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A. Applied Ecology & Conservation

Updated and Revised Checklist of the Mammals of Oklahoma, 2019

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Abstract: An updated list of the mammals of Oklahoma was compiled from literature records, sight records, and museum specimens. A total of 108 native species, 4 extirpated species, and 5 introduced/exotic species are reported.

Introduction

In a checklist of mammals of Oklahoma (Caire et al. 1989), a total of 106 species of mammals were listed as occurring in Oklahoma, including 4 extirpated and 4 introduced species. In 1998, an updated checklist was published (Choate and Jones 1998) listing 111 species of mammals including 4 extirpated and 7 introduced/exotic species. Since the publication by Caire et al. (1989) and the updated checklist of Choate and Jones (1998), there have been several changes in distributional occurrences and nomenclature of mammal species found in Oklahoma that have been documented in various scientific and lay literature. The checklist below updates the earlier lists and contains 117 species of mammals, including 4 extirpated species and 5 introduced/exotic species. Both scientific and common names are provided. Six species (*Blarina brevicauda*, *Sorex longirostris*, *Eumops perotis*, *Ovis canadensis*, *Geomys jugossicularis*, and *Perognathus merriami*), not included in the most recent checklist of Choate and Jones (1998), have been verified as occurring in the state. Choate and Jones (1998) included the domestic dog and cat as introduced/exotic species which we did not. This document has been created in part to assist those working with the many different and varied aspects related to the state's mammals. It will provide a common point of reference and terminology.

Methods

To compile the updated list, we began with Caire et al. (1989) and examined the literature for any occurrence of a species not previously reported from the state. We also examined museum specimens from many university and institution holdings for any new occurrences. In the checklist, the sequence of orders are presented following Wilson and Reeder (2005). The families, genera, and species are listed alphabetically. Bradley et al. (2019) suggested a nomenclatural change of *Peromyscus*

maniculatus to *Peromyscus sonoriensis*. We are hesitant to accept this change until additional discussion of the recommendation has occurred. Several other taxonomic changes follow those suggested by Bradley et al. (2014). With minor exceptions, common names are adapted from those listed by Wilson and Cole (2000), Wilson and Reeder (2005), and Bradley et al. (2014).

Results and Discussion

CHECKLIST OF THE MAMMALS OF OKLAHOMA

E = Extirpated I = Introduced

Order: DIDELPHIMORPHIA – Opossums	
Family: Didelphidae – Opossums	
Didelphis virginiana	Virginia Opossum
Order: CINGULATA – Armadillos	
Family: Dasypodidae – Armadillos	
Dasypus novemcinctus	Nine-banded Armadillo
Order LAGOMORPHA – Pikas, Hares, and Rabbits	
Family: Leporidae – Hares and Rabbits	
Lepus californicus	Black-tailed Jackrabbit
Sylvilagus aquaticus	Swamp Rabbit
Sylvilagus audubonii	Desert Cottontail
Sylvilagus floridanus	Eastern Cottontail
Order SORICOMORPHA – Insectivores	
Family: Soricidae – Shrews	
Blarina brevicauda	Northern Short-tailed Shrew
Blarina carolinensis	Southern Short-tailed Shrew
Blarina hylophaga	Elliot's Short-tailed Shrew
Cryptotis parva	Least Shrew
Notiosorex crawfordi	Crawford's Desert Shrew
Sorex longirostris	Southeastern Shrew
Family: Talpidae – Moles	
Scalopus aquaticus	Eastern Mole
Order CHIROPTERA – Bats	
Family: Molossidae – Free-tailed Bats	
Eumops perotis	Western Bonneted Bat
Nyctinomops macrotis	Big Free-tailed Bat
Tadarida brasiliensis	Brazilian Free-tailed Bat
Family: Vespertilionidae – Vesper Bats	
Antrozous pallidus	Pallid Bat
Corynorhinus rafinesquii	Rafinesque's Big-eared Bat
Corynorhinus townsendii	Townsend's Big-eared Bat

Eptesicus fuscus	Big Brown Bat
Lasionycteris noctivagans	Silver-haired Bat
Lasiurus borealis	Eastern Red Bat
Lasiurus cinereus	Hoary Bat
Lasiurus seminolus	Seminole Bat
Myotis austroriparius	Southeastern Myotis
Myotis ciliolabrum	Western Small-footed Myotis
Myotis grisescens	Gray Myotis
Myotis leibii	Eastern Small-footed Myotis
Myotis lucifugus	Little Brown Myotis
Myotis septentrionalis	Northern Long-eared Myotis
Myotis sodalis	Indiana Myotis
Myotis velifer	Cave Myotis
Myotis yumanensis	Yuma Myotis
Nycticeius humeralis	Evening Bat
Parastrellus hesperus	American Parastrelle
Perimyotis subflavus	American Perimyotis

Order CARNIVORA – Carnivores

Family: Canidae – Dogs, Foxes, and Wolves

Canis latrans	Coyote
[E]*Canis lupus*	Gray Wolf
[E]*Canis rufus*	Red Wolf
Urocyon cinereoargenteus	Common Gray Fox
Vulpes velox	Swift Fox
Vulpes vulpes	Red Fox

Family: Felidae – Cats

Lynx rufus	Bobcat
Puma concolor	Mountain Lion

Family: Mephitidae – Skunks

Conepatus leuconotus	American Hog-nosed Skunk
Mephitis mephitis	Striped Skunk
Spilogale gracilis	Western Spotted Skunk
Spilogale putorius	Eastern Spotted Skunk

Family: Mustelidae – Weasels, Otters, and Badgers

Lontra canadensis	Northern River Otter
Mustela frenata	Long-tailed Weasel
[E]*Mustela nigripes*	Black-footed Ferret
Mustela nivalis	Least Weasel

Vison vison	American Mink
Taxidea taxus	American Badger
Family: Procyonidae – Raccoons, Ringtails, and Coatis	
Bassariscus astutus	Ringtail
Procyon lotor	Northern Raccoon
Family: Ursidae – Bears	
Ursus americanus	American Black Bear
[E]*Ursus arctos*	Grizzly (Brown) Bear
Order ARTIODACTYLA – Even-toed Ungulates	
Family: Antilocapridae – Pronghorn	
Antilocapra americana	Pronghorn
Family: Bovidae – Cattle, Antelope, Sheep, Goats, and African Exotics	
Bison bison	American Bison
Ovis canadensis	Bighorn Sheep
Family: Cervidae – Deer	
Odocoileus hemionus	Mule Deer
Odocoileus virginianus	White-tailed Deer
Cervus canadensis	Elk
Family: Suidae – Pigs	
[1]*Sus scrofa*	Feral Pig
Family: Tayassuidae – Peccaries	
Pecari tajacu	Collared Peccary
Order RODENTIA – Rodents	
Family: Castoridae – Beavers	
Castor canadensis	American Beaver
Family: Cricetidae – New World Mice, Rats, and Voles	
Baiomys taylori	Northern Pygmy Mouse
Microtus ochrogaster	Prairie Vole
Microtus pinetorum	Woodland Vole
Neotoma floridana	Eastern Woodrat
Neotoma leucodon	White-toothed Woodrat
Neotoma mexicana	Mexican Woodrat
Neotoma micropus	Southern Plains Woodrat
Ochrotomys nuttalli	Golden Mouse
Ondatra zibethicus	Common Muskrat
Onychomys leucogaster	Northern Grasshopper Mouse
Oryzomys texensis	Texas Marsh Rice Rat
Peromyscus attwateri	Texas Deermouse

Peromyscus boylii	Brush Deermouse
Peromyscus gossypinus	Cotton Deermouse
Peromyscus laceianus	Lacey's White-ankled Deermouse
Peromyscus leucopus	White-footed Deermouse
Peromyscus maniculatus	North American Deermouse
Peromyscus nasutus	Northern Rock Deermouse
Peromyscus truei	Piñon Deermouse
Reithrodontomys fulvescens	Fulvous Harvest Mouse
Reithrodontomys humulis	Eastern Harvest Mouse
Reithrodontomys megalotis	Western Harvest Mouse
Reithrodontomys montanus	Plains Harvest Mouse
Sigmodon hispidus	Hispid Cotton Rat
Family: Dipodidae – Jumping Mice	
Zapus hudsonius	Meadow Jumping Mouse
Family: Echimyidae – Coypus	
[1]*Myocastor coypus*	Nutria
Family: Erethizontidae – New World Porcupines	
Erethizon dorsatum	North American Porcupine
Family: Geomyidae – Pocket Gophers	
Cratogeomys castanops	Yellow-faced Pocket Gopher
Geomys breviceps	Baird's Pocket Gopher
Geomys bursarius	Plains Pocket Gopher
Geomys jugossicularis	Hall's Pocket Gopher
Family: Heteromyidae – Pocket Mice and Kangaroo Rats	
Chaetodipus hispidus	Hispid Pocket Mouse
Dipodomys elator	Texas Kangaroo Rat
Dipodomys ordii	Ord's Kangaroo Rat
Perognathus flavescens	Plains Pocket Mouse
Perognathus flavus	Silky Pocket Mouse
Perognathus merriami	Merriam's Pocket Mouse
Family: Muridae – Old World Mice and Rats	
[1]*Mus musculus*	House Mouse
[1]*Rattus norvegicus*	Norway (Brown) Rat
[1]*Rattus rattus*	Black Rat
Family: Sciuridae – Squirrels	
Cynomys ludovicianus	Black-tailed Prairie Dog
Glaucomys volans	Southern Flying Squirrel
Ictidomys tridecemlineatus	Thirteen-lined Ground Squirrel

Marmota monax	Woodchuck
Otospermophilus variegatus	Rock Squirrel
Sciurus carolinensis	Eastern Gray Squirrel
Sciurus niger	Eastern Fox Squirrel
Tamias quadrivittatus	Colorado Chipmunk
Tamias striatus	Eastern Chipmunk
Xerospermophilus spilosoma	Spotted Ground Squirrel

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Angular Substrate Preference and Molting Behavior in the Giant River Prawn, *Macrobrachium rosenbergii* and its Implications for Cannibalism Management

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Abstract: The Giant River Prawn is an important commercial species from southeastern Asia and has a large global market. Many arthropods require firm perches on which they undergo ecdysis. We investigated preference for substrate slope and its influence on ecdysis. Prawns occupied horizontal surfaces more than others, but they perched on high-sloped and vertical substrates to flex their shell and molt. We recommend cannibalism management include sufficient vertical or high-sloped surfaces to facilitate ecdysis, while providing much horizontal space for foraging. This should create separation between foraging and freshly-molted prawns, thus leading to reduced cannibalism-related mortality.

Introduction

The Giant River Prawn (*Macrobrachium rosenbergii* [De Man 1879]) is a large crustacean indigenous to southern and southeastern Asia, Oceania, and several islands in the South Pacific Ocean (New 2002). A global market for this species has developed since the 1990s, so it is commercially raised for food around the globe (FAO 2002). The species undergoes a complex life cycle involving spawning in brackish waters, hatching as planktonic larvae, and development into post-larvae followed by metamorphosis into juvenile prawns that migrate back into freshwater to mature and return to estuaries to spawn. Giant River Prawns feed on an assortment of live, dead and decaying plant and animal matter. They become aggressively cannibalistic in captivity, providing complications for producers.

Despite their widespread commercial appeal, their life history and behavior are not well detailed. This lack of information is important because these kinds of details form the foundation for appropriate husbandry practices and because life history details provide the outcomes of the evolutionary process, suggesting much about an organism's biology (McCallum and McCallum 2006; Bury 2006). One of these life history elements is their aggressive and cannibalistic nature. Producers confront this management problem by creating infrastructure in ponds

and aquaria to provide more habitable surface area. This is thought to dilute prawns, which limits encounters leading to improved growth rates and survivorship. Details vary about how this infrastructure should be constructed. Some producers place vertical mesh walls with enough separation to allow space for the size of prawn housed. Another common option is to create a series of horizontal mesh platforms stacked one over the other. Variations on these themes run the gamut, with many placing lines of snow fencing through ponds or bundling plastic mesh for placement in aquaria or ponds.

We asked if prawns use vertical, horizontal and angled surfaces similarly. The rationale is that investment in excess infrastructure that is not readily occupied is money and time spent unwisely on materials and maintenance. Likewise, if one uses only horizontal or vertical platforms, it may lower production potential if prawns have a biologically-based preference. We hypothesized that prawns may demonstrate preference for the angularity of the substrate. We predicted that if they do, they would be found more frequently on substrates of some angles than others.

Methods

Between 75-100 juvenile prawns (body mass range = 0.01 – 0.045 g) were housed communally in an aerated 40-L glass aquarium containing 30-L of medium toxicological hard water (U.S. EPA 2002). Prawns were fed ground shrimp pellets daily *ad libitum*. Four 6.35 x 30 cm long strips of plastic mesh (3 mm mesh size) were formed into 30-60-90 triangles to serve as perching structures for prawns. Two were placed with the short leg and two with the long leg as the horizontal base of the triangle. Each triangle was observed twice daily (N = 9 observation periods)and ~7 hrs apart, for five minutes and the number of prawns observed on each surface was noted. These data were converted to prawns/mm and then angle preference was analyzed using one-way ANOVA with a Tukey means comparison test. We also recorded the number of tail flexing prawns (Fig. 1) and the number that hung upside down versus right side up on each side. Pre-molt flexing behavior was analyzed using Chi Square.

We also housed 30 prawns in individual 50 ml flasks from an unrelated experiment. Seven of these were provided vertical perches made out of 3 mm^2 plastic mesh. Molting success, frequency and mortality were recorded over a four-week period. The data were analyzed using Chi square with an alpha = 0.05.

Results

Data for preferred perch angle was not normally distributed (Anderson-Darling: A^2 = 1.494, P = 0.001), so we transformed it using the "normal scores" function in MiniTab 13.0 to allow analysis by ANOVA. Although prawns used some surfaces more than others (Fig. 2; ANOVA: $F_{(3, 108)} = 5.60$, P = 0.002), there was no significant difference between use of 60 degree and 30 degree sloped surfaces (Tukey: -1.003, 0.3710). Thus, we pooled data to prawns resting on the hypotenuse, vertical or horizontal mesh

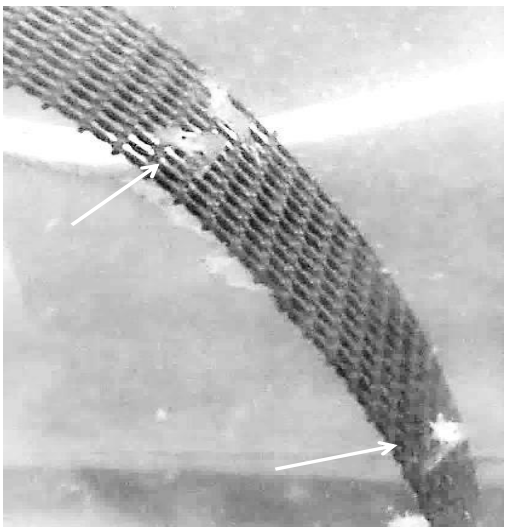

Figure 1. Flexing behavior of juvenile prawns on a slanted surface. Two prawns are flexing (see arrows). The third was flexing prior to taking the photograph. There are also two prawns hanging upside down.

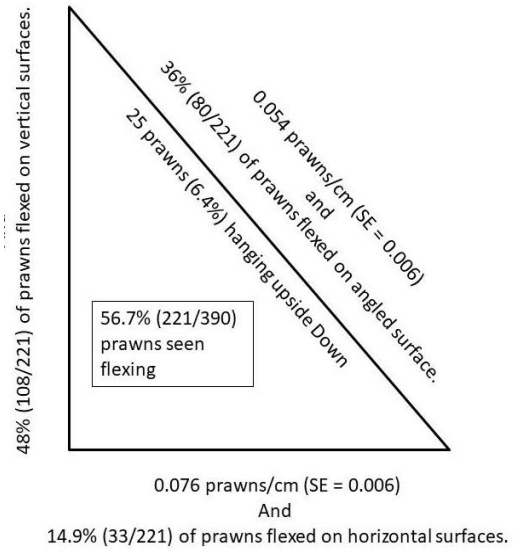

Figure 2. Distribution of juvenile prawns and flexing behavior on mesh infrastructure.

surfaces to simplify further analyses. Here, prawns used some surfaces more than others (ANOVA: $F_{(2, 105)} = 5.60$, P = 0.005). They used horizontal surfaces more frequently than vertical surfaces (Tukey: -1.23, -0.196). There was no difference in use between vertical and angled surfaces (Tukey: -0.747, 0.290). They occurred on angled surfaces marginally more often than vertical surfaces (Tukey: -0.032, 1.0051). Prawns seldom hung upside down (6.4%, 25/390 observations), preferring to remain upright.

Flexing behavior was not uniformly distributed among surfaces (Fig. 2; $\chi^2 = 7.14$, df = 2, P = 0.028). It occurred more frequently on vertical ($\chi^2 = 7.14$, df = 1, P = 0.0.008) and marginally more frequently on angled surfaces ($\chi^2 = 2.78$, df = 1, P = 0.096) than on horizontal surfaces. There was no significant difference in flexing behavior between vertical and angled surfaces ($\chi^2 = 1.32$, df = 1, P = 0.251). No prawns were observed flexing while hanging upside down.

Of the individually-housed prawns, 7 (100%) molted at least once when a perch was present. Among those not provided perches, 13/22 (59%) also molted, but the remainder failed to molt. Prawns that were not provided a perch molted less frequently than prawns that were provided a perch (Chi square = 4.15, P = 0.042).

Discussion

Mortality from post-larvae to adult in production systems ranges from 20 – 50% and appears related to molt state (Peebles 1978). Uniformly-sized prawns are especially susceptible to aggression and cannibalism during late pre-molt and early post-molt (Peebles 1978). Further, animals weakened by disease or environmental conditions succumb during ecdysis (Justo et al. 1991) Flexing behavior is known to precede molting in tailed decapods (Travis 1954; Tamm and Cobb 1978). Many molting insects must grasp a substrate firmly during ecdysis (Howard 1995; Fahrbach and Mesce 2005; White and Ewer 2014), much captive mortality arises when individuals in molt fall from their perch (Whitman 1986). Perhaps nowhere is this more familiar than with cicadas (Cicadidae) (Truman JW, III 2012; Truman 1983; Mantel 1971). Our results suggest a similar need in *M. rosenbergii*, and possibly other tailed decapods. Generally, the focus for captive prawn mortality has been aggression and cannibalism; however, our results beg to question if cannibalism is a symptom of lacking appropriate habitat for molting. Molting prawns occupy habitat used less frequently by non-molting individuals and molting frequency is inhibited when perches are lacking, but no increase in survivorship occurs when a perch was present. This suggests cannibalism results from the lack of non-horizontal perches that force molting prawns to occupy habitat that non-molting prawns frequent; leading to higher exposure to foraging prawns that can cannibalize newly molted individuals. However, a previous study in which surface area was increased 20% by installation of PVC frames with horizontal plastic mesh and vertical suspended seines did not significantly change mortality rates, but ponds without infrastructure had a higher proportion of small males, lower proportion of orange-clawed males, and larger body size in blue and orange clawed males, and

reproductive and virgin females (Tidwell et al. 2007). Our data support these findings and may provide evidence that increasing the surface area more than 20% may provide even better results. All producers and others raising prawns in captivity should ensure that both horizontal and non-horizontal surfaces (preferably vertical, since these are used most during molting and least during foraging) are available in culture chambers to reduce molt-related mortality due to cannibalism.

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Diet Evaluation of Large Blue Catfish and Flathead Catfish from Lake Ellsworth, Oklahoma

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Abstract: Blue Catfish (*Ictalurus furcatus*) and Flathead Catfish (*Pylodictis olivaris*) are the largest Ictalurids in Oklahoma's rivers and reservoirs. Their native ranges are within the Arkansas and Red River basins in Oklahoma, however they are found almost statewide due to introduction as a sportfish. Few studies have documented their diet composition within Oklahoma's waters, particularly for large fish. Winter diets were evaluated for Blue Catfish and Flathead Catfish captured using large mesh gillnets set overnight at Lake Ellsworth, Oklahoma. Sampling occurred during February and March of 2019. A total of 159 catfish were evaluated for stomach content analysis. Stomach contents were observed in 79 of the fish (63 Blue Catfish and 16 Flathead Catfish) and only six different prey fish species were observed in diets. The combined stomach content weight (from 206 prey items [182 from Blue Catfish and 24 from Flathead Catfish]) for both species was 17.1 kg (15.8 kg for Blue Catfish and 1.3 kg for Flathead Catfish). Of the six prey species consumed, Gizzard Shad (*Dorosoma cepedianum*) occurred most often in Blue Catfish diets, whereas Freshwater Drum (*Aplodinotus grunniens*) occurred most often in Flathead Catfish diets. Cannibalism among and within species was observed for Blue Catfish, but at low rates. Of the 182 fish consumed by Blue Catfish, 144 fish total lengths were reconstructed using the linear relationship between backbone length to total length or standard length to total length. These lengths were then plotted against Blue Catfish total length (for fish $\geq$ 600 mm), which suggested that Blue Catfish $\geq$ 600 mm TL consumed similar sized prey as the largest fish in the sample. An expansion of research to other Oklahoma reservoirs is needed to better understand catfish diets and the effects of large catfish on fish communities in Oklahoma.

Introduction

Blue Catfish (*Ictalurus furcatus*) and Flathead Catfish (*Pylodictis olivaris*) are both large-bodied predators that are relatively long-lived and can weigh in excess of 50 kg (Graham 1999, Jackson 1999, Boxrucker and Kuklinski 2006, Schmitt et al. 2017). These two catfish species are the largest members of Ictaluridae in Oklahoma. Due to their trophy potential, angling interest for these large-bodied catfish has increased in recent years (Boxrucker and Kuklinski 2006). Although Blue Catfish and Flathead Catfish are native to the Arkansas and Red River basins, they are now found in most of Oklahoma because the Oklahoma Department of Wildlife Conservation (ODWC) has introduced them into many reservoirs to create recreational angling opportunities (Miller and Robinson 2004).

In most aquatic systems, Blue Catfish

and Flathead Catfish occupy different tropic niches. Blue Catfish are considered omnivores, consuming vegetation, mollusks, insects, crustaceans, and fish (Bonvechio et al. 2011, Hogberg and Pegg 2016, Schmitt et al. 2017, Jennings et al. 2018). However, Flathead Catfish are almost exclusively piscivorous, transitioning to fish prey when they reach 250 mm TL (Turner and Summerfelt 1971, Layher and Boles 1980, Herndon and Waters 2002, Schmitt et al. 2017). Feeding strategy likely drives these differences, as Flathead Catfish are considered an ambush predator that are not gape limited (Slaugther and Jacobson 2008), foraging non-selectively with respect to prey abundance within microhabitats that they occupy (Pine et al 2005). Whereas Blue Catfish are a pelagic species that move up-river in spring for spawning and retreat back down-river into reservoirs when water temperatures cool in the fall, feeding opportunistically through these seasonal habitat shifts (Phflieger 1997, Graham 1999, Snow at el. 2018).

Diets of Blue Catfish and Flathead Catfish have been described for native and introduced populations (Turner and Summerfelt 1971, Layher and Boles 1980, Herndon and Waters 2002, Bonvechio et al. 2011, Hogberg and Pegg 2016, Schmitt et al. 2017, Jennings et al. 2018). However, few of these evaluations have described diets of large individuals (≥ 600 mm). Diet information in Oklahoma Reservoirs, particularly for Blue Catfish is limited. The ODWC standard sampling protocol for Blue Catfish and Flathead Catfish uses low-frequency pulsed DC electrofishing to sample these species. However, collection of large (≥ 600 mm) Blue Catfish or Flathead Catfish during these surveys is rare, which limits a meaningful description of diet across the entire size structure of the population due to small sample size of large individuals (Boxrucker and Kuklinski 2006, Ford et al. 2011, Bodine et al. 2013, ODWC *unpublished data*). In this paper we describe diets of large Blue Catfish and Flathead Catfish caught using large mesh gillnets during winter (February through March of 2019) at Lake Ellsworth, Oklahoma.

Methods

Study Area: Lake Ellsworth is a flood control reservoir that was formed in 1961 by impounding Chandler Creek, East Cache Creek, and Tony Creek, which are tributaries of the Red River in Caddo and Comanche Counties in Southwestern Oklahoma (Cofer 2011, Figure 1). At normal pool elevation, Lake Ellsworth is 2,069 ha with 86.1 km of shoreline. It is considered to be mesotrophic, but can shift to hypereutrophic

Figure 1. Map of Lake Ellsworth in Caddo and Comanche Counties in Southwestern Oklahoma.

during warm weather months (Oklahoma Water Resources Board 1994). Lake Ellsworth has a mean depth of 4.82 m and a maximum depth of 16.5 m. The water storage is managed by the City of Lawton and serves as a municipal water supply (Cofer 2011).

Blue Catfish were stocked into Lake Ellsworth in 1961 and 1979 (Cofer 2011). Reproduction was first documented by ODWC during sampling in 1968 (Bennett 1968). Reproduction appears to be consistent but growth is slow compared to other reservoir populations in Oklahoma (Boxrucker and Kuklinski 2006). Flathead Catfish were not stocked into Lake Ellsworth. A remnant population occupied the existing creek systems prior to impoundment, were introduced by anglers, and/or stocked unintentionally. Sampling conducted during 1991-1993 determined Flathead Catfish were abundant and reproducing (Cofer 2011).

Sampling: Fish sampling occurred at 29 sites selected randomly from areas associated with creek channels within Lake Ellsworth, however due to standing timber or large woody debris, some sites had to be adjusted to avoid entangling gear. Sampling occurred during February and March 2019 at water temperatures ranging from 3.9 – 11.2 °C and depths from 2.1 – 12.2 m. Single-panel sinking gillnets of two different bar mesh sizes (net 1 - 152.4 mm bar mesh x 182.9 m length x 7.3 m depth and net 2 - 127 mm bar mesh x 91.4 m length x 7.3 m in depth) were set overnight to capture both catfish species. Fish from each sampling event were transported to the Oklahoma Fishery Research Laboratory in Norman, Oklahoma for processing. Fish were weighed to the nearest kg, measured for total length (TL, mm), sexed, and stomachs excised.

Once stomachs were extracted, prey items were removed and identified, enumerated, and individual prey items were weighed to the nearest gram. All prey items were identified to species when possible using scientific taxonomic keys to identify aquatic invertebrates (Merrit et al.2008), fish fillets and scales (Oats et al. 1993), cleithra (Traynor et al. 2010), and fish dichotomous keys (Miller and Robison 2004) to identify fish prey items when possible. Once the prey was identified, we reconstructed TL of all prey fish (when possible) using the linear relationship between backbone and TL or standard length and TL (Table 1).

Diet analysis: Prey importance was assessed by using percent occurrence (O_i; total number of occurrences of a specific prey group/ total number of stomachs containing any prey items), percent composition by number (N_i; total number of a specific prey group/total number of prey items counted), and percent weight of prey items (W_i; total weight of each prey group/total weight of prey consumed; Bowen 1996). Stomach fullness was calculated (total stomach content weight/fish body weight x 100) and reported as a percent of both Blue Catfish and Flathead Catfish weight (Pine et al. 2005). To describe the relationship between predator and prey size, we fit quantile regression representing the 5th, 50th, and 95th percentile of reconstructed prey length for dominant prey groups relative to Blue Catfish TL (Cade and Noon 2003). ANCOVA was used to test the difference among slopes of quantile regressions. Quantile regressions relating prey size and Flathead Catfish TL could not be constructed because prey items were

Table 1. Linear relationships between backbone/total length and standard length/total length used to reconstruct total lengths of prey items consumed by Blue Catfish and Flathead Catfish during wintertime from Lake Ellsworth, Oklahoma.

				Slope			Y-intercept		
Species	Variable	*n*	r^2	Estimate	95% LCI	95% UCI	Estimate	95% LCI	95% UCI
Freshwater Drum	Back Bone Length	22	0.81	1.941	1.715	2.167	-63.244	-80.766	-45.721
	Standard Length	22	0.89	0.984	0.846	1.122	23.705	17.330	30.080
Gizzard Shad	Back Bone Length	24	0.88	1.283	1.211	1.354	35.039	23.910	46.167
	Standard Length	24	0.93	1.211	1.161	1.260	4.952	-3.699	13.603
White Crappie	Back Bone Length	16	0.85	0.947	0.752	1.142	111.277	76.825	145.729
	Standard Length	16	0.86	0.800	0.620	0.980	94.520	53.498	135.543

limited as a result of fish regurgitating upon capture (Richard Snow, visual observation). This also applies to the diet analysis of the Flathead Catfish, however we are reporting this information due to diet evaluations of large Flathead Catfish being limited in Oklahoma.

Results

A total of 159 catfish (82 Blue Catfish and 77 Flathead Catfish) was captured and analyzed for diet contents. The sizes and weights of Blue Catfish (263-1132 mm TL; 0.14 - 21.2 kg) and Flathead Catfish (635 to 1146 mm TL; 3 - 23.2 kg) evaluated in this study were similar. Prey items were found in 76.8% (63 of 82) of Blue Catfish stomachs and 20.8% (16 of 77) Flathead Catfish stomachs. The 79 catfish having diet items contained 206 individual prey items (182 items in Blue Catfish and 24 items in Flathead Catfish stomachs) and the combined stomach content weight was 17.1 kg (15.8 kg for Blue Catfish and 1.3 kg for Flathead Catfish). Only two prey items found in the catfish diets could not be identified. Both species were exclusively piscivorous during February and March 2019, foraging on six different fish species (Table 2). Mean stomach fullness for Blue Catfish was 3.6% and ranged from 0.02% to 22.6%. Flathead Catfish stomach fullness was lower and less variable with a mean of 0.7% and ranged from 0.03% to 2.2%.

Blue Catfish diets were largely composed of Gizzard Shad (*Dorosoma cepedianum*), which dominated diets by N_i (58.2%) and O_i (57.3%; Table 2). Gizzard Shad had the highest W_i (43.1%), followed closely by White Crappie (*Pomoxis annularis,* W_i = 41.6%) even though White Crappie only occurred in 27.8% of the diets (Table 2). White Crappie and Freshwater Drum (*Aplodinotus grunniens*) were both similar by N_i (18.7% and 19.2%). However, Freshwater Drum only occurred in 13% of the diets. Cannibalism of Blue Catfish and Channel Catfish (*Ictalurus punctatus*) was observed by Blue Catfish, but occurred infrequently (1.9% and 1.6% for Blue Catfish and Channel Catfish, respectively). Sunfish were also consumed by Blue Catfish, but at low rates (Table 2).

Flathead Catfish consumed similar prey items as Blue Catfish, however indices values differed. Freshwater Drum dominated Flathead Catfish diets by N_i (45.83%) and O_i (49.70%; Table 2), although White Crappie had the highest W_i (43.40%) followed by Freshwater Drum 36.30%. Gizzard Shad (25%) and White Crappie (16.70%) followed Freshwater Drum (45.83%) in N_i. Unidentified fish (4.7%) and sunfish (3.13%) occurred in Flathead Catfish diets at low rates.

Total lengths at time of consumption were estimated for 144 prey items using measurements taken from 62 backbone to TL or standard length to TL measurements (Table 1). These relationships were then used to build the three quantile relationships between total prey length and Blue Catfish TL. Blue Catfish consumed prey with a mean TL of 220 mm (range = 89 to 387 mm). Outcomes of linear regression models

Table 2. Diet composition (percent occurrence [%O_i], percent by number [%N_i], and percent by weight [%W_i]) of prey groups in the stomach contents of Blue Catfish (N = 63) and Flathead Catfish (N = 16) sampled from Lake Ellsworth, Oklahoma, during February - March 2019.

Species	Prey Species	%O_i	%N_i	%W_i
Blue Catfish	Blue Catfish *Ictalurus furcatus*	1.90	1.10	0.32
	Channel Catfish *Ictalurus punctatus*	1.58	0.55	0.48
	Freshwater Drum *Aplodinotus grunniens*	13.02	19.23	13.65
	Gizzard Shad *Dorosoma cepedianum*	57.32	58.24	43.10
	Sunfish *Lepomis* sp.	1.58	2.20	0.89
	White Crappie *Pomoxis annularis*	27.76	18.68	41.58
Flathead Catfish	Freshwater Drum	49.70	45.83	36.30
	Gizzard Shad	14.40	25.00	16.50
	Sunfish	3.13	4.20	2.70
	White Crappie	21.90	16.70	43.40
	Unidentified fish	4.70	8.33	1.15

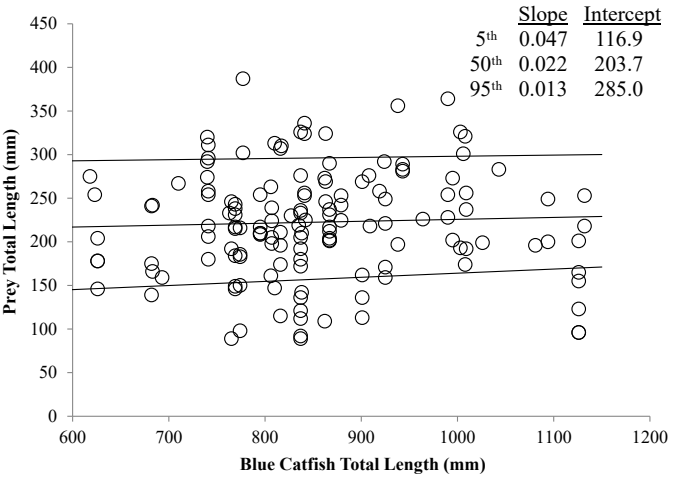

Figure 2. Quantile regressions representing the 5th, 50th, and 95th percentiles of TL of all prey sizes consumed by Blue Catfish ≥ 600 mm TL from Lake Ellsworth, Oklahoma. All prey TL were reconstructed from backbone/total length and standard length/total length linear relationships.

suggest that the 5th, 50th, and 95th quantiles of prey sizes consumed by Blue Catfish ≥ 600 mm were not significantly greater than zero (5th P = 0.31, 50th P = 0.68, and 95th P = 0.35; Figure 2). Further, we found no significant difference between the slope of prey size against Blue Catfish TL for the three quantile regressions ($F_{0.32,}$ df = 29, P = 0.73).

Discussion

The large (≥ 600mm TL) catfish collected during February and March 2019 in this study were exclusively piscivorous. This is consistent with the Flathead Catfish literature, which suggests that Flathead Catfish transition to piscivory when they are > 250mm TL (Turner and Summerfelt 1971, Layher and Boles 1980, Herndon and Waters 2002, Schmitt et al. 2017). Blue Catfish in this evaluation also only consumed fish. Blue Catfish are typically considered omnivores, consuming vegetation, mollusks, insects, crustaceans, and fish (Bonvechio et al. 2011, Hogberg and Pegg 2016, Schmitt et al. 2017, Jennings et al. 2018). Jennings et al. (2018) found that mussels, fish, and insects dominated diets of Blue Catfish during winter and spring in Lake Oconee, Georgia. Although our sampling also occurred in late winter and early spring, we found no evidence of invertebrate consumption by Blue Catfish in Lake Ellsworth. The differences in prey consumption between these studies may be related to the length distribution of Blue Catfish evaluated, which ranged from 150 to 1050 mm TL in Jennings et al. (2018), and diets were not presented by fish length groups. Conversely, Bonvechio et al. (2011) found that Blue Catfish ≥ 600 mm TL consumed mussels (50% by occurrence) and fish were present in 25% of the diets, although only nine fish of this size class were evaluated and the sample was collected during summer. Blue Catfish experience diet shifts throughout the year (Jennings et al. 2018), which could explain the lack of invertebrates in diets from Lake Ellsworth. However, Schmitt et al. (2017) found as Blue Catfish size increased the occurrence of fish in their diets also increased.

Differences in habitat and foraging behavior between the two catfish species may explain variations in diet observed in this study. Although, the prey types consumed by Blue Catfish and Flathead catfish were similar, Flathead Catfish consumed Freshwater Drum at a higher rate. Turner and Summerfelt (1971) found Freshwater Drum to be the second most preferred prey species in an evaluation of Flathead Catfish diets in six Oklahoma reservoirs. Turner and Summerfelt (1971)

speculated that the benthic habitat preference of these two species resulted in niche overlap, which resulted in the consumption of Freshwater Drum by Flathead Catfish. Our observation of Flathead Catfish being caught consistently within 1 m of the bottom of the net supports the findings of Turner and Summerfelt (1971). Conversely, Blue Catfish were often captured in the top half off the gill nets. In reservoirs, Blue Catfish prefer open water habitats. Shifts in habitat use occur seasonally when Blue Catfish reside in upper ends of reservoirs during summer, and move to the lower portion of reservoirs as water temperatures cool in the fall (Graham 1999, Grist 2002). Gizzard Shad also return to deeper water in the lower end of reservoirs in the fall when water temperature decreases (Porath 2006, Jennings et al. 2018), allowing for habitat overlap between these species that may be driving higher consumption rates of Gizzard Shad by Blue Catfish.

The large catfish captured and evaluated for diet consumed a substantial biomass of fish prey. Little is known about how these large bodied catfish influence fish communities in Oklahoma reservoirs. Where Flathead Catfish are invasive, food web simulation modeling suggests that Flathead Catfish can reduce native species biomass by 50% (Pine et al. 2007). Blue Catfish are considered generalists that can adapt to a wide range of habitats and prey resources, so they may compete with native species without directly consuming them (Schmitt et al. 2017). However, large Blue Catfish appear to be more piscivorous as their size increases (Schmitt et al. 2017). Although our sample size of Blue Catfish used for diet analysis was fairly small (63), they consumed 15.8 kg of fish. For example, White Crappie comprised 42% of Blue Catfish diets by weight, however they only made up 18.7% of the sample by number. This finding makes us curious about the impacts that large catfish have on shaping fish communities in Oklahoma reservoirs and is a need for further research.

Although we were not able to construct quantile regressions relating prey size to Flathead Catfish TL because samples sizes were low due to regurgitation, previous research suggests that Flathead Catfish are not gape limited and can eat prey of almost any size (Slaughter and Jacobson 2008). For example, the world record Flathead Catfish (1549.54 mm TL) caught in Elk City Reservoir, Kansas contained a 711.2 mm TL Bigmouth Buffalo, which was 46% of the Flathead Catfish TL (Neely and Lynott 2016). The quantile regressions suggests that once Blue Catfish reach ≥ 600 mm they consume similar sized prey as the largest fish found in the sample. However, we could not find anything in the literature to compare our results, so this could be specific to Lake Ellsworth. Size structure of Blue Catfish in Lake Ellsworth is considered slow growing and maximum growth potential is smaller when compared to other reservoirs in Oklahoma (Boxrucker and Kuklinski 2006, Cofer 2011). Diet studies from other reservoirs in Oklahoma with a large Blue Catfish size structure would help to gain a better understanding of predator-prey dynamics.

We used gillnets (set overnight) to capture Blue Catfish and Flathead catfish for diet analysis. However, Bowen (1996) suggests that this technique could result in loss of diet items through regurgitation caused by capture stress. It was apparent to us that Flathead Catfish were regurgitating at high rates. Upon dissection we found that their swim bladders were inflated (likely from lifting fish in gillnets out of deep water), which pushed the stomach and contents out of most fish, and in some cases, the stomach was observed inverted in the mouths of fish. To avoid fish regurgitating, Bowen (2006) recommends setting gillnets for a shorter amount of time or using trammel nets. However, we did not observe the same effect on Blue Catfish, as only 23% of fish had empty stomach, which was similar to empty stomach rates in other studies (Bonvechio et al. 2011, Schmitt et al. 2017, Jennings et al. 2018). Jennings et al. (2018) speculated that using gillnets during warmer months influenced the number of Blue Catfish containing stomach contents. The use of gillnets is a potential bias in our study, however electrofishing is not effective during wintertime (Bodine and Shoup 2010), does not capture many large catfish (≥ 762 mm; Boxrucker and Kuklinski 2006), and could therefore be equally

biased, just in different ways. Gillnets were our only option to describe winter catfish diets for large individuals. However, comparison of our results with diet studies collected by electrofishing should be made with caution given the possibility of different biases related to gears.

Our results describe the diet composition of large Blue Catfish and Flathead Catfish from a single Oklahoma Reservoir. This improves our knowledge regarding the diets of large catfish in Oklahoma, which was previously not well understood. Large Blue Catfish may have the potential to consume a large biomass of fish prey (250.4 g/fish). If the current ODWC Blue Catfish regulation (harvest of one fish ≥762 mm) is effective at increasing the number of large catfish in Oklahoma reservoirs, our results suggest that they may shape the fish communities through predation. Further research should expand diet analysis across several Oklahoma reservoirs and other times of the year to better understand seasonal and size structure effects, predator-prey relationships, ontogenetic shifts, and prey selectivity of large catfishes. Also, a multiple gear approach may be necessary to fully describe catfish diets, as a single gear type is not effective at collecting catfish across seasons.

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Body Size Estimation and Identification of Twelve Fish Species Using Cleithrum Bones

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Abstract: Diet evaluations are conducted to understand predator-prey dynamics of fish communities. However, unless prey items are extracted from fish immediately after consumption, items can be observed at various stages of decomposition due to digestion. Thus, the ability to accurately measure or identify prey fish is difficult. Fortunately, some skeletal structures, such as the cleithrum bone, are not easily digested and remain in fish stomachs. Cleithra have been used to estimate the total length of a fish by determining the linear relationship between the total length, horizontal length, or vertical height of a cleithrum against known-sized fish from which the structure was taken. We used linear regression to develop equations to estimate body size for twelve common forage species found in Oklahoma reservoirs using cleithrum bones. The relationships between total fish length:cleithrum length (r^2= 0.94-0.99), total fish length:horizontal cleithrum length (r^2= 0.90-0.98), and total fish length:vertical cleithrum length (r^2= 0.88-0.98) were significant. Additionally, we also described cleithrum characteristics for each of the twelve fish species, such that fish can be identified even when prey items are heavily digested. When used collectively, the regression equations and diagnostic features of cleithra will provide a more accurate description of fish diets and a better understanding of predator-prey relationships.

Introduction

Dietary analysis is an important aspect to understanding predator-prey dynamics in fish communities. Diets are typically evaluated using stomach content analysis, which relies on identification of prey remains, but depending on digestion rate, prey items can be in various stages of decomposition making it difficult to accurately identify prey items, or get an accurate measure of their length and weight (Hansel et al. 1988, Scharf et al. 1998, Tarkan et al. 2007, Snow et al. 2017). Accurately identifying and measuring prey items is critical when attempting to understand bioenergetics, feeding ecology, predator consumption rates (Hansel et al.1988, Scharf et al. 1998, Snow et al. 2017), predation influences on fish recruitment (Ball and Weber 2018), and when managing fisheries at the community level (Knight et al. 1984), so overcoming issues associated with digested prey items is important to thoroughly describe fish diets.

Skeletal remains (cleithra, dentaries, operculum bones, otoliths, pharyngeal arches, and vertebrae) found in stomach contents have been used to identify different prey species, and reconstruct length and weight of prey items in both marine and freshwater systems (Hansel et

al. 1988, Scharf et al. 1998, Radke et al. 2000, Dietrich et al. 2006, Tarkan et al. 2007, Snow et al. 2017, Yazicioglu et al. 2017, Assis et al. 2018). Cleithra (bones associated with the pectoral girdle) are often used because they are one of the largest and most robust bones in the skeletal system of a fish, persist in diets because they are not easily digested, and are morphologically distinct (Figure 1; Hansel et al. 1988). In addition, they are one of the first diagnostic bones to form during fish development, making this structure useful for young prey fish (Hansel et al.1988). A linear relationship exists between cleithrum dimensions and fish size, which allows for back-calculation of fish total length and weight using a cleithrum measurement (Hansel et al. 1988, Scharf et al. 1998, Wood 2005, Dietrich et al. 2006, Snow et al. 2017).

Because cleithra are useful for reconstruction of fish size and identification of fish species, the objectives of this study were to evaluate the linear relationship between cleithra dimensions and fish size (length and weight) for twelve common prey fish species in Oklahoma aquatic systems. Further, we will describe diagnostic features of cleithra to aid in identification of these species. This information will benefit future diet studies in Oklahoma, or other systems where these prey species are common, by allowing for a more comprehensive description of fish diets.

Methods

A total of 737 fish were collected from twelve species, which consisted of 30 Black Crappie (*Pomoxis nigromaculatus*), 88 Bluegill (*Lepomis macrochirus*), 89 Gizzard Shad (*Dorosoma cepedianum*), 30 Golden Shiners (*Notemigonus crysoleucas*), 78 Green Sunfish (*Lepomis cyanellus*), 75 Inland Silversides (*Menidia beryllina*), 62 Largemouth Bass (*Micropterus salmoides*), 86 Longear Sunfish (*Lepomis megalotis*), 36 Red Shiners (*Cyrpinella lutrensis*), 79 Redear Sunfish (*Lepomis microlophus*), 47 saugeye (female Walleye [*Sander vitreus*] and male Sauger [*S. canadensis*], and 37 White Crappie (*Pomoxis annularis*). Fish were collected opportunistically during fall 2017 through spring 2019 using boat electrofishing, seining, or fyke netting from eight reservoirs in Oklahoma (Table 1). Once fish were collected, they were put on ice and transported to the Oklahoma Fishery Research Laboratory in Norman, Oklahoma, where they were frozen until processing.

