnivore detections in summer (O'Connell et al. 2006, Hackett et al. 2007, Crimmins et al. 2012). Additionally, our summer season was from May to August, which was a comparatively shorter survey period for particularly elusive species like mesocarnivores. Therefore, longer study periods along with repeated surveys are required (Gompper et al. 2006).

Even though coyotes were ubiquitous, their activity peaked during sunrise and sunset in both seasons. Mammals are known to change their daily rhythmic activity according to their thermoregulatory energetic requirements (Pavey et al. 2016). In winter, homoeothermic animals will alter their foraging and other related activities in a way that they will maximize their foraging activities. Therefore, all detected mesocarnivore species had high activity peaks during sunset and hence are nocturnal, as the potential for finding prey at night is higher in winter (Symmank et al. 2014). This is specifically true for Coyotes because they travel significantly higher distances at night in winter when they are not breeding or rearing pups (Andelt and Gipson 1979). Therefore, higher nocturnal activity during winter can be expected. Our results agree with other related studies about winter activity patterns of mesocarnivore species that are mostly nocturnal (Lesmeister et al. 2015; Tigasa et al. 2002). The activity patterns of raccoons in winter showed high-density peaks at sunrise and lower activity during the daytime. Raccoons' breeding season starts in March leading to an increase in movement as much as twice normal movement, both diurnally and nocturnally (Greenwood 1982). Therefore, less activity during the daytime in winter could be expected. Opossums were highly nocturnal with comparatively low activity peaks during sunrise in winter.

In contrast, coyotes and raccoons had increased diurnal activity patterns in summer than in winter. Coyotes and raccoons have their breeding season during spring; therefore, they should be active throughout the day while nurturing their pups (Ozoga and Harger 1966). This is specifically true for coyotes as the lactating coyote females tend to travel as far as males during summer (Ozoga and Harger 1966). Our results of coyotes' activity patterns during summer supported the results of previous studies.

Bobcat males and females usually live separately with wider home ranges, but they live nearby during the breeding season (Lawhead 1984). The breeding season of the bobcat typically spans from February to March, immediately following our winter sampling period. Therefore, it is plausible that bobcats exhibit elevated activity levels in preparation for breeding, aligning with the increased activity observed during winter (Lawhead, 1984). During the kitten-rearing season, male and female bobcats do not demonstrate significant separation; instead, they adapt their movements within their respective home ranges. Male bobcats typically have larger seasonal home ranges than females, with males' territories approximately three times larger than those of females (McNitt et al., 2020; Janečka et al., 2007). Despite variations in reproductive investments, both sexes maintain relatively consistent seasonal home ranges, indicating constraints imposed by territorial behavior (Janečka et al., 2007). Female bobcats increase their movements during the kitten-rearing period, engaging in intensive foraging and frequent returns to den sites (Plowman et al., 2006). In contrast, male bobcats exhibit increased movements during the dispersal period, potentially reflecting heightened territorial behavior before breeding. These findings suggest that seasonal fluctuations in home range selection and movements are influenced by reproductive activities and prey availability.

Contradictory to what we expected, coyotes and bobcat activity patterns largely overlapped during winter. Since bobcats and coyotes are sympatric across their distribution range, we expected that they would coexist by partitioning resources either by selecting different prey species or being active at different times of the day (Flores-Morales et. al 2019). However, coyotes and bobcats had activity overlap throughout the day. Similar studies have found that bobcats do not avoid coyotes, and they largely coexist (Fedriani et al. 2000; Lesmeister et al. 2015; Melville et al., 2020; Lombardi et al., 2020). Bobcats are solely carnivorous, and their diet mainly consists of rodents and lagomorphs (Lesmeister et al. 2015; Neale et al. 2001; Wilson et al. 2010). Coyotes are mostly carnivorous, but their diet has seasonal variations (Andelt and Gipson 1979; Turner et al. 2011). Coyotes mainly depend upon deer carcasses and in some cases invertebrates. Therefore, these two mesocarnivores could co-exist by separating their resource use. Consequently, this type of resource use could result in realized niche partitioning other than competition-driven niche partitioning (Lesmeister et al. 2015; Neale et al. 2001; Wilson et al. 2010).

Due to the ubiquitous nature of coyotes, we expected that their activity would overlap with all the other small mesocarnivores. According to the Mesopredator Release Theory (MRH), large mesocarnivores have a profound impact on smaller mesocarnivores. The intensity of the impact may depend upon the defensive mechanisms coupled with the intensity of competition between smaller mesocarnivores and coyotes

(Prange and Gehrt 2007). According to MRH coyotes can significantly reduce the population numbers of skunks, opossums, and raccoons; but a considerable amount of research around the United States has still failed to identify skunks or raccoons in the coyotes' diet (Prange and Gehrt 2007). Therefore, predation could not be the major reason for skunks, raccoons, and opossums to avoid coyotes. Indeed, there is evidence that they do not temporally avoid coyotes (Crooks and Soulè 1999; Prange and Gehrt 2007; Sovada et al. 2000).

Coyotes and bobcats are more carnivorous than raccoons who are more generalized in their diet. Raccoons have larger body sizes than all the other potential prey species of coyotes and bobcats. Also, there are observations that a raccoon can successfully defend deer carcasses from coyotes (Lesmeister et al. 2015), because of that there should be less resource competition between coyotes, bobcats, and raccoons allowing them to co-exist and have high temporal activity overlap. These findings could be further strengthened by our findings of activity overlap between these three species.

The activity of opossum highly overlaps with coyotes and bobcats. According to similar studies, there is evidence that coyotes and bobcats are not major predators of opossums (Cove et al. 2012; Prange and Gehrt 2007; (Troyer et al. 2014). Our results of opossums with coyotes and bobcats observed here support strongly the current findings on mesocarnivore resource partitioning and co-existence.

Skunks' activity overlaps with both coyotes and bobcats are slightly higher than opossums. Skunks are predominantly nocturnal in winter with low activity during the daytime. A possible reason would be to avoid sympatric large mesocarnivores. However, similar research has shown skunks and coyotes can co-exist with slight interspecific avoidance due to conspicuous coloration in the pelage of skunks and their defensive noxiousness (Aleksiuk and Stewart 1977; Lesmeister et al. 2015; Prange and Gehrt 2007).

Contradictory to the winter season, summer had minimal detections from bobcats, opossums, and skunks (Plowman et al. 2006). Our results with low detections support similar studies, however, there are no adequate references specifically from different regions of Oklahoma to compare our results obtained from the southcentral region. During the summer season, the most influential event that happened in the preserve was cattle grazing. Abundant cattle may not have had a significant influence on coyote behavior. According to our data, which had increased diurnal activity in summer than winter, coyotes may not have been affected by the presence of cattle to the same degree as other mesocarnivores. Coyotes can be a predator of calves, but not an adult cow (Danner and Smith 1980; Bradley and Fagre 1988). Similar studies have found that coyotes can overlap home ranges with cattle ranches, but they aggregate more around carcasses of cattle than live cattle (Bradley and Fagre 1988, Danner and Smith 1980). Therefore, the presence of cattle may not have affected the activity of coyotes during summer. Southcentral Oklahoma summer weather could be another possibility for low activity detections because most species prefer cover and the preserve mostly consists of open grassland with less cover. At the same time, there was abundant rainfall in summertime causing more vegetation growth in the preserve which could lead to a considerable number of false detections and low detection of small species like opossums and skunks (Gompper et al. 2006).

In conclusion, this study supported the fact that strong interference competition may not always happen within carnivore communities; rather species tend to coexist and structure the community in a way that allows co-existence. The mesocarnivore species identified in this study did not exhibit significant dietary overlap or inferred competition with one another. Consequently, they are capable of coexisting within their shared habitat. We recognize that we were unable to detect any seasonal variations in the activity patterns of mesocarnivores within this preserve. However, our findings mark the first of their kind reported in southcentral Oklahoma. Looking ahead, it's essential to conduct additional research to delve into the implications of this intraguild co-existence among mesocarnivores, particularly concerning the management and conservation plans for Oka' Yanahli Preserve.

Acknowledgments

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Possible Red Imported Fire Ant (*Solenopsis Invicta* Buren) Ingestion-induced Mortality to Stocked Trout in the Lower Mountain Fork River, Oklahoma

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Abstract: The introduction of hatchery-reared trout into Oklahoma's Lower Mountain Fork River (LMFR) has enhanced angling opportunities. However, both Brown Trout (*Salmo trutta*) and Rainbow Trout (*Oncorhynchus mykiss*) have recently behaved erratically, with subsequent mortality, thought to be the result of Red Imported Fire Ant (*Solenopsis Invicta* Buren) flotilla consumption during flood events. The objectives of this paper are to: (1) enumerate diets, (2) compare physical characteristics and diets between trout species, and (3) determine if there is a relationship between diet and physical characteristics of dead trout collected after two flooding events (February 2023, June 2024) at the LMFR. Analysis of nine dead trout revealed that Fire Ants comprised 99% of their diet by frequency of occurrence, percent composition by number, and percent composition by weight. Statistical analysis showed no significant difference between Brown Trout and Rainbow Trout physical characteristics (length, weight) or diet characteristics (diet weight, number of Fire Ants consumed). Significant positive correlations were observed between fish size (length, weight) and diet weight, though no relationship was found between fish size and number of fire ants consumed. We found larger trout generally consumed a greater mass of Fire Ants, suggesting a potential threshold effect where high ingestion of Fire Ants may lead to mortality. However, more work needs to be done to determine the mechanistic cause and mortality threshold for Red Imported Fire Ant consumption by trout.

Introduction

For more than a century, the introduction of hatchery-reared salmonid fishes into streams and reservoirs has been used to provide angling opportunities where native fish have been extirpated, or to enhance angling opportunities where natural trout populations were reduced or extirpated due to anthropogenic impacts (Hanisch et al. 2012). Trout are typically found in cold-water streams, reservoir tailwaters, or seasonally in small impoundments where water temperatures

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remain suitable for their survival (Snow et al. 2019). Brown Trout (*Salmo trutta*) and Rainbow trout (*Oncorhynchus mykiss*) are not native to Oklahoma (Miller and Robison 2004), but the Oklahoma Department of Wildlife Conservation (ODWC) stocks them in certain waters to provide angling opportunities (ODWC 2023). In 1965 ODWC started a put-and-take trout stocking program on the lower Illinois River below Lake Tenkiller, followed by trout stockings on the Blue River Public Fishing area in southern Oklahoma in 1968 (Gilliland 1989). Since the 1960s ODWC has expanded the Lower Mountain Fork River (LMFR) to a year-round trout fishery and created several other seasonal trout put-and-take fisheries (ODWC 2023). Trout stockings are primarily conducted from November through the end of February when water temperatures are cooler. Both Brown Trout and Rainbow Trout are considered cold-water species with a critical thermal maximum ranging from 23 to 30°C (Beitinger et al. 2000; Carline and Machung 2001; Chen et al. 2015). However, critical thermal maximum for these species can vary based on factors such as acclimation, size, and genetic adaptation to local environmental conditions (Beitinger et al. 2000).

Hatchery-raised trout are opportunistic feeders that quickly adapt to a natural diet, predominantly consuming invertebrates (Tay et al. 2007, Odenkirk and Estes 1991, O'Rouke 2014). Although typically small, the magnitude and severity of impacts on other native fish and invertebrate populations vary considerably from system to system (Rodger et al. 2021, Rodger and Stark 2022). For example, Fenner et al. (2004) found that stocked Rainbow Trout rarely compete with other top predators in these systems, though they might compete with insectivorous fish such as darters, sculpins, and cyprinids. Likewise, Metcalf et al. (1997) suggested stocked trout can potentially influence the recruitment of Smallmouth Bass (*Micropterus dolomieu*) through competition and Snow et al. (2019) illustrated that stocking predatory fish like trout can negatively influence age-0 Gizzard Shad (*Dorosoma cepedianum*) biomass. Given their broad diet and adaptability, trout are able to consume a broad range of diet items. However, consumption of invasive species such as Red Imported Fire Ants (*Solenopsis Invicta* Buren; Figure 1) may have negative consequences on their survivability (*sensu* Hutchins 1960, Prather 1960, Crance 1965).

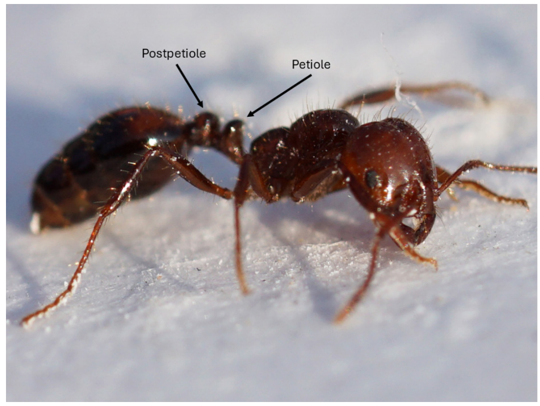

Figure 1. Photograph of a Fire Ant collected in July of 2024. The distinguished feature of a Red Imported Fire Ant is the two nodes on the waist portion of the abdomen called the petiole and postpetiole. Picture courtesy of Brandon Brown.

The Red Imported Fire Ant is a global invader that has been introduced to at least seven countries, including the United States in the mid-1930s, where it has spread across fourteen states (Mooney and Cleland 2001, Robbin et al. 2012). They have successfully invaded the southern United States, specifically Oklahoma, due to the lack of interspecific competition with other ant species and the absence of more diverse aggressive ant fauna found in South America (Allen et al. 2004, Morrison et al. 2004). Anthropogenic soil disturbance increases the density of Red Imported Fire Ants, while they can achieve high densities in both dry and wet undisturbed habitats, their populations are substantially influenced by soil disturbance, with densities decreasing over time as disturbed areas return to conditions similar to undisturbed ones (LeBrun et al. 2012). This pattern of fire ant density related to soil disturbance is a novel finding and highlights the significant impact of anthropogenic activities on their population dynamics as it relates to the highly developed LMFR area.

Anthropogenic influences, such as the development of infrastructure like parking lots, have created the perfect habitat for introduced Red Imported Fire Ants, and the colonization of these areas of the LMFR (LeBrun et al. 2012). The LMFR cuts through an area with shallow rocky soils which allow little rainfall to be absorbed into the ground. During heavy or prolonged rainfall this often results in flooding of low-lying areas. Heavy rain events wash out some Red Imported Fire Ants into sounding tributaries that are within the mound portion of their nest that can range from 30 cm to 90cm tall (Green et al. 1999), into the LMFR. Red Imported Fire Ants survive flooding events by creating a flotilla (Figure 2), a behavioral response in which ants connect to one another to form a structure that allows them to float on the surface of the water (Roeder et al. 2018). While flotillas are present in the river, predation by trout or other fishes in the river is likely occurring. Although anecdotal, angler observational records indicate that when Red Imported Fire Ant flotillas are observed floating in the LMFR, trout are also observed acting erratically or are found dead.

Figure 2. This photograph shows a Red Imported Fire Ant flotilla formed by ants linking together (shown in the close-up photograph of the structure) to float on the water's surface. This behavior enables them to survive flooding events. These flotillas can become prey for fish, including trout, as they drift into aquatic habitats.

Reports of dead trout were received from anglers on February 23, 2023, and June 17, 2024, from the LMFR. Anglers observed Red Imported Fire Ants in a stomach removed from one of the dead trout (Figure 3). Afterward, the angler collected a subsample of dead trout was for diet analysis to verify stomach content. The objectives of this study were to: (1) enumerate diets of dead trout, (2) compare physical (i.e., length, weight) and diet characteristics of expired Rainbow Trout and Brown Trout to determine if there are differences between each species, and (3) determine if there is a relationship between diet (i.e, weight, Red Imported Fire Ants consumed) and physical characteristics of dead trout.

Figure 3. Photograph taken by an angler of a dead trout that had consumed Red imported Fire Ants at the Lower Mountain Fork River, OK, on June 17, 2024.

Methods

A total of five trout (3 Brown Trout and 2 Rainbow Trout) were collected by anglers on February 23, 2023. An additional four trout (2 Brown Trout and 2 Rainbow Trout) were collected by the ODWC on June 17, 2024. All trout were frozen upon capture or received frozen from anglers and remained frozen until processing. Trout were thawed and processed at the Oklahoma Fisheries Research Lab in Norman, Oklahoma.

Fish were identified using information present in Oats et al. (1993) and Miller and Robison (2004). Fish were measured for total length (mm) and weight (g). Stomachs were extracted, prey items were removed, identified and enumerated, and prey items were weighed (g). All prey items were identified to species when possible, using scientific taxonomic keys to identify aquatic invertebrates (Merrit et al. 2008). Due to the presence of ants, we used a key in Hung et al. (1977) to determine the species of Red Imported Fire Ants. Stomach samples were analyzed by percentage of empty stomachs, frequency of occurrence (O_i), percent composition by number (N_i), and percent composition by weight (W_i; Bowen 1996; Chipps and Garvey 2007).

Several species-specific comparisons were made between Rainbow Trout and Brown Trout. We compared Rainbow Trout versus Brown Trout weights and TLs, and recorded if they exhibited differences in the number of Red Imported Fire Ants consumed present in the stomachs or the weight of their diet samples. Weights for both species were $\log_{10}$ transformed prior to analysis (Zar 1999). If data were normal and variance was equal between species samples a Student's t-test was used (Student 1908), if data were normal and variance was unequal between species samples a Welch's t-test was used (Welch 1951), and if data were nonnormal but variance was equal between species samples a Mann-Whitney U test (Mann and Whitney 1947) was used ($\alpha = 0.05$). Normality was assessed for each species using a Shapiro-Wilks test (Shapiro and Wilks 1965) and equality of variance between each species sampled determined via an F-test ($\alpha = 0.05$).

We then compared diet and fish size metrics. Pearson correlations were used to assess and test the strength of the association between weights and TLs of fish and the number of Red Imported Fire Ants consumed and diet weight (Pearson 1896; $\alpha = 0.05$). Fish weight, TL, number of Red Imported Fire Ants consumed, and diet weight were all $\log_{10}$ transformed for these analyses (Zar 1999). Normality of each variable was determined via a Shapiro-Wilks test and homoscedasticity was determined via a studentized Breusch-Pagan test (Breusch and Pagan 1979, Koenker 1981; $\alpha = 0.05$). Outliers for each variable were assessed by transforming each $\log_{10}$ transformed variable into a z-score and determining if they fell within the range of -3.29 – 3.29. All analyses were conducted in program R version 4.4.1 (R Core Team 2024).

Results

Of the 9 specimens collected, 0% had empty stomachs. Brown Trout and Rainbow Trout ranged in TL from 193 - 246 mm and 176 - 307 mm (Table 1). Brown Trout and Rainbow trout ranged from 60 - 322 g in weight (Table 1). Diets by O_i, W_i, and N_i of these fish contained 99% Red Imported Fire Ants and <1% other, which consisted of a rubber grub lure, crawfish claw, Isopod, Leafhopper, and Odonate. The weight of ants consumed ranges from 1.13 – 10.76 g with a mean of 3.41 g of Red Imported Fire Ants per fish with total weight 31.23 g of total ants consumed by these 9 trout. In total, 1,285 Red Imported Fire Ants were enumerated, ranging from 27 – 426 ants per trout stomach (Figure 4A).

Table 1. Species, total length (TL; mm), and weight (g) of expired trout from the Lower Mountain Fork River, OK. Included are the number of Red Imported Fire Ants (Fire Ants) within each trout stomach and the weight of the diet items from each stomach (Diet Weight).

Species	TL	Weight	Fire Ants	Diet Weight
Brown	193	80	86	1.77
Brown	205	84	83	2.14
Brown	216	88	35	1.19
Brown	241	148	51	1.47
Brown	246	150	48	2.29
Rainbow	176	60	211	2.48
Rainbow	191	70	27	1.13
Rainbow	301	278	318	8.00
Rainbow	307	322	426	10.76

Log_{10} transformed weight, TL, number of Red Imported Fire Ants consumed, and diet sample weight were all normal for samples from Rainbow Trout (W range = 0.80 – 0.98, all $p > 0.05$).

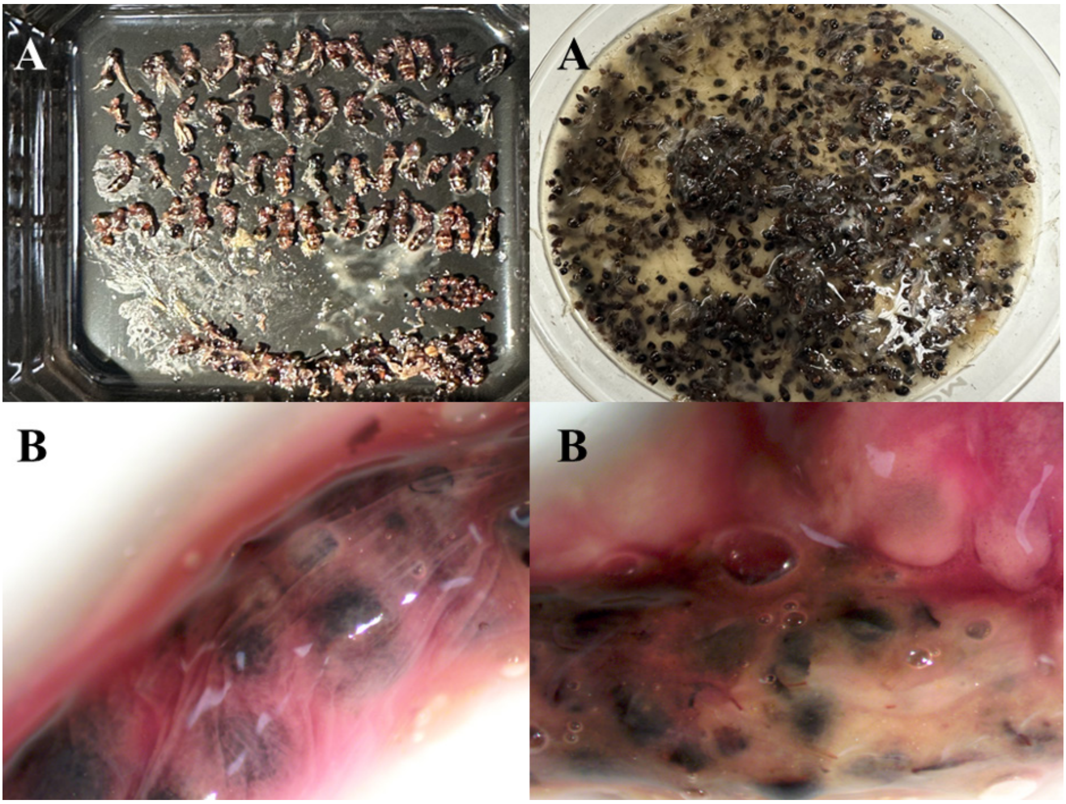

Figure 4. Images show the stomach and intestinal contents of trout sampled from the Lower Mountain Fork River. The upper panel (A) depicts a trout stomach filled primarily with Red Imported Fire Ants, while the lower panel (B) shows a section of the trout's intestines containing Red Imported Fire Ants and other minor dietary items.

Log_{10} transformed TL, number of Red Imported Fire Ants consumed, and diet sample weight were normal for samples all Brown Trout (W range = 0.87 – 0.96, all p > 0.05); however, log_{10} weight was not normal (W = 0.77, p = 0.04). Variances were unequal between species for number of Red Imported Fire Ants consumed (F = 0.02, p < 0.01) and diet weight (F = 0.01, p < 0.01). Welch's t-tests suggested that the two species consumed a similar number of Red Imported Fire Ants (t = -2.16, p = 0.12) and that diet weights were similar between species (t = -1.67, p = 0.19). Variance was similar between species TLs (F = 0.11, p = 0.06) and student's t-tests suggested TLs were similar between species (t = -1.90, p = 0.10). Variance was similar between log_{10} transformed weights (F = 0.13, p = 0.08) and Mann-Whitney U tests suggested log_{10} transformed weights were similar between species (W = 10, p = 1.00).

Statistically similar sizes of expired Rainbow Trout and Brown Trout were sampled from the Lower Mountain Fork River, OK. Both species appeared to consume a statistically similar number of Red Imported Fire Ants, and their stomach contents exhibited statistically similar weights. Though a small number of other organisms were present in diets, most of the matter within each stomach consisted of Red Imported Fire Ants. These tests suggest that there was not a species-specific bias in weight or TL of observed expired trout. It also suggests that no species-specific preference for consuming Red Imported Fire Ants was exhibited in the Lower Mountain Fork River. However, these findings are likely driven by low sample size.

All data were normally distributed after $\log_{10}$ transformations based on our Shapiro-Wilks test (W range = 0.84 – 0.92, all $p > 0.05$). Likewise, the relationships between $\log_{10}$ transformed weight and TL was homoscedastic for both $\log_{10}$ transformed number of Red Imported Fire Ants consumed and diet weight (BP range = 0.09 – 1.20, all $p > 0.05$). No outliers were detected in any $\log_{10}$ transformed variable (z-score range = -1.27 – 1.83). The Pearson correlation between $\log_{10}$ transformed weight and number of Red Imported Fire Ants consumed was 0.57 and was determined to be insignificant ($t = 1.85$, $df = 7$, $p = 0.11$). The Pearson correlation between $\log_{10}$ transformed TL and number of Red Imported Fire Ants consumed was 0.51 and was determined to be insignificant ($t = 1.56$, $df = 7$, $p = 0.16$). The Pearson correlation between $\log_{10}$ transformed weight and diet weight was 0.81 and was significant ($t = 3.70$, $df = 7$, $p < 0.01$). The Pearson correlation between $\log_{10}$ transformed TL and number of diet weight was 0.77 and was significant ($t = 3.22$, $df = 7$, $p = 0.02$).

Number of ants consumed did not appear to be significantly correlated with fish weight or TL (Figure 5). However, the weight of diet items was significantly positively correlated with fish weight and TL (Figure 5). Given Red Imported

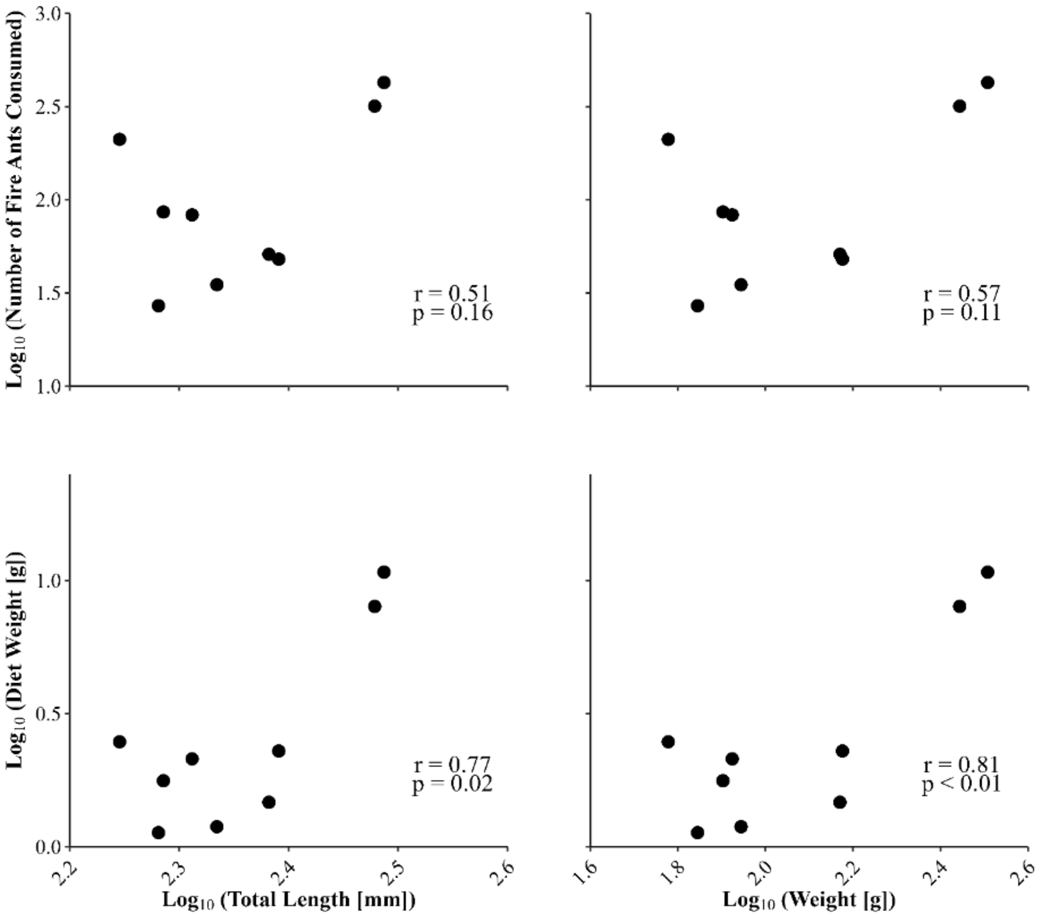

Figure 5. $\log_{10}$ transformed number of Red Imported Fire Ants consumed and diet weights plotted against $\log_{10}$ transformed total lengths and weights from dead trout collected from the Lower Mountain Fork River, OK (black circles). Included are Peterson correlation coefficients (r) and significance of each correlation (p).

