PROCEEDINGS
of the
OKLAHOMA ACADEMY OF SCIENCE
Volume 98
2018

EDITOR: Mostafa Elshahed

Production Editor: Tammy Austin

Business Manager: T. David Bass

The Official Organ of

the

OKLAHOMA ACADEMY OF SCIENCE

Which was established in 1909 for the purpose of stimulating scientific research;
to promote fraternal relationships among those engaged in scientific work in Oklahoma;
to diffuse among the citizens of the State a knowledge of the various departments of science;
and to investigate and make known the material, educational, and other resources of the State.

Affiliated with the American Association for the Advancement of Science.

Publication Date: January 2019

POLICIES OF THE *PROCEEDINGS*

The *Proceedings of the Oklahoma Academy of Science* contains papers on topics of interest to scientists. The goal is to publish clear communications of scientific findings and of matters of general concern for scientists in Oklahoma, and to serve as a creative outlet for other scientific contributions by scientists. ©*2018 Oklahoma Academy of Science*

The *Proceedings of the Oklahoma Academy of Science* contains reports that describe the results of original scientific investigation (including social science). Papers are received with the understanding that they have not been published previously or submitted for publication elsewhere. The papers should be of significant scientific quality, intelligible to a broad scientific audience, and should represent research conducted in accordance with accepted procedures and scientific ethics (proper subject treatment and honesty).

The Editor of the *Proceedings* expects that the author(s) will honor the following principles of the scientific community, which are also policies of the *Proceedings*.

1. Each author shall have contributed substantially to major aspects of the planning, execution, and /or interpretation of the study and to the preparation of the manuscript. It is assumed that all listed authors concur in the submission, that final copy of the manuscript has been seen and approved by all authors, and that all authors share responsibility for the paper.

2. Submission of manuscripts based on unique material (e.g. cloned DNAs; antibodies; bacterial, animal, or plant cells; hybridomas; viruses; and computer programs) implies that these materials will be available for noncommercial purposes to all qualified investigators.

3. Authors of manuscripts reporting nucleic acid sequence(s) must be prepared to submit relevant data to GenBank. Authors of manuscripts reporting crystallographic studies must be prepared to submit the relevant structural data to the Protein Data Base and/or other appropriate repository. Information necessary for retrieval of the data from the repository will be specified in a reference in the paper.

4. Manuscripts that report research involving human subjects or the use of materials from human organs must be supported by a copy of the document authorizing the research and signed by the appropriate official(s) of the institution where the work was conducted. Research involving humans and animals must have been performed in accordance with the recommendations from the Declaration of Helsinki and the appropriate guidelines of the National Institutes of Health.

5. Submission of manuscripts that report research on/with recombinant DNA implies that the relevant physical and biological containment levels conformed to the guidelines of the National Institutes of Health; the Editor may request proof to substantiate this implication.

6. The *Proceedings* does not publish statements of priority or novelty, or mere descriptions of work in progress or planned. The dates of manuscript receipt, revision, and acceptance will be published.

7. A primary research report must contain sufficient details and reference to public sources of information to permit other scientists to repeat the work.

8. Any unusual hazard encountered or anticipated in using chemicals, equipment, or procedures in the investigation should be clearly identified in the manuscript.

PROCEEDINGS OF THE OKLAHOMA ACADEMY OF SCIENCE

Volume 98

CONTENTS

REPORTS

A. Applied Ecology & Conservation

Miocene Proboscidean Tooth Found in Evaporite Karst Sinkhole near Gate, Oklahoma

Bryce L. Bonnet
University of Oklahoma, Department of Biology, Norman, OK 73019

Nicholas J. Czaplewski
Oklahoma Museum of Natural History, Norman, OK 73072

Kent S. Smith
Oklahoma State University Center for Health Sciences, Office of American Indians in Medicine and Science, Tulsa, OK 74107

Abstract: Fragments of a proboscidean tooth were found in Neogene sediments of the Ogallala Formation within an evaporate karst sinkhole formed in Permian redbeds outside Gate, Oklahoma. The pieces were reconstructed and identified by comparison with museum specimens and literature. The tooth was determined to belong to the family Gomphotheriidae, and the species complex *Gomphotherium "productum."* The species is known by other records in the Late Miocene of Oklahoma and surrounding areas. Although a number of collapse sinkholes with fillings of Ogallala Formation sediments are known in Oklahoma, very few of them have been found to contain identifiable fossils such as this one.

Introduction and Methods

Gomphotheres were elephant-like proboscideans that were globally distributed from the Miocene epoch to the Pleistocene. Gomphotheres (Family Gomphotheriidae) were a diverse and paraphyletic group, but were distinguished by their two upper and two lower tusks. In the Miocene, gomphotheres were distributed all across the North American continent, especially the Great Plains. Specifically in Oklahoma, their fossil record spans from about 11 - 6 Ma (million years ago), where it includes the genera *Amebelodon, Gomphotherium, Rhynchotherium,* cf. *Serbelodon*, and *Stegomastodon* (Lambert and Shoshani 1998; Schultz 2002; Czaplewski and Smith 2003; Czaplewski 2008).

In this paper we describe a proboscidean tooth fragment found in an evaporite karst sinkhole in Beaver County, Oklahoma. Evaporite karst is common in western Oklahoma; Permian redbeds in this region include layers of gypsum and other evaporate rocks that are occasionally prone to subsurface dissolution, forming sinkholes and caves of various types (Meyers 1962; Johnson 1989, 1996; Johnson and Neal 2003; Gutiérrez et al. 2007). These sinkholes are occasionally filled with buff to yellow to brown Neogene sands and silts of the Ogallala Formation, and they sometimes preserve vertebrate fossils in the region (e.g., at the Rowe-Lewis Ranch quarries in Texas with late Miocene horse bones and leaf fossils; Schultz 2002). At times, these local structural collapses can be larger and develop sizable fillings of Ogallala Formation sediments with abundant vertebrate fossils. In the Miocene they may have held small lakes and ponds with fish, turtles, and alligators, as well as horses and other terrestrial animals on the surrounding uplands, as evidenced by fossils of the Beaver local fauna, Oklahoma (Schultz 2002).

The sinkhole in which the fossil tooth occurred was located along an approximately 4 m-high bluff that runs horizontally for about 45 m and was possibly exposed by local faulting

as well as subsurface collapse; in this bluff, the strata are dipping to the east, possibly indicating a larger-scale collapse outside the boundaries of the small, vertical-walled sinkhole in the central portion of the exposed bluff. The small vertical-walled sinkhole is well exposed in cross-section within the bluff (Figure 1), and has a semicircular surface expression (seen in plan view in Google Earth imagery) truncated by the exposure of the bluff face. In cross-section the Permian beds dip from both vertical walls of the sink toward the center of the sink (Fig. 1; partly obscured by modern trees). In this part of Beaver County, widespread Permian rocks are overlain by Neogene rocks of the Ogallala Formation. According to Oklahoma Geological Survey surface geology maps, the Ogallala Formation unconformably overlies the Permian Rush Springs Formation in the immediate vicinity of the sinkhole (Stanley et al. 2002). Most beds of the Ogallala Formation in Oklahoma preserve fossils of Miocene age. On the land surface on top of the collapse we observed a thin veneer of pale buff-colored deposits of the Ogallala Formation only 30 m away from the sinkhole, and there is detritus of Ogallala within the sinkhole. The tooth fragment is clearly of Miocene age, and it could have weathered out of the overlying Ogallala Formation deposits and fallen into this sinkhole more recently. Alternatively, Ogallala Formation deposits containing the fossil tooth might have once filled part of the sinkhole in the Miocene, but mostly weathered away since.

The sinkhole is partially filled with Permian breakdown and paler Miocene sediments. Cheek tooth fragments of a mastodont proboscidean were found in this sinkhole by Albert Laverty while surface prospecting for fossils in 2014. The fossil site is designated as Oklahoma Museum of Natural History (OMNH) locality V1748, and is about 9.5 km southwest of the town of

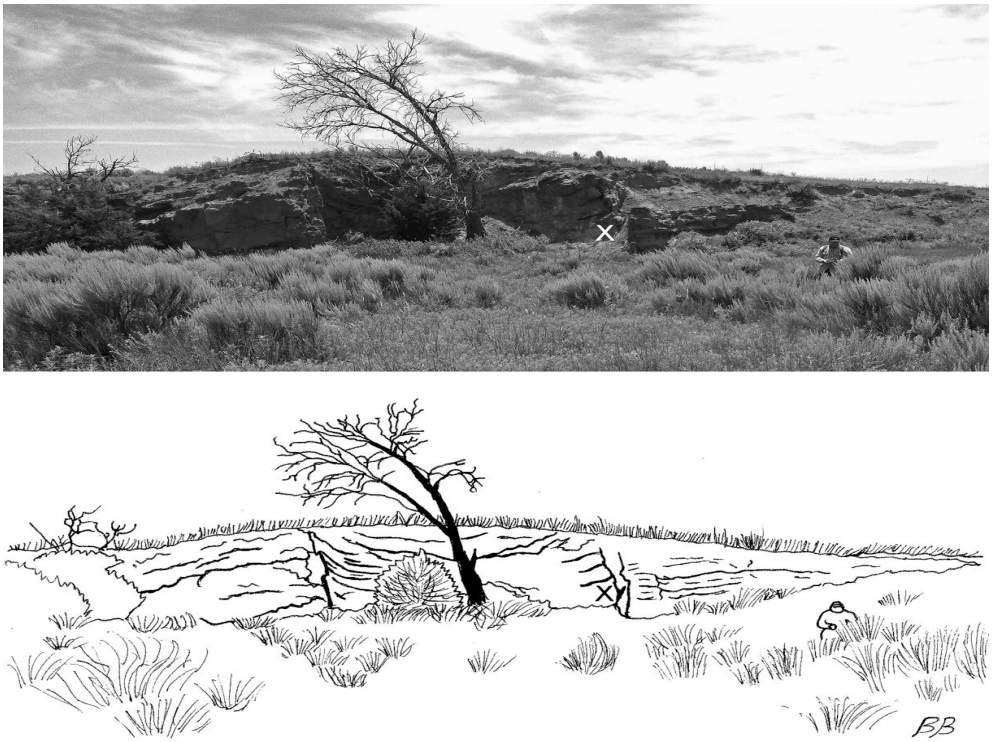


Figure 1. Photograph and interpretive sketch of the fossil locality and collapse sink (with trees growing at the center of the base) in which the partial gomphothere tooth (Oklahoma Museum of Natural History 79124) occurred, Beaver County, Oklahoma. X indicates the spot where the tooth was found. View is toward the south.

Gate, Beaver County, Oklahoma. The site was visited by an OMNH crew in 2015, when we used picks and shovels to dig through more of the sinkhole fill sediments. In the process, a few more fragments of the tooth and a small chip of bone were recovered. The fragments were transported to the Vertebrate Paleontology Lab of the OMNH where they were reconstructed into a partial tooth, now cataloged as OMNH 79124. The tooth was measured with a dial caliper to the nearest 0.01 mm.

Results and Discussion

The tooth is part of a lower left molar, either m2 or m3 (Figure 2). Our initial uncertainty about whether it is an m2 or m3 stems from the fact that at least half of the tooth is missing. Its size matches better that of m3s and is larger than m2s of other gomphothere teeth in the OMNH vertebrate paleontology collection. In addition, it shows a wear facet on one end caused by interdental contact with an adjacent molar. We interpret that end as the anterior end of an m3, because the m3 is the last tooth to appear in adult gomphotheres and the posterior end could not have been in contact with another tooth. The m3 is at a relatively light stage of wear, and has very thick enamel with a simple enamel pattern composed of single trefoils. Due to the incompleteness of the molar, we were only able to make one meaningful measurement; the width of the first lophid is 76.6 mm.

We adopted the dental terminology proposed by Tassy (2014:fig. 2; translated to English). Using a model describing the degrees of zygodonty developed by Wang et al. (2016), we determined that the sinkhole tooth displayed level 0 zygodonty, indicating a relatively primitive level of complexity compared to other gomphotheres and mastodons. Specifically, the m3 shows the following characteristics: blunt pretrite main cuspid, singular non-crestlike pretrite mesoconelet, weak non-crestlike pretrite central conules, blunt non-crestlike postrite main cusp that is anteroposteriorly compressed, large and blunt non-crestlike postrite mesoconelet that is not subdivided, weak postrite central conules, and anteroposteriorly narrow interlophids.

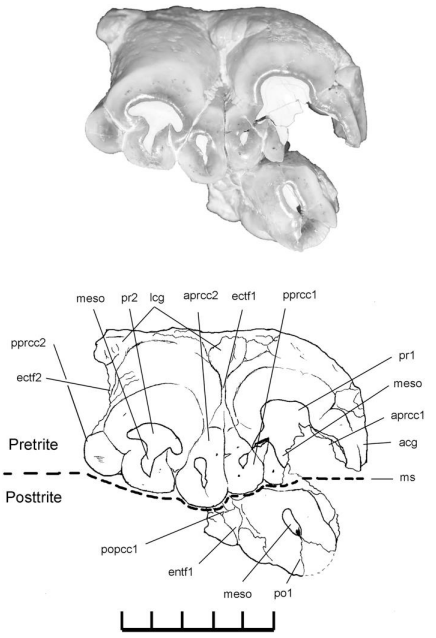


Figure 2. Dental nomenclature for the lower left molar of a gomphothere adapted from Tassy (2014: fig. 2b) and applied to Oklahoma specimen (OMNH 79124). Heavy dashed line indicates the median sulcus, which separates the pretrite and posttrite aspects of the tooth. Abbreviations: acg, anterior cingulum; aprcc1, anterior pretrite central conule of 1st lophid (paraconid); aprcc2, anterior pretrite central conule of 2nd lophid; ectf1, ectoflexus of 1st interlophid; ectf2, ectoflexus of 2nd interlophid; entf1, entoflexus of 1st interlophid; lcg, lingual cingulum; meso, mesoconelet of each half-lophid on either side (pretrite or posttrite) of the median sulcus; ms, median sulcus; po1, posttrite main cusp of 1st lophid (metaconid); popcc1, posterior posttrite central conules of 1st lophid; pprcc1, posterior pretrite central conule of 1st lophid (protoconulid); pprcc2, posterior pretrite central conule of 2nd lophid; pr1, pretrite main cuspid of 1st lophid (protoconid); pr2, pretrite main cuspid of 2nd lophid. Scale bar = 5 cm.

These morphological characteristics demonstrate that the tooth belongs to the genus *Gomphotherium*, which has a fossil record in North America, Eurasia, and Africa. The only species of *Gomphotherium* currently recognized in North America is *Gomphotherium*

"productum." We also compared the sinkhole tooth with other proboscidean teeth identified as *G. "productum"* from the Arnett locality (OMNH locality V54, early Hemphillian). *Gomphotherium "productum"* molars from Arnett that are at a similar stage of wear also very closely match the morphological characteristics of our sinkhole tooth.

The species *G. "productum"* was recognized by Wang et al. (2017) as a species-complex that needs further study, because the taxon might include undiagnosed additional species. For this reason, we place quotation marks around the name "*productum*." The species spans geologic time from 16 million to 4 million years ago (Wang et. al 2017), and accordingly, confirms a Miocene age for the molar from the sinkhole deposit. Mammalian fossils found elsewhere in western Oklahoma in sediments of the Ogallala Formation indicate that the formation there reflects the Late Miocene, and the fossils represent both the Clarendonian and Hemphillian North American Land Mammal Ages (NALMA; Schultz 2002). Typical fossils of the Ogallala Formation in western Oklahoma represent megafauna or large vertebrates (few microvertebrates) including giant tortoises, alligators, scimitar-toothed cats, nimravids (false cats), bone-cracking dogs, shovel-tusked gomphotheres, diverse camelids (up to four genera during the Clarendonian), other extinct artiodactyl families, diverse horses (up to 10 genera during the Clarendonian), and rhinoceroses (Schultz 2002). Relatively few small collapse sinkholes filled with Ogallala sediments are recognizable in western Oklahoma, and fewer still yield vertebrate fossils (Czaplewski 2008; personal observation). This find is a rare and distinctive occurrence of a single identifiable Neogene fossil from a small collapse sink in Oklahoma. Because these sinkholes sometimes form a part of the surface drainage (draining into the subsurface voids) and have been present since at least the Miocene, they probably provided waterholes during the Neogene at which animals like gomphotheres could drink, possibly become trapped, or into which their remains could have been washed. It is not possible to assign this occurrence of *G. "productum"* to one of the two of the Late Miocene NALMAs, because no other faunal remains or taxa were recovered in the collapse sinkhole that could help narrow the age range. Nevertheless, a Late Miocene age is consistent with other Miocene localities and faunas in western Oklahoma (Schultz, 2002).

Acknowledgments

We gratefully acknowledge Alvie Laverty for finding, helping with fieldwork, and donating the specimen to OMNH, and landowners Britt and K'Dawn Hilton and Stanly Barby for access to their property.

Our fieldwork was generously funded by the Chickasaw and Cherokee Nations, and Native Explorers Foundation. We thank the participants in the Native Explorers expedition of 2015; in particular, we appreciate the assistance of Jared Wahkinney, Sara Hofferber, Masheli Billy and the Billy family, Lisa, Phillip, and Anoli, in the fieldwork. Kyle Davies and Brandon Trueblood helped to reconstruct the gomphothere tooth from fragments. We extend a special thanks to Gary S. Morgan for his helpful comments on the specimen and discussion of gomphotheres. Amina M. Switzer provided logistical support.

References

Czaplewski NJ. 2008. Miocene vertebrates from Ogallala Formation sites in western Oklahoma. New Mexico Museum of Natural History and Science Bulletin 44: 1-14. http://econtent.unm.edu/cdm/compoundobject/collection/bulletins/id/624

Czaplewski NJ, Smith KS. 2003. The gomphothere *Stegomastodon* (Mammalia: Proboscidea) in the late Pliocene or early Pleistocene of Oklahoma. Oklahoma Geology Notes 63: 104-111. http://ogs.ou.edu/docs/geologynotes/GN-V63N3.pdf

Gutiérrez F, Cooper AH, Johnson KS. 2007. Identification, prediction, and mitigation of sinkhole hazards in evaporite karst areas. Environmental Geology 53: 1007-1022. https://link.springer.com/article/10.1007/s00254-007-0728-4

Johnson KS. 1989. Salt dissolution, interstratal karst, and ground subsidence in the northern part of the Texas Panhandle. In: Beck BF, editor. Proceedings of the 3rd Multidisciplinary Conference on Sinkholes. Rotterdam: Balkema. p. 115-121.

Johnson KS. 1996. Gypsum karst in the United States. International Journal of Speleology 25: 183–193. http://scholarcommons.usf.edu/cgi/viewcontent.cgi?article=1317&context=ijs

Johnson KS, Neal JT. 2003. Evaporite Karst and Engineering/Environmental Problems in the United States. Oklahoma Geological Survey Circular 109, US Geological Survey and National Cave and Karst Research Institute, National Park Service, 353 p. http://ogs.ou.edu/docs/circulars/C109.pdf

Lambert WD, Shoshani J. 1998. Proboscidea. In: Janis CM, Scott KM, Jabcobs LL, editors. Evolution of Tertiary Mammals of North America. Volume 1: Terrestrial Carnivores, Ungulates, and Ungulatelike Mammals. Cambridge: Cambridge University Press, p. 606-621

Myers AJ. 1962. A fossil sinkhole. Oklahoma Geology Notes 22: 13–15. http://ogs.ou.edu/docs/geologynotes/GN-V22N1.pdf

Schultz GE. 2002. Clarendonian and Hemphillian Vertebrate Faunas from the Ogallala Formation (Late Miocene - Early Pliocene) of the Texas Panhandle and Adjacent Oklahoma. Society of Vertebrate Paleontology Field Trip Guidebook 62nd Annual Meeting, Norman, OK. Oklahoma Geological Survey Open-File Report 10-2002: 35-71. http://ogs.ou.edu/docs/openfile/OF10-2002.pdf

Stanley TM, Suneson NH, Standridge GR. 2002. Geologic map of the Beaver 30' x 60' quadrangle, Beaver, Ellis, Harper, and Texas counties, Oklahoma. OGQ-37, map at 1:100000 scale. http://ogs.ou.edu/docs/OGQ/OGQ-37-color.pdf

Tassy P. 2014. L'odontologie de *Gomphotherium angustidens* (Cuvier, 1817) (Proboscidea, Mammalia): données issues du gisement d'En Péjouan (Miocène moyen du Gers, France). Geodiversitas 36(1): 35-115. https://doi.org/10.5252/g2014n1a2

Wang S-Q, Ji X-P, Jablonski NG, Su DF, Ge J-Y, Ding C-F, Yu T-S, Li W-Q, Duangkrayom J. 2016. The oldest cranium of *Sinomastodon* (Proboscidea, Gomphotheriidae), discovered in the uppermost Miocene of southwestern China: implications for the origin and migration of this taxon. Journal of Mammalian Evolution 23: 155-173. https://link.springer.com/article/10.1007/s10914-015-9311-z

Wang S-Q, Duangkrayom J, Yang X-W, He W, Chen S-Q. 2017. A new species of *Gomphotherium* (Proboscidea, Mammalia) from China and the evolution of *Gomphotherium* in Eurasia. Journal of Vertebrate Paleontology 37(3): e1318284. https://doi.org/10.1080/02724634.2017.1318284

Submitted April 20, 2018 Accepted November 2, 2018

Macroinvertebrate Community Structure and Physicochemical Conditions of a Northwestern Oklahoma Spring

David Bass
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Bobbie Gaskin
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Kinsey Tedford
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Abstract: Little Boiling Spring is a small rheocrene spring located in northwestern Oklahoma emerging from a sandy substrate and eventually flowing into the North Canadian River. Macroinvertebrate collections and water quality measurements were recorded seasonally for an annual period at the springhead and in the springbrook. A total of 31,376 individuals representing 49 taxa were collected in Surber net samples, while an additional three taxa were collected using a dip net. The spring fauna was dominated by worms, crustaceans, and dipterans. Shannon's species diversity values at the springhead differed significantly from those in the springbrook during all seasonal collections, most likely due to increasing levels of oxygen and detritus documented during each collection in the springbrook. Collectors were the most abundant trophic group present in the spring throughout the investigation.

Introduction

Springs are described as naturally occurring sources of emerging groundwater having unique properties, such as discrete habitats with relatively constant conditions with respect to temperature, dissolved oxygen concentration, and flow (van der Kamp 1995). Only a few ecological studies of these special environments have occurred in Oklahoma. Matthews et al. (1983) attempted to determine whether macroinvertebrate community compositions could be useful indicators of groundwater quality, but low similarities between the springs in the study led to inconclusive results. Varza and Covich (1995) concluded that limited food availability and predation by crayfish limited macroinvertebrate abundance in Buckhorn Spring. Bass (2000) reported a combined total of 39 taxa of macroinvertebrates from two springs in the Pontotoc Ridge Nature Preserve sampled during 1995. Based on samples of invertebrates from six springs along a southeast to northwest gradient across Oklahoma, Gaskin and Bass (2000) concluded a unique spring fauna was generally not present in Oklahoma and the spring inhabitants were associated with populations from other nearby stream habitats. Rudisill and Bass (2005) reported 64 taxa, dominated by dipteran larvae, during a year-long investigation from three adjacent springs in Roman Nose State Park. Graening et al. (2006) compiled a checklist of all amphipods known from subterranean habitats, including springs, in Oklahoma. Three springs in south-central Oklahoma yielded 114 invertebrate taxa over an annual period, dominated by the species complex *Hyalella azteca* and *Tanytarsus* (Brown and Bass 2014). Bass et al. (2017) found 49 invertebrate taxa, dominated by insects, especially larval Chironomidae, in another year-long investigation inhabiting Desperado Spring, a small, rocky spring flowing into the Blue River

in south-central Oklahoma.

During the same annual period as the Desperado Spring investigation (Bass et al. 2017), a parallel study was being conducted at Little Boiling Spring in northwestern Oklahoma. The macroinvertebrate community of Little Boiling Spring had been previously sampled by Gaskin and Bass (2000).

Purposes of the current investigation were to 1) describe the macroinvertebrate community composition of Little Boiling Spring over an annual period, 2) compare composition of these communities to previously collected data on this and other springs, and 3) determine selected physicochemical conditions of Little Boiling Spring.

Methods

Little Boiling Spring is a rheocrene spring located in Boiling Springs State Park of northwestern Oklahoma in Woodward County (36.4541°N, 99.2876°W). The spring emerges from a sandy substrate and flows approximately 500 meters before reaching the North Canadian River. The emergence pool at the head is approximately 2 m in diameter and less than 0.5 m deep while the run measured about 0.5 m in width and almost 0.1 m in depth. The substrate of the run is composed primarily of sand, with an abundance of aquatic vegetation, as well as decomposing leaf and wood debris (Gaskin and Bass 2000).

Little Boiling Spring was sampled seasonally (October, January, April, and July) beginning in 2002. Both physicochemical and macroinvertebrate samples were collected during each quarterly visit. Two sampling sites (springhead and approximately 25 m downstream in springbrook) were established and three Surber net samples were collected from each site. Qualitative samples were also collected, by examining microhabitats using a dip net, to capture species that may have been missed by the Surber net. All samples were washed in a number 60 (0.250mm) U.S. standard sieve bucket and preserved with a 10% mixture of formalin and Rose Bengal dye. The preserved macroinvertebrates were returned to the laboratory to be sorted, identified, and counted. Identification of macroinvertebrates was determined primarily using keys by Wiederholm (1983), Epler (1995), Smith (2001), Merritt et al. (2008), and Thorp and Covich (2009). All specimens were deposited in the Invertebrate Section of the University of Central Oklahoma Natural History Museum.

Shannon's (1948) diversity index was calculated for springhead samples and springbrook samples during each collection period. Sorenson's index of similarity (Brower et al. 1997) was used to make comparisons between the species present at the springhead and the springbrook sites for each collection. Chi-square contingency analyses were performed to determine if there was a relationship in the number of individuals between the springhead and the springbrook during the different seasons. Finally, a Hutcheson *t*-test was used to compare species diversity between the springhead and the springbrook samples (Zar 2010).

Water temperature, dissolved oxygen concentration, and pH were measured at both the springhead and the springbrook, while alkalinity and flow were measured only at the springhead (American Public Health Association 1999). In addition, a water sample collected from the springhead was used to determine turbidity, conductivity, and concentrations of ammonia, nitrites, nitrates, and orthophosphates in the laboratory using a Bausch & Lomb Spectrophotometer 20 (Hach 1987).

Results and Discussion

Results from the analysis of the physicochemical conditions taken at each collection date indicated the water quality is sufficient to support aquatic macroinvertebrates (Table 1). Both dissolved oxygen concentration and percent oxygen saturation values increased between the springhead and springbrook sites as atmospheric oxygen diffused into the water below the emergence point (van der Kamp 1995). The values of most other parameters

Table 1. Physicochemical conditions measured at the springhead in Little Boiling Spring, Boiling Springs State Park, Woodward County, Oklahoma. Values in parentheses describe conditions in springbrook.

Parameter/Collection	Oct. 2002	Jan. 2003	Apr. 2003
Water Temperature °C	17.5 (17.3)	15.3 (15.5)	16 (16.4)
Dissolved Oxygen (mg/l)	4.5 (7.4)	4.6 (7.2)	5.1 (8.5)
DO Percent Saturation	45 (75)	43 (75)	43 (85)
pH	6.9 (7.3)	7.4 (7.6)	7.5 (7.5)
Alkalinity (mg/l)	175	185	169
Carbon Dioxide (mg/l)	38	13	9
Turbidity (JTU)	3	2	3
Specific Conductivity (umhos/cm)	620	668	610
Ammonia (mg/l)	0.27	0.27	0.23
Nitrates (mg/l)	0.88	0.73	0.88
Nitrites (mg/l)	4	11	2
Orthophosphates (mg/l)	0.33	0.17	0.17
Flow (m/sec)	0.2	0.2	0.2

were relatively constant.

A total of 31,376 individuals representing 49 species were collected in the Surber net samples during the four seasonal sampling periods from Little Boiling Spring (Table 2). In addition, a single species of decapod and two species of coleopterans were collected only in the qualitative samples, so they were not included in the statistical analyses. Hexapods made up 39.9% of the individuals and 36 taxa, while non-hexapods formed 61.1% of the individuals and 13 species. This overwhelming dominance by crustaceans, particularly the species complex *Hyalella azteca,* is similar to what was reported by Brown and Bass (2014) in the Pontotoc Ridge springs of southeast Oklahoma. It is well known that springs often support more non-insects than insects (Webb et al. 1995; Brown and Bass 2014), and this was certainly the case with Little Boiling Spring. Although the number of individuals was lower among the hexapods, there were almost three times more species of hexapods than non-hexapods in the spring. This is most likely because the springbrook flows into the nearby North Canadian River, an environment supporting many aquatic insects, so its fauna may easily colonize the spring environment (Gaskin and Bass 2000).

Six genera dominated the Little Boiling Spring macroinvertebrate community with each one constituting greater than five percent of the total number of individuals and collectively making up almost 70% of the individuals present in the collections. These included the annelid *Limnodrilus* sp. (7.3%), an unidentified nematode (7.9%), amphipods of the *Hyalella*

azteca species complex (12.0%), Podocopida ostracods (25.8%), and the chironomid larvae *Eukiefferiella claripennis* (9.9%) and *Larsia* sp. (6.9%). *Eukiefferiella claripennis and Larsia* sp. always occurred in higher numbers at the springhead, while *Limnodrilus* sp., unknown Nematoda, *Hyalella azteca* species complex, and Podocopida were consistently more abundant in the springbrook (Table 2). It is possible these distributions reflected preferences for different microhabitats – the springhead substrate was composed primarily of sand, while the springbrook substrate contained a greater amount of plant detritus overlying the sand. It is likely inhabitants of the springhead preferred the sand for burrowing and the springbrook species required the greater organic content present with the detritus.

Comparisons of the species present at the springhead and those present at the springbrook sites using Sorensen's index of similarity yielded values ranging from 0.37 - 0.41 during the four collection periods. Specifically, those numbers were 0.41 during October, 0.41 during January, 0.37 during April, and 0.40 during July. Although some species did occur at both sites, other species were limited to either the springhead or the springbrook (Table 2).

Results from the chi-square analyses indicated there was a statistically significant relationship between the number of individuals in the springhead and springbrook collections during all four seasons (χ^2 contingency test, $p<0.00001$). Shannon's diversity values at the springhead ranged from 1.985 - 2.656 while these values in the springbrook were 1.642 - 2.351 (Table 2). Hutcheson *t*-test showed a significant difference in species diversities between the springhead and springbrook for each collection month (October $t=12.564$, $p<0.0001$; January $t=4.146$, $p<0.0001$; April $t=7.991$, $p<0.0001$; July $t=8.244$, $p<0.0001$) (Fig. 1). As noted in other Oklahoma spring studies (Bass 2000; Rudisill and Bass 2005; Bass et al. 2017), dissolved oxygen concentrations increased, presumably from atmospheric diffusion, as water flowed from the springhead into the springbrook, allowing the springbrook to support different species of macroinvertebrates. There was also more detritus present in the springbrook serving as food and microhabitat for these species. Although the species richness during each sampling period showed little variation between the springhead and springbrook, a greater number of individuals were found in the springbrook, leading to lower Shannon's species diversity values for the springbrook collections (Brower et al. 1997).