When processing samples, each fish was measured for total length (TL; nearest mm) weighed (nearest g), and both cleithra removed (Figure 1). Cleithra were cleaned by placing them into a beaker filled with water and boiled on a hot plate (Thermolyne Type 1900; 107.2°C). Cleithra were removed from small fish (<50 mm) by boiling them whole. Similarly, fish with fragile cleithra (Gizzard Shad and Inland Silverside) were cut into a section that encapsulated the cleithra and boiled, which lessened the risk of damaging diagnostic features of the cleithra. Cleithra were boiled until they could be easily cleaned (30 to 90 sec, depending on size). Cleaned cleithra were placed into an envelope to dry and stored until measuring.

Cleithra were measured (nearest .01mm) under a dissecting scope, using AmScope 3.7 software. If cleithra were too large to be measured under the microscope, a digital caliper

Table 1. Sampling locations of the twelve fish species collected for cleithra evaluation.

	Lakes							
Species	Arcadia	Dahlgren	Elmer	New Spiro	Pawhuska	Sparks	Stilwell City	Thunderbird
Black Crappie (*Pomoxis nigromaculatus*)				X	X		X	
Bluegill (*Lepomis macrochirus*)			X	X	X	X	X	
Gizzard Shad (*Dorosoma cepedianum*)	X		X	X				
Golden Shiner (*Notemigonus Crysoleucas*)					X		X	
Green Sunfish (*Lepomis cyanellus*)		X	X		X			
Inland Silverside (*Menidia extensa*)	X			X			X	
Largemouth Bass (*Micropterus salmoides*)	X		X	X	X	X	X	
Longear Sunfish (*Lepomis megalotis*)	X				X		X	
Red Shiner (*Cyprinella lutrensis*)	X							
Redear Sunfish (*Lepomis microphus*)			X	X	X	X	X	
Saugeye: female Walleye [*Sander vitreu* s] and male Sauger [*S. canadensis*]	X							X
White Crappie (*Pomoxis annularis*)			X	X		X		

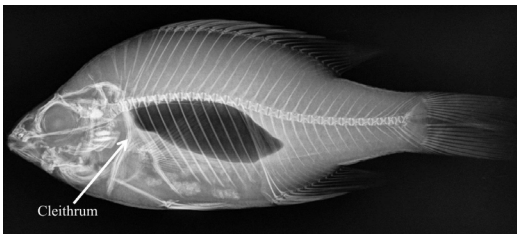

Figure 1. X-ray image showing the location of the cleithrum in a Redear Sunfish.

(Griffon Corporation, New York, NY) was used to measure cleithra (±0.03 mm). Three measurements: cleithrum length (CL), vertical length (VL), and horizontal length (HL), were recorded for each cleithrum from the inside lateral view (ILV) side for both the left and right cleithrum (Figure 2). Only CL could be measured for Gizzard Shad and Inland Silversides because of the shape of their cleithra. If a cleithrum was damaged any measurement that would have been affected by the damage was not taken. For example, if a spine was broken neither CL nor VL were measured.

Linear regression models relating fish total length and cleithrum measurements were

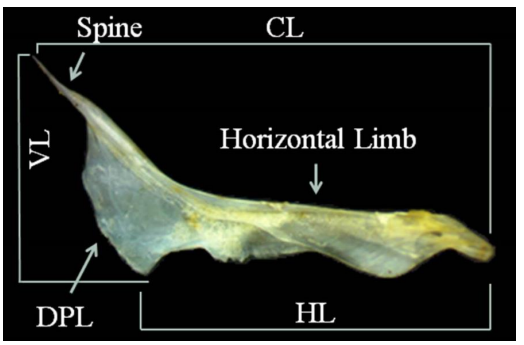

Figure 2. Photograph of a Redear Sunfish cleithrum with labels describing morphological features and measurements taken for regression analysis. Cleithra are viewed from the inside lateral view. Cleithrum length (CL) is measured from tip of spine to posterior end of the horizontal limb. Vertical length (VL) is measured from tip of spine to posterior end of the vertical limb, also known as the dorsoposterior lobe (DPL). Horizontal length (HL) is measured from the posterior end of the DPL to the posterior end of the horizontal limb.

calculated using Microsoft Excel. Models were developed for the left and right cleithra, using all three cleithrum measurements. A paired t-test was used to compare the predicted total fish length from the right and left cleithrum for each measurement (CL, VL, and HL) and species. Test outcomes were considered significant at $P \leq 0.05$. If t-tests were not statistically different, the left and right cleithra were pooled for regression analysis. Each correlation was tested for strength using r^2 values and mean percent prediction error [(Observed - Predicted)/Predicted*100] for each model and averaging the percent prediction error for each observation (Wood 2005, Scharf et al. 1998, Snow et al. 2017). Lastly, an exponential equation was developed to predict fish weight using the observed length of the fish.

Results

Estimation of Original Prey Length

Linear regression models indicated significant relationships between fish total length and all cleithrum measurements ($P < 0.001$; Table 2- 4). The predicted fish lengths using measurements from the left and right cleithrum for each species were not significantly different ($P > 0.05$), so samples were pooled. On average, fish total length:CL (left, right, and pooled) had the strongest linear relationships (r^2=0.95-0.99;Table 2), although strong relationships existed for fish total length: HL (r^2 = 0.90-0.98; Table 3) and fish total length: VL (r^2 = 0.88-0.98;Table 3).The mean percent predictive error range was smallest for measurements of CL (-0.9-0.26%; Table 1), followed by HL (-1.79-0.52%; Table 3) and VL (-2.72-1.74%; Table 4).The exponential equations relating length and weight were highly correlated ($r^2 \geq 0.91$; Table 5).

Cleithrum Morphology

The cleithra were diagnostic for all species studied, although some are more difficult to differentiate than others (Figure 3). Cleithra morphology is very similar across species in the family Centrarchidae. In general, members of this family have cleithra with a short spine at the tip of the vertical limb, an elongated horizontal limb, and an unserrated dorsoposterior lobe (DPL; Hansel et al. 1988). Distinguishing among

Table 2. Linear regression equations for predicting total length from the right cleithrum length (CLR), left cleithrum length (CLL), and pooled cleithrum length (CLP) with the related r^2, P-value, and mean predictive error (% Error).

Species	Range (mm)	Equation	n	R^2	P-value	% Error
Black Crappie	65-157	TL = 5.1936CLR + 11.69	30	0.97	<0.001	0.26
		TL = 5.3621CLL + 8.0177	29	0.97	<0.001	-0.8
		TL = 5.27CLP + 9.9957	59	0.97	<0.001	-0.24
Bluegill	25-203	TL = 5.1173CLR + 5.9752	80	0.98	<0.001	-0.73
		TL = 4.943CLL + 6.5757	81	0.98	<0.001	-0.9
		TL = 5.0246CLP + 6.3331	161	0.98	<0.001	-0.82
Gizzard Shad	65-217	TL = 6.8994CLR - 14.278	84	0.98	<0.001	-0.1
		TL = 6.907CLL - 14.709	82	0.99	<0.001	-0.12
		TL = 6.9028CLP - 14.484	166	0.98	<0.001	-0.11
Golden Shiner	78-221	TL = 7.3608CLR + 25.481	26	0.95	<0.001	-0.24
		TL = 7.8963CLL + 14.854	25	0.95	<0.001	-0.08
		TL = 7.6329CLP + 20.053	51	0.95	<0.001	-0.18
Green Sunfish	25-171	TL = 4.4246CLR + 11.517	75	0.98	<0.001	-0.31
		TL = 4.4922CLL + 8.8259	72	0.98	<0.001	-0.05
		TL = 4.4464CLP + 10.421	147	0.98	<0.001	-0.18
Inland Silverside	24-110	TL = 11.428CLR - 5.9938	70	0.97	<0.001	0.21
		TL = 11.453CLL - 5.0227	69	0.98	<0.001	0.11
		TL = 11.455CLP - 5.5879	139	0.97	<0.001	0.16
Largemouth Bass	65-197	TL = 5.8924CLR + 13.093	55	0.97	<0.001	-0.12
		TL= 5.8796CLL + 12.257	52	0.96	<0.001	-0.08
		TL = 5.8945CLP + 12.544	107	0.97	<0.001	-0.1
Longear Sunfish	55-142	TL = 3.8577CLR + 23.621	84	0.97	<0.001	-0.09
		TL= 4.0157CLL + 19.305	74	0.97	<0.001	-0.08
		TL = 3.9239CLP + 21.79	158	0.97	<0.001	-0.09
Red Shiner	37-75	TL = 6.6307CLR + 9.767	33	0.95	<0.001	-0.04
		TL = 6.5405CLL + 9.5064	36	0.96	<0.001	-0.02
		TL = 6.5747CLP + 9.6915	69	0.95	<0.001	-0.03
Redear Sunfish	54-183	TL = 4.5682CLR + 10.9	78	0.98	<0.001	-1.65
		TL = 4.679CLL + 7.5378	76	0.98	<0.001	-1.74
		TL = 4.6197CLP + 9.3033	154	0.98	<0.001	-1.7
Saugeye	56-130	TL= 9.2917CLR - 7.4933	43	0.99	<0.001	0
		TL = 9.2475CLL - 7.8239	43	0.98	<0.001	-0.02
		TL = 9.2709CLP - 7.6717	82	0.98	<0.001	-0.1
White Crappie	91-174	TL= 5.0061CLR + 24.1	37	0.95	<0.001	-0.05
		TL= 5.3132CLL + 17.439	35	0.94	<0.001	-0.04
		TL = 5.146CLP + 21.074	72	0.94	<0.001	-0.05

genera (*Lepomis*, *Micropterus,* and *Pomoxis)* within Centrarchidae is not difficult, but it can be challenging to distinguish species within a genus. In *Lepomis*, Bluegill and Longear Sunfish have morphologically similar cleithra, however Bluegill have a more rounded DPL, and Longear Sunfish cleithra have a more robust spine. Redear Sunfish have a long, thin spine with a rectangular-shaped DPL. Green Sunfish cleithra spines taper abruptly and have a notch in the DPL. Black and White Crappie (*Pomoxis spp*.) and Largemouth Bass (*Micropterus*) cleithra all have thick spines, which easily distinguish them from *Lepomis spp.* However, Largemouth Bass cleithra are distinguished from Crappie by having a flatter HL. *Pomoxis spp*. are difficult to distinguish from each other using cleithra, but these species can be separated because Black Crappie have a depression at the top of the DPL where it intersects with the spine. Black Crappie cleithra also have a trapezoidal-shaped notch where the DPL transitions to the horizontal limb, whereas this notch is rounded in White Crappie cleithra. Saugeye (Percidae) cleithra are similar

Table 3. Linear regression equations for predicting total length from the right cleithrum horizontal length right (HLR), left cleithrum horizontal length (HLL), and pooled cleithrum horizontal length (HLP) with the related r^2, P-value, and mean predictive error (% Error).

Species	Range (mm)	Equation	n	R^2	P-value	% Error
Black Crappie	65-157	TL = 7.0368HLR + 14.961	30	0.96	<0.001	0.52
		TL = 7.2972HLL + 11.538	29	0.97	<0.001	-0.63
		TL = 7.1591HLP + 13.349	59	0.96	<0.001	-0.03
Bluegill	25-203	TL = 7.3123HLR + 6.582	81	0.97	<0.001	-0.8
		TL = 7.213HLL + 6.1171	84	0.98	<0.001	-0.7
		TL = 7.2608HLP + 6.3529	165	0.98	<0.001	-0.75
Golden Shiner	78-221	TL = 10.526HLR + 27.791	26	0.94	<0.001	-0.28
		TL = 11.186HLL + 18.841	26	0.94	<0.001	-0.22
		TL = 10.868HLP + 23.151	52	0.94	<0.001	-0.26
Green Sunfish	25-171	TL = 6.2311HLR + 12.158	76	0.98	<0.001	-0.32
		TL = 6.0848HLL + 13.279	74	0.98	<0.001	-0.13
		TL = 6.1593HLP + 12.695	150	0.98	<0.001	-0.23
Largemouth Bass	65-197	TL = 7.3355HLR + 10.661	58	0.97	<0.001	-0.07
		TL = 7.2502HLL + 12.198	53	0.97	<0.001	-0.05
		TL = 7.2985HLP + 11.356	111	0.97	<0.001	-0.06
Longear Sunfish	55-142	TL = 5.2714HLR + 23.683	84	0.96	<0.001	-0.09
		TL = 5.3853HLL + 20.84	74	0.96	<0.001	-0.09
		TL = 5.3163HLP + 22.498	158	0.96	<0.001	-0.09
Red Shiner	37-75	TL = 8.5844HLR + 12.419	32	0.90	<0.001	-0.06
		TL = 8.4246HLL + 12.171	36	0.92	<0.001	-0.04
		TL = 8.4773HLP + 12.413	70	0.91	<0.001	-0.05
Redear Sunfish	54-183	TL = 6.4133HLR + 14.566	79	0.98	<0.001	-1.79
		TL = 6.7244HLL + 8.3065	75	0.98	<0.001	-1.48
		TL = 6.5484HLP + 11.747	154	0.98	<0.001	-1.68
Saugeye	56-130	TL= 9.0398HLR + 3.4672	43	0.98	<0.001	0.02
		TL= 8.8001HLL + 6.9112	43	0.98	<0.001	0.03
		TL = 8.914HLP + 5.2417	82	0.98	<0.001	0.03
White Crappie	91-174	TL= 6.9388HLR + 27.71	37	0.93	<0.001	-0.07
		TL= 7.1534HLL + 24.612	35	0.91	<0.001	-0.05
		TL = 7.0395HLP + 26.263	72	0.92	<0.001	-0.06

in shape to those of centrarchids, but differ in that they have a short, wide DPL that can be serrated (Figure 3; Traynor et al. 2010).

Gizzard Shad and Inland Silverside have very distinct cleithra compared to all other fish evaluated in this study. Gizzard Shad cleithra are fragile and have a distinct sickle-shape with a large medial process. Inland silverside cleithra are claw shaped with holes in the DPL. Cleithra in Cyprinidae can be very difficult to distinguish among species, but in general, they have cleithra with an expanded lateral shelf, and some will have a hook-like process on the anterior end of the horizontal limb (Traynor et al. 2010). In this study, only Golden Shiners and Red Shiners were common enough in reservoirs to include. Cleithra of both species are L-shaped, with a hook-like process on the anterior end of the horizontal limb, but the Golden Shiner has a

Table 4. Linear regression equations for predicting total length from right cleithrum vertical length (VLR), left cleithrum vertical length (VLL), and pooled cleithrum vertical length (VLP) with the related r^2, P-value, and mean predictive error (% Error).

Species	Range (mm)	Equation	n	R^2	P-value	% Error
Black Crappie	65-157	TL = 9.9602VLR + 24.528	30	0.96	<0.001	0.14
		TL = 9.6133VLL + 26.93	30	0.95	<0.001	1.18
		TL = 9.7827VLP + 25.752	60	0.96	<0.001	0.66
Bluegill	25-203	TL = 10.311VLR + 13.781	81	0.94	<0.001	-1.33
		TL = 10.057VLL + 13.739	83	0.96	<0.001	1.74
		TL = 10.176VLP + 13.794	163	0.95	<0.001	-1.31
Golden Shiner	78-221	TL = 9.804VLR + 37.261	26	0.88	<0.001	-0.23
		TL = 10.319VLL + 29.472	27	0.91	<0.001	-0.21
		TL = 10.077VLP + 33.129	53	0.90	<0.001	-0.22
Green Sunfish	25-171	TL = 8.0022VLR + 19.315	75	0.95	<0.001	-0.31
		TL = 8.1371VLL + 16.267	72	0.96	<0.001	0.66
		TL = 8.0425VLP + 18.079	146	0.95	<0.001	-0.19
Largemouth Bass	65-197	TL = 11.129VLR + 33.69	57	0.93	<0.001	-0.15
		TL = 11.636VLL + 27.535	60	0.94	<0.001	-0.2
		TL = 11.363VLP + 30.745	117	0.94	<0.001	-0.17
Longear Sunfish	55-142	TL= 7.5075VLR + 30.099	85	0.95	<0.001	-0.09
		TL= 7.8644VLL + 26.001	81	0.95	<0.001	-0.09
		TL = 7.6713VLP + 28.217	166	0.95	<0.001	-0.09
Red Shiner	37-75	TL = 8.0008VLR + 16.834	33	0.92	<0.001	-0.03
		TL = 7.8596VLL + 16.956	36	0.93	<0.001	-0.03
		TL = 7.9252VLP + 16.906	69	0.93	<0.001	-0.03
Redear Sunfish	54-183	TL = 8.1726VLR + 19.406	78	0.97	<0.001	-2.72
		TL = 8.4527VLL + 15.612	77	0.97	<0.001	-1.73
		TL = 8.1726VLP + 19.406	155	0.97	<0.001	-2.24
Saugeye	56-130	TL = 17.79VLR- 4.0251	45	0.96	<0.001	0.1
		TL= 17.563VLL - 4.073	44	0.98	<0.001	0.03
		TL = 17.661VLP - 3.9634	87	0.97	<0.001	0.07
White Crappie	91-174	TL = 9.43VLR + 40.056	37	0.89	<0.001	-0.01
		TL= 9.3281VLL + 39.717	37	0.94	<0.001	-0.01
		TL = 9.3692VLP + 39.975	74	0.92	<0.001	-0.01

cleithrum with a more elongated DPL, while the DPL on a Red Shiner cleithrum approximates a 90° angle, and also has a rounded anterior end on the horizontal limb (Figure 3).

Discussion

Reconstruction of original sizes and identification of prey from digested remains is essential to thoroughly characterize fish diets. While use of external features can be a quicker method to identify a prey item (Scharf et al. 1997, Ball and Weber 2018), it may not be possible due to digestive decomposition. Numerous bony structures of fish have been used to identify the species or reconstruct the original size of the fish, including dentary, premaxilla, and maxilla bones (Hajkova et al. 2003, Wood 2005), otoliths (Tarkan et al. 2007, Snow et al. 2017, Assis et al. 2018), pharyngeal bones (Mann and Beaumont 1980, Hansel et al. 1988, Radke et al. 2000, Snow et al. 2017), opercula (Hansel et al.

1988, Scharf et al. 1998, Hajkova et al. 2003, Wood 2005), vertebrae (Trippel and Beamish 1987, Hajkova et al. 2003), and cleithra (Hansel et al. 1988, Scharf et al. 1998, Wood 2005, Snow et al. 2017). Hansel et al. (1988) found that cleithra and dentaries were found most often in stomach contents of piscivores and are the most reliable structures for identifying prey fish. Further, cleithra measurements can be used to predict the original length of a fish (Hansel et al. 1988, Scharf et al. 1998, Wood 2005, Snow et al. 2017).

In our study, a significant linear relationship existed between the total length of a fish and the three cleithrum measurements (CL,

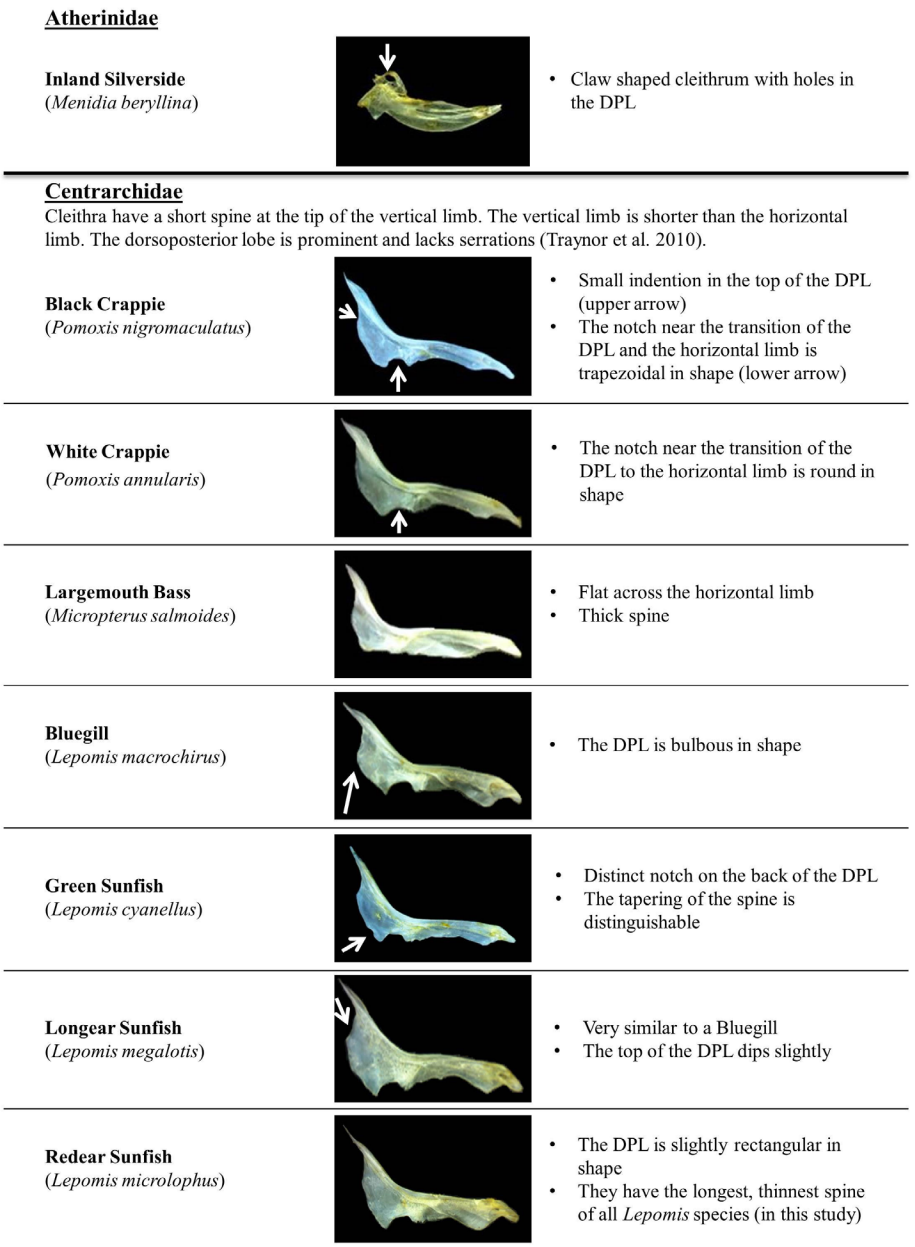

Figure 3. Photographs and description of cleithra diagnostic characteristics from twelve species of common forage species collected from Oklahoma reservoirs.

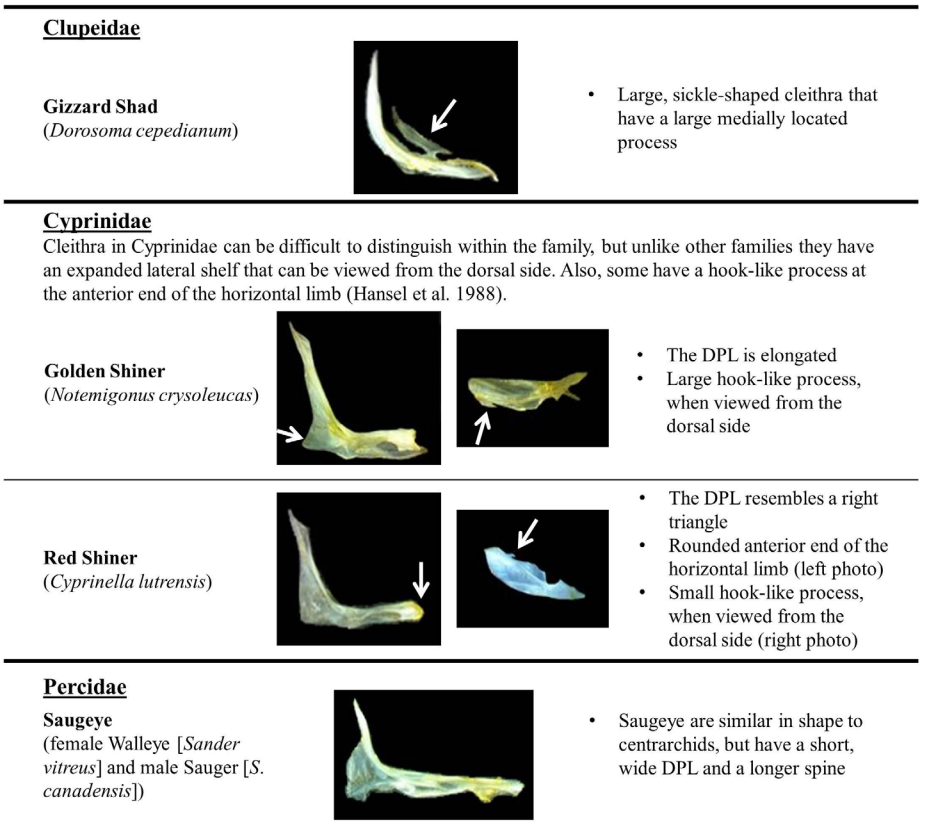

Figure 3. (Continued).

VL, and HL). Similarly, Wood (2005) found linear relationships between dentary, maxilla, premaxilla, opercle, and cleithrum measurements and live total length of prey fish found in Bluefish diets. Hansel et al. (1988) found slightly more accurate estimates of fish length predicted using cleithrum and opercle measurements than with measurements from pharyngeal arches or dentaries for 14 species. In a comparison of boney structures to predict total length for 10 fish species, cleithrum length was the most reliable measurement for predicting live fish length (Scharf et al.1998). Snow et al. (2017) found that measurements of cleithra, pharyngeal teeth, and otoliths accurately predicted total length of Central Stonerollers. Our results suggest that cleithra measurements can be used reliably to estimate the original fish length of the twelve species evaluated in this study.

In this study, all predictive equations from the three cleithra measurements produced reliable estimates of fish total length. This suggests that even if a spine or part of the horizontal limb is broken, an accurate predicted fish length can still be attained using a measurement from the intact portion of the cleithrum. When fresh from a diet, cleithra are soft and can be easily torn, so it is important to handle them with care. Although our results suggest that all may not be lost if a structure is damaged, several considerations should be made when using cleithra dimensions to reconstruct original prey size. The cleithra used in our study were boiled and cleaned. Scharf et al. (1998) suggested that boiling to remove soft tissue might cause bones to shrink or deform if an excess of time elapses between boiling and measuring. The cleithra in this study were only boiled long enough to loosen excess tissues, given time to dry, and were measured immediately after drying. Although not used

Table 5. Exponential equations for predicting weight (W) from total length (TL), and the resulting r^2 value for twelve fish species.

Species	Equation	R^2
Black Crappie	$W = 0.4307e^{0.0317(TL)}$	0.98
Bluegill Sunfish	$W = 0.1019e^{0.0534(TL)}$	0.93
Gizzard Shad	$W = 0.7342e^{0.0244(TL}$	0.96
Golden Shiner	$W= 0.7411e^{0.0244(TL)}$	0.94
Green Sunfish	$W = 0.42e^{0.0348(TL)}$	0.94
Inland Silverside	$W = 0.0431e^{0.0504(TL)}$	0.91
Largemouth Bass	$W = 0.9201e^{0.0244(TL)}$	0.97
Longear Sunfish	$W = 0.6265e^{0.0333(TL)}$	0.98
Saugeye	$W = 0.1405e^{0.0372(TL)}$	0.98
Redear Sunfish	$W = 0.479e^{0.0334(TL)}$	0.96
Red Shiner	$W = 0.0725e^{0.0581(TL)}$	0.91
White Crappie	$W = 0.838e^{0.0248(TL)}$	0.96

in this study, preservatives also alter bone dimensions if used to store stomach contents (Hansel et al. 1988, Scharf et al. 1998, Snow et al. 2017). It is imperative that cleithra are handled cautiously to ensure diagnostic features are preserved, so accurate measurements can be taken.

Significant relationships were found between the three cleithra measurements and fish total length and weight of the twelve prey species evaluated in this study. Because the linear relationships reported in this study are for fish within a particular range of sizes, caution should be taken before applying lengths to fish outside of this range, as allometric relationships may change depending on fish size (Scharf et al. 1998). Although differences among genera can be subtle, cleithrum morphology can be used to identify fish remains for the twelve fish species in this study. Identification of prey items in piscivore diets using cleithra will allow for a more accurate depiction of fish diet breadth, which is important when investigating diets of top predators. When used collectively, the regression equations and diagnostic features described in this study from cleithra will provide a more accurate description of fish diets and a better understanding of predator-prey relationships.

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#GARWEEK: Insights from a Social Media Outreach Campaign about Alligator Gar in Oklahoma

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Abstract: Alligator gar (*Atractosteus spatula*) populations have declined throughout much of their range, and one of the reasons for the decline is misconceptions by fisheries managers and the general public. In recent years, directed research and renewed interest by anglers has increased appreciation of alligator gar, but fisheries managers still lack an understanding of how the general public perceives this species. Using social media, managers can characterize the public's sentiment towards alligator gar and identify misconceptions. Biologists and the social media specialist with the Oklahoma Department of Wildlife Conservation joined Texas Parks and Wildlife in their social media campaign, #GARWEEK, to educate the public about alligator gar and gain a better understanding of the public's opinion of alligator gar in Oklahoma. Five posts were made to a variety of social media platforms with Facebook being the most heavily used. Social media analytics and sentiment analysis were used to evaluate the public's perception of alligator gar. Overall, the sentiment expressed by the public was positive indicating a major shift in public perception towards alligator gar. However, misconceptions relating to alligator gar and management remain, though these may be overcome via communication efforts that include social media. In time, this will lead to a better understanding by the public of alligator gar and the role of alligator gar in the environment and more effective management.

Introduction

Historically, alligator gar (*Atractosteus spatula*) has been viewed through a lens of hostility, criticism, and distaste (Scarnecchia 1992). The negative public perception towards gar originates from their toothy, threatening appearance and the misconception that gars prey primarily on sportfish. These sentiments resulted in the targeted removal of gar from aquatic systems by recreational anglers (Scarnecchia 1992; Garcia de Leon et al. 2001; O'Connell et al. 2017). Historically, many state natural resources agencies have directed efforts to eradicate gar with the motivation of promoting sportfish population growth (Scarnecchia 1992;

Binion et al. 2015; David et al. 2018). Local reductions in population abundance caused by targeted removal and harvest, coupled with habitat alteration and loss, has resulted in the extirpation of alligator gar from most of their historic range (Buckmeier et al. 2016; Kluender at al. 2016; David et al. 2018). The American Fisheries Society's Endangered Species Committee currently recognizes the alligator gar as "vulnerable" because of range reduction and over-exploitation (Jelks et al. 2008).

In recent years, however, the perception of alligator gar has apparently shifted in a positive direction. An increase in directed research and renewed interest by recreational anglers has led to an increased appreciation of alligator gar among fisheries biologists and anglers (David et al. 2018). Despite unregulated harvest of the species historically (DiBenedetto 2009, Smith et al. 2018), recent management efforts have recognized the commercial and recreational value of alligator gar by regulating harvest, promoting sustainable harvest, and reintroducing the species in portions of its former range (Buckmeier et al. 2016, Smith et al. 2018). Compared to other members of Lepisosteidae, alligator gar presently garners the most attention from recreational anglers because of their potential to reach "trophy" sizes (≥ 2438 mm total length; Buckmeier et al. 2016, Smith et al. 2018).

Even with increasing interest from biologist and anglers towards alligator gar, managers lack an understanding of how the general public currently perceives alligator gar. Management and conservation actions, and the research that informs such efforts, are often directed toward species that are charismatic or well-known by the public (Reimer et al. 2013). Would the public support restoration projects or management efforts directed at conserving alligator gar populations? This question is a complex one that has yet to be addressed. Alligator gar have been traditionally viewed as an unfavorable species, but how have perceptions of alligator gar among the general public changed as more research, management, and angling effort is directed towards the species?

Prior to widespread adoption of social media, communication among recreational anglers occurred primarily through word of mouth when they would gather to discuss fishing at local coffee shops, baits shops, boat ramps or access areas (Claussen et al. 2013; Kopaska and Fox 2013; Midway and Cooney2013; Taylor and Sammons 2019). Therefore, researchers often used creel surveys, phone surveys, or questionnaires to gauge the attitudes and opinions of constituents about a given management topic. Within the last decade, however, social media platforms have provided anglers the ability to exchange information in real time via the internet (Martin et al. 2012; Kopaska and Fox 2013). In response, biologists have adopted social media platforms to improve public outreach of management and conservation topics (Claussen et al. 2013; Kopaska and Fox 2013; Midway and Cooney 2013; Taylor and Sammons 2019). Social media outreach efforts can result in quantifiable increases in public perception; for example, Reimer et al. (2013) found that providing the public with facts about the rarity, uniqueness, habitat, and local importance resulted in an increased positive perception of the eastern hellbender (*Cryptobranchus alleganiensis alleganiensis*) a rare, but uncharismatic, aquatic giant salamander that has been killed intentionally by humans in the past. Furthermore, analyzing the public's comments made on social media outreach posts via "opinion mining" could help managers characterize the public's sentiment towards a given topic (Palomino et al. 2016).

Because modern anglers now rely heavily on social media to obtain information about fishing (Martin et al. 2014) and social media platforms provide an instant path of communication where constituents can exchange information (Claussen et al. 2013; Midway and Cooney 2013; Martin et al. 2014), the Oklahoma Department of Wildlife Conservation (ODWC) has been using social media platforms to educate the general public about the state's natural resources and the science that informs resource management. The ODWC joined the Texas Parks and Wildlife Department's (TPWD) #GARWEEK campaign to inform followers about alligator gar and to gain

a better understanding of the public's perception of alligator gar in Oklahoma. Biologists and social media specialists with ODWC crafted outreach-oriented #GARWEEK posts about the conservation and management of alligator gar in Oklahoma with the following objectives: 1) to increase ODWC's target audience and impact on its social media pages on Facebook, Twitter and Instagram; and 2) to quantify the public's sentiment towards these messages about alligator gar via "opinion mining" of comments made on these social media posts. Results gleaned from this study can be used to improve the breadth and impact of future social media outreach efforts related to alligator gar, while also providing an important baseline for public sentiment towards alligator gar that can be monitored over time.

Methods

Social media outreach posts were made by ODWC biologists and social media specialists during a 5-day period designated as "Gar Week" from June 11 to 16, 2018. A total of 5 posts were created, each consisting of informative text about alligator gar and at least one original video or photograph intending to garner more engagement among followers (Osborne-Gowey 2014; Taylor and Sammons 2019). Post #1 consisted of a 1-minute video clip that featured a large alligator gar and texts that detailed their large size, diet of primarily scavenging on non-game fishes, and status as a Species of Special Concern in Oklahoma (Figure 1). Post #2 featured a photograph of the snouts of an alligator gar, a hybrid alligator

1.) Oklahoma's largest fish, the alligator gar, is something seen as a freshwater monster. But, these ancient fish mostly scavenge for nongame fish like gizzard shad, carp, and buffalo species. In Oklahoma they are a Species of Special Concern. #GARWEEK

2.) Four species of gar are found in Oklahoma. Pictured here are two species and a hybrid. Researchers have recently discovered hybridization between alligator gar and other species of gar occurs in their natural habitat. This photo shows the snout differences between an alligator gar (left), hybrid (middle) and longnose gar (right). #GARWEEK

3.) Have you ever wondered about the early development of alligator gar? Perhaps this will help... During spawning, alligator gar eggs are deposited on vegetation that is submerged in water (1), where they attach for a period of 2-5 days until they hatch. After hatching, the alligator gar are in a lecithotrophic stage (they rely on a yolk sac for feeding), which lasts for 1-4 days after hatching. During this period, the snout tip of larval gar is an adhesive disk that acts like a suction cup (2)….. See More

4.) To best understand alligator gar population characteristics including growth, mortality and reproduction, it is essential to age these fish. Biologists have used numerous methods to estimate the ages of gar, however otoliths (three pairs of ear bones) are the preferred structure for estimating age because they are considered more accurate compared to other aging structures. Knowing the age of a fish allows fisheries biologists to evaluate growth rates of fish in a population….. See More

5.) Notice the green tag near this alligator gar's dorsal fin? This tag provides fisheries biologists valuable data to help them better estimate alligator gar population size. The tag also allows biologists to track changes in growth between when the fish was tagged and then recaptured at a later data. Even if an alligator gar does have a tag, it must be reported to the Wildlife Department. #GARWEEK

Figure 1. #GARWEEK posts shared on ODWC's Facebook, Twitter, and Instagram pages in ascending order (1 - 5). Due to Twitter's character limits, Post #3 was separated into four tweets (3a, b, c, and d). A single post (#2) was made to Instagram.

gar x longnose gar, and a longnose gar, along with text describing hybridization among the four gar species native to Oklahoma. Post #3 highlighted alligator gar development along with photographs of sac fry and free-swimming fingerlings. Post #4 illustrated how age can be estimated from harvested alligator gar otoliths, and how that information can provide insights into the environmental conditions that favor reproduction. Finally, Post #5 showed a photograph of a tagged alligator gar and explained how reporting tagged fish helps ODWC to estimate population size.

These #GARWEEK posts were shared on ODWC's Facebook, Twitter, and Instagram pages. One post was made each day of "Gar Week" on Facebook. Because of Twitter's character limits, Post #3 was separated into four tweets (3a, b, c, and d) on Twitter, therefore, Twitter's posts included Post #'s 1, 2, 3a, 3b, 3c, 3d, 4 and 5. A single post (#2) was made to Instagram. Across all three platforms, each post included the hashtag #GARWEEK, which allowed social media users to follow this specific topic (Palomino et al. 2016). In addition to ODWC's posts, concurrent #GARWEEK social media posts were made by TPWD and several other scientists active in alligator gar research; however, analysis of those posts are beyond the scope of this study.

Each social media platform provided different metrics towards quantifying the reach and engagement of #GARWEEK posts. Facebook provided numbers of likes, shares, reach, impressions, and engagement for Facebook (Table 1; Facebook 2019). Twitter utilized a similar metrics to Facebook with retweets, impressions, engagement, and engagement rate (Table 1; Twitter 2019). Instagram reported hearts, comments, and collections (Table 1; Instagram 2019). Because of differences in audience, engagement metrics, and algorithms used to display content to users across platforms, we calculated platform-specific measures of reach and engagement for #GARWEEK posts. We used analytics tools available to administrators of Facebook and Twitter accounts to download data for the #GARWEEK posts that had accumulated over a nine-day window (June 11-19), and manually compiled data from Instagram. To quantify how #GARWEEK posts performed relative to other posts on each platform, we calculated the percent increase of these metrics as compared to a 30-day average for posts made by ODWC (calculated from the 30 days of data on each platform prior to the first #GARWEEK post). Facebook, Twitter, and Instagram averages encompass a timeline from 5/13/2019 – 6/8/2019. The number of posts varied between medial forum ranging from 13 - 25 (Table 2, 3, 4). Percent increase was calculated by (Gar Week mean – previous 30-day's mean = increase), then (increase / previous 30-day's mean) x 100 = percent increase.

Table 1. Descriptions of each metric used to quantify increases of ODWC's target audience and reach on its social media pages.

Media forum	Metric	Description
Facebook	Engagement	Performing actions on your page (post clicks, likes, shares and comments).
	Impressions	The number of times a post from your page is displayed.
	Reach	The number of people who visited or saw your page or one of your posts in a news feed.
	Comments	A follower submits a comment to a post on your page.
	Likes	A feature that allows followers to show their support for specific posts, comments, and statuses.
	Shares	A follower shares a post with their Facebook friends, possibly adding commentary.
Twitter	Engagement	Total number of times a user interacted with a Tweet (retweets, replies, follows, likes, or hashtags).
	Engagement Rate	Is the percentage of users who saw a tweet and engaged with it.
	Impressions	The number of times a tweet shows up in somebody's timeline.
	Comments	A user submits a comment to a tweet.
	Likes	A user showing appreciation for a tweet.
	Retweets	Re-posting (sharing) of a Tweet.
Instagram	Collections	Allows you to save the post into a private collection, where it can be accessed at any time.
	Comments	A submits a comment to a post on your page.
	Hearts	Liking any picture posted or showing interested in a post

Table 2. #GARWEEK Facebook post analytics compared to previous 30-day analytics.

Facebook	Number of posts	Engagement	Impressions	Reach	Comments	Likes	Shares
Mean 30 Day Comparison	25	1,457	28,560	19,662	44	242	102
https://www.facebook.com/wildlifedepartment/videos/1847811505268453/	1	3,612	55,723	40,553	255	622	235
https://www.facebook.com/wildlifedepartment/posts/1849254468457490	1	65,996	670,961	418,266	2,941	6,907	2,941
https://www.facebook.com/wildlifedepartment/posts/1850462848336652	1	1,796	23,747	17,171	43	235	49
https://www.facebook.com/wildlifedepartment/posts/1851506881565582	1	1,943	25,911	17,115	11	123	26
https://www.facebook.com/wildlifedepartment/posts/1852878341428436	1	2,811	32,894	21,059	32	219	137
Mean of Gar Week Posts	5	15,232	161,847	102,833	656	1,621	678
% Increase		945	260	423	1,397	570	565

Table3. #GARWEEK Twitter post analytics compared to previous 30-day analytics.

Twitter	Number of posts	Engagements	Engagement rates	Impressions	Comments	Likes	Retweets
Mean 30 Day Comparison	14	30	0.016	1,827	0.07	4	1
https://twitter.com/OKWildlifeDept/status/1006309913428156416	1	152	0.047	3,262	0	30	10
https://twitter.com/OKWildlifeDept/status/1006680110953529352	1	951	0.106	8,977	5	70	31
https://twitter.com/OKWildlifeDept/status/1006978571984830466	1	87	0.026	3,299	1	13	1
https://twitter.com/OKWildlifeDept/status/1006978575755595776	1	148	0.059	2,511	1	6	1
https://twitter.com/OKWildlifeDept/status/1006978578721005568	1	64	0.032	1,978	1	6	1
https://twitter.com/OKWildlifeDept/status/1006978582147629059	1	43	0.029	1,481	1	12	5
https://twitter.com/OKWildlifeDept/status/1007633186317389824	1	536	0.068	7,935	1	59	24
Mean of gar week posts	7	283	0.052	4,206	1.43	28	10
% Increase		848	226	130	1,902	684	668

Table 4. #GARWEEK Instagran post analytics compared to previous 30-day analytics.

Instagram	Number of posts	Collections	Comment	Heart
Mean 30 Day Comparison	13	3	2	172
https://www.instagram.com/p/Bj7_HH_HxZ4/	1	24	22	419
% Increase		845	853	143

We then conducted sentiment analysis, "opinion mining," of public comments to the #GARWEEK Facebook posts at two relevant data resolutions using the R programming language (v.3.5.1; R Core Team 2019). First, we conducted an analysis of overall sentiment by pooling all comments and posts. We used the *tidytext* (v. 0.2.0; Silge and Robinson 2016) and *dplyr* (v. 0.8.0.1; Wickham et al. 2019) packages to tokenize individual words and assign them to a positive or negative sentiment based on the "Bing" lexicon (Silge and Robinson 2016). The overall sentiment of the entire dataset of tokenized words was then calculated as the sum of positive words minus the sum of negative words. We created a word cloud to compare the most frequently used words associated with positive versus negative sentiments using the *wordcloud* package (v. 2.6; Fellows 2018).

Second, we conducted a comment-level analysis to compare the sentiments of comments among #GARWEEK Facebook posts. We tokenized words within each comment, calculated the sentiment of each comment based on its tokenized words compared to the "Bing" lexicon, and then compared these comment-level sentiments among posts. For each post, we calculated the mean, standard deviation, minimum, and maximum of the comment-level sentiments. We visualized differences in mean sentiment among posts with a boxplot created with the *ggplot2* package (v. 3.0.0; Wickham 2016), and we performed pairwise t-tests to identify significant differences in mean sentiment among posts at $P \leq 0.05$, with a Bonferroni adjustment to control for Type I rates across multiple tests.

Results

In terms of engagement on social media, the #GARWEEK posts were successful across all three platforms. On Facebook, #GARWEEK posts saw a 456% increase in reach, 260% increase in impressions, 945% increase in engaged users and an impressive 1,397% increase in comments (Table 2). Likes and

shares had a similar percent increase. In fact, all five posts were among the Facebook page's top one-third best performing posts for the year. Post #2 was ODWC's second best performing post of the year, reaching 418,266 people and receiving 2,941 shares, 6,907 likes and 1,693 comments (Table 2).