Fire Ants constituted most of the diet weight in each stomach, it is likely larger fish consumed a greater mass of Red Imported Fire Ants prior to expiring. Given the phenotypic variation in Fire Ant colonies this is likely the reason that the number of Red Imported Fire Ants did not exhibit a relationship with either weight or TL of expired fish.

Discussion

This is the first study in Oklahoma to demonstrate a connection between the consumption of Red Imported Fire Ants by trout in the LMFR and the observed deaths of these fish. Red Imported Fire Ants were found in the highest abundance of all prey items in the diets of both Brown and Rainbow Trout found dead within the LMFR. Although the sample size is limited, there is little variation between Brown and Rainbow Trout in terms of the number and weight of ants consumed. Despite this, the number of Red Imported Fire Ants consumed did not exhibit a significant correlation with trout weight or total length. Instead, the weight of diet items, including Red Imported Fire Ants, was significantly correlated with trout size. This suggests that while the proportion of Red Imported Fire Ants in the diet was high across all sampled fish, larger trout may be consuming a greater total mass of these ants. The finding of similar diet composition between Brown and Rainbow Trout indicates that both species are equally affected by the presence of Red Imported Fire Ants, but it also highlights the need for further investigation into how different factors, such as prey availability and trout size, influence the effects of consuming Red Imported Fire Ants.

Despite the high percentage of Red Imported Fire Ants found in the trout stomachs, the study did not observe a direct statistical correlation between the number of Red Imported Fire Ants consumed and fish size, suggesting that other factors might also play a role in the mortality of the trout. However, the significant correlation between diet weight and fish size implies that larger fish are more likely to have ingested a greater mass of Red Imported Fire Ants, which could exacerbate the impact of consuming these invasive ants. This underscores the potential for a threshold effect, where exceeding a certain amount of ingested Red Imported Fire Ants might result in acute health issues leading to the trout's death. For example, based on the weight of the diet sample divided by the number of ants counted, each ant weighed ~.024 g. Extrapolating out the percentage of total Fire Ant weight to trout weight results in an estimated value of 2.3% (Table 1). This suggests that an individual trout consuming ≥ 2.3% of it is weight in Red Imported Fire Ants may die. However, further experimentation would be needed to confirm this value. This is especially true as trout examined in this study consumed 66.2 to 304.7 g of Red Imported Fire Ants and that value on average is 1.5 times higher than Red Imported Fire Ants enumerated in diets. Further investigation into the digestive tract of the fish showed that Red Imported Fire Ants were prevalent throughout the entire digestive tract of all dead fish (Figure 4B). This suggests that investigation of the total digestive tract may be more representative of the total number of Red Imported Fire Ants consumed by the fish resulting in a better estimate of the number of Red Imported Fire Ants that would potentially cause trout mortality.

This is the first documented event of Red Imported Fire Ants causing death to fish in Oklahoma waters; however, it is not the first documented fish death due to Red Imported Fire Ants in the United States. Green and Hutchins (1960) fed Red Imported Fire Ants to a variety of sunfish in ponds after reports of Red Imported Fire Ants causing fish kills and did not record any mortality; however, when sunfish were force-fed 1-2 millimeters of macerated Red Imported Fire Ants in a lab setting, fish died ≤1 hr. Furthermore, Prather (1960) fed gelatin pills to sunfish containing 100 Red Imported Fire Ants twice daily for 4 weeks with no fish mortalities being observed. Crance (1965) examined 153 sick or dead bluegills, with 151 of those individuals containing Red Imported Fire Ants in diet samples. In 1963 the Alabama Department of Conservation received reports of several fish kill events. During the ensuing investigation, 183 sick or dead fish were taken from

26 ponds and examined, with 96.7% of the fish stomach content containing whole or pieces of winged Red Imported Fire Ants (Crance 1965).

Anglers and ODWC staff have observed interesting behaviors in trout after ingesting Red Imported Fire Ants, such as loss of equilibrium and swimming ability. During these observations, some fish have been seen recovering from this state after some time, and others that are dying or dead are collected. This behavior is likely due to the consumption of a small amount of Red Imported Fire Ants, although this antidotal observation it is supported by examining the stomachs of expired trout in the same area where trout were observed with loss of equilibrium and swimming ability. Although this is extrapolated from our data with a low sample size, it does provide a plausible reference on where to start to understand what the threshold is for what amount of Red Imported Fire Ants a trout can consume until death may occur. For example, Crance (1965) documented fish becoming sick or distressed after being exposed to swarms of Red Imported Fire Ants. In one observation, hundreds of fish in ponds were affected, but most recovered within 12 to 18 hours (Crance 1965). Further experiments documented seven sick or distressed bluegills from ponds that were brought to the lab; all seven recovered within 12 hours and remnants of Red Imported Fire Ants were found at the bottom of the holding tanks (Crance 1965).

Data from this study indicate that mortality due to the consumption of Red Imported Fire Ants is likely occurring on the LMFR. However, the mechanistic cause remains unknown. The physical damage caused by consuming many Red Imported Fire Ants might interfere with the trout's digestive system, leading to complications or even death. Likewise, the potential toxicity of consuming many Red Imported Fire Ants may cause complications or mortality. The mechanistic cause of these symptoms should be an area of future study. Regardless, Red Imported Fire Ant consumption could become a critical concern for the management of trout fisheries in the LMFR. For example, given Red Imported Fire Ants appear to be consumed when flooding occurs on the LMFR and flooding is becoming more common, mortalities due to Red Imported Fire Ant consumption may increase in frequency. Investigation into the mechanism behind mortality due to Red Imported Fire Ants consumption may become important to fisheries managers in other areas where Red Imported Fire Ants are common or increasing as they expand their geographic range. Understanding the precise mechanisms behind this mortality is crucial for mitigating the impacts of invasive species on aquatic ecosystems and developing strategies to protect both the trout populations in the LMFR and the overall health of the environment.

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Assessing the Population Dynamics and Sampling Efficacy of Skipjack Herring (*Alosa Chrysochloris*) in Oklahoma's Arkansas River: Growth, Mortality, and Recruitment

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Abstract: Skipjack Herring (*Alosa chrysochloris*) can be found throughout the Atlantic coastal waters of North America along with several inland river systems. In Oklahoma, Skipjack Herring are found in the Arkansas River and the Red River drainages. Little is known about the population dynamics of Skipjack Herring across their range and no information is available for populations occurring in Oklahoma. Therefore, our objectives were to: (1) qualitatively evaluate hook-and-line sampling to capture Skipjack Herring, and (2) assess population dynamics (i.e., age and growth, length-weight relationship, size structure, recruitment) of Skipjack Herring in the Arkansas River, Oklahoma. Targeted angling using Sabiki rigs was conducted in July and August 2023 to capture Skipjack Herring in active feeding areas, resulting in the collection of 157 individuals. Age estimates ranged from 0.5 to 3.5 years, and lengths ranged from 107 to 485 mm TL. The von Bertalanffy growth model estimated an asymptotic length of 430.5 mm TL, a Brody growth coefficient (*k*) of 1.1, and a theoretical length at age zero (t_0) of 0.0. The annual survival rate was 47.8%, and the instantaneous total mortality rate was 0.7. Recruitment stability, indicated by a Recruitment Variability Index of 0.8, suggests a relatively stable recruitment pattern. This study confirms that hook-and-line sampling, with Sabiki rigs, is an effective method for capturing Skipjack Herring, and may outperform previously reported traditional gears such as gill nets and electrofishing. Our study provides the first detailed demographic data for

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Skipjack Herring in Oklahoma, including growth rates, mortality, and recruitment patterns. These findings are crucial for managing Skipjack Herring populations and highlight the need for further research into sampling methods and population trends of this species, particularly in the context of climate change and its potential effects on the species.

Introduction

Skipjack Herring (*Alosa chrysochloris*; Figure 1) are members of the Clupeidae family, which comprises herrings, shads, menhaden, sardines, and other economically important species (Page and Burr 2011, Bloom and Lovejoy 2013). The Clupeidae family contains ~210 species, of which seven are found within or along the contiguous United States, and four species reside in Oklahoma (Waldman 2003, Miller and Robison 2004, Page and Burr 2011). North American Clupeids are broadly distributed and found in marine and freshwater environments across the majority of the United States (Page and Burr 2011). Skipjack Herring inhabit the Atlantic coastal waters of North America, ranging from the Gulf of St. Lawrence to the Gulf of Mexico; including river systems along the Atlantic coast, such as the St. Lawrence River, Hudson River, and Mississippi River (Pigg et al. 1991, Pflieger 1997, Miller and Robison 2004). In Oklahoma, Skipjack Herring are known to inhabit the Arkansas River and the Red River drainages (Pigg et al. 1991, Miller and Robison 2004).

Figure 1. Picture of a Skipjack Herring captured from the Arkansas River in Oklahoma (photo courtesy of Brandon Brown).

There is currently a lack of information about the population dynamics of Skipjack Herring across their range (Waldman and Limburg 2003), however; there is some research available regarding their growth, fecundity, and diet (Mettee and O'Neil 2003). There has been no research conducted on Skipjack Herring population dynamics and vital rates in Oklahoma. However, Pigg et al. (1991) suggested their abundance appeared to be increasing within Oklahoma based on their increased relative abundance in standardized samples. This coincides with the observation that they are generally abundant in Tennessee and the Missouri River (Pflieger 1975, Etnier and Starnes 1993), though their populations appear to be decreasing in far inland portions of the Mississippi River (Mette and O'Neil 2003). In May of 2023, a Skipjack Herring weighing 1573.4 g with a length of 517.5 mm was captured by an angler using hook and line and declared a new Oklahoma state record fish (ODWC 2023). This state record fish highlighted the need for additional information on their population dynamics (i.e. age and growth, mortality, and recruitment) and life history in Oklahoma.

The lack of research and information on Skipjack Herring may be attributed to the difficulty of capturing individuals resulting in low catch rates using fisheries sampling gears (Miller and Robison 2004) . For example, Skipjack Herring were not captured in pre-impoundment surveys conducted by the Oklahoma Department of Wildlife Conservation (ODWC) of the Arkansas and Cimarron rivers (Linton 1961) potentially suggesting a sampling gear bias. Standardized fish surveys conducted post-impoundment by ODWC and the Oklahoma Department of Health captured 0 to 45 Skipjack Herring annually from 1979 to 1989 (Pigg et al. 1991). Over the past 40 years, ODWC has captured Skipjack Herring in 20 of their standardized sampling years using electrofishing, experimental gill nets, and seining (~18 individuals per year) indicating an irregular capture history and low per-sample abundance of this species (OFAA 2024). Due to the low sample sizes of individuals captured in standardized sampling events, researchers have been unable to quantify Skipjack Herring population dynamics (age and growth, mortality, and recruitment) and vital rates. However, local Oklahoma anglers have reported abundant Skipjack Herring catch rates using hook and line angling methods in late summer along the Arkansas Basin, indicating that increased sample sizes of these fish can be obtained with this method.

Characterizing fish population dynamics (e.g., age and growth, length-weight relationships, recruitment) is critical to understanding and properly managing fish populations (Campana et al. 1995, Neumann et al. 2012). All fishery gear types have sampling biases, so when considering sampling options it is important to identify methods that most accurately and precisely sample the fish population of interest (see Pope et al. 2010, Hayes et al. 2012, Hubert et al. 2012). Given the poor performance of traditional fishery gears in sampling Skipjack Herring and subsequent lack of population information for Oklahoma populations (Pigg et al. 1991), the objectives of this study were to: (1) qualitatively evaluate the effectiveness of hook-and-line sampling to capture Skipjack Herring, and (2) assess age and growth, length-weight relationship, size structure, and recruitment of Skipjack Herring in the Arkansas River, Oklahoma. The results of this study will provide necessary baseline population metrics that will be beneficial to fisheries managers to understand their population dynamics in Oklahoma.

Methods

Skipjack Herring were captured between July and August 2023 above Robert S. Kerr Reservoir at the confluence of the Lower Illinois and Arkansas Rivers, United States (35°29'38.9" N, 95°06'14.1" W). Local anglers reported high hook-and-line catch rates of Skipjack Herring in this location, which guided our sampling efforts. Initial hook-and-line sampling trips were conducted from a boat using small (~10 - 25 mm) soft-plastic artificial lures, but this failed to capture any Skipjack Herring. Therefore, Sabiki rigs (Ocean Cat, Quality Tackle for All Anglers, and handmade rigs) consisting of either six 1/32-oz white feather jigs (with size 4 or 6 hooks) tied in a series, with a ½ oz weight affixed to the end of the mainline were used to target groups of actively feeding (i.e. feeding and leaping near the surface) Skipjack Herring (Pflieger 1997). Sabiki rigs were most effective when casted into areas of actively feeding fish and allowed to sink to the reservoir bottom, then retrieved with a slow repetitive sweeping motion. Sampling trips were conducted for four hours on four dates with 2 to 3 anglers each trip.

Once captured, Skipjack Herring were placed into a 1:1 ice water slurry to be euthanized (Blessing et al. 2010) and transported to the ODWC office in Porter, OK. Each fish was weighed to the nearest gram and their total length (TL) was measured to the nearest millimeter. Sagittal otoliths were extracted and dried (> 24 hours) for age estimation. Whole otoliths were placed in a viewing dish concave side up, covered with water, and viewed with a dissecting microscope (4–45x) using an external light source to illuminate annuli. When illuminated, annular marks on otoliths appeared as opaque bands on a lighted background (Snow et al. 2018). Annular marks were counted to assign an age estimate to each fish. A double-blind counting approach was used to confirm age estimates from each otolith (Dunlop et al. 2023). A half-year was added to each age estimate to account for growth since annuli formation occurs in the spring, and these fish were sampled in the later summer. (*sensu* Sammons and Macenia 2009; Hanson and Stafford 2017).

A common von Bertalanffy growth model was fit to the adjusted estimated ages and total lengths using the equation:

$$L_t = L_\infty\left(1 - e^{\left(-k(t-t_0)\right)}\right)$$

where L_t represents estimated length at time t, $L\infty$ represents the asymptotic theoretical length, k is the Brody growth coefficient, and t_0 represents the theoretical time at which length is zero (Quist et al. 2012). The von Bertalanffy growth curve was fit via nonlinear least squares estimation using the FSA package (Ogle et al. 2023) in program R. We obtained 95% confidence intervals (95% CI) and 95% predictive intervals (95% PI) from our von Bertalanffy growth model using the propagate package (Spiess 2022) in program R (R Core Team 2022).

We estimated annual survival (S) for Skipjack Herring using the Chapman-Robson method (Chapman and Robson 1960, Robson and Chapman 1961):

$$S = \frac{T}{n + T - 1}$$

Where T represents the sum of the recorded estimated ages of fish on the descending limb of the catch curve (i.e., log(abundance) versus age) and n represents the number of fish observed on the descending limb of the catch curve. Instantaneous total mortality rate (Z) was then estimated by taking the negative natural logarithm of S (*sensu* Miranda and Bettoli 2007). Estimates of S, Z, and 95% CIs for S and Z were obtained using the FSA package (Ogle 2023) in program R (R Core Team 2022).

Variation in recruitment for Skipjack Herring was estimated using the recruitment variability index (RVI):

$$RVI = \left(\frac{S_n}{(N_m + N_p)}\right) - \left(\frac{N_m}{N_p}\right)$$

where S_n represents the sum of cumulative relative age frequencies across all age classes, N_m represents the number of missing year-classes in the sample, and N_p represents the number of present year-classes within the sample. The values of the RVI range from -1 to 1, with values close to 1 representing more stable recruitment (Maceina and Pereira 2007). The assumptions of RVI are that more year classes are present than absent (i.e., $N_p > N_m$), fish are fully recruited to the sampling gear, catch-at-age represents year-class strength, and there are no year-classes beyond the last age-group represented (Guy and Willis 1995). RVI is not recommended when there are less than three year-classes in a sample (Guy 1993).

The length-weight relationship of Skipjack Herring was estimated using a least-squares linear regression of the Log10 transformed TL (mm) and weight (g; Neumann et al. 2012). We then estimated a 95% CI and 95% PI for the weight-length regression in program R (R Core Team 2022). After this, we back transformed the length-weight regression for visual interpretation.

Results

We found that angling for Skipjack Herring was an effective sampling method averaging ~9.8 fish per hour over 16 boat-angling hours across four days (i.e., 157 individuals). Counter-intuitively, based on length at age data, size 6 Sabiki rigs anecdotally appeared to be more useful for capturing smaller individuals (107-220 mm TL), and size 4 Sabiki rigs were more useful for capturing larger individuals (>220 mm TL). Both sizes of Sabiki rigs caught multiple individuals per cast when targeting areas where Skipjack Herring were surface feeding.

Of the 157 fish captured, we successfully estimated the age of 148 and used these fish in generating our population assessments. We found that the Skipjack Herring sagittal otoliths were very small and fragile resulting in seven individuals having their otoliths damaged during the removal or aging processes (Figure 2). Skipjack Herring used for aging ranged from 0.5 to 3.5 years old (Figure 3) and TL ranged from 107-485 mm (Figure 4). Length-weight regression parameters (on Log10 scale) were log(a) = -4.9 (95% CI = -5.0 – -4.8) and b = 2.9 (95% CI = 2.9 – 3.0, Figure 6). The fit of this model was excellent (r^2 = 0.99).

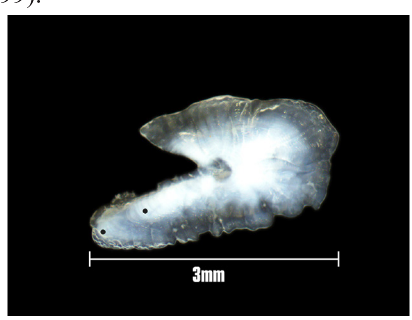

Figure 2. Image of a sagittal otolith obtained from a Skipjack Herring measuring 306 mm TL and age estimated to be 2.5 years captured from the Arkansas River, Oklahoma. Black dots indicate annuli counted.

Our von Bertalanffy growth model suggested asymptotic theoretical length was 430.5 mm (95% CI = 413.3 – 456.8 mm), the Brody growth coefficient (k) was 1.1 (95% CI = 0.8 – 1.3), and t_0 was 0.0 (95% CI = -0.1 – 0.1; Figure 5). The Champman-Robson estimate of annual survival was 47.8% (95% CI = 42.0 – 53.7%) resulting in an instantaneous total mortality of 0.7 (95% CI = 0.4 – 1.1). Our estimated RVI for Skipjack Herring was 0.8, suggesting recruitment in the population is relatively stable.

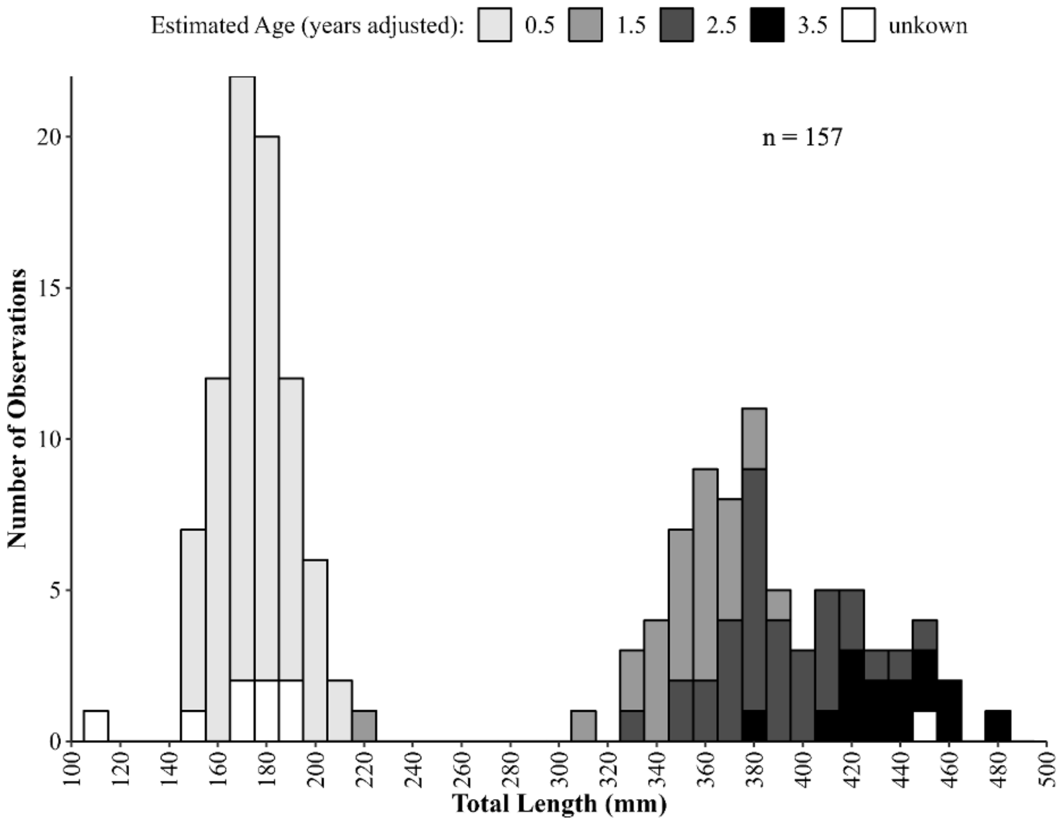

Figure 3. Length frequency histogram (10-mm bins) of Skipjack Herring collected using Sabiki rigs from the Arkansas River basin, Oklahoma in late summer and early fall of 2023. Bar height represents the number of observations for each length group and shading represents the proportion of adjusted age estimates within each length group. The unknown fish are individuals that we were unable to determine an age.

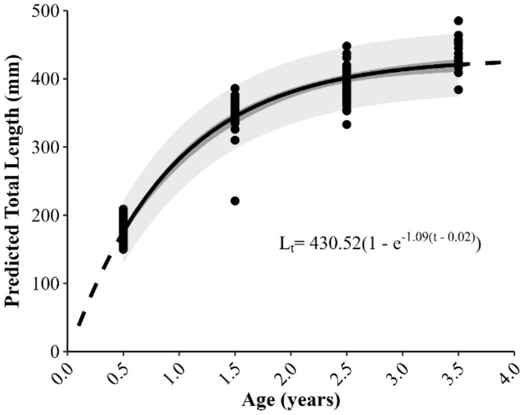

Figure 4. Mean predicted total length at age (solid and dashed black lines) for Skipjack Herring based on the von Bertalanffy growth model. The solid and dashed lines represent mean predicted total length for observed and unobserved ages, respectively. Included are 95% CI (dark grey) and 95% PI (light grey) estimates for observed ages, along with observed total length and age estimates for Skipjack Herring (black circles). Parameter estimates for the von Bertalanffy growth model are included.

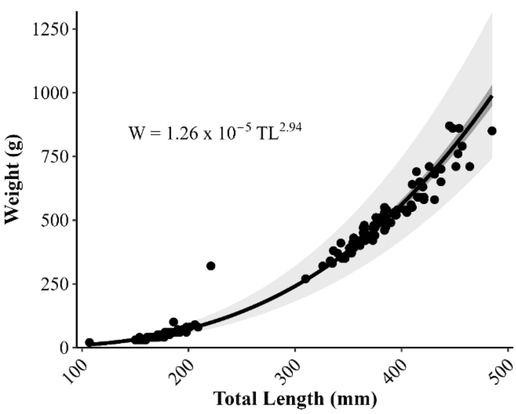

Figure 5. Mean weight-length relationship (black line) for Skipjack Herring along with the corresponding parameter estimates. Included are 95% CI (dark grey) and 95% PI (light grey) estimates for the relationship, along with observed total lengths and weights (black circles).

Discussion

This study increased our knowledge of Skipjack Herring population characteristics in Oklahoma and expanded on the limited amount life history information currently available for the species across their range (Mettee and O'Neil 2023, Waldman 2023, Waldman and Limburg 2023). To the best of our knowledge, this is the first study to estimate population dynamics and vital rates of Skipjack Herring in Oklahoma. Furthermore, this is the first study to use sagittal otoliths to estimate growth, mortality, and recruitment of Skipjack Herring whereas previous studies that have estimated ages for Skipjack Herring have used scales (Wolfe 1969). Although otoliths of Skipjack Herring have not been validated directly, other members of the family Clupeidae (e.g., American Shad [Alosa sapidissima], Duffy et al. 2012) have been validated for accuracy and used to characterize vital rates. Finally, this paper represents the first assessment of this method for sampling Skipjack Herring in Oklahoma. Hook-and-line sampling has been used to collect Skipjack Herring in Florida (Wolfe 1969) and has also been used to capture other members of the family Clupeidae (e.g., American Shad, Ely et al. 2008).

Hook-and-line sampling was effective at capturing Skipjack Herring in the Arkansas River, Oklahoma. Sabiki rigs (equipped with #4 and #6 hooks) fished near actively feeding Skipjack Herring was more effective than using soft plastics. A total of 157 Skipjack Herring were captured over 16 total angling hours using Sabiki rigs while targeting areas where they were actively feeding. In general, this method was more efficient (higher catch rates) than previous ODWC gill netting and electrofishing efforts on Robert S. Kerr reservoir (a slack-water portion of the Arkansas River formed by a lock and dam). For example, gillnet samples from 2019 yielded 18 Skipjack Herring with 15 net nights of effort, and samples from 2022 yielded 4 Skipjack Herring with 20 net nights of effort (OFAA 2024). Furthermore, the ODWC boat electrofishing standardized sampling survey on Robert S. Kerr Reservoir did not catch any Skipjack Herring. In addition, seining efforts having failed to produce any Skipjack Herring (OFAA 2024). Past collection efforts on the Arkansas River also failed to capture as many Skipjack Herring as were captured via hook-and-line angling (Pigg et al. 1991). These findings are consistent with results from Wolfe (1969), who captured 279 Skipjack Herring in 6 days of effort using hook-and-line sampling on the Apalachicola River, Florida.

There has been an increase in hook-and-line sampling in the scientific literature because this gear can be an effective way to collect specimens when traditional fisheries sampling gears are unable to capture them (i.e., size and species selectivity, Gabelhouse and Willis 1986, Pope et al.2005, Bonvechio and Rydell 2016, Hall et al. 2019). Fisheries managers are relying more on angler catch data because these data can be collected passively and cost effectively compared to biased electrofishing sampling surveys (e.g., Gabelhouse and Willis, 1986; Bonvechio and Rydell 2016). For instance, Hall et al. (2019) found that angling collected 8 times more trophy bass than the creel methods and 24 times more than electrofishing methods. Bonvechio and Rydell (2016) used angling to encounter larger fish due to relative scarcity of this size class in electrofishing samples. Furthermore, hook-and-line sampling is commonly used and widely accepted for obtaining information on the size, structure, and abundance of Rainbow Trout *Oncorhynchus mykiss* in southwest Alaska (Heterick and Bromghin 2006) and Largemouth Bass *Micropterus nigricans* in central Illinois (Santucci and Whal 1991).

Hook-and-line sampling efficiently captured Skipjack Herring, although there is potential for bias in our samples. The use of different sized Sabiki rigs allowed for the capture of different sized Skipjack Herring, which is not surprising as hook size is known to influence catchability of fishes via angling (e.g., Cooke et al. 2005). Anecdotally, observations from our targeted angling suggest Skipjack Herring feeding groups consisted of similarly sized individuals. Therefore, to obtain a broad range of sizes some active feeding events were ignored, or abandoned, to seek feeding events with larger individuals. To the best of our knowledge, no investigations into size bias between feeding schools of Skipjack Herring exists. Though anecdotal information on separation of age groups exists for the species (see Wolfe 1969), further study is needed to determine its potential effects on catchability and the implications for standardized sampling designs.