Collectors dominated Little Boiling Spring based on trophic catagories in Merritt et al. (2008) and Thorp and Covich (2009). Collectors were composed of 29 taxa and made up 81.3% of the individuals present. Predators (15 species, 17.8% of the total individuals) and detritivores (five species, 0.9% of the total individuals) composed the remaining trophic groups in the community. It should be noted that in addition to emergence of adults from the spring, some of these species have different trophic roles as they grow and mature (Merritt et al. 2008), so these proportions may change through time.

A large fraction of insect nymphs and larvae were found in the samples (Table 2). Gaskin and Bass (2000) also observed many immature

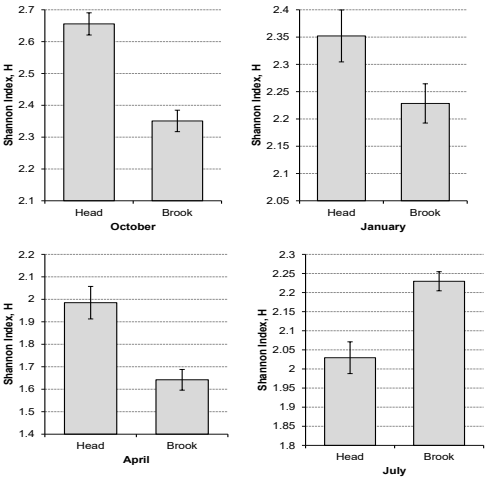


Figure 1. Box charts displaying the variation in diversity between springhead and springbrook samples in Little Boiling Spring for each month of data collection.

Table 2. Macroinvertebrates collected in Little Boiling Spring, Boiling Springs State Park, Woodward County, Oklahoma.

Taxa	Oct. 2002 Springhead	Oct. 2002 Springbrook	Jan. 2003 Springhead	Jan. 2003 Springbrook	Apr. 2003 Springhead	Apr. 2003 Springbrook	Jul. 2003 Springhead	Jul. 2003 Springbrook	Totals
Platyhelminthes									
Dugesia sp.	48	65	86	44	25	40	109	180	597
Gastropoda									
Physa sp.	66	97	59	116	5	24			367
Bivalvia									
Sphaerium sp.		148		107	2	123	4	172	556
Annelida									
Dero sp.	45	24		2	6	8		202	287
Helobdella triserialis				1	1				2
Limnodrilus sp.	108	505	9	981	40	260	9	377	2289
Lumbriculus sp.	13	48	4	1	4	5			75
Nematoda									
Nematoda	93	1445	38	471	11	107	27	300	2492
Crustacea									
Harpacticoida	148	12	15	40		1			216
Hyalella azteca complex	258	477	225	326	125	415	389	1564	3779
Podocopida	4	279	22	1410	25	2882	403	3078	8103
Cambaridae *									*
Collembola									
Isotomidae	3		3		2		4	1	13
Sminthuridae	1				1	1		4	7
Ephemeroptera									
Baetis sp.	1	8	25	37	2	196	1	494	764
Stenonema sp.				2					2
Odonata									
Argia sp.	87	93	142	90	145	195	107	163	1022
Coenagrionidae	344	170	401	238			49	25	1227
Orthoptera									
Acrididae					1			1	2
Hemiptera									
Aquarius sp.						1		3	4
Belostoma sp.							1		1
Coleoptera									
Agabus sp.					1				1
Celina sp.							1		1
Hydraenidae						1	5		6
Laccophilus sp.*									*
Matus sp.*									*
Paracymus sp.	1					1			2
Tropisternus sp.			2						2

Table 2 Continued

Taxa	Oct. 2002 Springhead	Oct. 2002 Springbrook	Jan. 2003 Springhead	Jan. 2003 Springbrook	Apr. 2003 Springhead	Apr. 2003 Springbrook	Jul. 2003 Springhead	Jul. 2003 Springbrook	Totals
Diptera									
Chironomus sp.				2			9		11
Corynoneura sp.	106	111	43	2	102	90	9	9	472
Cricotopus sp.			5	7	22	39	7	28	108
Cryptochironomus sp.		5	5	168	4	15	7	133	337
Dasyhelea sp.	113	395	10	29	12	72	8	103	742
Dicrotendipes sp.				2					2
Dixella sp.			4	1	1		3	10	19
Djalmabatista sp.				2					2
Eukiefferiella claripennis	453		247	43	670	81	935	684	3113
Labrundinia sp.	3	2				17	10	30	62
Larsia sp.	186	144	374	139	135	36	880	284	2178
Nemotelus sp.						1			1
Paratendipes sp.						2			2
Polypedilum halterale	228	217	117	40	40	6	143		791
Simulium sp.	8	44	4	92	1	7	3	607	766
Stratiomyidae	35						2		37
Stratiomys sp.	32		2		19				53
Tanytarsus sp.	15	1	28	2	24	49	53	269	441
Tipula sp.	5	18	1	13	8	4		2	51
Trichoptera									
Hydroptila sp.	4	21	8	11		4	21	33	102
Setodes sp.		66	1	53		15	7	2	144
Lepidoptera									
Crambidae		1			1		19	2	23
Acarina									
Hydrachnidiae 1	74	4	1				14		93
Hydrachnidiae 2			1	2			5	1	9
Number of Individuals	2482	4400	1882	4474	1435	4698	3244	8761	31376
Species Richness	28	26	29	32	29	31	31	29	49 + (3*)
Species Diversity	2.656	2.351	2.352	2.228	1.985	1.642	2.029	2.230	2.625

*** indicates taxa were not present in Surber net samples; found only in qualitative samples and not used in the statistical analysis.**

insects present in Little Boiling Spring and suggested reproduction must have been occurring there. This may have been the case, but another possibility was suggested by Bass et al. (2017) regarding a similar situation in Desperado Spring. Individuals making up the spring populations may have originated in the nearby river and later moved into the springbrook, using it as a refuge from larger predators, such as fishes, that would have difficulty existing in the spring's shallow water. It is unknown which, if either, of these hypotheses correctly explains the high number of immature individuals in Little Boiling Spring, but they are both possibilities.

It was mentioned previously that this Little Boiling Spring investigation was being done concurrently with the study of Desperado Spring (Bass et al. 2017). Although the same number of taxa, 49, was collected in the Surber net samples from both Little Boiling Spring and Desperado Spring, there was not much species similarity between the two springs. They shared only 23 taxa, resulting in a Sorenson's similarity value of 0.235. Besides these springs existing at opposite ends of Oklahoma geographically, Little Boiling Spring is located in the Central Great Plains ecoregion while Desperado Spring occurs in the Cross Timbers ecoregion (Oklahoma Forestry Services 2018), resulting in considerable physical differences between the two sites. Similarity values are reduced as different species are adapted to those variations in the local environments, such as substrate type, detrital composition, and water quality parameters.

It may be of interest to note Gaskin and Bass (2000) reported 14 species from Little Boiling Spring during 1999, many of which were also collected in the present investigation. However, the 1999 study sampled that site only once and used different methodology for collecting. The present investigation sampled a larger section of the spring site and made seasonal collections, resulting in finding a total of 52 taxa. This shows the value of increasing sample size, employing several sampling methods, and making collections over an annual period to assure obtaining a more complete description of community structure.

Conclusion

Springs are unique and often over-looked aquatic environments. While many springs are reported to contain fauna found nowhere else, the Little Boiling Spring invertebrate community contains many species also found in the nearby North Canadian River. Crustaceans are the dominant group in many springs and this phenomenon was also observed in Little Boiling Spring, However, due to its close proximity to the North Canadian River, chironomids were abundant as well. Results of the physicochemical conditions in Little Boiling Spring indicated the water quality is capable of supporting a diverse biota and this was confirmed by the intolerant taxa present in the samples.

Acknowledgments

The University of Central Oklahoma provided financial support to complete this study. Permission to access and sample the spring was granted by Boiling Springs State Park personnel. J. McDonald assisted sorting samples in the laboratory.

References

American Public Health Association. 1999. Standard Methods for Examination of Water & Wastewater. 20th Edition.

Bass D. 2000. A preliminary study of aquatic macroinvertebrates from two springs in the Pontotoc Ridge Nature Preserve, Oklahoma. Proc Okla Acad Sci 80:105-109.

Bass D, Gaskin B, Tedford K. 2017. Macroinvertebrate community structure and physicochemical conditions of Desperado Spring. Proc Okla Acad Sci 97:41-46.

Brower JE, Zar JH, von Ende CN. 1997. Field and laboratory methods for general ecology. Boston (MA): WCB/McGraw-Hill. 273 p.

Brown K, Bass D. 2014. Macroinvertebrate community structure and physicochemical conditions of three southeastern Oklahoma springs. Proc Okla Acad Sci 94:28-38.

Epler JH. 1995. Identification manual for the larval Chironomidae (Diptera) of Florida. Bureau of Water Resources Protection, Florida Department of Environmental Protection Tallahassee (FL): 317 p.

Gaskin B, Bass D. 2000. Macroinvertebrates collected from seven Oklahoma springs. Proc Okla Acad Sci:17-23.

Graening GO, Holsinger JR, Fenolio DB, Bergey EA, Vaughn CC. 2006. Annotated checklist of the amphipod crustaceans of Oklahoma, with emphasis on groundwater habitats. Proc Okla Acad Sci 86:65-74.

Hach Chemical Company. 1987. Procedures for water and wastewater analysis. 2nd edition. Loveland (CO): Hach Chemical Co. 119 p.

Matthews WJ, Hoover JJ, Milstead WB. 1983. The biota of Oklahoma springs: natural biological monitoring of ground water quality. Final Report. Stillwater (OK): Oklahoma Water Resources Research Institute. 64 p.

Merritt RW, Cummins KW, Berg MB. 2008. An introduction to the aquatic insects of North America. 4th edition. Dubuque (IA): Kendall/ Hunt Publishing. 1158 p.

Oklahoma Forestry Services. 2018. The ecoregions of Oklahoma [online]. Available from: http://www.forestry.ok.gov/ecoregions-of-oklahoma (Accessed July 11, 2018).

Rudisill T, Bass D. 2005. Macroinvertebrate community structure and physicochemical conditions of the Roman Nose spring system. Proc Okla Acad Sci 85:33-42.

Shannon CE. 1948. A mathematical theory of communication. Bell Syst Tech J 27:379-423, 623-656.

Smith DG. 2001. Pennak's freshwater invertebrates of the United States. 4th edition. New York (NY): John Wiley & Sons, Inc. 638 p.

Thorp J, Covich A. 2009. Ecology and classification of freshwater invertebrates. 3rd edition. San Diego (CA): Academic Press. 1021 p.

Varza, D, Covich A. 1995. Population fluctuations within a spring community. J Kan Entomol Soc 68:42-29.

Webb DW, Wetzel MJ, Reed PC, Phillips LR, Harris MA. 1995. Aquatic biodiversity in Illinois springs. J Kan Entomol Soc 68:93-107.

Wiederholm T. (ed.). 1983. Chironomidae of the Holarctic region. Keys and diagnoses. Part 1-Larvae. Ent Scand Supple No. 19. 457 p.

van der Kamp G. 1995. The hydrogeology of springs in relation to the biodiversity of spring fauna: a review. J Kan Entomol Soc 68:4-17.

Zar JH. 2010. Biostatistical analysis. 5th edition. Upper Saddle River (NJ): Pearson. 960 p.

Submitted July 16, 2018 Accepted November 2, 2018

Potential Longnose Darter Population in the Kiamichi River of Oklahoma

Colt T. Holley
Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078

James M. Long
U.S. Geological Survey, Oklahoma Cooperative Fish and Wildlife Research Unit, Oklahoma State University, Stillwater, OK 74078

One of Oklahoma's rarest fish species (Robison 1992; Miller and Robison 2004), the Longnose Darter, *Percina nasuta* (Bailey 1941), is historically known to have occurred in only two river systems in Oklahoma: Lee Creek and the Poteau River (Cross and Moore 1952; Lindsey et al. 1983; Wagner et al. 1985) (Figure 1). In Oklahoma, Longnose Darters are designated as a state-endangered species and are considered of conservation concern throughout their range (Jelks et al. 2008; ODWC 2016). The Poteau River and its tributaries hosted a population of Longnose Darters that was recorded in some of the first historical expeditions in this region (Jordan and Gilbert 1886; Cross and Moore 1952), but construction of Wister Reservoir in 1949 may have contributed to its decline in this system (Lindsey et al. 1983; Wagner et al. 1985). Only one specimen has been observed in the Poteau River since impoundment of Wister Reservoir; a single Longnose Darter upstream near the Oklahoma-Arkansas border in 2015 (OWRB 2015). The only other known instance of Longnose Darters in the Poteau River system post-impoundment of Wister Reservoir involved translocation of 164 individuals from Lee Creek into Blackfork Creek (O'Donnell 1991, 1992) where prior populations were thought to be absent. Subsequent surveys in 2016 for Longnose Darters in Blackfork Creek failed to find any (C. Holley, Oklahoma State University, unpublished data), suggesting that the population in Lee Creek remains the only robust population in Oklahoma (Burns & McDonnell Engineering Company 1990; Gatlin and Long 2011).

While developing a range-wide ecological niche model for Longnose Darters to identify areas outside of Lee Creek and the Poteau River in Oklahoma with potentially suitable habitat, we compiled a record of all the Longnose Darter occurrence locations in Oklahoma (Figure 1). During this process, three disparate records of Longnose Darter occurrence in the Kiamichi River, outside of the known range of the species (Miller and Robison 2004), were identified and scrutinized. To scrutinize these

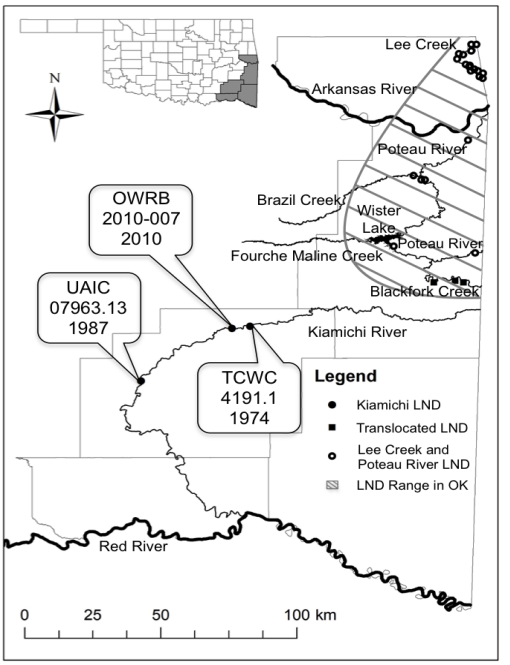


Figure 1. Distribution of Longnose Darter (*Percina nasuta*; LND) in Oklahoma with disparate occurrence records in the Kiamichi River.

specimens housed in museum collections, we either obtained the specimens via loan or asked the curator to measure certain aspects. We used measurements from dichotomous keys for Oklahoma (Miller and Robison 2004) and Arkansas (Robison and Buchanan 1988) that were diagnostic and easy to measure. The Oklahoma key indicates that Longnose Darters have "width of snout less than ¾ its length" (Miller and Robison 2004) and the Arkansas key uses snout "length 9 percent or more of standard length" (Robison and Buchanan 1988). If a population of Longnose Darters exists in the Kiamichi River, it would represent a significant extension of the range in Oklahoma, outside the Arkansas River watershed.

The earliest occurrence of Longnose Darter in the Kiamichi River was field identified in 1974 and is housed at Texas A&M University's Biodiversity Research and Teaching Collection (specimen number: TCWC 4191.1). This specimen was received on a specimen loan and measured by the authors. According to both keys, this specimen is a Longnose Darter (Table 1; Figure 2).

The second record came from 1987 and is housed at the University of Alabama's Ichthyological Collection (catalogue number: UAIC 07963.13). This catalogue number constitutes a lot of five specimens, which were measured by the curator. All five specimens key out as Longnose Darters with the Fishes of Oklahoma key, but not using the Fishes of Arkansas key (Table 1). Furthermore, tissue samples from these specimens (GenBank KM209995, KM 210049; Benson et al. 2012) were used in a genetic analysis (Robison et al. 2014) that considered them to be Slenderhead Darters *Percina phoxochephala*.

The third and most recent record of Longnose Darter was obtained from a website of fishes collected during routine monitoring by the Oklahoma Water Resources Board (OWRB) (http://owrb.maps.arcgis.com/apps/webappviewer/index.html?id=33cca35f06e64c4f8f84fc9bf0228218), but no voucher specimen was kept (L. Kimmel, University of Central Oklahoma; pers. comm.). As a result, we cannot independently verify the accuracy of this record, but other vouchered specimens from this database (2015P-033 from Poteau River and OWRB2013-006 from Lee Creek) were measured according to the characteristics identified in Table 1 and verified as Longnose Darters, making this record from the Kiamichi River credible. Related, the Poteau River specimen is the first record in this river since impoundment of Wister Reservoir suggesting this population is not extirpated. Whether this specimen represents a remnant population since impoundment or is a result of the translocation from Lee Creek into Blackfork Creek is unknown.

From these records, it seems plausible that a previously undiscovered population of Longnose Darters occurs in the Kiamichi River. This new information, if correct, would benefit management agencies that are tasked with monitoring endangered species. However, because of disagreement in species identification between dichotomous keys and the paucity of records of this species in the Kiamichi River, it is also plausible that these specimens represent anomalies or a misidentification of other common species known to inhabit the system, such as Slenderhead Darter. Because Longnose Darter

Table 1. Table of Longnose Darter (*Percina nasuta*) occurrence records in the Kiamichi River of Oklahoma with identifying characteristics from the Fishes of Oklahoma (Miller and Robison 2004) and Fishes of Arkansas (Robison and Buchanan 1988) taxonomic keys.

Date	Collection	Specimen #	Snout Length/SL (>9 %)	Snout Width/Snout Length < 0.75
1974	TCWC 4191.1	1	9.4*	0.48*
1987	UAIC 07963.13	1	6.8	0.49*
1987	UAIC 07963.13	2	7.3	0.48*
1987	UAIC 07963.13	3	7.5	0.53*
1987	UAIC 07963.13	4	6.1	0.64*
1987	UAIC 07963.13	5	7.4	0.52*
2010	OWRB 2010-007	1	NA	NA

**Percina nasuta* according to taxonomic key

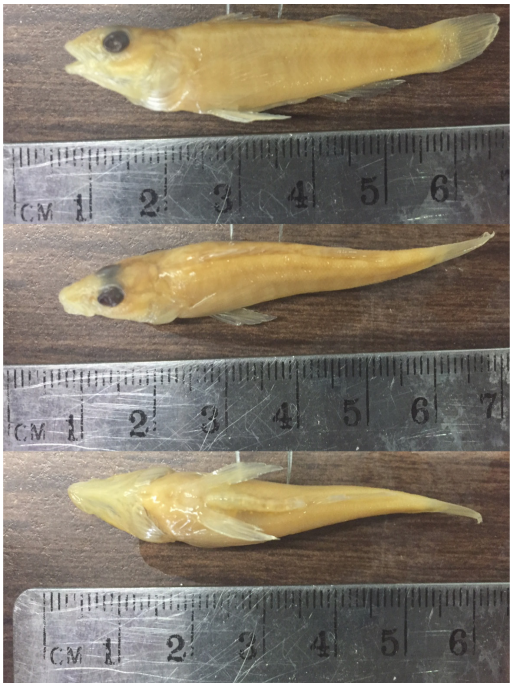


Figure 2. Photographs of a Longnose Darter (*Percina nasuta*) specimen (TCWC 4191.1) from the Kiamichi River of Oklahoma. Photograph courtesy of Heather Prestridge (Texas A&M University).

is state-endangered, correctly documenting their extant range is crucial for proper management. Surveys for Longnose Darters in the Kiamichi River near these collection records would thus be beneficial as would curation of specimens in museum collections that are accessible for verification.

Acknowledgments

We thank Worth Pugh (University of Alabama), Heather Prestridge (Texas A&M University), and Laura Kimmel (University of Central Oklahoma) for allowing us access to specimens and we thank Curtis Tackett (Oklahoma Department of Wildlife Conservation) and Lance Phillips (Oklahoma Water Resources Board) for help researching historical records in Oklahoma. Dustin Lynch (Arkansas Natural Heritage Commission) provided comments that improved this manuscript. This project was funded by the Oklahoma Department of Wildlife Conservation through State Wildlife Grant F15AF01196 (OK T-87-1). The Oklahoma Cooperative Fish and Wildlife Research Unit is supported by the Oklahoma Department of Wildlife Conservation, U.S. Geological Survey, the Wildlife Management Institute, and the U.S. Fish and Wildlife Service.

References

Bailey RM. 1941. *Hadropterus nasutus*, a new darter from Arkansas. Occas Pap of the Mus of Zool, Univ Mich. 440:1-8.

Benson DA, Karsch-Mizrachi I, Clark K, Lipman DJ, Ostell J, Sayers EW. 2012. GenBank. Nucleic Acids Res. 40:D48-D53.

Burns & McDonnell Engineering Company. 1990. Report on the Longnose Darter survey of Black Fork Creek, Oklahoma. Report to the City of Fort Smith, Arkansas. 33p.

Cross FB, Moore GA. 1952. The fishes of the Poteau River, Oklahoma and Arkansas. Am Midl Nat. 47:396–412.

Gatlin MR, Long JM. 2011. Persistence of the Longnose Darter (*P. nasuta*) in Lee Creek, Oklahoma. Proc Okla Acad Sci. 91:11–14.

Jelks HL, Walsh SJ, Burkhead NM, Contreras-Balderas S, Diaz-Pardo E, Hendrickson DA, Lyons J, Mandrak NE, McCormick F, Nelson JS, et al. 2008. Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries. 33:372–407.

Jordan DS, Gilbert CH. 1886. List of fishes collected in Arkansas, Indian Territory, and Texas, in September, 1884, with notes and descriptions. Proc US Natl Mus. 9:1–25.

Lindsey HL, Randolph JC, Carroll J. 1983. Updated survey of the fishes of the Poteau River, Oklahoma and Arkansas. Proc Okla Acad Sci. 63:42–48.

Miller RJ, Robison HW. 2004. Fishes of Oklahoma. Norman (OK):University of Oklahoma Press. 450p.

O'Donnell S. 1991. Report on the translocation of the Longnose Darters (*Percina nasuta*) from the inundation zone on Lee Creek to the Black Fork Creek. Oklahoma Fishery Research Laboratory, Oklahoma Department of Wildlife Conservation. 6p.

O'Donnell S. 1992. Report on the 1992 translocation of the Longnose Darters (*Percina nasuta*) from the inundation zone on Lee Creek to Black Fork Creek. Oklahoma Fishery Research Laboratory, Oklahoma Department of Wildlife Conservation. 4p.

[ODWC] Oklahoma Department of Wildlife Conservation. 2016. Oklahoma comprehensive wildlife conservation strategy: a strategic conservation plan for Oklahoma's rare and declining wildlife. Oklahoma Department of Wildlife Conservation. 422p.

[OWRB] Oklahoma Water Resources Board. 2015. Beneficial use monitoring program. Interactive Maps, Fish Monitoring Sites. Available from: https://owrb.maps.arcgis.com/home/index.html (Accessed: June 01, 2018).

Robison HW. 1992. Distribution and status of the Longnose Darter, *Percina nasuta* (Bailey), in the Ozark National Forest, Arkansas. Final report to US Forest Service Ozark National Forest, Russellville, Arkansas. 56p.

Robison HW, Buchanan TM. 1988. Fishes of Arkansas. Fayetteville (AR): University of Arkansas Press. 544p.

Robison HW, Cashner RC, Raley ME, Near TJ. 2014. A new species of darter from the Ouachita Highlands in Arkansas related to *Percina nasuta* (Percidae: Etheostomatinae). Bull Peabody Mus Nat Hist. 55:237–252.

Wagner BA, Echelle AA, Maughan OE. 1985. Status and distribution of the Longnose Darter, *Percina nasuta*, and the Neosho Madtom, *Noturus placidus*, in Oklahoma. Proc Okla Acad Sci. 65:59–60.

Submitted July 24, 2018 Accepted November 23, 2018

Shoreline Foraging Activity by Gray Bats (*Myotis grisescens*) and Northern Long-eared Bats (*Myotis septentrionalis*) on Grand Lake, Oklahoma

Keith Martin
Department of Biology, Rogers State University, Claremore, OK 74017

Craig R. Zimmermann
Department of Biology, Rogers State University, Claremore, OK 74017

Abstract: Shoreline foraging activity of the endangered gray bat (*Myotis grisescens*) and threatened northern long-eared bats (*Myotis septrionalis*) on Grand Lake, Oklahoma was assessed using acoustic sampling. Activity was surveyed in summer 2015 and 2016 along six mobile boat transects using Anabat acoustic detectors. Four transects using stationary detectors were also used in 2016. A total of 34,593 calls were detected for 9 bat species. The tri-colored bat (*Perimyotis subflavus*) and gray bat were the most frequent, combining to make up ≈ 90% of the total calls. The gray bat was recorded in five of the six mobile routes with call abundance highest within 8 km of maternity caves, specifically Drowning Creek, Elk River, and Three Fingers Cove. In contrast, most calls on stationary transects were found on Duck Creek and Honey Creek. The northern long-eared bat was the least detected species, comprising <0.4% of the total calls. A single call was detected during mobile surveys, occurring in 2015 on the northern shore of Drowning Creek. Stationary transects were more successful with calls for this species with most calls found on Drowning Creek and Honey Creek. In total, 293 locations were found to support foraging activity across nine species. More specifically, 48 locations were identified as foraging habitat for the two imperiled bat species (28 gray; 20 northern long-eared). Such spatial data provides the potential for identifying habitat factors needed for effective conservation for these species.

Introduction

Data are lacking for at-risk bat species in Oklahoma that use mesic, forest, or aquatic habitats. Additionally, few studies within the state have assessed the effects of anthropogenic disturbance on bats that forage in riparian habitats on manmade reservoirs. The volant nature of bats allows them access to multiple habitats, which may decrease their dependence on any single habitat. How specific bat populations respond to changing anthropogenic landscapes, therefore, is needed for effective habitat management (Arnett, 2003). The goal of this study was to assess the foraging activity of bats along shoreline habitat of Grand Lake, Oklahoma. Data garnered from this analysis will guide future landscape management aimed at conserving these species. Particular focus is directed toward two imperiled bat species – the endangered gray bat (*Myotis grisescens*) and threatened northern long-eared bats (*Myotis septrionalis*). The gray bat was once widespread in the southeast, but has been listed as endangered since 1976. The species is cave-obligate and many caves in the region serve as refugia. Maternity caves are used by adult females and their newborn pups. Bachelor caves are used by adult males and yearlings of both sexes and are typically within 35 km of maternity caves. Females and juveniles join males in July and August. This aggregation prior to fall migration may serve to aid young bats in learning routes to foraging areas and hibernacula caves (Tuttle, 1976). Gray bats disperse nightly from caves and may travel over long distances to forage on

flying aquatic insects along streams and lakes (Decher and Choate 1995). Bats from maternity caves in Delaware and Ottawa Counties likely travel to Grand Lake to feed. The northern long-eared bat is widely distributed in eastern North America. Due to declines caused by white-nose syndrome, the species was listed as threatened in 2015. This species roosts singly or in colonies within cavities and crevices of living and dead trees during the summer months. Rare individuals have also been found roosting in manmade structures, like barns and sheds. Winter months are spent hibernating in caves and mines (Caseres and Barclay, 2000). The species was first reported in Oklahoma in the mid-20th century in Adair, Delaware, and LeFlore Counties (Glass and Ward, 1959). Our knowledge of its distribution in Oklahoma, however, remains largely anecdotal.

Methods

Study site. Grand Lake o' the Cherokees (aka Grand Lake) is a manmade reservoir located on Grand River in northeastern Oklahoma. Formed in 1940 by construction of Pensacola Dam, the reservoir comprises a 19,000-hectare impoundment with around 2,100 kilometers of shoreline. It is the first of two successive reservoirs located along the river created and operated by the Grand River Dam Authority (GRDA). The Ozark Plateau covers about 103,000 km^2 in the central United States with elevations between 260-460 m above mean sea level (Huffman 1959). The area is dominated by outcrops of alternating layers of limestone and flint and sandstone conducive to cave formation. Multiple caves are located within 20 km of the lake perimeter with four found within 2.5 km. Vegetation on upland slopes is predominantly blackjack oak (*Quercus marilandica*), post oak (*Quercus stellata*), black hickory (*Carya texana*), and winged elm (*Ulmus alata*). Coralberry (*Symphoricarpus orbiculatus)* and sassafras (*Sassafras albidum*) comprise a sparse shrubby understory. Riparian areas in lowlands are dominated by silver maple (*Acer saccharium*), river birch (*Betula nigra*), American elm (*Ulmus americana*), cottonwood (*Populus deltoides*), sycamore (*Platanus occidentalis*), and various oak species (*Quercus* spp.). Sporadic openings of managed grasslands are used for agriculture (Blair and Hubbell 1938; Harvey et al. 1981). The Grand Lake ecosystem has been impacted by a long history of human influence. Much of the region surrounding this 100 km-long lake has been a popular residential and tourist destination since the lake's creation. Privately-owned land is generally located within feet of the water's edge and much shoreline habitat has been altered by residences, boat docks, and other structures. This is compounded by thousands of tourists that visit the lake annually months for water sports, fishing tournaments, and other recreation. Water quality issues have also figured prominently in recent years. The watershed is dominated by a variety of crop and animal agriculture which produce chemical and organic waste that drain into the lake. Heavy metals in mine drainage from Picher, OK have also been detected in lake sediments.