Twitter posts about #GARWEEK saw similar success, with an 848% increase in engagement, 226% engagement rate. Comments had the highest increase among the three media platforms at 1,902% (Table 3). Likes and retweets experienced similar percent increases as seen on Facebook. Also, like Facebook Post #2 was the second-best performing tweet of the year (Table 3). While only one post (#2) was made to Instagram, it too was highly successful with 143% increase in hearts and 853% increases in comments (Table 4). This post was ranked 4 out of 104 public posts with #GARWEEK on Instagram. Compared to the previous 30-day's posts, collections had an 845% increase (Table 4).

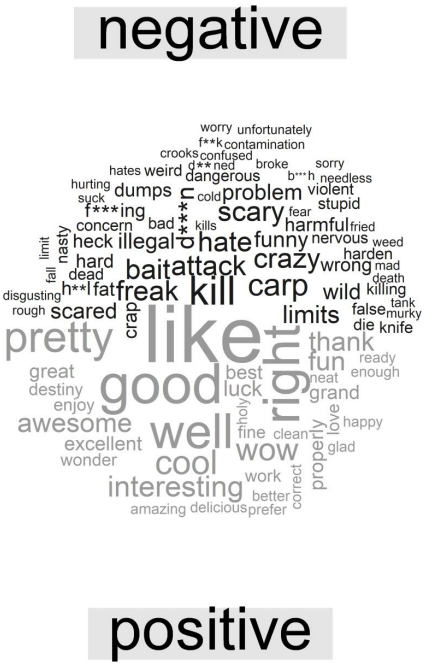

Figure 2. Word cloud illustrating the most frequently used words in comments of #GARWEEK Facebook posts. Frequency is indicated by font size, sentiment is indicated by font color (positive is grey, negative is black).

The overall sentiment of comments made on the five #GARWEEK Facebook posts was positive (+54), with 263 positive and 209 negative words identified in the overall pool. Some of the most frequently used positive words were "like," "good," "right," and "well," whereas frequently used negative words included "kill," "attack," "freak," "scary," and "crazy" (Figure 2). For the comment-level analysis, the number of comments with a sentiment score varied from $\underline{n} = 4$ for Post #5 to $\underline{n} = 218$ for Post #2 (Table 5). Mean sentiment of the comments among the five Facebook posts ranged from 0.00 (i.e., neutral) for Post #1 to 1.27 (i.e., slightly positive) for Post #4 (Figure 3). The most negative comment-level sentiment (-3) was recorded for Post #1, whereas the most positive sentiment (+6) was recorded for Post #4. Pairwise t-tests identified two statistically significant differences between Post #'s 1 and 4 and between Post #'s 2 and 4 (both with $P = 0.02$); however, Post #4's relatively low sample size ($n = 15$), coupled with a potential outlier of the single highest sentiment score (+6) observed, may have influenced these test results.

Discussion

Our reflective look at #GARWEEK revealed that ODWC's social media outreach posts about alligator gar were widely popular and increased ODWC's audience and engagement in all three social media platforms evaluated. All five posts during gar week were well received by followers with many of the posts ranking in the top third of all posts for the year. Specifically, post #2 ranked as one of the top posts of the year across all ODWC social media forums. Furthermore, the overall sentiment of comments made on the five Facebook posts during Gar Week were positive (+54). Going into Gar Week, ODWC was unsure of the general public's sentiment towards alligator gar, so it is promising that most Oklahoma constituent's sentiments were positive.

Positive sentiments could mean a major

Table 5. Comment-level sentiment measures for #GARWEEK Facebook posts. Post numbers relate to information in Figure 1. Sample size reflects the count of comments with a sentiment scored.

Post	Mean	SD	Min	Max	n
1	0.00	1.41	-3	4	44
2	0.14	1.32	-4	4	218
3	0.50	1.22	-1	2	8
4	1.27	1.57	-1	6	15
5	0.25	0.83	-1	1	4

shift in public perception towards alligator gar. Historic perceptions towards alligator gar were likely driven by their menacing exterior (shielded with large ganoid scales and rows of sharp teeth), combined with the belief that they negatively impacted more appealing species. In general, humans treat animals that they find most attractive with the greatest respect (Estren 2012). However, the public's perception towards wildlife species once viewed as ominous or unattractive appears to be changing. For example, George et al. (2016) found a positive increase in public attitudes toward coyotes, wolves, vultures, sharks, bats, and rats from 1978 to 2014. Further, social media was used to clarify misconceptions towards hellbenders that resulted in improved perception towards this species and garnered support for their conservation in Indiana, Missouri, and North Carolina (Reimer et al. 2013, Mullendore et al. 2014, Perry-Hill et al. 2014, Williams et al. 2019). Our results suggest that page followers were overall receptive of conservation-oriented messages about alligator gar in Oklahoma, suggesting that continuing directed outreach efforts could instill an improved perception of the species to a broader audience.

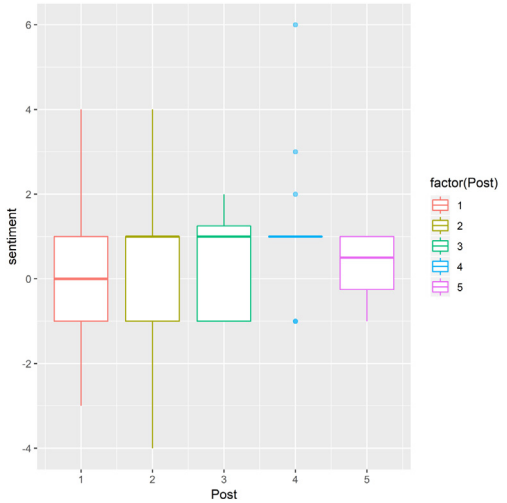

Figure 3. Boxplot of comment-level sentiment as compared across five #GARWEEK Facebook posts.

The ability to utilize open-source statistical packages, instead of subjective sentiment ratings in this study, establishes a repeatable workflow that can be used to objectively quantify sentiment and monitor trends over time. Despite these positive aspects of word-level sentiment analysis, the methodology is not perfect and some results should be interpreted with caution. For example, a post like "The killing of alligator gar is disgusting" would have an overall negative sentiment of -2 based on the tokenized words "killing" and "disgusting." However, the overall context of this comment suggests the individual may be receptive to management and conservation actions that limit the mortality or harvest of alligator gar. "Deep learning" text analysis methods attempt to identify keywords, concepts, sentiment, and subject-action-object relationships (Turian 2013; IBM 2019). These deep learning tools, like AlchemyAPI and IBM Watson, are business-oriented, typically require a subscription, and necessitate additional coding language expertise beyond open-access coding (e.g., the R language). Therefore, we suggest that social media page managers and biologists also apply their own understanding of posted topics when they review social media interactions, understanding that some degree of subjectivity may be necessary to best evaluate the overall attitude and sentiment of each comment.

While the overall sentiments were positive and agreed with ODWC's message, glimpses of historical negative perspectives towards alligator gar did surface. The topics of bowfishing or killing alligator gar were "hot button" issues that sparked vulgar language (as evidenced in the word cloud), and revealed differing opinions among interest groups – particularly, differences arose between those that favored killing gar and those that promoted a complete moratorium on the harvest of alligator gar. Bow anglers are a

dedicated constituency that participate only in this fishing activity (Bennet et al. 2015), which may explain some of the passionate comments observed in this study. Future ODWC alligator gar social media outreach campaigns could promote a balance between the importance of gars in aquatic ecosystems, while also highlighting that sustainable harvest provides sporting opportunities without increasing the conservation risk of the overall population.

A potential bias associated with the study is a lack of understanding of the users that follow the various ODWC social media pages. It can be assumed if an individual is following the ODWC social media forums they are involved, or interested, in hunting, fishing or other outdoor activities. Outdoor enthusiasts may be more informed about alligator gar, which may have biased the sentiment related to alligator gar in a more positive direction than would be observed among the general public. Further, followers of ODWC pages may also be more prone to follow other resource agency pages. If so, this could make them more educated on natural resource topics in general, this may result in differing perceptions of alligator gar than the general public.

Future research should be directed towards surveying social media followers to understand some of their background information including, their age, education level, residency, outdoor hobbies (ex. fishing, hunting, nature watching), overall interests, and views on natural resources in Oklahoma. Having this background information for different social media audiences may provide resource agencies with insight into what is driving the various perspectives towards natural resource topics on their social media pages. Further, this information can be used to direct specific outreach campaigns designed to clarify misconceptions about natural resource topics that a particular audience may have. Although we are confident in our findings, it is possible that sentiments towards alligator gar (negative or positive) are a reflection of how some members of the public feel towards the agency (ODWC), agency personnel, or outdoor activities. Further research is needed to tease this potential bias out of future social media evaluations.

This study was a first step at trying to understand public attitudes towards a unique, but misunderstood species, in Oklahoma. While most comments were positive, misconceptions towards alligator gar remain with some page followers. For example, the perception that all gar are alligator gar results in a lack of understanding why alligator gar are a species concern in Oklahoma. To remedy this misconception, future outreach efforts could be directed towards educating the public on gar species diversity.

A much more difficult topic to navigate and address in the future is the "hot button" issue of bowfishing for alligator gar. A subjective look at the comments suggests that many followers do not understand why bowfishing or other angler harvest is allowed if the alligator gar is a Species of Special Concern in Oklahoma. ODWC may slowly work towards changing this perception towards harvesting alligator gar by working with bowfishing groups to limit distasteful photographs, irresponsible dumping of harvested fish, and other posts that put bowfishing in a bad light.

In conclusion, #GARWEEK achieved its objectives and goals, and utilizing the hashtag propelled our message beyond ODWC's typical social media audience. This campaign not only allowed ODWC to educate and inform a larger audience, it also allowed us to better understand the public's perception towards alligator gar and other gar species. The information gained in this study suggests that future posts should highlight Oklahoma's gar diversity, the importance of alligator gar in aquatic environments, ongoing gar research, and regulations. Perhaps the most important topic to address is the angler's role in alligator gar conservation and research. Continued engagement of constituents will become increasingly critical as management biologists continue to learn more about gar species and consider implementation of conservation strategies.

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Examination of Three State Records for Spotted Gar in Oklahoma

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Abstract: Spotted Gar are found throughout Oklahoma and managed as a non-game species. This designation allows the species to be recreationally harvested using any legal methods. We examined three-state record (unrestricted division; harvested by bowfishing) Spotted Gars to estimate age, back calculate length-at-age (growth rates), and identify sex and stomach contents. Spotted Gars age estimates ranged from 32-43 years old. Back-calculated length-at-age displayed similar growth among the three specimens. All Spotted Gar examined were females, and contained no stomach contents. Although this study is limited in scope to the largest of Oklahoma specimens, it improves our knowledge of an understudied species in Oklahoma.

Introduction

The Spotted Gar (*Lepisosteus oculatus*) is one of four species of Lepisosteidae found in Oklahoma (Miller and Robison 2004, Frenette and Snow 2016). Lepisosteidae represents an ancient lineage of fishes that are widespread in central and eastern North America and throughout Central America (Echelle and Grande 2014, Snow et al. 2017, Davis et al. 2018). Gars are large-bodied, top-level piscivores that are important components of aquatic food webs (David et al. 2015). Historically, however, gars were viewed as nuisance species, so many aspects of their biology remain understudied (Scarnecchia 1992). Populations of several gar species have declined as a result of habitat loss or intentional removal efforts and are now in need of conservation (Scarnecchia 1992, Alfaro et al. 2008, Staton et al. 2012, NatureServe 2019). Spotted Gar, while globally secure, is a species of conservation concern at the northern edge of its range and is listed as critically imperiled in Canada (Glass et al. 2011, Staton et al. 2012, David et al. 2015, NatureServe 2019, Ontario Ministry of Natural Resources and Forestry 2016), several U.S. states (Kansas, Ohio, and Pennsylvania), and are thought to be extirpated in New Mexico (NatureServe 2019).

In Oklahoma, Spotted Gar are managed by the Oklahoma Department of Wildlife Conservation (ODWC) as a non-game fish (allowing harvest by any legal method) and currently no regulations protect this species (bag or size regulations), but populations are considered stable. The current state record Spotted Gar (and the four previous state records) was harvested via bowfishing, which is a method of take in the unrestricted division (bow and arrow, gig, spear, trotline, jug-line, limb-line) of the state record fish program (ODWC 2019). The objective of the state record fish program is to increase awareness of fishing opportunities for species that are regularly sought after and routinely found in reservoirs and rivers throughout Oklahoma.

Angler preference for gar fishing in Oklahoma has remained consistent across the last 35 years, ranking 16-20 of the most preferred species list (Jager 2016, Elizabeth York, ODWC personal communication). Although gar popularity

among anglers has stayed consistent, the state record Spotted Gar has been broken 4 times in the last 17 years (all via bowfishing), suggesting growing popularity within a specialized angling group (bow anglers). The most recent two state record Spotted Gars (held by Dustin Satton and Chasey Nease) were donated to ODWC in the last two years for research purposes. Additionally, a previous Spotted Gar state record holder (Brandon Taber) allowed examination of his taxidermy specimen and agreed to allow ODWC to remove a sagittal otolith from the mount. The objective of this paper is to examine these three-state record Spotted Gars to estimate age, back calculate length-at-age to understand growth rates, and to identify sex and stomach contents.

Methods

The most recently designated two state record Spotted Gars were donated to the Oklahoma Department of Wildlife Conservation for research purposes. These fish were brought to the Oklahoma Fishery Research Laboratory (OFRL) in Norman, Oklahoma where they were measured for total length (TL, mm) and weighed (nearest g). Sagittal otoliths were removed from each fish through the ventral side of the brain case. A third specimen (harvested by Brandon Taber in 2003) in the form of a taxidermy mount, was brought to the OFRL for otolith removal. A hole was drilled into the non-viewed side of the taxidermy mount and one sagittal otolith was removed. Otoliths were cleaned and dried before processing.

Otoliths were processed following methods in Buckmeier et al (2018), where otoliths were ground in a plane transverse to the nucleus using a rotary tool fixed with a grinding bit (#85422, Dremel, Racine WI). The rotary tool was attached to a table, and forceps coated in Tool Dip (Plasti Dip International, Blaine MN) were used to securely hold the posterior portion of the otolith during the grinding process. Sagittal otoliths were ground on a plane perpendicular to the anterior–posterior axis. Otoliths were then polished using wet 2000 grit sand paper. Prepared otoliths were stood polished-side up in a dish containing modeling clay, immersed in water, and viewed with a variable-power stereomicroscope (capable of 130× magnification) using a fiber-optic filament attached to an external light source.

Otolith annuli were defined as the distal edge of the opaque zones, because these margins appear as very bright fractures when illuminated with intense light from a fiber-optic filament (Buckmeier et al. 2012, Buckmeier et al 2018). Age estimates were performed independently by two readers experienced with gar otoliths and who had no knowledge of the other's age estimates or specific information about the fish (length or weight) being examined. If the two readers did not agree on an age estimate, the readers viewed the otolith together and determined a final consensus age. Once age estimates were finalized, the Dahl-Lea method was used to back-calculate length-at-ages (mm) for each fish (Quist et al 2012).

To determine sex, pruning shears were used to cut the ventral side of the fish from the vent to the isthmus exposing the internal organs. Once exposed, sex was determined by examining the gamete release pathways following a standardized procedure for determining sex in Lepisosteids (Ferrara and Irwin 2001). After sex was determined, the stomach was extracted, and stomach contents removed. On the third specimen that was mounted a stomach sample was not taken during harvest or observed.

Results & Discussion

Ages estimated from the Spotted Gars in this evaluation ranged from 32-43 years old (sagittal otoliths; Table 1, Figure 1), considerably higher longevity than what has been reported in the literature. Buckmeier et al. (2018) reported Spotted Gars up to age 27 (sagittal otoliths) from two Texas reservoirs. Similarly, King et al. (2018) found Spotted Gar that reach age 24 (sagittal otoliths) from twelve Illinois water bodies. Prior to these studies, the previous maximum known age of Spotted Gar was 18 years (COSEWIC 2005). Similarly, Redmond (1964) estimate ages of Spotted

Table 1. Information from state record Spotted Gar harvested in Oklahoma.

Angler	Lake Harvested	Date	Age (yrs)	Weight (g)	TL (mm)	Girth (mm)	Sex	Method of Take
Dale Starry	Lake Arbuckle	4/28/2002	N/A	3,230	946	298	N/A	Bow and Arrow
Brandon Taber	Lake Arbuckle	3/15/2003	39	4,136	972	349	F	Bow and Arrow
Jimmy Nelson	Lake Arbuckle	4/19/2008	N/A	4,141	946	394	N/A	Bow and Arrow
Chasey Nease	Lake Texoma	4/21/2016	32	4,595	968	394	F	Bow and Arrow
Dustin Satton	Lake Arbuckle	5/11/2018	43	5,171	1029	349	F	Bow and Arrow

Gar to 18 years old using brachiostegal ray. Using three structures (otoliths, pectoral ray, and branchiostegal rays), Glass et al. (2011) estimated the maximum age of Spotted Gar to be 14 years old. Frenette and Snow (2016) found Spotted Gar in Lake Thunderbird, Oklahoma up to age 12 (sagittal otoliths), but this population may have recently become established. The longevity of Spotted Gars from estuarine populations appears shorter (6-10 years) than those from reservoir populations (branchiostegal rays; Love 2004, Smith 2006). Differences in maximum age estimates in these studies may be due to the aging structure (otoliths, spines, fin rays, and branchiostegal rays) that was used or the aging technique, which can result in varying age estimation precision.

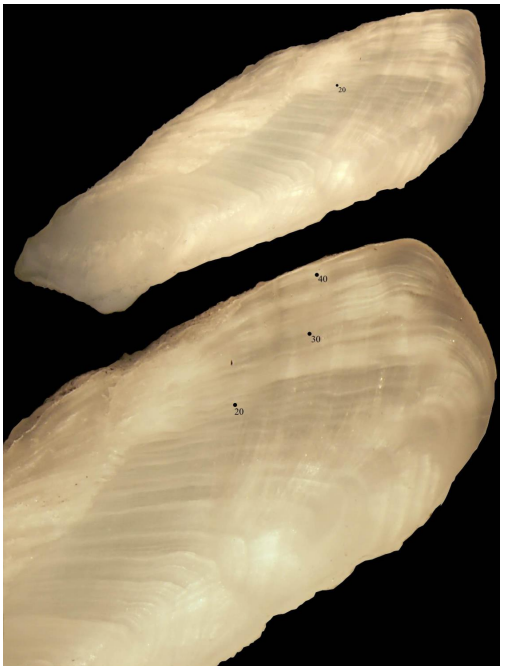

Figure 1. Photograph of a sectioned sagittal otolith from the current state record Spotted Gar (age 43) harvested on 11 May 2018. Age reference points indicate years.

Spotted Gars have been found to grow rapidly during their first year of life (Matthews et al. 2012, David et al. 2015, Frenette and Snow 2016). Back-calculated Spotted Gar lengths were similar for the three Spotted Gars evaluated in this study. Back-calculated lengths-at-age suggest that the three Spotted Gar in this study grew to 38% of their TL in the first year and 81% of their TL by age 6, although growth plateaued after age 16 (Figure 2). Similarly, Frenette and Snow (2016) found Spotted Gar grew to half of their maximum TL in the first year and approached maximum TL by age 4.

Gars are known to be sexually dimorphic in body size, with females being larger, on average, than males (Love 2002, McGrath and Hilton 2012, McDonald et al. 2013). Frenette and Snow (2016) found female Spotted Gars were larger than males in Thunderbird Reservoir, Oklahoma. The three Spotted Gars in this study were determined to be female. However, only the 2016 and 2018 state record fish were dissected to visually examine sex. The gar harvested in 2003 was not dissected, but the angler described grayish, egg-like objects falling from the fish through the exit wound of the arrow on the ventral side of the fish. This account accurately describes the gametes in mature female Spotted Gar. Based on their size, it is likely that the two Spotted Gar records that were not examined (Table 1) are also female, as this species is sexually dimorphic (Love 2002, McGrath and Hilton 2012, McDonald et al. 2013). Of the two Spotted Gars observed for diet, both had empty stomachs. However, this is not surprising as

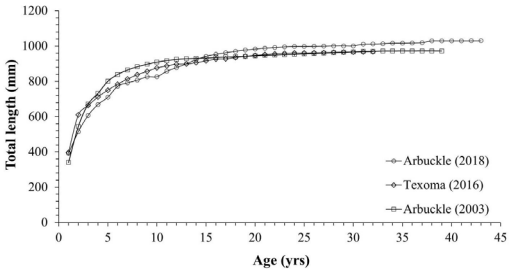

Figure 2. Back-calculated total length-at-age (mm) for three state record Spotted Gar harvested in Oklahoma.

both fish were harvested during the expected spawning time of Spotted Gar (April-June in Oklahoma; Tyler and Granger 1984, Miller and Robinson 2004, Frenette and Snow 2016). Tyler and Granger (1984) found that 82% (of 172 Spotted Gar evaluated for diets) were empty, suggesting that foraging slows or ceases during the spawning period.

Spotted Gars in this study were all large, mature females that may be very important to these populations. In general, older and larger females are more fecund, and produce larger, higher quality eggs and larvae that have a greater potential to survive compared to those that smaller females produce (Daugherty et al. 2019), which could be important for population sustainability. On the whole, little is known about Spotted Gar populations in Oklahoma, however evaluations like this improve our knowledge of this species, and provide ODWC with data and results to better manage the species. Further, increased efforts to understand population dynamics of this species in Oklahoma waters are underway.

Although this study is limited in nature, it does document considerably higher longevity than previously described for Spotted Gars. Additionally, observation of the largest recorded specimens in Oklahoma is rare. Biologists with ODWC were privileged that anglers enabled us to examine these specimens for further evaluation. Findings reported in this study demonstrate the ability of Spotted Gar to grow rapidly and live up to 43 years old. Furthermore, they provided information to develop a better understanding of Spotted Gar natural history and assist with conservation and management of this species.

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A Novel Food Item (Diptera: Stratiomyidae) of Spotted Gar, *Lepisosteus oculatus* and Bowfin, *Amia calva*, from Southeastern Oklahoma

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Abstract: Predatory fishes such as Spotted Gar (*Lepisosteus oculatus*) and Bowfin (*Amia calva*) are top predators that feed primarily on fish. Both also occasionally take crustaceans, mollusks, and various aquatic insects. Here, we provide an instance where both species from a private lake in Oklahoma fed on an unusual novel food item, larval soldier flies, *Stratiomys* sp. (Stratiomyidae). It is unknown how or why these fish fed on this insect but suggests these predators may also occasionally feed opportunistically.

Introduction

Gars (Lepisosteidae) and Bowfins (Amiidae) are top ambush predators that ingest a variety of prey items, the majority being fishes, including Gizzard Shad, Golden Shiner, bullheads, and sunfishes (Lee and Wiley 1980; Robison and Buchanan 1988; Etnier and Starnes 1993; Pflieger 1997; Miller and Robison 2004; Walker et al. 2013). Other food ítems reported from these fishes include: crustaceans, various aquatic insects, crayfish, mollusks, and frogs (Goodyear 1967; Dugas et al. 1976; Burgess and Gilbert 1980; Tyler and Granger 1984).

While obtaining fishes for parasitic examination, we discovered an unusual food ítem in the stomachs of a Spotted Gar, *Lepisosteus oculatus* Winchell and a Bowfin, *Amia calva* L. from the same collection site. To our knowledge, what we observed was a novel prey ítem that had not been previously reported from either fish species. Here, we document an instance where both fishes were found to contain a large quantity of this novel food ítem.

Methods

Fishes were taken by bowhunting at night on 28 October 2018 from a private lake on the Turner Ranch in the Little River drainage north of Idabel in McCurtain County, Oklahoma (33° 55' 56.93"N, 94° 43' 43.22"W). One specimen was a 670 mm total length (TL) female *L. oculatus* and the other a 470 mm TL female *A. calva.* Both were placed on ice and necropsied within 12 h. A midventral incision was made from anus to throat and the gastrointestinal tract, including the esophagus, stomach and intestines were cut, placed in separate dishes, and rinsed in 0.9% saline. The stomach of each was split lengthwise and food items examined and identified. Insect larvae were identified using the key in MacFadden (1967) and voucher specimens were deposited in the collection of the Louisiana State Arthropod Museum (Louisiana State University, Baton Rouge, LA). Photovouchers of fish were deposited in the Henderson State University Collection (HSU),

Arkadelphia, Arkansas.

Results and Discussion

Both stomachs were found to be completely distended and contained the remains of >250 larval soldier flies, *Stratiomys* sp. (Figs. 1A–D) measuring 15 to 20 mm long. The identification was based on the presence of a caudal whorl of respiratory setae, location of the antennae on the ocular lobes, and various integumental characters. North American *Stratiomys* larvae are not identifiable to species using available resources, thus the designation "sp.". The genus includes about 92 species with 30 species found in the Nearctic (James and Steyskal 1952; Woodley 2001). These larvae play a role as essential decomposers in breaking down organic substrates and returning nutrients to the soil and aquatic systems. Members of this genus are not only aquatic, but occur in the water column and are variously reported as filter feeders (Stehr 1987). Other larval members of the subfamily are detritivores in shoreline debris, so this finding, albeit unusual, is not completely unexpected. *Stratiomys* larvae may represent an under reported forage base for predatory fish.

Although little detailed research has been done, all indications are that *Stratiomys* have annual life cycles in temperate regions. The related black soldier fly, *Hermetia illucens* (L.) has three generations a year in Georgia from April to November (Shepard et al. 2002). It is interesting to note that on several return visits to the same locality (April-June 2019), none of the other *L. oculatus* or *A. calva* that were necropsied were found to contain *Stratiomys* larvae in their stomachs. Based on scant evidence, we can speculate that (1) *Stratiomys* had a productive year at this locality, and (2) toward the end of the warm season, the larvae had reached maximum size during their annual growth cycle and would have attracted the attention of the larger predators.

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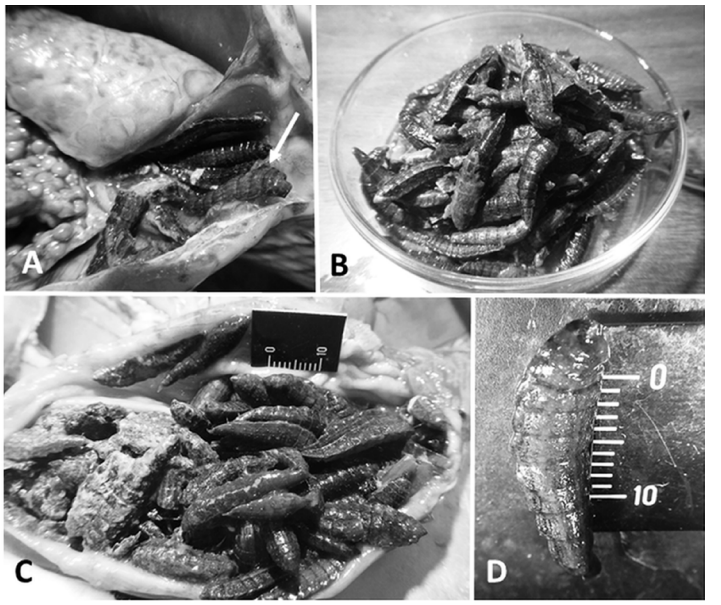

Figures 1A–D. Soldier fly larvae (*Stratiomys* sp.) from *Lepisosteus oculatus* and *Amia calva*. A. Stomach of *L. oculatus* showing larvae *in situ* (arrow). B. Standard-sized Petri dish with larvae collected from stomach of *L. oculatus*. C. Stomach of *A. calva* showing larvae *in situ*. D. Single larval *Stratiomys*. Note scale bars (mm) for Figs. C–D.

properties as well as assistance with collecting.

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A Species of *Trichodina* (Ciliophora: Mobilida: Trichodinidae) Infesting Channel Catfish (*Ictalurus punctatus*) from the Verdigris River, Oklahoma

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Abstract: A Channel Catfish (*Ictalurus punctatus*) from the Verdigris River, Oklahoma, was found to harbor a ciliate, *Trichodina* sp., on its gills. There are three previously reported species of *Trichodina* from North American *I. punctatus*, but none from fish hosts in Oklahoma. We therefore document a new geographic record for a trichodinid from any fish in the state.

Introduction

Trichodina Ehrenberg, 1838, is the largest genus within the family Trichodinidae Raabe, 1959. Over 100 species have been described, but a further 69 species have been inadequately described (Van As and Basson 1989; Lom and Dyková 1992). They are protistan peritrichous ciliated parasites of marine, brackish, and freshwater species of fish and amphibians (Lom 1958). In large numbers they can often potentially cause skin and gill disease, leading to the death of the host (Hoffman 1999). These organisms are saucer-shaped and possess a prominent denticular internal cytoskeleton ring or aperture. Reproduction is by simple binary fission and most species are host specific. They infest fish and spread to others by incidental contact between infested fish and a susceptible host fish or through contact with the parasite in the water column. *Trichodina* spp. feed on the epithelium covering the surface of the skin and gills of the fish and heavy loads can directly result in abrasions, lesions and ulcers which allow for secondary bacterial infections to develop at the affected site (Lom 2006; Smith and Schwarz 2009). The clinical signs of the infestation usually present as superficial white lesions on the body and the fins can become frayed. Scales eventually loosen and opportunistic microorganisms may lead to secondary bacterial infection causing ulceration and erosion. In the end, fish may have respiratory compromise from gill damage.

The Channel Catfish, *Ictalurus punctatus* (Rafinesque) is native to the Mobile, Rio Grande, and Mississippi drainages of the United States and northeastern México (Page and Burr 2011). In Oklahoma, *I. punctatus* occurs over most of the state except for the western part in the Panhandle (Miller and Robison 2004). It is a piscivorous predator found in lakes, rivers, and large streams, and may enter brackish waters. In recent decades it has been widely introduced along the Atlantic Slope and western drainages for sport and commercial fish management (Glodek 1980; Jackson 1999).

The parasites of this catfish are fairly well known (Hoffman 1999); however, only three valid species of *Trichodina* have been previously reported from the gills of North American *I.*

punctatus as follows: *T. discoidea* Davis, 1947, from five species of hosts from Illinois, Iowa, and West Virginia (Davis 1947; Blecka 1972); *T. fultoni* Davis, 1947, on *I. punctatus* from West Virginia (Davis 1947); and *T. vallata* Davis, 1947, from *I. punctatus* from Iowa (Davis 1947). Another species, *Trichodina symmetrica* Davis, 1947, was described from *I. punctatus* from Iowa but was later considered to be a mixture of two species placed in different genera (*Trichodinella symmetrica* (Davis, 1947) Lom, 1959 (Lom 1959, Hoffman 1999) and *Tripartiella symmetricus* (Davis, 1947) Lom and Haldar, 1977 (Lom and Haldar 1977; Hoffman 1999). Because they were a mixture of two species, these three taxa are considered *nomina nuda* (Lom and Haldar 1977; Hoffman 1999). Trichodinids have also been reported from cultured *I. punctatus*, including *T. pseudoheterodentata* Fahui, Zhang, and Zhao, 2017 from China (Fahui et al. 2017) and *Trichodina heterodentata* Duncan, 1977 from Brazil (Martins et al. 2010). In the largest survey to date on trichodinids, Wellborn (1967) examined 936 fish of 46 species from 36 geographic locations in 10 states (Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, and Tennessee) but did not report any species from *I. punctatus*. Therefore, no trichodinid species have been reported from *I. punctatus* from Oklahoma. We document the first report of a species of *Trichodina* from a Channel Catfish of the state and, to our knowledge, from any fish in Oklahoma.

Methods

A single adult Channel Catfish was collected on 13 March 2017 by boat electrofisher from the Verdigris River at McClellan-Kerr lock and dam 17, Arkansas River Drainage, Cherokee County, Oklahoma (35°52'17.3712"N; 95°23'16.4184"W). We followed accepted guidelines for the use of fish in research (AFS 2014). The specimen was placed on ice and overdosed in a concentrated chloretone (chlorobutanol) solution; it was subsequently preserved in 10% formalin. Gills were removed and examined for ectoparasites under a stereomicroscope at 20–30×. Parasites, picked directly from the gills of their host with small needles, was placed in tap water and observed as temporary wet mounts or mounted in Grey and Wess medium stained with Gomori's trichrome (Kritsky et al. 1978). Observations were made from digital images taken with an Accu-scope Ecelis HDS camera mounted on an Accu-scope LED series phase-contrast microscope (Accu-Scope®, Commack, New York). Measurements were made to the nearest micrometer (μm) according to Wellborn (1967). A voucher specimen was deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results

An unknown species of *Trichodina* was found on the gills of *I. punctatus*. We provide a description of its characteristics below.

Ciliophora: Mobilida: Trichodinidae

***Trichodina* sp. (Fig. 1)**

Description (*n* = 4): Small trichodinid, body diameter 21 (19–24). Number of denticles 21 (19–24), denticle ring diameter 14 (11–17), denticle length 20 (20–21). Rods (pins)/denticle 6 (5–6).

Specimens deposited: HWML 139864, slide of the monogenean *Ligictalurus pricei* (Mueller, 1936) Klassen and Beverley-Burton, 1985 with single *Trichodina* sp. near haptor.

Pathology: None observed.

Remarks: The body diameter (21 [19–24]) and number of rods/denticles (6 [5–6]) of *Trichodina* sp. are less than those of *T. discoidea* (35–50, 6–8), *T. fultoni* (75–90, 12–14), and *T. vallata* (38–48, 10), but were similar to those of the *nomen nudum Trichodina symmetrica* (= *Trichodinella symmetrica* and *Tripartiella symmetricus*) (24–35, 5) (Davis 1947). Unfortunately, because we did not use the impregnating silver staining method of Klein (1958), it was impossible to see some important morphological characteristics for a specific identification. Furthermore, morphological

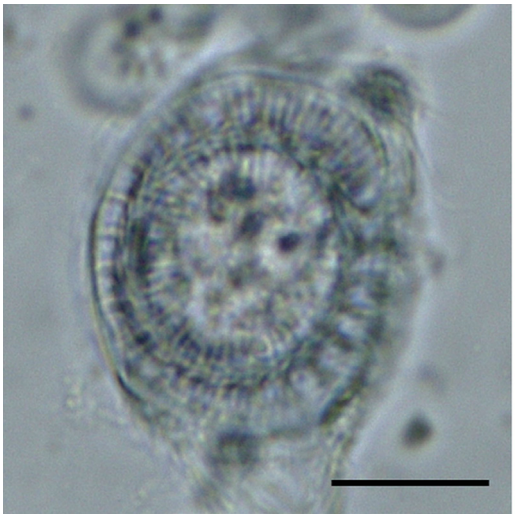

Figure 1. Adhesive disk of *Trichodina* sp. on the gills of *Ictalurus punctatus* from the Verdigris River, Oklahoma. Scale bar = 10 µm.

characteristics are insufficient for identifying species of *Trichodina* and molecular and phylogenetic analyses are considered to be the most promising and useful tools for identifying these species.

Discussion

Further knowledge of parasites, including trichodinids, may be important in management of catfishes, especially under crowded culture conditions. For example, in addition to what has already been mentioned herein, trichodinids have been reported to cause other pathological conditions such as immunogenic alterations and inflammatory responses (increased il-1β expression and decreased il-8 and tgf-β expression) in some species of infested fish (Abdelkhalek et al. 2018). Knowing this information should help improve our understanding of the responses of teleost fish to trichodinid parasite infestation and will be helpful in the development of new control strategies.

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Myxobolus sp. cf. *angustus* Kudo, 1934 (Cnidaria: Myxosporea: Myxobolidae) on the Gills of *Dionda* sp. cf. *flavipinnis* (Cope) (Cypriniformes: Cyprinidae) from the South Concho River, Texas

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Abstract: An undescribed species of roundnose minnow, *Dionda* sp. cf. *flavipinnis* (Cope), is a small endemic cyprinid that inhabits northern tributaries (San Saba and upper Concho rivers) of the Colorado River drainage in southcentral Texas. Nothing is known of the protistan or metazoan parasites of this fish. Therefore, we examined a small sample of this minnow from the South Concho River for parasites. One species of myxozoan, morphometrically very similar to *Myxobolus angustus* Kudo, 1934, was found on the gills of one of four (25%) *D.* sp. cf. *flavipinnis*; no other parasites were found. As such, this is the first time any parasite has been reported from this fish and represents a new host and geographic record for a myxozoan from this endemic species.

Introduction

Myxozoans are a group of microscopic, oligocellular, obligate parasites that belong to the Phylum Cnidaria. They are parasites of aquatic poikilothermic invertebrates (annelids and oligochaetes) and vertebrates but are most commonly encountered in wild and cultured freshwater teleost and marine fishes of the world (Lom and Dyková 2006). Those cnidarian species belonging to the genus *Myxobolus* Bütschli, 1882, are known from a wide variety of fish, including many minnows and shiners of the family Cyprinidae (Eiras et al. 2005, 2014). These parasites are found on the fins, gills and skin of these hosts, as well as internally, generally infecting the gall bladder and various organs. However, many cyprinids have yet to be surveyed for these parasites, including some in the genus *Dionda* (Thomas et al. 2007; Page and Burr 2011). One example is an undescribed species of roundnose minnow, *Dionda* sp. cf. *flavipinnis* (Cope) = (*Dionda* sp. 3) of Schönhuth et al. (2012). It is a small minnow endemic to the headsprings and upper reaches of the northern tributaries of the upper Colorado River (the Concho and San Saba river drainages) in Texas (Schönhuth et al. 2012). This minnow is locally common in pools and runs of headwaters, creeks, rocky pools, and small rivers where it is usually found associated with filamentous algae. It was formerly synonymized with the Roundnose Minnow, *D. episcopa* Girard (Gilbert 1988; Mayden et al. 1992; Nelson et al. 2004), but is now considered a distinct undescribed species (Schönhuth et al. 2012). There are no published reports concerning any parasite of this species. This paper reports the presence of a myxozoan parasite on the gills of *D.* sp. cf. *flavipinnis*.

Methods

On 22 June 2018, 4 adult (67–75 mm total length [TL]) *D.* sp. cf. *flavipinnis* were collected by backpack electrofisher from the South Concho River at Christoval, Tom Green

County, Texas (31.187341°N, 100.501214°W). Fish were placed in aerated habitat water and killed by immersion in a concentrated tricaine methanesulfonate solution following accepted guidelines (Use of Fishes 2014). A mid–ventral incision was made to expose the gastrointestinal (GI) tract and internal viscera. The GI tract was split longitudinally from esophagus to anus and all internal organs were placed in Petri dishes containing 0.9% saline and examined under a stereomicroscope at 20–30×. The gills were also removed and examined for parasites similarly. Myxozoan plasmodia were picked directly from the gills with small forceps or needles and placed in a drop of tap water on microscopic slides. They were photographed from these wet smears. Plasmodia were measured (to the nearest μm) and then ruptured to release spores for observation as wet mounts. Spores were measured to the nearest 0.1 μm with a calibrated ocular micrometer and identified by comparing its morphometrics with other North American freshwater myxozoans (Landsberg and Lom 1991; Cone and Raesly 1995; Eiras et al. 2005, 2014).

Prevalence and intensity of infection were calculated according to Bush et al. (1997). A photovoucher of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Photovouchers of fish were deposited in the Henderson State University Collection, Arkadelphia, Arkansas.

Results

One species of myxozoan, morphometrically very similar to *Myxobolus angustus* Kudo, 1934, was found on a 75-mm TL *D.* sp. cf. *flavipinnis* and is described below. No other parasites were found.

Cnidaria: Myxosporea: Myxobolidae
***Myxobolus* sp. cf. *angustus* Kudo, 1934 (Figs. 1A–C)**

***Site of infection*:** Gill lamellae.

Prevalence: 1/4 (25%).

***Intensity*:** 2 plasmodia, spores too numerous to count.

***Specimens deposited*:** Photovoucher (HWML 216064).

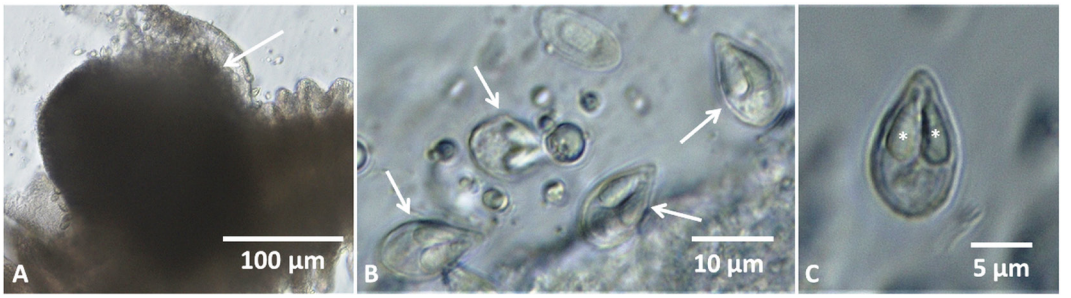
***Description*:** Plasmodia (cyst) spheroidal, diameter 213–234 (n = 2). Myxospores (n = 20): pyriform, widest in region near tips of polar capsules, length 12.9 (12.3–14.4), width 7.5 (6.7–8.2). Two polar capsules, equal or one usually slightly longer than the other; length of longer polar capsule 7.9 (7.2–9.0), width 3.2 (2.8–3.5); length of shorter polar capsule 7.6 (7.2–8.5), width 3.1 (2.8–3.6). Sutural ridge distinct.

Remarks: The pyriform spores of *Myxobolus* sp. cf. *angustus* are very similar to those of *M. angustus*, but are slightly smaller (12.9, range = 12.3–14.4 *vs.* 14.0–15.0) (Kudo 1934). The polar capsules of both species are pyriform and extend approximately halfway through the spores, but those of *M*. sp. cf. *angustus* (width 3.2, range = 2.8–3.6) are more robust than those of *M. angustus* (width 2.5–3.0) (Kudo 1934). The cysts of the two species differ in shape, spheroidal in *M*. sp. cf. *angustus vs.* ellipsoidal in *M. angustus* (Kudo 1934).

Myxobolus sp. cf. *angustus* is also very similar to *M. spalli* (Spall, 1974) Landsberg and Lom, 1991 (Syn: *Myxosoma cyprini* Spall, 1974, a junior homonym of *Myxobolus cyprini* Doflein, 1898 (Landsberg and Lom 1991). The spores and polar capsules of *M. spalli* are pyriform as in *M*. sp. cf. *angustus*, but are larger (length of fixed specimens 14.5, range = 13.5–15.5) (Spall 1974).

Discussion

The majority of species of *Myxobolus* display strict site and host specificity, as they are often found on or in only one organ/tissue and a single host species (Landsberg and Lom 1991; Cone and Raesly 1995; Eiras 1995, 2014; Hoffman 1999). Contrary to this trend, *M. angustus* had been previously reported from the gills of several cyprinids, including Bullhead Minnow,



Figures 1A–C. ***Myxobolus* sp. cf. *angustus* from the gills of *Dionda* sp. cf. *flavipinnis*. A. Plasmodium showing myxospores along the edge of cyst (arrow). B. Frontal view of four myxospores (arrows). C. Single myxospore showing polar capsules (*).**

Pimephales vigilax (Baird and Girard) from Illinois (Kudo 1934); Common Shiner, *Luxilus cornutus* (Mitchill) from Ontario, Canada (Desser and Paterson 1978, as *Myxobolus* sp.; Hoffman 1999, probably *M. angustus*); and Golden Shiner, *Notemigonus crysoleucas* (Mitchill) and Fathead Minnow, *Pimephales promelas* Rafinesque, both from North Carolina (Hoffman 1999). *Myxobolus spalli* has been reported from gills of Red Shiner, *Cyprinella lutrensis* (Baird and Girard) and *N. crysoleucas* from Oklahoma (Spall 1974). The very close similarity between *M. angustus* and *M. spalli* was revealed when Hoffman (1999) stated that *M. cyprini* is probably *M. angustus* or *M. spalli*, perhaps hinting that they may be synonyms.

Whether our *M.* sp. cf. *angustus* is synonymous with a variable wide-ranging *M. angustus* that may include *M. spalli*, or a separate undescribed species, remains to be resolved. The most definitive approach to identifying species of myxozoans is utilization of small-subunit ribosomal DNA (SSU rDNA) gene sequences. Such sequences are needed to help resolve the identification of *M.* sp. cf. *angustus* and the taxonomic status of *M. angustus* and *M. spalli* on a variety of hosts.

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Acanthocephalans (Eoacanthocephala: Neoechinorhynchida: Neoechinorhynchidae) of Common Snapping Turtles (*Chelydra serpentina*) from Arkansas and Oklahoma, with Observations on Host Suitability

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Abstract: Eighteen common snapping turtles (*Chelydra serpentina*) were collected from Arkansas (*n* = 5) and Oklahoma (*n* = 13) and examined for acanthocephalans. Three (17%) were found to harbor infections, including one from Arkansas with a *Neoechinorhynchus* sp., and two from Oklahoma with *Neoechinorhynchus emyditoides* Fisher, 1960. We document one of the few instances of *C. serpentina* harboring acanthocephalans and discuss host suitability.