All fisheries sampling gears are have biases associated with them. Therefore it is important to identify the biases present within each gear to either account or correct for its influence (Shoup and Ryswyk 2016, Montague and Shoup 2022). While choosing a sampling gear with high sampling efficiency may be beneficial for collecting demographic information (Zentner et al. 2023), it does not guarantee relative abundance estimates reflect true population size, size structure is accurately represented, or that fisheries metrics are precise enough to monitor populations (Montague and Shoup, 2022). Given the age structure present in our samples reflects all but the highest recorded age for Skipjack Herring (i.e., Age 4, Wolfe 1969), it is unlikely strong bias was present in our size structure data. Furthermore, prior comparisons of size structure indices and relative abundance obtained via angling and electrofishing for Largemouth Bass found angling and electrofishing to provide statistically similar estimates of both metrics (Santucci and Whal 1991, Isaak et al. 1992). Given the difference between species, a multi-gear (e.g., angling, electrofishing, seining) approach would likely be needed to confirm that these findings translate to Skipjack Herring. Regardless, even if a size bias exists for capturing Skipjack Herring with hook-and-line angling, so long as sampling precision is high this method may be accurate enough to track changes in relative abundance of the species (Pope et al. 2010) as size bias is present across a multitude of fisheries sampling gears (Hubert and Fabrizio 2007). Future work should focus on understanding bias and precision for hook-and-line sampling for Skipjack Herring to quantitatively assess its potential as a sampling gear and to provide information needed to develop standardized sampling protocols. However, we currently recommend the use of hook and line angling with methods described in this study to sampled Oklahoma populations of Skipjack Herring.

Skipjack Herring showed rapid growth in their first year, achieving a mean total length of 176 mm at the end of their first summer and three-quarters of their predicted TL by the time they reached age 1. Wolfe (1969) found Skipjack Herring TLs on the Apalachicola River were approximately 197-205 mm at age 1, 240-378 mm at age 2, 293-445 mm at age 3, and 310-482 at age 4. Wolfe (1969) estimated age using scales whereas we used sagittal otoliths to derive age estimates. Currently the accuracy (i.e., bias and reader precision) of otoliths has not been validated for Skipjack Herring. Nevertheless, sagittal otoliths have been found to have high precision and accuracy between readers for other members of the family Clupeidae (Maceina 1988, Hendricks et al. 1991, Lessa et al. 2008, Duffy et al. 2012). This combined with the fact that similar estimates were obtained from scales by Wolfe (1969) suggest our results are robust. Scales are commonly used because they are easy to sample and inexpensive to analyze compared to other structures (e.g., otoliths, spines, vertebrae), the removal of scales is generally non-lethal. Furthermore, the process of preparing sagittal otoliths is time-consuming and sometimes requires advanced equipment (Davies et al. 2017, Davies et al. 2015, Koch and Quist, 2011). Regardless, future studies should compare the scale and otolith derived age estimates for Skipjack Herring especially considering scales often over-age young fish and under-age older fish (Lowerre-Barbieri et al. 1994, McBride et al. 2005, Maceina and Sammons 2006). The accuracy of age estimates from scales can be negatively influenced by periods of nutritional scarcity during which fish will resorb nutrients present in scales (Kerr and Campana 2014).

Our study provided information into length-weight, mortality, and recruitment information for Skipjack Herring in Oklahoma. To the best of our knowledge, no prior study has produced a length-weight relationship for any Skipjack Herring population. However, Wolfe (1969) observed fishes from 197 – 482 mm TL exhibited weights ranging from 58 – 1,078 g. Recruitment and mortality of Skipjack Herring has not been documented prior in the peer reviewed literature. Our results suggest that less than half of the population survives each year. However, Skipjack Herring within the Arkansas River, Oklahoma exhibit relatively stable recruitment as indicated by the derived RVI index. This information combined with the growth rates observed for the species suggest Skipjack Herring may be a fast-growing shorter-lived species (see Winemiller and Rose 1992, Winemiller 2005), though more information is needed to confirm this hypothesis.

This study provides baseline population dynamics information for Skipjack Herring from the Arkansas River basin, the first use of otoliths to derive population dynamics for Skipjack Herring, and a novel method of capturing this species. Due to a lack of available literature, we cannot say whether this represents a typical population within their range, although our results are similar to the Skipjack Herring population described by Wolfe (1969). Future research should focus on additional validation of the findings from this study and the applicability of these estimates for other populations of Skipjack Herring in Oklahoma. This is especially important as information on Skipjack Herring is lacking statewide and is critical to understanding and properly managing this species. Future research efforts should focus on quantifying the sampling biases associated with hook and line sampling for Skipjack Herring and aim to identify a standardized sampling protocol for these fish where they are found. Developing a standardized sampling survey will also provide insights into Skipjack Herring range and whether this species can inhabit various systems throughout Oklahoma (Pigg et al. 1991, Galat et al. 2005, Neebling and Quist 2008, Dunn et al. 2018). For example, Skipjack Herring are a euryhaline species thought to be anadromous in the Gulf of Mexico that is also capable of completing its life cycle solely in freshwater (Etnier and Starnes 1993), therefore the increase in salinity in the Mississippi River basin caused by climate change may create a competitive advantage for Skipjack Herring over other native species, thereby increasing their range. Given this species is native to the Gulf of Mexico, it may also benefit from increasing water temperatures caused by climate change.

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Effects of Turbidity on Growth of Young-of-Year and Juvenile Spotted Gar (*Lepisosteus oculatus*)

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Abstract: This study examined the impact of turbidity on the growth of young-of-year and juvenile Spotted Gar (*Lepisosteus oculatus*), a key predator in North American freshwater systems facing conservation threats due to habitat degradation. Conducted in controlled outdoor aquaria, the experiment exposed Spotted Gar to high (Secchi depth ≤ 20 cm) and low (Secchi depth > 54 cm) turbidity conditions to assess their growth in total length (TL) and weight. Initial TL and weight measurements did not differ significantly between turbidity treatments within each age group. Our results suggest turbidity affected growth in TL, but there was no effect on growth in weight. Increased turbidity appeared to result in slower growth in TL for young-of-year spotted gar, but higher growth in TL for juvenile spotted gar. Growth in weight was significantly greater in juvenile Spotted Gar compared to young-of-year Spotted Gar. Turbidity may not have influenced growth in weight due to an inability to account for individual variation during statistical analysis or due to Spotted Gar investing more energy in growth in TL at the life stages studied. These findings suggest that increased turbidity may impair young-of-year growth, but potentially benefits older juveniles. Future work should confirm these findings in a field setting. The study highlights the importance of maintaining clear, vegetated habitats for the early life stages of Spotted Gar and suggests that conservation efforts should address habitat quality and turbidity to support Spotted Gar populations, particularly in northern regions where habitat degradation is increasing.

Introduction

Gars (family: Lepisosteidae) are a primitive group of fishes distributed widely in central and eastern North America and throughout Central America (Echelle and Grande, 2014). Gars are large-bodied, top-level piscivores, and are important components of aquatic food webs (David et al. 2015). Gar biology is poorly understood in comparison to other fish species, largely because they are considered nuisance fish across many areas where they are found (Scarnecchia 1992). Populations of several species within Lep-

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isosteidae have declined because of habitat loss and removal efforts which has resulted in conservation challenges (Scarnecchia 1992, Staton et al. 2012, NatureServe 2024). Over the last 15 years, however, there has been a concerted effort to gain a better understanding of the fundamental biology of these species (Smith et al. 2020).

The Spotted Gar (*Lepisosteus oculatus*) is one of four species of gar native to Oklahoma (Miller and Robison 2004, Frenette and Snow 2016). While populations are considered stable in Oklahoma, the Spotted Gar is a species of conservation concern at the northern edge of its range and is critically imperiled in Canada, Kansas, Ohio, and Pennsylvania (Glass et al. 2011, Staton et al. 2012, David et al. 2015, Ontario Ministry of Natural Resources and Forestry 2016, NatureServe 2024). Furthermore, Spotted Gar are presumed to be extirpated in New Mexico (NatureServe 2024). The recovery strategy for Spotted Gar in Canada emphasizes the importance of early life history on population growth rates (Staton et al. 2012, Ontario Ministry of Natural Resources and Forestry 2016). This relatively large ambush predator prefers clear, heavily vegetated near-shore waters, habitats that are especially susceptible to human activities that can negatively impact water quality, such as near-shore development, erosion, and nutrient loading (COSEWIC 2015).

Turbidity is a measure of water cloudiness caused by suspended particles (Wetzel 2001). Turbidity influences both the aquatic ecosystem and the biota within it (Henley 2000, Owens et al. 2005, Prestigiacomo et al. 2007, Rodriguez-Blanco et al. 2013, Merten et al. 2014). Turbidity reduces light penetration into the water, affecting the growth of aquatic plants and algae which can result in bottom-up influences on the food web (Treweek and Morgan 1980, Zingel and Paaver 2010, Jia Du et al. 2023). Reduced visibility due to increased turbidity has been shown to influence foraging behavior and how efficiently fish capture food (Shoup and Wahl 2009, Carter et al. 2010, Higham et al. 2015, Snow et al. 2018), potentially driving changes in growth and condition (Zingel and Paaver 2010, Lowe et al. 2015). Though turbidity effects have been studied on traditional game fishes such as Largemouth Bass (*Micropterus salmoides)*, Shoup and Wahl 2009, Shoup and Lane 2015), little information is available for native nongame species such as Spotted Gar. Turbidity's effects on the hatching success of Spotted Gar has been implicated to explain their population decline at the northern extent of their range (Gray et al. 2012).

Turbidity has been shown to negatively affect hatching success by approximately 24% for Spotted Gar eggs held in turbid conditions compared clear water conditions (Gray et al. 2012). Turbidity does not appear to effect growth of young-of-year Spotted Gar (Frenette 2014). However, mortality of young-of-year Gar was high (Frenette 2014), particularly in smaller individuals (measured as initial TL), which resulted in limited replication. These results suggested the study should be repeated to better understand turbidity's influence on early life survival of Spotted Gar. Therefore, the goal of this study is to test the effects of high (Secchi depth ≤ 20 cm) and low (Secchi depth ≥ 53 cm) turbidity on growth of young-of-year (Age 0) and juvenile (Ages 1 to 3) Spotted Gar. The specific objectives of our study were to determine if high and low turbidity changed growth based on (1) length or (2) weight for young-of-year and juvenile Spotted Gar.

Methods

Young-of-year Spotted Gar were captured in the river-reservoir confluence of Texoma Reservoir, Oklahoma, using mini-fyke nets (0.6 m x 6.35 m with 3.18 mm mesh, 0.6 m x 1.92 m rectangular cab, and 510 mm metal throat) with 9.14 m leads. Mini-fyke nets were set perpendicular towards the shoreline where herbaceous vegetation and woody debris were abundant (Brinkman 2008, Snow et al 2016). Juvenile Spotted Gar were collected from Sparks Reservoir, Oklahoma, using boat electrofishing (pulsed DC, high voltage, Smith Root GPP). Electrofishing was used to sample the entire shoreline, and all Spotted Gar were captured; however, only individuals <450 mm total length (TL) were retained. Both reservoirs experience a wide range of turbidity levels that overlap and range from 30 to 130 cm (Sager et al. 2011, ODWC unpublished data), All Spotted Gar collected were placed in a livewell on each boat and transferred to a hauling box where dissolved oxygen (DO) was maintained at or above 7 mg/L and water temperature mirrored that of the body of water where collection occurred. Spotted Gar were then transported to the Oklahoma Fisheries Research Laboratory, where they were acclimated to the temperature of one of four 946-L round fiberglass tanks. All four tanks shared the same water supply which resulted in consistently similar temperatures for all tanks during the duration of the study.

Fathead Minnows (*Pimephales promelas*) that had been raised in outdoor ponds were used as forage for Spotted Gar. All Fathead Minnows were donated by the Matt McBride fish farm (Wetumka, Oklahoma). Fathead Minnow TL were selected based on the TL of sampled Spotted Gar to provide forage of optimal size (optimum point of handling time divided by prey weight; Hoyle and Keast 1987), resulting in forage being 10 to 25% of predator TL. All Fathead Minnows were transported to the Oklahoma Fisheries Research Laboratory, where they were placed into a 946-L round tank with slow water exchange and aeration. Fathead Minnows were allowed to acclimate for at least 10 days prior to use in the experiment.

Growth trials were conducted outdoors in oval 378.5-L aquaria. At the start of the trial, each aquarium was randomly assigned a turbidity level based on Secchi depth of either ≤ 20 or > 54 cm. Turbidity in each tank was produced using bentonite clay. Clay and water were first stirred together in a separate container until thoroughly mixed. The clay mixture was then added to aquaria until the desired turbidity was achieved. Turbidity in each tank was measured using a Secchi tube (Myre and Shaw 2006). To maximize precision, the same observer always measured Secchi depth. All aquaria were equipped with aeration to keep the clay suspended (Shoup and Wahl 2009). The turbidity measurement of ≥ 54 cm were chosen based on the maximum water depth in the aquarium. With the ≤ 20 cm turbidity measurement being chosen to ensure consistent turbidity levels throughout the trial, following the approach used by Snow et al. (2018).

Once the desired turbidity level was achieved in aquaria, 25 Fathead Minnows were added to each tank. After forage was added a single Spotted Gar was netted out of the holding tank and TL (mm) and weight (g) were recorded. Spotted Gar were then randomly assigned to aquaria. During trials turbidity was measured daily along with temperature (^{o}C) and DO (mg/L). If the Secchi depth was not within 10% of the assigned level within a 48-hour period, the turbidity level was adjusted to within 10%. A total of 38 young-of-year and 16 juvenile Spotted Gar were available for trials, allowing for 19 and 8 individuals to be assigned to each turbidity treatment, respectively. Since there were not enough aquaria to run all trials simultaneously, two separate trials were conducted resulting in isolative segregation between age groups (Hurlbert 1987). Trials for young-of-year Spotted Gar were conducted between 6/16/2017 and 7/11/2017, whereas trials for juvenile Spotted Gar were conducted between 7/20/2017 and 8/28/2017. Spotted Gar TL was measured during trials once every half to full fortnight, and additional Fathead Minnows were added so that 25 individuals were available in each aquarium. This occurred at 8, 14, 19, and 25 days after the start of trials for young-of-year Spotted Gar and at 7, 20, 29, and 39 days for juvenile Spotted Gar. After trials, tanks were drained, and TL and weight were recorded for each Spotted Gar.

To determine if initial TLs or weights were different between Spotted Gar that received each turbidity treatment, we used two-sample Kolmogorov-Smirnov tests (KS-tests; Kolmogorov 1933, Smirnov 1939) to compare initial measurements of each individual separately for each age group (i.e., young-of-year, juvenile). Kolmogorov-Smirnov tests were confirmed via distributional overlap ($\hat{\eta}$; Pastore and Calcagnì 2019) estimated using the overlapping package (Pastore 2020). Thresholds for low, moderate, and high distributional overlap were equivalent to estimates of $\hat{\eta}$ = 0.20, 0.50, and 0.80, respectively (see Pastore 2020). To determine if turbidity level influenced Spotted Gar growth based on TL or weight, we estimated growth rates for each fish. Growth in TL was estimated as the difference in TL at each sample period relative to the initial TL of each Spotted Gar. Weight was estimated as the difference in weight at the end of the study relative to the initial weight of each Spotted Gar. We then used a generalized linear mixed model (GLMM) or generalized linear model (GLM) to determine if turbidity significantly influenced the change in TL or weight of Spotted Gar, respectively. All analyses were conducted in program R version 4.4.1 (R Core Team 2024) and significance was always assessed at $\alpha = 0.05$.

A Poisson distributed GLMM with a log link function was used to model growth based on TL via the lmerTest package (Kuznesova et al. 2017). Individual Spotted Gar were used as the random effect to account for each fish receiving multiple TL measurements over the course of the study. Fixed effect predictors were turbidity, time since start of trial (days), and age group (i.e., young-of-year or juvenile). Time since start of trial was centered and scaled prior to analysis (R Core Team 2024). A backwards selection approach was used to determine if any of our predictors or their interactions exhibited a significant relationship with growth based on TL (James et al. 2013). Insignificant predictors ($p > 0.05$) were removed starting with the three-way interaction, then second-order interactions, and then main effects, if necessary (Jeter et al. 2023). Appropriateness of the final GLMM from the backwards selection process was assessed via a nonparametric dispersion test using the DHARMa package (Hartig 2022) along with diagnostic plots displaying a posterior predictive check, variance inflation factors, scale-location relationships, leverage, and normality within our random effects using the performance package (Lüdeke et al. 2021). Coefficients of determination for fixed (R^2_F) and random (R^2_R) effects for the final GLMM were estimated via the rsq package (Zhang 2020, Zhang 2023). Predictions from the final GLMM from the backwards selection process were then used with coefficient estimates to determine how significant predictors or interactions influenced growth based on TL.

A Gamma distributed GLM with a log link function was used to model growth based on weight over the course of the study via the lmerTest package (Kuznesova et al. 2017). Random effects were not included as each Spotted Gar only had only one measurement (i.e., the final measurement) taken to estimate growth in weight. Likewise, the effect of time since start of trial was not included as it was confounded with age group, and age group was of more interest. Therefore, predictors used for this model were turbidity and age group. The same backward selection approach was taken as described prior for GLMMs investigating growth in TL. However, removal of insignificant predictors included the two-way interaction (i.e., turbidity level × age group) and main effects until only significant predictors remained. Appropriateness of the final GLM from the backwards selection approach was determined via a nonparametric dispersion test using the DHARMa package (Hartig 2022) along with diagnostic plots displaying a posterior predictive check, scale-location relationships, and leverage using the performance package (Lüdeke et al. 2021). McFadden's pseudo-r^2 (ρ^2) was used as the coefficient of determination for the top model, with a $\rho^2 = 0.20$ indicating an 'excellent' model fit (McFadden 1974, 1979). Predictions from the final GLM from the backward selection process were then used with coefficient estimates to determine how significant predictors or interactions influenced growth based on weight.

Results

Across all trials tank temperatures ranged from 21°C to 27°C daily and DO ranged between 6.8 – 8.7 mg/L. Forage densities appeared to be appropriate as all fish grew during the trials (Table 1). Young-of-year Spotted Gar TLs increased 1 to 95 mm over the course of the study, and weights increased by 4 to 13 g. Likewise, juvenile Spotted Gar TLs increased 1 to 29 mm over the course of the study and weights increased by 9 to 43 g. No Spotted Gar mortalities were observed during trials.

Initial TLs and weights were similar between turbidity treatments for young-of-year and juvenile Spotted Gar (Table 1). Our KS-tests did not detect a significant difference in starting TLs (D = 0.21, p = 0.74) or weights (D = 0.37, p = 0.07) between turbidity treatments for young-of-year Spotted Gar. Overlap tests suggested there was high overlap between initial TLs ($\hat{\eta}$ = 0.82) and moderately high overlap between initial weights ($\hat{\eta}$ = 0.76) for young-of-year Spotted Gar. Similarly, KS-tests detected no significant differences in starting TLs (D = 0.38, p = 0.66) or weights (D = 0.38, p = 0.57) between turbidity treatments for juvenile Spotted Gar. Overlap tests suggested high overlap between initial TLs ($\hat{\eta}$ = 0.80) and moderately high overlap between initial weights ($\hat{\eta}$ = 0.79) for juvenile Spotted Gar.

Table 1. Mean, 95% quantiles (95%Q), and ranges of total lengths and weights for each age group (i.e., young-of-year [YOY] and juvenile) of Spotted Gar in each turbidity treatment (based on Secchi Depth) at the beginning (Time = 0) and end (YOY Time = 25, Juvenile Time = 39) of each trial.

Age Group	Secchi Depth	Time (days)	Total Length (mm)			Weight (g)		
			Mean	95%Q	Range	Mean	95%Q	Range
YOY	≤ 20 cm	0	131	115 - 147	112 - 147	5	3 - 8	3 - 8
YOY	≤ 20 cm	25	184	172 - 205	164 - 206	14	11 - 19	10 - 19
YOY	> 54 cm	0	131	111 - 158	108 - 163	4	2 - 8	2 - 8
YOY	> 54 cm	25	179	167 - 204	157 - 205	13	8 - 19	7 - 19
Juvenile	≤ 20 cm	0	402	353 - 449	347 - 450	192	127 - 275	123 - 284
Juvenile	≤ 20 cm	39	417	375 - 458	373 - 458	220	159 - 301	154 - 308
Juvenile	> 54 cm	0	383	355 - 422	352 - 424	160	127 - 219	123 - 223
Juvenile	> 54 cm	39	401	377 - 442	374 - 446	187	160 - 240	155 - 245

The final model from the backwards selection process investigating growth in TL for Spotted Gar included a two-way interaction between age group and time since start of trial, a two-way interaction between turbidity and age group, and their associated main effects (Table 2). The dispersion test suggested that the Poisson distribution was appropriate for the data (p = 0.80) and diagnostic plots suggested good fit of fixed and random effects, low multicollinearity between predictors, and no influential points or leverage. Coefficients of determination suggested fixed effects (R^2_F = 0.71) explained more variation than the random effect (R^2_R = 0.19) in our final model, resulting in a relatively good overall fit to the data ($R^2_F + R^2_R$ = 0.90). Coefficient estimates for the two-way interaction between age group and time since start of trial suggested that juvenile Spotted Gar growth in TL increased slower than young-of-year (Table 2). Interestingly, the two-way interaction between turbidity and age group suggested that turbidity had a positive overall effect on growth in TL of juveniles relative to young-of-year Spotted Gar (Table 2). Predicted output from the final GLMM from backward selection showed that young-of-year Spotted Gar growth in TL was faster than that of juvenile Spotted Gar (Figure 1). Likewise higher turbidity (Secchi depth ≤ 20 cm) resulted in slower growth of young-of-year Spotted Gar and faster growth in TL of juvenile Spotted Gar (Figure 1). However, it is important to note that the growth in TL was not statistically different within each age group due to turbidity. Instead, the effect of turbidity on growth in TL varied between the age groups.

Table 2. Mean and standard error (SE) estimates along with resulting z- and p-values for each fixed parameter from the final Poisson distributed GLMM obtained via backward selection predicting growth in TL. Parameters include the intercept, turbidity (i.e., Secchi depth ≤ 20 or > 54 cm), age group (i.e., young of year, juvenile) and time since start of trial (Time).

Predictor	Mean	SE	z-value	p-value
Intercept	2.43	0.06	43.21	$< 2.00\times10^{-16}$
Turbidity	0.03	0.05	0.62	0.54
Age Group	-0.62	0.06	-11.02	$< 2.00\times10^{-16}$
Time	0.74	0.02	29.88	$< 2.00\times10^{-16}$
Turbidity × Age Group	0.11	0.05	2.13	0.03
Age Group × Time	-0.28	0.02	-11.41	$< 2.00\times10^{-16}$

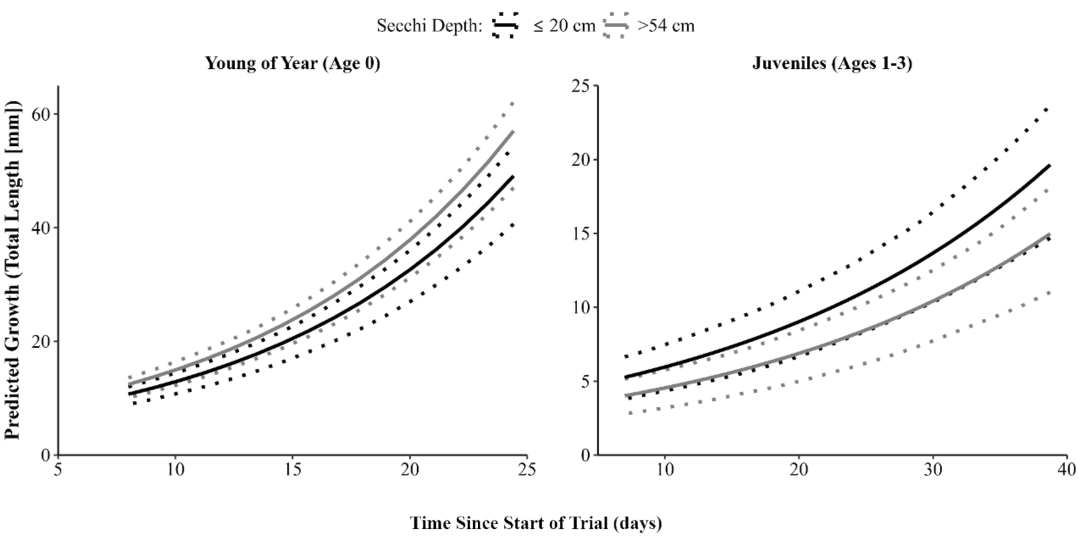

Figure 1. Predicted growth based on total length plotted against time since start of trial for age groups in each turbidity treatment (based on Secchi Depth) from the final Poisson distributed GLMM obtained via backward selection. Solid lines represent means and dotted lines represent 95% confidence intervals. Note that different scales have been used for each age group to better display trends.

The final model from the backwards selection process investigating growth in weight for Spotted Gar only included the main effect age group (Table 3). The dispersion test suggested that the Gamma distribution was appropriate for the data (p = 0.70) and diagnostic plots suggested good fit and no influential points or leverage. The coefficient of determination suggested this model had "excellent" fit to our data (ρ^2 = 0.23). Coefficient estimates for the effect of age group suggested that juvenile Spotted Gar growth in weight was higher relative to young-of-year (Table 3). Predicted output from the final GLM from backwards selection confirmed this trend with juvenile Spotted Gar growth in weight being roughly three times higher than young-of-year (Figure 2). Though there was a 14-day difference in trial length, the large difference in growth in weight between age groups suggests that this difference was primarily due to age group. For example, when predated growth in weight for each age group was divided by the duration of each study there was still a substantially higher daily growth rate for juvenile Spotted Gar (mean = 0.71 g/day [95%CI = 0.59 – 0.81 g/day]) compared to young-of-year Spotted Gar (mean = 0.34 g/day [95%CI = 0.31 – 0.37 g/day]). However, this hypothesis cannot be confirmed due to the confounding effects of trial length and age group.

Table 3. Mean and standard error (SE) estimates along with resulting z- and p-values for each parameter from the final Gamma distributed GLM obtained via backward selection predicting growth in weight. Parameters include the intercept and age group (i.e., young of year, juvenile).

Predictor	Mean	SE	z-value	p-value
Intercept	2.73	0.04	62.71	$< 2.00\times10^{-16}$
Age Group	0.59	0.04	13.61	$< 2.00\times10^{-16}$

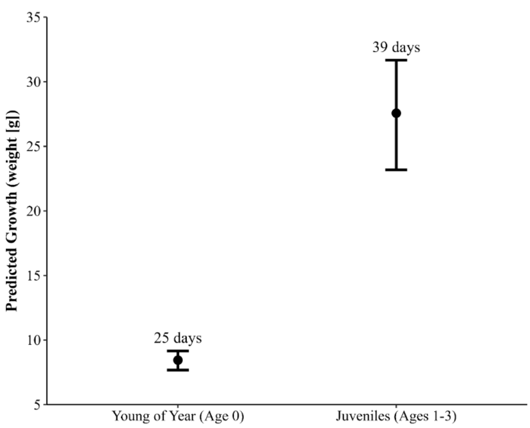

Figure 2. Predicted growth based on weight plotted against age groups from on the final Gamma distributed GLM obtained via backward selection. Black circles represent means and error bars represent confidence intervals. Included above each estimate is the total duration of the trial for each age group.

Discussion

Our results suggest that turbidity levels have varying effects on different age groups of Spotted Gar. Growth in TL was less for young-of-year Spotted Gar and more for juvenile Spotted Gar under increased turbidity. However, growth in TL was not statistically different between turbidity treatments within age groups (i.e., young-of-year, juvenile) even though data showed trends. Though no studies comparing the effects of turbidity on different age groups of Spotted Gar were available, Wellington et al. (2010) determined that young-of-year and juvenile Yellow Perch (Perca flavescens) exhibited differences in foraging success due to different levels and types of turbidity likely influencing growth. Prior work found no statistical difference in growth for young-of-year Spotted Gars under turbid and clear conditions (Frenette 2014). Frenette (2014) noted higher growth under clear conditions and attributed the lack of statistical difference to insufficient statistical power caused by a low sample size due to high mortality during the study. Predicted output from our GLMM also suggested higher but insignificant growth of young-of-year Spotted Gar in our lower turbidity treatment. Our results suggest that turbidity improves growth in TL of juvenile Spotted Gar, though this improvement was not statistically significant. Further study into the effects of turbidity on growth in TL for young-of-year and juvenile Spotted Gar and other gar species (e.g., Longnose Gar [*Lepisosteus osseus*], Alligator Gar [*Atractosteus spatula*]) is warranted given the lack of literature available on this topic.