Acoustic surveys. Bat shoreline activity by bats was assessed in the summer of 2015 and 2016 using mobile and stationary acoustic sampling. Acoustic sampling employs special frequency detectors to record the subsonic echolocation calls used by bat species during nightly foraging (O'Farrell et al., 1999). Each species possess a unique set of calls that can be subsequently identified by computer software with high accuracy. Acoustic sampling techniques for assessing bat activity have been used for over 40 years and are now well established. Mobile boat surveys adapted methodology used for auto-based road surveys (Roche et al., 2011; Whitby et al., 2014). Six routes in proximity to known bat cave colonies were selected for surveying – Three Fingers Cove, Drowning Creek, Elk River, Duck Creek, Honey Creek, and Horse Creek (See Figure 1 and Appendix 1). Routes were sampled once each summer within the period from late May through July for a total of 12 mobile surveys. Two Anabat receivers (Titley Electronics, Australia) were employed during each mobile survey. An Anabat SD2 ultrasound bat detector was mounted on the boat bow with the microphone directed forwards, 5-15° off vertical. Another Anabat detector was mounted in the stern with a detached microphone

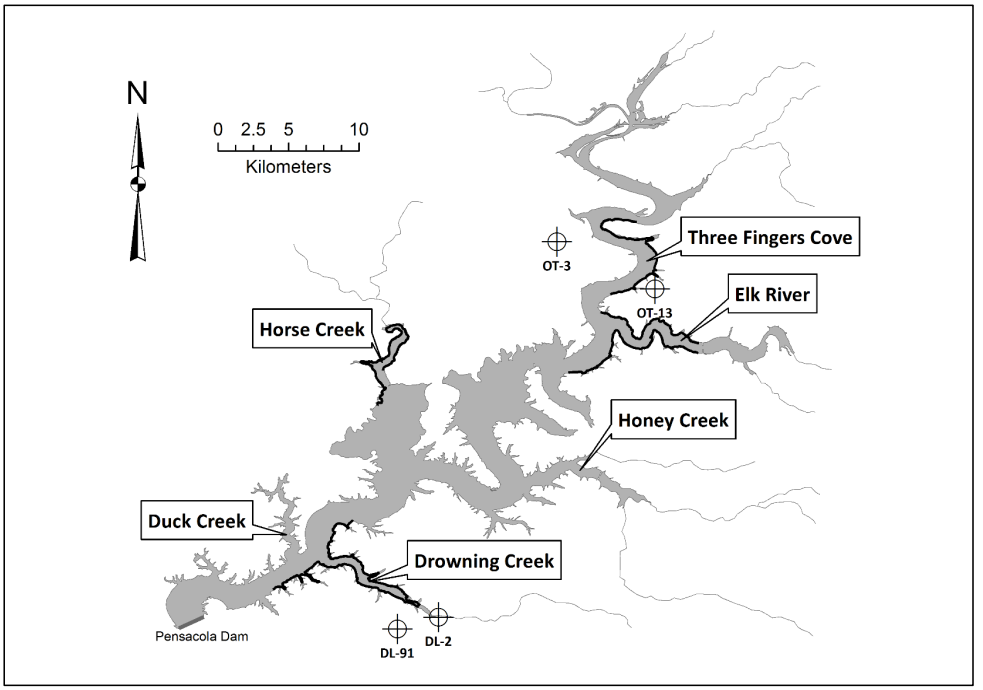


Figure 1. Schematic of Grand Lake showing the locations of 2015-2016 acoustic bat surveys. Dark black lines indicate boat routes used for mobile sampling transects. Bat roosting caves are denoted with cross-hairs symbol and U.S. Fish and Wildlife identifier code.

directed vertically. GPS antennas recorded the geographic coordinates for each call. Surveys were conducted on nights with low wind (< 24 km/h), no rain, and ambient temperatures >60°F. Recording was started 20 minutes after sunset in 2015, but was increased to 60 minutes in 2016. The later start time was adopted to potentially increase bat encounters. Routes were driven at 9.5-11 km per hour with each detector recording continuously for a 90 minute duration. This speed was chosen to lessen the effect of wind and wave noise on detections. Boats steered a course as close to the shoreline as surface and subsurface features allowed. Most routes required significant maneuvering around boat docks and other manmade structures. Larger tributaries along the main route were explored, if possible. Stationary Anabat Express units were also deployed in 2016 at three locations. Two of these, Duck Creek and Honey Creek, were not surveyed by mobile routes in either survey year. The southern shore of Drowning Creek was surveyed using both mobile and stationary units in 2016, but surveys were not performed concurrently. Stationary surveys monitored activity for six consecutive nights (1800-0600 hr) at five locations along each transect separated by at least 400 m (See Appendix 2). Call data files were processed with Analook software (Titley Electronics) to filter ambient noise. Echolocation calls were identified to species using BCID 2.7 software (Bat Call Identification Inc.). This computerized application interacts with the ANABAT recorders and allows users to automate the identification process of a high volume of calls of North American bats.

Results

A total of 34,593 identifiable echolocation calls were recorded for nine bat species during the two years of surveys. Of these, 274 calls were recorded on mobile surveys (Table 1) and 34,319 calls were recorded on stationary surveys (Table 2). Stationary surveys detected nine species: big brown bat (*Eptesicus fuscus*), silver-haired bat (*Lasionycteris noctivagans*), eastern red bat (*Lasiurus borealis*), hoary bat (*Lasiurus cinereus*), gray bat, little brown bat (*Myotis lucifugus*), northern long-eared bat, evening

Table 1. Summary of bat echolocation calls by species and transect recorded on mobile shoreline surveys in summer 2015 and 2016.

Transect Location	Species									Total
	Gray	*Northern Long-eared*	*Tri-colored*	*Silver-haired*	*Red*	*Evening*	*Hoary*	*Big Brown*	*Little Brown*	
Drowning Creek S.	3	0	26	0	0	0	0	0	0	29
Drowning Creek N.	5	1	80	4	2	0	0	0	0	92
Elk River S.	5	0	21	1	2	3	0	0	0	32
Elk River N.	1	0	11	0	0	0	0	0	0	12
Horse Creek	0	0	18	0	0	0	0	0	0	18
Three Fingers Cove	29	0	50	0	8	4	0	0	0	91
Total	**43**	**1**	**206**	**5**	**12**	**7**	**0**	**0**	**0**	**274**
% of Calls	**15.7**	**0.4**	**75.2**	**1.8**	**4.4**	**2.6**	**0**	**0**	**0**	**100**

Table 2. Summary of bat echolocation calls by species and transect recorded on stationary shoreline surveys in summer 2015 and 2016.

Transect Location	Species									Total
	Gray	*Northern Long-eared*	*Tri-colored*	*Silver-haired*	*Red*	*Evening*	*Hoary*	*Big Brown*	*Little Brown*	
Drowning Creek	870	30	13,141	80	123	2,596	81	72	42	17,035
Duck Creek N.	1,468	2	5,573	45	141	131	21	10	25	7,416
Duck Creek S.	146	4	1,388	56	232	150	12	12	23	2,023
Honey Creek	1,397	24	5,264	77	602	386	15	18	62	7,845
Total	**3,881**	**60**	**25,366**	**258**	**1,098**	**3,263**	**129**	**112**	**152**	**34,319**
% of Calls	**11.3**	**0.2**	**74.0**	**0.8**	**3.2**	**9.5**	**0.4**	**0.3**	**0.4**	**10.0**

bat (*Nycticeius humeralis)*, and tri-colored bat (*Perimyotis subflavus*). Mobile surveys recorded six of these, failing to detect any calls for the big brown bat, hoary bat, or little brown bat. The tri-colored bat and gray bat were the most frequently recorded species for both survey methods. The tri-colored bat comprises 75.2% and 74.0% of the calls for the mobile and stationary surveys, respectively. The gray bat comprised 15.7% and 11.3% of these calls. The northern long-eared bat was the least detected species in both survey methods, comprising only 0.2-0.4% of the total calls.

The northern shore of Drowning Creek recorded the highest call abundance of the six mobile survey routes. Two routes, Drowning Creek North and Elk River South, recorded the highest species richness, recording five species and six species, respectively. The gray bat was recorded in five out of the six mobile routes. Call abundance for this species was highest within 8 km of maternity caves, specifically Drowning Creek, Elk River, and Three Fingers Cove. The northern long-eared bat, on the other hand, was only detected once among the two seasons of mobile surveys. This call was recorded in 2015 on the northern shore of Drowning Creek. The southern shore of Drowning creek recorded the most calls of the four stationary transects, nearly equaling the number of calls of the other three transects combined. All stationary transects detected calls for the nine species recorded during the study. Gray bat calls were most abundant on Duck Creek North and Honey Creek, with 1,468 and 1,397 calls, respectively. For the northern long-eared bat, a total of 60 calls were recorded at nine of the 19 stationary sample points. These calls were most abundant on Drowning Creek and Honey Creek, with 30 and 24 calls, respectively.

Conclusions

The efficacy of mobile versus stationary acoustic methods for bat detection has been shown to vary in different habitats (Fischer-Phelps et al., 2017; Tonos et al., 2014; Whitby et al., 2014). In this study, stationary detectors positioned at 19 fixed locations for a period of 6 nights detected a total of nine bat species. Mobile surveys detected three fewer species, but required fewer sampling events than stationary transects (12 versus 19). The three species not detected by mobile surveys were highly rare in the stationary surveys, each comprising no more than 0.4% of the total call volume. Stationary surveys also detected a much higher volume of calls, recording more than a 120-fold increase over mobile surveys. The frequency of calls by species, however, was generally the same for both survey methods. The tri-colored bat (74-75.2%) and gray bat (11.3-15.7%) were the most frequently detected species for the mobile and stationary surveys. Similarly, the northern long-eared bats was the least frequent (0.2-0.4%) in both methods. A key advantage of mobile surveys for bat conservation is that spatial location can be recorded for each call, thus allowing for a more comprehensive assessment of bat activity with spatial habitat variability.

Foraging activity along riparian habitat was highly different among the two imperiled species. The endangered gray bat was highly ubiquitous among recorded calls, second only to the tri-colored bat. Four caves within 2.5 km of the lake perimeter serve as refugia for this cave-obligate species, with three of these confirmed to house maternity colonies. Gray bat call volume was found to be highest within 8 km of these maternity caves. The high prevalence of bat detections in proximity to these caves substantiate management stakeholder predictions that riparian habitat on Grand Lake is essential foraging grounds for these colonies. These three maternity caves are currently monitored and protected by GRDA as part of the agency's conservation efforts for this species, but the spatial extent to which the bat forages along the Grand Lake shoreline was heretofore speculative. Gray bats exhibit strong competitive-adaptive habits to foraging on night-flying aquatic insects along streams, rivers, wooded riparian habitats, and edges of impoundments (Tuttle, 1976; Decher and Choate, 1995; Brack and LaVal, 2006). Gray bats predate largely on mayflies which swarm in enormous numbers during the warm months in portions of Grand Lake. Preferred bat foraging habitat may, therefore, correlate closely with conditions that promote high mayfly abundance.

The threatened northern long-eared bat, in contrast, was the least detected species in the study. Six caves within 20 km of Grand Lake provide some form of refugia for this species. Northern long-eared bats forage in a variety of habitats including forests, forest edges, and riparian zones. Consequently, suitable foraging grounds are likely found closer to cave locations than Grand Lake. Whether the scarcity of calls detected for this species reflect habitat preference or a general rarity of this species in the region, however, is not known.

In total, 293 shoreline locations were found to support foraging activity across nine bat species. More specifically, 48 locations were identified as foraging habitat for the two imperiled bat species (28 gray bat; 20 northern long-eared bat). The association of specific habitat parameters with these preferred feeding grounds, however, has not yet been determined. Of key interest for conservation-related measures is the effect of human-caused habitat alteration on bat activity. Quantitative measures of shoreline alteration for Grand Lake is challenging, if non-existent. It is evident, however, that significant portions of the shoreline have been altered for a variety of residential and commercial purposes. Consequently, a spatial analysis of foraging activity relative to natural and manmade habitat parameters is recommended for effective shoreline management. Such parameters could include: 1) vegetation species composition, 2) vegetation structure (canopy density, stem height, stem density, basal area), 3) land use types (natural/developed), 4) water properties (depth, temperature, movement, dissolved oxygen, nutrient loads, pollution), and 5) artificial light intensity.

These GPS referenced calls can be used to potentially define spatial habitat variations that are preferred or avoided by different species of bats in general, and federally imperiled species specifically. We recommend using a GIS-based approach that combines spatial data from ground surveys and remote sensing to index habitat parameters. GIS-habitat mapping indices coupled to GPS referenced call data presented in this study, can ultimately be used for predictive shoreline habitat management decisions for federally imperiled species of bats by the Grand River Dam Authority and its stakeholders.

Acknowledgments

The authors would like to thank the GRDA Ecosystems and Education Center for financial and logistical support.

References

Arnett EB. 2003. Advancing science and partnerships for the conservation of bats and their habitats. Wildlife Society Bulletin 31:2-5.

Brack, V Jr, LaVal RK. 2006. Diet of the Gray Myotis (*Myotis grisescens*): variability and consistency, opportunism, and selectivity. Journal of Mammalogy 87: 7–18.

Caseres C, Barclay RMR. 2000. *Myoits septentrionalis*. Mammalian Species Account No. 634. American Society of Mammalogists.

Decher J, Choate, JR. 1995. Mammalian Species, No. 510, *Myotis grisescens*. American Society of Mammalogists.

Fischer-Phelps M, Schwilk D, Kingston T. 2017. Mobile acoustic transects detect more bat activity than stationary acoustic point counts in a semi-arid and agricultural landscape. Arid Environments 136:38-44.

Glass BP, Ward, CM. 1959. Bats of the genus *Myotis* from Oklahoma. Journal of Mammalogy 40:194-201.

Harvey MJ, Cassidy JJ, O'Hagan GG. 1981. Endangered bats of Arkansas: distribution, status, ecology, and management: Arkansas Game and Fish, United States Forest Service, and United States National Park Service-Buffalo National River. Arkansas Game and Fish Commission, Little Rock, AR.

Huffman GG. 1959. Mississippian stratigraphy and tectonics of the Oklahoma Ozark area. Tulsa Geological Society Digest 27:104-176.

O'Farrell,MJ, Miller BW, Gannon, WL. 1999, Qualitative identification of free-flying bats using the Anabat Detector. Journal of Mammalogy 80: –18.

Roche N, Langton S, Aughney T, Russ JM, Marnell F. Lynn D, Catto C. 2011. A car-based monitoring method reveals new information on bat populations and distributions in Ireland. Animal Conservation, 14:642-651.

Tonos JM, Pauli BP, Zollner PA, Haulton GS. 2014. A comparison of the efficiency of mobile and stationary acoustic bat surveys. Proceedings of the Indiana Academy of Science 123(2):103-111.

Tuttle MD. 1976. Population ecology of the gray bat (*Myotis grisescens*): philopatry, timing and patterns of movement, weight loss during migration, and seasonal adaptive strategies. Occasional Papers of the Museum of Natural History, University of Kansas, 54:1-38.

Whitby MD, Carter TC, Britzke ER, Bergeson SM. 2014. Evaluation of mobile acoustic techniques for bat population monitoring. Acta Chiropterologica, 16(1):223-230.

Submitted August 16, 2018 Accepted November 20, 2018

Appendix 1. Geographic coordinates for starting and ending points for each mobile survey route.

Transect	Point	Latitude	Longitude
Drowning Creek South	1	36.484	-94.892
	2	36.492	-94.913
	3	36.509	-94.934
	4	36.510	-94.951
	5	36.501	-94.928
Duck Creek North	1	36.537	-94.971
	2	36.556	-94.985
	3	36.549	-94.970
	4	36.562	-94.975
	5	36.525	-94.967
Duck Creek South	1	36.554	-94.983
	2	36.549	-94.975
	3	36.536	-94.974
	4	36.555	-94.980
	5	36.524	-94.971
Honey Creek	1	36.575	-94.787
	2	36.580	-94.778
	3	36.577	-94.784
	4	36.573	-94.786
	5	36.578	-94.779

Appendix 2. Geographic coordinates for each stationary sampling point by transect.

Transect	Point	Latitude	Longitude
Drowning Creek South	Start	36.482	-94.888
	End	36.493	-94.981
Drowning Creek North	Start	36.483	-94.887
	End	36.538	-94.930
Elk River South	Start	36.645	-94.710
	End	36.633	-94.793
Elk River North	Start	36.652	-94.709
	End	36.672	-94.772
Three Fingers Cove	Start	36.683	-94.766
	End	36.730	-94.749
Horse Creek	Start	36.612	-94.915
	End	36.634	-94.911

Population Characteristics of Gizzard Shad Introduced into a Small Western Oklahoma Impoundment

Matthew T. Lyons
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Richard A. Snow
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Michael J. Porta
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Abstract: Gizzard Shad (*Dorosoma cepedianum*) are often considered a vital forage species in many aquatic systems. However, when populations of Gizzard Shad become dominated by large (> 200 mm) overabundant individuals they can have negative direct and indirect effects on sportfish populations. In September 2016, 198 Gizzard Shad were collected from Lake Carl Etling in far northwest Oklahoma to evaluate population characteristics. Total length (TL; mm) and weight (g) were recorded and sagittal otoliths were removed for aging. Gizzard Shad ranged from 56-308 mm TL with a mean age estimated at 2.63 years and a maximum age of 12 years. This Gizzard Shad population has rapid growth rates within the first year, slowing with increasing age, poor relative body condition, low mortality, and is comprised primarily of small adults. We speculate that the fast growth rate of the Gizzard Shad population may be affecting the sportfish populations in Lake Carl Etling, as indicated by low proportional size distribution (PSD) and below average relative weight (W_r) of Walleye (*Sander vitreus*) and Largemouth Bass (*Micropterus salmoides*). Information about Gizzard Shad populations in Oklahoma is limited, and this study provides baseline characteristics to which other Gizzard Shad populations in small impoundments can be compared.

Introduction

Gizzard Shad (*Dorosoma cepedianum*) are one of four clupeid species found in Oklahoma's reservoirs and rivers (Miller and Robison 2004). Gizzard Shad are widely distributed in eastern North America from the central Dakotas to Quebec, south to Florida and southwest to Texas and northeast Mexico (Page and Burr 1991, Miller and Robison 2004). The range of Gizzard Shad has expanded through anthropogenic influences (dams and canals; Miller 1957), introductions via accidental and intentional stocking (Mueller and Brooks 2004, DeVries and Stein 1990), and climate change (VanDeHey et al. 2014). However, the expansion of Gizzard Shad has slowed northward due to their critical thermal minima (4 ºC) being reached during winter months (Porath 2006).

When a population of Gizzard Shad becomes established, it can dominate the biomass of a system (Noble 1981, Stein et al. 1995). Jenkins (1949) suggested that all large Oklahoma reservoirs have Gizzard Shad populations, and are typically the most abundant species. Gizzard Shad are filter feeders that use gill rakers to strain detritus and plankton (Miller 1960, Miller

and Robison 2004). This foraging behavior can influence zooplankton and phytoplankton densities, resulting in interspecific competition with juvenile and adult sportfish (Jenkins 1957, Aday et al. 2003, Michaletz 2017, Neely et al. 2018). Furthermore, Gizzard Shad can considerably increase phytoplankton, nutrient levels, and suspended solids, which may increase turbidity and ultimately impact foraging ability of piscivores (Schaus and Vanni 2000, Aday et al. 2003).

Gizzard Shad can grow very rapidly and can reach large sizes (>200 mm TL) in the first year (Michaletz 1998, Evens at el. 2014). This rapid growth limits most predators to consuming young-of-the-year Gizzard Shad (Miller 1960, Evens at el. 2014), and in some cases young-of-the-year Gizzard Shad may become too large to be utilized as prey if they outgrow predator gape limits (Cyterski & Ney 2005). Therefore, fast growth improves survival of Gizzard Shad to adulthood and minimizes the time they are vulnerable to piscivores (Evans et al. 2014). Conversely, if predator populations are in balance with Gizzard Shad prey populations and if predator size distribution is relatively even, a greater size range of Gizzard Shad can be consumed, keeping these respective populations in balance.

Clearly, the presence of Gizzard Shad in aquatic systems has both negative and positive effects on sport fisheries. Information about Gizzard Shad population characteristics in Oklahoma is limited. Furthermore, previous Gizzard Shad studies relied on scale-based ages to describe population characteristics. It is well established in the literature that scales ages are less precise when compared to otolith estimated ages. This has been observed for many freshwater fish species including Largemouth Bass (*Micropterus salmoides*), Spotted Bass (*Micropterus punctulatus*), Smallmouth Bass (*Micropterus dolomieu;* Long and Fisher 2001), Bluegill (*Lepomis macrochirus;* Edwards et al. 2005), Walleye (*Sander vitreus;* Kocovsky and Carline 2000), Saugeye (*S. vitrues x S. Canadensis*; Koch et al. 2017), Yellow Perch (*Perca flavescens;* Niewinski and Ferreri 1999), and White Crappie (*Pomoxis annularis;* Boxrucker 1986). Imprecise age estimates can result in biased population parameters, leading management biologists to make misguided management decisions that may negatively impact those fisheries. Therefore, the objective of this study was to assess age (using sagittal otoliths), as well as growth, mortality, condition, and size structure of Gizzard Shad collected from Lake Carl Etling, Oklahoma.

Methods

Sample Area – Lake Carl Etling was formed in 1958 by impounding South Carrizo Creek, a tributary of the Cimarron River (Snow et al. 2017) in the far northwestern tip of Oklahoma in Cimarron County. Lake Carl Etling is 159 acres at normal pool with 8 kilometers of shoreline. It is a hyper-eutrophic system with a mean depth of 1 meter and maximum depth of 5.5 meters (Snow et al. 2017). Water temperature can range from 1.6 - 33.4ºC depending on time of the year. Lake Carl Etling is a turbid system with mean secchi depth measuring 23.4 cm (Snow et al. 2017). The reservoir is managed by the Oklahoma Department of Wildlife Conservation and is surrounded by Black Mesa State Park.

Sampling – Ten Gizzard Shad per 10-mm length group were collected in September 2016 using boat electrofishing (pulsed DC, high voltage, Smith Root 7.5 GPP) to ensure that all size and age classes were represented in the sample (Michaletz 1994, DeVries et al. 1995, DiCenzo et al. 1996, Michaletz 1998, Aday et al. 2003, Wuellner et al. 2008, Michaletz 2017). During these efforts, the entire shoreline was sampled. Fish were placed on ice immediately after capture, and processed at the Oklahoma Fishery Research Laboratory in Norman, Oklahoma. Fish were measured for total length (TL; mm), weight (g) and sagittal otoliths were removed for aging.

Otolith aging - After otoliths were removed they were allowed to dry for at least 24 hr before mounting. Clayton and Maceina (1999) validated that one annulus forms yearly (via marginal increment analysis) in Gizzard Shad otoliths

and that fish <3 years old can be estimated using whole otoliths, however, otoliths from fish age 3 and older require sectioning in the sagittal plane for precise age estimation. Sagittal otoliths of Gizzard Shad are very delicate and require embedding in epoxy prior to sectioning. Otoliths were embedded by placing them in a 21-cell latex mold (12 mm x 5 mm x 6 mm; Electron Microscopy Sciences, Hatfield, PA), then immersed in West Systems epoxy (105 resin and 206 harder; Gougeon Brothers Inc., Bay City, Michigan). After the epoxy cured, otoliths were sectioned in a sagittal plane using a low speed Buehler IsoMet® saw (127 mm x 0.4 mm diamond wafering blade) and polished using 2000-grit sandpaper, as described by Maceina (1988). Otoliths were positioned polished-side up in modeling clay and covered with water to reduce glare.

To estimate ages, otoliths were viewed with a dissecting microscope (3.6-90x) using a fiber optic light and a reflective light source when needed. Annuli, which appeared as opaque bands on a light background, were counted to assign an age estimate to each fish. Each otolith was evaluated in random order by two independent readers (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.

Analysis - A length-frequency histogram (for aged fish and all fish combined) and proportional size distribution (PSD, stock ≥ 180mm, quality ≥280 mm; Anderson and Gutreuter 1983) was used to visualize and quantify Gizzard Shad size structure. A $\log_{10}$ weight to $\log_{10}$ TL regression was used to describe the weight:length relationship of the population. Gizzard Shad condition was evaluated by calculating relative weight (W_r) using the standard weight equation (W_s = -5.376 + 3.170 × log10 TL) presented by Anderson and Gutreuter (1983) where 100 = the 75th percentile of the national average weight of Gizzard Shad. A von Bertalanffy growth model was used to describe growth of Gizzard Shad (Cerrato 1990) and catch-curve-regression was used to assess total annual mortality (Ricker 1975). Age-0 fish are typically not recruited to sampling gears (Miranda and Bettoli 2007), so young-of-year Gizzard Shad were not included in the catch-curve analysis. Total annual mortality was calculated by regressing the $\log_e$ number of fish caught at each age to estimate instantaneous total mortality (Z), which was then converted to total annual mortality ($A = 1 - e^{-Z}$; Ricker 1975).

Results

A total of 198 Gizzard Shad were utilized for population assessment. Gizzard Shad used for aging purposes ranged from 0 to 12 years old and 56-308 mm TL (Figure 1A). This population was dominated by sub-stock sized (< 180 mm; Figure 1B) Gizzard Shad (n=120; 61%), although stock (n=78) and quality (n=12) sized fish were collected. This resulted in a PSD of 15. The weight-length relationship of Gizzard Shad was $\log_{10}(W) = -4.5974+2.7872 \log_{10}(TL)$ ($R^2 = 0.98$; Figure 2). This weight-length relationship results in a mean W_r of 78, which is well below the average of 100. When evaluated by size classes, W_r of stock sized

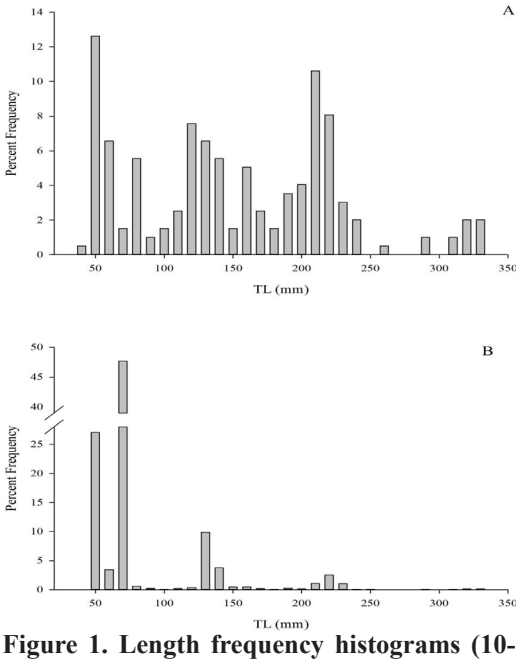


Figure 1. Length frequency histograms (10-mm bins) of (A) aged Gizzard Shad (n=198) and (B) all Gizzard Shad collected (n=2,886) collected from Lake Carl Etling, Oklahoma in September 2016.

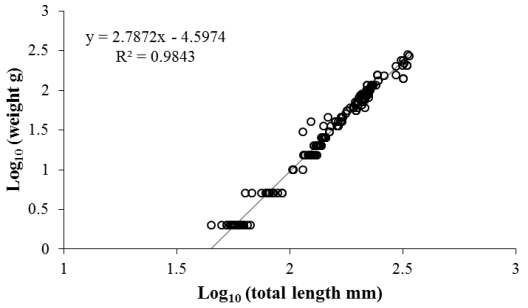


Figure 2. Weight-length relationship for Gizzard Shad collected from Lake Carl Etling, Oklahoma in September 2016. The logarithmically-transformed weight-length equation is $\log_{10}$ (W) = -4.5974+2.7872 $\log_{10}$ (TL).

Gizzard Shad was 82, however, W_r of quality sized Gizzard Shad was substantially lower at 58. The modelled von Bertalanffy growth curve indicates that Gizzard Shad approach maximum length steadily (K=0.31), with individuals in the population reaching approximately half (49%) of the predicted maximum TL by age-1 and growing to 73% of their predicted TL (L_∞ =295) by age-3 (Figure 3). The total annual mortality estimate for the Gizzard Shad population in Lake Carl Etling was 27% (Figure 4).

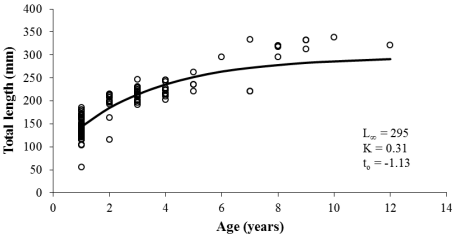


Figure 3. Von Bertalanffy growth curve calculated from otolith age estimates for Gizzard Shad collected from Lake Carl Etling, Oklahoma in September 2016. L_∞ = predicted maximum total length, K = growth constant, and t_0 = theoretical time when TL = 0.

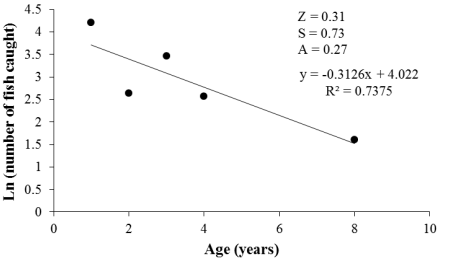


Figure 4. Catch curve regression and total annual mortality (A) for Gizzard Shad collected from Lake Carl Etling, Oklahoma in September 2016. Z = instantaneous total mortality and S = annual survival.

Discussion

To our knowledge this is the first study to use sagittal otoliths to gain a better understanding of population dynamics of a Gizzard Shad population in Oklahoma. Results suggest that Gizzard Shad from Lake Carl Etling experience fast growth in their first year, slowing as age increases, which is a characteristic of Gizzard Shad in eutrophic systems (DiCenzo et al. 1996, Michaletz 1998, Michaletz 2017). The body condition (Wr = 82-93) of Gizzard Shad from Lake Carl Etling was lower than previous studies, particularly when compared to studies from mesotrophic reservoirs (DiCenzo et al. 1996, Michaletz 1998, Michaletz 2017). Typically, in eutrophic systems W_r is stable for all size classes of Gizzard Shad (DiCenzo et al. 1996), however, condition of Gizzard Shad from Lake Carl Etling decreased as size increased. Despite poorer condition with size (and age) total annual mortality was low (27%). Michaletz (2017) reported mean annual mortality rates of 65% for small impoundments in Missouri. Using only shad > age 3, Wuellner et al. (2008) found an annual mortality rate of 27%. Gizzard Shad are characterized as a short-lived species (< 8 yrs) in eutrophic system (DiCenzo et al. 1996, Michaletz 2017), but are longer lived in mesotrophic systems (8-14 years; DiCenzo et al. 1996, Wuellner et al. 2008). We found longevity of Gizzard Shad in Lake Carl Etling to be high

(12 years) with low mortality.

The Lake Carl Etling Gizzard Shad population was composed primarily of small adults (< 180 mm). Gizzard Shad populations with low PSD values, which indicate a population dominated by small adults, often experience poor reproduction (Willis 1987). Conversely, Willis (1987) suggested that a population of Gizzard Shad with a high PSD value results in more successful reproduction and produces a greater biomass of age-0 fish. Gizzard shad populations with high PSDs are also typically in good condition (Michaletz et al. 1998). Because the Lake Carl Etling Gizzard Shad population has a low PSD and relatively poor condition, reproduction and resulting age-0 fish production may be limited. This is cause for concern because most piscivores only consume age-0 Gizzard Shad (<100 mm TL), with older shad exceeding the predators preferred size (Evans et al. 2014). Only larger piscivores can consume shad ≤ 200 mm TL (Evans at el. 2014).