Introduction

Acanthocephalans are highly specialized, dioecious metazoan parasites of the intestinal tract of a variety of vertebrates. The life cycle requires either an aquatic intermediate host (amphipods, copepods, isopods, and ostracods) or a terrestrial intermediate host (insects, crustaceans, and myriapods) and a vertebrate definitive host (Crompton and Nickol 1985). These "spiny- or thorny-headed worms" are relatively common in a variety of fresh and brackish water fishes (Pinacho-Pinacho et al. 2018) as well as aquatic turtles, and 10 species of *Neoechinorhynchus* have been previously reported from North American chelonian hosts (Barger 2004, 2005; Barger and Nickol 2004). One turtle species that has often been surveyed for helminth parasites is one of the most widespread native North American reptiles, the common snapping turtle, *Chelydra serpentina* L. (see summary in Ernst and Ernst 1977; Moravec and Little 2004; Zelmer and Platt 2009; Bursey and Brooks 2011; McAllister et al. 2015), but it has rarely been reported to harbor acanthocephalans. Four species have been reported from this host and states, including *Neoechinorhynchus chrysemydis* Cable and Hopp, 1954 from one of 26 (4%) from Indiana (Fisher 1960); *N. emydis* (Leidy, 1851) Van Cleave, 1916 from three of 30 (10%) from Oklahoma (Williams 1953); *N. emyditoides* Fisher, 1960 from Virginia (Zelmer and Platt 2009); *N. pseudemydis* Cable and Hopp, 1954 from one of 17 (6%) from Illinois (Martin 1973); two of 15 (13%) from Tennessee (Limsuwan and Dunn 1978); and immature specimens of a *Neoechinorhynchus* sp. from one of 13 (7%) from Louisiana (Acholonu 1969). Here, we document the second report of a certain species of acanthocephalan from *C. serpentina* from Oklahoma, with insight regarding the scarcity of reports.

Methods

Between April 2013 and October 2019, 18

juvenile and adult *C. serpentina* (Table 1) were collected from five counties of Arkansas (n = 5) and four counties of Oklahoma (n = 13) by hand, with hoop nets baited with fish, or collected opportunistically as dead on the road (DOR) specimens. Turtles were overdosed with an intraperitoneal injection of sodium pentobarbital (Nembutal®), and an electric saw was used to open their plastron to expose the internal organs. The intestinal tract was removed, sectioned, and placed in Petri dishes containing 0.9% saline. Intestinal contents were scraped from the lumen of the tract and examined with a stereomicroscope. When acanthocephalans were found, they were rinsed of mucus and placed in dishes containing distilled water for 24 h in a refrigerator to evert their proboscides. Each worm was placed on a glass slide, and a wet mount was prepared by adding a drop of tap water and a coverslip. All worms were examined at 100 to 400× with an Olympus BX-51 upright research microscope configured for Brightfield (BF) and Differential Interference-Contrast (DIC) microscopy. Digital images were taken of all worms using an Olympus 5-megapixel digital camera, and total length and greatest width of each worm were measured with ImageJ software (Schneider et al. 2012). All measurements are reported in millimeters (mm), including the mean ± 1 standard deviation.

We follow the reptile database (Uetz et al. 2019) for all turtle common and scientific names. We also follow Amin's (2013) classification of the Acanthocephala and Barger and Nickol (2004) for further species identification. Voucher specimens of acanthocephalans were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. A voucher host was deposited in the Arkansas State University Museum of Zoology (ASUMZ), Herpetological Collection, State University, Arkansas.

Results and Discussion

Three of 18 (17%) *C. serpentina* were infected with acanthocephalans (Table 1). A single *C. serpentina* from St. Francis County, Arkansas, harbored immature *Neoechinorhynchus* sp., with an unknown intensity. Worms from two *C. serpentina*, collected from McCurtain County, Oklahoma, were identified as *Neoechinorhynchus emyditoides* Fisher, 1960 (HWML 111443), based on posterior end morphology of non-gravid females (Fig. 1) (Barger and Nickol 2004). Each of these two turtles were infected with two individual acanthocephalans, for a total of four worms, with lengths of 8.3 ± 0.42 mm (range = 8.0–8.6) and widths of 0.51 ± 0.04 mm (range = 0.48–0.54).

Neoechinorhynchus emyditoides was originally described by Fisher (1960) from red-eared sliders (*Trachemys scripta elegans*) from Arkansas. To date, *N. emyditoides* has been reported from at least 14 species and/or subspecies of turtle hosts from four families across the United States and México (Table 2), including a *C. serpentina* from Virginia (Zelmer and Platt 2009). Barger (2004) previously reported *N. emyditoides* from *T. s. elegans* from McIntosh and Pittsburg counties, Oklahoma. In addition, McAllister et al. (2015) reported a *Neoechinorhynchus* sp. from an eastern river cooter (*Pseudemys concinna concinna*) from McCurtain County, Oklahoma, thought to represent either *N. emyditoides* or *N. pseudemydis*; however, because no fully developed eggs were present in specimens

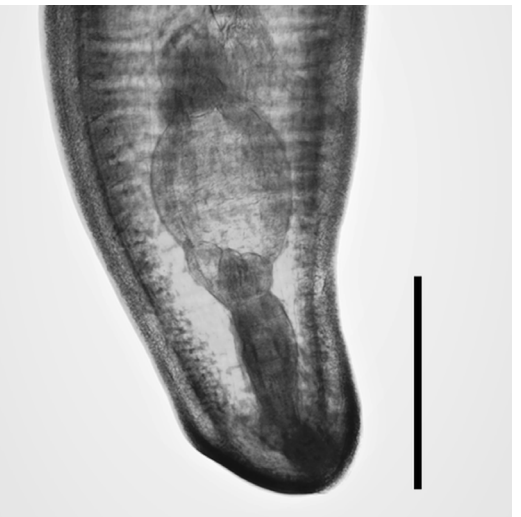

Figure 1. Posterior end of non-gravid female *Neoechinorhynchus emyditoides* from *Chelydra serpentina*. Scale bar = 100 µm.

Table 1. Eighteen common snapping turtles collected between 2013 and 2019 from Arkansas (AR) and Oklahoma (OK) and examined for acanthocephalans.

Date collected	Carapace length (mm)	Sex	Locality	Infected?
20 April 2013	92	F	AR:Franklin Co., off St. Hwy. 23	No
9 May 2013	225	F	AR:Union Co., El Dorado	No
14 May 2013	260	F	OK:McCurtain Co., Yashau Creek	No
15 May 2015	235	M	OK:McCurtain Co., vic. Idabel	No
30 April 2016	195	M	OK:McCurtain Co., N of Broken Bow	No
21 April 2017	165	M	AR:St Francis Co., jct. St. Hwys. 255/261	Yes
20 June 2018	220	M	AR:Sevier Co., Mill Creek at Horatio	No
8 July 2018	265	M	OK:McCurtain Co., Hochatown	No
21 March 2019	205	F	OK:McCurtain Co., Yashau Creek	Yes
15 May 2019	219	F	OK:Payne Co., Stillwater	No
16 May 2019	195	M	OK:McCurtain Co., E of Broken Bow	Yes
24 May 2019	295	F	OK:Payne Co., Stillwater	No
10 June 2019	320	M	OK:McCurtain Co., 3.2 km N Broken Bow	No
12 June 2019	200	M	AR:Polk Co., vic. Board Camp	No
	218	F	OK:Payne Co., Stillwater	No
19 June 2019	225	F	OK:Lincoln Co., Stillwater	No
18 July 2019	275	M	OK:Payne Co., Stillwater	No
1 October 2019	260	M	OK:Choctaw Co., Ft. Towson	No

(HWML 91960), species identification was problematic and, unfortunately, remains unknown. Here, we document the second report of *N. emyditoides* in *C. serpentina*, comprising a total of four species of *Neoechinorhynchus* reported from common snapping turtles.

Of the seven previous reports of *Neoechinorhynchus* spp. from common snapping turtles, prevalence and intensities of infections were low, and all worms were non-gravid or immature females. This is in contrast to reports of species of *Neoechinorhynchus* from aquatic emydid turtles where prevalence and mean intensity can range from one to 100% (average 50.2%) and one to 2,337 (average 82.2), respectively (Fisher 1960; Martin 1973; Esch et al. 1979; Lindeman and Barger 2005). Results from the present study also report low prevalence (17%) and mean intensity (two) of *Neoechinorhynchus* spp. from snapping turtles from Arkansas and Oklahoma. Additionally, none of the *Neoechinorhynchus* spp. from this study were mature and/or gravid, and the four female *N. emyditoides* averaged 8.3 ± 0.42 mm in length and 0.51 ± 0.04 mm in width, much smaller than the original species description (females, length = 34.3 mm; females, width = 0.94 mm), but from red-eared sliders (Fisher 1960). Taken together, these results suggest that common snapping turtles are unsuitable hosts for acanthocephalans.

There are various factors that may influence host suitability for parasites. However, one likely factor could be due to the food habits of *C. serpentina*, as they are more carnivorous, specifically, piscivorous, compared to other species of emydid turtles (Lagler 1943). Additionally, the foraging behavior of *C. serpentina* shifts from opportunistic predation in juveniles to ambush predation in adults (Ernst et al. 1994). However, because four of the 10 turtle acanthocephalan species have

Table 2. Previous reports of *Neoechinorhynchus emyditoides* from four families of North American turtles.

Family/Host	Locality	Prevalence*	Reference
CHELYDRIDAE			
Chelydra serpentina	Virginia	–	Zelmer and Platt (2009)
	Oklahoma	2/13 (15%)	This study
EMYDIDAE			
Chrysemys picta	Indiana	–	Barger (2004)
Emydoidea blandingii	Massachusetts	–	Fisher (1960)
Graptemys geographica	not reported	–	Barger (2004)
Graptemys pseudogeographica	Tennessee	–	Barger (2004)
Pseudemys concinna	Louisiana	8/12 (67%)	Acholonu (1969)
Pseudemys floridana	Louisiana	–	Acholonu (1969)
Trachemys gaigeae	New Mexico	3/5 (60%)	McAllister et al. (2008)
	México (Nuevo León)	–	Garcia-Varela et al. (2011)
Trachemys ornata	México (Tabasco)	–	Bravo-Hollis (1946)
Trachemys scripta elegans	Arkansas†	17/24 (71%)	Fisher (1960)
		91/94 (97%)	Rosen and Marquardt (1978)
		–	Barger (2004)
	Alabama	–	Johnson (1969)
	California	–	Fischer (1960)
	Indiana	–	Barger (2004)
	Louisiana	6/12 (50%)	Fisher (1960)
		44/78 (56%)	Acholonu (1969)
	Mississippi	12/12 (100%)	Fisher (1960)
		–	Barger (2004)
	New York	–	Fisher (1960)
	North Carolina	–	Johnson (1969)
	Oklahoma	–	Barger (2004)
	South Carolina	–	Aho et al. (1992)
	Tennessee	–	Barger (2004)
	Texas	–	Fisher (1960)
		10/10 (100%)	Little and Hopkins (1968)
	Virginia	–	Fisher (1960)
	México (Veracruz)	–	García-Varela et al. (2011)
Trachemys scripta scripta	South Carolina	42/106 (40%)	Esch et al. (1979)
Trachemys scripta troostii	not reported	–	Barger (2004)
TRIONYCHIDAE			
Apalone spinifera	Louisiana	9/18 (50%)	Acholonu (1969)
KINOSTERNIDAE			
Kinosternon sp.	not reported	–	Barger (2004)

*Number infected/number examined (%).

†Type locality.

been reported from *C. serpentina*, and because worms were never reported as gravid or mature, these data suggest that this host must also have various physiological constraints preventing acanthocephalans from establishing in the intestine. Given that various aquatic turtles, in addition to *C. serpentina*, have been reported with *Neoechinorhynchus* spp. in North America (Barger 2004, 2005), it appears that *C. serpentina* remains an outlier host, regardless of the overlap in habitat. Interestingly, a report (West et al. 2000) of three *Neoechinorhynchus* species, including *N. chrysemydis*, *N. emydis*, and *N. pseudemydis*, from alligator snapping turtles (*Macroclemys temminckii*) from Arkansas and Louisiana indicates a low prevalence of 9% and mean intensity of 26, suggesting that other chelydrid turtle species may also be unsuitable hosts for *Neoechinorhynchus* species.

The exact reason for the unsuitability of *C. serpentina* for acanthocephalans remains unknown yet hints toward both historical events of host-parasite evolution and current ecological conditions, such as host diet. Finally, although the intermediate and paratenic hosts are not known for *N. emyditoides*, other turtle *Neoechinorhynchus* species use ostracods and snails as intermediate and paratenic hosts, respectively (Hopp 1954). This suggests that snapping turtles do not ingest as many suitable ostracods and/or snails compared to red-eared sliders. Future work should aim to survey turtles for acanthocephalans, keeping *C. serpentina* in mind due to the rarity of reports.

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Helminth Parasites (Trematoda, Cestoda, Nematoda) of Select Mammals (Didelphimorpha, Chiroptera, Carnivora) from McCurtain County, Oklahoma

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Abstract: Between February 2019 and September 2019, five individual mammals were collected in McCurtain County, Oklahoma, and examined for helminth parasites as follows: Virginia opossum (*Didelphis virginiana*), two eastern red bats (*Lasiurus borealis*), bobcat (*Lynx rufus*), and gray fox (*Urocyon cinereoargenteus*). Eight taxa of parasites were recovered, including three trematodes, *Paralecithodendrium* sp., *Rhopalias coronatus* and *Fibricola cratera*, two cestodes, *Vampirolepis* sp., and *Taenia rileyi*, and three nematodes, *Physaloptera rara*, *Turgida turgida*, and *Cruzia americana*. We document seven new geographic records; all of these helminths (except *T. turgida*) are reported from Oklahoma for the first time.

Introduction

Oklahoma supports about 101 native species of mammals (Caire et al. 1989) in nine physiognomic regions that range from Piñon-Juniper Mesas in the Panhandle to the Oak-Hickory-Pine Ouachita Highlands and bottomland forest with loblolly pine (*Pinus taeda*) in the southeast corner of the state. The latter region of Oklahoma includes McCurtain County, the third largest in the state, with total area of 4,930 km^2 (1,902 mi^2). It contains a varied topography that extends, in the north, from the rocky foothills of the Ouachita Mountains to the fertile Coastal Plain region along the Red River, which forms the southern boundary with Arkansas and Texas.

A recent survey of the mammals of Red Slough Wildlife Management Area in southeastern McCurtain County (Roehrs et al. 2012) has shown that a wide variety of species occur there and throughout much of the county, but little to nothing is known about their parasites. Here, we document some distributional records for several helminth parasites of select mammals from the county.

Methods

Between February 2019 and September 2019, five individual mammals were collected opportunistically in various parts of McCurtain County as follows: Virginia opossum (*Didelphis virginiana*), two eastern red bats (*Lasiurus borealis*), bobcat (*Lynx rufus*), and gray fox (*Urocyon cinereoargenteus*). All specimens, except *D. virginiana*, were found recently dead on the road (DOR) without necrosis, salvaged, and processed within one hour; the opossum was euthanized with an intraperitoneal injection

of sodium pentobarbital (Nembutal®) following accepted guidelines (Sikes et al. 2011). A mid-ventral incision was made from the throat to anus of each specimen to expose the viscera and the entire gastrointestinal tract and other organs were placed in Petri dishes containing 0.9% saline, and examined for helminths under a stereomicroscope at 20–30×. Trematodes were fixed without coverslip pressure in near boiling water and transferred to 95% (v/v) molecular grade ethanol. Cestodes were detached from the host's intestine, gently rinsed in 0.9% saline, and fixed in hot 4% formaldehyde solution (formalin) with subsequent transfer to 95% molecular grade ethanol. Both were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were fixed in near boiling water and preserved in 70% (v/v) ethanol. They were later cleared and identified in temporary mounts of lactophenol and then returned to the preservative.

Voucher specimens of parasites (except those retained for molecular analysis) were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Photovouchers of the hosts were deposited in the Henderson State University Museum (HSU), Arkadelphia, Arkansas.

Results and Discussion

Eight taxa of parasites were found, including three trematodes, two cestodes, and three nematodes; no acanthocephalans were observed. An annotated list of the specific parasites recovered and their host data follows.

Trematoda: Digenea: Echinostomatidae

***Rhopalias coronatus* (Rudolphi, 1819) Stiles and Hassall, 1898** – Several *R. coronatus* (HWML 216096) were found in the intestine of *D. virginiana* collected on the EOSC-Idabel campus (33°55'13.926" N, 94°46'40.8792" W) on 13 March 2019. Chandler (1932) reported this trematode from *D. virginiana* in Texas, and it has also been reported from various other didelphid hosts from México, Panama, Argentina, Bolivia, Paraguay, and Venezuela (Monet-Mendoza 2005; Haverkost and Gardner 2008; Acosta-Virgen et al. 2015). Interestingly, recent molecular evidence from López-Caballero et al. (2019) suggests that specimens originally reported as *R. coronatus* found in the intestine of common opossum (*D. marsupialis*) from Yucatán, México, may actually represent a new species. In addition, a similar species, *R. macracanthus* Chandler, 1932 has been reported from *D. virginiana* from Oklahoma (Self and McKnight 1950) as well as several other states (Alden 1995). The life cycle is unknown, but based on the known life cycles of related taxa and phylogenetic data by Tkach et al. (2016) definitive hosts likely acquire the parasite by the ingestion of metacercariae encysted in amphibians or fish. We document *R. coronatus* from Oklahoma for the first time.

Lecithodendriidae

***Paralecithodendrium* sp.** – Here, we follow Lotz and Font (1983) using the name *Paralecithodendrium*, which has priority over the name *Prosthodendrium* at the rank of genus. Three specimens of a *Paralecithodendrium* sp. were found in the anterior third of the intestine of *L. borealis* collected on 26 June 2019 from Idabel (33°55'0.4152"N, 94°51'01.8648"W). Eastern red bats have been reported to harbor *P. transversum* (Byrd and Macy, 1942) Lotz and Font, 1983 from Tennessee, Iowa, and Indiana, respectively (Byrd and Macy 1942; Kunz 1968; Pistole 1988). Representatives of this trematode genus was also reported from northern long-eared bat (*Myotis septentrionalis*) from adjacent Arkansas (McAllister et al. 2004). In addition, *L. borealis* from Minnesota and Iowa have been reported to harbor *Paralecithodendrium nokomis* (Macy, 1937) Lotz and Font, 1983 (Macy 1937; Blankespoor and Ulmer 1970) and red bats from Iowa are hosts of *Paralecithodendrium swansoni* (Macy, 1936) Lotz and Font, 1983 (Blankespoor and Ulmer 1970). *Paralecithodendrium naviculum* (Macy, 1936) Lotz and Font, 1983 was reported from Arkansas in American perimyotis (*Perimyotis subflavus*) (McAllister et al. 2011). In the life cycle, snails and anopheline mosquitoes serve as intermediate hosts, and the adult worm is found

in the intestinal tract of bats, which have most likely ingested intermediate hosts (Abdel-Azim 1936). Molecular analysis will be required to provide a specific identity for our specimens.

Diplostomatidae

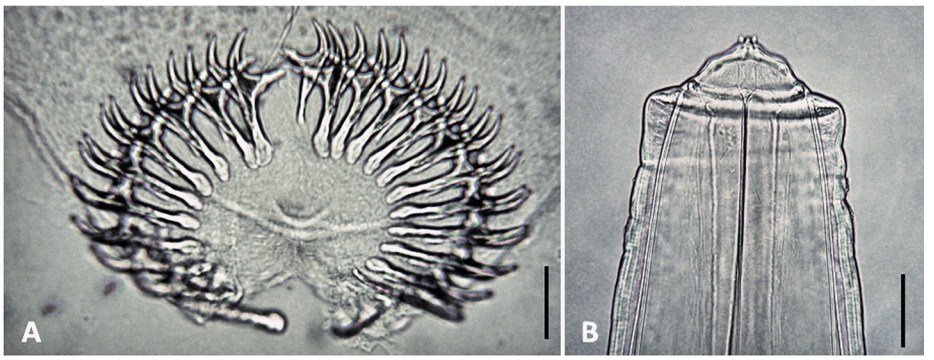
***Fibricola cratera* (Barker and Noll, 1915) Dubois, 1932** – Several of these trematodes (HWML 216095) were found in the small intestine of the same *D. virginiana* reported above. The life cycle involves aquatic snails (*Physa* spp.) as first intermediate hosts and anurans as second intermediate hosts (Hoffman 1955). This digenean has been previously reported from *D. virginiana* from Florida, Louisiana, Michigan, Tennessee and Wisconsin (Alden 1995). It is reported here for the first time from Oklahoma as well as the initial documentation from a geographic locality west of the Mississippi River.

Cestoda: Cyclophyllidea: Hymenolepidae

***Vampirolepis* sp.** – Two tapeworms were taken from the small intestine of a pregnant *L. borealis* female collected on 10 June 2019 from off St. Hwy. 152 at Cerrogordo (35°17' 27.276"N, 98°43'0.7284" W) and a single individual was collected from another eastern red bat from Idabel (33°55'0.4152"N, 94°51'01.8648"W) on 26 June 2019. Tapeworms of the genus *Vampirolepis* Spassky, 1954 are commonly reported from bats in North America (Nickel and Hansen 1967; Rausch 1975; Vaucher 1992); however, this is the first report from eastern red bats and to our knowledge, the first time this genus has been reported from any bat from Oklahoma. Blankespoor and Ulmer (1970) and Pistole (1988) reported the tapeworm, *Cycloskrjabinia taborensis* (Loewen, 1934) from *L. borealis* from Iowa and Indiana, respectively. In addition, McAllister et al. (2005, 2006, 2017) reported a *Vampirolepis* sp. from Rafinesque's big-eared bat (*Corynorhinus rafinesquii*), *Vampirolepis decipiens* (Diesing, 1850) from Brazilian free-tailed bats (*Tadarida brasilensis*), and *Vampirolepis* sp. from eastern small-footed bats (*Myotis leibii*) from neighboring Arkansas, respectively. Specimens are being retained for molecular analyses.

Taeniidae

***Taenia rileyi* Loewen, 1929** – Various specimens (HWML 111234) of these tapeworms matched the description of *T. rileyi* (Rausch 1981) and were found in the intestinal tract of a pregnant female *L. rufus* collected on 16 April 2019 in Idabel (33°53'37.41"N, 94°51'09.8352" W). Measurements of specimens of *T. rileyi* are as follows: the rostellum (Fig. 1A) has 42 hooks (21 large, 21 small) that measured 235 and 188 µm, respectively. Surveys of bobcat parasites have been conducted from Canada to México, as well as in between in several US states, including specimens from Alabama, Arkansas, Georgia, Kansas, Illinois, Massachusetts, Nebraska, New Mexico, North Carolina, Oklahoma, South Carolina, Texas, Utah, Virginia, and West Virginia (Marchiondo et al.



Figures 1A–B. Some parasites of Oklahoma mammals. (A) Rostellar hooks of *Taenia rileyi* from *Lynx rufus*; scale bar = 100 µm. (B) Anterior end of *Physaloptera rara* from *Urocyon cinereoargenteus*; note: large cephalic collarette and two large, simple triangular lateral pseudolabia. Scale bar = 100 µm.

1986; Reichard et al. 2004; see also references in Hiestand et al. 2014). *Taenia rileyi* has been reported in bobcats from Georgia, Illinois, Minnesota, Nebraska, "New England", North Carolina, South Carolina, Texas, Utah, Virginia, and West Virginia (Hiestand et al. 2014). Larval *T. rileyi* have been reported in rodents in Florida and Georgia and may serve as intermediate hosts of this tapeworm (Kinsella 1974, 1988, 1991). In Illinois, Hiestand et al. (2014) reported *T. rileyi* occurred in high prevalence (70%) and caused intense infections in bobcats; it is also considered to be a bobcat-specific helminth. We here document *T. rileyi* from Oklahoma for the first time.

Nematoda: Spirurida: Physalopteridae

***Physaloptera rara* Hall and Wigdor, 1918** – Three *P. rara* (HWML 111237, Fig. 1B) were found in the lower esophagus and stomach of an adult male *U. cinereoargenteus* collected on 25 September 2019 from Idabel (33°55'0.4152''N, 94°51'01.8648''W). There are about 100 species or more in the genus (Pereira et al. 2013). Ubelaker et al. (2015) noted that *P. rara* has been reported previously from gray foxes from Florida, Illinois, Minnesota, New Mexico, and Texas. The life cycle involve insects as intermediate hosts, including beetles, cockroaches, and crickets. This nematode shows little host specificity as it has also been reported from other North American carnivores (domestic dogs, coyotes, wolves, raccoons, domestic cats, and bobcats), including kit foxes (*Vulpes macrotis*) and swift foxes (*V. velox*) from New Mexico (Ubelaker et al. 2014a, b). Here, for the first time, we report *P. rara* from *U. cinereoargenteus* from Oklahoma.

***Turgida turgida* (Rudolphi, 1819)** – Specimens (HWML 111233) were found attached to the stomach wall of *D. virginiana*. This nematode is a relatively common parasite of *D. virginiana* and other didelphids in both North and South America. It has been reported in *D. virginiana* from México (Acosta-Virgen et al. 2005; Monet-Mendoza 2005) and at least 15 U.S. states (Alden 1995; Matey et al. 2001; Richardson and Campo 2005; Nichelason et al. 2008), including Oklahoma (Hill 1939).

Kathlaniidae

***Cruzia americana* Maplestone, 1930** – Several specimens of *C. americana* (HWML 111232) were found in the cecum of *D. virginiana*. This nematode feeds on the intestinal mucosa, taking nutrients and ingesting blood and has been reported to cause significant illness in opossums (Jones 2013). It has been reported in *D. virginiana* from California (Nichelason et al. 2008; Jones 2013), Connecticut (Richardson and Campo 2005), Georgia, Illinois, North Carolina, Ohio, Pennsylvania, Texas, and Virginia (Alden 1995), and México (Monet-Mendoza 2005). This is the initial report of *C. americana* from Oklahoma.

In conclusion, we document seven new geographic records for these mammalian parasites as well as the initial report of *F. cratera* being found in a host from a geographic locale west of the Mississippi River. Although this was a small, opportunistic survey, it shows that there are many mammalian parasites yet to be reported from the state. With additional examinations, we expect additional geographic as well as new host records will be documented, including the possibility of discovering new species.

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Novel Natural History and Ecological Information on Select Oklahoma Biota

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Abstract: In this contribution on the subject, we include noteworthy observations on the natural history and ecology of select biota of Oklahoma. Here, additional biological records on 15 species of invertebrates and vertebrates from the state are documented. Novel information is provided for an asellid isopod, three cambarid crayfishes, a stonefly, three fishes, one turtle, three snakes, one bird, and two mammals. Our purpose is to help complement and fill gaps in our limited biological knowledge of this biota that should help in future studies and observations conducted in the state.

Introduction

Oklahoma's biota is comprised of a very diverse group of organisms that inhabit the state's dynamic ecosystems. Therefore, when data on the natural history and ecology of the various taxa is noteworthy, it should be documented when available. Novel information has been provided by our previous community collaborative efforts (McAllister and Robison 2016, 2017; Robison et al. 2018) and, here, we continue that effort and provide new biological information on five invertebrates and nine vertebrates of the state.

Methods

Crayfishes were collected by hand or dipnet and preserved in 70% (v/v) isopropyl alcohol. Fish were taken with gill nets or by bowhunting, measured for total length (TL), preserved in 10% formalin, and stored in 45% isopropanol; they were also examined for stomach contents. A turtle and three snakes, collected by hand, were measured for carapace length (CL) and snout-vent length (SVL), respectively. Feces from the rectum of snakes were collected and placed in a vial containing 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$) and, after flotation in Sheather's sugar solution (sp. gr. 1.30), examined for coccidians. A single bird, bat and bobcat each were found dead without necrosis on the road (DOR) and examined. The feathers of the bird was brushed vigorously over a white enamel tray to observe ectoparasites and those found were placed in individual vials of 70% (v/v) ethanol; selected specimens were cleared in 10% potassium hydroxide, dehydrated through an ethanol series, further cleared in xylene, and slide-mounted in Canada balsam (Price et al. 2003). Localities for

all sites herein are reported as GPS (latitude and longitude) coordinates.

Crayfish voucher specimens were deposited in the Southern Arkansas University (SAU) Collection, Magnolia, Arkansas. Photovouchers of fish and mammals were deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas, and photovouchers of reptiles were deposited in the Arkansas State University Museum of Zoology (ASUMZ), Herpetological Collection, State University, Arkansas. The bird was deposited in the Eastern Oklahoma State University-Idabel collection, Idabel, Oklahoma. Voucher specimens of bird lice were deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia, under an individual accession number. Voucher specimens of other parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

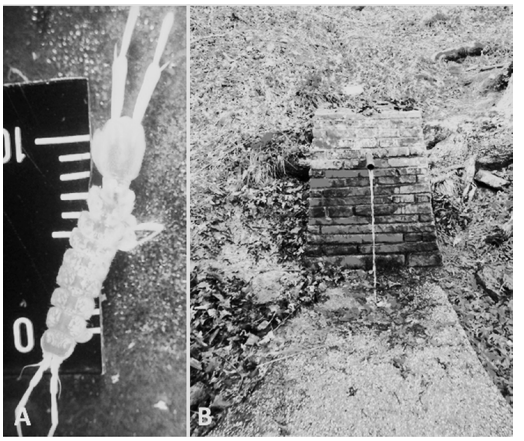
We follow the Reptile Database for all common and scientific names of reptiles (Uetz et al. 2019), and/or Burbrink and Guiher (2014) who synonymized the copperhead subspecies *Agkistrodon contortrix contortrix*, *A. c. mokasen*, and *A. c. phaeogaster* (in part) into *A. contortrix*.

Results and Discussion

The collections described herein represent important records of geographic distribution or previously unknown observations of their natural history and ecology, and are reported below in an annotated format as follows.

Arthropoda: Crustacea: Malacostraca: Isopoda: Asellidae

***Caecidotea* sp.** – an unknown species of asellid isopod (Fig. 1A) was collected by aquatic dipnet on 29 December 2018 from Pipe Spring, 3.2 km N of Big Cedar off US 259, Le Flore County (34°41’53.35”N, 94°38’41.21”W) (Fig. 1B). It possessed small eyes and moderate pigmentation (Fig. 1A) and was determined to belong to the genus *Caecidotea*. There are at least 21 species of *Caecidotea* in Oklahoma (Graening et al. 2007). Additional specimens will be required to provide a specific identity. However, *C. montana* (Mackin and Hubricht, 1938) has been reported by Graening et al. (2007) from a site just to the south of the collection locality above a “stream near Big Cedar.” The voucher was retained in the personal collection of Julian J. Lewis.



Figures 1A–B. Isopod from Le Flore County. (A) Specimen of *Caecidotea* sp. showing moderate pigmentation and without eyes; scale bar increments = 1 mm. (B) Pipe Spring study site looking south.

Decapoda: Cambaridae

***Fallicambarus fodiens* (Cottle, 1863) – Digger Crayfish.** In Oklahoma, *F. fodiens* is known from only three localities in Le Flore and McCurtain counties, and from “one locality in north central portion of the state” that was not specified (Reimer 1968; Morehouse and Tobler 2013). This crayfish is a primary burrower, but constructs one of the least complex burrows of any species in its genus (Hobbs and Robison 1989). We document herein two new collection sites of *F. fodiens*. On 16 March 2000, a single form II male was collected from a roadside ditch at the jct. of US 70 and St. Hwy. 98, ca. 8 km E of Valliant, McCurtain County (33°59’27.4812”N, 95°02’6.6696”W). In addition, a female *F. fodiens* was collected from a simple burrow ca. 6.4 km E of Hugo on US 70, Choctaw County (33°59’52.674”N, 95°23’58.9416”W). The latter specimen represents a new county record of *F. fodiens* in the state and extends the range of this species westward to the Hugo area.

***Fallicambarus schusteri* Taylor and Robison, 2016 – Carmel Crayfish.** Recently, Taylor and Robison (2016) described *F. schusteri* from four locations in the flatlands draining south into the Red River from Idabel in southcentral McCurtain County to Ashdown, southcentral Little River County, Arkansas. These authors believed continued sampling of roadside ditches for burrowing crayfishes in this area would likely yield additional localities for this crayfish. In Oklahoma, only three localities are known for this species. We herein report a new collection site of *F. schusteri* in addition to the collection of a single ovigerous female captured on 17 March 2000 from a burrow in a roadside ditch along St. Hwy. 3 at Bokhoma, McCurtain County (33°49'20.73''N, 94°34'58.4256''W). This is the first report of *F. schusteri* with eggs; unfortunately, no egg counts were made. The specimen was dug from a burrow composed of a single shaft 10 cm deep within a low chimney.

***Procambarus clarkii* (Girard, 1852) – Red Swamp Crayfish.** This crayfish is a wide-ranging species which occurs naturally along the Gulf Coastal Plain from northeastern México to the Florida panhandle, extending northward into southeastern Missouri and southwestern Illinois (Page 1985; Pflieger 1996; Walls 2009). In Oklahoma, *P. clarkii* occurs naturally in the southeastern corner where the Gulf Coastal Plain enters the state (Morehouse and Tobler 2013). On 4 September 2000, a single ovigerous 83 mm TL female with 106 ova (1.0–1.4 mm in diameter, wet weight = 1.1 g) was collected from a flooded roadside ditch at the jct. of US 259 and co. rd. 2250 in Harris, McCurtain County (33°46'16.3632" N, 94°43'48.5328" W). In Louisiana, females with eggs have been collected in September (Penn, 1943). Page (1985) reported females with eggs from June through early September in Illinois. Morehouse and Tobler (2013) reviewed the distribution and natural history of *P. clarkii* in Oklahoma, but reported no ovigerous females were known from the state. The discovery of this female with eggs is the first report of an ovigerous *P. clarkii* taken in Oklahoma.

Insecta: Plecoptera: Leuctridae

***Zealeuctra claasseni* (Frison, 1929) – Common Needlefly.** A nymphal specimen of *Z. claasseni* was collected with an aquatic dipnet on 29 December 2018 from the same Pipe Spring site noted above. There are currently 12 species of *Zealeuctra* distributed in central and eastern North America (Grubbs et al. 2013; Verdone et al. 2019). *Zealeuctra claasseni* ranges widely and has been previously reported from Alabama, Arkansas, Illinois, Indiana, Kansas, Kentucky, Missouri, Oklahoma, Ohio, Tennessee, Texas, and West Virginia (DeWalt et al. 2019). In Oklahoma, *Z. claasseni* has been reported from Comanche, Johnston and Latimer counties (Grubbs et al. 2013). A voucher specimen was deposited in the C. P. Gillette Museum, Colorado State University, Fort Collins, Colorado. We document a new county record for this stonefly in Oklahoma.

Actinopterygii: Lepisosteiformes: Lepisosteidae

***Lepisosteus oculatus* Winchell, 1864 – Spotted Gar.** An adult female *L. oculatus* (670 mm TL) was collected by bowfishing on 28 October 2018 from a private lake (Little River drainage) on the Turner Ranch just north of Idabel, McCurtain County (33°55'56.93"N, 94°43' 43.22"W). Examination of the body revealed two fish lice, *Argulus americanus* C. B. Wilson, 1902 (HWML 111302, Fig. 2A). The genus *Argulus* (Crustacea: Branchiura) has a worldwide distribution and about 32 species and subspecies are considered valid (McLaughlin et al. 2005). However, relatively little is known of the distribution and species composition of the crustacean ectoparasite genus *Argulus* in Oklahoma. McAllister et al. (2016) reported *A. americanus* from Flathead Catfish (*Pylodictis olivaris*) and Largemouth Bass (*Micropterus salmoides*) from Broken Bow Lake (McCurtain County), ca. 32 km N of the current study site. The geographic distribution of records of this louse from Bowfin (*Amia calva*) and Longnose Gar (*Lepisosteus osseus*) include: Florida, Illinois, Indiana, Iowa, Louisiana, Michigan, Oklahoma, Wisconsin, and Québec, Canada (Wilson 1916; Meehan 1940; Shimura and Asai 1984; Poly 1998; McAllister et al. 2016). In

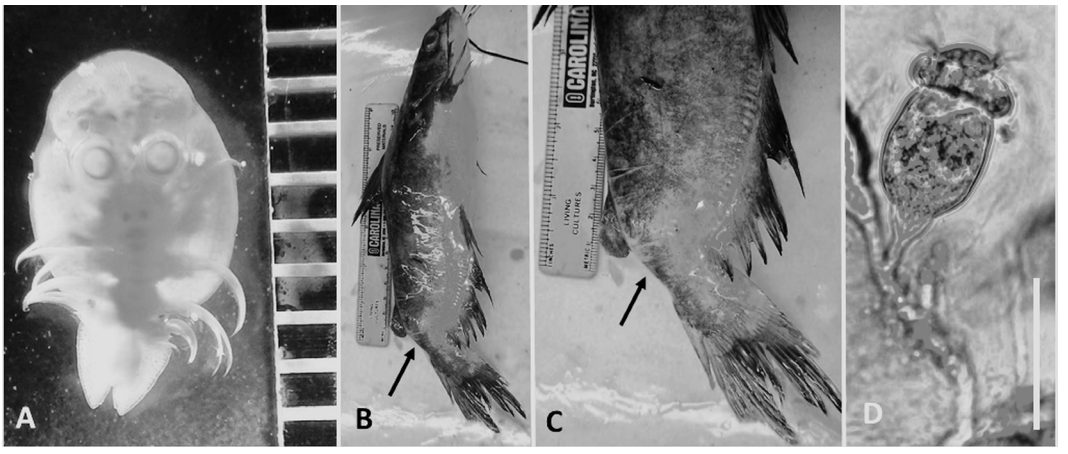
Arkansas, *L. oculatus* has been reported as a host of *A. nobilis* Thiele, 1904 (Hoffman 1999) and a specimen of *A. mississippiensis* Wilson, 1916, deposited in the United States National Museum (now National Museum of Natural History) as USNM 191113, was taken from a *L. oculatus* from Oates Creek at Bradford (White County), Arkansas. Branchiurans are primarily ectoparasites of fishes (Poly 2008) and these ectoparasites can be destructive due mainly to secondary fungal infections that attach at the puncture sites, and they can also transmit viral diseases and other fish parasites. This is the first report of *A. americanus* from a Spotted Gar from Oklahoma, and the second report of the parasite from the state.

Hiodontiformes: Hiodontidae

***Hiodon alosoides* (Rafinesque, 1819) – Goldeye.** The diet of the Goldeye is reported to be composed primarily of aquatic and terrestrial insects (Johnson 1963) and studies from Lake Texoma have supported this notion (Shelton 1969; Miller and Robison 2004). In addition, Tumlison et al. (2018) reported a *H. alosoides* that fed on cottonwood leaf beetles, *Chrysomela scripta* in the Mississippi River, Arkansas. To our knowledge, there are no reports of specific foods consumed by specimens of *H. alosoides* from Oklahoma. On 6 February 2018, an adult Goldeye (360 mm TL) was collected by gill net from the vicinity of the Willis Bridge, Lake Texoma, Marshall County (33°52'31.9224"N, 96°50'01.2804"W). While it was being necropsied for parasites, the stomach was found to contain a menagerie of terrestrial insects as follows: a spider, stink bugs (Hemiptera), a carabid and a cerambycid beetle, and hymenopterans, but no aquatic taxa. This further supports *H. alosoides* food habit data that suggests this fish feeds on insects, terrestrial in origin, via frequent surface feeding in shallow water.

Siluriformes: Ictaluridae

***Ictalurus punctatus* (Rafinesque, 1818) – Channel Catfish.** A 265 mm TL *I. punctatus* collected in a gill net on 10 June 2019 from a tributary of the Little River on the same Turner Ranch site above exhibited an unusual skeletal deformation that appeared to show the typical facies of kyphosis-scoliosis of the vertebral column (Fig. 2B–C). It is not known what caused this deformity but nutritional factors such as deficiencies in phosphorus and vitamins C and K, and hypervitaminosis A can lead to twisted neural and hemal spines, development of soft bones, decreased bone mass, vertebral fusion, lordosis, kyphosis, and scoliosis (Berillis 2015). Although this fish was a wild caught specimen, scoliosis has been observed in cultured Channel Catfish and is associated with severe vitamin C deficiency (Andrews and Murai 1975). In addition, Lim and Lovell (1978) reported that



Figures 2A–D. Fish louse, scoliosis, and commensal ciliate. (A) *Argulus americanus* from *Lepisosteus oculatus*; scale bar increments = 1 mm. (B) Scoliosis in *Ictalurus punctatus* (arrow); note ruler scale. (C) Close-up view of scoliosis (arrow) in *I. punctatus*. (D) *Epistylis* sp. from *Chelydra serpentina*; scale bar = 50 µm.

I. punctatus with vitamin C deficiency had a decreased bone collagen content and developed vertebral column malformations (kyphosis, lordosis, scoliosis). We document the first report of scoliosis in a non-cultured Channel Catfish, including, to our knowledge, the initial specimen from Oklahoma afflicted with this skeletal disorder.

Reptilia: Testudines: Chelydridae

***Chelydra serpentina* (L., 1758) – Common Snapping Turtle.** An individual *C. serpentina* (CL = 265 mm) collected on 8 July 2018 from off US 259 in Hochatown, McCurtain County (34°09'53.2656"N, 94°45'20.2608"W) was found to be partially covered with ectocommensal *Epistylis* sp. (Fig. 2D) on its carapace. These ciliates are sessile peritrichous organisms often present as a branching colony with a short oral disc and collar, and non-contractile rigid stalk (Dias et al. 2006). *Epistylis* spp. have been reported on various turtles (Bishop and Jahn 1941; Bovee 1976) and from two species of emydid turtles from Arkansas (Tumlison and Clark 1996). This is the first report of an *Epistylis* sp. on *C. serpentina* from Oklahoma.

Ophidia: Colubridae

***Lampropeltis holbrooki* Stejneger, 1902 – Speckled Kingsnake.** On 16 July 2019, a juvenile male (425 mm SVL) *L. holbrooki* was collected by hand in Hochatown, McCurtain County (34°09'55.152"N, 94°45'35.8776"W), and held at room temperature. Within 12 hrs, it regurgitated a 215 mm SVL rough earth snake (*Haldea striatula*) (Fig. 3). Interestingly, the prey item made up 51% of the SVL of the *L. holbrooki*. Speckled kingsnakes are reported to feed on a variety of vertebrate prey including small mammals, birds, venomous and nonvenomous snakes, lizards, turtles, frogs, as well as the eggs of birds and reptiles (Werler and Dixon 2000). Konvalina et al. (2015) documented an instance *L. holbrooki* eating a *H. striatula* in adjacent Arkansas. In addition, McAllister (2016) reported a *L. holbrooki* from the same Oklahoma locale above that had eaten both a flat-headed snake (*Tantilla gracilis*) and a smooth earthsnake (*Virginia valeriae elegans*). Here, we report a species of snake that has not previously been reported as prey of an Oklahoma *L. holbrooki*.

***Lampropeltis calligaster calligaster* (Harlan, 1827) – Prairie Kingsnake.** An adult *L. c. calligaster* (550 mm SVL) collected on 5 May 2018 from Smithville, McCurtain County (34°28'0.4794"N, 94°38'37.6794"W) was passing sporulated oocysts and sporocysts of a *Sarcocystis* sp. (HWML 216103) and sporulated oocysts of *Caryospora lampropeltis* Anderson, Duszynski, and Marquardt, 1968 (HWML 216104) in its feces. Sporocysts of the former (Fig. 4A) measured (L × W) 11 × 7 μm; the latter oocysts (Fig. 4B) measured ca. 22–25 μm in diameter with a single polar granule, and their sporocysts were 18–20 × 12–13 μm with a button-like Stieda body and a plump subStieda body, all fitting the description of *C. lampropeltis*. Anderson et al. (1968) reported a *Sarcocystis* (syn. *Cryptosporidium lampropeltis*) from *L. c. calligaster* from Illinois. In addition, the type

Figure 3. Speckled kingsnake (above) with rough earth snake prey (below). Note ruler scale.

host of *Ca. lampropeltis* is *L. c. calligaster* from Illinois (Anderson et al. 1968). McAllister et al. (2017) also reported the coccidian from *L. c. calligaster* from Arkansas. This is the second report of a *Sarcocystis* sp. in this host from the state (McAllister et al. 2013) and the first report of *Ca. lampropeltis* from Oklahoma.