The differential influence of turbidity on growth in TL for young-of-year and juvenile Spotted Gar may be the result of juvenile Spotted Gar being better adapted to foraging in turbid conditions. Though we were unable to locate other published study results for Spotted Gar, higher turbidities are thought to generally result in reduced foraging success for fishes (Ginetz and Larkin 1976, Gardner 1981, Huenemann et al. 2012, Snow et al. 2018). However, size-specific variation in foraging success has been observed for Walleye (Sander vitreus, Vandenbyllaardt et al. 1991), Yellow Perch (Perca flavescens, Wellington et al. 2010), and Pikeperch (Zingel and Player 2010). Interestingly, during this study, we observed both young-of-year and juvenile Spotted Gar which exhibited discoloration in the high turbidity treatment (Figure 3). Given we did not observe differences in growth rates within the age group, this discoloration may have allowed study subjects to better blend in with the turbid water, potentially resulting in higher capture efficiency; however, this was beyond the scope of our study. Color variation such as this should be studied further to understand the mechanisms behind depigmentation and any potential effects it may have on foraging success. Likewise, turbidity's effects on foraging success in Spotted Gar should be studied to determine if this caused the observed differences in growth in TL between young-of-year and juveniles.

Turbidity appeared to have no effect on growth in weight for young-of-year or juvenile Spotted Gar in our study. We were unable to locate studies comparing growth in weight and turbidity for young-of-year or juvenile Spotted Gar, though findings were available for other species. Weight gain in larval Northern Pike (*Esox Lucius*) and juvenile Silver Seabream (*Pagrus auratus*) was reduced at higher turbidity levels (Salonen and Engström-Öst 2013; Lowe et al. 2015). Zingel and Paaver (2010) noted that turbidity had no relationship with Fulton's condition factor (i.e., 100,000 × weight/TL^3) for Pikeperch (*Sander Lucioperca*) ranging from 31 – 75 mm TL and a negative relationship with Fulton's condition factor for Pikeperch 76 – 90 mm TL, suggesting the effect of turbidity on condition varied with size. Our findings that turbidity has little to some effect on growth in weight for young-of-year and juvenile Spotted Gar contradicts most other available literature for other species (e.g., Pikeperch [*Sander Lucioperca*]: Zingel and Paaver 2010, Rainbow Trout [*Oncorhynchus mykiss*]: Ginetz and Larkin 1976; salmonids: Berg 1982; juvenile Walleyes [*Sander vitreus*]: Vandenbyllaardt et al. 1991; Bluegill [*Lepomis macrochi*], Gardner 1981, Largemouth Bass [*Micropterus salmoides*]: Huenemann et al. 2012; juvenile Yellow Perch [*Perca flavescens*]: Wellington et al. 2010). This may be due to Spotted Gar exhibiting different relationships with turbidity than other species of fish. Though this may be the result of species-specific differences, further study into the effects of turbidity on growth in weight for young-of-year and juvenile Spotted Gar is warranted. This is especially true as, to the best of our knowledge, this study constitutes the only investigation into the effects of turbidity on growth in weight available for the gar family.

Contrasting results for turbidity's effect on growth in TL and weight for young-of-year and juvenile Spotted Gar may be due to differences in the statistical analysis or differences between indexes of growth. Individual variation in growth was accounted for when comparing turbidity's effect on growth in TL. However, we were unable to account for induvial variation when comparing turbidity's effect on growth in weight due to having only one observation per individual. Conversely, turbidity may have had little effect on growth in weight due to more energy being invested in growth in TL at the early life stages studied (Moyle and Cech 2004, Won and Borski 2013). The effects of turbidity on growth in weight should be studied further and compared while accounting for individual variation to determine if the lack of effect observed in this study is an artifact of the statistical process used.

Reduced growth in TL due to increased turbidity in young-of-year Spotted Gar may have significant population-level consequences. The first season of growth is likely when Spotted Gar are most vulnerable (Staton et al. 2012). Early life survival in fishes is often influenced by their ability to grow large enough before facing their first period of resource scarcity, typically occurring during their first winter (Gray et al. 2012, Frenette 2014). This could explain why Spotted Gar populations appear most vulnerable at the northern extent of their range, where winter resource scarcity generally persists for longer durations (Hurst 2007). Differences in winter resource scarcity between northern and southern latitudes may also explain why Spotted Gar in Oklahoma appears to exhibit stable recruitment in reservoirs that are turbid (ODWC unpublished data, Frenette and Snow 2016). This study does suggest that if Spotted Gar can survive through their first year they may acclimate to turbid conditions and such conditions may improve growth, which

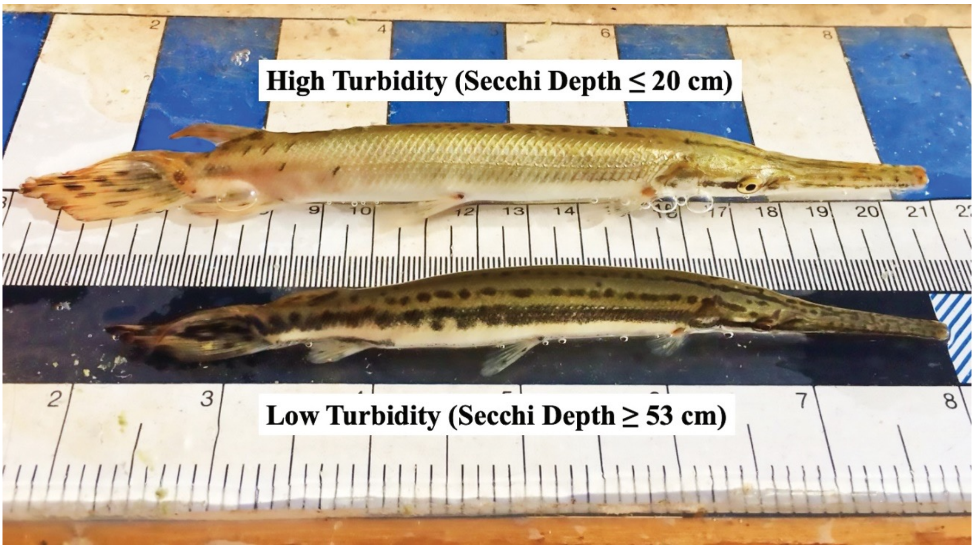

Figure 3. Example of discoloration observed in Spotted Gar due to high turbidity (top) relative to those in low turbidity treatments (bottom).

may be influenced by transitioning from clear, near-shore refugia at early life history stages to more open, turbid environments as they grow. However, these results should be confirmed in a field setting as prior investigations into turbidity's influences on foraging success and growth have varied between laboratory and field studies in other species (Spier and Hidinger 2002, Shoup and Lane 2015). Increased turbidity may also indirectly affect Spotted Gar through the reduction of vegetated habitats upon which these fish are thought to be reliant (Bouvier and Mandrak 2010, Gray et al. 2012). Early in life Spotted Gar are thought to use aquatic vegetation to forage and as refuge from predators (Snedden et al. 1999). However, there is still a need to identify critical habitats as young-of-year Spotted Gar transition into juveniles (Glass et al. 2012). Future studies should focus on a better understanding of direct and indirect effects of turbidity on gar populations at broader spatial scales along with a better understanding on turbidity's effects on foraging and predation, especially at early life stages.

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Occurrence of Goldfish, *Carassius auratus* (Cypriniformes: Cyprinidae) in the Little River System of Southeastern Oklahoma

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Abstract: Goldfish (*Carassius auratus*) are native cyprinids in eastern Asia. In the United States, individuals have been collected from every state as well as three Canadian Provinces. In Oklahoma, there are sporadic records of *C. auratus* known from the state but none reported in the refereed literature from any southeastern watershed. Here, we document the occurrence of goldfish from a creek in the Little River watershed of McCurtain County, southeastern Oklahoma.

Introduction

The goldfish, *Carassius auratus* L., 1758, is native to eastern Asia and parts of Europe, thought to have originated in rivers of China and/or Japan, and was first introduced into the USA in the late 1600's (DeKay 1842; Page and Burr 2011). The species is now established in many states as well as southern parts of Ontario, Alberta, and British Columbia, Canada (Hensley and Courtenay, 1980). Although populations can be distributed sporadically, they can also be locally common. This fish inhabits shallow, muddy pools, and backwaters of sluggish rivers, ponds, and lakes. It prefers warm still turbid waters usually with emergent vegetation and is tolerant of pollution. It is an omnivore as adults feed mostly on phytoplankton, whereas young feed mostly on zooplankton and larval insects (Hensley and Courtenay, 1980).

In Oklahoma, there are sporadic geographic records of *C. auratus* from watersheds in Cherokee-Sequoyah, Cimarron, Craig, Marshall, Payne, and Wagoner counties (Moore and Cross 1950; Miller and Robison 2004, see their p. 88). Here, we document the first published geographic record for *C. auratus* in the Little River system of McCurtain County.

Methods

On 8 June 2024 and again on 20 June 2024, fish were collected by backpack electrofisher from a tributary to Yashau Creek off

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Airport Road at Broken Bow (34°01′08.04″N, −94°45′24.51″W). This locality (Fig. 1) is a drainage ditch containing warm turbid water with muddy substrate and some aquatic vegetation which feeds the main creek about 91 m east. It quickly fills with rainwater during precipitous months of the year but can nearly dry up in summer months. Specimens were transferred to an aquarium with treated (chlorine-free) tap water for 24 hr and killed in a concentrated solution of tricaine methanesulfonate (TMS-222), placed in 70% (v/v) ethanol, and deposited as voucher specimens in the Sam Noble University of Oklahoma Ichthyology Collection, Norman, Oklahoma. Fish scientific names follow Page et al. (2023).

Figure 1. Study site in McCurtain County, Oklahoma. Note the drainage culvert at top of photograph.

Results and Discussion

Three *C. auratus* (total length [TL] = 69, 83, and 86 mm; Fig. 2) were collected on 8 June 2024. On 20 June 2024, three additional *C. auratus* (87, 90, and 95 mm TL) were collected. As far as the fish community at this site, there were several other species from eight families collected by CTM over the last decade, including: Leuciscidae: *Campostoma spadiceum*, *Notemigonus crysoleucus*, *Semotilus atromaculatus*; Catostomidae: *Erimyzon claviformis*; Ictaluridae: *Ameiurus melas*, *Ameiurus natalis*; Esocidae: *Esox americanus vermiculatus*; Aphredoderidae: *Aphredoderus gibbosus*; Fundulidae: *Fundulus olivaceus*; Poeciliidae: *Gambusia affinis*; Centrarchidae: *Lepomis cyanellus*, *Lepomis gulosus*, *Lepomis macrochirus*, *Lepomis megalotis*, *Micropterus nigricans*; and Percidae: *Etheostoma radiosum*.

Figure 2. One of six goldfish collected from McCurtain County, Oklahoma.

Miller and Robison (2004) reported that populations of *C. auratus* are scattered across the state of Oklahoma and specifically noted that some have possessed large numbers in the panhandle of Cimarron County (Upper South Carrizzo Creek, Upper Cimarron drainage; Fig. 3). The distributional map shown by Miller and Robison (2004, see p. 88) shows shading in several other watersheds. However, there are none shaded in the southeastern part of the state. In adjacent Arkansas, Robison and Buchanan (2020) show spotty records for *C. auratus* within six counties of all the major river drainages, except the Red River. We therefore document a new geographic record for *C. auratus* in the Little River system of the Red River drainage of McCurtain County (Fig. 3).

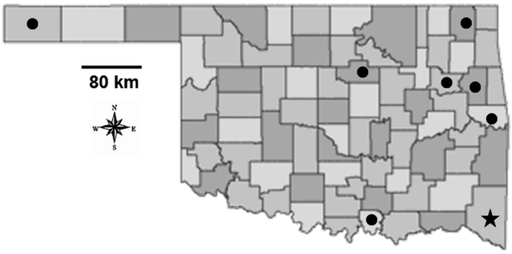

Figure 3. Records of goldfish collected in watersheds of various counties of Oklahoma. Dots = previous records; star = new record.

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Non-native land snails in Oklahoma: results from urban surveys and citizen science

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Abstract: Urban habitats within Oklahoma contain diverse assemblages of land snails, including several poorly documented non-native species. Using published accounts, museum collections, and iNaturalist, we list 25 species that include 16 exotic species, 7 extralimital species, and 2 species that are native to a portion of the state but have expanding ranges. The European-native milk snail, *Otala lactea*, is a new Oklahoma record, which was likely introduced in a rural site on trucks carrying the snails from Texas, whereas many non-native snails are dispersed through the plant trade. Verified iNaturalist records were assessed for four non-native species that can be easily identified from photographs (*Cornu aspersum*, *Otala lactea*, *Bradybaena similaris*, *Polygyra similaris*) and these records expanded the known ranges of all of four species, demonstrating the utility of iNaturalist records in updating distributions of land snails, especially urban snails. To be useful, iNaturalist photographs of snails need to be in sharp focus and show distinguishing features (which generally requires more than one image) and a scale. Many iNaturalist snail records were not identifiable or were misidentified. Identification issues can be improved by better photographs, greater input by taxonomists, adding the distinguishing features in comments, and the availability of non-technical regional identification guides.

Introduction

Oklahoma has a diverse land snail fauna and was cited for the state's extensive snail collection efforts (Hubricht 1985) because of numerous, mostly historical surveys. However, these historical surveys, like most land snail surveys, primarily targeted natural areas. More recent land snail surveys in Oklahoma have included urban areas (Bergey 2019; Bergey and Boonmachai 2023; Bergey and Figueroa 2016; Bergey et al. 2014; Bergey and Whipkey 2020) and these urban-based surveys have resulted in numerous new state records of land snails.

Non-native snails are often found in urban areas (Herbert 2010; Horsák et al. 2009; Perez et al. 2021), where they occur in yards (Arruda 2018; Bergey and Figueroa 2016; Bergey and Whipkey 2020; Perez et al. 2021), parks (Hodges and McKinney 2018), in greenhouses and plant nurseries (Bergey et al. 2014; Cowie et al. 2008; Gutiérrez Gregoric et al. 2020; Horsák et al. 2004; Richling and von Proschwitz 2021; Yeung et al. 2019), and even on green roofs (McKinney et al. 2019). Recent snail records from urban areas include multiple non-native species – either originating within the United States but occurring outside their native range (extralimital) or originating from other countries (exotic). Many non-native snails are small and are unlikely to become pests or even nuisance species (Hausdorf 2023) and even large species may not pose se-

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rious problems, although there are certainly exceptions.

The widespread occurrence of non-native snails in urban areas results largely from human-assisted dispersal of land snails and habitat modifications that benefit snail establishment in urban areas. Although snails disperse slowly by themselves, human-assisted movement allows rapid dispersal, sometimes over long distances. Mechanisms of human-assisted dispersal can be intentional, as when snails are moved as pets, food, or biocontrol agents, or unintentional, as when snails are moved on potted plants, on produce, in firewood, or attached to or within shipped goods (Cowie and Robinson 2003). A wide diversity of snails can be dispersed, as evidenced by the 369 species of freshwater and terrestrial snails found on commodities entering the United States over a 5-yr period by US Department of Agriculture inspectors (Robinson 1999). Within the United States, the plant trade is commonly implicated in the dispersal of land snails (Bergey et al. 2014; Cowie et al. 2008; Yeung et al. 2019). Snails found in yards may have arrived in association with potted plants, where snails commonly occur on undersides of the pot, in drain holes, and on the soil surface, where they may be hidden by leaf litter. Finding the species in plant nurseries bolsters the possibility that movement occurred through the plant trade. Nurseries may source plants from multiple other places, many of which are in distant states (Bergey et al. 2014). Other possibilities for introduction include the movement of firewood or landscape materials, snails in plant material such as vegetables or flowers, the release of pet snails, and snails being carried on vehicles (Cowie and Robinson 2003).

Habitat modifications that can aid in the establishment of non-native snails include watering and the provision of thermal microhabitats. Snails are prone to desiccation and watering provides a water source that not only supplements rain but regular watering prevents local drought conditions and can promote snail populations (Bergey and Whipkey 2020). Non-native species that do not tolerate freezing or the hot summers often burrow and may benefit from proximity to buildings, especially poorly insulated ones (Bergey 2019).

In this project, we (1) provided a list of known non-native land snails in Oklahoma and (2) provided detailed accounts of the known distributions of four non-native land snail species in Oklahoma. Two of the four species – the brown garden snail *Cornu aspersum* and the milk snail *Otala lactea* are of particular interest because of their large size, and hence visibility. These two species are easily identified from photographs and survey records can be augmented with photographic records from iNaturalist. The two other species, the southern flatcoil *Polygyra cereolus* and the Asian tramp snail *Bradybaena similaris,* also have distinctive shell morphology. Identification of these latter two species is possible from photographs, but is more challenging than for the two larger species as photographs may not show the necessary distinguishing characteristics.

Methods

Land snail records were obtained from three sources: previously published surveys by the authors, museum records from the Herbarium and Zoological Collection at the University of Science and Arts of Oklahoma, and iNaturalist (iNaturalist 2023; www.inaturalist.org). iNaturalist is an online network in which people post images of organisms for identification. Images and the accompanying data, particularly collection date and location, can be searched to find distribution records. A second online source, GBIF (Global Biodiversity Information Facility; www.gbif.org) was consulted but did not add additional records. GBIF is an online international tool based on amassed databases, including the iNaturalist database. Urban surveys that included records of the four target species were a survey of Oklahoma plant nurseries and three surveys of land snails in residential yards.

iNaturalist records for the four target species were searched in December 2023 using the following searches (by genus; but only one species in each genus is known in Oklahoma):

Cornu:https://www.inaturalist.org/observations?place_id=12&subview=table&taxon_id=87634

Otala: https://www.inaturalist.org/observations?-place_id=12&subview=table&taxon_id=202861

Bradybaena:https://www.inaturalist.org/observations?place_id=12&subview=table&taxon_id=172111

Polygyra:https://www.inaturalist.org/observations?place_id=12&subview=table&taxon_id=91217

All photographs resulting from these queries were copied into PowerPoint and examined for distinguishing features for the corresponding species, without regard to the locality of the record (photographs were numbered and the database information for each photograph was kept in a separate file). Photographs were given one of three scores: (1) correct – the photograph included enough features of the shell to positively identify the target species, (2) not sure – the photographs were inadequate to identify the target species because identifying features were not visible (e.g., features were obscured by the orientation of the shell, the aperture (especially) was not visible because of the snail's body, or the photograph was too blurry to see the identifying features), and (3) incorrect – shells lacked the appropriate identifying features and were sometimes identifiable to a different taxon. In addition, two records were discounted because they were evidently not locally sourced – one was photographed in a (commercial?) snail collection box and the other was described as a purchased pet.

Maps of collection sites were made with BatchGeo (www.batchgeo.com), an online program that maps locations based on address or coordinates. Sites are listed as towns rather than counties because, with one exception, all sites were urban (including plant nurseries, yards, and parks). In cases of multiple iNaturalist records from the same location on different dates, only one record was considered an occurrence.

Results and Discussion: species' accounts

The currently known list of non-native snails in Oklahoma comprises both exotic and extralimital species (Table 1). Although some taxa have long been known from the state (e.g., the slugs *Limacus flavus* and *Ambigolimax valentianus*), many are more recent records resulting from urban surveys. *Otala lactea* is recorded here as a new state record. Two species (*Anguispira alternata* and *Ventridens demissus*) are considered extralimital because, although their native range includes eastern Oklahoma (Hubricht 1985), these species are now much more widely distributed within the state. *Deroceras laeve* is reported as a native species, but recent genetic work has shown that the species is a non-native that originated in Europe (David Robinson, personal communication). Unlike nearly all of the other non-native species listed in Table 1, *D. laeve* has expanded its range beyond urban areas.

Table 1. List of non-native land snails in Oklahoma. 'exotic' species are native outside the United States and 'extralimital' species are native USA species but are not native in Oklahoma (parentheses indicate extralimital species that are native to the eastern one-third of Oklahoma but now occur elsewhere in the state). Documentation of records are indicated ('USAO' = University of Science and Arts Museum; 'plant nurseries': Bergey et al. (2014); 'Norman yards' survey: Bergey & Figueroa (2016); *'Cornu survey'*: Bergey (2019); yards in 'nine towns' in Oklahoma survey: Bergey & Whipkey (2020); 'OKC Zoo' survey: Bergey & Boonmachai (2023). iNaturalist records were only checked for the four target species and records were present for all four species (not listed in the Table).

Family/Species	Native?	USAO Museum	plant nurseries	Norman yards	*Cornu* survey	nine towns	OKC Zoo
Achatinidae							
Allopeas gracile (Hutton, 1834)	exotic	x	x	x		x	x
Rumina decollata (Linnaeus, 1758)	exotic	x				x	
Subulina octona (Bruguière, 1789)	exotic		x				
Agriolimacidae							
Deroceras laeve (Müller, 1774)	exotic	x	x	x		x	x
Deroceras reticulatum (Müller, 1774)	exotic		x			x	
Arionidae							
Arion fasciatus (Nilsson, 1823)	exotic		x			x	
Arion rufus (Linnaeus, 1758)	exotic		x				
Camaenidae							
Bradybaena similaris (Férussac, 1822)	exotic		x	x		x	x
Helicidae							
Cornu asperum (Müller, 1774)	exotic	x	x	x	x	x	
Otala lactea (Müller, 1774)	exotic						
Discidae							
Anguispira alternata (Say, 1817)	(extralimital)	x	x	x		x	x
Gastrodontidae							
Ventridens demissus (Binney, 1843)	(extralimital)	x	x			x	x
Limacidae							
Ambigolimax valentianus (Férussac, 1821)	exotic	x	x	x		x	x
Limacus flavus (Linnaeus, 1758)	exotic	x	x	x		x	
Limax maximus Linnaeus, 1758	exotic					x	
Milacidae							
Milax gagates (Draparnaud, 1801)	exotic		x	x		x	
Oxychilidae							
Oxychilus cellarius (Müller, 1774)	exotic		x				
Polygyridae							
Patera appressa (Say, 1821)	extralimital		x	x		x	
Polygyra cereolus (Megerle von Muhlfeld, 1816)	extralimital		x	x		x	x
Triodopsis hopetonensis (Shuttleworth, 1852)	extralimital	x	x	x		x	x
Xolotrema fosteri (Baker, 1932)	extralimital	x				x	x
Valloniidae							
Vallonia excentrica Sterki, 1893	extralimital					x	
Vallonia pulchella (Müller, 1774)	extralimital					x	x
Veronicellidae							
Angustipes ameghini (Gambetta, 1923)	exotic		x				
Vertiginidae							
Vertigo teskeyae Hubricht 1961	extralimital					x	

Cornu aspersum (brown garden snail; family Helicidae; Fig. 1A and Fig. 2A)

This is a large snail, with a globose shell of 25-40 mm diameter (Herbert 2010). The shell is brown and usually patterned with broken darker brown spiral bands (Fig. 1A). Adults have a white recurved aperture lip. *C. aspersum* is native to the Mediterranean area of southern Europe and northern Africa. The species is raised for food in Europe, as it is a commonly eaten escargot species, known as escargot petit gris (= small gray). *Cornu aspersum* now occurs in many countries and continents (summarized in Herbert 2010). The species first introduction in the USA was thought to be in California the 1850's, when it was imported as an edible species by a Frenchman (Stearns 1900), an origin shared with South Africa (e.g., Herbert 2010).

Cornu aspersum is known from multiple states in the United States, including three states bordering Oklahoma: New Mexico, Colorado, and Texas (NatureServe 2024). In California, the species can reach high densities and can be a pest in citrus and a nuisance in yards but elsewhere it causes little problem.

The first known record of *C. aspersum* in Oklahoma was in a yard in Nichols Hills (Oklahoma County) in June 1994 (USAO #9452)(Fig. 2B). The second record was also in the greater Oklahoma City area, in Edmond (Oklahoma County) in Dec 2011 (USAO #12927). Land snail surveys in urban areas and in plant nurseries documented several more recent locations. In a survey of 28 plant nurseries, a single live specimen was found in a plant nursery in Davis, Oklahoma (Bergey et al. 2014). Subsequent yard surveys found C. aspersum in yards in Norman (Bergey 2019; Bergey and Figueroa 2016) and Altus (Bergey and Whipkey 2020). One of the sites in Norman was an extensive population of 281 counted live snails that ranged over 19 yards (Bergey 2019). iNaturalist had 10 records, of which 3 were correct identifications: two in Oklahoma City and one in Norman.

***Otala lactea* (milk snail; family Helicidae; Fig. 1B and Fig. 2B)**

Otala lactea is a large snail (shell diameter of 20 to 40 mm) originating from the Mediterranean region (Pfleger and Chatfield 1988). The shell is thicker and somewhat flatter than the shell of *C. aspersum*. The dark brown, shiny colored area that completely surrounds the aperture is characteristic. Other than the aperture color, shell coloration is variable, ranging from cream colored to a variable number of brown bands to brown, with the color interspersed with lighter marking (Herbert 2010) (Fig. 1B).

The species has been introduced in some South American countries, Bermuda, and possibly Mexico (summarized by Hayes et al. 2023). Within the United States, *O. lactea* occurs in two states that border Oklahoma (Texas and Missouri), most of the Gulf Coast states, California, Kentucky, Virginia, Ohio, Pennsylvania New York, and Maryland (NatureServe).

The first known record of *O. lactea* in Oklahoma was a set of photographs sent by Cody Rhodes from Pontotoc County in 2021 (Fig. 2B). The site was disturbed rangeland that had previously been a rock mining site. That same year, we received shells and found living snails at the Master Gardener demonstration garden in Norman. Although the gardeners have tried to eradicate this population using snail bait, snails were still present in 2023. One of the gardeners transplanted some plants from the Garden to her yard and subsequently found live snails in her yard in Norman. Both the Pontotoc and Norman populations are small and are considered established.

***Bradybaena similaris* (Asian tramp snail; family Camaenidae; Fig. 1C and Fig. 2C)**

The shell of this medium-sized species is pyramidal with rounded body whorl. The shell is light in color with a single well defined brown band, which can sometimes be indistinct (Fig. 1C). Similar to *C. aspersum*, and as it's common name implies, this species has spread widely across the globe from its origin in Asia (Herbert 2010).

In the United States, the known distribution of *Bradybaena similaris* includes all the Gulf Coast states (Texas, Louisiana, Mississippi, Alabama, and Florida), as well as Georgia, South Carolina, North Carolina (although this state's INaturalist records are unconfirmed), and two 2022 reports in Wisconsin (both in Milwaukee) (Gladstone et al. 2020; iNaturalist; NatureServe).

As far as we are aware, the first records of *B. similaris* in Oklahoma were in eight plant nurseries in six towns (Davis, Edmond, Enid, Inola, Oklahoma City, and Owasso) (Bergey et al. 2014), with a recent finding in the University of Oklahoma greenhouses (where it was apparently introduced since the earlier survey). Our urban surveys found the species in Ardmore and Woodward yards (Bergey and Whipkey 2020) and the Oklahoma City Zoo (Bergey and Boonmachai 2023) (Fig. 2C).

iNaturalist had 75 records of *B. similaris* in Oklahoma. Ten records were misidentified, most commonly these were polygyrids, and 21 records could not be positively identified (to the authors' satisfaction). Of the confirmed *B. similaris* records, 36 were repeated observations, with 29 records from one street and 7 from another – all during 2023; these were considered as one location for each street. An additional record lacked site location beyond the state and was not further considered. This left 9 records (including the two multiple-record sites) and comprised further sites in Norman and Oklahoma City, and added new locations in Broken Bow, Tulsa, and Moore.

***Polygyra cereolus* (southern flatcoil; family Polygyridae; Fig. 1D and Fig. 2D)**

Polygyra cereolus is the smallest of the four included species (Fig. 1E). The shell is characterized by being flattened, with close spirals, transverse ridges on dorsal shell surfaces, a shouldered body whorl, and a large umbilicus. Adults have a thick white recurved lip on the aperture, which has a single tooth - features that are not present in juveniles. Shells do not have an obvious color pattern, although some specimens, especially juveniles, appear mottled because of coloration of the body (Fig. 1D).

Unlike the other three target species, *P. cereolus* is native to the United States. Hubricht (1985) mapped the then-known distribution as all of Florida and along coastal areas from South Carolina to Texas, with few records inland within these states, noting the species apparent affinity to slightly salty conditions. More recently, this species has greatly extended its range, although NatureServe indicates these same states. By 2008, the species was also found in North Carolina, Kentucky, and Hawaii (Perez and Cordeiro 2008) and the species has become invasive, as *P. cereolus* is now known from southern Europe and northern Africa (Hausdorf 2023), the Caribbean (Charles and Arnaud 2020), and the Middle East (Neubert 1998), and has been intercepted re-entering the United States from Europe (Robinson 1999).