Gizzard Shad are typically the main forage source for the predatory fishes in Lake Carl Etling. Black Bullhead (*Ameiurus melas*), Largemouth Bass, Walleye, Hybrid Striped Bass (*Morone saxatilis x M. chrysops*; last stocked 2010), Tiger Muskellunge (*Esox masquinongy x E. Lucius*; if present in very low numbers; Snow et al. 2017), and stocked Rainbow Trout (*Oncorhynchus* mykiss) all consume Gizzard Shad. Walleye and Largemouth Bass are known to prefer soft rayed-fishes, like Gizzard Shad, over spiny rayed-fishes (Gillen et al. 1981, Knight et al. 1984, Storck 1986, Einfalt and Wahl 1997, Shoup and Lane 2015). When Walleye are able to forage on soft rayed-fishes, growth rates are typically higher (Knight et al. 1984). We found that Gizzard Shad in Lake Carl Etling grow to almost half their full size in the first year, and by year three they approach 75% of their maximum size. This rapid growth early in life means that Gizzard Shad are only accessible by piscivores for a short period of time due to gape limitations. Because age-0 Gizzard Shad are only seasonally available due to their rapid growth, Largemouth Bass, Walleye and other piscivores may be forced to feed on less desired spiny rayed-fish, like Bluegill, which may slow piscivore growth rates.

The Largemouth Bass and Walleye populations in Lake Carl Etling have poor growth rates, resulting in low PSD and below average body condition (mean W_r; Table 1, ODWC unpublished data). A combination of limited age-0 Gizzard Shad biomass and rapid growth of young Gizzard Shad (age 0-2) may be affecting predator populations that experience reduced growth rates and below-average condition. Size-specific differences in condition were apparent for Largemouth Bass in this population; as Largemouth Bass size increased, mean W_r increased (stock = 90, quality = 97, and preferred = 103). This suggests that larger Largemouth Bass may take advantage of larger bodied Gizzard Shad as forage, resulting in better body condition. Conversely, smaller Largemouth Bass had poorer condition, likely because most Gizzard Shad have outgrown the gape limits of smaller Largemouth Bass. The majority (86%) of Largemouth Bass in this population are < 300 mm TL, which have a gape width allowing them to consume shad 126 – 136 mm TL (Lawerence 1957). Similarly, previous studies that evaluated the relationship between Largemouth Bass TL and TL of Gizzard Shad consumed found that Largemouth Bass measuring 300 mm typically consume Gizzard Shad < 150 mm TL (Lewis et al. 1974, Shepherd 2008). The condition of Walleye was also well below average in Lake Carl Etling, which may also be attributed to the size of Gizzard Shad. It is not surprising that condition of Largemouth Bass and Walleye are below average in the spring. During winter, Rainbow Trout are stocked into Lake Carl Etling

Table 1. Proportional size distribution (PSD) and relative weight (W_r) of two sportfish collected from Lake Carl Etling, Oklahoma in spring 2017 (ODWC unpublished data).

Species	PSD (quality)	PSD (preferred)	W_r (stock)	W_r (quality)	W_r (preferred)	Mean W_r
Largemouth Bass	11	5	90	97	103	91
Walleye	11	N/A	85	87	N/A	85

to create an additional sport fishing opportunity. Snow et al. (*in-review*) found that Rainbow Trout consume a large portion of the age-0 Gizzard Shad biomass during winter months. During this time, Largemouth Bass, Walleye, and Rainbow Trout are all competing for the same resources. By spring, most of the Gizzard Shad remaining are too large to be consumed by the resident piscivores, likely resulting in poor body condition of the predators.

Lake Carl Etling is not well suited for Gizzard Shad, which were stocked with the intention of providing forage for sport fishes. Michaletz (1998) found that Gizzard Shad are best suited for deep clear mesotrophic impoundments and are undesirable in shallow eutrophic impoundments such as Lake Carl Etling. The Lake Carl Etling fish community is dominated by predators, which is limiting the number of age-0 Gizzard Shad recruiting to adulthood. This heavy cropping of age-0 Gizzard Shad by predators results in fast growth within the first-year allowing survival to sizes that are too large to be consumed by piscivores.

This study provides baseline population dynamics information for Gizzard Shad from a single small impoundment in Oklahoma, and it represents the first use of otoliths to derive population dynamics for Gizzard Shad in Oklahoma. Because we do not have data on other Oklahoma populations for comparison, we cannot say whether this represents a typical Gizzard Shad population in Oklahoma. However, population characteristics are important because, without these data, fisheries managers do not know if or how sport fish populations are impacted by Gizzard Shad, whether or not a small impoundment can withstand stocking of an additional predator biomass, or if the majority of fish biomass is comprised of Gizzard Shad. Future research should focus on gaining more knowledge about Gizzard Shad populations in Oklahoma reservoirs and small impoundments, as this information is lacking statewide and is critical to managing sportfish populations where shad serve as the primary forage base. We further recommend that Gizzard Shad population assessments be based on the use of sagittal otoliths.

Acknowledgments

The authors thank those individuals that assisted with sampling including Amie Robison, Dakota Schoeling, Jeff Tibbets and Rusty Menefee. We thank Kurt Kuklinski (ODWC) and Dr. Dan Shoup (OSU) for reviewing an earlier draft of this manuscript. Financial support for this publication was provided by the Sport Fish Restoration Program grant [F-50-R-25], [F-86-D-1] and [F-65-D-7] to the Oklahoma Department of Wildlife Conservation.

References

Aday, D.D., R. John, H. Hoxmeier, and D.H. Wahl. 2003. Direct and indirect effects of gizzard shad on bluegill growth and population size structure. Trans. Amer. Fish. Soc.132: 47-56.

Anderson, R.O., and S.J. Gutreuter. 1983. Length weight and associated structural indices. Pages 284-300 *in*: L.A. Nielsen and D. Johnson, editors. Fisheries techniques. Am. Fish. Soc., Betheda, MD.

Boxrucker, J. 1986. A comparison of the otolith and scale methods for aging white crappies in Oklahoma. North Am. J. Fish. Manage. 6: 122-125.

Clayton, D.L., and M.J. Maceina. 1999. Validation of annulus formation in Gizzard Shad otoliths. North Am. J. Fish. Manage. 19: 1099-1102.

Cerrato, R.M. 1990. Interpretable statistical tests for growth comparisons using parameters in the von Bertalanffy equation. Can. J. Fish. Aquat. Sci. 47: 1416–1426.

Cyterski, M.J., and J.J. Ney. 2005. Availability of clupeid prey to primary piscivores in Smith Mountain Lake, Virginia. Trans. Amer. Fish. Soc. 134: 1410–1421.

DeVries, D.R., and R.A. Stein. 1990. Manipulating shad to enhance sport fisheries in North America: an assessment. North Am. J. Fish. Manage. 10: 209-223.

DiCenzo, V.J., M.J. Maceina, and M.R. Stimpert. 1996. Relations between reservoir trophic state and gizzard shad population characteristics in Alabama reservoirs. North Am. J. Fish. Manage. 16: 888-895

Edwards, K.R., Q.E. Phelps, J.L. Shepherd, D.W. Willis, and J.D. Jungwirth. 2005. Comparison of scale and otolith age estimates for two South Dakota bluegill populations. Proc. S. Dak. Acad. Sci. 84: 181-186.

Einfalt, L.M., and D.H. Wahl. 1997. Prey selection by juvenile walleye as influenced by prey morphology and behavior. Can. J. Fish. Aquat. Sci. 54: 2618-2626.

Evans, N.T., D.E. Shoup and D.C. Glover. 2014. A simplified approach for estimating age-0 Gizzard Shad supply and predator demand. Fish. Manage. Ecol. 21: 140-154.

Gillen, A.L., R.A. Stein, and R.F. Carline. 1981. Predation by pellet-reared tiger muskellunge on minnows and bluegills in experimental systems. Trans. Amer. Fish. Soc. 110: 197-209.

Hoff, G.R., D.J. Logen, and M.F. Douglas. 1997. Otolith morphology and increment validation in young lost river and shortnose suckers. Trans. Amer. Fish. Soc. 126: 488-494.

Jenkins, R.M. 1949. A fish population study of Claremore City Lake. Proc. Okla. Acad. Sci. 30: 84-93.

Jenkins, R.M. 1957. The effect of gizzard shad on the fish populations of a small Oklahoma Lake. Trans. Amer. Fish. Soc. 85:58-74.

Knight, R.L., J.F. Margraf, and R.F. Carline. 1984. Piscivory by walleye and yellow perch in western Lake Erie. Trans. Amer. Fish. Soc. 113: 677-693.

Koch, J., B. Neely, and B. Sowards. 2017. Precision of three structures for saugeye age estimation. North Am. J. Fish. Manage. DOI:10.1002/nafm.10019.

Kocovsky, P.M. and R.F. Carline. 2000. A comparison of methods for estimating ages of unexploited walleyes. North Am. J. Fish. Manage. 20: 1044-1048.

Lawrence, J.M. 1957. Estimating sizes of various forage fishes largemouth bass can swallow. Proc. Southeastern Assoc. Game and Fish Comm. 11: 220-225.

Lewis, W.H., R. Heidinger, W. Kirk, W. Chapman, and D. Johnson. 1974. Food intake of the largemouth bass. Trans. Amer. Fish. Soc. 103: 277-280.

Long, J.M. and W.L. Fisher. 2001. Precision and bias of largemouth, smallmouth, and spotted bass ages estimated from scales, whole otoliths, and sectioned otoliths. North Am. J. Fish. Manage. 21: 636-645.

Maceina, M.J. 1988. Simple grinding procedure to section otoliths. North Am. J. Fish. Manage. 8: 141-143.

Michaletz, P.H. 1994. Comparison of electrofishing and gillnetting for sampling Gizzard Shad. Proc. Annu. Southeast Assoc. Fish. Wildl. Agencies 48:533-541.

Michaletz, P.H. 1998. Population characteristics of gizzard shad in Missouri reservoirs and their relation to reservoir productivity, mean depth and sport fish growth. North Am. J. Fish. Manage. 18: 114-123.

Michaletz, P.H. 2017. Variation in characteristics among gizzard shad populations: The role of impoundment size and productivity. Fish Manag Ecol: 24: 361–371.

Miller, R.J. and H.W. Robison. 2004. Fishes of Oklahoma. University of Oklahoma Press, Norman, Oklahoma.

Miller R.R. 1960. Systematics and biology of the gizzard shad (Dorosoma cepedianum) and related fishes. Washington (DC): United States Fish and Wildlife Service. Fish. Bull. 173: 371-388.

Miller, R.R. 1957. Origin and dispersal of the alewife, *Alosa pseudoharengus*, and the gizzard shad, *Dorosoma cepedianum*, in the Great Lakes. Trans. Amer. Fish. Soc. 86: 97-111.

Miranda, L.E. and P.W. Bettoli. 2007. Mortality. Pages 229-277 in C.S. Guy and M. L. Brown, editors. Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, MD.

Mueller, G.A. and J.L. Brooks. 2004. Collection of an adult gizzard shad (*Dorosoma cepedianum*) from the San Juan River, Utah. West N. Am. Nat. 64: 135-136.

Neely, B.C., J.D. Koch. and S.T. Lynott. 2018. Effects of gizzard shad reduction on relative abundance, growth, and body condition of bluegills and largemouth bass in a small kansas impoundment. North Am. J. Fish. Manage. 38: 856-866.

Niewinski, B.C., and C.P. Ferreri. 1999. A comparison of three structures for estimating the age of yellow perch. North Am. J. Fish. Manage. 19: 872-877.

Noble, R.L. 1981. Management of forage fishes in impoundments of the southern United States. Trans. Amer. Fish. Soc. 110: 738-750.

Page, L.M., and B.M. Burr. 1991. A field guide to freshwater fishes of North America north of Mexico. The Peterson Field Guide Series, volume 42. Houghton Mifflin Company, Boston, MA.

Porath, M.T. 2006. Climate and habitat factors related to a localized extirpation of gizzard shad (Dorosoma cepedianum). Great Plains Res. 16: 127-135.

Ricker W.E. 1975. Computation and interpretation of biological statistics in fish populations. Ottawa: J. Fish. Res. Board Can. 191.

Schaus, M.H., and M.J. Vanni. 2000. Effects of gizzard shad on phytoplankton and nutrient dynamics: role of sediment feeding and fish size. Ecol. 81: 1701–1719.

Shepherd, M.D. 2008. Effects of striped bass stocking on largemouth bass, and spotted bass in Lewis Smith Lake, Alabama. [MSc thesis]. Auburn, (AL): Auburn University

Shoup, D.E., and W.D. Lane. 2015. Effects of turbidity on prey selection and foraging return of adult largemouth bass in reservoirs. North Am. J. Fish. Manage. 35: 913-924.

Snow, R. A., C.P. Patterson, D.E. Shoup, and M.J. Porta. 2017. An evaluation of tiger muskellunge introduced into Lake Carl Etling, Oklahoma. Proc. Okla. Acad. Sci. 97: 33-40.

Snow, R.A., D.E. Shoup, M.J. Porta, and C.P. Patterson. *In review*. Effects of wintertime stocking of rainbow trout on the forage community of an Oklahoma impoundment. North Am. J. Fish. Manage.

Stein, R.A., D.R. DeVries, and J.M. Dettmers. 1995. Food web regulation by a planktivore: exploring the generality of the trophic cascade hypothesis. Can. J. Fish. Aquat. Sci. 52: 2518–2526.

Storck, T.W. 1986. Importance of gizzard shad in the diet of largemouth bass in Lake Shelbyville, Illinois. Trans. Amer. Fish. Soc.115: 21-27.

VanDeHey, J.A., D.W. Willis, J.M. Harris and B.G. Blackwell. 2014. Effects of gizzard shad introduction on walleye and yellow perch populations in prairie glacial lakes. Fish. Sci. 150: 49-59.

Willis, D.W. 1987. Reproduction and recruitment of gizzard shad in Kansas reservoirs. North Am. J. Fish. Manage. 7: 71-80.

Wuellner, M.R., B.D.S. Graeb, M.J. Ward, and D.W. Willis. 2008. Review of gizzard shad population dynamics at the northwestern edge of its range. Pages 637-653 *in*: M.S. Allen, S. Sammons, and M.J. Maceina, editors. Balancing fisheries management and water uses for impounded river systems. Am. Fish. Soc., Betheda, MD.

Submitted August 23, 2018 Accepted November 18, 2018

New Ectoparasite (Phthiraptera; Siphonaptera; Diptera) Records from Birds (Strigiformes: Passeriformes) and Mammals (Lagomorpha; Rodentia) in Southeastern Oklahoma

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Lance A. Durden
Department of Biology, Georgia Southern University, Statesboro, GA 30458

Kylie N. Brecheisen
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Will K. Reeves
USDA-APHIS-BRS, Fort Collins, CO 80526

Abstract: Little is known about many of the ectoparasites occurring on higher vertebrate hosts in Oklahoma. Here, we report nine taxa of ectoparasites from Oklahoma for the first time. These include five species of chewing lice [*Degeeriella fulva* from *Buteo lineatus* (red-shouldered hawk), *Strigiphilus otus* from *Megascops asio* (eastern screech owl), *Philopterus osborni* and *Myrsidea interrupta* from *Corvus brachyrhynchos* (common crow), and *Stachiella octomaculatus* from *Sylvilagus floridanus* (eastern cottontail)], three species of fleas [C*ediopsylla simplex* and *Odontopsyllus multisinosus* from *S. floridanus,* and *Polygenis gwyni* from *Neotoma floridana* (eastern woodrat)], and a dipteran (*Ornithoctona erythrocephala* from *B. lineatus*).

Introduction

Over the past half-decade, our research consortium has provided information on various ectoparasites occurring on hosts in Oklahoma for the first time (McAllister et al. 2013a, b, 2014, 2016, 2017a, 2018; McAllister and Durden, 2014a, b, 2017; Connior et al. 2015). One of these reports presented information on a road-killed great-horned owl (*Bubo virginianus*) from the state that yielded information on five taxa of parasites. This showed that utilizing road-killed raptors for parasite surveys was an excellent way to conduct research in the name of conservation rather than having to collect and then euthanize live specimens. Here, we continue that research by reporting new state records for nine taxa of ectoparasites from select birds and mammals.

Methods

Three avians from McCurtain County, including a common crow (*Corvus brachyrhynchos*), eastern screech owl (*Megascops asio*), and red-shouldered hawk (*Buteo lineatus*) were found dead on the road. In addition, an eastern cottontail (*Sylvilagus floridanus*) was shot and an eastern woodrat (*Neotoma floridana*) was found dead. All

were brought to the laboratory for ectoparasitic examination. Those specimens that appeared to be recently killed showed no sign of putrefaction. The feathers and hair were vigorously brushed over a white enamel tray to help see 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). Hosts were deposited as photovouchers and are housed in the Eastern Oklahoma State University-Idabel collection, Idabel, Oklahoma. Voucher specimens of ectoparasites were deposited in the General Ectoparasite Collection in the Department of Biology at Georgia Southern University, Statesboro, Georgia, or the G. P. Gillette Museum of Arthropod Diversity Colorado State University (CSUC), Fort Collins, Colorado, under individual accession numbers.

Results and Discussion

All birds and mammals examined were infested with various live ectoparasites. Nine taxa, including five species of chewing lice, three species of fleas, and a dipteran, were collected. All ectoparasites are reported from Oklahoma for the first time. The nine ectoparasite species recovered are presented below in annotated format.

Insecta: Phthiraptera: Ischnocera: Philopteridae

***Degeeriella fulva* Giebel.** – Three males and seven females (accession no. L3820) were removed from a *B. lineatus* collected on 2 March 2018 from Wright City (34° 04'5.9088"N, 95° 00' 19.368"W). This louse is a common ectoparasite of a wide variety of raptors (Emerson 1972; Price et al. 2003) although Emerson (1940) did not record it from Oklahoma.

***Strigiphilus otus* Emerson.** – One male, one female, and two nymphs of *S. otus* (L3814) were taken from *M. asio* collected on 22 January 2018 from Smithville (34° 28' 0.4794"N, 94° 38' 37.6794"W). This louse is mainly an ectoparasite of the screech owl and the genus is restricted to owls. Previous records from *M. asio* are from Arizona, Georgia, Indiana, Maryland, Minnesota, New York, Oregon, Texas, and British Columbia, Canada (Emerson 1955). There are records from four additional species of New World owls from Coahuila and Michoacan, México, and Arizona (Clayton 1990; Price et al. 2003).

***Philopterus osborni* Edwards.** – One female *P. osborni* (L3816) was found on *C. brachyrhynchos* collected from Smithville (34° 28' 0.4794"N, 94° 38' 37.6794"W). This louse is widespread in North America and has been recorded from five species of *Corvus* but mainly from *C. brachyrhynchos* (Price et al. 2003). Philopterid chewing lice are ectoparasites of birds and some species serve as vectors of filarial nematodes (Durden 2018).

Menoponidae

***Myrsidea interrupta* Osborn.** – Two males and one female *M. interrupta* (L3816) were removed from *C. brachyrhynchos* collected on 8 February 2018 from Smithville (34° 28' 0.4794"N, 94° 38' 37.6794"W). This chewing louse was originally described from an American crow from Nebraska and is widespread in North America (Emerson 1972). However, Emerson (1940) did not record it from Oklahoma. This louse appears to be genus specific as it has only been recorded from birds of the genus *Corvus* (Price et al. 2003).

Trichodectidae

***Stachiella octomaculatus* Paine.** – Two males, one female, and two nymphs of *S. octomaculatus* (L3819) were taken from a *S. floridanus* collected on 10 March 2018 from Haworth (33° 50' 47.3994"N, 94° 39' 9.7194"W). This louse is typically found on the raccoon, *Procyon lotor*. The host association recorded here might be considered accidental and/or could be the result of both mammals cohabitating in a nest or burrow.

Siphonaptera: Pulicidae

***Cediopsylla simplex* (Baker) (rabbit flea).** – Three males and two females of *C. simplex* (L3817) were found on *S. floridanus*

collected on 10 March 2018 from Haworth (33° 50' 47.3994"N, 94° 39' 9.7194"W). This is a widespread flea of lagomorphs and their predators in the eastern two-thirds of North America (Holland 1985; Durden et al. 2012).

Rhopalopsyllidae

***Polygenis gwyni* (C. Fox).** – A single male *P. gwyni* (L3815) was found on a *N. floridana* collected on 29 January 2018 in Smithville (34° 28' 0.4794"N, 94° 38' 37.6794"W). This is an unusual occurrence since woodrats tend to be uncommon hosts of this flea. It is more often reported from hispid cotton rats (*Sigmodon hispidus*), the most commonly recorded host of *P. gwyni* (McAllister et al. 2017b). In addition, there are also several records of *P. gwyni* from Virginia opossum (*Didelphis virginiana*) and some other mammals throughout its range in the southern U. S. (Smit 1987; Durden et al. 2012).

Leptopsyllidae

***Odontopsyllus multispinosus* Baker.** – One male and one female *O. multispinosus* (L3817) were found on *S. floridanus* collected on 10 March 2018 from Haworth (33° 50' 47.3994"N, 94° 39' 9.7194"W). This is a large flea associated with leporids and their predators in eastern North America (Holland 1985; Durden et al. 2012).

Diptera: Hippoboscidae

***Ornithoctona erythrocephala* (Leach).** – A single female of *O. erythrocephala* (CSUC) was taken from *B. lineata* collected on 2 March 2018 from Wright City (34° 04'5.9088"N, 95° 00' 19.368"W). This louse fly infests raptors and other medium to large-sized birds (Bequaert 1954). It has a wide geographic distribution and has been recorded from seven Canadian Provinces, 24 U. S. states, and México, as well as Central and South America as far south as Chile. To date, 16 genera, 25 families, and 14 orders of birds are known as hosts. The broad geographic distribution and wide host range could indicate that the nominal taxon *O. erythrocephala* actually includes several cryptic species, molecular studies might clarify this. Louse flies (213 species) belong to a family of pupiparous dipterans, which in their adult stage are ectoparasites of birds and mammals (Bequaert 1954; Maa 1969; Dick 2006).

In conclusion, we document nine new distributional records for ectoparasites of some common birds and mammals from Oklahoma. Once again (see Nelder and Reeves 2005; McAllister et al. 2017), this survey illustrates the significance of salvaging road-killed raptors and other birds and vertebrates which can yield knowledge on their parasites when data could not be otherwise obtained because of state and federal restrictions on collecting and euthanizing live birds, all in the spirit of conservation. In addition, these ectoparasites can be screened for significant tick, louse, and flea borne pathogens as shown by Reeves et al. (2005).

Acknowledgments

The Oklahoma Department of Wildlife Conservation and U.S. Fish and Wildlife Service for provided Scientific Collecting Permits to CTM to salvage migratory birds, permit numbers 7297 and MB84782C-0**,** respectively.

References

Bequaert JC. 1954. The Hippoboscoidea or louse flies (Diptera) of mammals and birds. Part II. Taxonomy, evolution and revision of American genera and species. Entomol Amer 34 (n.s.):1–232.

Clayton DH. 1990. Host specificity of *Strigiphilus* owl lice (Ischnocera: Philopteridae) with description of new species and host associations. J Med Entomol 27:257–265.

Connior MB, Durden LA, McAllister CT. 2015. New county record for the least shrew, *Cryptotis parva*, and notes on an ectoparasite, *Haemogamasus longitarsus*. Proc Okla Acad Sci 95:33–34.

Dick CW. 2006. Checklist of world Hippoboscidae (Diptera: Hippoboscoidea). Chicago (IL): Field Museum of Natural History. 7 p.

Durden LA. 2018. Lice (Phthiraptera). In: Mullen GR and Durden LA, editors. Medical and veterinary entomology, 3rd ed. San Diego (CA): Elsevier Press. p 79–106.

Durden LA, Wilson N, Eckerlin RP, Baker WW. 2012. The flea (Siphonaptera) fauna of Georgia, USA: Hosts, distribution and medical-veterinary importance. Ann Carnegie Mus 80:83–113.

Emerson KC. 1940. A preliminary list of the Mallophaga (biting lice) of Oklahoma. Proc Okla Acad Sci 20:103–104.

Emerson KC. 1955. A new mallophagan from the screech owl (Philopteridae). Proc Entomol Soc Wash 57: 241–242.

Emerson KC. 1972. Checklist of the Mallophaga of North America (north of Mexico). Part IV. Bird host list. Dugway (UT): Deseret Test Center. 216 p.

Holland GP. 1985. The fleas of Canada, Alaska and Greenland (Siphonaptera). Mem Entomol Soc Canada No. 130. 631 p.

Maa TC. 1969. A revised checklist and concise host index of Hippoboscidae (Diptera). Pacific Insects Monogr 20:261–299.

McAllister CT, Bursey CR, Connior MB, Durden LA, Robison HW. 2014. Helminth and arthropod parasites of the ground skink, *Scincella lateralis* (Sauria: Scincidae), from Arkansas and Oklahoma. Comp Parasitol 81:210–219.

McAllister CT, Connior MB, Bursey CR, Durden LA, Seville RS, Robison HW, Trauth SE. 2016. Parasites (Coccidia, Trematoda, Acari) of tri-colored bats, *Perimyotis subflavus* (Chiroptera: Vespertilionidae): new geographical records for Oklahoma. Proc Okla Acad Sci 96:63–69.

McAllister CT, Durden LA. 2014a. New records of chiggers and ticks from an Oklahoma amphibian and reptile. Proc Okla Acad Sci 94:40–43.

McAllister CT, Durden LA. 2014b. *Haemogamasus harperi* Keegan (Arthopoda:Acari: Laelapidae): New to the mite fauna of Oklahoma. Proc Okla Acad Sci 94:70–72.

McAllister CT, Durden LA. 2017. Parasitic Acari from four Oklahoma vertebrates (Aves, Mammalia), including new state records for mites (Laelapidae, Listrophoridae, Macronyssidae). Proc Okla Acad Sci 97:21–24.

McAllister CT, Durden LA, Connior MB. 2013a. New state records for the sucking louse, *Hoplopleura sciuricola* (Anoplura: Phthiraptera: Hoplopleuridae) and flea, *Foxella ignota* (Siphonaptera: Ceratophyllidae), from Oklahoma. Proc Okla Acad Sci 93:25–28.

McAllister CT, Durden LA, Connior MB, Robison HW. 2013b. Parasitism of reptiles by the blacklegged tick (*Ixodes scapularis*) and western blacklegged tick (*Ixodes pacificus*) with new records of *I. scapularis* from Arkansas and Oklahoma lizards: implications for Lyme Disease epidemiology. Herpetol Rev 44:572–579.

McAllister CT, Durden LA, Richardson DM, Hnida JA. 2017a. Some parasites (Apicomplexa, Trematoda, Nematoda, Acanthocephala, Phthiraptera) of the common great horned owl, *Bubo virginianus* (Aves: Strigiformes: Strigidae), from southeastern Oklahoma. Proc Okla Acad Sci 97:83–90.

McAllister CT, Durden LA, Robison HW, Connior MB. 2017b. The fleas (Arthropoda: Insecta: Siphonaptera) of Arkansas. J Ark Acad Sci 71:69–76.

McAllister CT, Mitchell JC, Durden LA. 2018. Life history notes: *Acris blanchardi* (Ectoparasites). Herpetol Rev 49:297–298.

Nelder MP, Reeves WK. 2005. Ectoparasites of road-killed vertebrates in northwestern South Carolina, USA. Vet Parasitol 129:313–322.

Price RD, Hellenthal RA, Palma RL, Johnson KP, Clayton DH. 2003. The chewing lice: World checklist and biological overview. Illinois Nat Hist Surv Spec Pub 24. 501 p.

Reeves WK, Nelder MP, Korecki JA. 2005. *Bartonella* and *Rickettsia* in fleas and lice from mammals in South Carolina, USA. J Vector Ecol 30:310–315.

Smit FGAM. 1987. An illustrated catalogue of the Rothschild collection of fleas (Siphonaptera) in the British Museum (Natural History) with keys and short descriptions for the identification of families, genera, species and subspecies of the Order. Volume VII. Malacopsylloidea (Malacopsyllidae and Rhopalopsyllidae). Oxford and London: Oxford University Press and The British Museum (Natural History). 380 p.

Submitted September 4, 2018 Accepted December 3, 2018

Anthropogenic Influence on American Black Bear Diet in the Western Ozark Mountains in Eastern Oklahoma

Joseph P. Connor
School of Natural Sciences, University of Central Missouri, Warrensburg, MO 64093

Victoria L. Jackson
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Jennifer R. Mittelhauser
School of Natural Sciences, University of Central Missouri, Warrensburg, MO 64093

W. Sue Fairbanks
Department of Natural Resource Ecology and Management, Oklahoma State University, Stillwater, OK 74078

Abstract: American black bears (*Ursus americanus*) are returning to eastern Oklahoma from Arkansas, where they were re-introduced in the 1950s. This movement back into human-occupied areas can lead to conflict. Black bears are known to use anthropogenic food sources. To determine the extent of anthropogenic influence, we analyzed scat for anthropogenic food items. We collected 38 scat samples from 16 May 2014 through 17 October 2014 in the Ozark Plateau of Adair, Cherokee, and Sequoyah counties. Once collected, scat was dried for preservation. The samples were rehydrated and filtered for undigested items, which were categorized into 6 groups. We measured percent volume and percent frequency of occurrence of each item that was > 1% of the volume of the entire sample. The volumes of diet groups were significantly different (Kruskal-Wallis, $p < 0.001$) and were separated into 3 statistically distinct groups: Anthropogenic food > Reproductive plant parts > Animal matter, Herbage, Debris, and Unidentified (Tukey's Honest Significant Difference test, alpha = 0.05). There was no significant difference between the volumes of anthropogenic versus natural food (Mann Whitney U, $p = 0.368$). Though black bears will eat anthropogenic food sources if they encounter them, anthropogenic food sources do not make up a significant portion of their diet when compared against all natural forage.

Introduction

Human activity can affect wildlife populations in negative ways such as transportation of invasive species, over-harvest of species, habitat loss, and habitat fragmentation (Forester and Machlis 1996; Woodroffe 2000; McKinney 2001). Furthermore, human influence is changing specific animal behaviors, such as diet preference (Breck et al. 2009).

Food abundance and distribution can affect movements and behavior of mammals (Isbell et al. 1998), and often more so in carnivores (Jepsen et al. 2002). For some species, such as the raccoon (*Procyon lotor*), many of the effects of anthropogenic influence are positive, such as increased food supply through anthropogenic means and a decrease in natural predators (Prange et al. 2003). For other species, such as American

black bears (*Ursus americanus)*, anthropogenic influence is almost always negative mainly due to the fragmentation of habitat and human-bear conflict (Mattson 1990). Conflict occurs when bears try to utilize anthropogenic food sources such as trash containers, gardens, orchards, apiaries, and corn (Merkle et al. 2013). This problem is exacerbated when humans purposely provide anthropogenic food sources to wildlife, such as deer feeders.