Viperidae

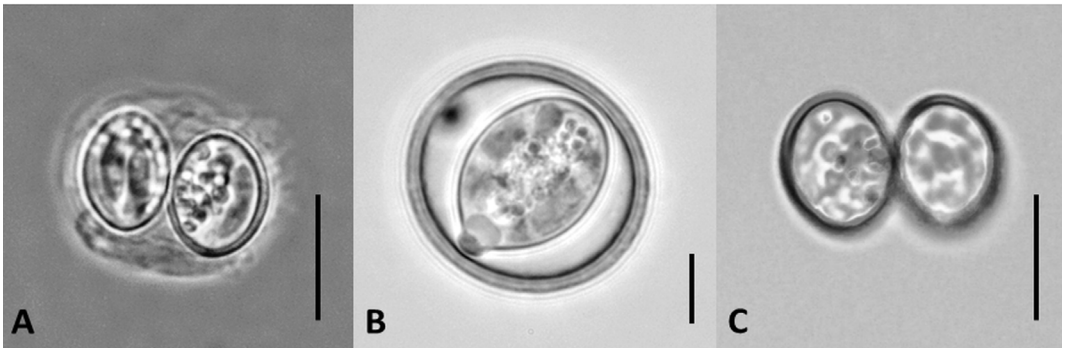
***Agkistrodon contortrix* (L., 1766) – Eastern Copperhead.** Although coccidian parasites are relatively common in snakes, copperheads in general have rarely been reported as hosts (Duszynski and Upton 2009). Wacha and Christiansen (1975) were the first to report an isosporan from eastern copperheads (formerly Osage copperheads, *A. contortrix phaeogaster*) in Iowa that was later determined to represent a *Sarcocystis* sp. Lindsay et al. (1991) and Robison et al. (2018) reported *Sarcocystis montanaensis* and a *Choleoeimeria* sp., from *A. contortrix* from Arkansas and Oklahoma, respectively. A juvenile eastern copperhead (400 mm SVL) collected on 25 June 2019 from Hochatown, McCurtain County (34°09'55.152"N, 94°45'35.8776"W) was found to be passing sporulated oocysts and sporocysts (Fig. 4C) of a unknown *Sarcocystis* sp. (HWML 216105). Unfortunately, there were not enough sporocysts to attempt to establish experimental infections in rodents and therefore, a description of a new or previously known species using microscopy alone is not possible. This represents the third time *Sarcocystis* sp. has been reported from copperheads as well as the third species of snake (McAllister et al. 2013) harboring this coccidian genus in Oklahoma.

Aves: Passeriformes: Turdidae

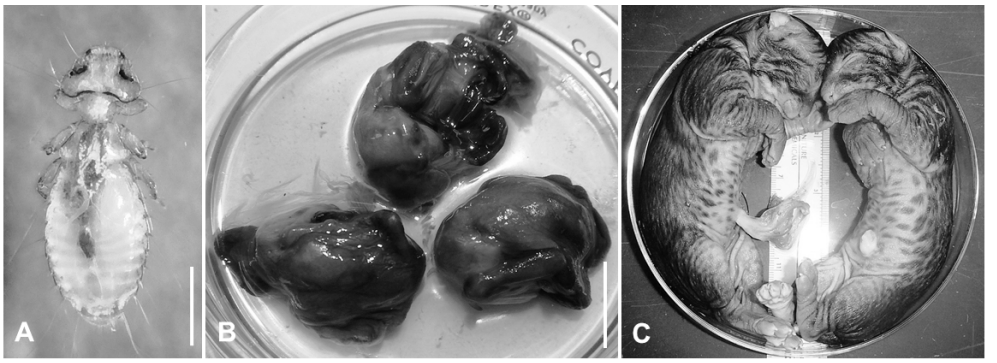
***Hylocichla mustelina* Baird, 1864 – Wood Thrush.** An adult *H. mustelina* found DOR on 14 May 2019 in Hochatown, McCurtain County (34°09'55.152"N, 94°45'35.8776"W) was found to be infested with one male, six females, and 11 nymphs of *M. eurysternus* (L3832) (Fig. 5A). *Menacanthus eurysternus* feeds on host blood obtained by piercing the quill of pin feathers and by gnawing through the epidermis (Agarwal 1983). Therefore, this louse could potentially be harmful to populations of wood thrushes. The host list for *M. eurysternus* is extensive, and includes at least 20 families, 70 genera, and 118 species (Price 1975). The wood thrush is included as a host by Price (1975) but; unfortunately, he did not specify the host locality (state) beyond USA. In addition, the louse was not listed by Emerson (1940) as occurring in Oklahoma. We document *M. eurysternus* for the first time in Oklahoma.

Mammalia: Chiroptera: Vespertilionidae

***Lasiurus borealis* Müller, 1776 – Eastern Red Bat.** A pregnant female *L. borealis* collected DOR on 1 June 2019 from off St. Hwy. 152 at Cerrogordo, McCurtain County (35°17'27.276'' N, 98°43'0.7284'' W) contained three fully-developed embryos (Fig. 5B) with crown-rump lengths of ca. 30 mm. Caire et al. (1989) and Ammerman et al. (2012) noted that *L. borealis* is one of the few bats that regularly give birth to more than two young (since they possess four teats), which enables the females to successfully raise three or four young. We document the first report of the young of *L. borealis* from



Figures 4A–B. Coccidians from snakes. (A) *Sarcocystis* sp. from *Lampropeltis calligaster calligaster*. (B) *Caryospora lampropeltis* from *L. c. calligaster*. (C) *Sarcocystis* sp. from *Agkistrodon contortrix*. Scale bars = 10 µm.



Figures 5A–C. Bird louse and mammal reproduction. (A) *Menacanthus eurysternus* female. Scale bar = 500 µm. (B) Three embryos of *Lasiurus borealis*. Scale bar = 15 mm. (C) Two fetuses of *Lynx rufus*. Note ruler scale.

Oklahoma.

Carnivora: Felidae

***Lynx rufus* Schreber, 1777 – Bobcat.** A pregnant female *L. rufus* collected DOR on 16 April 2019 in Idabel, McCurtain County (33°53'37.41''N, 94°51'09.8352'' W) was found to contain two fetuses with crown-rump lengths of 135 and 145 mm (Fig. 5C). Given a gestation period of about 50 days, these young were near birth since their parents probably mated in January or February (Caire et al. 1989). In an unpublished thesis, Rolley (1983) reported that bobcats in Oklahoma give birth to two to four young with a mean *in utero* litter size of 2.25 for yearlings and 2.66 for adults. We document the first published report on an aspect of reproduction in a *L. rufus* from the state.

In summary, we provide additional information on the biology of five invertebrates and 10 vertebrates from Oklahoma. We suggest that future novel observations should be documented to help further our knowledge of the natural history and ecology of biota of the state.

Acknowledgments

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Potential to Improve Growth of Bluegills Using Supplemental Feeding

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Abstract: Bluegills are a popular sport fish in many parts of the United States, but some populations demonstrate a reduced size structure. Numerous attempts have been made by natural resource agencies to improve bluegill size structure through management manipulations, but these attempts have had variable success. Supplemental feeding has been used to improve growth of bluegills in small impoundments. In May 2019, large bluegills were observed in a hatchery pond at the United States Fish and Wildlife Service, Tishomingo National Fish Hatchery. These bluegills may have benefited from pelletized feed that was applied to the pond to provide additional forage for brood stock alligator gar and largemouth bass. The goal of this study is to evaluate the potential for supplemental feeding to improve growth of bluegills in Oklahoma. Bluegill growth, in both length and weight metrics, was superior to growth of bluegills from five quality populations in Oklahoma. These results suggest that supplemental feeding can result in growth of bluegills to large sizes, but further research is needed to determine if supplemental feeding can produce similar growth affects in larger, natural environments.

Introduction

Bluegill *Lepomis macrochirus* is a ubiquitous sunfish species (Centrarchidae) found in most aquatic systems across the United States. In many parts of the country, bluegill populations create some of the most popular recreational fisheries (U.S. Fish and Wildlife Service and Bureau of the Census 2011). However, some bluegill populations can demonstrate reduced size structure (Drake et al. 1997). Causes of stunted bluegill growth include overharvest (Coble 1988, Drake et al. 1997, Rypel 2015), male social dynamics (Drake et al. 1997, Jennings et al. 1997, Aday et al. 2006, Peterson et al. 2010), insufficient predator populations (Guy and Willis 1990), and prey resource availability (Berger 1982, Aday et al. 2006).

Bluegill populations with reduced size structure (many fish < 152 mm TL) are undesirable to recreational anglers, as they are considered too small to harvest (Paukert et al. 2002). Fisheries management efforts to create quality bluegill populations or reclaim stunted populations have typically relied on predator introductions to control numbers and recruitment of small bluegills (Otis et al. 1998, Schneider and Lockwood 2002) and harvest regulations (bag or length limits; Ott et al. 2001, Paukert et al. 2002, Sammons et al. 2006, Rypel 2015). However, attempts to create high quality sunfish angling opportunities through these management manipulations have produced variable results (Beard et al. 1997, Sammons et al. 2006).

Supplemental feeding has been used successfully to improve growth of bluegills in small impoundments (Berger 1982, Woodard et al. 2013, Henderson et al. 2019). In spring 2019, Oklahoma Department of Wildlife Conservation

(ODWC) personnel observed large bluegills in a hatchery pond at the United States Fish and Wildlife Service (USFWS) Tishomingo National Fish Hatchery (TNFH) while collecting alligator gar brood stock. Bluegill growth may have benefited from pelletized food that was applied to the pond, therefore growth of bluegills collected from the hatchery pond will be described. Further, growth of these bluegills will be compared to bluegill growth from five high quality sunfish populations in Oklahoma (Porta 2019). This case study will evaluate the potential for supplemental feeding to improve growth of bluegills in Oklahoma.

Methods

Bluegill were raised in a 0.4 ha earthen pond at TNFH, Tishomingo, Oklahoma. The intended purpose of this pond was to hold adult alligator gar *Atractosteus spatula* that are used for annual hatchery production. Besides bluegill and alligator gar, the predominant fish species in the pond included largemouth bass *Micropterus salmoides* and redear sunfish *Lepomis microlophus*. Bluegills were stocked into the hatchery pond to provide forage for the largemouth bass, and largemouth bass served as forage for alligator gar. Additionally, alligator gar and largemouth bass were provided 2.72 kg of commercial fish feed (6.4 mm pellets) twice weekly (equivalent to 1.93 kg ha/day; R. Simmons, USFWS, personal communication).

Bluegills were collected from the hatchery pond using a bag seine (12.2 m L x 1.8 m H with 6 mm mesh) in May 2019. Bluegills were collected from five Oklahoma small impoundments (Elmer Lake, New Spiro Lake, Pawhuska Lake, Sparks Lake, and Stilwell City Lake) during April-May 2019 using boat electrofishing to sample the entire perimeter of each lake. Once captured, bluegills were measured for total length (TL; mm). We attempted to collect a sample of ten fish per 10 mm length group for age and growth purposes, to ensure that all size and age classes were represented in the sample. All fish collected for age assessment purposes were kept on ice until they were returned to the ODWC Oklahoma Fishery Research Laboratory, Norman, Oklahoma.

In the laboratory, each fish was measured for TL and weight (g), sex identified, and sagittal otoliths were removed for age estimation. Otoliths were prepared for age estimation using methods similar to Maceina (1988). Otoliths were viewed in random order by two independent readers and an age estimate was assigned to each fish (Hoff et al. 1997). When there was a disagreement on an estimated age, a concert reading was conducted by both readers and a final age estimate was determined.

The length frequency was graphed to visualize the size structure of hatchery pond bluegills. Mean length and standard deviation of bluegill in each age class was calculated. Growth of bluegills from the hatchery was described using von Bertalanffy growth models. Growth of bluegills from the TNFH pond was plotted against growth curves from the five quality sunfish populations (Porta 2019) to compare growth patterns.

Results and Discussion

A total of 163 bluegills ranging 56-258 mm TL and weighing 2-470 g were collected from the TNFH pond (Figure 1). A total of 654 bluegills ranging 27-213 mm TL and weighing 0.1-246 g collected from five wild populations were used for comparison. Bluegills exposed to supplemental food in the TNFH pond attained a larger length (L_∞ = 349 mm TL) and weight (W_∞ = 406 g) than wild fish from five quality sunfish lakes (L_∞ = 179-227 mm TL, W_∞ = 118-216 g, Figure 2; Porta 2019). Differences in length and weight between bluegills from the TNFH pond and those from five quality bluegill populations in Oklahoma suggests that supplemental feeding can result in improved growth of bluegills in small aquatic systems, which has been observed in previous supplemental feeding evaluations (Berger 1982, Woodard et al. 2013, Henderson et al. 2019).

The intended purpose of applying supplemental feed to the TNFH pond was to provide additional forage for adult alligator gar

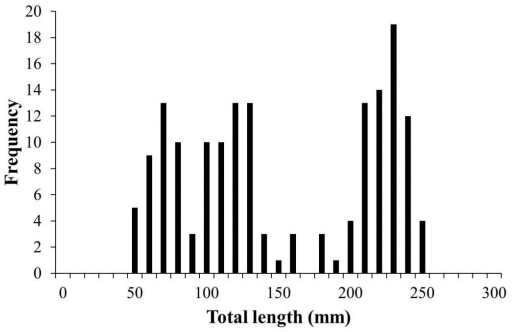

Figure 1. Length frequency diagram for bluegills collected from a pond at Tishomingo National Fish Hatchery during May 2019.

brood stock and largemouth bass (also serving as forage for the alligator gar) that were held in this pond. However, it appears that bluegills were able to take advantage of supplemental feeding, particularly when they reached 3 years old (Figures 2 and 3). Variability in length-at-age was greatest at age-3 (Figure 3), which likely resulted in the gap (140-200 mm) in the length frequency distribution. Presumably, this is when bluegill gape was large enough to begin consuming the large pellets (6.4 mm) that were applied to the pond, giving some age-3 fish a growth advantage. This suggests that earlier growth benefits of bluegills could occur if they were provided feed of a smaller pellet size. Bluegills were provided a smaller pellet size (3 mm) in previous supplemental feeding evaluations where positive growth was observed (Berger 1982, Woodard et al. 2013, Henderson et al. 2019).

Positive bluegill growth in this study was observed when applying a feeding rate of 1.93 kg/ha/day. Previous research suggests a feed rate of 2.7-11 kg/ha/day has a positive growth effect on bluegills (Woodard et al. 2013, Henderson et al. 2019). Although, Berger (1982) found that supplemental feeding at lower rates improved the sizes of bluegills in a Kansas reservoir (4.54 kg*6 feeders/30.4 ha = 0.89 kg/day maximum feeding rate). This study suggests that supplemental feeding can result in growth of bluegills to large sizes (> 250 mm TL; Figure 4). However, further research is necessary to determine whether supplemental feeding can result in similar growth effects of bluegills in larger, natural environments in Oklahoma.

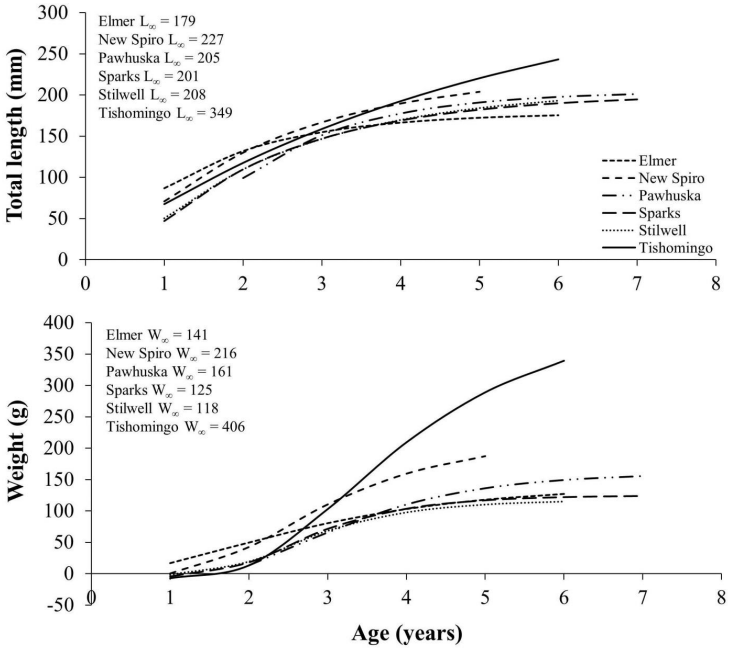

Figure 2. von Bertalanffy growth curves comparing growth of bluegills (by length [top] and weight [bottom] collected from a hatchery pond at Tishomingo National Fish Hatchery (TNFH) to growth of bluegills collected from five Oklahoma small impoundments (Porta 2019).

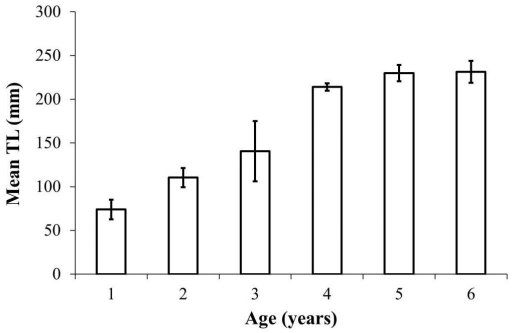

Figure 3. Mean length-at-age of bluegills collected from a pond at Tishomingo National Fish Hatchery during May 2019. Error bars represent the standard deviation of the mean.

Figure 4. Photograph comparing a bluegill captured from the pond at TNFH (246 mm TL, 364 g) to a typical quality-sized bluegill from the five small impoundments (180 mm TL, 110 g).

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Seven Novel Hemiptera (Miridae; Pentatomidae; Reduviidae; Rhyparochromidae) Records from Southeastern Oklahoma

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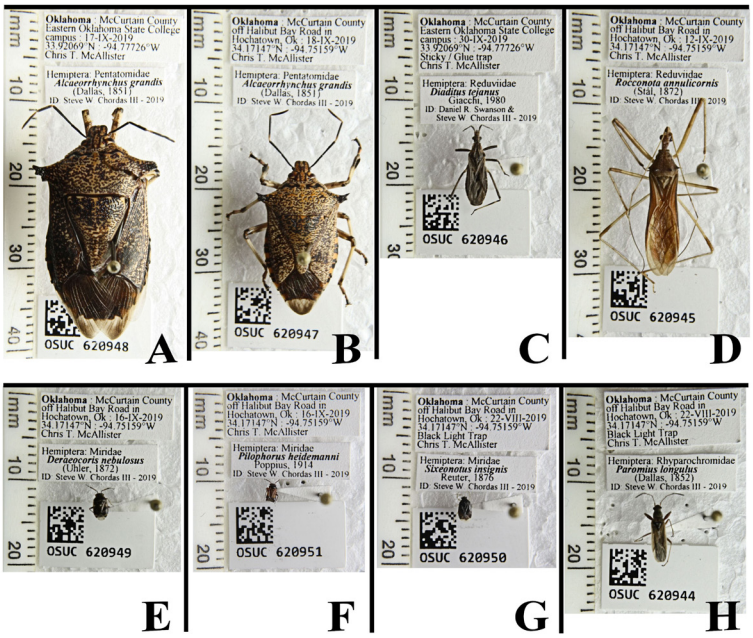
Abstract: Our knowledge of the true bugs (Hemiptera) of Oklahoma has grown over the last decade. Several reports from our lab have provided new records of hemipterans in the state for the first time. Here, we continue our efforts by providing seven new state records for species of hemipterans within the families Miridae, Pentatomidae, Reduviidae, and Rhyparochromidae from McCurtain County.

Introduction

Over the last decade, several new true bug (Hemiptera) records have been reported for Oklahoma; including six species reported by the authors for the first time (see refs in Chordas and McAllister 2018). Further, over the same period, Henry et al. (2010) reported *Corixidea major* from Latimer County, Swanson (2011) documented the assassin bug, *Empicoris orthoneuron* from Marshall County, and Henry and Sweet (2015) provided a description of a new species of chinch bug, *Wheelerodemus muhlenbergiae*, from the Arbuckle Mountains of Oklahoma. Here, we continue to provide new distributional records for seven true bugs within four families in the state.

Methods

Between May and September 2019, various true bugs were collected at two localities in McCurtain County with an insect aspirator under a porch light or from black light pan traps at a residence in Hochatown (34° 10' 17.0286"N, 94° 45' 5.7414"W) and with Trapper® Max glue traps (Bell Laboratories, Inc., Madison, WI) at the Eastern Oklahoma State College Campus (EOSC), Idabel (33° 55' 16.3272''N, 94° 46' 41.43''W). Habitat of the area consisted of various hardwoods (*Carya* and *Quercus* spp.) and pines (*Pinus* spp.) in Ouachita uplands. Specimens were placed in individual vials containing 70% (v/v) ethanol and forwarded to the senior author for laboratory identification. Blatchley (1926), Knight (1941), Schuh and Schwartz (1988), and Blinn (2009) were consulted for species identifications. Henry and Wheeler (1988), Schuh and Schwartz (1988), Snodgrass (1991), Maw et al. (2000), Boyd et al. (2002), Henry et al. (2005), Chordas et al. (2011), Swanson (2011), Packauskas (2012), and Sites et al. (2012) were used as literature distributional references. Voucher specimens (Figs. 1A–H) were deposited in the C. A. Triplehorn Collection at The Ohio State University, Columbus, Ohio. Image H (Fig. 2) was created via stacking digital photographs (using CombineZP) of the curated voucher specimen captured with a Cannon EOS DLSR through an Olympus SZ60 dissecting microscope processed with Corel PaintShopPro 2020 (Corel Corporation 2019a). Maps of literature records (Figs. 2 A–G) were created with CorelDraw 2019 (Corel Corporation 2019b) and all other images (Fig. 1) were captured using a10× close-



Figures 1A–H. Museum vouchers of new Oklahoma Hemiptera state records. Contents of each image top to bottom: location label, identification label, voucher specimen, unique museum number and code: (A) *Alcaeorrhynchus grandis* female; (B) *Alcaeorrhynchus grandis* male; (C) *Diaditus tejanus*; (D) *Rocconota annulicornis*; (E) *Deraeocoris nebulosus*; (F) *Pilophorus heidemanni*; (G) *Sixeonotus insignis*; (H) *Paromius longulus*. Scale in millimeters (mm) on left side of each image.

up lens attachment on a Cannon EOS DLSR.

Results and Discussion

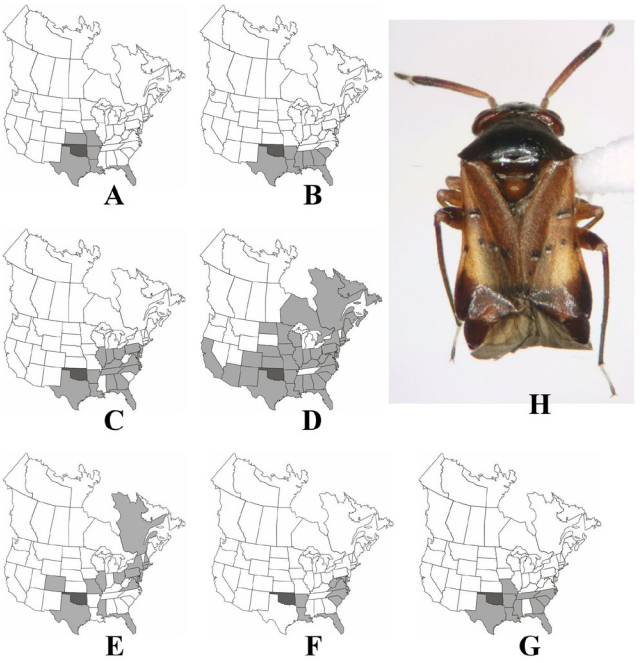
The following seven species, all collected in a four month period in 2019, representing new Oklahoma records, were identified. Species are listed alphabetically by family then by subfamily within family below in an annotated format.

Hemiptera: Miridae: Bryocorinae

***Sixeonotus insignis* Reuter, 1876.** – Several *Sixeonotus* specimens were encountered; however, most had missing parts or were damaged hindering an accurate identification. However, one specimen taken in a black light trap on 22-VIII-2019 was intact and we report it here (see data label, Fig. 1G; unique museum code = OSUC 620950). We suspect the other *Sixeonotus* specimens were also this species. It has been recorded from the middle and eastern portions of North America (Fig. 2E). However, this mirid had not been previously documented from Oklahoma.

Deraeocorinae

***Deraeocoris nebulosus* (Uhler, 1872).** – A single specimen was taken on 16-IX-2019 (see data label, Figure 1E: unique museum code = OSUC 620949). It is found in southern and eastern Canada and is widespread in the United States (Henry and Wheeler 1988) (Fig. 2D). This plant bug is also common in the eastern states (Knight 1941), and has been reported in every state surrounding Oklahoma (Fig. 2D). However, it had not previously been documented in the refereed literature for Oklahoma; thus, it our record fills a distributional gap. Adults of *D. nebulosus* are about 3.5 to 4.0 mm long and 1.8 to 2.0 mm wide and possess an ovate, shiny, dark, olive body with pale markings. Wheeler et al. (1975) provided a critical review of the literature of *D. nebulosus* and summarized the various host (many common pests) and habitat associations (more than 75 species of ornamental trees and shrubs) of this well-known predator on plant-



Figures 2A–H. Species distribution maps (A–G; north of México) of new Oklahoma Hemiptera state records: (A) *Alcaeorrhynchus grandis*; (B) *Diaditus tejanus*; (C) *Rocconota annulicornis*; (D) *Deraeocoris nebulosus*; (E) *Sixeonotus insignis*; (F) *Pilophorus heidemanni*; (G) *Paromius longulus*. Light shade = prior literature record, dark shade = new Oklahoma record. (H) Dorsal view of *Pilophorus heidemanni* voucher specimen (unique museum code = OSUC 620951); specimen is ca. 2 mm long.

feeding insects; whiteflies (Aleyrodidae) and other sessile hemipterans (aphids, scale insects) were prominently mentioned. In addition to those insects, it is a generalist predator of mites (Wheeler et al. 1975; Jones and Snodgrass 1998). In Mississippi, *D. nebulosus* has been observed in commercial cotton (*Gossypium*) fields in association with aphids, even when fields were sprayed with heavy insecticide use (Westigard 1973; Snodgrass 1991) and, more recently, has been associated with whitefly infestations in cotton. Boyd et al. (2002) studied digestive enzymes and stylet morphology from a South Carolina population.

Phylinae

***Pilophorus heidemanni* Poppius, 1914. –** A single specimen was encountered on 16-IX-2019 (see data label, Fig. 1F: unique museum code= OSUC 620951). We include an image of the curated museum specimen of this stunning little uncommon species (Fig. 2H). Previously recorded for neighboring Arkansas (Schuh and Schwartz 1988), a record Chordas (2017) overlooked in the recent Arkansas list, this Oklahoma record constitutes a slight western range extension (Fig. 2F). The species is dark-colored and about 3.0 mm long with a slightly constricted mid-body; its middle region is often brightly colored orange with an anterior small black stripe and four posterior black disconnected and disjointed dots topped with white markings on each wing (Schuh and Schwartz 1988) (Fig. 2H). Schuh and Schwartz (1988, see p. 119) provided an excellent illustration of this species and a superb image of it from Virginia that was posted on iNaturalist (https://www.inaturalist.org/) by pbedell.

Pentatomidae: Asopinae

***Alcaeorrhynchus grandis* (Dallas, 1851). –** At two separate locations, only a single day apart (17 and 18-IX-2019), a male and a female of this species were encountered (see data label, Fig. 1A = female and Fig. 1B = male; unique

museum codes = OSUC 620947 [male] and OSUC 620948 [female]). This is one of the larger stink bug species and both sexes have unique bifid humeral projections (Figs. 1A–B). Having been reported for all states to the north, south and east of Oklahoma, this new record fills a gap in the species' distribution (Fig. 2A). It has been previously reported from Arkansas, Florida, Kansas, Louisiana, Missouri and Texas (Barton and Lee 1981; Packauskas 2012; Sites et al. 2012). The giant strong-nosed stink bug is a very large (females up to 25 mm, males up to 21 mm) predator which occurs in several row crops and preys on other insects, especially lepidopteran caterpillars.

Reduviidae: Stenopodainae

***Diaditus tejanus* Giacchi, 1980.** – A single specimen of this assassin bug was taken in a glue trap on the campus of EOSC on 30-IX-2019 (see data label, Fig. 1D; unique museum code = OSUC 620946). This species was previously thought to be limited to the coastal plain of the southeastern United States, but Swanson (2011) showed this was not the case with many inland records of the bug. It is distributed mainly in the south and southeast, but also occurs north to Arkansas and now in Oklahoma (Fig. 2B).

Harpactorinae

***Rocconota annulicornis* (Stål, 1872).** – A single specimen of this assassin bug was taken on 12 IX 2019 in Hochatown (see data label, Fig. 1D; unique museum code= OSUC 620945). Widespread throughout the eastern portion of the United States and the bordering states of Arkansas and Texas (Fig. 2C), this Oklahoma record was not unexpected and helps fill a gap in the western portion of the species' distribution. The ringed-horn assassin bug is a relatively small (16–20 mm) stout, elongate-oval, reddish to yellowish, member of the family with four prominent spines on pronotum (Fig. 1D).

Rhyparochromidae: Rhyparochrominae

***Paromius longulus* (Dallas, 1852).** – This dirt-colored seed bug is distributed mainly in the mid, south and southeastern United States (Fig. 2G). Having been previously reported from both Texas and Arkansas (Chordas et al. 2011), it was not unexpected that this hemipteran would be found in Oklahoma (Fig. 2G). We encountered a single specimen in a black light trap on 22-VIII-2019 (see data label, Fig. 1H; unique museum code= OSUC 620944).

During the same four-month period, 15 other hemipterans within seven families were collected and all have been reported previously from Oklahoma including the following taxa (descriptor's omitted): **Alydidae:** *Megalotomus quinquespinosus*; **Berytidae:** *Jalysus spinosus*; *Metacanthus multispinus*; **Cydnidae:** *Pangaeus bilineatus*; **Miridae:** *Lygus lineolaris*; *Pilphorus crassipes*; **Reduviidae:** *Repipta taurus*; *Zelus luridus*; *Zelus tetracanthus*; **Rhopalidae:** *Arhyssus lateralis*; *Harmostes reflexulus*; **Rhyparochromidae:** *Antillocoris pilosulus*; *Pseudopachybrachius basalis*; *Pseudopachybrachius vinctus*; *Ptochiomera nodosa*.

Additional collections of hemipterans in the state should be conducted including the search for several uncommon reduviids (assassin bugs) recently reported from adjacent Arkansas (Chordas and Tumlison 2016) that would eventually become new distributional records for Oklahoma as well as other families of hemipterans that could lead to additional novel records. More surveys of aquatic taxa should be conducted as several that occur in surrounding states should also be found in watersheds of Oklahoma.

Acknowledgments

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Two New Distributional Records for Caddisflies (Trichoptera: Philoptamidae, Helicopsychidae) in Eastern Oklahoma

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Abstract: Our knowledge of the caddisflies (Trichoptera) of Oklahoma is in its early stages. Previous reports have provided some initial foundational information but there remains a need for additional surveys of this state's caddisfly biota. Here, we provide new state records for two species of caddisflies from Robber's Cave State Park, Latimer County. Our purpose is to help fill gaps in our limited biological knowledge of this fauna that should help in future surveys and observations conducted in the state.

Introduction

Caddisflies (Trichoptera) are an integral part of the biota of many regions of North America (see Holzenthal et al. 2007) and those that occur in Oklahoma are no exception. However, up to 1970, only about 50 species had been reported from the state, most by H. H. Ross (Ross 1938a, b, c, 1941). Since then, four studies have increased our knowledge of this group of Oklahoma insects, including Resh et al. (1978) from Lake Texoma, Bowles and Mathis (1992) with emphasis from the mountainous regions of the state, Moulton and Stewart (1996) from the Interior Highlands, and Zuellig et al. (2006) from Fort Sill. In toto, these studies brought to about 165 species within 16 families of caddisflies known from Oklahoma. Here, we document two species of caddisflies in the state for the first time.

Methods

On 8 August 2019, five blacklight (15 watt long-wave fluorescent bulb) traps were set along the banks of Fourche Maline Creek in Robber's Cave State Park, Latimer County (35° 00' 17.5206"N, 95° 20' 05.6934"W). The site is dominated by oak-pine-hickory woodlands of the Sans Bois Mountains. Traps were set at sunset and picked up about one hr later. Specimens were placed in containers of 70% (v/v) ethanol and shipped to BCK. Identifications were confirmed using Ross (1944), Morse (1975), Lago and Harris (1987), Moulton and Stewart (1996), and Johanson (2002). Voucher specimens were deposited in the C. P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado.

Results

At least nine species of caddisflies were

collected including two males and two females of *Chimarra obscura* (Walker, 1852), one male of *C. parasocia* Lago and Harris, 1987, one male of *Helicopsyche mexicana* Banks, 1901, two males and nine females of *Hydropsyche arinale* Ross, 1938, a female of *Cheumatopsyche* sp., two males of *Macrosternum carolina* (Banks, 1909), two males and one female of *Ceraclea transversa* (Hagen, 1861), two females of *Setodes* sp., and four females of *Triaenodes* sp. All of these have been reported previously from Oklahoma, except *C. parasocia* and *H. mexicana*. The two new state records described herein are reported below in an annotated format as follows.

Arthropoda: Insecta: Trichoptera: Philoptamidae

***Chimarra parasocia* Lago and Harris, 1987 (Little Black Sedge) –** A single male specimen was collected. The male terminalia is identical to original illustrations provided by Lago and Harris (1987) and expert identified voucher material at the C. P. Gillette Museum of Arthropod Diversity. This is primarily a southeastern species but regionally reported from Arkansas (Lago and Harris 1987; Bowles and Mathis 1989; Moulton and Stewart 1996; Cooper and Morse 1998; Etnier 2010), Missouri (Lago and Harris 1987; Armitage 1991; Mathis and Bowles 1992; Moulton and Stewart 1996; Cooper and Morse 1998), and Texas (Bowles et al. 1993; Abbott et al. 1997; Moulton and Stewart 1997; Cooper and Morse 1998).

Helicopsychidae

***Helicopsyche mexicana* Banks, 1901 (Snail-Case Caddisfly) –** a single male specimen was collected. The male terminalia of this specimen is identical to the concept presented by Johanson (2002). Regionally, this species has been reported from New Mexico by Johanson (2002) and Texas by Ross (1944), Edwards (1973), Wiggins (1996), and Meyerhoff and Lind (1987), and Moulton and Stewart (1997).

Discussion

Previous studies on the caddisfly fauna of Oklahoma include an early study by Resh et al. (1978) at Lake Texoma near the University of Oklahoma Biological Station, Marshall County. They reported 26 species of caddisflies within six families. Bowles and Mathis (1992) reported 145 species within 15 families from 26 counties of the state. Moulton and Stewart (1996) documented caddisflies from the Interior Highlands (Ozarks and Ouachitas) of eastern Oklahoma and listed 146 species. The most recent survey was conducted by Zuellig et al. (2006) at Fort Sill Military Reservation in Comanche County in southwestern Oklahoma. In all, these surveys have brought the total number of caddisflies of the state to about 165 species within 16 families.

Bowles and Mathis (1992) reported 16 species of caddisflies from two sites (their sites 25–26) within Robber's Cave State Park. Of these, we did not share similar collections except for the possibility that two females of *Cheumatopsyche* and 73 females of *Hydroptila* could represent *C. analis* (Banks, 1903) and *H. grandiosa* Ross, 1938, respectively, that was collected in the park by Bowles and Mathis (1992).

In summary, we provide new state records for two species of caddisflies from Latimer County, Oklahoma, that brings to about 167, the number known from the state. Because the state contains nine major physiognomic regions, several areas are quite diverse climatologically, vegetatively, and topographically (Caire et al. 1998). Therefore, we suggest that additional surveys should be conducted in various parts of the state where little collecting has been done which should result in additional new geographic records for caddisflies.

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New Ectoparasite (Diptera; Phthiraptera) and Helminth (Trematoda; Cestoda; Nematoda) Geographic Records from Three Species of Owls (Strigiformes) in Southeastern Oklahoma

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Abstract: We are just now beginning to learn about the ectoparasites and helminth parasites of some owls of Oklahoma. Some recent contributions from our lab have attempted to help fill a previous void in that information. Here, we report, four taxa of ectoparasites and five helminth parasites from three species of owls in Oklahoma. They include two species of chewing lice (*Strigiphilus syrnii* and *Kurodeia magna*), two species of hippoboscid flies (*Icosta americana* and *Ornithoica vicina*), a trematode (*Strigea elegans*) and a cestode (*Paruterina candelabraria*) from barred owls (*Strix varia*), and three nematodes, *Porrocaecum depressum* from an eastern screech owl (*Megascops asio*), *Capillaria* sp. eggs from *S. varia*, and *Capillaria tenuissima* from a great horned owl (*Bubo virginianus*). With the exception of *Capillaria* sp. eggs and *I. americana*, all represent new state records for Oklahoma and extend our knowledge of the parasitic biota of owls of the state.

Introduction

Over 455 species of birds have been reported from Oklahoma and several are species of raptors or birds of prey that make up an important portion of the avian fauna of the state (Sutton 1967; Baumgartner and Baumgartner 1992). However, until recently, little was known about their parasites. Over the last few years, novel information on the parasites of raptors has been gained by our research group from examination of salvaged road-killed specimens (McAllister et al. 2017, 2018, 2019a, b). Here, we continue to opportunistically examine raptors from the state and document new geographic records for their parasites in Oklahoma.

Methods

Between January 2018 and September 2019, three owls were found dead on the road in McCurtain County, including an eastern screech owl (*Megascops asio*) collected on 22 January 2018 from Smithville (34° 28’ 0.4794”N, 94° 38’ 37.6794”W), a barred owl (*Strix varia*) collected on 1 February 2019 from Hochatown at the jct. of US 259/259A (34° 06’ 52.506’’N, 94° 44’ 23.28’’W), and another *S. varia* collected

on 21 September 2019 from Holly Creek (33° 58' 40.494'' N, 94° 49' 03.8892'' W). These specimens appeared to be recently killed and showed no sign of putrefaction. In addition, an injured great horned owl (*Bubo virginianus*) was a captive specimen in rehab at the Hochatown Rescue Center (34° 08' 22.074''N, 94° 44' 47.328''W) and, after four days in captivity, died on 26 August 2019 and was subsequently donated to CTM. All were placed in individual plastic bags on ice and immediately brought to the laboratory at Eastern Oklahoma State College (EOSC) for parasitic examination. Their feathers were vigorously brushed over a white enamel tray to observe ectoparasites and those found were placed in individual vials of 70% (v/v) ethanol; selected specimens were cleared in 10% potassium hydroxide, dehydrated through an ethanol series, further cleared in xylene, and slide-mounted in Canada balsam (Price et al. 2003). Hippoboscidae were passed though 100% acetone for 24 hr, air dried, and point mounted. The specimens were identified using the keys by Maa (1966, 1969a). A midventral incision was made of each owl from the cloaca to throat to expose the viscera and the gastrointestinal tract and associated organs were placed in individual Petri dishes containing 0.9% saline. Contents were examined at 20 to 30× under a stereomicroscope and parasites found were rinsed of mucus. Feces from the rectum from each owl was collected and placed in individual vials containing 2.5% (w/v) potassium dichromate ($K_2Cr_2O_7$) and, after flotation in Sheather's sugar solution (sp. gr. 1.30), examined for coccidians and parasite ova by brightfield microscopy. Trematodes were fixed without coverslip pressure in near boiling water and transferred to 95% (v/v) molecular grade ethanol. Cestodes were detached from the host's intestine, gently rinsed in 0.9% saline, and fixed in hot 4% formaldehyde solution (formalin) with subsequent transfer to 95% molecular grade ethanol. Both were stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were fixed in near boiling water and preserved in 70% (v/v) ethanol. They were later cleared and identified in temporary mounts of lacto-phenol and then returned to the preservative.

Hosts were deposited as photovouchers and/or housed in the EOSC collection, Idabel, Oklahoma. Voucher specimens of parasites (except those retained for further work) were deposited as follows: (1) helminths in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska; (2) specimens of Phthiraptera in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia, under individual accession numbers; and (3) Hippoboscidae in the C. P. Gillette Museum of Arthropod Diversity, Colorado State University, Fort Collins, Colorado. Common and scientific names of owls follow König and Wieck (2008).

Results and Discussion

A single owl each was infested with two species of lice and two dipterans, and all four owls were infected with various helminths. Nine taxa, including two species of chewing lice, one trematode, one cestode, and two nematodes were collected as well as nematode eggs and two dipterans; no coccidians or acanthocephalans were found. All parasites, except for *Capillaria* sp. ova and *I. americana*, are reported from Oklahoma for the first time. The parasite species recovered are presented below in annotated format.

Trematoda: Strigeidae

***Strigea elegans* Chandler and Rausch, 1947.** – Several specimens were taken from the intestinal tract of *S. varia* from the Hochatown site. The life cycle is a four-host obligatory one that involves snails as first intermediate hosts, anurans (bufonid and ranid tadpoles) as second intermediate hosts, watersnakes and ducks as third intermediate hosts (with tetracotyles), and owls as final hosts (Pearson 1959; Miller et al. 1965). Kinsella et al. (2001) previously reported *S. elegans* from *S. varia* and *B. virginianus* from Florida, and McAllister et al. (2019a) reported it from *B. virginianus* from Arkansas. Specimens are being retained for further work.

Cestoda: Cyclophyllidea: Parauterinidae

***Paruterina candelabraria* (Goeze, 1872) Fuhrman, 1906. –** Several *P. candelabraria* (HWML 216091) were found in the intestine of *S. varia* from the same site above. One of the major characteristics defining the family is the presence of a single paruterine organ and our specimens clearly possessed this structure (Figs. 1A–B). In North America, larval stages of *P. candelabraria* have been reported from shrews, deer mice, voles and squirrels (Freeman 1957; Baron 1971; Kinsella 2007), and possibly bats (de Souza 2019), which are regularly eaten by birds of prey (Johnsgard 1990). This tapeworm genus is restricted to a group of three species parasitic in owls; *P. candelabraria* has a Holarctic distribution (Europe, North Asia and North America). It has also been previously reported from little owls (*Athene noctua*) from China (Guo et al. 2019), Sunda scops owl (*Otus lempiji*) from islands of the Malay Archipeligo (Iwaki et al. 2012), and tawny owls (*Strix aluco*) from Moldavia, the Ukraine (Kornyushin 1989), and Spain (Campillo et al. 1994; Sanmartin et al. 2004; Santoro et al. 2012). Hoberg et al. (1989) and Richardson and Kinsella (2010) reported the similar *Paruterina rauschi* Freeman, 1957 from northern spotted owls (*Strix occidentalis*) from Oregon and S. varia in Connecticut, respectively.

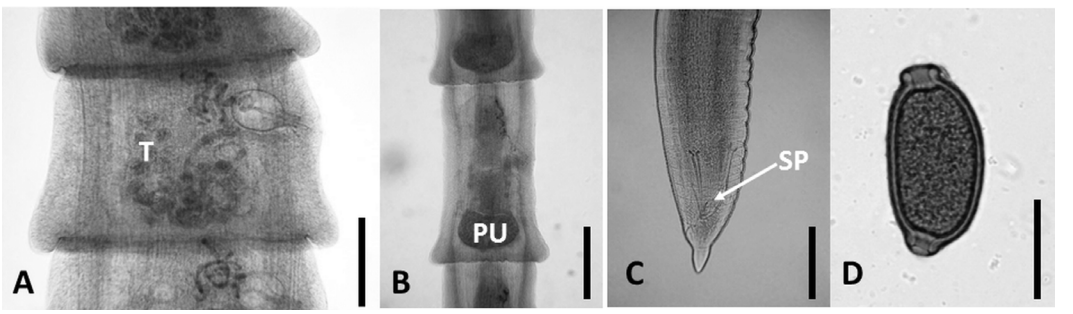
Nematoda: Ascaridida: Ascaridae

***Porrocaecum depressum* (Zeder, 1800).** – Nematodes from the intestinal tract of a *M. asio* were identified as *P. depressum* (Fig. 1C) based on the length of the esophagus and length of the spicules (425 µm). Previous reports of *P. depressum* from owls include *S. varia* from Florida (Kinsella et al. 2001) and Louisiana (Nadler and Hudspeth 1998), long-eared owl (*Asio otus*) and Eurasian eagle-owl (*Bubo bubo*) from Czech Republic (Sitko 1994), *B. virginianus* from Florida and Alberta and Manitoba, Canada (Wong et al. 1990; Kinsella et al. 2001), and northern spotted owl (*Strix occidentalis*) from Oregon (Hoberg et al. 1989).