Our surveys found 62 unique site records (a few records were duplicated sites). *Polygyra cereolus* was found in all 28 of the surveyed plant nurseries (Bergey et al. 2014), in 23 of 61 surveyed yards in Norman (Bergey and Figueroa 2016), and in yards in Altus, Elk City, Woodward, and Miami (Bergey and Whipkey 2020), as well as the Oklahoma City Zoo (Bergey and Boonmachai 2023).

iNaturalist listed 72 records of *P. cereolus* in Oklahoma. Thirty records were misidentified as *P. cereolus*, with identifiable taxa including *Ventridens* sp., *Triodopsis hopetonensis*, *Linisia texasiana*, *Anguispira* sp., and *Euchemotrema*/*Stenotrema* sp. We were unable to confidently confirm the identifications of 23 records. Of the remaining 19 records, 3 lacked specific location information and 1 was a repeat record, leaving 15 usable records. These 15 records were all recent (2019-2023), duplicated some city records (e.g., Norman, Oklahoma City), and added five new cities to the known distribution within the state – namely, Bartlesville, Tulsa, Owasso, Stillwater, and Edmond (Fig. 2D).

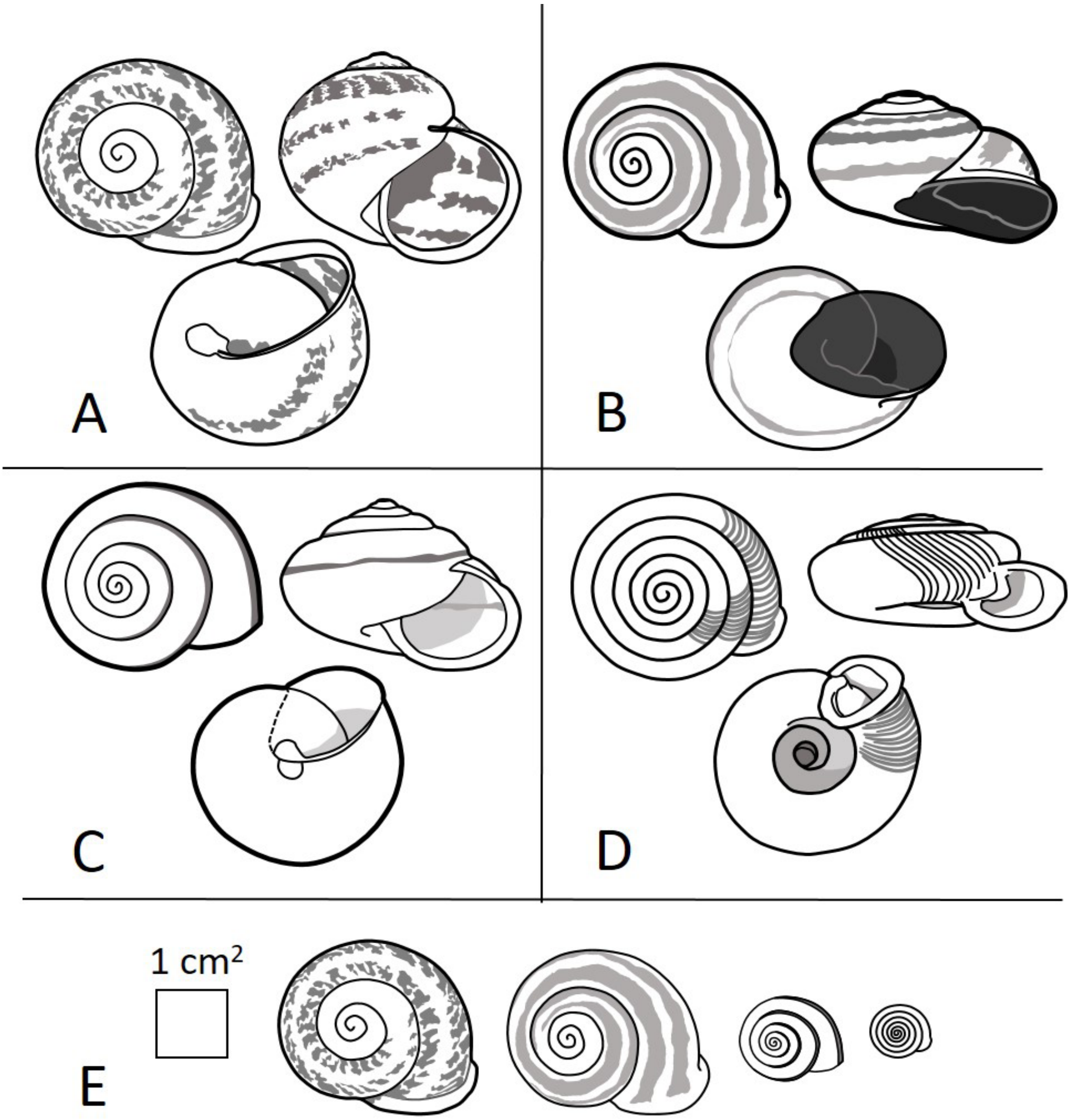

Figure 1. Illustrations (dorsal, lateral and ventral views) of four land snail species. A = *Cornu aspersum,* B = *Otala lactea*, C = *Bradybaena similaris*, D = *Polygyra cereolus*. Shell sizes are shown in E. Drawings were made from shells collected in Oklahoma.

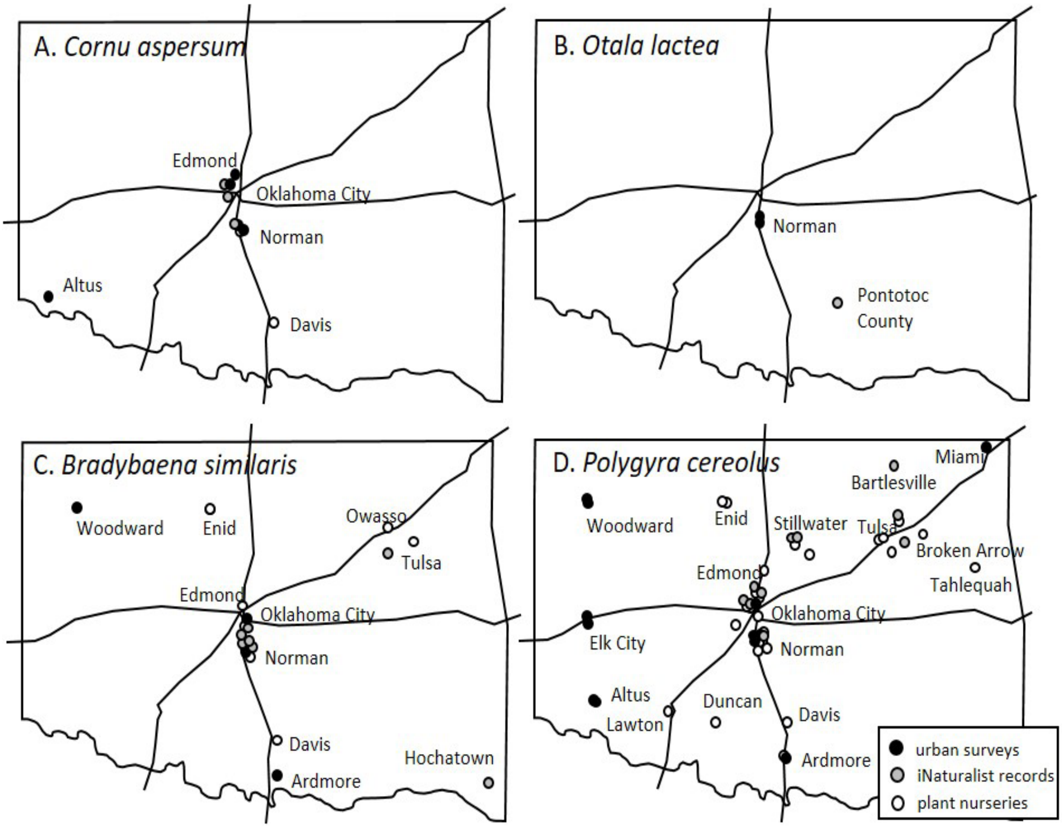

Figure 2. Distribution maps of four land snail species in Oklahoma. The panhandle of Oklahoma is not mapped; there are no records for these species from this part of the state. A = *Cornu aspersum*, B = *Otala lactea*, C = *Bradybaena similaris*, D = *Polygyra cereolus*. The shading in the circles indicates the origin of the records (urban surveys, iNaturalist records, or plant nursery surveys).

Discussion

Surveys in urban areas have been very productive in documenting the presence and distribution of non-native species and species with expanding ranges within the state. These surveys highlight the role that humans play in the distribution and establishment of land snails – both native and non-native species. This is especially evident in the distribution of *Polygyra cereolus* in Oklahoma. Although there were no records of this species in Oklahoma before 2014, *P. cereolus* was found in all 28 surveyed greenhouses (Bergey et al. 2014), indicating that the species is frequently dispersed by the commercial plant trade and its widespread presence in yards and other urban areas (Table 1) is consistent with establishment from transported plants. We know of no records for this species in natural areas away from urban areas and human habitations in the state. Like *P. cereolus*, the *Otala lactea* records in Norman are consistent with introduction through the plant trade (including by landscaping materials) – by its presence in a municipal demonstration garden and then being moved during plant transplanting to a gardener's yard (Georjana Mauldin, personal communication). However, in contrast to *P. cereolus*, the *O. lactea* record in Pontotoc County is the only record in this study that is in a non-urban area. The site is on a large cattle ranch, specifically in an area that had a history of rock mining and it is thought that the snails were transported on trucks from Texas (Cody Rhodes, personal communication), where the species is widely established (Elliott and Pierce 1992). Like some other Mediterranean snails, *O. lactea* climbs above ground to escape hot, dry conditions (Moreno-Rueda et al. 2009). Such climbing behavior and its role in dispersal was documented when *O. lactea* snails were found attached in the wheel wells and undercarriage of a car imported to Hawaii from southern California (Hayes et al. 2023), where the species is established (La Pierre et al. 2010).

Citizen science contributions through iNaturalist provide a mechanism to greatly expand our knowledge about the distribution of land snails (Rosa et al. 2022), as demonstrated in our study by the new iNaturalist distribution records for each of the four target species. iNaturalist is especially useful in areas of the country with the limited personnel and funding for snail surveys by experts, as occurs in Oklahoma and many other states. iNaturalist can also be used as a platform for more defined citizen science surveys, such as in specific areas or for certain taxa. SLIME is a successful program in southern California (Vendetti et al. 2018) (www.inaturalist.org/projects/slime), in which snail records are verified by experts and may result in specimen collection.

Land snails provide some challenges for the iNaturalist community – especially in users taking photographs useable for snail identification (Rosa et al. 2022), and the availability and willingness of snail experts to make and confirm identifications. The small size of most species of snails makes them difficult to photograph. As a result, there is a bias toward larger species (Barbato et al. 2021), such as *C. aspersum* and *O. lactea* in our study. In order to visualize distinguishing shell characteristics, records should include at least three photographs, with the top view, the side view clearly showing aperture features such as teeth, and the bottom view – along with some sort of scale, such as a coin. The photos should have good focus, which is challenging for small shells when using cell phone cameras (Barbato et al. 2017). An additional photograph of the live snail would be a good addition and provides a record of the presence of living snails (Rosa et al. 2022) but is much less important for identification than shell features (note: most aperture characteristics are not visible when the snail is extended out of its shell). Even with perfect photographs, not all species can be determined, as some require additional features such as microscopic texture on the shell, internal shell ridges or dissection of the reproductive systems (nearly all land snails are hermaphrodites).

As with many invertebrate groups, land snails are a diverse group with few taxonomists. As a result, many identifications in iNaturalist are best guesses – some are correct, but others are either unidentifiable to species or are identified incorrectly. Other identifications are based on iNaturalist artificial intelligence, which also introduces errors (Barbato et al. 2017). Unfortunately, incorrect identifications may be corroborated and one such misidentification among the target species was elevated to 'Research grade' and appeared as a record in GBIF. It would help greatly if identifiers would include the characteristics used to make their determinations; this would not only help substantiate identifications but would be useful to others learning to identify snails.

Urban areas, especially yards, are well represented in iNaturalist snail records (Barbato et al. 2017; Rosa et al. 2022; this study) – likely because of the attention paid in yards during yard care and the convenience of searching near home. This makes iNaturalist an excellent tool for the documentation of urban snails, including non-native species, without the requirement for physical surveys and the necessity for getting permission to survey each site. Indeed, iNaturalist can be an important data source for documenting the spread of non-native species of snails (Rosa et al. 2022).

Land snail identification in Oklahoma is hampered by not having a good snail identification resource, as is available for some states have (e.g., New Mexico: Metcalf and Smartt 1997). However, when guides are available, they are often quite technical, require microscopic examination, and may not include many of the non-native species found in urban areas. Instead of comprehensive guides, there are multiple published lists of Oklahoma snails (e.g., Branson 1961; Branson 1962) and the extensive snail collection data from the OSAO Herbarium and Zoological Collection, which is in the process of being added to OBIS (Oklahoma Biodiversity Database System) database hosted at the University of Oklahoma (https://obis.ou.edu/taxa/search/main). The key by Burch (1962) is helpful but does not include all possible species, including many of the non-native species. A pictorial flow chart key to some of the common snails (exclusive of slugs) in urban areas of Oklahoma is found in the associated OSF database (Bergey et al. 2024). This key is especially useful for people using iNaturalist, but like other resources, does not includes all species that may be encountered.

Acknowledgements

We thank the individuals who alerted us to the presence of *Otala lactea*: Cody Rhodes and Georjana Mauldin. Laura Figueroa made the original version of the identification key found in the OSF materials. We thank the users of iNaturalist who contributed land snail photographs and who identified these photographs.

Data availability

USAO Museum, urban survey and iNaturalist records for *Cornu aspersum*, *Otala lactea*, *Bradybaena similaris*, and *Polygyra cereolus* are found in an Open Science Framework database (Bergey et al. 2024) at www.osf.io/4pvxs.

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A Dilepid Tapeworm (Cestoda: Cyclophyllidea: Dilepididae) from Willow Flycatcher, *Empidonax traillii* (Passeriformes: Tyrannidae) from Nevada County, Arkansas

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Abstract: During August 2024, a single willow flycatcher, *Empidonax trailli* was found dead in Nevada County, Arkansas, salvaged, and examined for parasites. A single dilepid tapeworm was found in the small intestine. Unfortunately, it was an immature dilepid that could not be identified to genus. The specimen had 14 hooks in a single row on the rostellum (26 µm long), irregularly alternating genital pores, and about 10 testes. There are no previous reports of dilepids from *E. trailli*. We document the first report of a tapeworm from *E. trailli*.

Introduction

The willow flycatcher, *Empidonax trailli* (Audubon) is a small insectivorous neotropical migrant bird of the tyrant flycatcher family native to North America (Sibley 2016). The North American range extends from southern Canada throughout the USA. There are four subspecies and the taxon that occurs in Arkansas is the eastern nominate subspecies, *E. t. trailli* that breeds from the eastern coast of the USA to the western Rocky Mountains (Dunn and Alderfer 2011). It occurs in brushy habitats of wetlands as well as pastures and mountainous meadows. They may also be found in semi-arid landscapes, the borders of forests, dry, upland areas, mountain meadows and riparian forests. Willow flycatchers inhabit a variety of areas, their preferred habitat is within low growing willow thickets, but they also favor short, shrubby areas.

This bird is parasitized by quill mites (*Syrinogophilopsis*), ticks (Ixodida), and brood-parasitic brown-headed cowbirds, *Molothrus ater* (Boddaert) (Skoracki et al. 2008; Uyehara and Narins 1995; Hamer et al. 2012; Hendricks et al. 2013). There are two species of cestodes (Parauterinidae) from tyrant flycatchers, *Anonchotaenia prolixa* Phillips, Georgiev, Waeschenbach,

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and Mariaux, 2014 found in a single white-crested elaenia, *Elaenia albiceps chilensis* Hellmayr flycatcher in Chile (Phillips et al. 2014) and *A. vaslata* Phillips, Georgiev, Waeschenbach, and Mariaux, 2014 found in a tropical kingbird, *Tyrannus melancholicus* (Vieillot) and streaked flycatcher, *Myiodynastes maculatus* (Statius Müller) from Paraguay. However, we are unaware of any reports of helminth parasites of *E. trailli*. Here we document the first report of a dilepid tapeworm from a willow flycatcher.

Methods

Host collection and processing

During August 2024, a single willow flycatcher was found dead on the road off co. rd. 210, 9.0 km N of Prescott, Nevada County, Arkansas (33º53'18.69"N, -93º22'16.37"W). It was salvaged and taken to the laboratory for necropsy. The feathers were vigorously brushed over a white enamel tray to collect any ectoparasites. A mid-ventral incision was made from the cloaca to throat to expose the trachea, lungs, air sacs, esophagus, proventriculus, gizzard, gallbladder, liver, kidneys, and intestines. Feces from the lower intestine were placed in a vial containing 2.5% (w/v) aqueous potassium dichromate ($K_2Cr_2O_7$) for examination of coccidia. Organs were placed in individual Petri dishes containing 0.9% (v/v) saline, opened, and their contents rinsed of mucus. Several 100 mm sections of the tissues were cut, split lengthwise, and examined under a stereomicroscope at 20 to 30× to aid in locating endoparasites. A live cestode was fixed in nearly boiling tap water without coverslip pressure, transferred to 70% (v/v) DNA grade ethanol, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate or xylene, and mounted in Canada balsam.

Voucher specimens (photomicrograph and slide) of the cestode was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska-Lincoln, Lincoln, NE. A host voucher specimen (in ethanol) was deposited in the Northeast Texas Community College Vertebrate Collection (NTCCVC), Mt. Pleasant, TX.

Results

A single cestode parasite (Fig. 1) with characters of the order Cyclophyllidea van Beneden *in* Braun, 1900 and the family Dilepididae Fuhrmann, 1907 was found in the small intestine of the willow flycatcher. Because it was an immature specimen, a generic diagnosis was not possible. However, a morphometric description is provided below.

Brief Description (Fig. 1; HWML photovoucher 217891; slide HWML 217895)

Immature specimen, no uterus or parauterine organ observed; individual with 14 hooks in single row on rostellum (26 µm long), irregularly alternating genital pores, and ~10 testes.

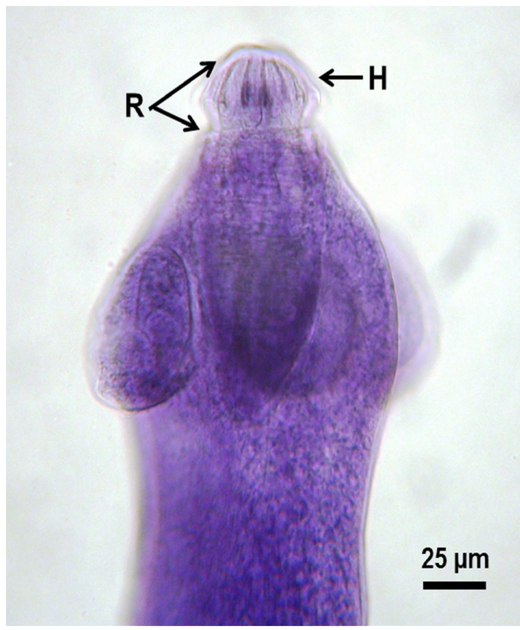

Figure 1. Dilepid tapeworm from willow flycatcher, *Empidonax trailli* from Arkansas. Whole mount of scolex showing armed rostellum (R) with hooks (H).

Discussion

Cestodes of the family Dilepididae have a cosmopolitan distribution and occur in most orders of birds; however, they are particularly diverse in the Passeriformes (Mariaux et al. 2017). The general life-cycle of dilepids includes a single intermediate host, usually an arthropod although annelids and molluscs also serve as intermediate hosts. The finding of this parasite in *E. traillii* represents a new host record and the first report, to our knowledge, of any helminth in this host.

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Batrachochytrium dendrobatidis (Chytridiomycota: Rhizophydiales) from Blanchard's Cricket Frog, *Acris blanchardi* (Anura: Hylidae) and American Bullfrog, *Rana catesbeiana* (Ranidae) from Arkansas

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Abstract: Between August 2023 and August 2024, 42 amphibians including, 14 many-ribbed salamanders, *Eurycea multiplicata*, three Blanchard's cricket frogs, *Acris blanchardi*, seven spring peepers, *Pseudacris crucifer*, four American bullfrogs, *Rana catesbeiana*, and four green frogs, *Rana clamitans* from Arkansas, as well as nine Woodhouse' toads, *Anaxyrus woodhousii*, and one southern leopard frog, *Rana sphenocephala utricularia* from Texas were examined for the skin fungi, *Batra-*

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chochytrium dendrobatidis (*Bd*), *B. salamandrivorans* (*Bsal*), and frog virus 3-like Ranavirus (FV3). Swabs from one of four (25%) *R. catesbeiana* and one of three (33%) *A. blanchardi* from Arkansas tested positive for *Bd*: the other amphibians sampled were negative for all three pathogens. Neither host showed any obvious pathological signs of infection. This is the second time *A. blanchardi* and *R. catesbeiana* from Arkansas has been reported with *Bd*.

Introduction

Chytrids belong to the Kingdom Fungi, Phylum Chytridiomycota, Class Chytridiomycetes, and Order Rhizophydiales. The fungus causes the disease chytridiomycosis, originally generated by *Batrachochytrium dendrobatidis* Longcore, Pessier, and Nichols (1999) (*Bd*), an amphibian chytrid (Lips et al. 1999; Longcore et al. 1999). The skin infection has a predilection to anurans (frogs and toads) and has been reported on every amphibian inhabitant of the continent. Unfortunately, the disease has resulted in a serious decline and extinction of more than 200 species of amphibians worldwide and poses the greatest threat to their biodiversity of any known amphibian disease (Stuart 2004; Skerratt et al. 2007; Pounds et al 2016; Collins 2020).

In Arkansas, 10 to 20% of endangered Ozark hellbenders, *Cryptobranchus alleganiensis bishopi* Grobman sampled from the Eleven Point River watershed in Randolph County were reported to harbor *Bd* (Briggler et al. 2008; Hardman et al. 2020). The fungus was also found in Blanchard's cricket frogs, *Acris blanchardi* Harper from Wapanocca National Wildlife Refuge in Turrell (Crittenden County) (Hanlon et al. 2014). However, no frog exhibited pathological signs of a *Bd* infection (e.g., lethargy, skin sloughing), indicating that *Bd* in this population of *A. blanchardi* may endure in populations without dying from *Bd*-induced mortality. In another study, *A. blanchardi* collected from the Bald Knob National Wildlife Refuge in White County and southern leopard frog, *Rana sphenocephala utricularia* (Cope) tadpoles from Felsenthal National Wildlife Refuge in Union County harbored *Bd* (Rothermel et al. 2008). A more recent study by McAllister et al. (2023) reported *Bd* in a single *R. catesbeiana* from Polk County.

A similar species, *B. salamandrivorans* Martel, Blooi, Bossuyt, and Pasmans, 2013 *(Bsal)* also causes chytridiomycosis but primarily occurs in caudate amphibians (salamanders) (Martel et al. 2013). It was initially discovered in captive and native fire salamanders, *Salamandra salamandra* (L.) in Belgium and the Netherlands, where it led to significant declines in salamander populations. Since then, *Bsal* has also been noticed in captive salamanders in the United Kingdom and Germany. To date, it has not been reported from any salamander from North America.

Another emerging disease include members of the genus *Ranavirus* (an iridovirus), capable of infecting a wide variety of poikilothermic vertebrate hosts including fish, amphibians, and reptiles (Whittington et al. 2010; Marschang 2011; Miller et al. 2011). It is known to have caused amphibian die-offs on five continents (Gray et al. 2009; Miller et al. 2011) and is capable of infecting amphibians from at least 14 families and over 72 individual species, with the majority of cases in members of the anuran family Ranidae (Miller et al. 2011; see their Table 1). While ranavirus infections have been documented in numerous areas across the USA (Allender et al. 2011; Gray et al. 2012; Goodman et al. 2013; Duffus et al. 2015), infections have been rarely detected in herps in Arkansas. Hardman et al. (2020) discovered ranavirus in 6% of *C. a. bishopi* in the state. However, Hanlon et al. (2016) surveyed the prevalence of ranaviruses in five species of turtles of the Wapanocca Wildlife Refuge and none were infected. In neighboring Oklahoma, ranavirus was found in 12 species of amphibians, some in eastern counties bordering the western border of Arkansas (Davis et al. 2019).

The two-fold purpose of the present study is: (1) survey several amphibians from Arkansas and Texas for these emerging amphibian pathogens, and (2) to document additional reports of *Bd* in two common anurans of the state. We hope to spur others in examining additional amphibians of Arkansas for pathogens, particularly populations that are listed as endangered, threatened, or are species of special concern or greatest conservation need (see: https://www.agfc.com/education/arkansas-wildlife-action-plan/).

Methods

Host collection and processing

Between August 2023 and August 2024, 14 many-ribbed salamanders, *Eurycea multiplicata* (Cope) from Montgomery, three *A. blanchardi* from Fulton, seven spring peepers, *Pseudacris crucifer* (Wied-Neuwied) from Polk, four *R. catesbeiana* from Fulton (*n* = 2) and Nevada (*n* = 2), and four green frogs, *Rana clamitans* (Latrielle) from Columbia (*n* = 1) and Polk (*n* = 3) counties, Arkansas, were collected by hand or dipnet. In addition, a *R. s. utricularia* from Lamar, and nine juvenile Woodhouse' toads, *Anaxyrus woodhousii* (Girard) from Morris counties, Texas, were also collected in a similar manner. We followed the sampling protocols of Livo (2004) and Standish et al. (2018) for collecting samples from potential hosts for *Bd*, *Bsal*, and 3-like Ranavirus. After processing, some specimens were photographed and released unharmed at their collection site while others were deposited as vouchers in the Northeast Texas Community College Vertebrate Collection, Mt. Pleasant, Texas (NTCCVC). We prefer the use of the genus *Rana* versus *Lithobates* for North American ranid frogs following Yuan et al. (2016).

Molecular Techniques

Swab DNA was extracted using 150 µL of the PrepMan™ Ultra Sample Preparation Reagent (Thermo Fisher Scientific, Waltham, Massachusetts) following the manufacturer's instructions. Multiplex quantitative PCR (qPCR) was performed, targeting FV3, *Bd*, and *Bsal* as detailed by Standish et al. (2018). A synthetic gBlock® containing all three target amplicons was used as a positive control and standard curve. Serial dilutions (10×) ranging from 10^7 to one copy per reaction were used to quantify copies per µL (Standish et al. 2018). Sample concentration (copies/ng total DNA) was determined using a Qubit™ 3.0 Fluorometer (Thermo Fisher Scientific) and the Qubit™ DNA High Sensitivity Kit (Thermo Fisher Scientific) following the manufacturer's instructions.

Results

Batrachochytrium salamandrivorans and FV3 were not detected in any samples. Swabs from one of two (50%) *R. catesbeianus* (urostyle length [UL] = 140 mm) collected in March 2024 from Town Creek at Salem, Fulton County (36°06′4.3848″N, -91°05′7.9224″W), and one of three (33%) *A. blanchardi* (UL = 22 mm) collected in March 2024 from Big Creek S of Mammoth Spring off co. rd. 81, Fulton County (36°26′22.46″N, -91°29′21.85″W), tested positive for *Bd*. Both host voucher specimens were deposited in the NTCCVC. The other amphibians sampled from four other Arkansas and two Texas counties were negative for *Bd*. Across duplicate reactions, swabs contained an average of 4.5 *Bd* copies/ng and 297 *Bd* copies/ng of total DNA for *R. catesbeiana* and *A. blanchardi*, respectively. The ITS copy in zoospores can vary significantly from 10 to 144 copies/zoospore, so we estimated based on CT values, resulting copy number, and lack of disease signs as these detections are subclinical infections representing <10 zoospores (Longo et al. 2013).

Discussion

In our survey, both anurans found to be infected with *Bd* have the widest geographical distribution within the state (Trauth et al. 2004). Both species have also been previously reported to harbor *Bd* in Arkansas (Rothermel et al. 2008; Hanlon et al. 2014; McAllister et al. 2023). The fungus has now been reported in amphibians from six of 75 (8%) counties of the state (Fig. 1). The apparent gaps in *Bd* distribution among amphibians in other counties (especially in the Ozarks of Northwest Arkansas) may be biased due to the lack of surveys conducted on them. In addition, as far we know no anuran from the state has been reported with FV3 although the endangered caudate *C. a. bishopi* from the Ozark highlands was found to be infected (Hardman et al. 2020).

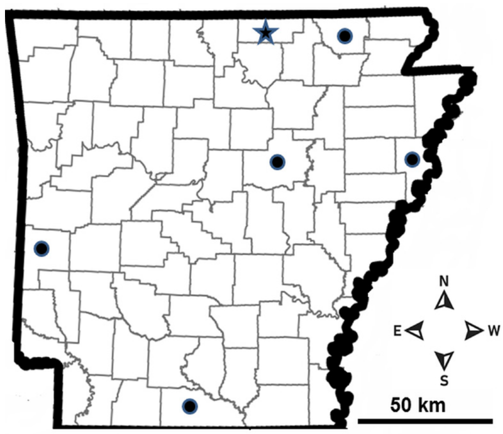

Figure 1. Counties in Arkansas where anuran hosts have been reported with Bd. Dots = previous records; star = new record.