Understanding how wildlife populations expand to recolonize former habitats is important for management and conservation (Swenson et al. 1998). American black bears can be found in relative abundance within much of the northern U.S. but are rare in the southern states. However, bears are beginning to recolonize southern areas in the U.S., including Oklahoma. Black bears were extirpated from Oklahoma by 1915 but they were reintroduced in Arkansas in the 1950s and 1960s (Smith and Clark 1994). The population has expanded back into Oklahoma and southern Missouri (Bales et al. 2005). The southern population in Oklahoma, found in the Ouachita Mountains, is well established and growing (Pfander 2016). In 2009, the Oklahoma Department of Wildlife Conservation opened a black bear hunting season for the counties of Le Flore, McCurtain, Pushmataha, and Latimer. The east-central population in Adair, Cherokee, and Sequoyah counties is not yet self-sustaining and is not hunted (Lyda et al. 2016).

The east-central black bear population is recolonizing in an area that already has some human habitation, with 22,098 people in Adair County (14.8 people/km^2), 48,700 people in Cherokee County (24.2 people/km^2), and 41,294 people in Sequoyah County (22.3 people/km^2) (U.S. Census Bureau 2016). As these animals move in, they may begin to use anthropogenic food sources such as apiaries, deer feeders, orchards, and trash bins (Merkle et al. 2013). In Oklahoma, it is legal to bait deer and other wildlife on private land. Availability of this bait, usually corn, may have a major influence on the overall diet of the bears and other wildlife in the area. Bears have been known to break into cars if they think food is inside (Breck et al. 2009). Conflicts might also include predation on livestock, a problem that is on the rise in Colorado (Baruch-Mordo et al. 2008). Based on reports of depredation to the Colorado Department of Wildlife from 1979 to 2003, complaints of depredation by bears mostly involved sheep, but also included goats, chickens, pigs, and cows (Baruch-Mordo at al. 2008). Bears are more likely to seek out anthropogenic food sources when there is a shortage of natural forage (Clark et al. 2005). In West Virginia, from 1980-2004, black bear mortality increased in years when there was mast failure. These deaths were mainly due to road kills and landowners destroying bears that damaged property (Ryan et al. 2007).

American black bears require a variety of habitats that produce seasonal foods, as well as extensive and secluded areas for denning (Landers et al. 1979). Habitat selection by bears varies seasonally and is governed by presence of food (Clark et al. 1994; Fuller and Keith 1980). Though they are part of Order Carnivora, black bears are omnivorous. These animals prefer heavily wooded areas with mast species like oak (*Quercus* spp.), hickory (*Carya* spp.), and various species of berries (Benson and Chamberlain 2006). Black bears rarely hunt but have been known to take neonate ungulates (Schlegel 1976). When available, they will also eat carrion (Arner 1948).

In the fall, black bears prefer a greater proportion of natural foods due to the availability of hard mast such as acorns, hickory nuts, walnuts (*Juglans* spp.) and beechnuts (*Fagus* spp.) (Sara Lyda, [Oklahoma State University, Stillwater, Oklahoma], personal communication, [September 2013]). The hard mast species are still important in the spring when females emerge from their dens with cubs. Quantity and quality of acorns determines the quality and quantity of milk produced by sows (McDonald and Fuller 2005). Milk is high in fat (220 g/kg) and low in water (670 g/kg), helping altricial cubs to gain weight quickly during nursing (Oftedal et al. 1993).

Our research addressed the proportion of bear diet comprised of anthropogenic resources

in the Ozark Plateau of eastern Oklahoma. We also determined the general composition of the diet based on scat analysis. Because of the availability of deer feeders, we predicted that anthropogenic foods would account for the greatest volume compared to natural foods.

Methods

As part of a larger project addressing population demography, both live capture and non-invasive genetic methods were used to identify individual bears. This project followed the American Society of Mammalogists (ASM) guidelines for the humane use of mammals (Sikes et al. 2011) and was approved by OSU's Institutional Animal Care and Use Committee (IACUC) under Protocol # AG-13-6.

The area of study was the Ozark Plateau in Adair, Cherokee, and Sequoyah counties in Oklahoma. From 1 May 2014 to 1 September 2014, average temperature for Cookson, OK, was 23.05 ˚C (73.5 ˚F) with a range of 1.1 -36.6 ˚C and the average precipitation was 0.28 cm/ per day (Oklahoma Climatological Survey 2015). Cookson, OK, is located in the eastern portion of the state and is 16 km from where these 3 counties intersect. Our study site was predominantly an oak-hickory forest with some pine associations (Duck and Fletcher 1943). In addition to the public wildlife management areas, much of the private land is used for wildlife management, though some landowners raise livestock as well (Sara Lyda [Oklahoma State University, Stillwater, Oklahoma] personal communication [August 2015]).

As part of the larger study, bears were lured into barrel traps with donuts and other baked goods. Corn was also set outside of traps. Traps were checked daily unless the forecast called for temperatures higher than 32.2 ˚C, in which case the doors were removed from the traps.

For scat analysis we followed the same methods as Greenleaf et al. (2009) and Graber (1981). From May through November 2014 we searched for scat near roads, trap sites, hair snares, natural forage areas, and potential trap sites. All samples were placed into zip-lock bags for storage. We recorded information about the scat such as: date collected, collector name, sample ID number, whether the sample was whole or partial, estimate of sample age, sample color, distance to anthropogenic food source, visible solid matter, UTM or latitude and longitude, county of collection, canopy cover, and a description of the collection site. Samples were considered partial if only a small amount of scat was collected or if the sample collected consisted of 2 or more scats and could not be separated. The partial samples were not included in the volume and frequency analysis because they do not represent a single whole sample. Samples were removed from bags later that day and dried by heat lamp to preserve for future analysis. In October 2014, we began rehydrating the samples. Samples were placed into a rubber tub with enough water and Dawn™ dish soap to submerge the samples and then were left to soak for approximately 1 hour (Graber 1981; Greenleaf et al. 2009). The Dawn™ dish soap served as a surfactant to lower the surface tension of the water. This was important to not only break up the scat, but also so particles could not stay dry by floating on the water's surface. We then washed the samples through a series of sieves (0.4 mm and 1 mm, H & C Sieving systems, Models 6998 and 7003, Columbia, Maryland, USA) to separate particles to equal size and remove any unwanted matrix. Food items were identified macroscopically as well as with a dissecting microscope. The identified items were then placed in film containers for storage. We categorized the items into 6 forage groups: anthropogenic food (corn), reproductive plant parts (fruit, seeds, and flowers), herbage (roots, stems, and leaves), animal matter (bones, hair, and insect parts), debris (rocks, wood, bark, and pine needles), and unidentifiable matter.

We identified all matter to species when possible. Insects were identified to class due to time constraints. To identify all plant material, we used *Field Guide to Oklahoma Plants* (Tyrl et al. 2002) and the United States Department of Agriculture Plant Database (USDA NRCS 2015). Many of the dietary items, such as a majority of the grass specimens, were too

degraded to identify further than family. The majority of seeds were keyed to species. Bone fragments were identified to species, genus, or family. The other fragments were labeled as large animal bones or unknown bones. We then measured percent volume and percent frequency of occurrence of each food item in scat samples that were > 1% of the volume of a sample (Graber 1981; Graber and White 1983, Greenleaf et al. 2009). We measured percent volume of the 6 classes using water displacement to the nearest 1%. Volumetric analysis tends to overestimate the amount of herbage and underestimate easily digested items such as reproductive plant parts like blackberries and animal tissue (Hatler 1972; Mealey 1980; Graber 1981). To attempt to more accurately assess dietary habits, we also calculated the percent frequency of occurrence of food items as the percent of total scat samples in which an item composed at least 1% of the volume of a sample (Graber 1981; Graber and White 1983; Greenleaf et al. 2009).

All scat that resembled bear scat was collected. Bear scat is variable in shape and is based on what the bear has eaten. If the animal has eaten more berries, the scat is more globular and not as solid. If the scat is more herbage or animal matter, the sample is more tubular and solid. Size is also variable depending on the size of the animal. Coyotes are omnivorous and eat many of the same kinds of soft mast such as blackberries, black cherries, and persimmons (McVey et al. 2013), and their scat can look similar. Bear scat is more variable in shape, but tubular scats are common. Coyote scat is tubular and of a size similar to a small bear. This is a potential source of error.

The Mann-Whitney U test, a non-parametric 2-tailed test (Zar 2009), was used to compare the volume of anthropogenic food items to the volume of natural food items. We also used the Mann-Whitney U test to compare food amounts from possible coyote scat against known bear scats. Using SPSS (2009), we also compared the volumetric amounts of the 6 diet groups against each other using the Kruskal-Wallis One-way Analysis of Variance, incorporating Tukey's Honest Significant Difference (HSD) post hoc test (Zar 2009). This test was used for further analysis after running an ANOVA or Kruskal-Wallis test and it was used to determine which groups in the sample were significantly different. The alpha level for tests was set at 0.05.

Results

The majority of scat samples were found in Cherokee and Sequoyah counties with only 1 whole sample collected in Adair County. Six samples were found in or near bear traps, 5 samples were found near blackberry (*Rubus* spp.) patches, and 11 samples were collected near deer feeders. An estimated 306 hours were spent searching for scat samples.

From 16 May through 17 October 2014, a total of 38 whole samples and 2 partial samples were collected from 24 sites (8 samples in May, 8 samples in June, 16 samples in July, 5 samples in August, and 1 sample in October). The volume of each sample after being washed through the sieves ranged from 0.9 mL to 117 mL with an average volume of 35.3 mL per sample. There were 28 different dietary items collected from the scat. There were 12 samples of the 38 collected that could have been small bear or coyote based on size, shape, and contents. We used the Mann Whitney U test to compare these scat samples to the rest of the samples that resembled only bear, based on the volumetric amounts of each of the 6 food categories. These samples were found to be significantly different and they were excluded from further analyses (Mann Whitney U test, P=0.046).

Items in the anthropogenic category were the most abundant item in terms of volume (Table 1). However, anthropogenic food items ranked second in frequency of occurrence in scat samples collected. The only anthropogenic item identified was corn (*Zea mays*).

The next most abundant food category was the reproductive plant parts (Table 1). Reproductive plant parts were found most frequently in samples collected. Blackberry seeds, black cherry seeds *(Prunus serotina*), wild rye (*Elymus* spp.) inflorescence were the

most abundant.

The majority of animal matter found was insect parts, primarily ant and bee exoskeletons. White-tailed deer (*Odocoileus virginianus*) hair was found and Leporid and *Microtus* bones were also present, but in small amounts.

Herbage was not very abundant in terms of volume but was abundant in terms of frequency. Blackberry leaves were the most abundant in this category. The next most abundant category was unidentified grasses. Some of the grasses identified were panic grass (*Panicum* spp.), blue stem (*Andropogon* spp.), and wheatgrass (*Agropyron* spp.).

Debris was present in most scat samples. This category consisted of small rocks, bark, wood chips, and pine needles. Unidentifiable matter made up a very small portion and consisted of anything that was too decomposed to be identified.

The volume of anthropogenic food in scats did not differ from the volume of natural food (Mann Whitney U test, $P = 0.368$, $N=26$). By comparing median ranks by ranking food categories by volume in all samples, the 6 diet groups were significantly different (Kruskal-Wallis, $P < 0.001$) and were separated into 3 statistically distinct groups: Anthropogenic food > Reproductive plant parts > Animal matter = Herbage = Debris = Unidentified (Tukey's Honest Significant Difference test, alpha = 0.05).

Discussion

The anthropogenic food identified in the scat from bears in our study was corn most likely obtained from deer feeders rather than cropland. Many of the landowners manage and/or hunt deer over bait, and they do so with deer feeders. Bear-human conflict arises when the bears destroy feeders or break into the feeders. Landowners try to deter bears from breaking into the deer feeders using several methods such as raising feeders far off the ground by cables. Many times bears are still able to breach the container and obtain the corn. Despite the frequency of deer feeders on the landscape, the abundance of corn in scat may be biased by the fact that corn was used to attract bears to trap sites, and some samples were collected near trap sites. However, bears would have to be at the trap site long enough for corn ingested at the trap site to pass through the digestive system for this to bias our results.

Reproductive plant parts were also very abundant due to the fact that blackberries and black cherries begin producing mast throughout April-May (Tyrl et al. 2002). These plants are common and attractive to wildlife. Seeds and berries are common in the diet of brown bears (*Ursus arctos*) and black bears (Graber and White 1983). In Washington, berries are a major part of the bears' diet (Lindzey and Meslow 1977).

Animal matter was somewhat common. Though the amount of animal matter by volume was not very large, animal remains were frequently found. The majority of this was insect parts. Bears frequently eat ants (Chatelain 1950; Hatler 1972; Beeman and Pelton 1980) and wasps (Hatler 1972; Boyer 1976; Beeman and Pelton 1980). The majority of insects identified were ants but there were also bee and wasp remains. Bears could have been seeking out ants or they could have ingested the ants with food items picked up from the ground. White-tailed deer hair wasn't very prevalent (volume or frequency) in the scat samples. The seasonal abundance of soft mast in the environment could attract bears more so than cervid remains, making animal tissue less prevalent in scat samples. Cervid remains in black bear scat usually represent carrion and juveniles (Schwartz and Mitchell 1945; Tisch 1961; Hatler 1972), but black bears are capable of preying on adult white-tailed deer (Svoboda et al. 2011).

Herbage was not a major component in volume and frequency in the samples. We believe this is because of the time of the year the scats were collected. Graber and White (1983) determined the amount of herbage decreases from spring to fall. Herbage is a very important dietary item in the spring, and it can make up

Table 1 Contents of American black bear scat collected in Adair, Cherokee, and Sequoyah counties, May through November 2014. N=26

Food Resource	Volume	Frequency
Anthropogenic	**54.4%**	**61.2%**
Corn	54.4%	61.2%
Reproductive Plant Parts	**32.3%**	**69.2%**
Blackberry	26.2%	34.6%
Black Cherry	1.6%	15.4%
Wild Rye	3.7%	19.2%
Animal Matter	**4.2%**	**69.2%**
Insect	2.4%	61.5%
Deer hair	1.2%	11.5%
Herbage	**2.4%**	**46.2%**
Blackberry leaves	0.9%	15.4%
Grass	0.4%	15.4%
Bluestem grass	0.4%	3.9%
Debris	**6%**	**80.8%**
Unidentifiable	**0.8%**	**15.4%**

about half of their diet (Piekielek and Burton 1975; Boyer 1976; Eagle 1979). Our first sample was not collected until May, so herbage may be underrepresented because the ripening of some berry species may have already attracted the bears away from herbage.

This study was limited in terms of scat collection and therefore was not a complete representation of the bear diet in this region. Our findings cover only a few months out of the year, and more extensive sampling would be needed to determine spring and fall diet more completely. However, our results do give a first impression of the importance of various foods in black bear diets in eastern Oklahoma.

There is a possibility that multiple samples were collected from a single bear. Elfström et al. (2013) examined gut retention time (GRT) with captive brown bears and found GRT to be 5 h 47 min with berry diets and 14 h and 30 min with meat diets. They also found that activity rate does not affect GRT. There were 3 days when multiple samples were collected from a single site. Although cameras often indicated more than one bear visiting the trap sites in a 24-h period, our samples should not be considered independent.

Similar diet studies have been conducted in Yosemite Valley in Yosemite National Park, California (Graber 1981; Greenleaf et al. 2009). In Yosemite, bears have a large variety of anthropogenic food due to a large number of campers, hikers, and other tourists bringing food to the park. Anthropogenic sources are abundant in the form of trash containers, campsites, and vehicles (Breck et al 2009.) The bears in our study area may not have access to the same concentration of anthropogenic food, but their habits are still affected.

Corn, an anthropogenic food source, accounted for a substantial portion of the black bear diet in the Ozark area of eastern Oklahoma.

This could mean bears are searching out corn over natural food sources or that deer feeders are abundant and bears use them as encountered. If bears are searching for deer feeders, this may lead to conflict, which can contribute to conflict between humans and bears.

This study showed that bear diet in the Oklahoma Ozarks area was affected by the availability of anthropogenic food resources. The bear population in this area is expanding into a human-dominated landscape, which increases the likelihood of conflict. With this knowledge, public education about living with bears and ways to reduce human-bear conflict can be directed toward the specific issues in this region.

Acknowledgments

We would like to thank Sara Lyda (senior research associate, Oklahoma State University) along with ODWC and OSU for the opportunity and equipment to conduct this research. Thanks to Emily Artz, Brittany Hoback, Colby Farquhar, Curt Allen, Jessica Mitcham, Licelle Vicenzio, Laurie Luckritz, Kyle Wilgers, Randy Kramer, Robin Bowin, Kay Bowin, Barb Lynn, Dr. Stefan Cairns, and Schuyler Greenleaf for all their assistance.

References

Arner DH. 1948. Fall food of the black bear in Pennsylvania. PA. Game News 19:13.

Bales SL, Hellgren EC, Leslie DM, and Hemphill J Jr. 2005. Dynamics of a recolonizing population of black bears in the Ouachita Mountains of Oklahoma. Wildlife Society Bulletin 33:1342-1351.

Baruch-Mordo S, Breck SW, Wilson KR, and Theobald DM. 2008. Spatiotemporal distribution of black bear–human conflicts in Colorado, USA. J of Wildlife Manage 72:1853-1862.

Beeman LE, and Pelton MR. 1980. Seasonal foods and feeding ecology of black bears in the Smoky Mountains. International Conference on Bear Research and Management 4:141-147.

Benson JF and Chamberlain MJ. 2006. Food habits of Louisiana black bears (*Ursus americanus luteolus*) in 2 subpopulations of the Tensas River Basin. American Midland Naturalist 156:118–127.

Boyer KB.1976. Food habits of black bears (*Ursus americanus*) in the Banning Canyon area of San Bernardino National Forest. M.S. Thesis. California State Polytechnic University., Pomona. 63pp.

Breck SW, Lance N, and Seher V. 2009. Selective foraging for anthropogenic resources by black bears: minivans in Yosemite National Park. J Mammal 90:1041-1044.

Chatelain EF. 1950. Bear-moose relationships on the Kenai Peninsula. Trans. North American Wildlife Conference. 15:224-234.

Clark JD, Clapp DL, Smith KG, and Ederington B. 1994. Black bear habitat use in relation to food availability in the interior highlands of Arkansas. International Conference on Bear Research and Management 9:309–318.

Clark JD, Van Manen FT, and Pelton MR. 2005. Bait stations, hard mast, and black bear population growth in Great Smoky Mountains national park. J of Wildlife Manage 69(4):1633-1640.

Duck LG and Fletcher JB. 1943. The game types of Oklahoma. Report to the Oklahoma Game and Fish Commission.

Eagle TC. 1979. Foods of black bears in the Great Smoky Mountains National Park. M.S. Thesis. University of Tennessee, Knoxville. 104pp.

Elfström M, Støen O, Zedrosser A, Warrington I, and Swenson JE. 2013. Gut retention time in captive brown bears (*Ursus arctos*).Wildlife Biol 19(3):317-324.

Forester DJ and Machlis GE. 1996. Modeling human factors that affect the loss of biodiversity. Conserv Biol 10: 1253–1263.

Fuller TK, and Keith LB. 1980. Summer ranges, cover type use, and denning of black bears near Fort McMurray, Alberta. Can Field Nat 94:80–83.

Graber DM. 1981. Ecology and management of black bears in Yosemite National Park. Dissertation, University of California. Berkeley, California, USA.

Graber DM and White M. 1983. Black bear food habits in Yosemite National Park. International Conference on Bear Research and Management 5:1–10.

Greenleaf SS, Matthews SM, Wright RG, Beecham JJ, and Leithead HM. 2009. Food habits of American black bears as a metric for direct management of human–bear conflict in Yosemite Valley, Yosemite National Park, California. Ursus 20:94-101.

Hatler DF. 1972. Food habits of black bears in interior Alaska. Can Field Nat. 86:17-31.

Isbell LA, Pruetz JD, and Young TP. 1998. Movements of vervets (*Cercopithecus aethiops*) and patas (*Erythrocebus patas*) monkeys as estimators of food resource size, density, and distribution. Behav Ecol Sociobiology 42:123–133.

Jepsen JU, Eide NE, Prestrud P, and Jacobsen LB. 2002. The importance of prey distribution in habitat use by Arctic foxes (*Alopex lagopus*). Can J Zoolog 80:418–429.

Landers JL, Hamilton RJ, Johnson AS, and Marchington RL. 1979. Foods and habitat of black bears in southeastern North Carolina. J of Wildlife Manage 43:143–153.

Lindzey FG, and Meslow EC. 1977. Home range and habitat use by black bears in southwestern Washington. J of Wildlife Manage 41:413-425.

Lyda S, Leslie DM, and Fairbanks WS. 2016. Final report: status and distribution of black bears in east-central and northeastern Oklahoma. Oklahoma Department of Wildlife Conservation, Oklahoma City. Project number F10AF00127.

Mattson DJ.1990. Human impacts on bear habitat use. International Conference on Bear Research and Management 8:33-56.

Mcdonald JE Jr., Fuller TK. 2005. Effects of spring acorn availability on black bear diet, milk composition, and cub survival. J Mammal 86: 1022-1028.

McKinney ML. 2001. Role of human population size in raising bird and mammal threat among nations. Anim Conserv 4: 45–57.

McVey JM, Cobb DT, Powell RA, Stoskopf MK, Bohling JH, Waits LP, and Moorman CE. 2013. Diets of sympatric red wolves and coyotes in northeastern North Carolina. J Mammal 94:1141–1148.

Mealey SP. 1980. The natural food habits of grizzly bears in Yellowstone National Park, 1973–74. International Conference on Bear Research and Management 4:281–292.

Merkle, JA, Robinson HS, Krausman PR, and Alaback P. 2013. Food availability and foraging near human developments by black bears. J Mammal 94:378-385.

Oftedal OT, Alt GL, Widdowson EM, and Jakubasz MR. 1993. Nutrition and growth of suckling black bears (*Ursus americanus*) during their mother's winter fast. Brit J Nutr 70:59–79.

Oklahoma Climatological Survey. 2015. Climate of Oklahoma [online]. Available from:http://www.mesonet.org/index.php/weather/local/cook. (Accessed August 2, 2015)

Pfander MA. 2016. American black bear ecology in southeastern Oklahoma: Population status and capture methodology. PhD Dissertation, Oklahoma State University, Stillwater, Oklahoma, 91 pp.

Prange S, Gehrt SD, and Wiggers EP. 2003. Demographic factors contributing to high raccoon densities in urban landscapes. J of Wildlife Manage 67:324–333.

Piekielek W and Burton TS.1975. A black bear population study in northern California. Calif. Fish and Game 61:4-25.

Ryan CW, Pack JC, Igo WK, and Billings A. 2007. Influence of mast production on black bear non-hunting mortalities in West Virginia. Ursus 18:46-53.

Schlegel M. 1976. Factors affecting calf elk survival in northcentral Idaho. A progress report. *In* proceedings of the 56th Annual conference of the Western Association of State Game and Fish Commissioners, Sun Valley, Idaho, 26-29 July 1976. Pp. 342-355

Schwartz JE and Mitchell GE. 1945. The Roosevelt elk on the Olympic Peninsula, Washington. J of Wildlife Manage. 9:295-319.

Sikes RS, Gannon WL, and the Animal Care and Use Committee of the American Society of Mammalogists. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J Mammal 92: 235–253.

Smith KG, and Clark JD. 1994. Black bears in Arkansas: characteristics of a successful translocation. J Mammal 75:309-320.

SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc.

Svoboda NJ, Belant JL, Beyer DE, Duquette JF, Stricker HK, and Albright CA. 2011. American black bear predation of an adult white-tailed deer. Ursus 22:91-94.

Swenson JE, Sandegreen F, and Soderberg A. 1998. Geographic expansion of an increasing brown bear population: evidence for presaturation dispersal. J of Animal Ecol 67:819-826.

Tisch EL. 1961. Seasonal food habits of the black bear in the Whitefish Range of northwestern Montana. M.S. Thesis. Montana State University, Missoula. 108pp.

Tyrl RJ, Bidwell TG, and Masters RE. 2002. Field guide to Oklahoma plants, commonly encountered prairie, shrubland, and forest Species. Oklahoma State University. Stillwell, Oklahoma.

U.S. Census Bureau. 2016. Human population for counties in Oklahoma [online]. Available from: Census.gov. (Accessed January 26, 2018).

USDA [United States Department of Agriculture], NRCS [Natural Resources Conservation Service]. 2015. The PLANTS Database. Available from: http://plants.usda.gov. (Accessed 6 October 2015).

Woodroffe R. 2000. Predators and people: Using human densities to interpret declines of large carnivores. Animal Conserv 3: 165–173.

Zar JH. 2009. Biostatistical analysis. Prentice Hall, New Jersey 663 pp.

Submitted September 10, 2018 Accepted November 6, 2018

Early Life History Characteristics and Contribution of Stocked Juvenile Alligator Gar in Lake Texoma, Oklahoma

Richard A. Snow
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Michael J. Porta
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Ralph W. Simmons Jr.
US Fish and Wildlife Service, Tishomingo National Fish Hatchery, Tishomingo, OK 73460

Jory B. Bartnicki
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Abstract: Due to concerns of overexploitation and population decline caused by anthropogenic influences, fisheries managers have turned to hatchery produced Alligator Gar (*Atractosteus spatula*) stocking to supplement inconsistent wild year classes. Aquaculture can be a useful option to reestablish or supplement natural populations, as many states currently have stocking programs to reintroduce this species. Recent interest in Alligator Gar ecology and conservation has led the Oklahoma Department of Wildlife Conservation to attempt to better understand early life history characteristics of age-0 Alligator Gar in Lake Texoma by tracking growth, diet, habitat use, mortality and stocking contribution. A total of 33,900 Alligator Gar fingerlings were stocked into Lake Texoma in 2017. During June-September 2017, a total of 46 age-0 Alligator Gar were captured in 279 net nights of effort using mini-fyke nets. Mean CPUE (catch-per-unit-effort; number per net night) was highly variable with CPUE varying based on vegetation stem density in the location of the net set. Of the 46 Alligator Gar captured in 2017, 84.5% (39 of 46) were stocked (OTC mark present). During the 4-month sampling period, the daily mortality estimate for stocked age-0 Alligator Gar was 0.049%/day. After the first four months (4.7% mortality rate), 32,306 of the initial 33,900 Alligator Gar remained in the system. The annual estimated mortality rate is 16.7%. The mortality estimate of fish stocked into Lake Texoma is substantially lower than the 94.8% mortality rate observed in the hatchery over three months (1,043 of 20,001 age-0 Alligator Gar remained). Stocking Alligator Gar as fingerlings when water level and habitat availability is sufficient may be more beneficial than holding them in a hatchery setting. Conversely, stocking Alligator Gar early may not be beneficial in a marginal year when nursery habitat is limited and inflow is not consistent enough to maintain a constant pool elevation. During these years it may be better to grow-out fingerlings to larger sizes, such that when stocked they are not easily preyed upon.

Introduction

The Alligator Gar (*Atractosteus spatula*) is one of four species of gar (Lepisosteidae) found in Oklahoma and is the largest fish found in the state (Miller and Robison 2004). Historically, Alligator Gar were found throughout the Arkansas River and Red River drainage systems (Page and Burr 1991, Miller and Robison 2004, Brinkman 2008). Anthropogenic influences such as dams, dredging, habitat change, unlimited recreational harvest and commercial fishing (Robinson and Buchanan 1988, Etnier and Starnes 1993, Ferrara 2001, Brinkman 2008, Inebnit 2009, Snow and Long 2015) have resulted in the disappearance of Alligator Gar from the Arkansas River system from Robert S. Kerr Dam up river in Oklahoma (ODWC unpublished data), but the Red River basin is still a stronghold for Alligator Gar in Oklahoma. The Red River basin is highly altered (by the Denison Dam and agricultural practices) yet still exhibits periodic flooding that is critical for spawning and providing nursery cover (Brinkman 2008, Snow and Long 2015, Buckmeier et al. 2017).

These flooding events are sporadic. However, Alligator Gar exhibit a periodic life history strategy having increased longevity (>50 years), delayed maturation (>10 years), high fecundity (>4000 eggs/kg body weight) and variable recruitment (Ferrarra 2001, Brinkman 2008, Buckmeier et al. 2012, Buckmeier et al. 2017). Previous research has focused on adult Alligator Gar, providing limited information on early life history characteristics of this species. The information available regarding young Alligator Gar details the difficulty in capturing age-0 fish. For example, Pigg and Gibbs (1996) described accidental capture of 21 age-0 Alligator Gar using a siene from a large shallow backwater area below Robert S. Kerr Dam. In 2007, juvenile Alligator Gar were successfully collected from Lake Texoma during April through November using mini-fyke nets during a flood year (Brinkman 2008). Snow and Long (2015) used mini fyke nets to collect 9 age-0 Alligator Gar from Lake Texoma in 2013 that were used to back calculate hatch date using their otoliths. In that year, hatch date was associated with an increase in pool elevation caused by high flows from the Red River.

Due to concerns of overexploitation and population decline, fisheries managers have begun using hatchery-produced fish to overcome the variable year class production by wild Alligator Gar (Porta et al. in-press). Aquaculture can be a useful option to re-establish or supplement natural populations, as many states currently have successful stocking programs for Alligator Gar (Todd 2005, Mendoza et al. 2008, Militello 2013, Richardson 2015). A recent study found that Alligator Gar stocked into marsh and wetland habitats in Monopoly Marsh (Mingo National Wildlife Refuge, Missouri) exhibited site fidelity. These Alligator Gar occupied small areas ranging from 4.8 to 12.9 ha (Solomon et al. 2013). Consequently, strong site fidelity of stocked Alligator Gar allows managers to more consistently collect stocked juveniles, giving insight to their early life history that typically cannot be obtained because of low abundance of wild fish.

The Oklahoma Department of Wildlife Conservation (ODWC) began stocking age-0 Alligator Gar into inundated vegetation at the Red River-Lake Texoma interface in 2017. The impetus for this stocking effort was to gain an understanding of early life history characteristics of juvenile Alligator Gar in Lake Texoma. Therefore, the objectives of this study were to 1) evaluate early life history characteristics (growth, mortality, habitat use) and contribution of stocked juvenile Alligator Gar and 2) compare mortality estimates and growth rates of Alligator Gar stocked in Lake Texoma to those allowed to grow in a hatchery setting.