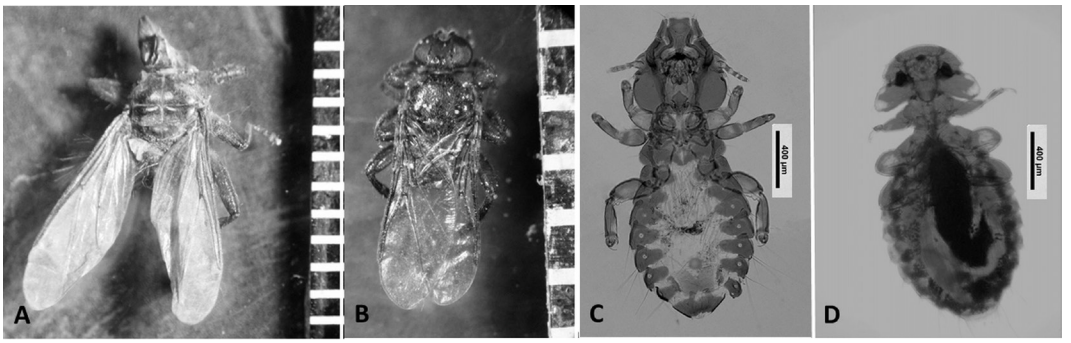
Trichinellidae

***Capillaria* sp. (ova)** – More than 250 *Capillaria* species have been reported from fish, amphibians, reptiles, birds, and mammals (Cross 1992). Ova of a *Capillaria* sp. (Fig. 1D) were recovered from the feces of *S. varia* from the Hochatown site. Unfortunately, it is not possible to provide a specific identity of these eggs. Kinsella et al. (2001) reported *Capillaria tenuissima* (Rudolphi, 1819) from *S. varia* from Florida. This is the second time *Capillaria* sp. ova has been reported in any owl from the state (McAllister et al. 2017).

***Capillaria tenuissima* (Rudolphi, 1819). –** Many specimens of this nematode were found in the small intestine of a *B. virginianus*. This nematode has been previously reported from the great horned owl from Florida (Read 1949; Ramalingam and Samuel 1978; Kinsella et al. (2001) and Connecticut (Richardson and



Figures 1A-D. Some helminth parasites from two owls from Oklahoma. (A) Photomicrograph of a mature proglottid of *Paruterina candelabraria* from *Strix varia* showing testes (T). (B) Pregravid proglottid of *P. candelabraria* showing parauterine organ (PU); scale bars = 500 µm. (C) Posterior end of male *Porrocaecum depressum* from *Megascops asio* showing spicules (SP). Scale bar = 400 µm. (D) *Capillaria* ova from *S. varia*. Scale bar = 400 µm.



Figures 2A–D. Some ectoparasites collected from *Strix varia* from Oklahoma. (A) *Icosta americana* female; note scale (1 mm intervals). (B) *Ornithoica vicina* female; note scale (1 mm intervals). (C) *Strigiphilus syrnii* female. (D) *Kurodaia magna* female.

Kinsella 2010). It has also been documented from the Megallanic horned owl (*Bubo megellanicus*) from Chile (Grandón-Ojeda 2018) and various other owls (Atkinson et al. 2008).

Insecta: Diptera: Hippoboscidae

***Icosta americana* (Leach, 1817)**. – One damaged female (Fig. 2A) was taken from *S. varia* from the Holly Creek site. Maa (1969) reports *I. americana* from across the Nearctic and parts of the Neotropical region from avians in the families Accipitridae (hawks and eagles), Phasianidae (ground-living birds), and Strigidae (owls). This species of hippoboscid is one of the most frequently collected from birds across the United States. It is one of the larger flies found on owls and since these birds are frequently studied it is well represented in most collections of hippoboscids. *Icosta americana* is also a potential vector of West Nile virus (Farajollahi et al. 2005). In addition several protistan blood parasites (*Haemoproteus* and *Trypanosoma*) are transmitted by *Icosta* spp. and other bird feeding hippoboscids (Reeves and Lloyd 2019). While not represented in published checklists from Oklahoma, there is a specimen from a bird hit by a car in Atoka County (Oklahoma) in the K. C. Emerson Entomology Museum at Oklahoma State University, Stillwater.

***Ornithoica vicina* (Walker, 1849)**. – One female (Fig. 2B) was taken off *S. varia* from the same site above. *Ornithoica vicina* is one of the smallest hippoboscids parasitizing birds in North America. It has a wide host range being found on over 80 genera and 10 orders of birds from Vancouver, Canada through Southern Chile into the Caribbean with an introduced population in Hawaii (Maa 1969b). There are no pathogens yet associated with this fly (Reeves and Lloyd 2019). Previous studies focusing on animals hit by cars have discovered *O. vicina* from *S. varia* (Nelder and Reeves 2005).

Phthiraptera: Ischnocera: Philopteridae

***Strigiphilus syrnii* (Packard, 1873).** – Lice are the most prevalent ectoparasites of raptors (Cooper 2002) and members within *Strigiphilus* represents the only genus with its taxa restricted to owl hosts (Clayton 1990). One female and two nymphs of *S. syrnii* (L3827A, Fig. 2C) were taken from *S. varia* from the Hochatown site. This louse is mainly an ectoparasite of the barred owl and it has been reported from specimens collected from Florida, Georgia, Minnesota, Pennsylvania, Virginia, Wisconsin, and British Columbia, Canada (Clayton and Price 1984). Other known hosts for *S. syrnii* are *B. virginianus*, great grey owl (*Strix nebulosa*), rufous-legged owl (*Strix rufipes*) and *S. occidentalis* from California, Connecticut, Maryland, Nebraska, Oregon, Texas, Washington (D.C.), and Québec and Saskatchewan, Canada (Clayton and Price 1984; Clayton 1990; Price et al., 2003). Emerson (1940) did not report this louse from Oklahoma, so we do here for the first time.

Amblycera: Menoponidae

***Kurodaia magna* Emerson, 1960.** – Three males, five females, and two nymphs of *K. magna* (L3827B, Fig. 2D) were removed from *S. varia* from the same site above. The species

was originally described from *S. varia* from Texas and paratypes were reported from the same host species from Alabama, Georgia, and Oregon (Emerson 1960). Other hosts include: *B. virginianus* and *S. occidentalis* from California and Oregon (Price and Beer 1963; Hunter et al. 1994; Price et al. 2003). This louse was not reported by Emerson (1940) in the state, so we report it, for the first time, from Oklahoma.

In conclusion, owls harbor a variety of ecto- and endoparasites, and the majority of them appear to be specific to raptors. Here, we document seven new distributional records for parasites of four owls from Oklahoma. We suggest that seven other species of owls that occur in the state, for which we know little or nothing about their parasites, should be examined including, northern saw-whet owl (*Aegolius acadicus*), short-eared owl (*Asio flammeus*), long-eared owl (*Asio otus*), burrowing owl (*Athene cunicularia*), snowy owl (*Bubo scandiacus*), western screech owl (*Megascops kennicottii*), and American barn owl (*Tyto furcata*). New geographic as well as the possibility of new host records would be expected when these owls are surveyed from Oklahoma.

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The Effects of Physical Activity on Salivary Stress Biomarkers in College Students

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Abstract: Physical exercise has been proven to have a positive impact on one's physical and mental health. Today's fast- paced, high tension lifestyles have led to an increase of chronic stress in individuals of society. Chronic stress is associated with many diseases and disorders such as hypertension, depression, heart attack, stroke, immune suppression, diabetes, and obesity. Exercise has been found to play a significant role in stress reduction. This research is focused on evaluation of the stress relief effect of physical exercise by measuring the levels of salivary stress markers: cortisol and α-amylase. Cortisol is a steroid hormone secreted by the adrenal gland and associated with the stress response in the human body. Levels of cortisol are increased during stimulation of the sympathetic nervous system and regulated by the hypothalamic-pituitary-adrenal axis (HPA). Salivary α-amylase is a correlate of sympathetic activity under conditions of physical or psychological stress. Levels of salivary α-amylase increase under a variety of stressful conditions in human subjects. Three groups of college students were studied: individuals who exercise regularly (active, athletes), students who exercise two to four times a week (active, non-athletes) and students who do not exercise (non-active). Quantitative measurements of cortisol and α-amylase variations were done using salivary analysis in enzyme immunoassay kits. Cortisol levels overall were reduced in individuals who exercise regularly, whereas α-amylase appeared useful in observance of various lifestyle activity levels. The implications of these findings suggest means of reducing stress by regular physical exercise, thus promoting overall health and wellbeing.

Introduction

College students experience constant psychological stress from being away from home, dealing with costs of college, often working at a job during the school year, having a heavy workload from academics, feeling pressure to obtain high grades in connection with career aspirations, their involvement in different scholar activities and other [Pariat, 2014]. Chronic stress affects the cardiovascular, immune [Gouin, 2008], nervous and endocrine [Cox, 1984] systems and may lead, among others, to diabetes [Dallman, 2010], hypertension, depression [Hammen, 2005], substance abuse, and antisocial personality disorder [Dohrenwend, 2000]. Physiological markers of stress like cortisol and α-amylase, provide an objective measure of changes in the stress response. Cortisol is the main glucocorticoid hormone in humans and is most commonly associated with stress in the human body. It is released in response to many psychosocial stimuli processed in the hypothalamic-pituitary-adrenal axis. The level of free cortisol in the blood is accurately reflected by the level of cortisol in saliva [Kirschbaum 1989, 1994]. Cortisol is secreted more slowly than other adrenal hormones such as catecholamines (like adrenaline) and remains in the body longer after initial stress. Cortisol is beneficial in times of stress because of its many regulatory functions. It aids in breaking down of glucose and fatty acids for energy, it has anti-inflammatory activity, is involved in fat storage, and immune functioning. However, high and prolonged levels of cortisol have been shown to have negative effects leading to serious medical conditions including diabetes, hypertension, depression, cancer, obesity, improper thyroid functioning,

and decreased bone density. Cortisol is well known for its role in storing of abdominal fat and its association with heart attacks, strokes, increased susceptibility to autoimmune disease and infection [Buford, 2008]. Cortisol levels vary with the circadian cycles, peaking during the first hour after awakening [Pruessner 1997] and decreasing for the rest of the day. Thus, careful choosing of the testing time is crucial as cortisol levels are dependent on the time of day. Repeated measurement of free cortisol levels within 60 minutes after awakening in the morning is considered a reliable biological marker of adrenocortical activity [Prussner 1997]. Cortisol can be measured in urine, plasma, and saliva. Salivary measures of cortisol are considered valid and reliable and provide distinct advantages including their non-invasive nature [Gozansky 2005]. Nonetheless, saliva samples can be affected, among others, by food and caffeine consumption, smoking, and timing of collection, so the protocol compliance is crucial to obtaining valid data. Increasing the validity of the results includes the standardized saliva sampling, consistent collection materials and methods, controlling the effects of food, drinks, and medications [Hanrahan 2006]. Another non-invasive biomarker that can be used to study stress in the body is α-amylase. **α-Amylase** is the most abundant enzyme found in human saliva and is responsible for breaking down carbohydrates and starch. α-Amylase is secreted in response to stimulation of the sympathetic nervous system, has been found to increase in response to a psychological stressor [Takai 2004] and may be a useful parameter for the measurement of stress. Gland secretion of α-amylase is regulated by the Autonomic Nervous System and has been shown to increase under sympathetic stimulation in conditions of stress [Nater, 2005, 2006, Shimazaki, 2008]. It was found that salivary α-amylase activity was higher in subjects suffering from chronic psychosocial stress as compared to non-stressed individuals and therefore may be used as a biomarker of chronic stress [Vineetha, 2014]. Increased α-amylase levels have been associated with aggression, impaired memory, and immune system suppression. Substantial research has been conducted on methods of lowering stress levels [Gordis, 2010, Rudolph, 2010, Ju-Yang, 2019]. Suggested methods include exercise (aerobics, yoga, strength training), meditation, listening to music, healthy diet, proper sleep as well as avoiding alcohol, caffeine, and tobacco. Physical exercise and activity are important contributors to a healthy lifestyle and have a wide range of health-related and psychological benefits including reduction of stress [Fleshner 2005]. This research examines the influence of the intensity of physical exercise on the stress level in college students. While exercise induces a rise in cortisol level initially, the long term effect of lowering overall cortisol levels may be seen leading to reduction of the negative effects of chronic stress. The levels of intensity of physical activity may be an important factor in evaluating the benefits of exercise. Surprisingly there is very little study done specifically on the potential benefits of exercise and its effects on lowering cortisol levels. If exercise could indeed be proven to affect stress biology in a positive manner, many medical implications could be made and the importance of lifestyle choices could be more greatly emphasized. The purpose of this pilot study was to examine reduction in stress measured by the levels of salivary stress biomarkers in response to different levels of physical activity.

Methods

Participants were Rogers State University college students 18 to 25 years old. Participant exclusions included: habitual smoking (tobacco), caffeine dependency, drug use, diagnosed psychological disorders such as depression, anxiety, psychosis, alcohol dependency, endocrine metabolic disorders, autoimmune disorders, severe allergies, major medical conditions. Institutional Review Board approval was obtained and a written informed consent was taken from each participant. Subjects were divided into three groups: Active-athletes group which consisted of 19 individuals who exercise regularly and are part RSU basketball and soccer teams, Active-nonathletes group which consisted of 19 students who exercise two to four times a week and Non-active group which consisted of 19 students who do

not exercise. Subjective evaluation (Perceived stress questionnaire) of the stress level of the individuals participating in the study was elicited using the PSS Scale reprinted with permission of the American Sociological Association from the Journal of Health and Social Behavior [Cohen 1983]. Saliva samples were collected from all participants at the same time of the day, within 60 minutes after awakening to minimize the effects of circadian variation. Participants were asked not to eat or drink before sample collections and refrain from physical activity the day before. Participants were also asked to wash their mouth before saliva collection and approximately 1 mL of unstimulated saliva was collected in a disposable plastic test tube and immediately stored at -20^{O}C. All samples were analyzed for the levels of cortisol and α-amylase with Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit and Salivary Alpha-Amylase Enzymatic Kit (Salimetrics LLC, State College, PA, USA). Cortisol concentrations were determined using a 4-Parameter Sigmoid Minus Curve Fit program from MyAssays.com. α-Amylase was calculated following Salimetrics kit instructions. All data were normally distributed and a single factor ANOVA was performed to detect intergroup differences. Values were considered to be statistically significantly different when $p<0.05$. Student's t-tests were computed for comparison of the means between the groups.

Results

Perceived stress questionnaire

The questionnaires completed by participants were used to evaluate perceived stress levels. The three groups were compared.

	Active-athlete	Active-nonathlete	Non-active
PSS level	17.50±6.23	12.88±6.14	16.55±7.61

Note: Data shown are means and standard deviations

It was found that the Active-athlete group had the highest scores in perceived stress, followed by the Non-active group, with the Active-nonathlete group showing the lowest scores of perceived stress. These differences however were not statistically significant (p=0.11)

Salivary stress markers analysis

	Active-athlete	Active-nonathlete	Non-active
Cortisol (μg/dL)	0.14±0.03	0.16±0.07	0.20±0.06
α-amylase (U/mL)	42.25±29.80	32.93±21.53	29.77±13.40

Note: Data shown are means and standard deviations

As shown by ANOVA analysis statistically significant difference (p=0.03) in cortisol concentrations between the three groups was observed. T-test computations showed statistically significant difference ($p<0.05$) in cortisol concentrations with lowest in the Active-athlete group (0.14 μg/dL) and the highest in the Non-active group (0.20 μg/dL). The Active-nonathlete group's mean cortisol concentration was 0.16 μg/dL.

In the α-amylase results, it was found that the enzyme activity in the saliva increased with participants increasing physical activity: Nonactive group (29.77U/mL), Active-nonathlete (32.93U/mL), Active-athlete (42.25U/mL). However, these results were not statistically significantly different (p=0.25).

Discussion

The purpose of the present pilot study was to examine reduction in stress in response to varying levels of physical activity in college students and to evaluate the usefulness of salivary cortisol and α-amylase as a biomarker of stress in chronically stressed individuals. Chronic stress, rather than acute stress, usually results in damage to the physical and mental wellbeing of an individual and may cause a number of pathologies. Individual's verbal or self-reporting questionnaires in stress evaluation usually provide inconsistent results and heavily depend on subject's mood and attitude at the time of testing. As shown here, by the results of "perceived stress questionnaire", there are no statistically significant differences in the stress level between the three groups studied. A more objective method in stress evaluation is necessary. Salivary cortisol and α-amylase provide a simple and non-invasive method in assessment of stress. In this study it was hypothesized that increased level of physical activity leads to the

significant overall decrease in amounts of stress biomarkers, cortisol and α-amylase in saliva. The findings partially support this hypothesis. Cortisol levels were statistically significantly different in all three groups studied with the lowest level in the most physically active group and the highest in the non-active group. It is well known that the salivary cortisol level increases under psychological stress [Hargreaves1990, Biondi 1990, Kirschbaum 1994], which college students are exposed to on regular basis. In the stressed individual, the cortisol secretion is elevated regardless of the time of day as stress overrides the circadian rhythm [Chaudhuri 1991]. The results presented show that salivary cortisol is a useful indicator of chronic stress and that intense physical activity leads to overall decrease in cortisol amounts in saliva. This indicates long term stress reduction and shows a great benefit of physical activity on individuals health and wellbeing. It is possible however, that the reduction in stress indicated by the cortisol data is not solely the result of physical exercise. Other aspects may play role in stress reduction, such as being in a group of similar individuals creating the atmosphere of acceptance, positive reinforcement, sense of accomplishment, and success. Nevertheless, the overall positive effect of physical activity on stress reduction is clearly shown. According to Noto [Noto 2005] the levels of salivary α-amylase change during exercise and psychological stress and increase after exposure to acute mental stress. However, the results of other studies of α-amylase reactivity to psychological stimuli have been inconsistent. Long term effect of the intensity of physical activity on the levels of salivary α-amylase was investigated in this study. It was found that the enzyme activity in saliva increased with participants increasing physical activity, the opposite effect to the one seen for cortisol, but the differences were not statistically significant. It has been suggested [Nater 2004] that α-amylase reflects the reaction of a different stress system than the Hypothalmic-pituitary-adrenal (HPA) axis which functioning and reactivity is assessed by the measurement of free cortisol in saliva. Salivary α-amylase has become established as a new biomarker of the psychosocial stress response within the sympathetic-adrenal-medullary (SAM) system. Even though SAM and HPA axis are closely intertwined, α-amylase and cortisol are not significantly correlated in the response to psychosocial stress [Nater 2004]. According to this study, α-Amylase activity can not be used to measure reduction of stress as an effect of intense physical exercise. Salivary α-amylase is used as one of the physiological parameters that are indicative for stress reactions in the body, however the mechanisms that lead to changes of the enzyme's activity due to stress are not entirely understood.

Conclusions

The findings of the present study show that the intense physical activity leads to the reduction of stress level in college students and is beneficial to their health and wellbeing. Cortisol, but not α-amylase, is a useful biomarker in measurements of stress reduction in college students as an effect of intense physical exercise. Some of the limitations of this study include a small sample size of 19 individuals in each group. Larger groups of participants should be studied in the future. The salivary biomarker levels were measured at the same time of day, however, there are typically individual differences in the diurnal cycle of cortisol and α-amylase, which were not controlled in this study. These results should be replicated in future studies before being generalized.

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Perceived Stress Scale

The questions in this scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

Date _____Age _____ Gender (*Circle*): **M F**

0 = Never 1 = Almost Never 2 = Sometimes 3 = Fairly Often 4 = Very Often

1. In the last month, how often have you been upset
because of something that happened unexpectedly?..... **0 1 2 3 4**
2. In the last month, how often have you felt that you were unable
to control the important things in your life?..... **0 1 2 3 4**
3. In the last month, how often have you felt nervous and “stressed”? **0 1 2 3 4**
4. In the last month, how often have you felt confident about your ability
to handle your personal problems?..... **0 1 2 3 4**
5. In the last month, how often have you felt that things
were going your way?..... **0 1 2 3 4**
6. In the last month, how often have you found that you could not cope
with all the things that you had to do? **0 1 2 3 4**
7. In the last month, how often have you been able
to control irritations in your life?..... **0 1 2 3 4**
8. In the last month, how often have you felt that you were on top of things?..... **0 1 2 3 4**
9. In the last month, how often have you been angered
because of things that were outside of your control? **0 1 2 3 4**
10. In the last month, how often have you felt difficulties
were piling up so high that you could not overcome them?..... **0 1 2 3 4**

Metal-Based Chemotherapy Drugs

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Abstract: Metal-based compounds have been used to treat disease since as early as the 5th and 4th century B.C. Cisplatin, a platinum-based chemotherapy drug, was one of the first metal-based compounds found to treat cancer. Other platinum drugs such as oxaliplatin and carboplatin have been the backbone of metal-based cancer treatment drugs as well. The potential therapeutic benefits of metal complexes, in particular, transition metals, has gained attention due to exhibiting unique characteristics including their capability to go through a redox reaction. Due to limitations like drug resistance and worsening side effects, ruthenium compounds have been developed that caused less severe and fewer side effects. Copper complexes have been found to exhibit cytotoxic properties with distinct mechanisms of actions and even have the ability to competitively bind to sites occupied by different metals. It has been suggested that gold complexes have the potential to possess antitumor properties if cisplatin cannot be used as a form of treatment. Silver complexes are another potential type of chemotherapeutic drug, found to exhibit greater selectivity against cancer cells and display better cytotoxic action than cisplatin with comparably low toxicity. The potential for metal-based chemotherapy drugs is continually expanding, and more research should be carried out in order to learn all of the benefits these unique drugs have to offer the world.

Introduction

> "Everything is poisonous, and nothing is harmless. The dose (amount) alone defines whether something isn't poison"
> Paracelsus, 1493-1541.

There is currently a broad scope of anticancer agents that targets numerous biological and cellular characteristics across multiple tumor types. The evolution of anticancer drugs has shifted away from traditional cytotoxicity and in the direction of selective agents which act on particular cellular targets. Nonetheless, considerable challenges linger, and the link between chemistry and structural biology may supply the most productive method for improving upon and discovering anticancer agents.

In nature, numerous biological systems utilize metal ions, for instance, copper and zinc, which perform crucial roles in the natural functioning of organisms. Transition metals including manganese, copper, and iron, among other transition metals, are involved in several biological mechanisms, from structural roles to catalysis to electron transfer, and are regularly associated with the active sites of enzymes. Dysregulation of several of these metals during regular biochemical processes has been involved with the incidence of numerous pathological disorders, including cancer. These cellular activities only need small, tightly regulated amounts of "trace metals." In comparison, other metals including nickel, chromium, cadmium, and arsenic are not as advantageous because those metals generate a broad range of severe toxic side effects, such as carcinogenesis (Arita and Costa, 2009, Rahman and Singh, 2019).

Throughout history, numerous metal-containing compounds have been used to treat a broad array of disorders. In medicinal chemistry,

which has generally been influenced by organic chemistry, metal-containing compounds have acquired favor as anticancer agents and diagnostic tools. The study of metal-containing anticancer agents began with the accidental discovery of the anticancer properties of cisplatin, cis-[PtII(NH_3)$_2$$Cl_2$]. The clinical use of cisplatin is limited as a result of resistance along with a confined spectrum of activity and dose-dependent toxicity. Because of these limitations, there has been an exploration for platinum-based complexes that demonstrate a wider spectrum of activity, higher selectivity, and lower toxicity; cisplatin and carboplatin emerged because of this research. Nonetheless, along with various platinum analogs, several other metal compounds consisting of metal ions, for instance, copper chelating agents, gold, silver, copper(II), and zinc(II) have gained abundant attention as possible anticancer agents. In addition, the analysis of ruthenium-containing complexes in clinical trials vouches for the vast potential of using non-platinum metal-based complexes for cancer treatment (Dabrowiak 2012, Franklin and Costello 2009, Ajmal 2017). This review examines the function of chosen metals in biological mechanisms within cells as they relate to malignancy as well as feature the medical uses of those metals and their structures in the development and design of metallodrugs for cancer treatment.

Exceptional Features of Metal-Based Complexes and Metal Ions

Medicinal inorganic chemistry is a promising and constantly evolving field that involves several processes including, but not limited to, the introduction (or expulsion) of an ionic metal into (or out of) an organism in order to diagnose or to provide therapeutic effects. Because metals have the ability to become cations when submerged in aqueous solution and bond to biological molecules with a negative charge, charge manipulation can be achieved contingent upon the coordination environment influencing the production of a cationic, ionic, or neutral biological molecule. Furthermore, metal ions displaying high electron affinity have the capability to polarize groups coordinated with them, which allows the production of hydrolysis reactions (Haas and Franz, 2009). Due to these properties of metals, the potential therapeutic use of medicinal inorganic chemistry in the construction of anticancer agents has recently gained significant interest.

Throughout ancient history, metals have been used in various medical treatments. The earliest written account of metals being used for medical treatment dates back to 1500 BC when Ebers Papyrus wrote about how iron could be used to treat anemia and how Copper could be used to decrease inflammation (Dabrowiak, 2012). Metals have the ability to treat cancer by being able to collaborate precisely with DNA and to specifically attack cancer cells. Since the phosphate backbone of DNA is negatively charged, the positive charge of almost all metals gives metals the ability to interact with DNA. A number of drugs manufactured that contain metals communicate precisely with separate metals that are already available at the protein active sites. Other drugs developed have the ability to make metals communicate with amino acids that have the largest reduction potential. Even though metals have been used since ancient times, the full potential of metal-based chemotherapy drugs was not realized until the discovery of cisplatin during the 1960s. It is well known that the existence of metals in cellular conditions is a heavily regulated process, therefore precise doses of drugs containing metals need to be established in order to attain an optimum therapeutic response. If precise administrations of metal-containing drugs are not established, then a deficiency or excess of metals could develop undesirable toxicity.

In comparison to conventional carbon-based compounds, metal-containing compounds present several benefits in the expansion of modern medicinal compounds. These benefits exist because of the metal-containing compounds capability to integrate ligands within a three-dimensional configuration, which allows the functionalization of groups which can be tailor-made to carefully defined molecular targets (Frezza et al., 2010). Metal-based compounds present a well-supplied environment to assemble a wide range of specific molecular structures

which deliberate an ample range of geometries and coordination numbers, including kinetic properties, which could not be accomplished with traditional carbon-based compounds (Frezza et al., 2010). Fascinating electronic features that have the ability to act as applicable probes in the composition of anticancer agents are due to the partly filled d orbitals that occur in metals. When looking at the design of coordination compounds, it is essential to recognize the oxidation state of a metal due to the fact that it grants participation in the biological redox chemistry and has a significant function in the optimum dosage and the bioavailability of agent distribution. In addition, the capacity of metals to participate in ligand-exchanged reactions proposes endless opportunities for coordination and interaction to occur between metals and biological molecules, exhibited by the broad medicinal use of cisplatin. When constructing metal-based drugs, one is not confined to choosing only certain metals; advantage can be taken of the special characteristics of nonessential metals, along with other first and second-row transition metals, including metals that are not found naturally (Haas and Franz, 2009). Most momentous is the composition of radiopharmaceuticals which have the ability to employ the radioactive features of metals and are frequently operated within diagnosing cancer and separate medicinal treatments.

Platinum-based analogs in chemotherapy

The history of the first metal-based chemotherapy drug begins not in 1965 as many people believe, but in 1844, during which time it was originally developed by Michele Peyrone and known as Peyone's chloride (Wheate and Apps, 2015). The extraordinary breakthrough was made in 1965 was by Barnett Rosenberg, a biophysical chemist, when he accidentally discovered cisplatin could be used as a treatment for cancer. The discovery of the therapeutic use of cisplatin in cancer therapy encouraged efforts to explore non-platinum and additional platinum metal-containing compounds that could potentially be employed in cancer treatment. Cisplatin has been broadly utilized to treat an assortment of tumors including testicular, non-small cell lung carcinoma, head, and neck, cervical and ovarian cancers, and is often employed in combination regimens (Kelland, 2007). The widespread clinical use of cisplatin has been impeded by the presence of acquired and intrinsic resistance and increased toxicity. In order to overcome these problems, there have been 2nd and 3rd generation platinum drugs developed, mainly oxaliplatin and carboplatin, which have the ability to maintain a much more controllable toxicity profile (Alama et al., 2009). Oxaliplatin has been clinically authorized as a treatment for colorectal cancer, which has shown to be resistant to cisplatin, while carboplatin has been shown to be an effective treatment for head and neck, lung, and ovarian carcinoma cancers (Frezza et al., 2010).

One of the main reasons platinum-based compounds have such a practical antitumor effect has to do with the platinum-based compounds ligand exchange kinetics. The ligand exchange behavior of platinum-based compounds is moderately slow, even though the platinum-ligand bond presents comparable thermodynamic ability and is considerably weaker than classic coordination bonds, like C-O, C-N, or C-C double and single bonds. Due to the ligand exchange behavior being rather slow, this gives platinum-based compounds a lofty kinetic stability and grants exceptionally prolonged ligand exchange reactions to the point where it can take minutes to days to complete instead of seconds (Reedijk, 2003). In addition, regarding Pt(II) compounds, ligands that are located in the trans arrangement are substituted much more quickly than ligands in the cis arrangement, which plays an important part in the antitumor efficiency of these compounds(Frezza et al., 2010). Cisplatin is known to go through ligand substitution reactions and rarely expands its coordination number. Through active or passive transport, cisplatin is absorbed through cells and its chloride ions will be replaced with molecules of water prior to interacting with DNA, thus making coordinative bonds to the nitrogen atoms in DNA (Fuertes et al., 2003). The resulting elevated chloride ion concentration in blood plasma allows cisplatin to be stable regarding hydrolysis, but the lower concentration of intracellular chloride facilitates accelerated

hydrolysis toward the activated cationic molecule that can bind DNA (Pizarro and Sadler, 2009). It has been found that carboplatin has a much more advantageous pharmacokinetic profile due to its delayed conversion rate to the reactive species of carboplatin (Frezza et al., 2010). Carboplatin has a similar mechanism of actions when compared to cisplatin, therefore problems remain when carboplatin is used to treat tumors that are resistant to cisplatin. Replacing the chloride group located on cisplatin by the cyclobutanedicarboxylate ligand located on carboplatin provides better stability and adequate aqueous solubility (Frezza et al., 2010). This then induces decreasing side effects, while maintaining a comparable degree of cross-resistance towards cisplatin and clinical activity.

At the time platinum-based compounds bind to cells in the body, miscellaneous signal transduction pathways undergo activation, which then acts to intervene with various cellular processes including DNA replication and transcription, thereby causing apoptotic cell death. In comparison to cisplatin and carboplatin, the hefty diaminocyclohexane (DACH) carrier ligand located on oxaliplatin has been thought to grant lower cross-resistance and a better toxicity profile (Frezza et al., 2010). In 2002 the FDA approved oxaliplatin, often combined with chemotherapy, for treating advanced colon cancer.

Throughout the years, research into platinum-based medicine has been heavily influenced by the medicinal use of carboplatin, cisplatin, and recently oxaliplatin. Researchers have been conducting innovative methods with the goal of creating the next era of platinum-based drugs due to continual issues with resistant and toxicity in current platinum-based drugs. The fundamental information learned from platinum(II) compounds, along with resistance mechanisms and cellular processing, harnessed with an enhanced insight towards mechanisms of action might assist in translating the upcoming generation of platinum-based medicinal compounds into clinical practice.

Two big concerns that started the pursuit of innovative platinum-based coordination compounds attention to oral administration were low bioavailability and poor solubility of clinically accepted platinum compounds (Frezza et al., 2013). While platinum(II) chemistry depends upon ligand exchange reactions, platinum(IV) has octahedral geometry that offers two excess ligand locations and the elevated kinetic inertness of platinum(IV) diminishes reactivity, which then has the ability to diminish off-target effects (Hall et al., 2007). Most evidence indicates platinum(IV) complexes are decreased in vivo to form platinum(II), which is the compound that is accountable for its stimulation and can be treated as a pro-drug (Frezza et al., 2010). Satraplatin, an octahedral platinum(IV) compound that is given orally and is in progressive clinical stages for the medicinal therapy of hormone refractory prostate cancer, is the most distinguished example originating from this group (Frezza et al., 2010). Platinum(IV) compounds demonstrate benefits due to their bioreductive activation and greater stability, hence letting a large amount of drug to arrive at its biological target. It has been shown that the anti-tumor action of satraplatin works similarly to cisplatin due to the development of inter and intrastrand DNA cross-links (Choy et al., 2008).

Powerful strides have been made regarding the field of platinum-based chemotherapy drugs, especially concerning mechanistic comprehension of these drug's pharmacological effects and design strategies. Advancing our current knowledge of platinum-based compounds, including resistance mechanisms, tumor uptake, and structure-activity-relationship (SAR) can assist the progress of the medicinal installation of the next era of platinum-based chemotherapy compounds.

Zinc

Zinc is known as a crucial trace element performs an integral function in a broad scope of cellular processes especially with regards to protection against free radicals, cell proliferation, and differentiation. Zinc operates as a crucial anatomical element in several enzymes and proteins, including DNA repair enzymes, cellular signaling proteins, and transcription

factors (Roohani et al., 2013).

It has been found that zinc performs a crucial function in regulating apoptosis within mammalian cells, although it is not completely understood how it does this. In several cell types, including ovarian epithelial cells, glial cells, prostate epithelial, and other cells, zinc has been shown to activate apoptosis, while in Hela cells, macrophages, renal cells, lung epithelial cells, and breast cells, zinc has been shown to have antiapoptotic effects (Franklin and Costello, 2009). These conflicting developments have been subjected to extreme investigation and still, remain unanswered.

Since zinc plays a crucial role in countless biochemical systems, it is unsurprising that altered amounts of zinc are correlated with systemic anomalies, including the incidence of cancer. Even though it has been found that concentrations of zinc are compromised in patients suffering from cancer in comparison to healthy patients, the relation between zinc levels and tumor developments lacks recognizable conclusions and depends on the type of tumor (Frezza et al., 2010). Patients suffering from prostate, digestive tract, gallbladder, or liver cancer have been found to have reduced zinc levels, while patients suffering from breast cancer exhibited elevated and decreased levels of zinc in malignant tissues and serum (Frezza et al., 2010). Emerging evidence has suggested that the expression levels of zinc transports are related to cancer progression (Zhao and Eide, 1996). Altered expression of zinc transporters could perform an important function in the incidence of cancer by interrupting function and intracellular distribution. Along with the crucial duty, zinc has in biological systems, its unique characteristics have granted it to acquire approval as probable anticancer agents.

Copper

Copper is a fundamental trace metal that is important in various biochemical processes including angiogenesis, development, cellular growth, and chemical redox reactions. In biological systems, copper is found as both (Cu+) or (Cu2+), which allows copper to act as a cofactor for redox reactions. In order to avoid unneeded binding to biomolecules, ensure distribution and proper uptake, the procurement and dispersion of copper is a highly controlled process. Notably, the coordination chemistry associated with copper is usually distinct dependent on its oxidation state: Cu+ displays favoritism towards sulfur donor ligands, for example, methionine or cysteine, while Cu2+ exhibits a preference for nitrogen donors, for instance, histidine or oxygen donors including aspartate or glutamate (Frezza et al., 2010).

The relationship between copper and carcinogenesis has been intensely investigated over the past two decades. The reason for this was because it was discovered that tumor-bearing humans and mice possess altered copper levels; studies found that elevated tissue and serum levels of copper were present in several human tumors including brain, lung, colon, prostate, and breast, in comparison to healthy individuals (Frezza et al., 2010). The reason for the elevated levels of copper has not yet been fully understood and no conclusions have been made.

The idea of antiangiogenic therapy utilizing copper chelators in the treatment of cancer has received abundant attention because of the discoveries concerning the significance of copper and angiogenesis in tumor development (Frezza et al., 2010). Considerable clinical trials have begun and have demonstrated encouraging results (Redman et al., 2003). It is known that elevated copper concentration and raised proteasome activity are distinct characteristics found in tumor-bearing humans. Frezza et al. (2010) found that these particular aspects associated with tumors cells have the ability to be utilized as distinct targets by previously clinically authorized drugs that perform as effective tumor cell killers when those drugs form a complex with copper.

Ruthenium

Because of limitations like drug resistance and worsening side effects to platinum-based drugs in the treatment of cancer, the focus has shifted toward the development and design of

practical non-platinum-based drugs, including gold and ruthenium that are less harmful to normal cells, with less resistance and enhanced efficiency (Ajmal, 2017). Ruthenium-based compounds have distinct characteristics, such as the ability to reside in a larger amount of spatial positions in comparison to cisplatin, a larger amount of probable accessory molecules which can be transported by the drug arrangement and in particular, the likelihood to prevail in the organic fluids of nearly all of the most crucial oxidation states ranging from oxidation state II to IV (Popolin et al., 2017). All of these unique properties have made ruthenium-based compounds encouraging antimetastatic and antitumor candidates. Popolin et al. (2017) discovered [$Ru(CH_3CO_2)$(dppb)(bipy)]PF6 [where bipy = 2,2'-bipyridine and dppb = 1,4-bis(diphenylphosphino)butane] as a possible treatment for triple-negative breast cancer (TNBC) due to its ability to more efficiently inhibit the proliferation of TNBC cells over normal, non-tumor cells, inhibit TNBC cells migration and invasion, adhesion, and to cause the death in TNBC cells, although this complex should be studied in vivo to establish its potential to help improve the treatment for breast cancer.

Silver

Since ancient times, it has been known that silver is an effective antimicrobial agent against a wide range of microorganisms. Currently, silver is used to regulate bacterial growth in medical settings, including the mending of burn wounds, catheters, and dental work (Jung et al., 2008). Silver nanoparticles (AgNPs) comprise a category of materials that range from 1-100nm. Recently AgNps have been reported to adjust Pgp activity and consequently improve the chemotherapeutic efficiency against cancer cells that are multi-drug resistant (Abdel-Fattah and Ali, 2018). Furthermore, the genotoxicity displayed by AgNPs is backed by the production of breaks in double-stranded DNA in addition to chromosomal instability which encourages the start of apoptotic execution (Abdel-Fattah and Ali, 2018). This acting mechanism indicates that AgNPs could be correlated with various DNA-targeting anticancer drugs.

Saratale et al. (2017) made AgNPs from Taraxacum officinale, also known as the dandelion, and demonstrated its enhanced cytotoxic impact against human liver cells stricken with cancer (HepG2). Biofunctionalized AgNps made within separate plant extracts of clove and guava displayed the satisfactory anti-cancer impact against four separate cancer cell lines including human chronic myelogenous, human colorectal adenocarcinoma, concerning the human kidney, leukemia, human cervix and bone marrow (Abdel-Fattah and Ali, 2018). Aydin et al. (2014) were able to demonstrate that two silver-containing metal complexes, $C_{16}H_{34}N_8O_5Ag_2$ Cd (AN1) and $C_{11}H_{16}N_7O_2Ag_3$ Ni (AN7), have the ability to act as effective anticancer drug candidates that demonstrate low cytotoxic, higher antiproliferative, strong apoptosis-inducing and efficient DNA topoisomerase inhibitory traits, although additional in vivo and in vitro studies must be carried out in order to verify the anticancer drug potential of AN1 and AN7.

Gold

In order to attain a more advanced cytotoxicity profile demonstrating a broader range of activity in comparison to platinum-based compounds, the chemotherapeutic ability of gold coordination complexes has been investigated. Studies have demonstrated that communications of DNA, the favorable destination of platinum, with gold(III) complexes failed to present an advantageous binding mode, accelerating the research into gold and protein interactions (Frezza et al., 2010). In the beginning, studies concentrated on an array of artificial gold(III) diothiocarbamate derivatives which were revealed to demonstrate 1-to-4-fold more cytotoxicity than cisplatin and had the ability to substantially overcome acquired and intrinsic resistance (Ranconi et al., 2005). Still, these studies were unable to demonstrate a molecular link among the gold compounds and their chemotherapeutic activity. A study done by Frezza et al. (2010) featured the proteasome as a crucial molecular destination of gold complexes but exhibited apparent mechanisms of action that are responsible for its fundamental biological actions, which rely upon the metal's oxidation state.

Conclusion

The crucial role metals perform in the maintenance and functioning of life features the extensive role nature plays in managing these essential components. The clinical achievements of cisplatin paved the road for researching metals, nonessential or essential, and those metals coordination complexes as possible anticancer agents. Since the chemotherapeutic effects of cisplatin were discovered, thousands of platinum-based compounds have been made, with only oxaliplatin and carboplatin accomplishing widespread clinical use. Construction methods of novel platinum compounds have been intensely investigated in order to focus on the defects of past generation platinum compounds.

Cisplatin was the first widely used metal-based chemotherapy drug, but due to drawbacks including the presence of acquired and intrinsic resistance, and increased toxicity, the search for other metal-based chemotherapy drugs has expanded. Oxaliplatin and carboplatin are 2nd and 3rd generation platinum drugs that have been developed in response to this and have a much more controllable toxicity profile. Several non-platinum-based drugs have been suggested including zinc, copper, gold, silver, and ruthenium, among others. The altered expression of zinc transporters could perform an important function in the incidence of cancer by interrupting function and intracellular distribution. Because it is known that elevated copper concentration and raised proteasome activity are distinct characteristics found in tumor-bearing humans, utilizing copper chelators in the treatment of cancer has received abundant attention as well. Due to limitations like drug resistance and worsening side effects, ruthenium compounds have been developed that caused less severe and fewer side effects. It has been suggested that gold complexes have the potential to possess antitumor properties if cisplatin cannot be used as a form of treatment. Silver complexes are another potential type of chemotherapeutic drug, found to exhibit greater selectivity against cancer cells and display better cytotoxic action than cisplatin with comparably low toxicity. Because metals are equipped with unique characteristics that are not present in common carbon-based medicines, the positive trend associated with the discovery of anticancer drugs can persist by advancing the fundamental knowledge acquired from the study of medicinal inorganic chemistry. The potential for metal-based chemotherapy drugs is continually expanding, and more research should be carried out in order to learn all of the benefits these unique drugs have to offer the world.

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Synthesis of a Potential New Internal Standard for the Analytical Determination of Dibutyl Phthalate (DBP) and Monobutyl Phthalate (MBP) in Water Samples

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Abstract: Dibutyl phthalate (DPB), a common plasticizer, and monobutyl phthalate (MBP), a DBP metabolite, are endocrine disruptors that leach into water sources through various pathways. Some of the most useful methods to find and quantify the concentrations of these pollutants in drinking water include using gas chromatography-mass spectrophotometry (GC-MS) with solid phase micro extraction (SPME), with the application of benzyl benzoate (BB) as an internal standard. However, since BB is also a common water pollutant and could interfere with practical analytical measurements, there is a need for a new internal standard. A new compound, dibutyl 4-chlorophthalate (Cl-DBP), is proposed as a potentially improved internal standard, because it shares characteristics with DBP and MBP, including the high ionizability of both the compounds as well as a common mass spectrometry fragmentation pattern. The synthesis, purification, and analysis of Cl-DBP as a potential internal standard for the quantitation of butyl derived phthalate plasticizers by GC-MS will be presented.

Introduction

Various phthalate esters are currently being used as plasticizers, including dibutyl phthalate (DBP). (Figure 1) More specifically, dibutyl phthalate is used as a plasticizer and softener to increase the flexibility, durability, transparency, and longevity in inks, plastics, adhesives, cosmetics, and countless other products humans come into contact with on a daily basis. Like other phthalates, DBP is physically, not chemically, incorporated into the polymer structures. (GreenFacts 2005) This means the ester can easily migrate from the polymer into the air, soil, food, and water; and in turn can easily absorb into the skin and gastrointestinal tract. The potential health effects of DBP has warranted growing concern and has led to the ban of DBP in products in Europe and Australia. The concerns involving DBP exposure stem from DBP being a suspected endocrine disruptor. An endocrine disruptor can suppress or overexpress the function of thyroid, androgen, and estrogen hormones; thereby disrupting normal functions of the body.-(Sundar 2016) DBP and one of its isomers has been linked to DNA damage in human mucosal cells and lymphocytes.(Kleinsasser 2001) Additionally,

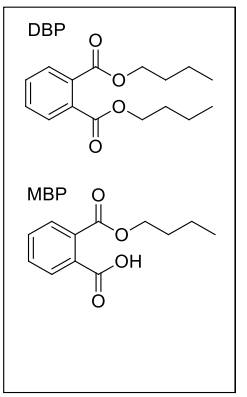


Figure 1. Structure of DBP and MBP

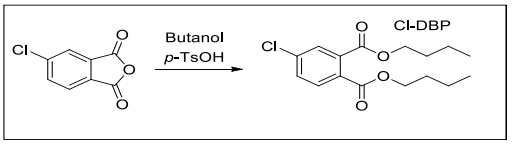
phthalate exposure, including DBP, has been shown to alter physical development in infants and toddlers by blocking hormone activity and reducing function in Leydig cells.(Braun 2013) Furthermore, monobutyl phthalate (MBP), which is a metabolite and hydrolysis product of DBP, is a known endocrine disruptor as well.