Currently, *Bsal* is not known to occur in North America, but entry for its introduction apparently exists. Although we did not find either *Bd* or FV3 in our small sample of frogs (*n* = 10) from Texas, studies in the state have confirmed the presence of *Bd* in several amphibians (Gaertner et al. 2009a, 2009b, 2010, 2012; Gascon et al. 2009; Saenz et al. 2010; Marshall et al. 2019).

We (McAllister et al. 2023) noted previously that American bullfrogs appear to be asymptomatic (resistant) to *Bd* and may act as vectors or reservoir hosts (Daszak et al. 2004; Hanselmann et al. 2004). Garner et al. (2006) detected *Bd* infections in introduced *R. catesbeiana* from six countries, including those introduced into Arizona. In addition, Standish et al. (2018) sampled adult *R. catesbeiana* in Wisconsin and identified *Bd* and FV3 with a prevalence of 33% and 17%, respectively; however, none of the American bullfrog tadpoles sampled tested positive for either pathogen. Rothermel et al. (2008) reported from a large sample of *R. catesbeiana* (*n* = 229), five (2%) individuals from Georgia, North Carolina, and Virginia harbored *Bd* although specimens from Florida, Louisiana, Mississippi, and South Carolina were not infected. In Oklahoma, *Bd* in *R. catesbeiana* was reported either rarely or in high prevalence in several surveys (Watters et al. 2016, 2018, 2019, 2021; Marhanka et al. 2017; Nichols et al. 2022).

As far as our other host species (*A. blanchardi*) is concerned, this small frog has been reported to be the host of *Bd* in several studies, including those in Arkansas (Rothermel et al. 2008; Hanlon et al. 2014), Oklahoma (Watters et al. 2016; 2018, 2019, 2021; Marhanka et al. 2017; Nichols et al. 2022), and Texas (Gaertner et al. 2009, 2012). Interestingly, none of these studies reported that specimens appeared moribund or lethargic with obvious skin lesions, so this frog may also share some vector or reservoir host attributes for *Bd* with *R. catesbeiana*.

Additional amphibians from the state should be surveyed for *Bd* as well as for other amphibian pathogens. Gaertner et al. (2009a) noted that because central Texas has a significant number of endangered and endemic species of amphibians (see Dixon 2013), an increase of positive environmental factors, specifically permanent water sources, could subsequently result in an increase of the abundance and transmission of *Bd*, and thus might have a negative effect from *Bd* on the amphibian populations in the region. Therefore, we predict that with additional surveys in Arkansas, more individuals will be found to harbor this fungus, including the possibility of finding any of these pathogens in Arkansas' protected amphibians.

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First Report of *Anchoradiscus triangularus* (Ancyrocephalidae) from Bluegill, *Lepomis macrochirus* (Perciformes: Centrarchidae) from Southeastern Oklahoma

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Abstract: During April 2024, seven bluegill, *Lepomis macrochirus* were collected with a backpack electrofisher from Yashau Creek, McCurtain County, Oklahoma. Fish were examined for gill parasites and a single (14%) *L. macrochirus* harbored a monogenean, *Anchoradiscus triangularis*. Mensural data is included as well as a photomicrograph of the specimen. This is the first time this parasite has been reported from Oklahoma. In addition, a summary of hosts and localities of *A. triangularis* is provided.

Introduction

Bluegill, *Lepomis macrochirus* (Rafinesque) have been reported as hosts for several parasites, including at least 35 monogeneans (Hoffman 1999). One species, *Anchoradiscus triangularis* (Summers, 1937) Mizelle, 1941 appears to be specific to centrarchid fishes, including *L. macrochirus* and other *Lepomis* spp. (Ta-

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ble 1). Here we document *A. triangularis* from a bluegill from southeastern Oklahoma, a new geographic distributional record for the parasite, and only the second time it has been reported from west of the Mississippi River.

Table 1. Previous reports of *Anchoradiscus triangularis* in fishes.

Host	Locality	Reference
Lepomis gibbosus	North Carolina	Mayes and Miller (1975)
Lepomis macrochirus	Alabama	Rawson and Rogers (1971)
	Arkansas	Becker and Cloutman (1975); Cloutman (1975)
	Florida	Mizelle (1941)
	Louisiana	Duobinis-Gray and Corkum (1985)
	North Carolina	Cloutman (1988)
	Oklahoma	This report
Lepomis microlophus	Florida	Mizelle (1941)
	Louisiana	Summers and Bennett (1938)*; Duobinis-Gray and Corkum (1985)
Lepomis symmetricus	Louisiana	Summers (1937)†

*Abstract.
†Original description.

Methods

Host collection and processing

During April 2024, seven *L. macrochirus* (mean ± SD total length [TL] = 79.4 ± 18.2, range 46–100 mm TL) were collected by backpack electrofisher from a tributary to Yashau Creek off Airport Road at Broken Bow (34°01′08.04″N, −94°45′24.51″W). Fish were transferred to containers with aerated habitat water and killed with a concentrated tricaine methanesulfonate solution. Gills were removed from the fish, placed in Petri dishes containing 0.9% (v/v) saline, and examined for parasites under a stereomicroscope at 20-30×. A single parasite was picked from the gills with minute needles, placed on a clean microscope slide in 0.9% (v/v) saline, cover-slipped, photographed alive with a Swift model M10 microscope (Microscope Central, Feasterville, PA), and fixed in 10% (v/v) neutral-buffered formalin (NBF). The specimen was permanently mounted on a microscope slide in Gray and Wess medium stained with Gomori's trichrome (Kritsky et al. 1978). Observations were made with an Accu-Scope 300-LED Series phase-contrast microscope (Accu-Scope®, Commack, NY). Digital images were taken with a camera mounted on the microscope. Measurements, in micrometers (μm), were made as presented by Mizelle and Klucka (1953). The specimen was deposited in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln. A host voucher specimen was deposited in the vertebrate collection of Northeast Texas Community College, Mt. Pleasant, TX.

Results

A single gill parasite with characters of the genus *Anchoradiscus* as diagnosed by Mizelle (1941) and Rawson and Rogers (1971) as well as conforming with the morphometric characters of *A. triangularis* described by Summers (1937) and Rawson and Rogers (1971) was found. A morphometric description is provided below.

Comparative Description (Fig. 1)

Body 720 long × 232 wide. Haptor discoidal, diameter 362. Two pairs of pigmented light receptors, anterior pair smaller and closer apart than posterior pair. Pharynx circular, diameter 54. Anchors with large triangular concave base. Dorsal anchors 148–150 long, 110–132 wide. Ventral anchors 150 long, base 114–120 wide. Dorsal bar 186 long. Ventral bar 194 long. Bars articulated by two pairs of knobs near midpoint. Dorsal bar consists of two heavily sclerotized arms and two lamellar lateral accessory plates. Ventral bar consists of two heavily sclerotized arms and two lamellar lateral accessory plates. Hooks 13–17 long. Male copulatory organ and accessory piece indiscernible among vitellaria.

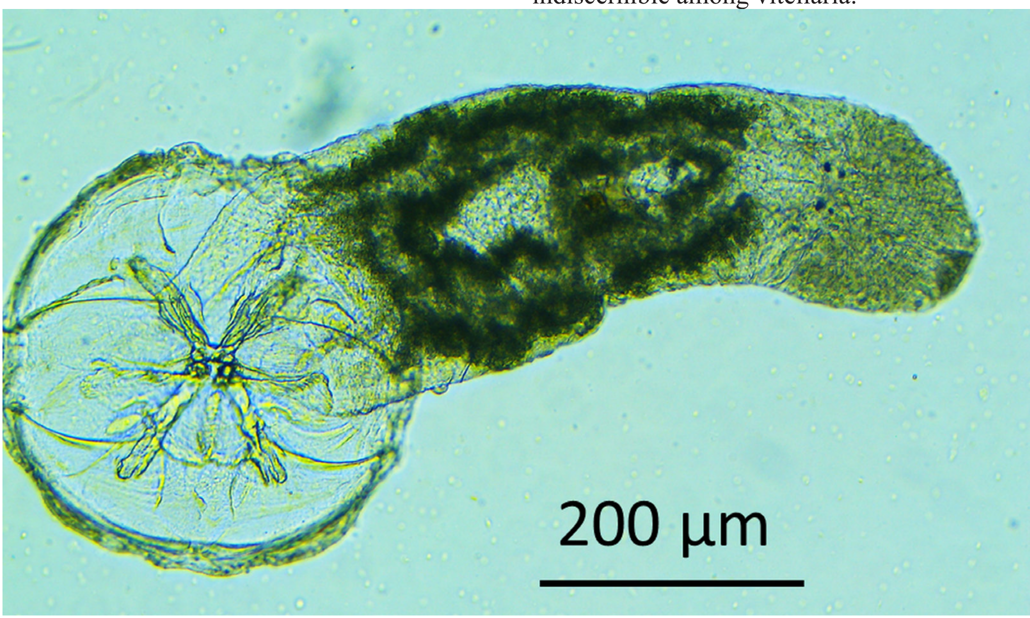

Figure 1. *Anchoradiscus triangularis*. Whole mount (ventral view) of live specimen showing entire haptor and transverse bars.

Discussion

Our specimen of *A. triangularis* conforms to those provided in the original description by Summers (1937) from bantam sunfish, *Lepomis symmetricus* (Forbes) from Louisiana. It has now documented from bluegill from Oklahoma (this report) and *L. macrochirus* from Alabama, Arkansas, Florida, Louisiana, and North Carolina (Table 1). The parasite has only been reported, to date, from other centrarchids, including pumpkinseed, *Lepomis gibbosus* (L.) and redear sunfish, *Lepomis microlophis* (Günther) (Table 1). However, one of us (EML; *unpubl. data*) has examined numerous centrarchids for the presence of monogeneans from Wisconsin waters of the upper Mississippi River and *A. triangularis* has not been observed despite the connected watershed. Nevertheless, it would not be too surprising to see future reports of *A. triangularis* from any of the other nine recognized species of *Lepomis* albeit we have surveyed several longear sunfish, *Lepomis megalotis* (Rafinesque) and green sunfish, *Lepomis cyanellus* Rafinesque from Arkansas and Oklahoma, and none harbored this parasite.

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The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit to CTM.. Usage of trade names does not imply endorsement by the U.S. Government. The findings and conclusions in this article are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

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Larval *Porrocaecum* sp. (Nematoda: Ascaridida: Ascarididae: Toxocarinae) in Northern Cottonmouth, *Agkistrodon piscivorus* (Ophidia: Viperidae) and Plain-Bellied Watersnake, *Nerodia erythrogaster* (Colubridae) from Western Arkansas

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Abstract: During June 2024, a northern cottonmouth, *Agkistrodon piscivorus* and plain-bellied watersnake, *Nerodia erythrogaster* were collected from Polk County, Arkansas, and examined for helminth parasites. Both snakes harbored larval *Porrocaecum* nematodes encapsulated in the dermis of their coelomic cavity and on organ surfaces. This is the first time this nematode genus has been reported to infect these semi-aquatic snakes. A summary of the larval *Porrocaecum* sp. in snakes is provided.

Introduction

Recently, helminth parasites were reported from the western cottonmouth, *Agkistrodon piscivorus* (Lacépède) from Arkansas (McAllister et al. 2023; see citations therein). There are several reports of helminth parasites from the plain-bellied watersnake, *Nerodia erythrogaster* (Forster) (see citations in Ernst and Ernst 2006). However, no report has documented larval *Porrocaecum* sp. from these snakes.

Normally, *Porrocaecum* nematodes belonging to the family Ascarididae Baird, 1853 occur as intestinal helminths of birds (Moravec

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and Kaiser 1995; Atkinson et al. 2009). The type species is *Porrocaecum crassum* Deslongchamps, 1824 which was reported from ducks (Deslongchamps 1824). In the life cycle, ova are eaten by earthworms in which they hatch in the intestine and migrate into blood vessels where they undergo molting and become infective third-stage larvae (Anderson 2000). When consumed by worm-eating avian (vermivore), the larvae are liberated and enter the submucosa of the bird's gizzard and then pass to the small intestine; however, when ingested by other vermivores, they can encyst in body tissues (Anderson 2000).

Larval *Porrocaecum* have been previously reported from fishes, often in the swim bladder (Moravec 1994), and from the dermis, organs, and coelomic cavities of numerous amphibians (salamanders, frogs, and toads) and reptiles (lizards and snakes but no turtles or crocodilians) (see summaries in Bursey and Brooks [2011] and McAllister et al. [2013]). The presence of larvae in any snake suggests that any vermivore could serve as a paratenic or accidental host. Here, we report, for the first time, *Porrocaecum* larvae in two species of semi-aquatic snakes from Arkansas.

Methods

Host collection and processing

On 20 June 2024, a 402 mm snout-vent length (SVL) *A. piscivorous* was collected with snake tong from the Ouachita Mountains Biological Station Pond, Polk County (34°27'43.4484"N, -93°59'54.3264"W). On 22 June 2024, a 475 mm SVL *N. erythrogaster* was collected by hand from the same locale. Both snakes were killed by an intraperitoneal injection of concentrated tricaine-methanesulfonate and a mid-ventral incision was made from the throat to vent. All major organs were removed and placed in Petri dishes containing 0.9% (v/v) saline and examined under a stereomicroscope. Encapsulated nematodes were observed in both hosts in the dermis of the coelomic cavity and on organ surfaces (mainly liver) and preserved in 10% neutral-buffered formalin. Specimens were further examined by placing them on microscopic slides and examined as temporary mounts in glycerol.

Parasites were deposited in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the vertebrate collection of Northeast Texas Community College, Mt. Pleasant, TX.

Results

Larval ascaridid nematodes with characters of the genus *Porrocaecum* Railliet and Henry, 1912 was found in both snakes. A basic generic character of the genus includes the presence of an intestinal cecum but without an oesophageal appendix. The northern cottonmouth harbored five larval *Porrocaecum* sp. (HWML 118838) while the plain-bellied watersnake possessed 19 larval *Porrocaecum* sp. (HWML 118839).

Discussion

McAllister et al. (2013) reported larval *Porrocaecum* sp. from a single adult Ouachita dusky salamander, *Desmognathus brimleyorum* Stejneger from Ouachita County, Arkansas. It represented the first report of *Porrocaecum* from a caudate host as well as from any herptile host in the USA. A summary of the 19 snake hosts of larval *Porrocaecum* sp. is provided in Table 1. The majority of hosts are reported from Costa Rica (n = 15) with single hosts from México and Turkmenistan, including our two new hosts from the USA (Table 1). In terms of snake families, the majority of hosts (n = 17) belong to the family Colubridae with single hosts in the families Elapidae and Viperidae (Table 1).

Table 1. Larval *Porrocaecum* sp. from snakes.

Host Family/Species	Locality	Reference
COLUBRIDAE		
Amastridium veliferum	Costa Rica	Goldberg and Bursey (2004a)
Chironius carinatus	Costa Rica	Goldberg and Bursey (2004a)
Coniophanes fissidens	Costa Rica	Goldberg and Bursey (2007)
Dendrophidion pericarinatum	Costa Rica	Goldberg and Bursey (2004a); Bursey and Brooks (2011)
D. vinitor	Costa Rica	Goldberg and Bursey (2004a)
Drymobius margartiferus	Costa Rica	Goldberg and Bursey (2005)
Erytrolampus bizona	Costa Rica	Goldberg and Bursey (2004a)
Imantodes inornatus	Costa Rica	Goldberg and Bursey (2009)
Leptodeira septentrionalis	Costa Rica	Goldberg and Bursey (2009)
Liophis epinephalis	Costa Rica	Goldberg and Bursey (2004a)
Mastigodryas melanolomus	Costa Rica	Goldberg and Bursey (2006)
Natrix tesselata	Turkmenistan	Velikanov (1982)
Nerodia erythrogaster	Arkansas, USA	This report
Oxybelis brevirostris	Costa Rica	Goldberg and Bursey (2004a); Bursey and Brooks (2011)
Pliocercus euryzonus	Costa Rica	Goldberg and Bursey (2007)
Rhadinea decorata	Costa Rica	Goldberg and Bursey (2007)
Thamnophis valida	México (Sinaloa, Sonora)	Goldberg and Bursey (2004b)
ELAPIDAE		
Micrurus nigrocinctus	Costa Rica	Goldberg and Bursey (2004a)
VIPERIDAE		
Agkistrodon piscivorus	Arkansas, USA	This report

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First Report of *Cotylaspis cokeri* (Trematoda: Aspidogastrea: Aspidogastridae) and *Fornixtrema elizabethae* (Polyopithocotylea: Polystomatidae) from Eastern Musk Turtle, *Sternotherus odoratus* (Testudines: Kinosternidae), with an Additional Records for *Polystomoidella oblongum* (Polystomatidae) and *Hapalorhynchus* sp. (Digenea: Schistostomatoidea), from Southeastern Arkansas

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Abstract: During June 2014, two eastern musk turtles, *Sternotherus odoratus* (Latreille *in* Sonnini and Latreille) were collected in Cane Creek Lake, Lincoln County, Arkansas, and examined for parasites. Found were an aspidogastrid, *Cotylaspis cokeri* Barker and Parson, 1914, in the intestine, a polystome, *Fornixtrema elizabethae* (Platt, 2000) Du Preez and Verneau, 2020 in the conjunctival sac of the eye, and *Polystomoidella oblongum* (Wright, 1879) Price, 1939 in the urinary bladder. In addition, a *Hapalorhynchus* sp. occurred in visceral wash and mesenteric blood vessels of both turtles. The former two taxa represent new host and geographic records for these parasites, and *P. oblongum* and *Hapalorhynchus* sp. are reported for the first time in Arkansas.

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Introduction

The eastern musk turtle or stinkpot, *Sternotherus odoratus* (Latreille *in* Sonnini and Latreille) is a common kinosternid species that ranges from southeastern Ontario, Canada, New England, and Wisconsin, south to southern Florida and west to Texas (Powell et al. 2016). In Arkansas, *S. odoratus* occurs statewide in almost any type of still or sluggish watershed that has a soft substrate such as ditches, lakes, oxbows, ponds, sloughs, and streams (Trauth et al. 2004). It is an omnivorous bottom feeder. A good bit of information is available on its natural history and ecology (Iverson and Iverson 1980; Ernst 1986), including data on several parasites (Ernst and Ernst 1975, 1979; McAllister et al. 2013, 2016; and others). Here, we add two additional parasite species to its host list as well as providing new geographic distributional records for each taxon.

Methods

Host collection

On 28 June 2014, two adult *S. odoratus* (carapace lengths [CL] = 80, 100 mm) were collected with baited hoop nets from Cane Creek Lake at Cane Creek Lake State Park, Lincoln County, Arkansas (33°55′01″N, −91°45′55″W). They were placed individually in collection bags, returned to the lab, measured for carapace length (CL) (mm) and processed within 24 hr for parasites.

Processing

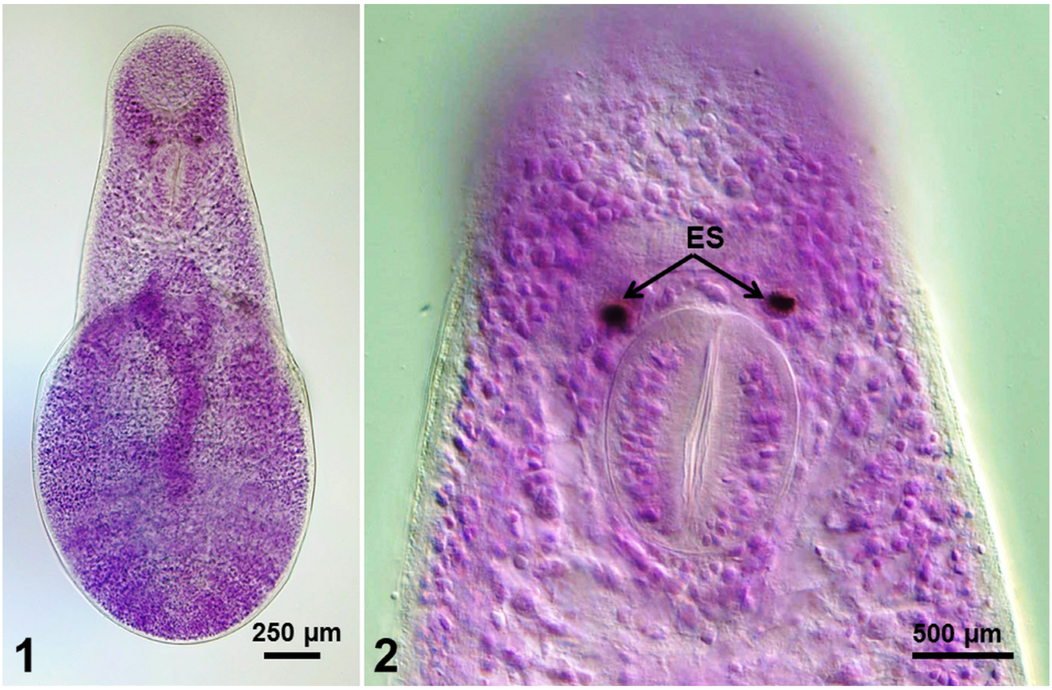
Turtles were killed with an intraperitoneal injection of a concentrated solution of tricaine methanesulfonate. Their mouth was examined as well as the conjunctival sacs of the eyes for polystomatid monogeneans. A bone saw was used to remove the plastron and a visceral wash from the blood vascular system of each was processed using a 7.0 g/L sodium citrate saline solution (to avoid blood clotting) following methods of Snyder and Clopton (2005). All internal organs from the throat to the vent was removed and placed in individual Petri dishes containing 0.9% (v/v) saline and examined. The entire intestinal tract was split lengthwise and cut into manageable sections (~50 mm) for examination of gastrointestinal parasites under a stereomicroscope. Four different parasites were found, heat-fixed in nearly boiling tap water without coverslip pressure, and placed in 95% DNA grade ethanol. They were later stained with acetocarmine, cleared in methyl salicylate, and mounted in Canada Balsam.

Voucher Specimens

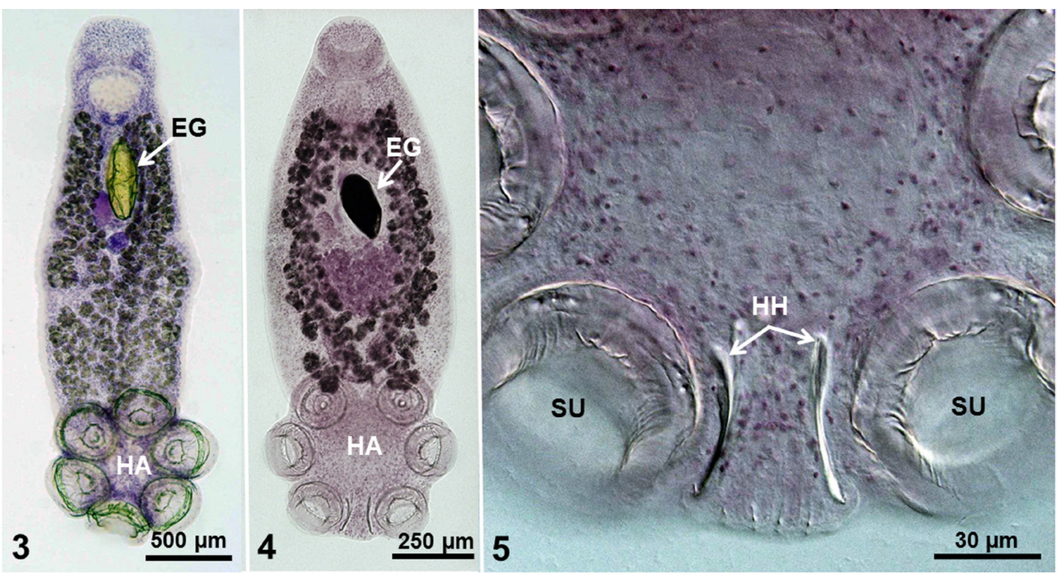
Parasite specimens were deposited in the Harold W. Manter Laboratory (HWML), University of Nebraska, Lincoln, Nebraska; some were retained for future molecular analyses. Host voucher specimens were deposited in the vertebrate collection of Northeast Texas Community College, Mt. Pleasant, TX. All turtle common and scientific names follow the Turtle Taxonomy Working Group (Rhodin et al. 2021).

Results

Three monogenean parasites were recovered, several aspidogastrid polystomes, *Cotylaspis cokeri* (Figs. 1–2) from the intestine matching the description of Barker and Parsons (1914), two *Fornixtrema* (=*Neopolystoma*) *elizabethae* (Fig. 3) from the conjunctival sacs conforming with the morphometric characters described originally described by Platt (2000) and redescribed by Du Preez and Verneau (2020), and a one and 57 individual *Polystomoidella* (=*Polystoma*) *oblongum* (Figs. 4–5) originally described by Wright (1879) from the urinary bladder of both turtles matching the description of Price (1939). In addition, both turtles harbored a *Hapalorhynchus* sp. from the visceral wash and mesenteric blood vessels.



Figures 1–2. Aspidogastrid, *Cotylaspis cokeri* from *Sternotherus odoratus*. (1) Entire worm. (2) Higher magnification showing eyespots (ES).



Figures 3–5. Polystomes from *Sternotherus odoratus*. (3) *Fornixtrema elizabethae* showing egg (EG) and six haptoral suckers (Type III of Du Preez and Theunissen [2021]) on haptor (HA). (4) *Polystomoidella oblongum*, entire worm showing egg (EG) and haptor (HA) with three pairs of haptoral suckers (Type III). (5) Higher magnification of haptor of *P. oblongum* showing large haptorial hooks (HH) and haptoral suckers (SU).

Discussion

Aspidogastrean polystomes are a small assemblage of flatworms with a cosmopolitan distribution. They are characterized by possessing a ventral holdfast organ with rows of alveoli or suckerlets, or just presenting a row of rugae or suckers (Rohde 2024). They infect molluscs as obligate hosts and various vertebrates (fishes, turtles) as facultative or obligate final hosts (Rohde 2024). Previous chelonian hosts of the families Trionychidae and Emydidae, respectively, of *C. cokeri* include: *Apalone ferox* (Schneider) and *Graptemys flavimaculata* Cagle, *Graptemys geographica* (Lesueur), and *Graptemys pseudogeographica pseudogeographica* (Gray) from Florida, Iowa (type locality), Mississippi, and Ohio (see summary in Alves et al. 2015). In addition, *C. cokeri* has been reported from paddlefish, *P. spathula* Bonaparte from Mississippi (Hoffman 1999). We document an additional family, Kinosternidae, as a host of this parasite.

De Preez and Verneau (2020) provided a revised classification of parasitic flatworms that infect the conjunctival sac of chelonians, introducing three new genera: *Apaloneotrema*, *Aussietrema*, and *Fornixtrema*, based on detailed morphological comparisons of these parasites across various turtle taxa. *Fornixtrema elizabethae* was originally described by Platt (2000) from the western painted turtle, *Chrysemys picta belli* (Gray) from Indiana, Michigan (type locality), and Wisconsin.

Polystomoidella oblongum was originally described by Wright (1879) from the urinary bladder of *S. odoratus* from Canada. It has also been reported from additional chelonian hosts from Iowa, Maryland, North Carolina, Texas, and Virginia, and México (Stunkard 1917; Price 1939; Mendoza-Garfias et al. 2017). We report this parasite from Arkansas for the first time, and from west of the Mississippi River for the second time.

Several *Haplorhynchus* sp. was found in the visceral wash of the blood vascular system. These blood digeneans are commonly found in turtles (Ernst and Ernst 1977), including *H. reelfooti* (Byrd, 1939) Platt and Snyder, 2007 from *S. odoratus* from Alabama, Indiana, Tennessee, and Virginia, (Byrd 1939; Platt and Snyder 2007; Roberts et al. 2017). To our knowledge, there are no previous reports of species of *Haplorhynchus* from an Arkansas chelonian host so we document a new distribution record and the second from west of the Mississippi River (see McAllister et al. 2015). Specimens are being retained for molecular analyses (V. V. Tkach, *pers comm*).

Although numerous parasites have been reported previously from *S. odoratus* over the past century or more, this is the first time either *C. cokeri* or *F. elizabethae* has been documented in this host. In addition, both of these parasites as well as *P. oblongum* and *Hapalorhrynchus* sp. are reported from Arkansas for the first time. This brief survey shows, even with a small sample size (n = 2), that it is possible to discover novel parasitological information in a well-examined turtle.