Methods

Study Area - This study was conducted in the river-reservoir interface of the Red River arm of Lake Texoma, which is largely comprised of backwater habitat (Brinkman 2008). Sampling areas were generally < 1 m in depth with varying densities of aquatic or terrestrial vegetation. Lake Texoma is a 36,000-ha reservoir on the Oklahoma-Texas border. During normal flow, the Red River is constrained within a river

channel, cut off from adjacent floodplains where terrestrial vegetation colonizes (Patton and Lyday 2008, Snow et al. 2016). When these areas are flooded, they are accessible to adult spawning Alligator Gar and the terrestrial vegetation is suitable spawning habitat for attachment of adhesive eggs and development of larval gar (Moore et al. 1973).

Marking and Stocking - Fingerling Alligator Gar (exogenous stage; actively feeding with yolk sac depleted) were obtained on May 23, 2017 from the Tishomingo National Fish Hatchery, Tishomingo, Oklahoma. Prior to stocking, fish were placed into hauling tanks containing 341 - 1,477.5 L of water in a concentration of 600 mg/L of Pennox 343 (Pharmgate, Omaha, Nebraska), of which each 1.32 g of powder contains 1 g of Oxytetracycline (OTC) HCl. Sodium phosphate dibasic was added at a rate of 62 mg/L per 100 mg/l of OTC to neutralize the pH. Alligator Gar were exposed to the OTC treatment for 4-6 hrs prior to stocking (Snow and Long 2017). OTC is incorporated into skeletal structures, which can be seen using ultraviolet light (Figure 1). In this study, otoliths were used to determine if Alligator Gar were stocked or wild.

After marking, Alligator Gar were transferred to a holding tank located on a boat for stocking into Lake Texoma. Water from Lake Texoma was slowly pumped into the holding tank to acclimate fish to in-lake water conditions. Once acclimated, fish were stocked by boat into nursery habitat consisting of flooded terrestrial and aquatic vegetation. All fish were stocked in the river-reservoir interface of the Red River in Lake Texoma.

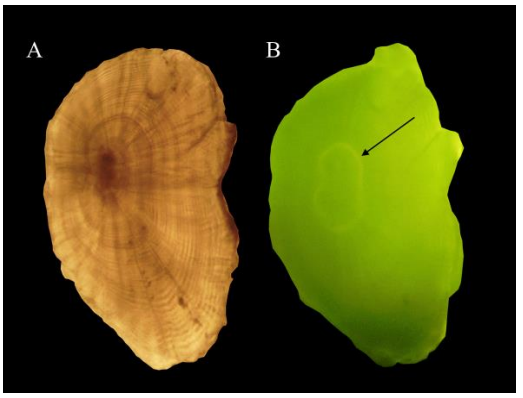


Figure 1. Photograph of a lapillus otolith (A) without fluorescence, and (B) with fluorescence, illuminating the OTC mark on lapillus otolith of a 67 mm stocked Alligator Gar captured using mini-fyke net on June 22, 2017 in Lake Texoma, Oklahoma. The OTC mark is identified with a black arrow.

Sampling – Sampling began two weeks after the last stocking event. Mini-fyke nets (0.6 m x 6.35 m; with 3.18 mm mesh, 9.14 m lead, 0.6 m x 1.92 m rectangular cab, and 510 mm metal throat; Snow et al 2016) were set in the Red River-Lake Texoma reservoir interface during June – September 2017. Sample sites were selected monthly by placing a 100- m grid over the map of all backwaters and shallow-water coves in the river-reservoir interface and individually numbered grids were randomly selected as fyke net sampling locations (Snow et al. 2016). Upon arriving at each sampling location, net leads were anchored with a T-Post, pulled tight, and anchored on the cod end. Nets were set perpendicular to the shoreline in water < 0.6 m in depth. Nets were allowed to fish for 24 hours. All Alligator Gar captured were preserved on ice until frozen and brought back to the Oklahoma Fisheries Research Laboratory where they were measured (mm total length (TL), weighed (nearest g), and otoliths were removed for aging and OTC mark detection purposes.

At the time of net deployment, depth measurements (cm) were taken at the opening of the fyke net and 3 replicate vegetation stem counts were taken in the area of the set net. This was done by haphazardly tossing a 0.32 m² hoop into the area around the lead of the net and stem density within the diameter of the hoop was counted for each of the three tosses (MacKenzie and Kaster 2004, MacKenzie 2005, Aikens and Roach 2014). To expedite stem density enumeration, a rating system was developed from average stem counts taken from 115 net sites during previous sampling efforts to estimate stem densities (Table 1). The rating system was tested by randomly selecting 5 sites out of 17 net sites (total net set in a day; totaling 44 sites)

Table 1. Criteria used to rate habitat sampled with mini-fyke nets for age-0 Alligator Gar from June through September of 2017 in Lake Texoma, Oklahoma. (SE = standard error).

Rating	Mean Stem Count (SE)	Stem Density m^2	Description
0	1.00 (0.68)	3.13	Vegetation very sparse
1	5.47 (1.33)	17.09	Vegetation patchy (but mainly open water)
2	13.93 (2.87)	43.53	Vegetation patchy (with moderate open water)
3	43.33 (4.87)	135.41	Even distribution of vegetation
4	87.35 (6.29)	272.97	Mostly even with small areas of dense vegetation
5	191.11 (28.67)	597.22	Vegetation very dense

that had already been enumerated, assigning a rating to each site, and ensuring that the ratings assigned to a particular site were within the range of stem densities in Table 1.

Otolith processing – Otoliths were allowed to dry for >24 hr prior to processing. Following drying, lapilli otoliths were embedded and sectioned near the otolith center in a frontal plane and mounted to slides for processing and viewing (Long and Snow 2016). Prior to viewing, otoliths were polished with 2000 grit wetted sandpaper to reveal the core. Otoliths were viewed for the presence of an OTC mark using an Olympus BH2 RFCA compound microscope equipped with a 100-W mercury lamp for fluorescence detection with a B3 filter cube. A single reader examined each otolith, independently three times to evaluate whether an OTC mark was present (Snow and Long 2017).

Analysis – Alligator Gar with an OTC mark present on the otolith were considered to be a stocked fish. Year class contribution was defined as the proportion of stocked to wild fish. Linear regression analysis was used to determine relationships for hatchery raised (held and grown at Tishomingo National Fish Hatchery) and stocked (growth in Lake Texoma post-stocking) Alligator Gar throughout the sampling season (TL at age [days]). Data pertaining to hatchery raised Alligator Gar was provided by Tishomingo National Fish Hatchery for comparison purposes. Catch-curve regression was used to assess daily mortality of stocked Alligator Gar over 4 months (June – September) using $\log_e$ transformed frequency of capture for each sampling day in 2017 (Miranda and Bettoli 2007). An analysis of variance (ANOVA) was used to determine difference in CPUE between the various habitat ratings at each net site. All data were $\log_e$+0.01 transformed to conform to the assumptions of normality and tests were performed at a significance level of $P \leq 0.05$. If significant, ANOVA results were conducted with a Tukey's HSD test.

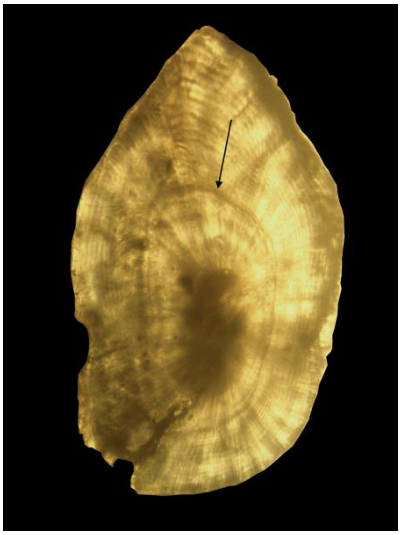


Figure 2. Photograph of a lapillus otolith from a 63 mm stocked Alligator Gar captured using mini fyke nets on June 23, 2017 with the presence of a stocking check. Stocking checks were observed in 28.2% of Alligator Gar sampled. The stocking check is identified with a black arrow.

Results

A total of 33,900 Alligator Gar fingerlings were stocked into Lake Texoma in 2017. Age-0 Alligator Gar were 46 mm mean TL at stocking. During June-September 2017, 46 age-0 Alligator Gar were captured in 279 net nights of effort. The mean catch-per-unit-effort (CPUE) was 0.17/net night with June having the highest CPUE (0.36 fish/net night) and September having the lowest CPUE (0.05 fish/ net night). Of the 46 Alligator Gar captured, 84.5% (39 of 46) were stocked (OTC mark present; Figure 1). Stocking checks were observed in 28.2% of stocked fish (Figure 2). The remaining 7 fish were wild fish (no OTC mark present).

Both terrestrial and aquatic vegetation stem density were enumerated from three separate samples taken at 115 net sites. Stem density ranged from 3 – 597 m². A subjective rating system was employed to reduce the time needed to enumerate vegetation density at each site. The rating system was on a 0-5 scale, with 0 having very sparse vegetation and 5 having very dense vegetation (Table 1). Forty-four sites were evaluated to ensure habitat categories were correctly assigned to areas of vegetation previously measured using the hoop method.

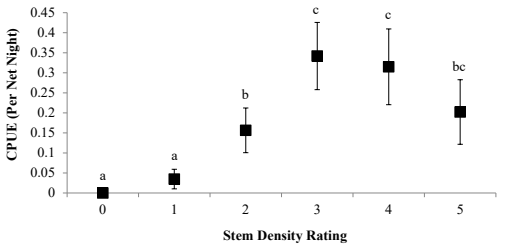


Figure 3. Post-hoc test results comparing mean CPUE among stem density ratings (0 = Vegetation very sparse, 1 = Vegetation patchy (but mainly open water), 2 = Vegetation patchy (with moderate open water), 3 = Even distribution of vegetation, 4 = Mostly even with small areas of dense vegetation, 5 = Vegetation very dense) sampled with mini-fyke nets during June-September in 2017 in Lake Texoma, Oklahoma. Different letters indicate significant differences of the post-hoc test.

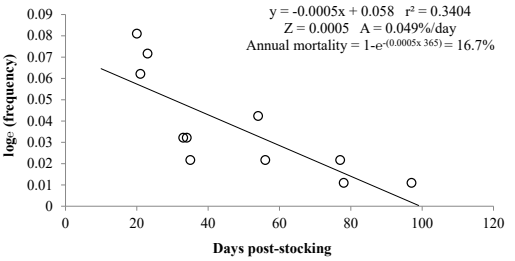


Figure 4. Catch curve regression and annual mortality (A) calculated from the 39 age-0 OTC marked Alligator Gar collected using mini-fyke nets from Lake Texoma, Oklahoma during June – September 2017.

Correct habitat density rating occurred for 88.6% of sites. The ability to rate categories 4 (mostly even with small areas of dense vegetation) and 5 (Vegetation very dense) proved difficult, as 5 (three category 4 and two category 5) sites were incorrectly rated. We found that stem density influences mean CPUE using mini-fyke nets (F_{279} = 3.65, P < 0.01; all Tukey's HSD comparisons P < 0.01; Figure 3). Catch rates were also lowest at sites with habitat ratings of 0. As the habitat rating increased, so did the mean CPUE until the stem density reached >135.41/m² when catch rates declined.

During the 4 month sampling period, the daily mortality estimate for stocked age-0 Alligator Gar was 0.049%/day (Figure 4). After the first four months (4.7% mortality rate), an estimated 32,307 of the initial 33,900 Alligator Gar remained in the system. The annual estimated mortality rate is 16.7%. The estimated mortality rates of Alligator Gar stocked into Lake Texoma are substantially lower than the mortality rate observed in the hatchery over three months 94.2%; 1,043 of 20,001 age-0 Alligator Gar remained). However, age-0 Alligator Gar grew 4.09 mm/day (r^2 = 0.98, P < 0.01) in the hatchery setting over three months. Age-0 Alligator Gar captured in summer of 2017 from Lake Texoma grew slower at 1.77 mm/day (r^2 = 0.66, *P* < 0.01; Figure 5).

Discussion

Contribution of hatchery produced Alligator Gar was high (84.5%) in this study. During

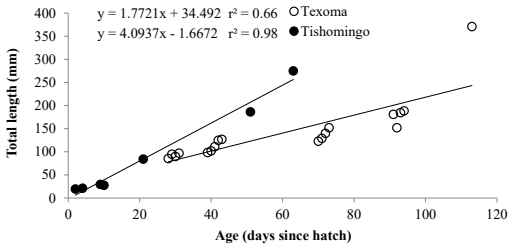


Figure 5. Relationship between total length and age of stocked age-0 Alligator Gar (open circle = ○) captured from Lake Texoma, Oklahoma in June – September of 2017 and hatchery raised age-0 Alligator Gar (filled circle = ●) from the Tishomingo National Fish Hatchery. Solid line is fit from linear regression.

spring and summer 2017, the pool elevation stayed high enough to maintain inundated vegetation in the river-reservoir interface of Lake Texoma. These conditions resulted in high survival and contribution of stocked juveniles, and also led to successful spawning of wild Alligator Gar that produced a low abundance of wild age-0 fish. Snow and Long (2015) found that drought kept water levels low in Lake Texoma until spring 2013, allowing herbaceous vegetation to establish exposed shorelines. This was followed by heavy rains that increased pool elevation, inundating these areas, which created spawning and nursery habitat for Alligator Gar. Habitat availability (for spawning and nursery cover) is probably the most critical variable affecting the early life history of wild Alligator Gar. Alligator Gar spawn in inundated vegetation (Brinkman 2008, Inebnit 2009, Snow and Long 2015, Buckmeier et al. 2017), which then serves as critical nursery habitat for young gar. Therefore, stocking fingerling Alligator Gar will likely produce the best results when water level and available habitat is sufficient, similar to conditions observed in Lake Texoma in 2017. Buckmeier et al. (2017) suggested that vegetation needs to be inundated for a period ≥ 5 days, which is enough time for eggs to hatch and fry to respond to receding waters. Furthermore, recruitment increased as days of inundation increased (Buckmeier et al. 2017). However, stocking fingerlings early may not be beneficial in years when nursery habitat is limited by low water levels. During years when these conditions are present, it would be better to grow-out fingerlings to larger sizes in a hatchery setting, such that when stocked they are not easily preyed upon.

Alligator Gar growth rates are fast in hatchery settings due to cannibalism, a high protein pellet diet, and little effort needed to forage in confined environments. Hatcheries provide optimal growing conditions in which fish get larger in a short period of time. However, rates of cannibalism are high in aquaculture facilities that raise Alligator Gar and cannibalism is one of the leading causes of mortality during production (Mendoza et al. 2002, Mendoza et al. 2008, Perschbacher 2011). Additionally, Alligator Gar can be impacted by infection in hatcheries because they are held in confined spaces, and this contributed to high mortality rates in this study (bacterial infection). Differences in mortality between the hatchery environment and post-stocking in Lake Texoma were substantially different. Habitat availability in 2017 likely resulted in the low observed mortality rates of juvenile Alligator Gar stocked into Lake Texoma. However, the higher mortality of juvenile Alligator Gar in a hatchery setting is acceptable, particularly in years when reservoir hydrology prevents the stocking of fingerling Alligator Gar.

The ability to sample juvenile Alligator Gar using mini-fyke nets may have affected mortality and growth rate estimation for juvenile Alligator Gar stocked into Lake Texoma. Based on catch rates, it appears that we can only effectively sample smaller, slower growing age-0 Alligator Gar that rely on the nursery cover sampled in this study. We speculate that age-0 Alligator Gar may move from nursery cover to open water flats or main lake coves when they reach a certain size, suggesting that mini-fyke nets are not effective for capturing larger juvenile Alligator Gar. It appears that mini-fyke nets are most effective at catching age-0 gar only when water levels inundate aquatic and terrestrial vegetation to maintain gar habitat. Brinkman (2008) captured juvenile Alligator Gar successfully

using mini-fyke nets in Lake Texoma during a flood year that allowed habitat to be accessed the entire growing season (April through November). As water level decreases mini-fyke nets become ineffective gear for sampling age-0 Alligator Gar. It appears that in years when water level decreases following a spawn, age-0 Alligator Gar movement increases, resulting in the need for future research to understand this movement and how it affects diet, growth, and survival. Future research should concentrate on understanding movement of juvenile Alligator Gar (ages 0 – 2). This information may provide important insight into sampling this size class of fish and may strengthen our knowledge of the early life history of this species.

A multiple gear approach may be necessary to sample juvenile Alligator Gar effectively. However, before that can be determined, a movement study needs to be conducted to evaluate habitats that various size classes of fish utilize. This will allow for refinement of a sampling approach. Alligator Gar < 100 mm do not recruit to mini-fyke nets (Snow et al. 2016). However, mini-fyke nets are effective for sampling Alligator Gar up to 630 mm if habitat stays consistent (Brinkman 2008). An active gear may be better at capturing gar <100 mm because their body movement is limited because of their reliance on the notochord appendage (Carpenter 1975). Effective sampling of individuals >100 mm may depend on lake elevation and available habitat. The high density of vegetation may not have allowed the nets to deploy correctly or Alligator Gar may not prefer habitat that dense, ultimately affecting catch rates. However, it is possible that a stem density >135.41/m² provide age-0 Alligator Gar with ideal ambush cover and movement is not needed to forage, thereby reducing capture with mini-fyke nets.

In this study, hatchery produced Alligator Gar were effectively used to evaluate early life history characteristics in Lake Texoma. We assumed that hatchery produced Alligator Gar would be easier to recapture post-stocking, similar to results of Solomon et al. (2013). However, habitat differences make Lake Texoma entirely different than those sampled by Solomon et al. (2013). In a typical year, habitat availability is low in Lake Texoma due to low summer water levels that limit habitat availability, whereas habitat was always available in the marsh system evaluated by Solomon et al. (2013). Alligator Gar in Lake Texoma did not appear to maintain small home ranges in 2017 (4.8-12.9 ha; Solomon et al. 2013), which may have led to the decreasing catch rates over time. Despite some difficulties in capturing juvenile Alligator Gar in Lake Texoma, important early life history information was collected for Alligator Gar in this system. The use of hatchery produced Alligator Gar provides a practical approach to evaluate early life history of this species in situations where they would otherwise be too rare to study. This approach will benefit other resource agencies that are evaluating stocking programs and early life history of Alligator Gar introduced into their jurisdictional waters. Although we are confident in our findings, these results should be interpreted with caution as they represent a single-year evaluation. Future evaluations should be conducted over an extended time period (8-10 years) to ensure that weather patterns (and resulting reservoir hydrology) affecting early life history traits of Alligator Gar are encompassed during the study period. A long-term evaluation will allow development of trend data, which will help better understand early life ecology of Alligator Gar.

Acknowledgments

The authors thank those individuals that assisted with sampling including Micah Waters, Shelby Jeter, Matthew Lyons, Parker Fleming and southcentral regional management staff. We thank Kurt Kuklinski (ODWC) and anonymous reviewers for OAS for reviewing an earlier draft of this manuscript. Financial support for this publication was provided by the Sport Fish Restoration Program grant [F-50-R-25] and [F-86-D-1] to the Oklahoma Department of Wildlife Conservation.

References

Aikens, M.L and D.A. Roach. 2014. Population dynamics in central and edge population of a narrow endemic plant. Ecol. 95: 185-1860.

Brinkman, E.L. 2008, Contributions to the life history of Alligator Gar, *Atractosteus spatula* (Lacepede), in Oklahoma. Master's thesis. Oklahoma State University, Stillwater, Oklahoma.

Buckmeier, D.L., N.G. Smith, D.J. Daugherty and D.L. Bennett. 2017. Reproductive ecology of Alligator Gar: identification of environmental drivers of recruitment success. J. Annu. Southeast Assoc. Fish. Wildl. Agencies 4: 8-17.

Buckmeier D.L, N.G. Smith and K.S. Reeves. 2012. Utility of Alligator Gar age estimates from otoliths, pectoral fin rays, and scales. Trans. Amer. Fish. Soc. 141: 1510-1519.

Carpenter, C.C. 1975. Functional aspects of the notochordal appendage of young-of-the-year gar (*Lepisosteus*). Proc. Okla. Acad. Sci. 55: 57- 64.

Etnier, D.A. and W.C. Starnes. 1993. Alligator gar. In: The fishes of Tennessee. Knoxville, Tennessee: University of Tennessee Press pp. 109-110.

Ferrara, A.M. 2001. Life-history strategy of Lepisosteidae: implications for the conservation and management of alligator gar. Doctoral Dissertation, Auburn Alabama: Auburn University.

Inebnit III, T.E. 2009, Aspects of the reproductive and juvenile ecology of alligator gar in the Fourche Lafave River, Arkansas. Master's thesis. University of Central Arkansas, Conway, Arkansas.

Long, J.M. and R.A. Snow. 2016. Ontogenetic development of otoliths in Alligator Gar. Trans. Amer. Fish. Soc. 145: 537-544.

MacKenzie, R.A. 2005. Spatial and temporal patterns in insect emergence from a Southern Maine salt marsh. Am. Mid. Nat. 153: 257-269.

MacKenzie, R.A. and J.L. Kaster. 2004. Temporal and spatial patterns of insect emergence from a Lake Michigan coastal wetland. Wetlands 24: 688-700.

May, E.B. and A.A. Echelle. 1968. Young-of-year alligator gar in Lake Texoma, Oklahoma. Copeia 629-630.

McDonald, D.L., J.D. Anderson, C. Hurley, B.W. Bumguardner and C.R. Robertson (2013) Sexual dimorphism in Alligator Gar. North Am. J. Fish. Manage. 33:811-816.

Mendoza, R., C. G. Aguilera, and A. M. Ferrara. 2008. Gar biology and culture: status and prospects. Aquac. Res, 39:748–763.

Mendoza, R., C. Aguilera, G. Rodriquez, M. Gonzalez and R. Castro. 2002. Morphophysiological studies on Alligator Gar (Atracosteus spatula) larval development as a basis for their culture and repopulation of their natural habitats. Rev. Fish Biol. Fisher. 12:133–142.

Militello, J. 2013. Juvenile Alligator Gar *Atracosteus spatula* home ranges, spatiotemporal movement patterns, and habitat use in the Clarks River, western Kentucky. Master's Thesis. Murray State University, Murray, Kentucky.

Miller, R.J. and H.W. Robison. 2004. Alligator Gar. In: Fishes of Oklahoma. Norman Oklahoma: University of Oklahoma Press pp. 52-53.

Miranda, L.E. and P.W. Bettoli. 2007. Mortality. Pages 229-278 in C. Guy and M. Brown [ed.] Analysis and interpretation of freshwater fisheries statistics. Am. Fish. Soc. Bethesda, Maryland.

Moore, G. A., M. B. Trautman, and M. R. Curd. 1973. A description of post larval gar (*Lepisosteus spatula* Lacepede, Lepisosteidae), with a list of with a list of associated species for the Red River, Choctaw County, Oklahoma. Southwestern Nat. 18: 343- 344.

Page, L.M. and B.M. Burr. 1991. A field guide to freshwater fishes. Boston, Massachusetts: Houghton Mifflin Company 432 pp.

Patton, T. and C. Lyday. 2008. Ecological succession and fragmentation in a reservoir: effects of sedimentation and habitats on fish communities. Pg 147-167. *In* M.S. Allen, S. Sammons, and M.J. Maceina [ed.] Balancing fisheries management and water uses for impounded river systems. Am Fish Soc, Sym 62, Bethesda, Maryland.

Perschbacher, P.W. 2011. Effects of structure, forage, and stocking density on juvenile production of Alligator Gars in outdoor pools. North Am. J. Aquac. 73: 21-23.

Pigg, J. and R. Gibbs. 1996. Observations on the propagation of two rare fish species in Oklahoma. Proc. Okla. Acad. Sci. 76: 89.

Porta, M.J., R.A. Snow, and K.G. Graves. In-press. Efficacy of spawning Alligator Gar in recreational-grade swimming pools. North Am. J. Aquac.

Post, D.M. 2003. Individual variation in the timing of ontogentic niche shifts in largemouth bass. Ecol. 84: 1298-1310.

Richardson, B.M. 2015. A dietary comparison of the reintroduced Alligator Gar *Atracosteus spatula* and three sympatric gar relatives (family Lepisosteidae) in the Clarks River, Kentucky. Master's Thesis. Murray State University, Murray, Kentucky.

Robison, H.W. and Buchanan, T.M. 1988. Fishes of Arkansas. Fayetteville, Arkansas: University of Arkansas Press 536 pp.

Schlechte, J.W., K.A. Bodine, and D.J. Daugherty. 2016. Size selectivity of multifilament gill nets for sampling Alligator Gar: Modeling the Effects on Population Metrics. North Am. J. Fish. Manage. 36: 630-638.

Snow, R.A. and J.M. Long. 2017. Otolith marking of juvenile Shortnose Gar by immersion in oxytetracycline. North Am. J. Fish. Manage. 37: 724-728.

Snow, R.A., J.M. Long, and C.P. Patterson. 2016. A Comparison of lead lengths for mini-fyke nets to sample age-0 gar species in Lake Texoma. Proc. Okla. Acad. Sci. 96: 28-35.

Snow R.A. and J.M. Long. 2015. Estimating spawning times of Alligator Gar (*Atractosteus spatula*) in Lake Texoma, Oklahoma. Proc. Okla. Acad. Sci. 95: 46-53.

Solomon, L.E., Q.E. Phelps, D.P. Herzog, C.J. Kennedy, and M.S. Taylor. 2013. Juvenile Alligator Gar movement patterns in a disconnected floodplain habitat in Southeast Missouri. Am. Mid. Nat. 169: 336-344.

Todd, R.M. 2005. Tennessee Alligator Gar management plan. Tennessee Wildlife Resources Agency, Nashville, Tennessee.

Submitted September 17, 2018 Accepted November 26, 2018

Observations of a Vertical Foraging Behavior of Blue Catfish in Lake Ellsworth, Oklahoma

Richard A. Snow
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Michael J. Porta
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Ryan G. Ryswyk
Oklahoma Department of Wildlife Conservation, J. A. Manning State Fish Hatchery, Lawton, OK 73507

Blue Catfish (*Ictalurus furcatus*) are the largest catfish found in Oklahoma and are native to the Red River and Lower Arkansas River (Miller and Robison 2004). Blue Catfish are now found in many areas of the state because the Oklahoma Department of Wildlife Conservation (ODWC) stocked Blue Catfish into many impoundments due to their popularity as a sport fish (Miller and Robison 2004, Snow et al. 2017). Blue Catfish are considered omnivorous due to their very diverse diet, often consuming vegetation, mollusks, insects, and crustaceans, with larger individuals shifting to piscivory (Schmitt et al 2017, Jennings et al. 2018). Their diverse forging ecology is likely caused by seasonal habitats shifts and opportunistic feeding strategy on abundant prey species (Magoulick and Lewis 2002, Jolley and Irwin 2003, Jennings et al. 2018).

Blue Catfish are a migratory species native to large rivers, moving up-river in the spring for spawning and retreating back down-river in fall when water temperatures cool (Pflieger 1997). In reservoirs, Blue Catfish remain in the upper end of the reservoir throughout summer eventually moving to the lower portion of the reservoir in fall where they remain throughout winter (Grist 2002). As Blue Catfish move towards the lower portions of reservoirs, a dietary shift to predominately Gizzard Shad (*Dorosoma cepedianum*) and Threadfin Shad (D. *petenense*) occurs (Graham1999, Grist 2002, Jennings et al. 2018). Overlapping habitat occupancy by both shad and Blue Catfish during fall and winter likely contributes to this diet shift.

Blue Catfish have been observed feeding on schools of Gizzard Shad in both reservoirs and in the tailwaters below reservoir dams. Blue Catfish were observed gorging on Gizzard Shad that were stressed after being discharged from a Missouri reservoir dam into a tailwater (Graham and DeiSanti 1999). Graham (1999) reported Blue Catfish suspending under a school of Gizzard Shad being preyed upon by Striped Bass (*Morone* saxatilis) and foraging on wounded and dead shad. On September 8, 2018, a potential fish kill was reported by an angler fishing at Lake Ellsworth, Oklahoma to ODWC officials. The report described Blue Catfish swimming vertically and bobbing up and down in the wave action near the Lake Ellsworth Dam in apparent distress. Attempts were made by ODWC officials to create a disturbance near the Blue Catfish, which caused individuals to flee (suggesting that the Blue Catfish were healthy) and those fish were quickly replaced with other Blue Catfish that continued to forage vertically on Gizzard Shad. On two occasions during this observation, ODWC officials also witnessed small schools of Blue Catfish swimming normally near the water surface around the outside perimeter of the group of vertically oriented Blue Catfish (also suggesting that the Blue Catfish were healthy).

Standard ODWC protocol requires an employee to investigate a reported fish kill or an ongoing fish kill on public waterways, particularly when reports describe thousands of fish in distress and potentially affected. When ODWC staff arrived at the Lake Ellsworth Dam, it was quickly recognized that the Blue Catfish were actively feeding on distressed Gizzard Shad that were pushed into the dam via wave action. ODWC staff observed several thousand Blue Catfish (Figure 1) positioned vertically in the water column foraging on Gizzard Shad (Figure 2). Blue Catfish predation on Gizzard Shad was confirmed by an angler catching these fish and ODWC staff observed age-0 Gizzard Shad (mean TL = 55 mm) being regurgitated once brought to shore (presumably due to Blue Catfish feeding until satiation). This behavior was observed for approximately one hour and no other fish species were observed.

We suggest there are two possible scenarios that triggered this vertical foraging behavior. First, in the southwest area of the dam where the observation occurred, shad were trapped between the dam face and a side wall (Figure 1). Wind and wave action may have forced Gizzard Shad into the corner of the dam, which prevented escapement and created an easy foraging opportunity for Blue Catfish. The confined, cube-like space at the corner of the Lake Ellsworth Dam where the Gizzard Shad were trapped likely improved foraging success of Blue Catfish and contributed to the observed vertical foraging behavior. Second, it is possible that Gizzard Shad were stressed by environmental events that de-stratified Lake Ellsworth, resulting in an easy foraging opportunity for Blue Catfish. This mixing of the epilimnion and hypolimnion occurs usually during the fall when the density of the water changes as a result of cooling water temperatures, which is caused by cooling air temperatures, wind action, or in-flow events, and results in oxygenated surface water mixing with anoxic water on the lakes bottom. When de-stratification occurs, the dissolved oxygen (DO) of the water usually decreases (Steichen et al. 1979, Boehrer and Schultze 2008) and trapped CO_2 (Carbon dioxide) and H_2S (Hydrogen sulfide) gases escape from the hypolimnion and can ultimately affect fish behaviors (Boehrer and Schultze 2008). Occasionally following a fall

Figure 1. Photographs taken on September 8, 2018 showing Blue Catfish foraging vertically on Gizzard Shad at the southwest corner of the dam at Lake Ellsworth, Oklahoma. Photographs depict A) the entire observation area, and B) a cutaway showing a group of Blue Catfish foraging on shad. Orange arrows show Gizzard Shad being launched from the water by Blue Catfish.