These concerns produce a heightened demand for dependable analytical methods that permit detection and quantitation of the phthalate esters DBP and MBP in water sources. The gas chromatography-mass spectrophotometry (GC-MS) and High-Performance Liquid Chromatography (HPLC) are commonly used methods for the analysis of phthalates. (Peñalver 2000) However, at low concentrations these esters are difficult to quantitate and require additional steps to reach a detectable threshold. Typically, the techniques employed for this are Liquid-Liquid Extraction (LLE) or Solid Phase Extraction (SPE). (Peñalver 2000) While these are employed out of necessity, there are problems with these additional steps, such as requiring large amounts of solvent, being time-consuming and labor-intensive, as well as increasing the potential error of the analytical method. (Liu 2008) On the other hand, solid phase micro extraction (SPME) greatly reduces the use of solvent, time, and labor. The SPME device has a 1-2 cm fused silica fiber with a polymer-coated tip that is shielded inside a hollow needle. The device has a plunger at the top that, once depressed, exposes the fiber for absorption and desorption of the analyte. SPME combines extraction and analysis into one step resulting in a SPME fiber that can be inserted directly into the GC-MS injection chamber. Furthermore, SPME allows determination at low concentrations and reduces the risk of secondary contamination. (Vas 2004)

While the direct detection of phthalates in water is possible, their quantitation will require that an internal standard be added to the sample before SPME extraction. This requires the internal standard be chemically similar to the analyte (DBP) in order to maintain the appropriate sensitivity in the instrumental response, yet it needs to be chemically unique enough to avoid potential overlap of the compounds during separation. Additionally, the standard employed must not interact or alter the analyte in a manner that could alter the outcome of the analysis. It should compensate for any variations that may occur during the process, such as sample preparation, quantitative errors, and variations in the GC separations since ideally whatever affects the analyte will equally affect the internal standard. Benzyl benzoate is used almost universally as the internal standard for the analytical determination of phthalate in water because it meets these criteria. (Ziembowicz, 2018) However, benzyl benzoate is used as a solvent, a chemical synthesis component, a perfume fixative, a food flavoring, a plasticizer, and in human and veterinary external medicine as a miticide and can be released into the environment through various waste streams. (O'Neil 2013, Larranaga 2016) This means that, in practice, water samples that are already contaminated with benzyl benzoate would cause an erroneously low measurement of DBP using this method. Therefore, it is necessary to design and test a new internal standard that can be used in this way, and that is not already present in the environment.

Materials and Methods

4-chlorodibutyl phthalate (Cl-DBP), was designed to be the new internal standard because of its similarity in structure to DBP. Cl-DBP was prepared by dissolving commercially available 4-chlorophthalic anhydride (0.5g, 1.6 mmol) in butanol (5mL, 54.6 mmol) with the addition of a catalytic amount of *p*-toluenesulfonic acid (0.104g, 0.6 mmol) (Scheme 1). The reaction mixture was heated to 65°C for four hours under an inert atmosphere. After evaporating the remaining ethanol, the crude product was purified by flash chromatography (silica) using 2 :3 ether:petroleum ether as the mobile phase.



Scheme 1. Synthesis of dibutyl-4-chlorophthalate

The procedure yielded 0.116 g (22.7%) of the desired compound. The identity of the pure compound was confirmed by ^{1}H NMR (Figure 2) and ^{13}C NMR (Figure 3) using a Bruker 300 MHz instrument. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.70 (d, *J*=8Hz, 1H), 7.65 (dd, *J*=2Hz, 1H), 7.49 (d, *J*=8Hz, *J*=2Hz, 1H), 4.31 (t, *J*=9Hz, 2H), 4.30 (t, *J*=7Hz, 2H), 1.71 (m, 4H), 1.42 (m, 4H), 0.96 (t, 6H). ^{13}C NMR (300 MHz, $CDCl_3$): δ 166.66, 166.57, 137.42, 134.40, 130.81, 130.48, 130.13, 128.85, 65.96, 65.78, 30.52, 19.16, 13.72.

The GC-MS used was an Agilent 7890A Gas Chromatogram, 5975C Mass Selective Detector (MSD). For this procedure, a 15 meter VF-5MS column was used with helium as the carrier gas. The mass spectrometer was set to SCAN/Single Ion Measurement (SIM) mode to collect both the ion count for a full range of mass/charge (m/z) ratios, which generally this relates to the mass of the ion with a charge of one (SCAN) and SIM mass spectra, which is just the ion count for only specific m/z ratios. After data collection, the raw results were processed using ChemStation software. The temperature program for the GC-MS procedure is a variation of the one used in the Peñalver study and is as follows.[6] The injection chamber temperature was 270°C and column temperature employed started at 60°C and increased at a rate of 70°C per minute up to 200°C. After reaching 200°C, the rate of the temperatre increase slowed to 15°C per minute until 225°C and was held there for four minutes. The resulting total run time was 7.667 minutes. (Peñalver 2000)

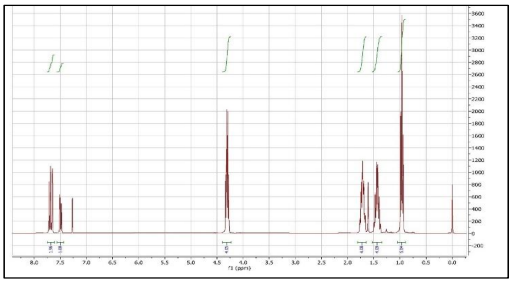


Figure 2. ^{1}H NMR of Cl-DBP

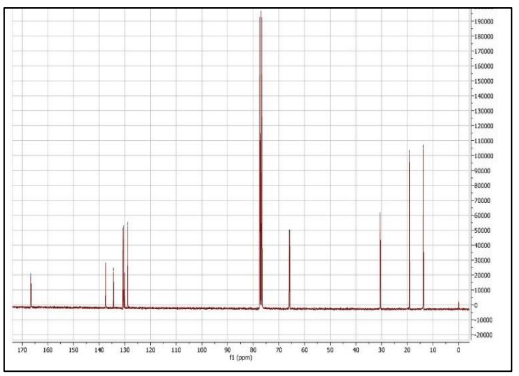


Figure 3. ^{13}C NMR of Cl-DBP

The SPME fiber used was an 85 µm polyacrylate SPME fiber. Before using it for absorption, the SPME fiber was conditioned in the GC-MS at a temperature of 270°C for 12 minutes in order to rid the fiber of any residual phthalates. The 4-chlorodibutyl phthalate samples were prepared by dispensing 1µL of product solution into a GC vial and diluting to 0.5mL using methanol, and the DBP and MBP samples were prepared in DI water at 0.77 ppm and 1.23 ppm, respectively. To increase the polarity of the solution and promote adsorption on the SPME fiber, approximately 1% by weight of sodium chloride was added to the water solution. Samples were then exposed to the SPME fiber for an absorption time of 15 minutes at the chosen standard stir rate. (Ormsby 2016) The SPME needle was then inserted into the GC-MS inlet, exposed for desorption for 1 minute, and then removed. (Peñalver 2000)

Results and Discussion

Utilizing the temperature program described earlier, the GC-MS analysis of the Cl-DBP sample yielded a chromatogram that showed ionizable compounds that eluted at approximately four minutes with an ion abundance of over $2x10^7$ (peak 1) and a second peak as the result of ionizable compounds that eluted from the column at approximately 5.5 minutes and had a ion abundance of $1x10^6$ (peak 2). (Figure 4). Analysis of ions that resulted in peak 1 showed two m/z ratios, or masses in this case as the charge was one. The first mass was 312.8 g/mol, which correlates to the Cl-DBP as expected and a base peak of 183.2, which correlates to 4-chlorophthalic anhydride.

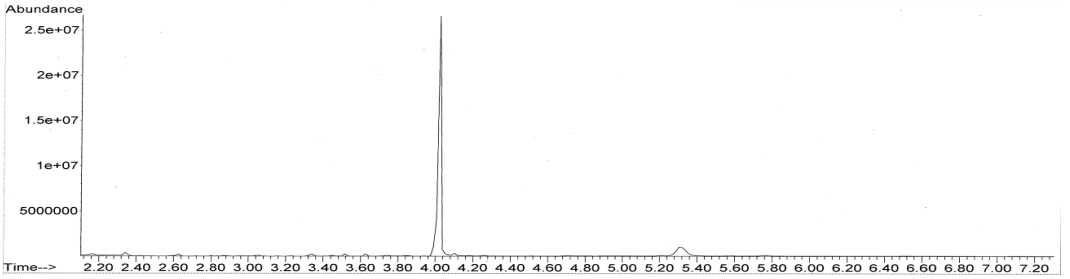


Figure 4. GC-MS of Cl-DBP using SPME Fiber

(Figure 5) The presence of the second mass can be explained as a fragmentation peak of the 4-DBP which results from the ion generation process within the detector.

After establishing the purity of the Cl-DBP sample, it was necessary to investigate its retention time in relation to DBP and MBP under the standard conditions described earlier. The GC-MS analysis revealed four peaks that resulted from ionizable compounds that eluted from the column at 3.22, 3.49, 3. 79, and 4.356 minutes. (Figure 6) Identifications of the compounds that give rise to each peak in the chromatograph were assigned by analyzing the m/z for the ions that eluted at the appropriate retention time. Analysis of the ions present with a retention time of 3.49 min. showed parent peak with a molar of 223.0 amu as expected for MBP and a base peak in of 148.9 amu which corresponds to phthaltic anhydride (Figure 7). As described earlier, the anhydride peak found within this ion set was the result of a fragment of the parent, MBP. The ions corresponding to a retention time of 3.79 min yielded parent peak with a ionic mass of 278.1 amu, as expected for DBP and, a base peak of 148.9 amu. (Figure 8) Again the ion with a m/z of 148.9 corresponds to phthaltic anhydride which would be a common fragment of either MBP or DBP. Analysis of the

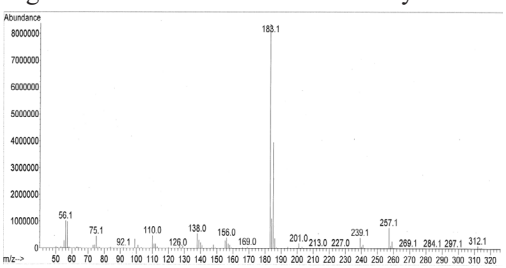


Figure 5. MS of ions eluting at 4.01 Minutes of GC-MS analysis of Cl-DBP

final peak at 4.356 to elute in this experiment demonstrated the highest total ion count and provided a parent peak with a ionic mass of 312.1 amu as expected for Cl-DBP. (Figure 9) The base peak among these ions is 183.0 amu which corresponds to 4-chlorophthaltic anhydride which was observed in the GC-MS of the purified compound. Therefore, the retentions times for Cl-DBP, DBP, and MBP under these conditions were 4.356, 3.787, and 3.493, respectively. Additionally, baseline separation was achieved for each of the compounds by GC in less than five minutes.

In summary, an efficient synthetic procedure and method of purification has been introduced for the preparation of 4-chlorodibutyl phthalate in high purity. GC-MS analysis has been presented to establish that Cl-DBP has chemical characteristics in line with butyl phthalate plasticizers. These characteristics include the high ionizability of both the samples as well as a common fragmentation pattern. Additionally, a GC protocol has been established that allows for the baseline separation of the potential internal standard and its possible target analytes. While future studies remain in order to establish and compare the standard curve of the ionization of both the Cl-DBP and the target analytes versus concentration, it seems that Cl-DBP may serve as a more suitable internal standard for the determination of the concentration of DBP and MBP in water samples compared to current literature examples.

Acknowledgments

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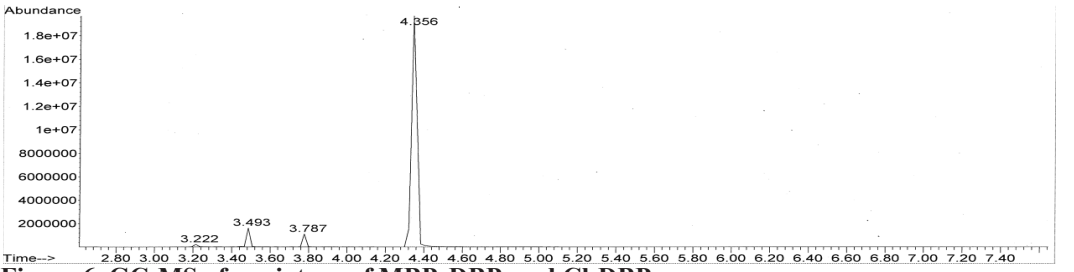

Figure 6. GC-MS of a mixture of MBP, DBP, and Cl-DBP

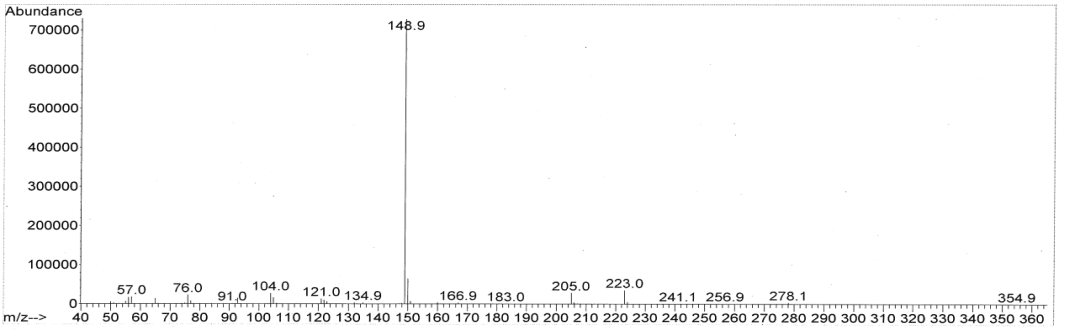

Figure 7. MS of ions eluting at 3.49 min. during GC-MS analysis of Cl-DBP, DBP, MBP mixture

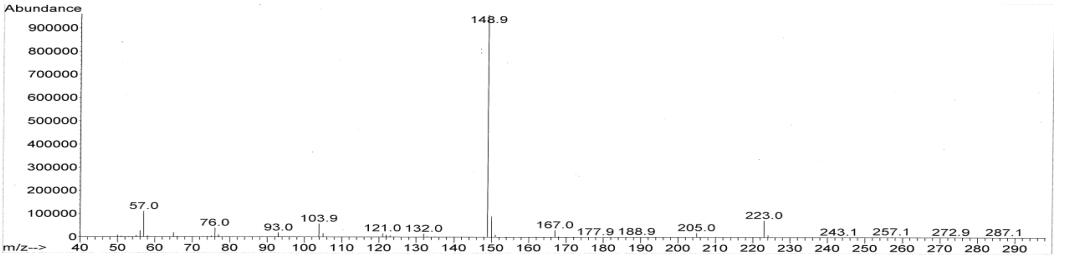

Figure 8. MS of ions eluting at 3.79 min. during GC-MS analysis of Cl-DBP, DBP, MBP mixture

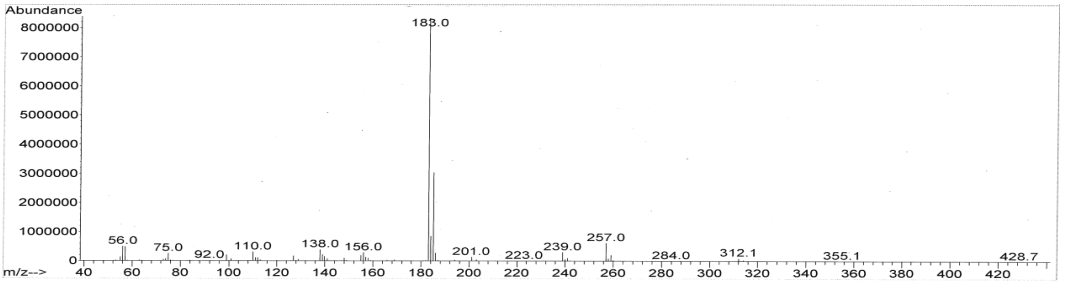

Figure 9. MS of ions eluting at 4.36 min. during GC-MS analysis of Cl-DBP, DBP, MBP mixture

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Analyzing Factors for First Semester General Chemistry Student Success at the University of Central Oklahoma

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Abstract: Student success in the first semester of general chemistry is crucial for not only STEM majors, but also all student's basic scientific understanding. The University of Central Oklahoma (UCO) Department of Chemistry, like many other regional universities, observes a historically high D, F, and Withdrawal (DFW) rate in this course. In order to address this DFW rate and increase student success and therefore retention, we implemented a presentation on metacognitive learning strategies during the course. Furthermore, we analyzed numerous variables that may affect the success of first semester general chemistry students using departmental and university data including the use of this presentation. This was compared to prior semesters, which did not receive this presentation. This presentation focused on learning strategies and study techniques that can be practically applied in the class. We fit a multiple linear regression model and a random forest model using these variables to predict the students' percentile on their final standardized American Chemical Society (ACS) General Chemistry First Term Exam. Neither model indicated that the presentation had a statistically significant effect on ACS exam percentile, while the Math ACT score had the largest effect.

Introduction

The first semester general chemistry course at the University of Central Oklahoma (UCO) historically has high D, F, and Withdrawal (DFW) rates not dissimilar to other regional institutions around the country. Generally, there is an interest in decreasing these rates and increasing student success without decreasing standards. As a means of combatting the high DFW rates, the Department of Chemistry at UCO incorporated a learning intervention into all first semester general chemistry classes aimed at giving students insights and tools for being more effective at learning and studying. Generally, instructors are reluctant to add

material to their already quick-paced, entry-level classes. However, department instructors suggested that students could benefit from explicit instruction on how to study effectively in addition to the subject material. This led the department to explore research into presentations created by Sandra McGuire in an attempt to address these issues. The presentations or learning interventions were based on *Teaching Students How to Learn: Strategies You Can Incorporate into Any Course to Improve Student Metacognition, Study Skills and Motivation.* (McGuire 2015) In the book, she suggests that arming students with the knowledge of how to learn in addition to providing "simple, straightforward strategies to use" empowers students to increase their learning retention and performance in any class. Most studies to-date focus on improving teaching methods and subsequently student performance. These practices are instructor dependent. This presentation aims to put the power back in the hands of the student (instructor independent), not only helping them in a specific class, but with their entire college education (Cook et al., 2013). These strategies are aimed at improving student metacognition or "students thinking about their own thinking". Improving student's metacognition has been shown to aid in deeper and more long-term retention of the subject matter (Bransford et al., 2004, Zhao et al.,2014). The presentation was given to first semester general chemistry students after they received their graded first exam of the semester, with the hope that having seen the presentation, the students would apply what they learned, and their success rates would improve over the semester. To determine whether the presentation on metacognitive strategies for studying has led to an improvement in the performance of students taking first semester general chemistry, we analyzed current and historical data collected by the UCO Department of Chemistry that have numerous categorical and quantitative variables. This includes the students' final exam percentile, which is the standardized American Chemical Society (ACS) General Chemistry First Term Exam and is a good predictor of student success.

Methods

Data Collection

Over the last few years, several professors in the Department of Chemistry gave the metacognitive strategies presentation to their students after having completed the first exam of the semester. Each student, as their final exam in the class, was required to take the ACS General Chemistry First Term Exam which is a nationally standardized exam. Each student's results were recorded by the UCO Department of Chemistry. Additionally, demographic information for the students was obtained through access to the students' enrollment profiles with UCO. The complete list of variables used in the analysis can be found in Figure 1. Several steps of data processing were required since the data was combined from multiple sources. Duplicate entries were removed and students without recorded ACS exam scores were deleted. This resulted in a total sample size of 1,010 UCO students over 6 consecutive semesters. Finally, the raw ACS exam scores were converted to percentiles using national data based on the year that the exam was administered (ACS Exam, 2016).

Model Creation

Two different models were used to predict ACS exam percentiles. These initial analyses both utilize multivariate techniques in order to account for the influence of potential confounding variables such as prior academic success and a variety of demographic attributes. First, a traditional multiple linear regression model was fit to the data. This approach enables one to perform hypothesis tests to examine the statistical significance of predictors, such as the indicator for the metacognitive strategies presentation, while controlling for other variables. Next, a random forest model was used to predict student performance using the same set of variables (Breiman 2001). Random forests are flexible, nonparametric models that inherently model complex interactions between predictor variables. The random forest procedure produces variable importance scores that are used to rank the predictor variables.

The full dataset was divided into training and test sets. The training set consisted of 80% of the observations while the test set contained the remaining 20%. The multiple linear regression model and random forest were both fit using the training data. The resulting models were then used to predict the ACS exam percentiles for the observations in the test set. Finally, the mean squared error (MSE) was calculated for each model using the test data. This allows for the comparison of the two models. A model with a smaller MSE is preferred.

Results and Discussion

For the multiple linear regression model results, the primary interest was in determining whether there was a significant difference in ACS exam percentiles between students who were exposed to the metacognitive strategies presentation and those who were not. The t test for the regression coefficient associated with the presentation indicator variable produced a p-value of 0.30, which is not statistically significant using $\alpha = 0.05$. Therefore, there was insufficient evidence to conclude a difference in mean ACS exam percentiles for students who did and did not receive the presentation. The multiple regression model had an adjusted R^2 value of 0.3472 and produced an MSE of 451.95 on the test set.

The random forest model more accurately predicted the ACS exam percentiles for the data in the test set with an MSE of 390.33. Unfortunately, traditional hypothesis tests are not readily available for random forest models. Nevertheless, the influence of predictor variables can still be assessed by calculating variable importance scores. These values measure the percentage of the increase in MSE after randomly permuting values for the predictor variables. Shuffling the values of a significant variable will cause a greater increase in MSE than shuffling values for a variable that is not significant. If the metacognitive strategies presentation is significant in predicting ACS exam percentiles, then randomly permuting that variable will increase the prediction errors thus resulting in that variable having a large importance score. The resulting variable importance scores from the random forest model can be seen in Figure 1. The presentation indicator variable was ranked eighth out of the fifteen independent variables. This middle-of-the-pack finish suggests that the presentation was not one of the most important variables for predicting student ACS exam percentiles.

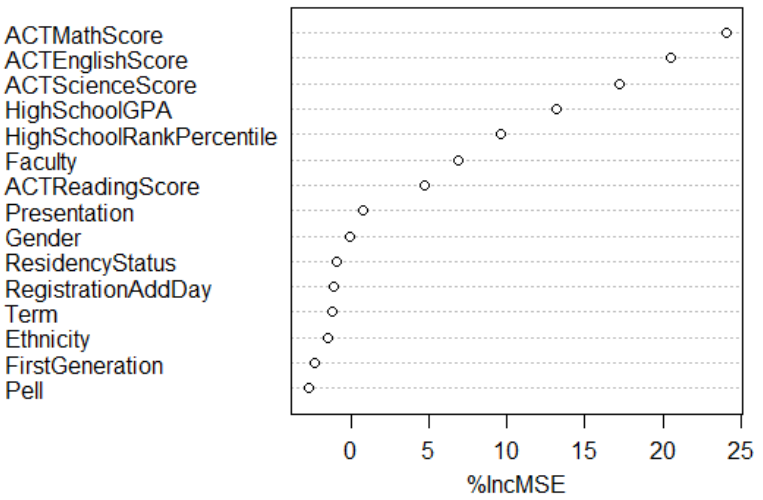

Figure 1. Plot showing the variable importance scores calculated from the random forest model. The percentage increase in MSE is shown on the horizontal axis. The vertical axis contains the list of independent variables ranked from most important at the top to least important at the bottom.

Neither model indicated that the metacognitive strategies presentation had a significant impact on ACS exam percentiles after controlling for the other variables. Box plots showing the distribution of the ACS exam percentiles for students receiving and those not receiving the presentation were created in order to get a simpler look at the effect of the presentation. These plots can be seen in Figure 2. From the box plots, it appears that the distributions of percentiles are nearly identical for both groups of students. The median percentile of students who received the presentation was marginally higher than the median of those in classes that did not have the presentation, but it is reasonable to conclude that difference is not statistically or practically significant. This does not mean that the presentations were not effective for individual students. While the overall trend is not statistically significant, anecdotal evidence from individual instructors with certain individual students has shown that the presentations were helpful. While we know this learning intervention helps individual students, we are still exploring ways to make it more effective for the entire general chemistry first semester population. One option we have considered is giving the initial metacognition talk after the first exam and then following it up every other week with a ten-minute refresher talk. This talk would remind students of metacognition and introduce an additional learning technique that they might find beneficial.

The score on the Math section of the ACT had the largest variable importance score from the random forest. This suggests that ACT Math score is among the most important variables in predicting student ACS exam percentiles. The results from the multiple linear regression model support this conclusion as the hypothesis test for the regression coefficient for ACT Math score showed statistical significance with $p < 0.001$. This result is not surprising as previous research has identified a relationship between ACT Math scores and success in first semester general chemistry and other Science, Technology, Engineering and Math (STEM) fields in general (Cook et al., 2013, Ralph et al., 2018, Elliott et al., 2001).

An additional simple linear regression model was fit using only ACT Math to predict ACS exam percentile in order to further explore the relationship between these variables. A scatterplot showing the positive relationship between these variables as well as the fitted regression line with corresponding standard errors can be seen in Figure 3. The resulting predicted value of ACS exam percentile can be

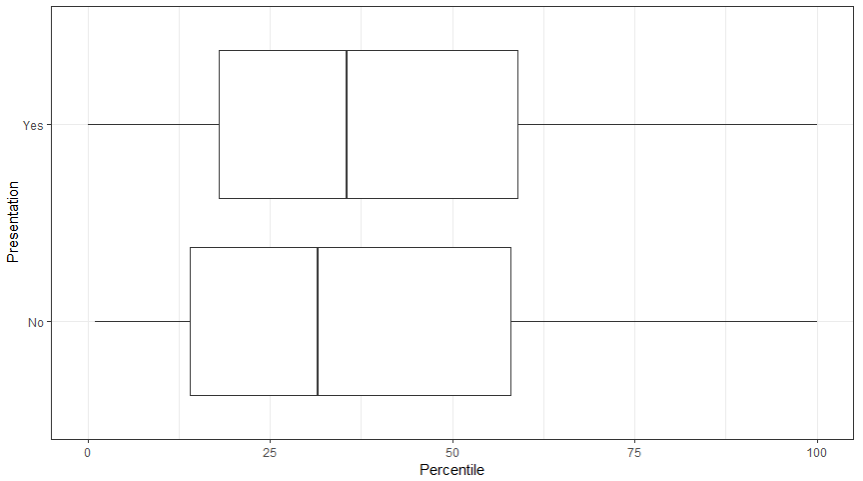

Figure 2. Box plots showing the distribution of ACS exam percentiles for students enrolled in classes that received the presentation (YES) and those enrolled in classes not receiving the presentation (NO).

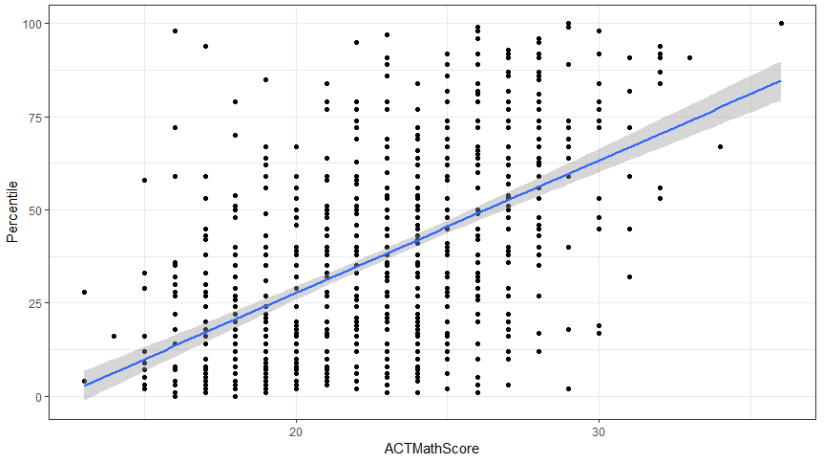

Figure 3. Scatter plot showing the relationship between ACT Math score on the horizontal axis and ACS exam percentile on the vertical axis. The resulting regression equation is included with corresponding 95% confidence band.

calculated from the following:

The model indicates that students with an ACT Math score of approximately 21 or greater can be expected to meet the 30th percentile cutoff on the ACS Exam. This cutoff was chosen based on historical student performance in first semester general chemistry at UCO. Students who performed under the 30th percentile on the ACS Exam were unlikely to be successful in completing the course with a passing grade.

The models showed that there were several variables that were significant in predicting ACS exam percentile. Both models indicate that ACT Math Score is the most significant predictor of ACS exam percentile. Other variables that were determined to be significant in both models are ACT Science, ACT English, High School GPA, and High School Rank Percentile. Notably, the presentation on metacognitive strategies was not deemed to be a significant predictor of ACS exam percentile in either model. While the presentation was not a significant difference for the average of the class, there may be individual students who benefited from this presentation. At the very least this data indicates that students do not perform worse on average despite using a significant amount of class time for these presentations. In the future probing individual student performance with and without the presentations could yield more specific results on which students are being positively or negatively affected. For example, we evaluated a student's final exam percentile as a proxy for student success without including other classroom assessments, like unit exams, which may tell a more nuanced story. It must also be mentioned that other methods of treating missing data, as opposed to deleting these entries, could allow for more data and may lead to different models for predicting student success. The addition of this missing data would include all students that are counted in the DFW rate, rather than only those that took the standardized ACS final. This may also help elucidate the connection between math proficiency and overall success in general chemistry.

Acknowledgments

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Investigating the Growth Characteristics and Infectivity of a Newly Isolated Bacteriophage Against *Mycobacterium smegmatis* mc^2 155

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Abstract: Bacteriophages or phages are specific viruses that are capable of infecting bacteria without harming eukaryotic cells. Mycobacteriophages are a specific type of phage that only infects bacteria in the genus *Mycobacterium*. Mycobacteriophages have been studied for their potential in killing virulent mycobacteria, such as the etiological agents of tuberculosis and leprosy. In this study, we used *Mycobacterium smegmatis* mc^2 155 to isolate a mycobacteriophage from Oklahoma [USA] soil. Mycobacteriophage OKCentral2016 was isolated from soil enrichment obtained at the University of Central Oklahoma. This phage produced transparent plaques and has a morphology consistent with the *Siphoviridae* morphotype. The phage remained stable at temperatures below 55°C and within the pH range of 6-8. The viral replication cycle took approximately 4 hours to complete under standard growing conditions. This phage only infected *M. smegmatis* and decreased biofilm formation in planktonic cultures.

Introduction

The genus *Mycobacterium* is composed of acid-fast, aerobic bacteria that are ubiquitous in soil and aquatic environments (Norby et al., 2007). Two of the more medically significant mycobacterial species are *M. tuberculosis* and *M. leprae*, which are the causative agents of tuberculosis and leprosy, respectively. The most common non-tuberculosis mycobacteria (NTM) agents that are medically relevant are: *M. avium* complex (*M. avium* and *M. intracellulare*), *M. kansasii*, *M. simiae*, and *M. abscessus*. These bacterial pathogens cause pulmonary disease, lymphadenitis, and soft tissue infections (Johnson and Odell, 2014). There has also been an increase in NTM infections in patients with cystic fibrosis (Leung and Olivier, 2013).

Biofilm formation is one of the key survival strategies used by bacteria to protect them from unfavorable conditions (Kiefer and Dahl, 2015). Biofilms are formed when bacteria aggregate together on a solid surface and become encased in a secreted matrix of an extracellular polymeric substance (EPS) (Harper et al., 2014). Bacteria encased in biofilms have increased resistance to disinfectants and antibiotics (Carter et al., 2003; Steed and Falkinham, 2006). This has become a problem in hospital settings due to the buildup of biofilms on medical equipment; biofilms have contributed to the rise of post-surgical nosocomial infections and chronic infections (Phillips and Reyn, 2001). The National Institute of Health estimates that 80% of all bacterial infections are associated with biofilms (Harro et al., 2010), and biofilm formation of *M. abscessus* has been linked to multiple cases of chronic pulmonary infections (Qvist et al., 2015).

Bacteriophages or phages are viruses that only infect bacteria, and regulate bacterial populations in the environment (Atterbury et al., 2005). Phages are considered the most abundant

and diverse group of organisms in the biosphere. It is estimated that there are approximately 10^{32} phages on our planet (Brussow and Kutter, 2005), with 10^8 different phage species (Rohwer, 2003).

Phage therapy is the therapeutic use of bacteriophages to treat bacterial infections. Currently, phages are being evaluated as an alternative treatment for antibiotic-resistant bacteria (Lin et al., 2017). Bacteriophages have also been used to treat and prevent the formation of biofilms (Fu et al., 2010; Harper et al., 2014).

Mycobacteriophages are bacteriophages that can infect mycobacteria. At the time of this study as per The Actinobacteriophage Database (phagesdb.org) 10,562 mycobacteriophages have been isolated and 1,766 have been sequenced. The bacteriophages on this database are taxonomically organized into clusters based on nucleotide similarity (Hatfull et al., 2010). Mycobacteriophages are studied for their therapeutic potential in treating NTM and *M. tuberculosis* infections (Broxmeyer et al., 2002; Danelishvili et al., 2006). Mycobacteriophages have been demonstrated to prevent mycobacterial contamination on solid surfaces (Kiefer and Dahl, 2015). *M. smegmatis* mc^2 155 is the model organism for studying the genus *Mycobacterium*, because of its non-pathogenicity and increased growth rate (Beltan et al., 2000; Gordon and Smith, 1953). In this study, we isolated a soil-dwelling phage using *M. smegmatis* mc^2 155. We characterized the virion and explored the therapeutic potential of the isolated phage against common NTM and biofilm formation in *M. smegmatis* mc^2 155.

Methods

Culturing bacteria

All mycobacterial strains were grown in 7H9 broth supplemented with albumin dextrose catalase (ADC) (10% V/V) and 1 mM $CaCl_2$. Liquid bacterial cultures were incubated in a shaking incubator set at 37°C. On solid media, the bacteria were grown on 7H10 agar supplemented with 0.2% glucose and 1 mM $CaCl_2$. The bacteria grown on 7H10 agar plates were incubated at 37°C. *Mycobacterium smegmatis* mc^2 155 was obtained from ATCC (# 700084™). *M. avium* (strain DJO-44271), *M. abscessus* subspecies bolletii (strain MC1518), *M. intracellulare* (strain 1956), *M. kansasii strain* (824), and *M. simiae* (MO-323) were obtained through BEI Resources, NIAID, and NIH.

Bacteriophage isolation

Soil was collected from the southwest corner of the University of Central Oklahoma (35.653371 N, 97.474118 W) near the Coyner Health Sciences building. The collected soil was enriched using a double enrichment method (Patton and Kotturi, 2018). Three grams of the collected soil was enriched with 10 mLs of 7H9 broth and 1 mL of host bacterium and was incubated at 37°C for 24 hours (h). Following incubation, 10 mLs of phage buffer (10 mM Tris pH 7.5, 10 mM $MgCl_2$, 68 mM NaCl, and 1 mM $CaCl_2$) was added to the mixture; the mixture was vortexed and centrifuged at 1,589 x g for 10 min. Following centrifugation, 9 mLs of supernatant was removed and added to 1 mL of 10X 7H9 broth, which was supplemented with ADC, and 1 mM $CaCl_2$. One mL of host bacterial culture with approximately 1.2 x 10^9CFU/mLs was also added, and the bacteria were incubated at 37°C for 24 h. Following enrichment, phage lysate was centrifuged at 12,000 x g for 10 min. The supernatant was filtered using a 0.2 µm filter and serially diluted. Fifty µL of diluted phage was added to 450 µL of host bacterium. The phage and host bacterium were mixed and incubated at room temperature for 10 min. Phage and bacteria were plated using the agar overlay method (Hockett and Baltrus, 2017) and incubated for 24 h at 37°C. Following incubation, the plates were examined for plaques and plaques were measured in mm.

Plaque purification

Once a single plaque was identified it was purified by removing the plaque and adding it to a pure culture of *M. smegmatis* mc^2 155 and incubated overnight. Following incubation, the culture was filtered and plated as previously described. This process was done three times to ensure purification of a single type of

bacteriophage.

Amplification and precipitation of phage

A single plaque was purified 3 times and the isolated phage was amplified by seeding 30 plates with a high titer phage of 10^8 plaque forming units (PFU)/mL. Approximately 7 mLs of phage buffer was added to the plates and plates were incubated for 8 h at room temperature. The phage lysate was pooled and centrifuged at 5500 x g for 10 min. at 4°C. The supernatant was transferred to a sterile flask and phage was precipitated by adding 1 M NaCl and polyethylene glycol 8,000 (10% V/V), and incubated overnight at 4°C (Colombet et al., 2007). Following incubation, the solution was centrifuged at 5500 x g for 10 min. at 4°C. The supernatant was decanted and the sediment containing the phage was resuspended in phage buffer. This solution was incubated at 4°C for 24 h with gentle agitation from an orbital shaker. The phage was centrifuged at 5500 x g for 10 min. at 4°C. The supernatant, containing the concentrated phage, was removed and was used as the phage stock. The phage stock's titer was determined, and the phage stock supplied the phage for all experiments.

Electron microscopy

High titer phage lysate of 10^9 PFU/mL was added to a carbon-coated electron microscope grid and negatively stained using 1% uranyl acetate. Micrographs of the virion were obtained using a JEM-2000FX scanning transmission electron microscope (TEM) at 80,000 X total magnification. Bacteriophage measurements of the head and tail were obtained using the ImageJ software package. The diameter of the icosahedral head was obtained by measuring the distance from one vertex to the opposing vertex. Phage tails were measured from the beginning of the tail, which was adjacent to the icosahedral head, to the bottom of the tail. Three measurements were taken for the head and tail and they were averaged. Five different virions were used when measuring the size of the tail and icosahedral head.

DNA extraction and restriction digest

The genomic DNA (gDNA) was extracted using the sodium dodecyl sulfate (SDS)/ phenol:chloroform:isoamyl alcohol (PCI) (25:24:1 V/V) extraction method as described by (Green and Sambrook, 2012). The extracted gDNA was treated with five different restriction enzymes (BamHI, ClaI, EcoRI, HaeIII, and HindIII,) per the manufacturer's recommendation (New England Biolabs). An undigested DNA sample was used as a control. The restriction digest results were confirmed by *in silico* analysis using the DNA Master Software package (http://cobamide2.bio.pitt.edu/).

Determining the thermal stability of phage

Thermostability of the phage particle was determined by incubating phage, at an initial concentration of 10^8 PFU/mL, at three different temperatures (50°C, 55°C, and 60°C). The incubated phage was diluted and plated at three specific time points (20, 40, and 60 min.). Plaques were counted, and the number of PFUs/ mL were determined.

Determining pH stability of phage

The stability of the virion was evaluated by incubating the phage in phage buffer with adjusted pHs (5, 6, 7, 8, and 9) for 1 h. Adjustment of the pH was done using a pH meter and the addition of HCl or NaOH to the phage buffer; the phage buffer, with an adjusted pH, was then autoclaved to ensure sterilization. Phage was incubated in adjusted phage buffer, at an initial concentration of 10^8 PFU/mL, at 37°C for 1 h. Following incubation, the phage was diluted, plated, and incubated using the agar overlay method described above.

One-step growth curve

This method was described by Catalao et al using a multiplicity of infection (MOI) of 1.0. Sulfuric acid (0.4%) was used to inactivate unattached phage. The solution was then neutralized using 0.4% NaOH (Catalao et al., 2010). Every 30 min. for a duration of 4 h, 1 mL of suspension was diluted and plated using the agar overlay method as previously described. Plates were incubated at 37°C for 24 h then plaques were counted. This experiment was repeated four times. Data was pooled and the mean ± standard error was graphed.

Effect of phage on biofilm formation

The biofilm forming media used in these experiments was composed of 7H9 broth (as previously described) supplemented with 100 µM $CuSO_4$ (Nguyen et al., 2010). A MOI of 1.0 was used by initially seeding 50 µL of phage (10^8 PFU/mL) onto a 96-well tissue culture plate. One hundred µL of *M. smegmatis* mc^2 155, at a 0.5 McFarland concentration, was added to a 96-well plate, and plates were incubated at 37°C for 72 h. Planktonic cells were removed and biofilms were washed with 200 µL of sterile nanopure water. Fifty µL of phage and 150 µL of biofilm forming media were added to the wells and incubated at 37°C for 72 h. Biofilms were quantified using crystal violet (CV) staining (Kang et al., 2013). Biofilm growth was analyzed using an ELx808 microplate reader (BioTek) at an OD_{570} nm. Phage-treated biofilms were compared to biofilms treated with phage buffer that lacked phage for both assays. In each experiment, 12 biological replicates were used, and experiments were repeated two additional times (n=36).

Determination of host range

The different hosts used to evaluate OKCentral2016's host range were: *M. avium* (strain DJO-44271), *M. abscessus* subspecies *bolletii* (strain MC1518), *M. intracellulare* (strain 1956), *M. kansasii* strain (824), and *M. simiae* (MO-323). The phage was enriched with the host bacterium for 48 h. Following incubation, the phage and host bacterium were filtered and plated using the agar overlay method as previously described. The plates were incubated at 37°C and plates were inspected for plaque formation every day for a duration of 30 days. Plates were inspected every day because of the diverse generation times of these mycobacterial species. *M. smegmatis* mc^2 155 was used as a positive control. These results were confirmed using a spot assay (Mirzaei and Nilsson, 2015).

Data collection and analysis

All experiments were performed using at least three biological replicates, and experiments were repeated at least two additional times to confirm the results. Student t-tests were used to perform the statistical analyses of the data using the analysis tool pack within the Microsoft Excel software package (2016).

Results

Using the methods described above, bacteriophage OKCentral2016 was isolated and purified from soil enrichment. We named the purified phage OKCentral2016. The name is derived from the location and year this phage was isolated. OKCentral2016 produced transparent plaques that had an average diameter of 4.0 mm (Figure 1). The size and transparency of the plaques remained consistent throughout this study. The phage was observed using TEM (Figure 2). OKCentral2016 possessed a *Siphoviridae* morphotype that contained an icosahedral head with a non-contractile tail. The average diameter of the icosahedral head was 56.152 (±5.187) nm, and the length of the non-contractile tail was 130.485 (±9.294) nm.

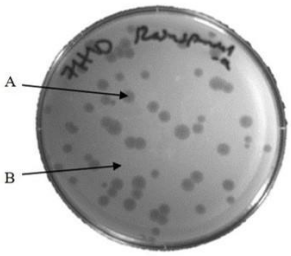

Figure 1. Plaques (A) formed by OKCentral2016 on a bacterial lawn of *M. smegmatis* (B).

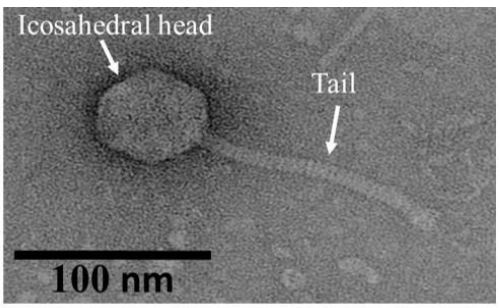

Figure 2. Micrograph of OKCentral2016 stained with 1% uranyl acetate.

The infectious cycle of this phage was characterized by constructing a one-step growth curve (Figure 3) based on the number of PFUs produced at a given time point. We found that the latent period and the rise period took 90 min. Following the rise period, the number of PFUs plateaued, which was indicative that the infectious cycle had concluded. These results are comparable to other mycobacteriophages such as SWU1, which had a latent period that lasted only 30 min (Fan et al., 2015). Whereas phages BO1 and BO2a had latent periods that lasted 150 min. and 260 min., respectively (Kraiss et al., 1973).

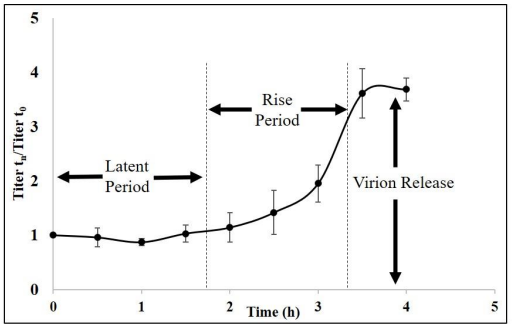

Figure 3. The one-step viral growth curve of OKCentral2016 infecting *M. smegmatis*. Error bars are expressed as ± SE.

A decrease in infectivity was observed after subjecting phage to various thermal (Figure 4A) and pH conditions (Figure 4B). There was a 1 log reduction of PFUs after 60 min. of incubation at 55°C. However, after 40 min. of incubation at 60°C no plaques formed. A significant decrease ($p<0.05$) in phage infectivity was also observed when phage was incubated at an acidic pH of 5.0 or an alkaline pH of 9.0. No reduction in phage infectivity was found within the 6.0 to 8.0 pH range.