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Enteric helminth infections in the red-eared slider turtle *(Trachemys scripta elegans)* from southern Oklahoma

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Abstract: Red eared sliders (n=50) were collected from four southern Oklahoma counties and one northern Texas county and examined for intestinal helminths. Eleven parasite species representing 3 phyla were discovered with no significant differences in parasite load occurring between locations or habitat types. The nematodes *Camallanus* sp, *Spiroxys* sp and the acanthocephalan *Neoechinorhynchus* sp. were the most prevalent helminths collected from the stomach and small intestine. Large intestine was also heavily parasitized primarily by the nematodes *Spironoura* sp., *Camallanus* sp. and the acanthocephalan, *Neoechinorhynchus emydis.* Minimal infections were detected from the urinary bladder and lungs. Total parasite load was positively correlated to carapace length and this relationship was also discovered for some individual species such as *Neoechinorhynchus emydis and N. psuedomydis.* The results of this study were compared to an older parasite study of sliders in Oklahoma with some notable differences in types of parasites, parasite load and organs affected.

Introduction

The red-eared slider (*Trachemys scripta elegans*) is a common, widely distributed turtle in the U.S. (Conant 1975, Ernst et al. 1994), ranging throughout Oklahoma (Sievert and Sievert 1993) and much of central through eastern Texas (Hibbitts and Hibbitts 2016), occupying a variety of aquatic habitats. The species has been studied extensively regarding life history (e.g., Cagle 1950, Gibbons 1990), food habits (e.g., Clark and Gibbons 1969, Hart 1983), nesting biology (e.g., Tucker 2000, Tucker 2001), genetics (e.g., Tucker et al. 1995, Lovich et al 1990, Thomas et al. 2020), and physiology (e.g., Hutton 1957, Reyes and Milsom 2010). However, only a few studies exist that characterize the incidence of parasitic infections in the species. For instance, McAllister and Upton (1988) studied infections of the protozoan *Eimeria* in Texas, Marquardt (1978) studied

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helminth infections in Arkansas, while Harwood (1931), and Everhart (1956, 1957), reported on helminth infections from Oklahoma. Since such a limited amount of data exists on the prevalence of parasitic infections in red-eared sliders from Oklahoma, the focus of this study was to assess helminth infections of red-eared sliders collected from five southern Oklahoma counties and one northern Texas county,

Methods

A total of 50 red- eared sliders were collected from 8 March through 21 August, 2005 from four southern Oklahoma Counties (Pontotoc, n=21; Garvin, n=2, Seminole, n=7, Pittsburg, n=13), and one north Texas county (Jack, n=5). Turtles were collected from one lake (Wintersmith Lake in Pontotoc County, Ok) several farm ponds and roadside canals either by hand or using chicken-wire funnel traps (Iverson 1979) baited with sardines or chicken livers. On capture, each specimen was numbered using the multiple peripheral scute notching method (Cagle 1939), then sexed, measured (plastron length and width) and weighed. After euthanization, each specimen was checked for ectoparasites before the plastron was separated from the carapace by cutting across the bridges. After peritoneal removal and inspection of cavities for cysts or free parasites, the organs were separated and placed in containers with physiological saline. The body cavity was then rinsed with 2% saline and both the washings and shells were examined for parasites. All organs were inspected using methods as described in Everhart (1956) and Schmidt (1971). Histozoic organs (heart, kidneys, spleen, liver, gall bladder) were inspected by teasing apart the tissue under a dissecting microscope and repeatedly washing in saline until clear. Coelozoic organs (alimentary canal, lungs, urinary bladder) were opened by a longitudinal slit to remove any parasites. To loosen any embedded parasites attached to the mucosal wall, a scapel was used for scraping. Lastly, each organ was repeatedly washed in saline and the washing examined for parasites. Total number of individual parasites per host and their specific location was recorded and each was placed in vials of 10% formalin for fixation.

Small to medium-sized trematodes and some hookworms were stained with Lynch's precipitated borax carmine method followed by a fast green counterstain (Galigher and Kozloff 1971). Larger trematodes and most nematodes were stained using Partsch's alum cochineal followed by a fast green counterstain.

Acanthocephalans were stained using Hoyer's Mounting Medium mixed with Giemsa stain (ratio of 19:1) (Everhart 1956, 1957; Galiher and Kozloff 1971), cured in a 45^{o} C oven for 2 weeks, then each slide was sealed with fingernail polish to keep out moisture. Some of the more delicate trematodes were prepared using Hoyer's mounting medium with aceto-carmine stain added to the mixture at a ratio of 1 to 10 parts Hoyer's (Everhart 1956, 1957). All parasite species were identified using the keys of Yamaguti (1958, 1961, 1963a, 1963b), Anderson et al (1974), Daily and Meyer (1996) Amin (2002) and Barger and Nickol (2004).

Before performing statistical analysis on the data using parametric statistical methods, all counts and measurements were log transformed to reduce the effects of unequal variances (Zar 2010).

Results

Eleven species of enteric helminths (from three phyla) were found in 50 turtles ($\bar{x}$= 481 per host) (Table 1). An analysis of variance indicated a significant difference in parasite load comparing geographic locations (4 Oklahoma counties, and 1 Texas County) ($P < 0.01$), but no differences in total number of species between locations ($P=4.01$). There was also no significant difference comparing aquatic habitats (lake vs pond vs roadside canals) in total parasites species per host ($P > 0.248$), and total number of species within each habitat ($P= 0.051$). A regression analysis showed no correlation of overall parasite abundance to date of capture ($P= 0.514$), but total number of parasite species infecting hosts was correlated to date of host capture ($R^2=0.04$, $F=0.15$, $P < 0.05$). In comparing parasite prevalence between male (n=19) and female (n=31) turtles, there was no significant difference ($X^2= 5.08$, $P= 0.6507$), even though some of the most heavily parasitized individuals were females.

Table 1. Prevalence, mean densities and organ infestations of parasitic helminths from *Trachemys scripta elegans* from Oklahoma (n= 4 counties, 45 turtles) and Texas Counties (n=1 county, 5 turtles).

Parasite	no. collected	% infected turtles	x ± SE	primary organ
Acanthocephala				
Neoechinorhynchus psuedomydis	12,937	57	453.9 ±392.6	small intestine (99.7%)
Neoechinorhynchus emydis	5,154	63	163.6 ±196.4	small intestine (99.0%)
Nematoda				
Camallanus sp.	3,062	92	66.6 ± 78.7	small intestine (94.2%)
Spiroxys sp.	1,278	78	32.0 ±40.3	stomach (92.2%)
Spironoura sp.	1,101	47	45.9 ±66.9	large intestine (67.1%)
Strongyloides sp.	1	2	1.0 ±0.5	small intestine (100%)
Digenea				
Telorchis sp.	456	22	41.5 ±64.7	small intestine (99.5%)
Schizamphisomoides sp.	24	14	3.4 ±1.2	large intestine (83.3%)
Spirorchis emydis	6	10	1.2 ±0.5	lungs (100%)
Diarmostorchis blandingi	3	2	3.0 ± 1.5	lungs (100%)
Monogenea				
Polystomoides coronatum	24	16	3.0 ±3.3	bladder (100%)

Nematodes such as *Camallanus sp.* (92%) and *Spiroxys sp.* (78%) and acanthocephalans of the genus *Neoechinorhynchus* (2 species) (60%) were the most prevalent enteric parasites, followed by the nematode *Spironoura sp.* (47%), a digenetic trematode, *Telorchis sp.* (22%), a monogenetic trematode, *Polystomoides sp.* (16%) and the digenetic trematode, *Schizamphistomoides sp.* (14%). All other parasites identified, *Spirorchis sp., Diarmostorchis sp.,* and *Strongyloides sp.* were recovered from 10% or less of the hosts sampled. All 50 turtles examined were infected with at least one parasite species, and all but two had multi species infections (range 2-8). The small intestine was the most heavily infected organ (n=21,797), primarily with *Neoechinorhynchus psuedomydis* (59.1%), *Neoechinorhynchus emydis* (23.3%) and *Camallanus sp.* (13.2%) (Table 1), and contained the highest number of parasites species per host (ẍ=3.0; range 2-5). The acanthocephalans, *Neoechinorhynchus emydis* and *N. psuedomydis* co-inhabited 28% of hosts with a heavy burden of individuals in the small intestine. However, the presence of one did not appear to affect the presence of the other as there were no difference in the population sizes of the two species in the intestines of co-inhabited turtles (t=0.429, P > 0.05).

The stomach was also heavily parasitized (n=1,355) with 80.4% of turtles containing stomach worms (ẍ=33), primarily *Spiroxys sp.* (87.0%), *Camallanus sp.* (12.3%), and *Neoechinorhynchus emydis* (0.51%). The large intestines contained 860 worms (ẍ=27), primarily *Spironoura sp.* (85.8%), *Neoechinorhynchus emydis* (5.3%), *Neoechinorhynchus psuedomydis* (4.0%), and *Schizamphistomoides sp.* (2.3%), with *Camallanus sp., Spiroxys sp.*, and *Telorchis sp.* making up less than 1% of the remaining sample.

The organs with the least worm burden was the urinary bladder (n=23), with 17.6% of turtles infected followed by the lungs (n=10) with 13.7% infected. The only parasite found in the urinary bladder was *Polystomoides coronatum* while the lungs were infected with the digenetic trematodes, *Spirorchis sp.* (60%), *Diarmostorchis sp.* (30%) and *Heronimus sp.* (10%). Only one individual turtle in the sample contained a dual infection of *Heronimus sp.* and *Spirorchis sp.*

Using simple linear regression to examine relationships to carapace length (ẍ=16.76 cm, range 4.4-30.4), we found a significant positive relationship of total parasite species load to carapace length in female turtles (R^2= 0.448, F=21.13, P < 0.001) (Fig. 1), but not in males (R^2= 0.053, F= 0.913, P > 0.05). For individual parasite species, a significant positive relationship of parasite load to carapace length was found for *Neoechinorhynchus psuedomydis* in female turtles (R^2= 0.479, F=14.75, P < 0.001) (Fig. 2) but not in males (R^2=0.093, F=1.89, P = 0.181 *). For N. emydis*, a significant positive relationship occurred in males (R^2=0.661, F=9.75, P < 0.05) (Fig. 3), but not in females (R^2= 0.048, F= 0.919, P = 0.350). Although other parasite species exhibited a positive correlation to carapace length (primarily in female turtles), none of these were significant (P > 0.05). Only *Diarmostorchis sp.* exhibited no correlation between parasite abundance and carapace length.

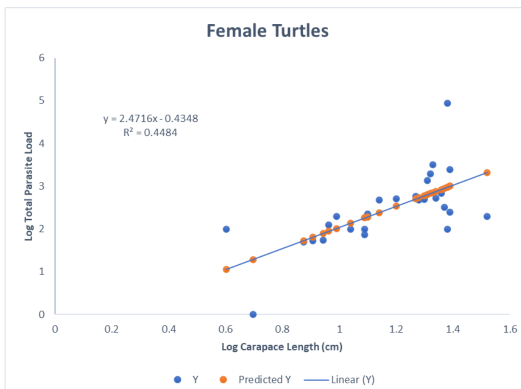

Figure 1. Parasite load plotted against carapace length for female red-slider turtles in Oklahoma (n=30).

Lastly, we compared the results of this study to the Oklahoma study of Everhart by parasite phyla and location in the host (Everhart 1956) (Fig. 4) and by the abundance and percent prevalence of infection (Everhart 1957) (Table 2). Helminth prevalence was considerably higher in this study with several multi-species infections for the red-eared slider, particularly *Neoechinoryhynchus emydis* and *N. psuedemydis* in the small intestine.

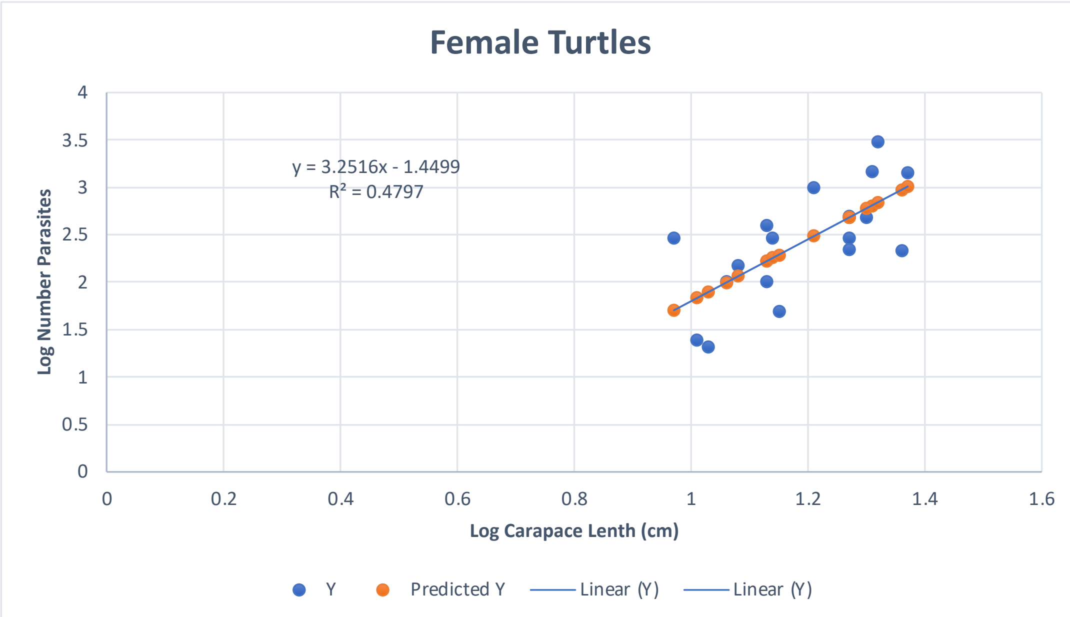

Figure 2. Relationship of parasite load to carapace length for *Neoechinorhynchus psuedomydis* in female turtles.

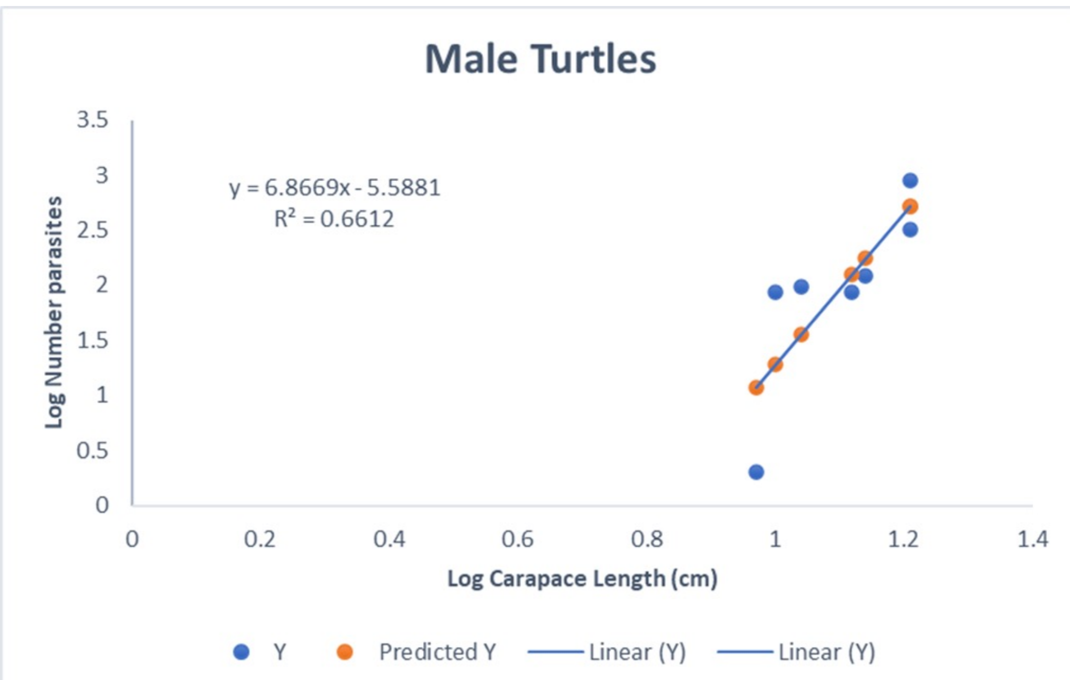

Figure. 3. Relationship of parasite load to carapace length for *Neoechinorhynchus emydis* in male turtles.

Parasite	Everhart 1957			this study		
	abundance (n)	prevalence (%)	ẍ (per host)	abundance (n)	prevalence (%)	ẍ (perhost)
Monogenea:						
Polystomoides coronatum	68	82.6	nr	24	16	3.0
Digenea:						
Telorchis sp.	802	30.6	52.5	456	22	41.5
Spirorchis sp.	28	8.3	5.0	6	10	1.2
Nemotoda:						
Camallanus sp.	1486	74	18.0	3062	92	66.6
Spiroxys sp.	no adults recovered only encysted larvae reported			1278	78	32.0
Acanthocephala:						
N. emydis	377	47.8	nr	5154	63	161.1

nr= not reported

Table 2. Prevalence of same parasites reported by the Oklahoma study of Everhart (1957) (n=23 turtles) compared to the results of this study (n=45 turtles).

Discussion

The prevalence of helminth infection was considerably higher in this study compared to the Oklahoma study of Everhart (1956, 1957). Similarly, the study of Rosen and Marquardt (1978) found infections of four acanthocephalan species of red- eared sliders in Arkansas. These findings do question whether competition occurs between these acanthocephalans and if it does, it may be evident in some of their shared intermediate hosts, particularly in the altering of host phenotype and behavior (Dezfuli et al. 2001, Rauque and Semenas 2011) or by occupying separate microhabitats within either the intermediate or definitive host. Though *N. emydis* was a slightly more frequent infection (63%) compared to *N. psuedemydis* (57%), no significant difference in mean population size of the two species occurred in co-inhabiting hosts. While we did not determine microhabitat differences between these two species when co-inhabiting hosts, we did find that while both species occasionally occurred in the large intestines, only *N. emydis* was found in the stomachs of a few hosts that were co-inhabited. Further studies about competing effects in these acanthocephalan species is needed.

Since most turtles carried a heavy infection of both species of *Neoechinorhynchus*, it's possible that large numbers of aquatic plants, could provide adequate habitat to support a substantial number of intermediate hosts like ostracods or gastropods (Esch et al. 1979). Even though there was no analysis of aquatic plants in this study, they were quite prevalent within most of the habitats where turtles were collected (pers. observation). Also, as turtles mature, their diet tends to shift towards herbivory (Clark and Gibbons 1969, Ernst and Barbour 1972), and this might explain why parasite load correlates to carapace length in females with *N. psuedomydis* and in males with *N. emydis*. If intermediate hosts are more prevalent among aquatic plants, then foraging turtles shifting to herbivory as they mature (Hart 1983), could be exposed to more infected intermediate hosts as they consume plants. The correlation of parasite load to shell length has been reported for other turtle species as well (e.g., Texas map turtle, *Graptemys versa*, Lindemann and Barger 2005), even though plastron was measured instead of carapace.

Other multi species infections were also observed in the stomachs (*Camallanus sp*. and *Sproxys sp*.) and large intestines (*Spironoura sp*., *N. emydis*, *N. psuedemydis* and *Schizamphistomoides sp*.) and rarely the lungs (one individual host with *Spirorchis sp*. and *Diarmostorchis sp*.) but these were much less common compared to the small intestine. The trematode *Spirorchis sp*. is primarily reported from circulatory organs (Williams 1953, Holliman and Fisher 1968) with some species in the brain (Platt 2000). While lung infections by certain species do occur (Roberts et al. 2016), there are scant reports in the literature for the species *Spirorchis emydis* and *Diarmostorchis* sp. Although the study of Oklahoma red-eared sliders by Everhart (1957) does not report any data for muli-species infections, some of the same species and/or genera were recovered, so its highly likely co-inhabitation of the same organs did occur. Further comparisons to Everhart (1956, 1957) were somewhat difficult since the data was not collected and reported as in this study. However, we were able to make some phyla and species comparisons and there were notable differences in the abundance of acanthocephalans, particularly of organs like the stomach and large intestine, which were not reported in the turtles examined by Everhart (1956, 1957). The present study reports far more nematode infestations in the stomach, small intestine, and large intestine but no infestation of the mouth, esophogus and heart as reported by Everhart (1956, 1957). Are these differences an indication of a shift in parasite prevalence in the host over time, or indicative of a change in the type and/or abundance of intermediate hosts? The answer lies with more intensive studies on red- eared slider enteric parasitism.

Acknowledgements

This paper is dedicated to the memory of Carol L. Bratt, student, colleague, and friend.

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ABSTRACTS OF THE 113TH OKLAHOMA ACADEMY OF SCIENCE TECHNICAL MEETING NOVEMBER 1, 2024 UNIVERSITY OF OKLAHOMA HEALTH SCIENCERS CENTER, OKLAHOMA CITY, OKLAHOMA

(sorted by presenter's last name)

A STUDY OF THE HIGH TEMPERATURE REACTION OF TITANIA, SILICA, AND YTTRIA

Hunter Allen and Dwight Myers, East Central University

The objective of this research is to investigate and catalog how titanium and silicon oxides (TiO_2, SiO_2) react when a ternary (third) metal is introduced at high temperatures. Previous work in this group confirmed that TiO_2 and SiO_2 do not react with one another at high temperatures. It is well known however that adding a third metal oxide can initiate a reaction between titania and silica at high temperatures. One example is the reaction caused by adding calcium oxide to form titanite ($CaTiSiO_5$). Our current ternary metal under investigation is yttrium. Other metals may be included in this study given an adequate time frame. Mixed oxide samples have been heated in a furnace at 1300°C. Sample characterization has been performed by X-ray diffraction. Results to date will be presented.

EXPLORING THE EXPRESSION PROFILE OF HIPSC-BMECS FROM MULTIPLE SCLEROSIS PATIENTS IN A BULK-RNASEQ DATASET

Evonn Darkoaa Annor, Oral Roberts University

Outstanding Undergraduate Paper in Biomedical Sciences

Multiple Sclerosis (MS) is a chronic disease affecting the central nervous system, where the immune system mistakenly attacks the myelin sheaths of nerve fibers, leading to slower nerve impulses, nerve damage, and signal distortion or blockage. A key feature of MS is the disruption of the Blood-Brain Barrier (BBB), which includes brain microvascular endothelial cells (BMECs). Developing BMEC lines can model healthy and diseased BBB conditions, facilitating the study of inflammatory changes. One such application of this is used in this experiment where hiPSC-derived BMECs and MS hiPSC-derived BMECs were developed using a new protocol. In this study, datasets of healthy BMECs and MS BMECs under normal and stimulated conditions (4 hours with TNF/IFN-γ) were examined. Additionally, healthy cells (HC) under normal and inflammatory conditions were also compared due to the importance of understanding cellular processes under inflammatory conditions. The datasets were reformatted for RStudio and organized in CSV files. These values were processed in the Integrated Differential Expression and Pathways Analysis (IDEP) to generate visualizations. DESEQ2 outputs were further analyzed in RStudio using various R packages including Bioconductor and clusterProfiler. Public data records were compared to primary and iPSC-derived BMECs, analyzed in IDEP to determine enrichment, and in GEO2R to generate visualizations and identify differentially expressed genes. Raw counts of healthy cells under normal and inflammatory conditions were placed in the Nucleic Acid Sequence Analysis Resource (NASQAR) to visualize differential expression of these groups. RStudio visualizations were compared to public datasets, showing differing enrichment patterns. The differential expression of MS and HCs revealed regulation of leukocyte chemotaxis and vascular smooth muscle cell proliferation were prominent. In inflammatory conditions, adaptive immune response, leukocyte mediated immunity and lymphocyte mediated immunity were also shown to be significant. These iPSC-derived BMECs do capture the key features we hope to see in primary BMECs.

DESIGN AND MANUFACTURE OF A VERTICAL AXIS WIND TURBINE FOR THE UNIVERSITY OF CENTRAL OKLAHOMA SCHOOL OF ENGINEERING MAKER-SPACE

Nelson Clements, Zeb Jandt, and Ben Drumm, University of Central Oklahoma

Outstanding Undergraduate Paper in Engineering Sciences

The UCO School of Engineering has expressed the desire to install a functional and aesthetic wind turbine on the roof of the new Makerspace building as a multi-purpose tool for recruiting new students, educating students on the increasingly important field of renewable energy, and to demonstrate the advanced manufacturing capabilities of the department. Wind energy production is particularly relevant to UCO graduates as wind energy provides the largest share of Oklahoma's net energy production and Oklahoma ranks third overall among all states in total amount of wind energy produced in the nation. Through research of the existing ideal vertical axis wind turbine design parameters and the use of rapid prototyping, this project will produce a small-scale prototype wind turbine and investigate the methods of production for a full-scale turbine. This project utilizes an axial flux generator to produce AC electric power which is rectified and stored in a large battery bank. Findings show that the most effective blade design utilizes a scale that is unfeasible in smaller prototypes, and therefore we must adjust our blade design to be an appropriate size. We have also found the small-scale vertical axis wind turbine in question does not have a large enough area to capture a sizable amount of energy from the wind, so the limitations must be kept in consideration. Utilizing a combination of design ideals from cutting-edge research publications, this vertical axis wind turbine combines aesthetic and efficiency considerations to result in a functional device to represent the School of Engineering.

REAL TIME AUDIOBOOK CREATOR

Annalise Dorety, Kien Nguyen, and Cobey Hixon, University of Central Oklahoma

Outstanding Undergraduate Poster

15% of all Americans are dyslexic, which impairs their ability to effectively use written information. The inability to keep up with peers creates a gap between students which takes years of specialized learning to correct. With the Real Time Audiobook Creator, listeners would be given a new chance to not only learn; but, add their findings to any subject. A small camera set up on a headband apparatus would scan the open page of a book or any text. Next a script would be created where an AI voice would output a humanistic sounding audio clip. A small earbud would connect to the user's ear creating a more discrete experience. Once all of the text on a page has been read, a soft sound will indicate the need for turning to the next page. Our product could be mass produced and sent to schools across the United States to create alternative and more inclusive learning experiences. Children learning to read would always have someone to read to them. People learning the English language would get a real time phonetic example of words and phrases. Those who develop or already have blindness would get the ability to experience the joy of reading a new book. These and so many others would have the resources to learn like neurotypicals or people with unimpaired vision do every day. With our product every article, essay, storybook, novel, textbook, and paper would become instantly available to anyone that wishes to learn.

FLUORESCENTLY LABELED UROPATHOGENIC E. COLI AS A WAY TO STUDY PATHOGENESIS

Victoria Espinoza[1], Tram-An Ho[2], Alejandro Lopez[1], and Janaki Iyer[1], [1]Northeastern State University; [2]Tulsa Community College

Outstanding Undergraduate Paper in Microbiology

Bladder cancer is the fourth most common cancer among men in the United States. The five-year survival for carcinoma in situ bladder cancer is 97% but this rate dramatically drops to 8% when the cancer becomes metastatic. Surgery, chemotherapy, and radiotherapy are common treatment options but more bladder cancers are becoming resistant to some of these strategies, thus highlighting the need for new therapies. Uropathogens are organisms that infect different organs of the urinary tract including the bladder. We previously found that *E. coli* strain CFT073 causes dramatic changes to bladder cells' shape indicative of cell stress and death in bladder cancer cells. To gain more insight into this process, we want to characterize the interactions of *E. coli* strain CFT073 with bladder cancer cells. We hypothesize that *E. coli* strain CFT073 adheres to bladder cancer cells resulting in cell death of bladder cancer cells. To test this hypothesis, we made chemically competent *E. coli* CFT073 and transformed a pGFP, a plasmid bearing the GFP gene. The expression of GFP in the bacteria was confirmed by fluorescence microscopy and we are now comparing the properties of fluorescently labeled *E. coli* CFT073 with untransformed *E. coli* CFT073. We will evaluate if the fluorescently labeled *E. coli* CFT073 can cause morphological changes to bladder cancer cells upon infection and determine the adherence to bladder cancer cells by confocal microscopy. Our findings will aid us in understanding how *E. coli* CFT073 causes damage to bladder cancer cells and enable us to identify factors produced by *E. coli* CFT073 that can be used as potential therapeutics.