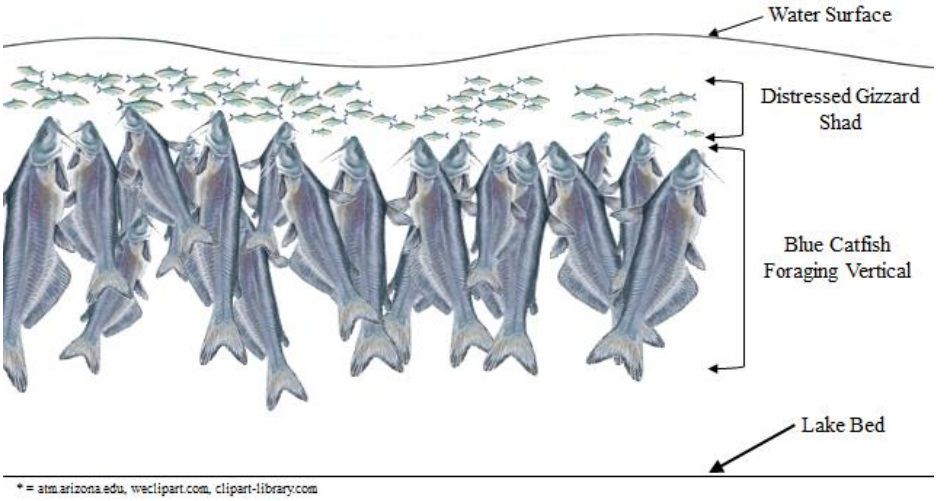


Figure 2. Depiction (in lateral view) showing observed vertical foraging behavior of Blue Catfish in Lake Ellsworth, Oklahoma on September 8, 2018. Blue Catfish were observed in the lower portion of the lake where presumably wave action had pushed distressed Gizzard Shad in to the southwest corner of the dam following a fall mixing event. *Acknowledgments for clipart illustrations.

turnover event, the reduction in DO or exposure to hydrogen sulfide gases can result in stress or death of fish (Steichen et al. 1979). A third option is a combination of the two; gizzard shad succumbed to reduced water quality conditions as a result of mixing, and when they surfaced they were transported by wind and wave action to the area at which they were observed.

Gizzard Shad are intolerant to swings in water temperature and DO, and stress associated with these environmental events can slow their movements increasing the risk of predation (Griffith and Tomljanovich 1976, Porath 2006, VanDeHey et al. 2012). Based on environmental data captured by the Apache, Oklahoma Mesonet Station (34° 54' 51" N, 98° 17' 31" W; Table 1) we concluded that mean air temperature decreased by 7.4°C from September 1 to September 8. A cold front entered the region resulting in 2.8 cm of rain over the same time period. Wind gusts from the north/northeast were 7.21 m/s with a mean wind speed of 3.36 m/s. Solar radiation during this time decreased by 16.83 MJ/m^2 as a result of increased cloud cover. A DO water column profile was collected at Lake Ellsworth

Table 1. Environmental data collected by the Apache, Oklahoma Mesonet Station (34° 54' 51" N, 98° 17' 31" W) from September 1 through September 8, 2018. This station is located 13.52 km to the north-northeast of Lake Ellsworth, Oklahoma.

Date	Mean Air Temp. (°C)	Wind Direction	Mean Wind Speed (m/s)	Wind Gust (m/s)	Daily Rain (cm)	Total Solar Radiation (MJ/m^2)
9/1/2018	28.5	SSE	5.11	13.39	0.00	22.36
9/2/2018	27.5	SSE	5.70	17.00	0.00	20.00
9/3/2018	24.6	SE	4.55	12.48	0.25	13.37
9/4/2018	23.7	S	4.94	11.56	0.05	8.68
9/5/2018	23.4	S	2.31	20.38	0.66	8.08
9/6/2018	23.6	NE	2.60	6.08	1.45	9.31
9/7/2018	22.3	NNE	3.49	7.48	2.77	5.11
9/8/2018	21.1	NNE	4.00	8.07	0.00	5.53

on September 11, 2018 to verify that the lake had mixed. We determined that DO concentrations ≥ 4.06 mg/L were present from the surface to the bottom in 12.2 m of water, suggesting that a fall turnover occurred, which likely affected Gizzard Shad.

The observation of vertical foraging of Blue Catfish is a novel and unique observation, which, to our knowledge, has not been described in the literature. Although diet of Blue Catfish has been well studied, mechanisms for obtaining their food are not well known. We will continue to monitor the Lake Ellsworth Blue Catfish population into the future to determine if this was an isolated event resulting from environmental conditions and a unique dam configuration or if this foraging behavior occurs in other reservoirs following a lake mixing event.

References

Boehrer, B., and M. Schultze. 2008. Stratification of lakes. Reviews of Geophysics 46:1-27.

Graham, K. 1999. A review of the biology and management of Blue Catfish. Pages 37-49 *in* E. R. Irwin, W. A. Hubert, C. F. Rabeni, H. L. Schramm, Jr. and T. Coon, editors. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Graham, K., and K. DeiSanti. 1999. The population and fishery of Blue Catfish and Channel Catfish in the Harry S. Truman Dam tailwater, Missouri. Pages 361–376 in E. R. Irwin, W. A. Hubert, C. F. Rabeni, H. L. Schramm, Jr., and T. Coon, editors. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Griffith, J. S, and D. A. Tomljanovich. 1976. Susceptibility of Threadfin Shad to impingement. Proceedings of the Annual Conference of Southeastern Association of Game and Fish Commissions. 29:223–234.

Grist, J. D. 2002. Movement and habitat use of Blue Catfish in Lake Norman, North Carolina. Master's Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Jennings, C. A., G. E. Mitchell, and C. Nelson. 2018. Seasonal food habits of introduced Blue Catfish in Lake Oconee, Georgia. Journal of the Southeastern Association of Fish and Wildlife Agencies 5:39-45.

Jolly, J. C., and E. R. Irwin. 2003. Food habits of catfishes in tailwater and reservoir habitats in a section of Coosa River, Alabama. Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies 57:124-140.

Magoulick, D. D., and L. C. Lewis. 2002. Blue Catfish predation on exotic zebra mussels: effects on predator and prey. Freshwater Biology 47:1-11.

Miller, R. J. and H. W. Robison. 2004. Fishes of Oklahoma. University of Oklahoma Press, Norman, Oklahoma.

Pflieger, W. L. 1997. The fish of Missouri. Missouri Department of Conservation, Jefferson City.

Porath, M. T. 2006. Climate and habitat factors related to a localized extirpation of Gizzard Shad (*Dorosoma cepedianum*). Great Plains Research. 16:127–135.

Schmitt, J. D., E. M. Hallerman, A. Bunch, Z. Moran, J. A. Emmel, and D. J. Orth. 2017. Predation and prey selectivity by nonnative catfish on migrating Alosines in an Atlantic slope estuary. Marine and Coastal Fisheries: Dynamic, Management and Ecosystem Science 9:108-125.

Snow, R. A., C. P. Patterson, and M. J. Porta. 2017. First evidence of Blue Catfish natural reproduction in Canton Reservoir, Oklahoma. Proceedings of the Oklahoma Academy of Science 97:54-60.

Steichen, J. M., J. E. Garton, and C. E. Rice. 1979. The Effect of lake destratification on water quality. American Water Works Association 71:219-225.

VanDeHey, J. A., D. W. Willis, and B. G. Blackwell. 2012. Survival, reproduction, and recruitment of Gizzard Shad (*Dorosoma cepedianum*) at the northwestern edge of its native range. Journal of Freshwater Ecology, 27:41-53.

Submitted September 24 Accepted November 20, 2018

Additional Information on the Natural History and Ecology of Select Fauna (Decapoda; Actinopterygii; Mammalia) from Oklahoma

Henry W. Robison
9717 Wild Mountain Drive, Sherwood, AR 72120

Chris T. McAllister
Division of Science and Mathematics, Eastern Oklahoma State College, Idabel, OK 74745

Donald G. Cloutman
P. O. Box 197, Burdett, KS 67523

Charles R. Bursey
Department of Biology, Pennsylvania State University, Shenango Valley Campus, Sharon, PA 16146

Trevor K. Turner
Division of Science and Mathematics, Eastern Oklahoma State College, Idabel, OK 74745

Abstract: In our third contribution on the subject, we include noteworthy observations on the natural history and ecology of three species of crayfishes, eight native fishes, and two mammals in Oklahoma. Here, additional records of three uncommon species of crayfishes, early spawning dates for five fishes, an unusual occurrence of the egg filaments of *Labidesthes sicculus* entangled among the gill filaments and rakers of *Menidia audens*, and notable information on ecto- and endoparasites from two species each of fishes and mammals are documented. Our purpose is to help complement and fill gaps in our limited biological knowledge of this biota that should help in future studies conducted in the state.

Introduction

The state of Oklahoma is presently inhabited by 31 species of crayfishes (Morehouse and Tobler 2013; Taylor and Robison 2016), over 180 species of fishes (Miller and Robison 2004), and 106 species of mammals (Caire et al. 1989). Updated information on the natural history and ecology of this biota is essential in understanding their biology. Here, in our third contribution on the subject (McAllister and Robison 2016, 2017), we provide biological information on three species of crayfishes, eight native fishes, and two mammals of the state.

Methods

Crayfishes were collected by hand and preserved in 70% isopropyl alcohol. Fishes were taken with 3.1 × 1.8 m or 6.1 × 1.8 m seines (3.2 mm mesh), gill nets, bowhunting, and/or with a back-pack electrofisher (DC current). They were killed by immersion in a concentrated tricaine methanosulfonate solution following accepted methods (Use of Fishes in Research Committee 2014), preserved in 10% formalin, and stored in 45% isopropanol. Fishes were also measured for total length (TL)

and specimens were examined for reproductive characters; some containing ripe ova had their egg sacs weighed to the nearest g. Leeches from fish were slowly relaxed by adding increasing concentrations of ethanol to its container water before being preserved in 95% (v/v) DNA grade ethanol. The Brook Silverside (*Labidesthes sicculus*) egg was identified using Wallus and Simon (2006). A single bat was found dead on the road, and two woodrats were collected with Sherman live traps baited with oatmeal. Woodrats were killed with an intraperitoneal injection of sodium pentobarbital following accepted guidelines (Sikes et al. 2016). A midventral incision was made on each mammal and the entire gastrointestinal tract was removed and placed in Petri dishes containing 0.9% saline and contents examined under a stereomicroscope at 20-30×. Nematodes were fixed in hot tap water and preserved in 70% (v/v) ethanol. They were later cleared by placing them in a mixture of 5% or 10% glycerin in 70% ethanol in an uncovered dish, and allowing the ethanol (and water) to evaporate. Cleared nematodes were studied as temporary mounts in glycerol. Localities are reported as GPS (latitude and longitude) coordinates. Crayfish and some fish voucher specimens were deposited in the Southern Arkansas University (SAU) Collection, Magnolia, Arkansas. Leeches were deposited in the Invertebrate Zoology Collections of the Peabody Museum of Natural History at Yale University (YPM), New Haven, Connecticut. Other fish and mammal vouchers were deposited in the Henderson State University (HSU) collection, Arkadelphia, Arkansas.

Results and Discussion

The results of our findings are detailed below in an annotated format and phylogenetic order.

Crustacea: Decapoda: Cambaridae (Cambarid Crayfishes)

***Cambarellus puer* Hobbs, 1945 – Swamp Dwarf Crayfish**. *Cambarellus puer* typically inhabits permanent, well vegetated, shallow, mud-bottomed swamps, sloughs, and lowland streams (Taylor et al. 2004). In Oklahoma, it is known from only a single location in McCurtain County along the Little River (Morehouse and Tobler 2013). On 27 November 1992, two female C. *puer* were collected by aquatic dip net in a roadside ditch on US 259, 10.1 km (6.3 mi) S of Broken Bow, McCurtain County (33° 46' 47.8086"N, 94° 46' 30.7704"W). We document this additional collection site for an uncommon Oklahoma crayfish which represents only the second documented geographic locale for C. *puer* in the state.

***Faxonius lancifer* (Hagen, 1870) – Shrimp Crayfish.** Until recently, this crayfish was placed in the genus *Orconectes*. The taxonomy of freshwater crayfishes was recently updated based on the last 20 years of phylogenetic studies that have called into question family, subfamily, genus, and subgenus affiliations for various taxa (Crandall and DeGrave 2017). This crayfish is most often found in permanent waters of oxbows, bayous, and lowland streams over substrates of mud or mixed mud and sand (Page 1985). Morehouse and Tobler (2013) reported this state crayfish was known from only two localities in McCurtain County. Herein, we report two additional collection sites of this uncommon crayfish in the state. On 27 November 1997, two female specimens of *F. lancifer* were taken by aquatic dip net from a roadside ditch along St. Hwy. 87, about 12.9 km (8 mi) SE of Tom, McCurtain County (33° 42' 29.7684"N, 94° 37' 53.6412"W). Three years later, on 30 November 2000, one additional male (form II) was collected from an unnamed swamp on US 259, 11.2 km (7.0 mi) S of Broken Bow, McCurtain County (33° 56' 42.093"N, 94° 45' 29.3214"W).

***Procambarus curdi* Reimer, 1975 – Red River Burrowing Crayfish.** In his original description of *P. curdi*, Reimer (1975) listed a number of collecting localities in southern Oklahoma, including three near Idabel, McCurtain County. Morehouse and Tobler (2013) later reported both males (form I and II) and females have been collected throughout the year in Oklahoma from burrows in the Red River drainage of the state. More recently, McAllister and Robison (2017) reported the first occurrence of an ovigerous female taken in Oklahoma.

We herein report the second collection of an ovigerous female in the state. On 25 March 2001 a single ovigerous female with 107 ova (1.0–1.5 mm in diameter) was dug from a burrow in a roadside ditch along U.S. Hwy. 70 (259), approximately 10.9 km (6.8 mi) NE of Idabel, McCurtain County (33° 57' 3.5742"N, 94° 45' 25.8798"W).

Actinopterygii: Lepisosteiformes: Lepisosteidae (Gars)

***Lepisosteus oculatus* Winchell, 1864 – Spotted Gar.** A 670 mm TL female *L. oculatus* was collected by bowhunting on 28 October 2018 from a private pond north of Idabel in McCurtain County (33° 55' 56.93"N, 94° 43' 43.22"W) and found to contain ripe eggs with a wet weight of 301 g. In Oklahoma, Spotted Gar are reported to spawn from about mid-April to early June (Tyler and Granger 1984). We document the earliest known date of potential spawning for *L. oculatus* in the state.

Amiiformes: Amiidae

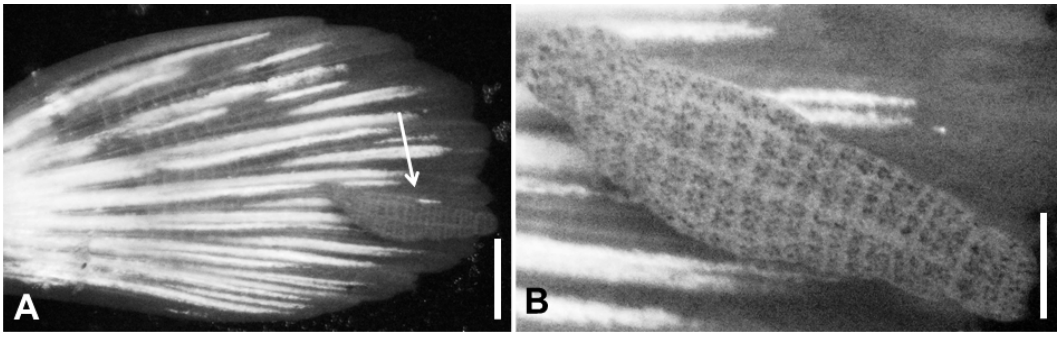
***Amia calva* Linnaeus, 1766 – Bowfin.** A 470 mm TL female *A. calva* was collected by bowhunting on the same date and site above and contained ripe ova with a wet weight of 43.7 g. Bowfin are reported to spawn in spring (early April into June) in Oklahoma (Miller and Robison 2004). We report the earliest known date of impending spawning for *A. calva* in the state.

Hiodontiformes: Hiodontidae

***Hiodon alosoides* (Rafinesque, 1819) – Goldeye.** A 143 mm TL *H. alosoides* was collected on 14 February 2018 with a gill net from near the Willis Bridge on Lake Texoma, Marshall County (33° 52' 32.4516"N, 96° 50' 2.3136"W) at a water temperature of 6.7°C (44°F). Examination of this female revealed it to be full of gravid (ripe) ova with a combined egg mass wet weight of 39.02 g. Spawning in *H. alosoides* occurs in early spring (April) in Oklahoma (Miller and Robison 2004). In other parts of its range, spawning has been reported in early spring in Illinois and in May to early July in Canada with a production of 6,000 to 25,000 eggs. We therefore document the earliest possible spawning of *H. alosoides* in Oklahoma.

Cypriniformes: Cyprinidae (Carps and Minnows)

***Campostoma pullum* (Agassiz, 1854) – Central Stoneroller.** A single leech, *Cystobranchus klemmi* (Williams and Burreson) was found on the pectoral fin of an 81 mm TL *C. pullum* (Figs. 1A–B) collected on 13 March 2017 from the Illinois River, Cherokee County (35° 57' 30.042"N, 94° 52' 10.0266"W). This represents a new host record for *C. klemmi*. *Cystobranchus klemmi* is primarily found on various stonerollers (Williams and Burreson 2005, Richardson et al. 2013) and the host list also includes other cyprinids such as Southern Redbelly Dace (*Chrosomus erythrogaster*), Bigeye Shiner (*Notropis boops*) and Creek Chub (*Semotilus atromaculatus*) (Richardson et al. 2013; Thigpen et al. 2015). This leech has now been reported from locales in Arkansas, Illinois, Missouri, and Oklahoma (Williams and



Figures 1A-B. Leech from *Campostoma pullum*. A. View of *Cystobranchus klemmi* (arrow) on pectoral fin; scale bar = 5.0 mm. B. Higher magnification of *C. klemmi* on pectoral fin. Scale bar = 2.0 mm.

Burreson 2005; Richardson et al. 2013; Thigpen et al. 2015; McAllister et al. 2018).

***Notropis nubilus* (Forbes, 1878) – Ozark Minnow.** Little is known concerning the actual time of spawning in Oklahoma of the Ozark Minnow. Miller and Robison (2004) did not specify dates and only stated that spawning occurs in the spring. Six nuptial males of *N. nubilus* collected on 7 April 2001 from the Illinois River at St. Hwy 51, S of Eldon, Cherokee County (35° 55' 16.5504"N, 94° 50' 14.8848"W) were in their orange breeding coloration and running milt. This is the first actual date of spawning condition in males reported for this species in Oklahoma.

***Erimystax x-punctatus* (Hubbs and Crowe, 1956) – Gravel Chub.** Miller and Robison (2004) reported spawning in this species occurs from March to early May; however, little is known concerning the reproductive period of this chub in Oklahoma. On 13 March 1996, three male *E. x-punctatus* in reproductive condition (running milt) were collected from the Illinois River at St. Hwy 51, S of Eldon, Cherokee County (35° 55' 16.5504"N, 94° 50' 14.8848"W). This is the earliest date in March reported for this species in spawning condition documented from Oklahoma.

Atherinopiformes: Atherinopsidae

***Menidia audens* Hay, 1882 – Mississippi Silverside.** Forty-four *M. audens* (mean TL = 75.7, range 63–96 mm) were collected by seine on 19 April 2018 from near the University of Oklahoma Biological Station, Lake Texoma, Marshall County (35° 52' 45.9906"N, 96° 48' 7.6068"W). While examining specimens for monogenean gill parasites (none found), a single specimen was found to possess filaments of two eggs (Fig. 2) of what we believe to be from a Brook Silverside (*Labidesthes sicculus*) tangled in its gills and gill rakers. We suggest the *M. audens* ate the eggs and the filaments got tangled in its rakers. This silverside has been reported to feed primarily on plankton (Wallus and Simon 2006), so this event seems plausible.

Perciformes: Percidae

***Percina phoxocephala* (Nelson, 1876) – Slenderhead Darter.** Little is known about the parasites of *P. phoxocephala* (McAllister et al. 2016). Two of seven (29%) *P. phoxocephala* collected on 25 October 2013 from Cow Creek Crossing on the Little River, McCurtain County (34° 31' 5.9658"N, 94° 30' 19.8288"W) were infested with leeches, *Myzobdella reducta* Meyer on the caudal fins (Figs. 3A–B). This leech has also been reported from other fishes from this locality (Richardson et al. 2014). Meyer (1940) originally described *M. reducta* (as *Piscicolaria reducta*) from *P. phoxocephala* from Illinois. There is an additional report of this leech being reported from *P. phoxocephala* in Minnesota (Erickson 1978), but this is first time Slenderhead Darters from Oklahoma have been documented with *M. reducta*.

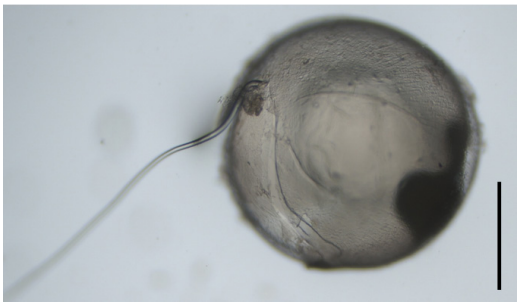


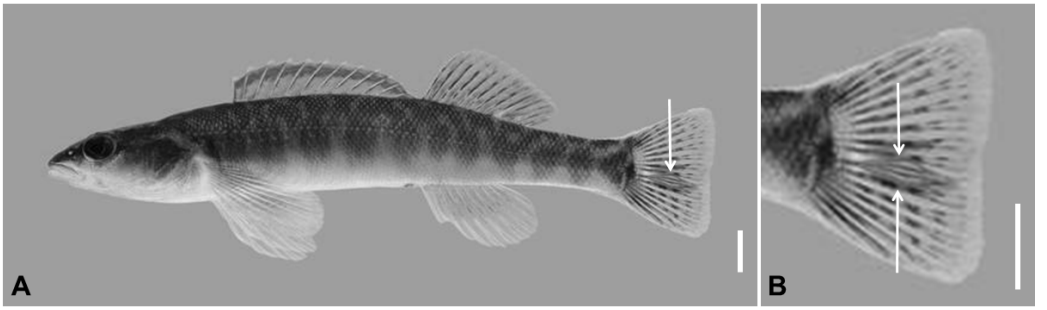
Figure 2. Egg of *Labidesthes sicculus* found on gill filament of *Menidia audens*; scale bar = 500 µm.

Mammalia: Chiroptera: Molossidae

***Tadarida brasiliensis* (I. Geoffroy, 1824) – Mexican free-tailed bat.** Three nematodes (two male, one female) of *Tadaridanema delicatus* (Schwartz, 1927) Falcon-Ordaz, Guzman-Cornejo, Garcia-Prieto, and Gardner, 2006 (HWML 110492) were found in the intestines of a single *T. brasiliensis* found dead on 1 November 2014 in Broken Bow, McCurtain County (34° 00' 23.66"N, 94° 45' 53.88"W). This nematode has been previously reported from *T. brasiliensis* from California, Florida, Louisiana, New Mexico, Texas, and México (Voge 1956; Jameson 1959; Cain 1966; Martin 1976; Falcón-Ordaz et al. 2006). We document *T. delicatus* in Oklahoma for the first time.

Rodentia: Cricetidae

***Neotoma floridana* (Ord, 1818) – Eastern**



Figures 3A-B. Leech from *Percina phoxocephala*. A. View of *Myzobdella reducta* (arrow) on caudal fin; scale bar = 10.0 mm. B. Closeup of same *M. reducta* (arrows) on caudal fin; scale bar = 10.0 mm.

woodrat. One of two *N. floridana* collected on 9 August 2018 from the Eastern Oklahoma State College Campus-Wilburton, Latimer County (34° 54' 54.3558"N, 95° 19' 51.9024"W) was found to harbor two murine whipworms, *Trichurus muris* (HWML 110493) in its cecum (Fig. 4). This species is a gastrointestinal parasite of mice which resides in the cecum and colon. In the life cycle, *Trichuris* eggs are unembryonated when passed in the host feces and take up to a month in a humid environment to become infective and require no intermediate host (Anderson 2000). The hosts obtain the infection by ingesting food items or drinking water contaminated with embryonated eggs. This whipworm has been reported from *N. floridana osagensis* from Oklahoma (Murphy 1952; Boren et al. 1993). Another species, *T. neotomae* Chandler has been reported from the southern plains woodrat, *N. micropus*, from Texas (Charles et al. 2012) and dusky-footed woodrat, *N. fuscipes* from California (Chandler 1945; Boren et al. 1993). We report an additional infection in *N. floridana* and the first photomicrograph (Fig. 4) of *T. muris* from an eastern woodrat.

Figure 4. *Trichurus muris* from *Neotoma floridana*. Scale bar = 1.0 mm intervals.

Acknowledgments

The Oklahoma Department of Wildlife Conservation (ODWC) provided a Scientific Collecting Permit to CTM. HWR thanks former Southern Arkansas University students for assistance in the collection of crayfishes and some fishes. CTM thanks Dr. Gary Wellborn, Director of the OU Biological Station, for allowing him to seine on the premises. We thank members of the ODWC (Caddo office), especially Cliff Sager, for collecting the *H. alosoides*, and Dr. David Neely (Tennessee Aquarium, Chattanooga, TN) and Uland Thomas (Chicago, IL) for assistance in collecting fish with the back-pack electrofisher at Cow Creek Crossing as well as the photograph of *P. phoxocephala*, and Kylie Brecheisen (EOSC-Idabel, OK) for assistance with seining at Lake Texoma. Drs. Dennis Richardson (Quinnipiac University, Hamden, CT) is acknowledged for depositing the leeches as well as Renn Tumlison (HSU) and Gabor Racz (HWML) for their expert curatorial assistance.

References

Boren JC, Lochmiller RL, Boggs JF, Leslie Jr DL. 1993. Gastrointestinal helminths of eastern woodrat populations in central Oklahoma. Southwest Nat 38:146–149.

Cain GD. 1966. Helminth parasites of bats from Carlsbad Caverns, New Mexico. J Parasitol 52:351–357.

Caire W, Tyler JD, Glass BP, Mares MA. 1989. Mammals of Oklahoma. Norman (OK): University of Oklahoma Press. 567 p.

Chandler AC. 1945. *Trichurus* species from California rodents. J Parasitol 31:284–286.

Charles RA, Kjos S, Ellis AE, Dubey JP, Shock BC, Yabsley MJ. 2012. Parasites and vector-borne pathogens of southern plains woodrats (*Neotoma micropus*) from southern Texas. Parasitol Res 110:1855–1862.

Crandall KA, De Grave S. 2017. An updated classification of the freshwater crayfishes (Decapoda: Astacidea). J Crust Biol 37:615–653.

Erickson JE. 1978. Parasites of the Banded Darter, *Etheostoma zonale* (Pisces: Percidae). J Parasitol 64:899.

Falcon-Ordaz, J, Guzmán-Cornejo C, Garcıa-Prieto L, Gardner SL. 2006. *Tadaridanema delicatus* (Schwartz, 1927) n. gen., n. comb. (Trichostrongylida: Molineidae) parasite of molossid bats. J Parasitol 92:1035–1042.

Jameson DK. 1959. A survey of the parasites of five species of bats. Southwest Nat 4:61–65.

Martin DR. 1976. New host and distribution records of helminth parasites of the Mexican free-tailed bat, *Tadarida brasiliensis*, from Texas and Louisiana. Proc Helminthol Soc Wash 43:85–86.

McAllister CT, Bursey CR, Robison HW, Connior MB, Trauth SE. 2016. New records of helminth parasites (Trematoda, Cestoda, Nematoda) from fishes in the Arkansas and Red River drainages, Oklahoma. Proc Okla Acad Sci 96:83–92.

McAllister CT, Robison HW. 2016. Natural history notes on select fauna (Decapoda, Actinopterygii) from southeastern Oklahoma. Proc Okla Acad Sci 96:58–62.

McAllister CT, Robison HW. 2017. Noteworthy natural history and ecological information on crayfishes (Decapoda) and fishes (Actinopterygii) from Oklahoma. Proc Okla Acad Sci 97: 25–32.

McAllister CT, Robison HW, Tumlison RT. 2018. Additional county records of invertebrates from Arkansas. J Ark Acad Sci 72:(in press).

Meyer MC. 1940. A revision of the leeches (Piscicolidae) living on fresh-water fishes of North America. Trans Amer Microsc Soc 59:354–376.

Miller RJ, Robison HW. 2004. The fishes of Oklahoma. 2nd edition. Norman (OK): University of Oklahoma Press. 450 p.

Morehouse RL, Tobler M. 2013. Crayfishes (Decapoda: Cambaridae) of Oklahoma: identification, distributions, and natural history. Zootaxa 3717:101–157.

Murphy MF. 1952. Ecology and helminths of the Osage woodrat, *Neotoma floridana osagensis*, including the description of *Longistrata neotoma* n. sp. (Trichostrongylidae). Amer Midl Nat 48:204–218.

Page LM. 1985. The crayfishes of Illinois. Ill Nat Hist Surv Bull 33:335–448.

Reimer RD. 1975. *Procambarus* **(***Girardiella***)** *curdi*, a new crawfish from Arkansas, Oklahoma and Texas (Decapoda, Astacidae). Tulane Stud Zool Bot 19:22–25.

Richardson DJ, Moser WE, McAllister CT, Tumlison R, Allen Jr JW, Barger MA, Robison HW, Neely DA, Watkins-Colwell G. 2014. New host and geographic distribution records for the fish leech *Myzobdella reducta* (Meyer, 1940) (Hirudinida: Piscicolidae). J Ark Acad Sci 68:167–169.

Richardson DJ, Tumlison R, Allen Jr JW, Moser WE, McAllister CT, Trauth SE, Robison HW. 2013. New host records for the fish leech *Cystobranchus klemmi* (Hirundinida: Piscicolidae) on cyprinid fishes from Arkansas and Oklahoma. J Ark Acad Sci 67:211–213.

Sikes RS and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. J Mamm 97:663–688.

Taylor CA, Robison, HW. 2016. A new burrowing crayfish of the genus *Fallicambarus* Hobbs, 1969 (Decapoda: Cambaridae) from the Red River drainage of the southcentral United States. Zootaxa 4144:575–583.

Taylor CA, Jones SN, Bergey EA. 2004. Crayfishes of Oklahoma revisited: New state records and checklist of species. Southwest Nat 49:250–255.

Thigpen CS, Trauth SE, Bagwell LI, Konvalina JD, Schratz SA, McAllister CT, Moser DE, Richardson DJ, Robison HW. 2015. New host and county records of the fish leech *Cystobranchus klemmi* (Hirudinida: Piscicolidae) in Arkansas. J Ark Acad Sci 69:147–148.