The gDNA of OKCentral2016 was approximately 49,000bp on agarose gel and contained restriction sites for two of the five enzymes tested, BamHI, and HaeIII (Figure 5). Digestion by BamHI resulted in the formation of 14 bands at various molecular sizes between 48,000 and 1,000 bp. HaeIII (lane 4)was the other enzyme that cleaved the gDNA with

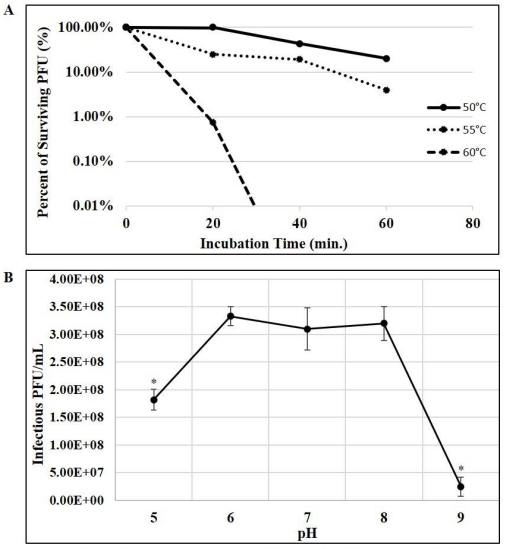

Figure 4. The effect that temperature (A) and pH (B) have on the infectivity of OKCentral2016. Error bars are expressed as ± SD.

***p<0.05**

multiple restriction sites. From the gel image, we can clearly see a DNA smear ranging from 49000 to 500bp in Figure 5. The gDNA did not possess the restriction sites for ClaI, EcoRI, and HindIII, and thus, the DNA was not cleaved. These results were compared to the untreated

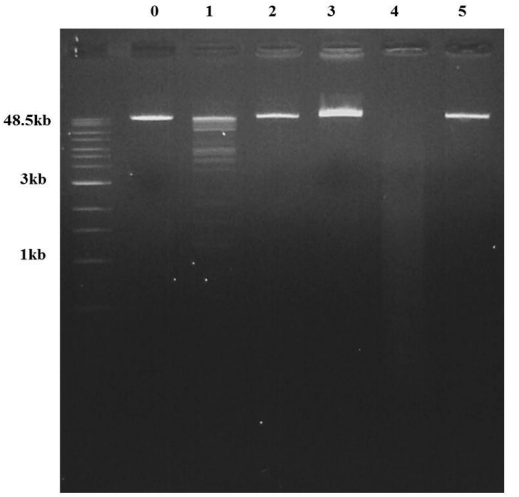

Figure 5. Treatment of OKCentral2016's gDNA with restriction enzymes BamHI (1), ClaI (2), EcoRI (3), HaeIII (4), and HindIII (5). Untreated gDNA served as a control (0).

gDNA. This restriction pattern is common among members of cluster A and subcluster A10. These results were confirmed through genomic sequencing and *in silico* analysis of the genome.

The presence of phage significantly decreased ($p<0.05$) biofilm formation in planktonic cultures of *M. smegmatis* (Figure 6A). However, when phage was added to cells already encased in a biofilm, the biofilm was significantly larger ($p<0.05$) than biofilms lacking phage treatment (Figure 6B). This phage did not infect any other mycobacterial species tested (Table 1).

Discussion

Due to the high pathogenicity and transmission of *M. tuberculosis*, mycobacteriophages have been studied as plausible phage therapy agents. It has been suggested that there is a relationship between the phage cluster and the phages ability to infect other mycobacterial species such as *M. tuberculosis*. The A2 and A3 subclusters have been shown to infect mycobacterial hosts other than *M. smegmatis*. For example, phages Bxb1 and U2 (A1 subcluster), L5 and D29 (A2 subcluster), and Bxz2 (A3 subcluster) infected *M. tuberculosis* (Jacobs-Sera et al., 2012; Rybniker et al., 2006). Treating OKCentral2016's gDNA with five different endonucleases determined that this phage belonged to cluster A (Gissendanner et al., 2014). Genomic sequencing confirmed that this phage belonged to cluster A and subcluster A10 (Patton and Kotturi, 2018). However, it has been shown that single amino acid substitutions on the putative tail fiber protein of mycobacteriophages allowed mutant phages to efficiently infect *M. tuberculosis* (Hatfull, 2014a). Phage infectivity of a specific host is postulated to be determined by the presence of specific bacterial receptors. However, very few phage receptors have been identified (Hatfull, 2014b). Therefore, identifying various phage receptors should be thoroughly investigated.

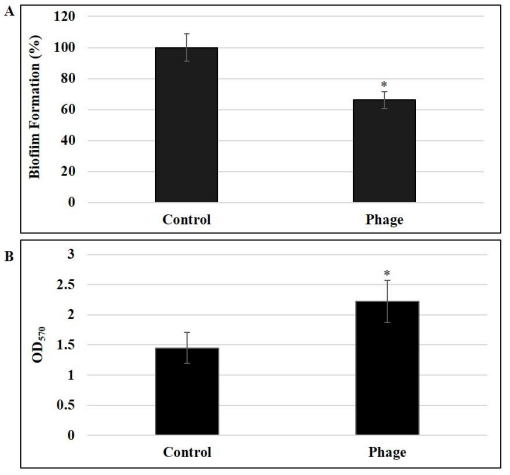

Figure 6. Adding OKCentral2016 to a planktonic culture of *M. smegmatis* significantly decreased biofilm formation (A). However, when OKCentral2016 was added to cells already encased in a biofilm, the biofilm increased significantly (B). These results were graphed ± SE. *$p<0.05$

Bacteriophages have been shown to effectively prevent and degrade various bacterial biofilms, such as those produced by *Campylobacter jejuni*, *Escherichia coli*, and *Proteus mirabilis* (Carson et al., 2010; Chibeu et al., 2012; Siringan et al., 2011). Phages capable of degrading biofilms may possess depolymerizing enzymes such as a glycanase, which breaks down EPS. For this to occur, the phage binds to biofilm polysaccharide, which serves as a secondary receptor. The glycanase degrades the biofilm by hydrolyzing β-glycosidic linkages until the phage has reached the primary receptor on bacterial cell's exterior surface. The phage then adsorbs to the bacterial receptor and begins replicating lytically or lysogenically (Hughes et al., 1998; Rieger-Hug and Stirm, 1981). Some mycobacteriophages have been shown to disrupt *M. smegmatis* biofilms (Kiefer and Dahl, 2015). Our results support the previous observations that treating established mycobacterial biofilms with phage can increase biofilm formation (Hughes et al., 2016). The underlying mechanisms related to enhancement or disruption of the biofilms by phages remain unknown and are not fully investigated. We can speculate that some of the annotated genes with no known function may contribute in some way. Our future work will examine some of these possible mechanisms.

Table 1. The host range of OKCentral2016.

Bacterial Strain	Phage Infectivity (+/-)
M. abscessus subspecies *bolletii* MC1518	-
M. avium DJO-44271	-
M. intracellulare 1956	-
M. kansasii 824	-
M. simiae MO-323	-
M. smegmatis mc^2 155	+

Acknowledgments

The following reagents were obtained through BEI Resources, NIAID, NIH: *Mycobacterium abscessus*, Strain MC1518, NR-44266, *Mycobacterium avium*, Strain DJO-44217, NR-49092, *Mycobacterium intracellulare*, Strain 1956, NR-44267, *Mycobacterium kansasii*, Strain 824, NR-44269, *Mycobacterium simiae*, Strain MO-323, NR-4434. We want to thank Mr. Ben Fowler at the imaging core facility at Oklahoma Medical Research Foundation for his help with electron microscopy. Funding for this project was acquired through the University of Central Oklahoma, Office of Research and Sponsored Programs – RCSA Grant program, and by the National Institute of General Medical Sciences of the National Institutes of Health under award number P20GM103447. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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THE COLONIZATION OF ARTIFICIAL LEAF SHELTERS BY LEPIDOPTERAN LARVAE AND OTHER ARTHROPODS ON *QUERCUS RUBRA*

Yongzhi Pan and H. George Wang, East Central University

Field manipulative experimentation is useful in discerning ecological community patterns and the mechanisms causing them. Such experiments often utilize artificially constructed structures to simulate variations in biological or environmental conditions. Leaf shelter building by Lepidopteran larvae is a type of ecosystem engineering that creates microhabitats utilized by many arthropods. We conducted an experiment to study the effect of forest edge on the distribution of leaf shelter building caterpillars by creating artificial leaf ties on red oak (Quercus rubra) trees using metal clips. We selected 20 trees at the Nature Conservancy's Pontotoc Ridge Preserve in Pontotoc County, OK, and constructed five artificial leaf ties on each tree by clipping two pieces of leaves together with a metal clip for each tie. The caterpillar and overall arthropod communities in these leaf ties were monitored over the summer of 2019. The majority (over 90%) of the artificial leaf ties were colonized by arthropods by late summer (August 2019).

EPIGENETIC REGULATION OF MICRORNA395 IN *ARABIDOPSIS* IN RESPONSE TO SULFATE DEPRIVATION

Pei Jia Ng and Ramanjulu Sunkar, Oklahoma State University

The chromatin remodeling, histone variants, DNA methylation and histone modifications, all bring changes in the chromatin state and play an essential role in regulating gene expression. In plants, microRNAs act as master regulators in various biological processes such as the plant growth and development and stress responses including nutrient deprivation. Previously we have shown that microRNA395 (miR395) is strongly induced by sulfate starvation in *Arabidopsis* and regulates the transcript abundances of a sulfate transporter (AST) and three ATP sulfurylases (APS) (Jagadeeswaran et al., 2014). In the present study, we propose to decipher the role of H2A.Z (histone variant) and epigenetic regulators in induction of miR395 during sulfate deprivation. Gene expression is positively correlated with the presence of H2A.Z at a locus. To address the role of H2A.Z in miR395 regulation, we are utilizing knockout mutants in genes encoding H2A.Z (hta9 and hta11) in Arabidopsis. Furthermore, H2A.Z promotes the binding of H3K4 methyltransferase (ATX) and promotes H3K4me3 deposition. To address the role of histone modifications (H3K4) in miR395 regulation, we are utilizing mutants defective in H3K4 methyltransferases (ATX1 and ATX2). Our methodology include measuring the expression levels of MIR395 loci using qPCR, and assaying for histone modifications using ChIP assay in H2A.Z mutants (hta9 and hta11) and H3K4 methyltransferase mutants (atx1 and atx2) under sulfate-deprivation. We will present our current progress on this project. Overall, the results will contribute to our understanding on the role of chromatin remodelers and epigenetic regulators in regulating miR395 expression under sulfate deprivation.

STUDYING YFAX IN *ESCHERICHIA COLI*

Brenna Hefley, Samantha Perry, and April Nesbit, East Central University

Outstanding Undergraduate Paper in Biochemistry and Molecular Biology Section

Even though *Escherichia coli* has been studied for over 100 years, the function of some proteins in *E. coli*, including YfaX, are still unknown. YfaX protein is predicted to be a transcription factor, and it is the first gene in the yfaXWVU operon. It has been suggested that other proteins in this operon interact with rhamnonate in vitro. Therefore, we tested the ability of *E. coli* to metabolize rhamnonate in vivo. We observed that *E. coli* cannot grow under aerobic in the presence of rhamnonate. Due to the inability of *E. coli* to metabolize rhamnonate in vivo, we looked at other possible sugars to interact with this operon. One sugar that was mentioned in the in vitro study was lyxonate, which is not commercially available, plays a role in vitro. In the place of lyxonate, we used ascorbate, which is predicted to degrade to lyxonate in the human intestines where *E. coli* often grows. We performed growth curves with ascorbate and found that *E. coli* could grow slowly with ascorbate only under anaerobic conditions. To test the effect on the yfaXWVU operon, we ran gene expression assays and discovered that yfaX promoter expression increased three-fold in the presence of ascorbate compared to glucose. However, there was no difference in gene expression with or without YfaX under conditions tested, indicating that YfaX does not act as a transcription factor to regulate the yfaX promoter. Furthermore, expression was increased in ascorbate compared to glycerol, suggesting that there is an ascorbate-specific response separate from catabolic repression. Based on these studies, we cannot determine whether YfaX is a transcription factor, but ascorbate does play a role in yfaX gene expression.

TRANSPORT AND RECOVERY OF IRON OXIDE, ALUMINUM OXIDE, AND TITANIUM DIOXIDE NANOPARTICLES THROUGH SEDIMENTARY ROCK

Dario Butler, Ricardo Buerra, George Wang, and Randall D Maples, East Central University

Metal oxide nanoparticles like aluminum oxide, iron oxide, and titanium dioxide have an interesting potential for use in subsurface characterization and modeling in the groundwater environment and as well are used along with other nanoparticles in various new materials due to their unique physiochemical properties. A unifying theme in the potential use of metal oxide nanoparticles as chemical tracers or the likelihood of metal oxide nanoparticles being potential contaminants in groundwater would be the transport characteristics and recovery of these nanoparticles. This study continues previous work looking at the transport and recovery of iron oxide nanoparticles through columns packed with limestone or dolostone and groundwater collected from the Arbuckle-Simpson aquifer, and extends this work to both aluminum oxide and titanium dioxide nanoparticles.

COMPARISON OF MICROHABITAT SELECTION BETWEEN RIFFLE DWELLING DARTERS, THE ORANGETHROAT DARTER (ETHEOSTOMA SPECTABILE) AND ORANGEBELLY DARTER (ETHEOSTOMA RADIOSUM) IN UPPER BLUE RIVER OF OKLAHOMA

Kourtney Myskey, East Central University

The Blue River of south-central Oklahoma is a spring-fed stream that drains much of the eastern Arbuckle-Simpson Aquifer, and is one of only two free-flowing rivers in Oklahoma with little to no anthropogenic influences on the natural flow. Not much is known about the riffle-dwelling fish communities in the upper reaches of the Blue River. In collaboration with The Nature Conservancy, assessments of fish inhabiting riffle mesohabitats of the Blue River were conducted in the summer of 2018. Individual fish were identified to species and biological metrics were calculated. Relative abundance from two data sets was measured for the Orangethroat darter (*Etheostoma spectabile*) and Orangebelly darter (*Etheostoma radiosum*). A total of eighteen species of fish were collected from the riffle mesohabitats in the upper reaches of the Blue River. The Orangethroat and Orangebelly darter were more likely to be found in areas in the riffles where river bed particles were in the small to large cobble size range. Looking at biota in this river could give insight into how different habitats function in a free-flowing river, and more specifically, in riffle habitats of the Blue River since these areas will be the first habitats affected if flows are reduced from withdrawals of water from the Arbuckle-Simpson Aquifer.

IDENTIFICATION OF BACTERIA THAT INHIBIT *ENTEROCOCCUS* GROWTH

Constance Green and April Nesbit, East Central University

Although there are many antibiotic-resistant bacteria now known, six species comprise the majority of the infections seen in healthcare settings. One of the six species that is of great interest is *Enterococcus faecium* (*E. faecium*). To find novel antibiotics that might be useful to treat E. faecium infections, we isolated bacteria from soil because soil has high levels of bacteria known to produce antibiotics. After testing sixteen sites for potential antibiotic-producing bacteria, we found four isolates, which show inhibition of the pathogen *E. faecium*. Inhibition was tested with both the patch-patch method and the top agar-cellophane method to verify that the isolated strains were capable of secreting an antibiotic to inhibit *E. faecium* growth. Identification of these isolates used staining and oxygen requirements indicates that all four isolates are Gram positive, endospore-forming bacilli in the Genus *Bacillus*. Future studies are to verify the species and isolate the secreted chemical causing inhibition of *E. faecium*.

*CHARACTERIZATION OF *DIAPORTHE* SPECIES IMPORTED ON GUATEMALA CANTALOUPES

Erin Dempsey, Sanam Kadel, Rita Ghale, Karuna Devkota, Charlie Biles, and Alisha Howard, East Central University

Melons (Cantaloupe; *Cucumis melo* var. *cantaloupensis*) were purchased from local grocers in 2016 through 2019 and observed for post-harvest diseases. Of the 80 melons purchased in 2019, 75% developed fruit rot symptoms caused by fungi. The diseased tissue indicated that the majority of lesions were caused by *Diaporthe* spp. (syn; *Phomopsis*), and to a lesser extent lesions were caused by *Alternaria* and *Fusarium* spp. Plant pathogens such as *Diaporthe* spp. enter the surface of the melon fruit early in development and remain latent until fruit maturity. While ripe fruit is harvested and imported with no external evidence of disease, internal fruit rot becomes evident as the fruit matures. The objective of this study was to characterize *Diaporthe* spp. imported in Guatemalan melons. Fungal isolates were characterized based on culture growth characteristics, spore morphology, and DNA analysis. Guatemalan isolates were morphologically similar to *D. sojae* and *D. curcurbitae*. Deoxyribonucleic acid (DNA) was extracted from fungal hyphae and purified polymerase chain reactions (PCR) products were Eurofin, Inc. for sequencing. Sequencing analysis demonstrated that some of the isolates were a match for *D. pterocarpi* and species within the *D. arecae* complex. Our finding of pathogenic *Diaporthe* spp. suggest that plant pathogens are carried across international borders and imported into the United States. Further analysis is being conducted on the melons collected in 2018 and 2019.

HIGH FREQUENCY STUDY OF THE ACOUSTIC AND MASS ATTENUATION COEFFICIENTS IN LEAD AND ALUMINUM

Karen A. Williams, East Central University

Previous work (Williams, 2017) revealed a high correlation between the acoustic attenuation coefficient at 1 MHz with the mass attenuation coefficient for calibrated lead and aluminum samples. In this work, the relationships of the two coefficients in two materials (lead and aluminum) were studied at 2 and 4 MHz. The mass absorption coefficient of these absorbers was calibrated by the manufacturer, so only the attenuation coefficient was determined for each material at both frequencies. Results at 1 MHz were brought into this study from 2017 data. The samples were: lead disks, square lead sample with aluminum on the back, and aluminum disks calibrated for gamma radiation studies. The Pearson correlation coefficient between the acoustic attenuation coefficient and the mass absorption coefficient at 1 MHz varied from 0.906 to 0.995; at 2 MHz from 0.846 to 0.973; and 4 MHz from 0.863 to 0.934. These correlations were higher than expected as the amplitude measurements depend on gel thickness and user technique. For round lead samples the slopes at 1 and 4 MHz transducer frequencies were 54140 and 51690 (db/cmMHz)/(cm2/mg) respectively, but at 2 MHz the slope was 24580 (db/cmMHz)/(cm2/mg). For the square lead (Al backed) samples the slope was 11300, 9334, 35910 (db/cmMHz)/(cm2/mg) for 1, 2 and 4 MHz transducer frequencies. For the aluminum samples the slopes were 7062, 7312 and 24450 (db/cmMHz)/(cm2/mg) at 1, 2 and 4 MHz. Curve fit analysis using frequency to the first and second power was selected. The curve fit yielded a root mean square error of zero. The slopes listed above versus frequency for all 3 samples appeared similar with the exception of 1MHz round sample. The first data point which represents the 2017 work for lead appears too high in magnitude, however the other two samples contain some aluminum which could lower their values. Temperature is also a variable that might change the acoustic attenuation coefficient.

READERS DIGEST: MYCOBACTERIOPHAGE AND YOU

Emily Hernandez and Greg Mullen, Oklahoma City University

Drug Resistant Tuberculosis (TB) is a worldwide health crisis. According to the World Health Organization, ~240,000 people died from drug resistant TB in 2016. In response to this global emergency, new methods of treatment are being developed and tested, one of which uses mycobacteriophage. Mycobacteriophage (phage) are viruses that specifically infect mycobacteria such as *Mycobacterium tuberculosis*. This form of therapy will use combinations of phage to avoid issues with resistance, which calls for identification of unique types of phage that enter the bacteria through different pathways. I have isolated phage that are capable of infecting all mycobacterium strains tested to date. I am currently cloning and sequencing the genomes of the phage to determine their relationships to existing phage. I have also isolated phage resistant mutants in *M. smegmatis* and *M. phlei*; three of these mutants are completely resistant to phage infection and the remaining five isolates have reduced susceptibility to infection or delayed phage maturation. I am going to use whole genome sequencing to identify candidate mutations in these resistant strains. I will also test phage isolated in other laboratories for their ability to infect the resistant mutants. My goal is to identify genes required for phage resistance and to develop a simple assay to determine which phage can be usefully combined for phage therapy.

CALCIUM CONCENTRATION AFFECTS THE HOST-PATHOGEN INTERACTIONS OF *PSEUDOMONAS AERUGINOSA* WITH LUNG EPITHELIAL CELLS

Deepali Luthra, Marianna Patrauchan, and Erika Lutter, Oklahoma State University

Outstanding Graduate Poster

Pseudomonas aeruginosa is an opportunistic human pathogen that form biofilms in airway mucosal epithelium in the lung cells of cystic fibrosis (CF) patients. Calcium (Ca^{2+}) levels in the lungs of CF patients are highly elevated and it is studied that Ca^{2+} acts as a trigger for expression of virulence factors in *P. aeruginosa*. Studies show that Ca^{2+} binds directly to the Ca^{2+}-binding protein EfhP of *P. aeruginosa*, but little is known about how Ca^{2+} regulates the virulence of *P. aeruginosa* during infection with A549 human lung epithelial cells. My research project focuses on determining how *Ca2+*, EfhP or other virulence factors such as adhesins, which includes flagella (fliC- flagellin Type B filament) and pili (pilA, Type IV fimbrial protein precursor) affect the adherence of *P. aeruginosa* to A549 cells. We focused on determining the effect of Ca^{2+} on initial adherence of *P. aeruginosa* in low and high Ca^{2+} conditions. The *P. aeruginosa* PAO1 and pulmonary isolates FRD1 strains, each having their corresponding Δefhp mutant and complemented background along with transposon mutants for fliC and pilA were used for this study. Adherence studies show that the wild type, efhP mutant and the complemented PAO1 and FRD1 strains exhibited significant increase in adherence to A549 cells in high Ca^{2+} condition compared to low Ca^{2+}. This indicates that Ca^{2+} plays significant role in enhancing adherence of *P. aeruginosa* to host cells. However, no significant difference was noted between in low and high Ca^{2+} conditions between the wild type and EfhP mutants of *P. aeruginosa* in PAO1 and FRD1 backgrounds. It was also observed that transposon mutants were not as adherent to A549 cells when compared with PAO1 wild type in high Ca^{2+}, suggesting that FliC and PilA play some role in affecting the adherence of *P. aeruginosa* in the presence of high Ca^{2+}.

CLONING, SEQUENCING, AND IDENTIFICATION OF PHAGE 16, AN UNKNOWN SALMONELLA OR EHEC (ENTEROHEMORRHAGIC *E. COLI*) BACTERIOPHAGE

Shrea Tyagi, Union High School, Tulsa, OK

Nayna Nambiar, Holland Hall, Tulsa, OK

B.J. Reddig and E.L. Blewett, Oklahoma State University – Center for Health Sciences, Dept. of Biochemistry and Microbiology, Tulsa, OK

P. Litt and D. Jaroni, Oklahoma State University – Dept. of Animal and Food Science

Bacteriophage are viruses that infect, replicate and kill bacteria. Salmonella and EHEC food poisoning are caused by Salmonella and *E. coli* bacteria. Bacteriophage can be used to prevent food poisoning by application to food products or processing machinery. Bacteriophage P16 specifically infects Salmonella and *E. coli* bacteria. We cloned fragments of the P16 genome, sequence the DNA and used bioinformatics to identify P16. Phage P16 is a Salmonella phage similar to "Stitch". A phylogenetic tree inferring relationships of P16 and other bacteriophage was created.

INHIBITION OF CLINICAL ENTEROVIRUS ISOLATES BY NATURAL COMPOUND OSW-1

Reddig, B.J. and Blewett, Earl, OSU-CHS, Dept. of Biochemistry and Microbiology, Tulsa, OK

Roberts, Brett and Burgett, Anthony, University of Oklahoma, Dept. of Chemistry and Biochemistry, Norman, OK

OSW-1 is a small, molecular compound isolated from the bulbs of the plant, *Ornithogalum saudersiae*. OSW-1 has been shown to kill cancer cells and to inhibit viral infection. OSW-1 interacts with cellular oxysterol-binding protein (OSBP) and reduces OSBP in the cell. OSBP is important in host cell cholesterol processing and traffic.

Enteroviruses belong to the *Picornaviridae* family and are single-stranded RNA (ssRNA) viruses. They cause many important human diseases including rashes, pleurodynia, encephalitis and aseptic meningitis. Many ssRNA viruses use host cell membranes to create replication organelles (ROs) in the infected cell. The virus uses ROs to concentrate and hide virus materials from the host cell, to avoid triggering anti-viral responses.

We hypothesize all ssRNA viruses create ROs in order to replicate in eukaryotic cells. In this project, we test clinical enterovirus isolates to see if these viruses use OSBP and cholesterol to create their ROs. We show that OSW-1 inhibits infection by Coxsackieviruses A and B, Echoviruses and Enterovirus-D68.

CLONING AND SEQUENCING OF THE DEPOLYMERASE-LIKE GENE FROM BACTERIOPHAGE J25

Nayna Nambiar, Holland Hall, Tulsa, OK

Shrea Tyagi, Union High School, Tulsa, OK

B.J. Reddig, and E.L. Blewett, Oklahoma State University – Center for Health Sciences, Dept. of Biochemistry and Microbiology, Tulsa, OK

P. Litt and D. Jaroni, Oklahoma State University – Dept. of Animal and Food Science

Bacteriophage are viruses that infect, replicate and kill bacteria. Salmonella and EHEC food poisoning are caused by Salmonella and *E. coli* bacteria. Bacteriophage can be used to prevent food poisoning by application to food products or processing machinery. Bacteriophage J25 specifically infects Salmonella and *E. coli* bacteria. We cloned fragments of the J25 genome, sequence the DNA and used bioinformatics to identify J25. We used genome data from similar bacteriophage in Genbank to design primers to amplify the depolymerase-like gene. We amplified and cloned this gene. When expressed, the gene product will be test with bacteriophage food treatment where it should augment bacteriophage killing.

CORRELATING TELOMERE LENGTH WITH DISEASES AND NOVEL GENETIC VARIANTS

Peter Gerstenberger and Celestino Velasquez, Oral Roberts University
Peter Gerstenberger, Patrick Allaire and Scott Hebbring, Marshfield Clinic Research Institute, Center for Human Genetics, Marshfield, WI.

Best Undergraduate Paper of Academy and Outstanding Undergraduate Paper in Biomedical Science Section

Telomeres are the repetitive non-coding short DNA segments that cap chromosome ends and function to protect vital genetic information. Telomere length correlates directly with the proliferative capacity of the parent cell, shortening by approximately 10 base pairs per replication cycle. When telomeres become too short, the DNA damage-response signaling pathway is triggered, causing cellular senescence. Shortened telomeres are associated with many age-related diseases as well as inheritance-related disorders, including type II diabetes and cancer. The goal of this project included two objectives: 1) find new associations between telomere length (TL) and various diseases via a Phenome-Wide Association Study (PheWAS), and 2) discover associations between TL and genetic variants via a Genome-Wide Association Study (GWAS). All genetic samples from the Personalized Medicine Research Project (PMRP) Biobank (includes ~20,000 patients) were genotyped to determine the relative average telomere length (raTL) using quantitative PCR, and then compared to each patient's electronic health record, containing codes for 8,989 phenotypic diseases (PheWAS). The telomere data was then correlated with over 8 million genomic single nucleotide polymorphisms (SNPs) to define associations between TL and genetic variants (GWAS). Preliminary results from the PheWAS show correlations between telomere length and conditions including atherosclerosis, heart disease, obesity, presbyopia, bronchitis, and diabetes. The strongest preliminary association signals in the GWAS were among variants already known to be linked with TL, including the genes RTEL1, TERC, and TERT. This tells us that our initial GWAS analysis was successful, and other discovered associations can be trusted. We successfully found phenotypic diseases and genetic variants associated with telomere length. The remaining data is still in process of being cleaned, adjusted, and associated with the correct health records, but preliminary data is promising. Follow-up studies will be performed to implement a PheWAS of the TL-associated SNPs.

CARBONIC ANHYDRASE, PSCA1 CONTRIBUTES TO THE VIRULENCE OF THE HUMAN PATHOGEN *PSEUDOMONAS AERUGINOSA*

Reygan E. Braga, Biraj B. Kayastha, and Marianna A. Patrauchan, Oklahoma State University

Outstanding Undergraduate Paper in Microbiology Section

Calcium deposition and calcification of soft tissue has been associated with several bacterial chronic infections including cystic fibrosis (CF). CF is associated with elevated levels of calcium in the body fluids resulting in calcification of organs. However, the exact molecular mechanisms of such calcification are not very clear. The opportunistic human pathogen *Pseudomonas aeruginosa* is the predominant cause of mortality and morbidity in CF patients. We hypothesized that this pathogen deposits extracellular calcium, a process that requires carbonic anhydrases (CAs). Previously, we have identified three β-class carbonic anhydrase genes, psCA1, psCA2 and psCA3 in *P. aeruginosa* PAO1. We showed that the expression of psCA1 is induced by elevated calcium and that this CA plays a major role in calcium deposition. We hypothesized that the ability of *P. aeruginosa* to deposit calcium enhances virulence of the pathogen and that psCA1 contributes to this process. To test this hypothesis, we used Galleria mellonella (wax worm) infection model. We observed that injection with PAO1 grown without added calcium resulted in death of up to 40% worms 20 hours post injection (hpi). However, injection with PAO1 grown at 10mM Ca2+ resulted in death of 80% worms 20 hpi. This supported the inducing effect of Ca^{2+} on *P. aeruginosa* virulence. The psCA1 deletion mutant failed to kill any worms even after 20 hpi, which demonstrated the importance of the enzyme in *P. aeruginosa* virulence. We also tested the effect of acetazolamide, earlier shown as an inhibitor of psCA1 enzymatic activity, but no significant impact on virulence was detected. We aim to use this model to study the effects of other CA inhibitors on PAO1 virulence. We also aim to determine the effect of calcium and other host factors on the transcription of psCA genes by using promoter activity approach both in vitro and in vivo.

A PRELIMINARY SURVEY OF FRESHWATER SPONGES IN OKLAHOMA

Emily Sample, Emily Boyer, Casie Hamill, Destiny Hamilton, Kyler Keef, Tyler McKenzie, Angela Spottedwolf, Rhonda Weigand, and Brenda Witt, Redlands Community College

Outstanding Undergraduate Paper in Applied Ecology and Conservation Section

Freshwater sponge distributions in Oklahoma have been understudied with only two minimal surveys published between 1922 and 1954. To expand upon this previous data, we surveyed littoral areas of selected water bodies throughout central and southern Oklahoma spanning January through March of 2018 and 2019. Water quality parameters including temperature, pH, salinity, specific conductivity, and dissolved oxygen were measured at each site using the In-Situ smarTROLL Multiparameter Handheld probe. Any substrate for which sponges would be likely to attach, such as rocks and logs, were visually examined and samples of adult sponges or reproductive gemmules were collected using sterile razor blades and stored in 70% ethanol to be identified via DNA barcoding. Of the sites sampled, 9 of 21 were positive for sponge presence and sites with and without sponges were marked on a state county map. A non-metric multi-dimensional scaling analysis (NMDS) indicated that sites with sponges were distinctly dissimilar from those where sponges were not found. Further analysis suggested that pH and specific conductivity are the main drivers of these differences, however a larger sample size inclusive of a wider variety of geological and ecological areas will better illustrate trends in preferred environmental conditions. Our study demonstrates that freshwater sponges are established in Oklahoma and that continued statewide surveying will further knowledge of their habitats and role in ecosystems.

THE EFFECTS OF LIGHT INTENSITY ON GROWTH AND CHLOROPHYLL PRODUCTION IN *CANNABIS*

Samantha Middleton and Stanley Rice, Southeastern Oklahoma State University

Outstanding Undergraduate Paper in Biological Science Section - Botany

In June of 2018 residents of Oklahoma effectively legalized the consumption and cultivation of medical cannabis for individuals who have a Medical Marijuana Patient Card. We conducted an experiment to determine the biomass allocation and chlorophyll production patterns in low light and high light *Cannabis indica* plants. Clones from the same mother plant were grown under an optic LED light source positioned at different distances from the light. High light plants were closer to the light source than low light plants, thus the light quality was the same in both treatments while the intensity received was different. We weighed stems, leaves, and roots and measured chlorophyll extracted in DMF to determine plant production and chlorophyll amount. We found low light plants grew taller relative to their total weight than high light plants and had more chlorophyll relative to leaf area. Relative leaf weight was not affected by growth light level. Middleton can cultivate up to 6 nonflowering cannabis plants legally within the state by being a registered medical marijuana patient with the OMMA.

SMART MEDICAL DEVICE

Erin Drewke, Jessica Petty, Mai Pham, and Nesreen Alsbou, University of Central Oklahoma

Outstanding Undergraduate Paper in Engineering Science Section

To provide accurate, non-invasive, real-time, and less painful monitoring of oxygenation and circulation for pediatric patients in hospitals. To optimize Cardiopulmonary Resuscitation (CPR) for patients utilizing non-invasive values: Cerebral and Renal Regional Oxygen Saturation (C-rSO2 and R-rSO2), End-Tidal Carbon Dioxide (EtCO2), Oxygen Saturation (SpO2), and Volume of CO2 (VCO2) signals. Signals are to be intercepted real-time from INVOSTM 5100C Cerebral/Somatic Oximeter and Respironics NM3 Profile Monitor. Values will be processed through algorithms to determine the likelihood of Return of Sudden Circulation (ROSC) using Youden Index. Oxygenation and circulation variables will be measured using Near Infrared Spectroscopy (NIRS). This will output to a monitor and be viewed by a medical official or technician to view along with generated plots of intercepted readings. This approach for non-invasive, real-time monitoring can be evaluated further by implementing algorithms of trending vitals in patients after using this device and gathering data in numerous patients. This can provide early detection of patients likely to undergo cardiac arrest so medical officials can provide medicine or medical attention as needed to address the issues that may lead to cardiac arrest or intervene during cardiac arrest.

PERCEIVING CHARACTERISTICS OF ABDUCTED CHILDREN

Taylor Pjesky and Robert Mather, University of Cental Oklahoma

Outstanding Undergraduate Paper in Social Science Section

The goal of the media is to get recognition and views. Due to this goal, the media does not always provide accurate information to the public. This especially happens when the media covers child abduction cases. This leads to the public misunderstanding a very important topic, our children. This study will aid in the correction of these misunderstandings, which can be used in prevention programs. Participants will be primed with facts and statistics concerning characteristics of abducted children. They will then complete an online questionnaire about the probable characteristics of abducted children. It is hypothesized that the four primed experimental groups will show more accurate knowledge of characteristics of abducted children, whereas, the control group will show beliefs of common myths, and less accurate information surrounding characteristics of abducted children. Data are still being collected.

NUMERICAL MODELING AND SIMULATION OF A MICROFLUIDIC PLATFORM FOR ENRICHMENT OF LOW ABUNDANCE PROTEINS

Frances Matthews, Mohammad Hossan, and Sanjeewa Gamagedara, University of Central Oklahoma

Outstanding Undergraduate Paper in Physical Science Section

Circulating TGF-β1 is one of the key regulators of cardiovascular health. The extremely low abundance of circulating TGF-β1 in blood is one of the major challenges in on-chip purification and extraction. This paper reports numerical modeling and simulation of more than 25000 folds concentration gain of TGF-β1 in a 2D cascade microchannel using isotachophoresis (ITP). The 4.3 cm long microfluidic channel with 250 times reduction in cross-sectional area from inlet to outlet was used for ITP simulation. The initial cross-sectional area was 1250 micrometer x 100 micrometer and the final cross-sectional area was 50 micrometer x 10 micrometer with 2D step changed. The reduction in cross-sectional area were used to complement ITP concentration gain. COMSOL Multiphysics 5.2 was used to simulate the separation and concentration of two proteins-TGF-β1 and albumin. The model used the Nernst-Planck equations to predict protein stacking and separation in the sample solution. Microchip with 1D and 2D step changed microfluidic channels were also explored using numerical simulations. In the simulation, the leading electrolyte (LE) was 10mM Hydrochloric acid (HCl) adjusted to a pH of 9.5 with Tris (1M) and the trailing electrolyte (TE) consisted of 60 mM DNP- epsilon -amino-n-caproic acid (EACA) adjusted to a pH of 10.0 with Tris (1M). A constant DC electric potential of 200 V was applied between anode and cathode reservoir. The proteins migration was observed under a fluorescence microscope and images of proteins band in different locations were taken. The initial concentration of TGF-β1 and alubumin was 1.25 microgram/ml and after ITP concentration, the each protein exhibited more than 25000 folds (~33 mg/ml) concentration gain. This is a significant improvement in protein concentration factor compared to our previous report in an ITP microchip. This demonstration can be utilized in the development of integrated microchip to detect low abundant proteins.

ANTI-PROLIFERATION EFFECT OF DANDELION'S EXTRACT ON HELA CELLS

Brooke Wiens, Chigozie Agu, Eleanor DeCelle, and Christina Hendrickson, University of Central Oklahoma

Outstanding Undergraduate Poster

We are currently working on a project that is determining whether or not dandelion root has the ability to stop cancer cell growth. This prospect originates from a man in Turkey who claimed to be cancer free after 40 days of drinking the root juice every morning. By studying the effects of this juice in varying concentrations, we are looking to investigate the validity of this claim.

GENERALIZED MODULAR POLYGONS

Effouehi Messou, University of Central Oklahoma

Outstanding Undergraduate Paper in Mathematics, Statistics, & Computer Science Section

Long before Sudoku people were interested in a different type of number puzzle called a "magic square" which is a square grid filled with distinct positive integers in the range such that each cell contains a different integer and all of the rows, columns, and diagonals must add to the same value. As early as 190BC, mathematicians have been fascinated by these magic squares and have discovered some amazing results concerning them as well as generalizations and modifications of them. In this talk we will describe one such generalization known as the "modular magic square" in with the rows, columns and diagonals must no longer add to the same value, but rather the remainder we obtain when we divide those rows, columns and diagonals sums by some fixed values must be equal. For instance, we could try to find a magic square in which all of the rows, columns and diagonals sum to an even number; where the remainder is 0 upon division by 2; or find a magic square in which all the rows, columns and diagonals sum to an odd number; when the remainder is 1 upon division by 2. We will also discuss how one could consider other polygonal shapes, such as rectangles and triangles(a magic triangle will be an arrangement of positive integers on the sides of a triangle with the same number of integers on each side, so that the sum of integers on each side add to the same value), resulting in "modular magic polygons". Finally, we will share a few of the results we have proven concerning modular magic rectangles; namely, we will describe conditions for which a modular magic rectangle can have rows and columns which all sum to even, or all sum to odd, values.

COMPUTATIONAL MODELING OF ADVANCED MATERIALS FOR PHOTOVOLTAIC AND BIOSENSING APPLICATIONS

Benjamin O. Tayo, University of Central Oklahoma

Quantum mechanics provides us with a complete set of equations that can be solved in order to determine the properties of any system made up of electrons and nuclei such as atoms, molecules, polymers, and crystals. With the revolution in modern computer technology, it has now become possible to apply quantum mechanics to different fields such as materials sciences, renewable energy, computational biology, drug design, molecular electronics, and genomics. The advantage of computational modeling lies in the fact that we can perform large scale exploratory studies in a fast and cost-effective manner. This can save thousands of dollars compared to trial and error exploratory experimental studies. In this presentation, we discuss how quantum calculations can be used for predicting the properties of redox-active polymers for light-harvesting applications, and 2D materials-biomolecules systems for biosensing applications. This research can shed useful insights that can guide the development of novel photovoltaic devices and DNA sequencing technologies.

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THE EFFECT OF NICOTINE AND COTININE ON THE DEVELOPMENT OF *COCHLIOMYIA MACELLARIA* (FABRICIUS) (DIPTERA: CALLIPHORIDAE)

Elise Hodges, Gautham Gautham, Heather Ketchum, and Eric Bright, University of Oklahoma

Outstanding Undergraduate Paper in Biological Science Section – Zoology

Nicotine, readily available in electronic nicotine delivery systems, poses a lethal threat as it is easily accessible and highly toxic in its liquid form. Seventy-five percent of nicotine is metabolized into cotinine in as little as 20 minutes in the blood plasma and has 10-times longer half-life than nicotine. With the growing prevalence of nicotine-related deaths comes the increased possibility of finding nicotine or cotinine in the tissues of a corpse, which could distort postmortem interval (PMI) estimates. Through entomotoxicology, the study of how drugs and toxins influence the development of insects present on a decomposing body, this study aimed to determine if varying concentrations of nicotine and cotinine affected the development of the forensically important *Cochliomyia macellaria* (Fabricius) [Diptera: Calliphoridae], secondary screwworm. In this study, *C. macellaria* maggots were reared on three different concentrations - 25% (QD), 50% (HD), and 100% (LD) of the lethal dose of nicotine. Knowing 75% of metabolized nicotine is cotinine, we used the 75% of the lethal nicotine dose to calculate the QD, HD and LD concentrations of cotinine. Maggot weights and lengths were determined every twelve hours until post-feeding. This study found that nicotine dosage affected the weight and length of maggots. Maggots reared on untreated liver weighed more and were longer than those maggots reared on liver treated with a lethal dose of nicotine. While there was no effect of cotinine on maggot length and weight, there was a significant difference in the growth rate. These results suggest a negative relationship with nicotine dosage and maggot length and weight and a positive relationship on growth rate with cotinine. These results could greatly affect PMI estimations and, consequently, lead to a wrongful conviction.

OKLAHOMA ACADEMY OF SCIENCE

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OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID
FOR THE YEAR ENDED DECEMBER 31, 2018

REVENUES COLLECTED		
Membership Dues:	$1,167.89	$1,167.89
Investment Income:	$47.99	$47.99
Meetings:		
Registration - Fall Meeting	$6,870.38	
Registration - Technical Meeting	$5,129.15	$11,999.53
Donations:	$224.00	$224.00
Woody Plants:	$25.00	$25.00
POAS:	$2,915.00	$2,915.00
Transfer from OJAS	$1,350.47	$1,350.47
Other Income:	$0.00	$0.00
Total Revenue Collected		**$17,729.88**
EXPENSES PAID		
Stipends and other Compensation:		
Stipends	$6,141.24	
Social Security	$618.45	
Medicare	$144.63	$6,904.32
Professional Fees:		
Audit	$500.00	
Tax Preparation	$1,070.00	$1,570.00
Meeting Expenses:		
Fall Meeting	$6,012.41	
Technical Meeting	$1,552.67	$7,565.08
Dues:	$315.00	$315.00
POAS:	$2,654.94	$2,654.94
Woody Plants:	$0.00	$0.00
Other Expenditures:	$2,755.57	$2,755.57
Total Expenses Paid		**$21,273.95**
Revenues Collected Over Expenses Paid		**$-3,544.07**

OKLAHOMA ACADEMY OF SCIENCE
STATEMENT OF ASSETS, LIABILITIES AND FUND BALANCE
ARISING FROM CASH TRANSCATIONS
DECEMBER 31, 2018

ASSETS

Cash:		
Checking Account	$25,400.03	
Savings Account	$3,276.77	
Endowment Savings Account	$2,132.55	$30,809.35
Investments:		
Certificate of Deposit	$60,000.00	$60,000.00
Total Assets:		**$90,809.35**

LIABILITIES AND FUND BALANCE

Liabilities:	$0.00	
Fund balance:		
Beginning operation fund balance	$94,353.42	
Excess revenues collected over expenses	$-3,544.07	
Total Funds:		**$90,809.35**

OKLAHOMA ACADEMY OF SCIENCE

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$40 Library/Institute

Section Affiliations: Number up to three areas of interest. 1=first choice; 2=second choice; 3=third choice.

_____A Biological Sciences_____E Science Education_____I Engineering Sciences _____M Environ. Sci.
_____ B Geology _____F Geography_____J Biochemistry/Biophysics_____N Biomedical Sci.
_____ C Physical Sciences_____G Fish and Wildlife_____K Microscopy_____Y Collegiate Acad.
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The corresponding author is notified of the receipt of a manuscript, and the Editor sends the manuscript to at least two reviewers, anonymous to the author(s). After the initial review, the Editor either accepts the manuscript for publication, returns it to the author for clarification or revision, sends it to another referee for further review, or declines the manuscript.

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

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A. Submission Process.

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Prospective authors should note carefully the policy statement "Policies of the *Proceedings*" on page ii.

The Editors review the MS and carefully select other reviewers as described in "Editorial Policies and Practices" (see p. 151); all referee and editorial opinions are anonymous. Send a resubmitted and/ or revised manuscript and a point-by-point response to the reviewers'/Editor's comments.

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

B. Types of Manuscripts.

A manuscript may be a paper (report), review, note (communication), a technical comment, or a letter to the editor. All manuscripts should be submitted as a Microsoft Word document, 12-point Times New Roman font, double spaced, and include line numbers.

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

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

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Proceedings. The quality or relevance of work may not be in question, but the printed material is still subject to scientific accuracy.

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

C. Manuscript Organization.

1. General organization.

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

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

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

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

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

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

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

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

VIII. The Discussion section should interpret the Results and how these observations fit with the results of others. Sometimes the combination 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.

1. References

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Examples of References in CBE Style and Format

Journal Articles

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

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

Books

Book with Authors:

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

Book with Editors:

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

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

Chapter in Book with Editors:

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).

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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.

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