ISOLATION AND IDENTIFICATION OF TWO NOVEL GENERA CAPABLE OF BREAKING DOWN TWO ANTHROPOGENIC CONTAMINANTS FROM LOCAL WASTEWATER

Jacobey King, Sam Miller, Nam Lu, Ralph Tanner, and Paul A. Lawson, University of Oklahoma-Norman)

As annual human production and intake of synthetic compounds surge alongside an ever-growing industrialized world population, anthropogenic contaminants have become harder to ignore. Some of these, such as sucralose, are relatively unchanged by their passage through wastewater treatment plants and make their way into distribution system water. Research into the impact of sucralose on human health has revealed mounting concerns, particularly about products formed under thermal degradation. The ability to break down two contaminants, sucralose and nitrilotriacetic acid, is rare among bacteria yet does happen at a quantifiable magnitude in wastewater. Which members of the community are undertaking this natural bioremediation is largely nebulous, with anaerobic degradation of sucralose being undocumented entirely. Taking great care to create enrichments where microbial communities from wastewater are forced to break down sucralose or nitrilotriacetic acid as the sole substrate allows for the eventual identification of microbes which are capable of this catabolism. Anoxic enrichments with the described conditions were conducted on a wastewater inoculum. Subsequent isolation yielded three strains of two novel species belonging to two novel genera, as assessed through a polyphasic approach. Strains 9ST and NTA5 are Gram-negative-staining rods which make up two closely related strains of a bacterial species able to grow on a meticulously crafted medium featuring sucralose as the sole substrate, and belong to the proposed taxon *Purgationimicrobium sucraloseivorans* gen. nov. sp. nov. located in the family Azonexaceae. Strain NTA1T is a Gram-positive-staining coccobacillus comprising the proposed taxon *Quisquiliimicrobium normanense* gen. nov. sp. nov. located within the family Actinomycetaceae.

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ELUCIDATING THE EFFECTS OF DOWNREGULATION AND OVEREXPRESSION OF CELL WALL BIOSYNTHESIS GENES ON STOMATAL DYNAMICS

Qimeng Li, Dr, Petrik, Brant Osbone, Maricela Espinosa, Northeastern State University

Outstanding Graduate Poster

Stomata, which are tiny pores on the surfaces of plants, allow intake of carbon dioxide for photosynthesis and release of water vapor for evaporative cooling. In grass plants, stomata are composed of two guard cells flanked by two subsidiary cells. To gain a better understanding of the stomata (guard and subsidiary cells), eleven cell wall biosynthesis genes that are expressed in stomatal cells of *Brachypodium distachyon* are being studied. These genes are involved in cellulose (CESA1, CESA3, CESA6), xylan (GT43B2, XAX1a/1b, GUX1, GUX2, GUX1/2, TBL29), and pectin (GAUT1, GAUT4) synthesis. Artificial microRNAs targeting each gene are being cloned into constructs under the expressional control of guard or subsidiary cell-specific promoters, allowing a decreased expression of each targeted gene by RNA interference. For overexpression of these genes, cDNAs of 7 essential genes will be PCR amplified from WT *Brachypodium distachyon* or synthesized by Thermofisher, followed by their cloning into overexpression constructs. Positive clones are identified by restriction enzyme digestion and DNA sequencing before being transformed into *Brachypodium distachyon*. These clones will be transformed using different methods of *Agrobacterium tumefaciens*-mediated plant transformation, such as the cut seed method or the callus method. Adult transgenic plants will then undergo a series of tests to identify stomatal differences between the transgenic lines exhibiting decreased expression of cell wall biosynthesis genes and wildtype control plants. Currently, stomata cell walls in plants have not been thoroughly studied. Through these experiments, we can better understand why the stomatal cells of plants show a faster response and rate of stomatal opening and closing than dicot plants, whose cell walls differ in chemical composition. This may allow the development of cereal crop plants with greater heat and drought tolerance.

CULTIVATION OF FASTIDIOUS ANAEROBIC BACTERIA ON CLARIFIED GUANO FILTRATE SUPPLEMENTED MEDIA

Nam Lu[1], Elizabeth Walker[1], Amber Nguyen[1], Jacobey King[1], Samuel Miller[2], and Paul A. Lawson[1], [1]The University of Oklahoma-Norman; [2]Oklahoma State University-Stillwater

Cultivation an essential tool for microbiology that provides access to the characterization of an organism's morphological, biochemical, physiological and chemotaxonomic traits. Although bats account for ~21% of mammal species, only a small fraction of all microorganisms have been identified and described, and even fewer have been successfully recovered from bat guano. In this study, a culture-dependent approach will be used to grow anaerobic fastidious organisms using a clarified guano filtrate supplement to aid in the recovery and identification of novel bacteria from bat guano. Often, bacteria fail to grow due to the lack of specific growth factors and substrates. Clarified guano filtrate supplementation will provide vital nutrients from the guano that further increasing the probability of continued growth when transferred to a range of different substrates. Guano filtrate supplemented media will then be inoculated with diluted guano sample and grown under anaerobic conditions. Candidate novel isolates (under 97% 16S rRNA sequence similarity) identified will have whole genome sequencing and be subjected to a panel of phylogenomic analysis, morphological, biochemical, physiological and chemotaxonomic (fatty acid, polar lipids, peptidoglycan). We envision that underrepresented microbes in bat guano will be further characterized and studied to understand key mechanisms in biogeochemical cycles.

ENVIRONMENTAL AND ANTHROPOGENIC EFFECTS ON TERRESTRIAL MAMMAL RICHNESS IN OKLAHOMA CITY METRO AREA

Chloe Lucas and Daniel G. Rocha, Southern Nazarene University

Urbanization is growing world-wide, and particularly in North America, posing challenges for wildlife. We investigated factors that affect wildlife distribution in the highly urbanized Oklahoma City (OK) Metro area to understand the relative importance of habitat quality, presence of domestic animals, or human activity intensity on wildlife, using medium-large terrestrial mammals as a model taxon. Here we report preliminary data on 21 camera trap sites (active for ~78 days during Summer of 2022 and 2023), in combination with site covariates (summer and winter NDVI, road intensity and number of domestic records) collected in a 250 m radius buffer around the camera locations. We used Poisson Generalized Linear models (GLM) to test the effect of site covariates on mammal species richness. Except for larger predators (i.e., black bear, cougar), we detected most mammal species expected to occur in the region. Sites varied greatly in species richness (3-9), being the white-tailed deer and the skunk the most and least detected species respectively. The summer NDVI model was the top ranked, followed by the winter NDVI model, suggesting that habitat quality, expressed in higher vegetation cover, positively influences mammal distribution in the study area. Data collection in the summer of 2024 and the inclusion of other covariates (e.g., habitat connectivity and human population density) will help us further understand the factors that influence mammal distribution in urban environments.

AN EXAMINATION OF THE EUTROPHICATION STATUS OF HAPPY LAKE IN CLAREMORE, OK

Ashli Mansour Ahmed Mohamed[1] and Chayanne Olson[2], [1]Rogers State University; [2]Northeastern State University

Cultural eutrophication is one of the most significant water quality issues worldwide. It occurs when water bodies, like lakes, become overloaded with nutrients, often from human activities, leading to harmful algal blooms (HABs). This process can severely impact ecosystems, causing low dissolved oxygen levels and mass fish die-offs. Cultural eutrophication affects millions of lakes and reservoirs globally and can lead to the premature decline of these water bodies. Claremore Lake in Rogers County, Oklahoma, serves as a drinking water source for the city of Claremore and is classified as impaired due to high chlorophyll concentrations, indicating hypereutrophic conditions. This study focuses on the contributions of nitrogen and phosphorus from Happy Lake. Happy Lake is a reservoir, constrained by an unmanaged rock and a concrete spillway constructed between 1953 and 1957, that feeds into Claremore Lake, particularly after rain events. Between April and August 2024, we examined water quality parameters— pH, oxygen, nutrient concentrations, and algal particles—at five sites along the gradient from Happy Lake to Claremore Lake. Results showed that Happy Lake exhibited greater fluctuations in nutrient levels, oxygen, and algal particles compared to sites deeper in Claremore Lake. This data is crucial for managing Claremore Lake's water quality and understanding nutrient sources. Future research should investigate the major nutrient inputs from northern tributaries, especially the upper portion of Dog Creek.

AN EXPLORATORY SURVEY OF DUNG BEETLES (COLEOPTERA: SCARABAEOIDEA) IN THE OKLAHOMA PANHANDLE

F.D. Noahubi[1], M.J. Cherry[2], M.C. Chitwood[3], M.M. Dart[2], R.W. DeYoung[2], W.S. Fairbanks[3], D.P. Hahn[3], L.J. Heffelfinger[2], R.C. Lonsinger[4], C.M.J. Rickels[2], E.P. Tanner[2], M.T. Turnley[3], and H.G. Wang[1], [1]East Central University; [2]Texas A&M University-Kingsville; [3]Oklahoma State University-Stillwater; [4]U.S. Geological Survey, Oklahoma Cooperative Fish & Wildlife Research Unit

Dung beetles potentially provide important ecosystem services in parasite control and nutrient cycling. Yet, little is known of the community composition of dung beetles in the Oklahoma panhandle. In June 2024, we sampled dung beetle assemblages using pitfall traps at four locations in Cimarron County, Oklahoma. The pitfall traps were arranged in a single transect at each location and baited with cattle dung. We checked the traps daily for four days and recorded the density and morphospecies of beetles collected in them. The traps were refilled with the baited dung as needed. A selected sample of the beetles collected were prepared into specimens and returned to East Central University to be labeled and photographed. We recorded a total of XXX beetles representing YYY morphospecies during the four-day sampling effort. There were noted differences in the distribution of dung beetles between the sampling locations. The pastures that were grazed by cattle at the time of sampling had a higher abundance of beetles but were dominated by a few species. The pastures lacking cattle grazing had fewer beetles overall but a higher diversity of beetle species. Our results suggest that land use can impact the community structure and abundance of dung beetles. One potential drawback of our study is that only cattle dung was used as bait. Future research should incorporate a variety of fecal material as bait to assess their difference in attracting dung beetles.

EFFECTS OF IN OVO CHRYSENE AND PHENANTHRENE EXPOSURE ON CHICKEN EMBRYO DEVELOPMENT AND CARDIAC FUNCTION: IS THERE EVIDENCE FOR SYNERGISM?

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

Outstanding Undergraduate Paper in Applied Ecology & Conservation

Polycyclic aromatic hydrocarbons (PAHs) are naturally occurring toxic chemicals found in crude oil and are known to transfer from the external eggshell surface to egg contents. Previously, we conducted an egg-injection study with White Leghorn chicken (*Gallus gallus*) eggs and identified two PAHs, chrysene (Chr) and phenanthrene (Phe), that increased embryonic heart mass and decreased embryonic heart rate. In this study, we investigated whether co-exposure to Chr and Phe resulted in additive or synergistic effects on chick embryo development. Chicken embryos were exposed to Chr (800 ng / g of egg mass), Phe (800 ng / g egg mass), and Chr and Phe in combination ($\sum$ PAH 1600 ng /g ng egg mass) via egg-injection, and we collected embryonic organ mass, heart rate, metabolic rate, and cardiac and hepatic mRNA expression of detoxification enzymes on embryonic day (ED)18. We observed a decrease in ED 18 heart rate across all treatments. We also saw an increase in ED 18 liver mass in eggs exposed to Chr and Phe simultaneously, and shifts in metabolic rate and mRNA expression of cardiac and hepatic detoxification enzymes. However, embryonic growth or morphology did not vary among treatments. Collectively, these data suggest in ovo exposure to PAHs may lead to congenital heart defects, which may have long-term implications for hatching success and hatchling survival.

IL7R MUTATION GENERATED IN HEMATOPOIETIC STEM CELLS VIA CRISPR/CAS9 TO DEVELOP HUMANIZED MOUSE MODEL OF B CELL ACUTE LYMPHOBLASTIC LEUKEMIA

Natalia Ramirez, Oral Roberts University

Outstanding Undergraduate Paper in Biochemistry & Molecular Biology

B-cell acute lymphoblastic leukemia (ALL), a hematologic disease characterized by an extensive proliferation of immature B cell precursors, is the leading cause of cancer-related deaths in children. Philadelphia chromosome-like B-ALL, which encompasses 15% of childhood ALL is associated with a high risk of relapse and poor outcome. One of the most frequently mutated genes involved in Ph-like ALL is interleukin 7 receptor (IL7R). Current models available for the study of Ph-like ALL are inadequate to fully recapitulate human leukemic development. Therefore, this study focuses on the creation of a humanized model for Ph-like ALL. Four different guide RNAs (gRNAs) were designed to target the IL7R gene, and homology directed repair (HDR) donors were established to replicate one of the most common IL7R mutations observed in B-ALL patients. A subpopulation of primary hematopoietic stem cells was selected via flow cytometry and edited via CRISPR-Cas9 with gRNAs and HDR against IL7R, achieving an IL7R mutation efficiency of 54%. IL7R edited cells were injected into mice after which they were allowed to engraft during a period of 12 weeks. IL7R edited cells kept in culture were selected for CD34+ expression and subjected to mutation analysis. Future directions include flow cytometry analysis and genotyping of the populations emerging from these mice which will determine whether or not an in vivo preleukemic model was created. In conclusion, this work successfully achieved the introduction of an IL7R mutation in human primary hematopoietic stem cells to develop a humanized mouse model of Ph-like B-ALL.

MOSQUITO-BORNE DISEASE SURVEILLANCE IN OKLAHOMA (MODSO)

Pranav Rao, Lillian Savage, Israel Gentry, Margaret Wojan, Aydin Read, and Caio Martinelle França, Southern Nazarene University

Central Oklahoma is an ecologically diverse region in the southern Great Plains, and it is crucial to identify potential nidi of infection; the spatiotemporal distribution of mosquitoes, where they can acquire pathogens, and their ability to transmit pathogens to hosts. We conducted mosquito surveillance during the early (May to July) and late (August to October) seasons in 2024 using adult host-seeking CDC Light and BG - Pro traps baited with CO2 as well as CDC's gravid traps baited with rabbit food pellets (0.250 lbs/gal). Female adult mosquitoes were sorted and identified to species using morphological keys; known vectors of disease were pooled by site, date of collection, and species containing 5-30 individuals. Purified nucleic acids from the pools were tested for the presence of West Nile Virus (WNV) and Saint Louis Encephalitis virus (SLEV). We sampled 17 trapping sites across three Oklahoma counties and generated more than 300 unique trapping events and collected approximately 12,200 mosquitoes from thirty one species representing 5 genera. *Culex pipiens* complex, which is one of the major WNV vectors, was the most abundant species, making up 54.4% of the total mosquito community. Other prominent regional species included *Aedes vexans* (10.6%), *Culex erraticus* (10.6%), and *Aedes albopictus* (6.0%). Singleplex TaqMan reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays detected WNV in 4.3% (12/279) and SLEV in 2.5% (7/279) of the mosquito pools. Statistical analysis such as analysis of similarities (ANOSIM) and non-metric multidimensional scaling (NMDS) will also be utilized to gauge the spatiotemporal relationship between the species that make up the mosquito community, their seasonality and land cover characteristics of the region sampled. Understanding the dynamics of the mosquito population has the potential to prioritize public health resources and inform mitigation strategies to combat mosquito borne diseases.

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DETERMINING DIVERGENCE ONE STEP AT A TIME

Trevor Rauch[1], Dysen House[1], Lindsey J. Long[1], and Laura Reed[2], [1]Oklahoma Christian University; [2]Genomics Education Program-University of Alabama

Outstanding Undergraduate Paper in Biological Sciences

The Insulin/Tor pathway in *Drosophila* is a signaling pathway that serves to control metabolism and growth. Genes along this pathway evolve at different rates due to the property of conservation. Two genes along different points in the signaling pathway, slmb and step, were compared to see how conservation varies for genes at different points along the pathway, as well as different levels of gene interaction. The two genes were annotated and sequenced throughout different species of *Drosophila*, and their divergence score was calculated. The divergence score was based on the similarity of the target gene to the baseline species, *D. melanogaster*, and the similarity of the genes surrounding the target gene. Due to the early location of slmb in the pathway and high connectivity to other genes, we hypothesized that it would be more conserved than step, which comes later in the pathway and is connected to fewer genes. Our annotation data as well as data from classmates led to the conclusion that generally, slmb is more conserved than step across different *Drosophila* species.

A NOVEL MICROBIAL BIOTHERAPEUTIC FOR MODULATION OF THE GUT-LIVER AXIS

Gwen Reilly and Crystal N. Johnson, Oklahoma State University-Center for Health Sciences

The gut-liver axis (GLA) refers to the interaction between gut microbes and the liver. It functions to relay signals initiated by diet, genetics, and environmental stimuli via the hepatic portal vein to the liver and intestines. This feedback loop plays valuable roles in health such as nutrient secretion and absorption, dissemination of toxins, and maintaining gut and liver homeostasis. Disruption of the GLA contributes to conditions such as obesity, fatty liver disease, and chronic inflammation, which have become abundant among society. One potential intervention is through the use of next generation probiotics to alter the composition of our gut microbiome. Specialized anaerobic and enrichment techniques have facilitated the discovery of a previously uncultivated organism from the Erysipelotrichaceae family, a new bacterial genus tentatively called GR63. While little is known about this organism, genome mining has revealed information on its taxonomy, phylogenetic relationships, and functional abilities that suggest this microbe could serve as a probiotic therapeutic for the reversal of GLA pathogenicity. 16S rRNA gene sequencing has revealed a low nucleotide similarity (89.57%) to the nearest phylogenetic neighbor of GR63, *Dubosiella newyorkensis*. Previous microbiome studies have indicated *D. newyorkensis* is involved in several mechanisms related to improved health, including increased longevity and cognitive function. In silico data gathered from our genomic insights have also suggested involvement in the protection against non-alcoholic fatty liver disease (NAFLD). Further polyphasic characterization of this novel genus is currently underway. Future work will be aimed at elucidating the potential probiotic mechanisms of GR63 using animal models. With the potential to enhance clinical care, microbial therapies offer a targeted solution to improve the interconnectedness of the gut-liver axis for improved functionality as the future of precision medicine.

ANALYSIS OF FISH DIVERSITY IN IMPAIRED STREAMS OF NORTHEASTERN OKLAHOMA

Ashleigh Ross[1] and Cheyanne Olson[2], [1]Rogers State University; [2]Northeastern State University

The growth of industrial and residential areas in Oklahoma have led to an increase of urbanization around small streams. This has led to a decline in quality of freshwater habitat and in turn a degradation in the aquatic biotic integrity. Using biotic diversity as an indicator for both biotic integrity and habitat health is a common technique used to analyze freshwater ecosystems functioning. Freshwater fish may be used as an indicator for stream impairment since they are heavily affected by various water quality parameters, are long lived, and are well-studied. Several small, urbanized streams in Northeastern Oklahoma are understudied, but are listed on Oklahoma's 303d list for a variety of impairments (*E. coli*, nutrient levels, etc.). This study used a habitat survey and fish collection in several small, impaired streams in Northeastern Oklahoma as a reference for their ecosystem health. The fish assemblages were collected using a combination of electrofishing and seining. The results showed low scores when assessed using the Shannon-Weiner Diversity Index, Simpson's Index, and the Oklahoma Index of Biotic Integrity. Additionally, urban streams received lower habitat scores. This aligns with the impairments noted by previous study of these same sites. These signs of biotic and habitat impairment showcase a need for restoration and conservation efforts regarding these types of urban streams.

OUT OF AFRICA: PALEONTOLOGICAL RESEARCH AND DISCOVERIES MADE WHILE RESIDING IN SOUTH AFRICA

Christen D. Shelton, Rogers State University

South Africa is known for its diverse fossil record spanning from the Paleozoic through to the Stone Age. I resided for two years in South Africa to study the animal group known as the Dinocephalians. This is a continuation of the paleohistological research started in Germany while investigating fibrolamellar bone (FLB) tissue in the basal mammal-like reptiles. Dinocephalians are a group of Therapsids (mammal like reptiles of Middle Permian that evolved from the Pelycosaurs of the Lower Permian). This work produced four publications showing results of consumptive sampling in *Anteosurus*, *Moschops*, *Keratocephalus*, *Struthiocephalus*, and *Jonkeria*. Findings revealed the vast presence of FLB which appears to be convergent in the mammalian line. This research revealed the first case of osteomyelitis in Dinocephalians. It has been previously discovered in *Pelycosaurs* and modern mammals. Additionally, participation in expeditions allowed further discoveries and contributions to South Africa's rich prehistory. I co-discovered half of a skull of one of the rarest dinocephalians, *Criocephalosurus*. The other half was found by the current curator of non-mammalian tetrapods at the London Natural History Museum. This discovery help extend the known biogeographic range of the *Tapinocephalus* Assemblage Zone from Abrahamskrall Formation up into the Teekloof Formation deposited when this land was part of Gondwana. Additionally, participation in two expeditions to KwaZulu Natal to recover fossil material of a rare Cretaceous sea turtle reported in the 1970's by an Ammonite researcher on the shores of St. Lucia Lake. This is the fourth known example of a dermochelyid turtle in all of the African continent. On the final expedition, the second known remains of a *Plioplatecarpine mosasaur* from South Africa were found. The first mosasaur fossil from South Africa was discovered in 1901. All fossil material is housed in the vertebrate paleontology collection of the Iziko Museum of Cape Town.

ECOLOGICAL DISTURBANCE IN THE ANTHROPOCENE: LEGACY EFFECTS OF ORPHANED WELLS ON METABOLIC PHENOTYPE OF FREE-LIVING RODENTS AND VEGETATIVE COMMUNITY

Jess Warr, Richard Dolman, and Christopher G. Goodchild, University of Central Oklahoma

Outstanding Graduate Paper

While catastrophic large marine oil spills often receive considerable media attention, smaller-scale inland spills occur much more frequently, resulting in legacy polycyclic aromatic hydrocarbon (PAH) contamination. Oklahoma currently has 15,965 documented orphaned oil rigs that were operated under less regulatory oversight. Oklahoma Energy Resources Board (OERB) is working diligently to plug orphaned wells. However, the toxic legacy effects on surrounding ecosystems are not well understood. Using two separate field sites in Payne County Oklahoma, we assessed site-specific disturbance by conducting vegetative surveys to generate Floristic Quality Assessments (FQAs) for each site. Further, we measured hematological damage, immune traits, and organismal metabolic rates in free-living deer mice (*Peromyscus maniculatus*) populations inhabiting sites with orphaned wells. Collectively, these data will allow us to evaluate the legacy effects of unplugged oil wells on the vegetative community and physiology of resident deer mice.

IMPLEMENTING BREWERY QUALITY CONTROL UTILIZING SPECTROPHOTOMETRIC TECHNOLOGY

Jamie Welsh, Oklahoma City University

Outstanding Undergraduate Paper in Physical Sciences

In this independent research opportunity, a spectrophotometer was used to measure vicinal diketones (VDK), international bitter units (IBU), and standard reference method (SRM, color) for a variety of beer styles at Stonecloud Brewing Co. Each of these parameters is unique to style thus giving a beer its distinct flavor. For the process of packaging and presenting the products to the public, quality analysis is crucial to maintain the flavor, olfactory, and visual characters unique to each style. During the process of fermentation, yeast synthesize the amino acid valine. 2,3 butanedione and 2,3 pentanedione are secreted by-products of this synthesis known collectively as VDKs which impart a buttery flavor to beer. As fermentable carbohydrates decrease during fermentation, yeast reuptake VDKs and utilize them as an energy source. At Stonecloud, the purpose of this assay is to find the point at which yeast has metabolized VDKs to a level below the capacity of organoleptic sensation. Early detection of below threshold VDK levels allows beer to be crashed (chilled to 35□), transferred out of fermenters, and into brite tanks for packaging thus freeing up fermenter space and allowing increased production. The purpose of this research presentation is to present lab findings on VDKs to explain the assay, how the assay works, and the consequences if the parameter is out of range. Stonecloud's core beers will be highlighted in this project. Alongside the VDK protocol, other spectrophotometer protocols and future projects will be highlighted and discussed to reveal how absorbance technology can be incorporated in a local brewery setting.

USING ULTRASOUND TO STUDY PROPERTIES OF RAW HONEY

Karen Williams, East Central University

Ultrasound is a non-destructive method of obtaining properties of raw honey. This study will obtain the velocity of 1MHz ultrasound waves through honey, the acoustic impedance of honey, and the acoustic attenuation coefficient of raw honey. Finally, water was introduced to the sample and tested to determine if adulterated honey could be detected easily. The velocity of raw honey was obtained using distance and time traveled. Velocity values ranged from 1830 m/s to 2030 m/s for a temperature range of 18.4 to 24.5 degrees C. The acoustic impedance is the product of the density times the velocity. The acoustic impedance was easily obtained using the mass and volume of the honey in the cell with the velocity obtained. Impedance ranged from 2.65 Marls to 2.94 MRayls. The acoustic attenuation of raw honey was obtained using a reflector block, the Gampt Echoscope, a 1MHz transducer, and Ascan software. The slope method (Williams, 2015) was used. The slope of the graph of attenuation in dB/ MHz versus the distance in mm is the acoustic attenuation coefficient. All were negative. They were -51.8, -147.5, -127.3, -45.69, and -57.77 dB/MHz mm for a temperature range from 18.1 to 24.5 deg C. Cooling overnight as a wider range of data was desired is thought to have caused the honey to crystallize; thus causing a wide range of acoustic attenuation values the next day (Jost and Peirong). It was very easy to tell from the velocity data the adulterated honey (18% water by volume) was not raw honey. The velocity of 1MHz ultrasound waves through the adulterated honey was 1590 m/s-far from the raw honey velocity values. This study shows promise in using ultrasound to accurately obtain properties of raw honey.

A NOVEL APPROACH TO WEST NILE VECTOR IDENTIFICATION AND SURVEILLANCE

Elijah Woodward, Luke Woodward, Alisha Howard, and Eric Howard, East Central University

Due to climate change, the risk of mosquito-borne diseases is increasing as mosquito habitats are changing. Our project aims to enhance the analysis of mosquito vector populations, specifically West Nile virus vectors, by developing a novel primer that can both amplify the DNA of multiple mosquito species and produce distinct products for each species that are visually distinguishable by gel electrophoresis. We are currently investigating if such sites exist within the mosquito genomes that a single primer set could both bind and amplify, giving products with enough size variation. We have designed and tested 20 unique primer sets in silico, but a functional, species-identifying primer set remains elusive. Despite this, the study establishes a foundation for future exploration using the proposed method. Future directions may involve multiplex PCR if a singular primer set is not found, and incorporating quantitative PCR to estimate species abundance in samples. We hope that, regardless of the success of our proposed methodology, our findings contribute to the advancement of mosquito population analysis techniques. As mosquito-borne disease threats intensify, the search for improved methods of vector population analysis remains crucial for public health.

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STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID
FOR THE YEAR ENDED DECEMBER 31, 2023

REVENUES COLLECTED:		
Membership Dues:		$1,930.00
Investment Income:		$18.00
Meetings:		$14,345.83
Registration – Fall Field Meeting	$5,204.95	
Registration – Technical Meeting	$9,140.88	
Donations		$230.00
POAS:		$5,007.08
Woody Plants:		$57.36
Dues/Meetings of NAAS/AJAS/AAAS:		$834.97
Other Revenue:		$257.01
Total Revenue Collected		**$22,680.25**
EXPENSES PAID		
Stipends and Other Compensation:		$7,158.68
Stipends	$6,141.24	
Social Security & Medicare	$1,017.44	
Meeting Expenses:		$9,357.90
Fall Field Meeting	$4,464.82	
Technical Meeting	$4,893.08	
POAS:		$4,912.55
Woody Plants:		$18.68
Dues/Meetings of NAAS/AJAS/AAAS:		$1,136.16
Other Expenditures:		$179.44
Total Expenses Paid:		**$22,763.41**
Revenues Collected Over Expenses Paid		**$-83.16**

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF ASSETS, LIABILITIES, AND FUND BALANCE
ARISING FROM CASH TRANSACTIONS
FOR THE YEAR ENDED DECEMBER 31, 2023

ASSETS		
Cash:		$22,146.36
Checking Account	$16,065.38	
Savings Account	$3,279.03	
Endowment Savings Account	$2,801.95	
Investments:		$60,000
Certificate of Deposit	$60,000	
Total Assets:		**$82,146.36**
LIABILITIES AND FUND BALANCE		
Liabilities:		$0.00
Fund balance:		
Beginning operation fund balance	$82,765.93	
Excess revenues collected over expenses	$-83.16	
Total Funds:		**$82,682.77**

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

C. Manuscript Organization.

1. General organization.

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

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

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

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

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

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

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

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

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

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

X. References are discussed below.

2. References

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

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

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

Examples of References in CBE Style and Format

Journal Articles

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

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

Books

Book with Authors:

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

Book with Editors:

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

Book with Organization as Author:

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

Chapter in Book with Editors:

Hamilton K, Combs DL, Randolph JC. 1985. Sportfishing changes related to hydro- power generation and non-generation in the tailwater of Keystone Reservoir, Oklahoma. In: Olsen FW,White RG, Hamre RH, editors. Proceedings of the symposium on small hydropower and fisheries. Bethesda (MD): American Fisheries Society. p 145-152.

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

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

D. Review Process.

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

E. Page Charges

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