Tyler JD, Granger MN. 1984. Notes on food habits, size, and spawning behavior of Spotted Gar in Lake Lawtonka, Oklahoma. Proc Okla Acad Sci 64:8–10.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. Bethesda (MD): American Fisheries Society. Available from: https://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf (Accessed September 30, 2018).

Voge M. 1956. A list of nematode parasites from California mammals. Amer Midl Nat 56: 423–429.

Wallus R, Simon TP. 2006. Reproductive biology and early life history of fishes in the Ohio River drainage. Aphredoderidae through Cottidae, Moronidae, and Sciaenidae. Volume 5. Boca Raton (FL): CRC Press. 360 p.

Williams JI, Burreson EM. 2005. *Gonimosobdella klemmi* n. gen., n. sp. (Hirudinida: Piscicolidae) from cyprinid fishes in Arkansas, Illinois, and Missouri, U.S.A. Comp Parasitol 72:166–172.

Submitted October 8, 2018 Accepted December 8, 2018

Species of *Ligictaluridus* (Monogenoidea: Dactylogyridae) Parasitizing Large Catfishes (Siluriformes: Ictaluridae) from Arkansas, Oklahoma, and Texas

Donald G. Cloutman
P. O. Box 197, Burdett, KS 67523

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Henry W. Robison
9717 Wild Mountain Drive, Sherwood, AR

Abstract: Three species of *Ligictaluridus* (Monogenoidea: Dactylogyridae) were found on large ictalurid catfishes in Arkansas, Oklahoma, and Texas. We provide the second report of *Ligictaluridus michaelalicea* from the gills of the Flathead Catfish (*Pylodictis olivaris*) but the first report from Lake Texoma, Oklahoma, and the Mississippi River, Arkansas. The species, originally described in 2018 from the upper Mississippi River in Iowa and Wisconsin, appears to be restricted to *P. olivaris*. *Ligictaluridus michaelalicea* was found in mixed infections with *L. mirabilis* and *L. pricei* on *P. olivaris*. We also document new distributional records for *L. mirabilis* and *L. pricei* on Blue Catfish (*Ictalurus furcatus*) from Lake Texoma, and Channel Catfish (*Ictalurus punctatus*) from Lake Texoma and the Verdigris River, Oklahoma. We suggest that previous records of *L. floridanus* from Arkansas, Oklahoma, and Texas be considered as *L. mirabilis*.

Introduction

Three species of large catfishes, Blue Catfish, *Ictalurus furcatus* (Lesueur), Channel Catfish, *Ictalurus punctatus* (Rafinesque), and Flathead Catfish, *Pylodictis olivaris* (Rafinesque) (Siluriformes: Ictaluridae), are native to the Mississippi, Mobile, and Rio Grande drainages of the United States and northeastern México (Page and Burr 2011). They are piscivorous predators found in large streams, rivers, and lakes, and have in recent decades been widely introduced along the Atlantic Slope and western drainages for sport and commercial fish management (Jackson 1999). General life history information for these species has been compiled in two International Ictalurid Symposia (Irwin et al. 1999; Michaletz and Travnichek 2011).

The parasites of these three species of catfish are fairly well known, including the Monogenoidea (Klassen and Beverley-Burton 1985; Hoffman 1999; Leis et al. 2018). To date, three species of monogenes of the genus *Ligictaluridus* Beverley-Burton, 1984, (Klassen and Beverley-Burton, 1985), have been reported from *I. furcatus*, *I. punctatus*, and *P. olivaris*, including *L. floridanus* (Mueller, 1936), *L. mirabilis* (Mueller, 1937), and *L. pricei* (Mueller, 1936) (Beverley-Burton 1984; Klassen and Beverley-Burton 1985; Hoffman 1999). Due to relatively few records, particularly from *I. furcatus* and for *P. olivaris*, previously undetected new species are possibly yet to be discovered, such as *L. michaelalicea*

Leis, Easy, MacLean, and Cone, 2018, recently described *P. olivaris* from the upper Mississippi River, Wisconsin and Iowa (Leis et al. 2018). *Ligictaluridus bychowskyi* (Price and Mura, 1969) has only been reported once, from *I. punctatus* in Louisiana (Price and Mura 1969), indicating need for further distributional studies. Also, there are questions remaining that concern the validity of early records of *L. floridanus* and *L. mirabilis*, often considered conspecific, until revisionary studies conducted by Klassen and Beverley-Burton (1985), particularly in the Mississippi River and western portion of the range of these catfishes (Leis et al. 2018). Here, we report on three species of *Ligictaluridus* found on the gills of *I. furcatus*, *I. punctatus*, and *P. olivaris* from Arkansas, Oklahoma, and Texas.

Methods

Host specimens were collected with a trawl in the Mississippi River, Arkansas, back-pack electrofisher from South Concho River, Texas, and with gill nets and boat-electrofisher from Lake Texoma and Verdigris River, Oklahoma, respectively. We followed accepted guidelines for the use of fish in research (AFS 2014). The fish were placed on ice and, if alive, were overdosed in a concentrated chloretone solution; all were subsequently preserved in 10% formalin (McAllister et al. 2015). Specimens were measured for total length (TL). Gills were removed from the fish and examined for ectoparasites under a stereomicroscope at 20–30×. Parasites, picked directly from the gills of their hosts with minuten nadeln, were mounted in Grey and Wess medium stained with Gomori's trichrome (Kritsky et al. 1978). Observations and measurements were made from digital images taken with an Accu-scope Ecelis HDS camera mounted on an Accu-scope LED series phase-contrast microscope. Measurements of haptoral sclerites, in micrometers (µm), and identifications of monogenes were based on Klassen and Beverley-Burton (1985), Klassen et al. (1985), and Leis et al. (2018). The curved male copulatory organ was measured as a straight line extending between the two most distant points of such structures. Ranges are followed by means and number of specimens measured in parentheses. Numbering of haptoral hooks follows Mizelle (1936). Prevalence, intensity, and range of infection were calculated according to Bush et al. (1997).

Voucher specimens of parasites 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

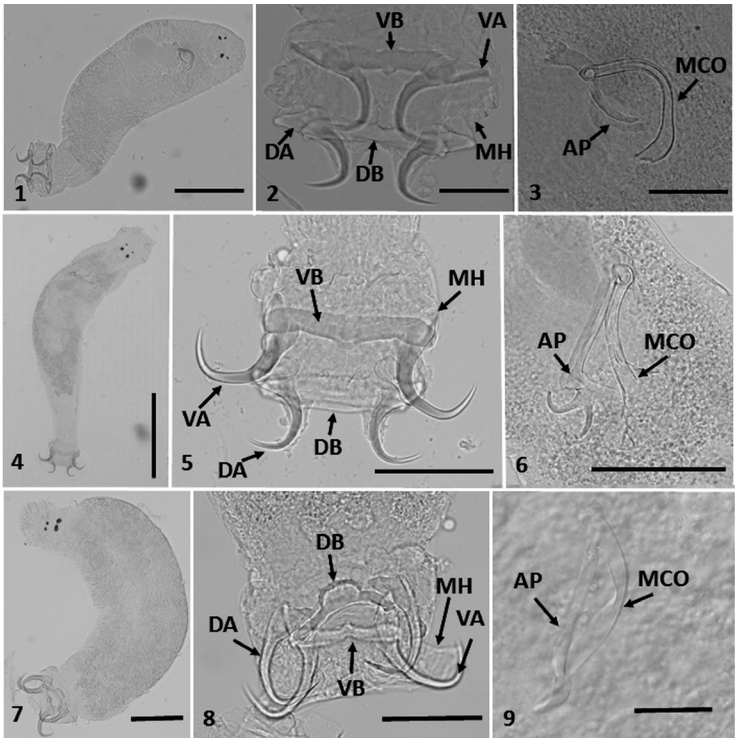
In this study, three species of Monogenoidea were found on the gills of *I. furcatus*, *I. punctatus*, and *P. olivaris*, including a recently described species that we provide morphological and mensural data on as follows:

Class Monogenoidea Bychowsky, 1937
Family Dactylogyridae Bychowsky, 1933
Genus *Ligictaluridus* Beverley-Burton, 1984
***Ligictaluridus michaelalicea* Leis, Easy, Maclean, and Cone, 2018**

***Host, localities, and dates collected*:** *Pylodictis olivaris* (one adult, 610 mm TL, host destroyed during necropsy, collected 21 February 2017), Lake Texoma in the vicinity of Willis Bridge, Red River Drainage, Marshall County, Oklahoma (33°52'31.9224"N, 96°50'01.2804"W). *Pylodictis olivaris* (2 juveniles, 118, 125 mm TL, hosts destroyed during necropsy, collected 16 October 2015), Mississippi River at Sans Souci Landing, Mississippi River Drainage, Mississippi County, Arkansas (35°39'21.387"N, 89°55'33.4956"W).

Prevalence, intensity, and material deposited: 2 of 3 (67%, 1 and 160 worms) overall; Lake Texoma in the vicinity of Willis Bridge, 1 of 1 (100%), 160 worms, HWML 139470 (1 specimen) and HWML 139471 (19 specimens); Mississippi River at Sans Souci Landing, 1 of 2 [HSU photovoucher, 125 mm TL] (50%), 1 worm, HWML 139472.

Site of infection: gills.



Figures 1-9. Monogeneans from ictalurids. Figs. 1-3. *Ligictaluridus michaelalicea* from *Pylodictis olivaris*, Lake Texoma, Oklahoma (HWML 139471). 1. Whole mount, ventral view. Scale bar = 200 µm. 2. Haptor showing dorsal anchor (DA), dorsal bar (DB), ventral anchor (VA), ventral bar (VB), marginal hook (MH). Scale bar = 50 µm. 3. Male copulatory organ (MCO) and accessory piece (AP). Scale bar = 50 µm. Figs. 4-6. *Ligictaluridus mirabilis* from *Ictalurus punctatus*, Lake Texoma, Oklahoma. 4. Whole mount, ventral view. Scale bar = 200 µm. 5. Haptor showing dorsal anchor (DA), dorsal bar (DB), ventral anchor (VA), ventral bar (VB), marginal hook (MH). Scale bar = 50 µm. 6. Male copulatory organ (MCO) and accessory piece (AP). Scale bar = 50 µm. Figs. 7-9. *Ligictaluridus pricei* from *Ictalurus punctatus*, Lake Texoma, Oklahoma. 7. Whole mount, ventral view. Scale bar = 100 µm. 8. Haptor showing dorsal anchor (DA), dorsal bar (DB), ventral anchor (VA), ventral bar (VB), marginal hook (MH). Scale bar = 50 µm. 9. Male copulatory organ (MCO) and accessory piece (AP). Scale bar = 15 µm.

***Description of specimens* (Figs. 1–3):** With characters of the genus *Ligictaluridus* as diagnosed by Beverley-Burton (1984) and Klassen and Beverley-Burton (1985). Body 490–1020 (752) (*n* = 20) long, greatest width 140–275 (214) (*n* = 20). Peduncle 0–100 (26) (*n* = 20) long, 66–137 (105) (*n* = 20) wide. Haptor 54–120 (97) (*n* = 20) long, 120–175 (152) (*n* = 20) wide. Cephalic lobes poorly developed, each lobe with a group of cephalic glands. Two pairs of eyes, anterior pair smaller and usually farther apart than posterior pair. Pharynx subcircular to ovate; 52–80 (68) (*n* = 20) long, 46–70 (60) (*n* = 20) wide. Gut smooth and confluent posteriorly. Two pairs of anchors; composed of solid base with short deep root, elongate superficial root, solid elongate, blade-like shaft curving to a sharp point; similar in shape, dorsal pair slightly smaller than ventral pair. Dorsal anchor 53–67 (60) (*n* = 20) long (total length measurement "a" of Klassen and Beverley-Burton (1985)-distance from tip of superficial root to curve of blade); greatest width of base 28–38 (34) (*n* = 20); length from curve of blade to notch between superficial and deep roots (measurement "b" of Klassen and Beverley-Burton (1985) 44–55

(51) (*n* = 20); superficial root 18–29 (22) (*n* = 20) long (measurement "d" of Klassen and Beverley-Burton (1985); distance from tip of blade to curve of blade 21–28 (24) (*n* = 20) long (measurement "e" of Klassen and Beverley-Burton (1985). Ventral anchor 62–76 (68) (*n* = 20) long (total length measurement "a" of Klassen and Beverley-Burton (1985); greatest width of base 33–44 (40) (*n* = 20); length from curve of blade to notch between superficial and deep roots (measurement "b" of Klassen and Beverley-Burton (1985) 47–65 (57) (*n* = 20); superficial root 21–30 (26) (*n* = 20) long (measurement "d" of Klassen and Beverley-Burton (1985); distance from tip of blade to curve of blade 23–30 (26) (*n* = 20) long (measurement "e" of Klassen and Beverley-Burton (1985). Dorsal bar broadly curved to straight with knobs on each end and broad median flange; 80–129 (100) (*n* = 20) long. Ventral bar broadly curved to straight with notched knobs on each end and flap-like median flange; 66–112 (89) (*n* =20) long. Fourteen marginal hooks (7 pairs), similar in size and shape. Each hook composed of solid base, solid slender shaft, sickle-shaped termination provided with opposable piece. Hook lengths: nos. 1, 13–21 (17) (*n* = 16); 2, 15–21 (19) (*n* = 19); 3, 17–23 (20) (*n* = 18); 4, 17–23 (20) (*n* = 15); 5, 17–23 (20) (*n* = 11); 6, 15–20 (18) (*n* = 8); 7, 14–19 (16) (*n* = 8). Copulatory complex composed of male copulatory organ and accessory piece. Male copulatory organ with proximal flap-like base bearing slender, curving tubular shaft, becoming bulbous distally; 61–108 (78) (*n* = 20) total length including base; shaft 49–98 (60) (*n* = 20) long. Accessory piece solid, slender, elongate, broadly curved uniramous rod, 29–54 (35) (*n* = 20) long; accompanied by elongate, lightly sclerotized membranous flap extending along and slightly past entire length. Testis ovoid, post ovarian. Two large prostatic reservoirs near male copulatory organ. Ovary ovoid, near middle of body. Vagina not observed. Vitellaria distributed from pharynx to peduncle.

Remarks: Our specimens of *L. michaelalicea* match the overall description of the monogenean provided by Leis et al. (2018). *Ligictaluridus michaelalicea* is easily distinguished from other species of *Ligictaluridus* by possessing a unique combination of the male copulatory organ becoming bulbous distally and a uniramous accessory piece. We extend the distributional range of the parasite about 900 km south of Lansing, Iowa, into the southern Mississippi River, Arkansas, and about 600 km upstream from the Mississippi River into the Red River, Oklahoma. This species has thus far been found only on *P. olivaris*.

***Ligictaluridus mirabilis* (Mueller, 1937) Klassen and Beverley-Burton, 1985**

Hosts, localities, dates collected, prevalence and intensity, range, specimens deposited: *Ictalurus furcatus,* Lake Texoma in the vicinity of Willis Bridge, Red River Drainage, Marshall County, Oklahoma (33°52'31.9224"N; 96°50'01.2804"W) collected 21 February 2017; 1 of 1 [host destroyed during necropsy; 850 mm TL] (100%, 6 worms) (HWML 139475, 2 slides). *Ictalurus punctatus*, Lake Texoma in the vicinity of Willis Bridge, Red River Drainage, Marshall County, Oklahoma (33°52'31.9224"N; 96°50'01.2804"W), collected 21 February 2017; 2 of 2 [hosts destroyed during necropsy; 310-375 mm TL] (100%, 4 and 72 worms) (HWML 139862, 3 slides). *Ictalurus punctatus*, 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), collected 13 March 2017; 1 of 1 [adult host destroyed during necropsy; not measured] (100%, 12 worms) (HWML 139863, 1 slide). *Pylodictis olivaris*, Lake Texoma in the vicinity of Willis Bridge, Red River Drainage, Marshall County, Oklahoma (33°52'31.9224"N; 96°50'01.2804"W), collected 21 February 2017; 1 of 1 [host destroyed during necropsy; 610 mm TL] (100%, 40 worms) (HWML 139473, 5 slides). *Pylodictis olivaris*, South Concho River at Christoval, Colorado River Drainage, Tom Green County, Texas (31°11'16.386"N; 100°30'0.0792"W), collected 23 July 2018; 1 of 1 [HSU photovoucher; 137 mm TL] (100%, 8 worms**)** (HWML 139859, 2 slides).

Remarks: *Ligictaluridus mirabilis* has been reported mostly on species of *Ictalurus* and *P. olivaris* (Klassen and Beverley-Burton 1985;

Hoffman 1999; Leis et al. 2018); its type host is *P. olivaris* (Mueller 1937). *Ligictaluridus mirabilis* was found in mixed infections with *L. michaelalicia and L. pricei*. This is the first confirmed report of *L. mirabilis* from Oklahoma and Texas, although previous reports of *L. floridanus* from these states are likely based on *L. mirabilis* (see Discussion).

***Ligictaluridus pricei* (Mueller, 1936) Klassen and Beverley-Burton, 1985**

Hosts, localities, dates collected, prevalence and intensity, specimens deposited: *Ictalurus furcatus,* Lake Texoma in the vicinity of Willis Bridge, Red River Drainage, Marshall County, Oklahoma (33°52'31.9224"N; 96°50'01.2804"W), collected 21 February 2017; 1 of 1 [host destroyed during necropsy; 850 mm TL] (100%, 3 worms) (HWML 139476, 2 slides). *Ictalurus punctatus*, Lake Texoma in the vicinity of Willis Bridge, Red River Drainage, Marshall County, Oklahoma (33°52'31.9224"N; 96°50'01.2804"W), collected 21 February 2017; 2 of 2 [hosts destroyed during necropsy; 310, 375 mm TL] (100%, 3 and 24 worms) (HWML 139862, 4 slides). *Ictalurus punctatus*, 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), collected 13 March 2017; 1 of 1 [host destroyed during necropsy; not measured] (100%, 5 worms) (HWML 139864, 1 slide). *Pylodictis olivaris*, Mississippi River at Sans Souci Landing, Mississippi River Drainage, Mississippi County, Arkansas (35°39'21.387"N; 89°55'33.4956"W), collected 16 October 2015; 1 of 2 [HSU photovoucher; 118–125 mm TL] (50%, 1 worm**)** (HWML 139474, 1 slide). *Pylodictis olivaris*, South Concho River at Christoval, Colorado River Drainage, Tom Green County, Texas (31°11'16.386"N; 100°30'0.0792"W), collected 23 July 2018; 1 of 1 [HSU photovoucher; 137 mm TL] (100%, 2 worms**)** (HWML 139860, 1 slide).

Remarks: *Ligictaluridus pricei* has been found on a wide variety of ictalurid catfishes, including *P. olivaris*, bullheads (*Ameiurus* spp.), madtoms (*Noturus* spp.), and *Ictalurus* spp., over a wide geographical range including Arkansas, Oklahoma, and Texas, and thus appears to be the least host specific species of *Ligictaluridus* (Klassen and Beverley-Burton 1985; Hoffman 1999; Leis et al. 2018). *Ligictaluridus pricei* was found in mixed infections with *L. michaelalicea* and *L. mirabilis*.

Discussion

In a cladistic analysis of *Ligictaluridus*, Klassen and Beverley-Burton (1987) hypothesized that *L. monticelli* (Cognetti de Martiis, 1924) and *L. pricei* are in a clade characterized by a slender, curved male copulatory organ. Because they share these characteristics, we hypothesize that *L. michaelalicea* and *L. bychowskyi*, which were not included in the analysis by Klassen and Beverley-Burton (1987), are also members of this clade, and these four species form a monophyletic group. We consider the bulbous distal terminus of the male copulatory organ of *L. michaelalicea* to be an autapomorphy derived within this clade. Because they share an elongate basal process directed opposite of the shaft of the male copulatory organ and a membrane running the length of the single-rod accessory piece (Price and Mura 1969; Fig. 3, present study), we hypothesize that *L. bychowskyi* and *L. michaelalicea* are sister species. Because *L. monticelli* and *L. pricei* both possess an accessory piece comprising two rod-like parts and an accompanying membrane (Cognetti de Martiis 1924; Klassen and Beverley-Burton 1985), we hypothesize that they are sister species. Leis et al. (2018) reported a segment of the 18S rRNA gene of *L. michaelalicea* was significantly similar (94%) to that of *L. pricei*, but cladistic analysis comparing data based on morphology and rRNA sequences cannot be performed because rRNA data are lacking for other species of *Ligictaluridus*.

We agree with Klassen and Beverley-Burton's (1987) analysis that *L. posthon* Klassen, Beverley-Burton, and Dechtier, 1985, possessing a male copulatory organ with an apomorphic enlarged diameter but lacking distal flairing, is in a clade by itself, and that *L. floridanus* and *L. mirabilis*, possessing an

enlarged diameter and distal flairing of the male copulatory organ, form another more recently derived monophyletic clade.

Species of *Ligictaluridus* are the only monogeneans that typically parasitize the gills of ictalurid catfishes (Klassen and Beverley-Burton 1985; Hoffman 1999). Some species, i.e., *L. bychowskyi*, *L. michaelalicea*, *L. monticelli*, and *L. posthon* have been found on only one host, whereas *L. floridanus*, *L. mirabilis*, and *L. pricei* have been found on several species (Klassen and Beverley-Burton 1985; Hoffman 1999; present study). Because of confusion over the identity of *L. floridanus* and *L. mirabilis* previous to the revisionary study by Klassen and Beverley-Burton (1985), further research is needed to clarify the host, prevalence and geographic distribution of these two species. Although reported several times from the Mississippi River (Hoffman 1999) previous to Klassen and Beverley-Burton (1985), *L. floridanus* has not been found in the drainage since (Leis et al. 2018; present study). We suspect that the natural range of *L. floridanus* is limited to east of the Mississippi drainage, as it was described from Florida (Mueller 1936) but it appears to have been introduced into northeastern México with *I. punctatus* (Rábago-Castro et al. 2014).

Further knowledge of parasites, including monogeneans, may be important in management of catfishes, especially under crowded culture conditions. For example, Rábago-Castro et al. (2014) reported that chronic exposure to *L. floridanus* reduced the mean weight of farmed Channel Catfish. In addition, mortality of *I. punctatus* due to heavy infections of *L. pricei* has been reported in culture ponds (Allison and Rogers 1970) and seems plausible under similar conditions with other species.

Acknowledgments

Scientific Collecting Permits were issued to CTM by the Arkansas Game and Fish Commission, Oklahoma Department of Wildlife Conservation (ODWC), and Texas Parks and Wildlife Department. We thank the following for assistance in collecting: Matt Mauck, David Routledge, and Cliff Sager (ODWC) at Lake Texoma; Tony Rodger, Matt Skoog, Trevor Starks, and Jon West (ODWC) on the Verdigris River; Robert Hrabik, David Ostendorf, and Dave Herzog (Missouri Department of Conservation), Uland Thomas (Chicago, IL), and Dr. David Neely (Tennessee Aquarium, Chattanooga, TN) on the Mississippi River, and Nikolas McAllister (Lukfata Elementary, Broken Bow, OK) on the South Concho River. We also thank Drs. Scott Gardner and Gabor Racz (HWML) and Renn Tumlison (HSU) for expert curatorial assistance.

References

Allison R, Rogers WA. 1970. Monogenetic trematodes of some Alabama freshwater fishes with descriptions of four new species and redescriptions of two species. Proc Helminthol Soc Wash 37:17–23.

Beverley-Burton M. 1984. Monogenea and Turbellaria. In: Margolis L, Kabata Z, editors. Guide to the parasites of fishes of Canada. Part I. Canadian Special Publication of Fisheries and Aquatic Sciences 74, Ottawa: Department of Fisheries and Oceans. 209 p.

Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583.

Cognetti de Martiis L. 1924. Nuovo Gyrodactylide parassita nella cavita olfattiva di *Amiurus catus* L. Boll Della Soc dei Nat Nap 36:76–81.

Hoffman GL. 1999. Parasites of North American freshwater fishes. Second edition. Ithaca (NY): Comstock Publishing Associates. 539 p.

Irwin ER, Hubert WA, Rabeni CF, Schramm HL Jr, Coon T (editors). 1999. Catfish 2000: Proceedings of the International Ictalurid Symposium. Amer Fish Soc Symp 24:1–516.

Jackson DC. 1999. Flathead Catfish: Biology, fisheries, and management. Amer Fish Soc Symp 24:23–35.

Klassen GJ, Beverley-Burton M. 1985. *Ligictaluridus* Beverley-Burton, 1984 (Monogenea: Ancyrocephalidae) from catfishes (Siluriformes: Ictaluridae) in North America with redescriptions of the type species, *Ligictaluridus pricei* (Mueller, 1936), and three others. Can J Zool 63:715–727.

Klassen GJ, Beverley-Burton M. 1987. Phylogenetic relationships of *Ligictaluridus* spp. (Monogenea: Ancyrocephalidae) and their ictalurid (Siluriformes) hosts: An hypothesis. Proc Helminthol Soc Wash 54: 84–90.

Klassen GJ, Beverley-Burton M, Dechtiar AO. 1985. *Ligictaluridus posthon* n. sp. (Monogenea: Ancyrocephalidae) from *Noturus flavus* Rafinesque (Siluriformes: Ictaluridae) in Ontario, Canada. Can J Zool 63:2071–2073.

Kritsky DC, Leiby PD, Kayton RJ. 1978. A rapid stain technique for the haptoral bars of *Gyrodactylus* species (Monogenea). J Parasitol 64:172–174.

Leis EM, Easy R, MacLean L, Cone D. 2018. *Ligictaluridus michaelalicea* n. sp. (Monogenea: Dactylogyridae) from Flathead Catfish (*Pylodictis olivaris*) in the upper Mississippi River, including remarks on taxonomy influencing monogenean treatment regulation in the United States. Parasitol Res 117:825–830.

McAllister CT, Font WF, Connior MB, Robison HW, Fayton TJ, Stokes NG, Criscione CD. 2015. Trematode parasites (Digenea) of the Slender Madtom *Noturus exilis* and Black River Madtom *Noturus maydeni* (Siluriformes: Ictraluridae) from Arkansas, U.S.A. Comp Parasitol 82:137–143.

Michaletz PH, Travnichek VH (editors). 2011. Conservation ecology, and management of catfish: The Second International Symposium. Amer Fish Soc Symp 77:1–780.

Mizelle JD. 1936. New species of trematodes from the gills of Illinois fishes. Amer Midl Nat 17:785–806.

Mueller JF. 1936. New gyrodactyloid trematodes from North American fishes. Trans Amer Micros Soc 55:457–464.

Mueller JF. 1937. Further studies on North American Gyrodactyloidea. Amer Midl Nat 18:207–219.

Page LM, Burr BM. 2011. Peterson field guide to freshwater fishes of North America north of Mexico. Second ed. Boston (MA): Houghton Mifflin Harcourt. 663 p.

Price CE, Mura A. 1969. The proposed synonymy of the monogenean genera *Cleidodiscus* Mueller, 1934 and *Urocleidus* Mueller, 1934 with the proposal of *Cleidodiscus bychowskyi* sp. n. Proc Helminthol Soc Wash 36:52–55.

Rábago-Castro JL, Genaro Sánchez-Martínez J, Pérez-Castañeda R, de la Luz Vázquez-Sauceda M, Ruiz-Orozco G. 2014. Chronic effects of a monogenean *Ligictaluridus floridanus* (Ancyrocephalidae) infection on Channel Catfish (*Ictalurus punctatus*) growth performance. Acta Vet Brno 83:83–87.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. Bethesda (MD): American Fisheries Society. Available from: https://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf (Accessed September 30, 2018).

Submitted October 8, 2018 Accepted December 3, 2018

Largemouth Bass Population Characteristics in a Densely Vegetated Small Impoundment

Michael J. Porta
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Richard A. Snow
Oklahoma Department of Wildlife Conservation, Oklahoma Fishery Research Laboratory, Norman, OK 73072

Clayton P. Porter
Oklahoma Department of Wildlife Conservation, J. A. Manning Hatchery, Lawton, OK 73507

Abstract: Elmer Thomas Reservoir is a small impoundment in southwest Oklahoma that contains a high abundance of the invasive plant Eurasian Milfoil. Because dense vegetation can negatively affect Largemouth Bass, population characteristics (condition, growth, and mortality) of Elmer Thomas Reservoir Largemouth Bass were described. Dense vegetation can impact Largemouth Bass foraging and may reduce angler success, so diet and presence of angler hooking wounds (index of angler catchability) were evaluated on a subset of Largemouth Bass. Condition (mean relative weight) of Largemouth Bass was below average (W_r = 88), which may explain the size distribution skewed towards smaller fish (most Largemouth Bass between 275-400 mm TL). Catch-curve analysis indicates that survival of Largemouth Bass is high (77%). Although high survival estimates suggest that angler exploitation (fishing mortality) is low, it appears that angler catchability is still high (35% of Largemouth Bass had hooking injuries; 45% when corrected for imperfect hooking wound detection). Largemouth Bass diets consisted mainly of fish (76% by number), more specifically age-0 Largemouth Bass comprised 28% by number and occurred in 20% of the diets. High incidence of age-0 Largemouth Bass in diets suggests a change in behavior as a result of dense vegetation and may help to explain the poor condition and growth of Largemouth Bass. This study suggests that Largemouth Bass are negatively affected by vegetation abundance at Elmer Thomas Reservoir. A vegetation management plan to reduce the vegetation to intermediate abundance levels (~30%) would likely benefit the Elmer Thomas Reservoir fish community and increase shoreline fishing opportunities for anglers.

Introduction

Largemouth Bass *Micropterus salmoides* are one of the most important recreational species in North America (USFWS 2016). Largemouth Bass are also ecologically important, as they are a keystone species in most aquatic systems and shape fish communities through top-down predation (Miranda and Pugh 1997). For these reasons, Largemouth Bass are intensively managed by many natural resource agencies. Therefore, fishery managers rely on consistent natural recruitment to maintain robust Largemouth Bass populations.

First year growth is an important factor affecting recruitment of age-0 Largemouth Bass to adulthood (Goodgame and Miranda 1993; Ludsin and DeVries 1997). Largemouth Bass that grow to larger sizes during their first year often have a competitive advantage over smaller fish of the same cohort (Goodgame

and Miranda 1993; Ludsin and DeVries 1997). Larger juvenile fish have an advantage over smaller fish because they can transition to piscivory sooner (Ludsin and DeVries 1997, Mesing et al. 2008) and may avoid predation, thereby reducing mortality rates (Santucci and Wahl 1993). Juvenile Largemouth Bass that attain a greater size prior to their first winter (an important survival bottleneck in their early life history), typically have increased recruitment to age-1 (Ludsin and DeVries 1997).

Survival of Largemouth Bass has been associated with increased habitat complexity (Miranda and Pugh 1997). Increased habitat complexity provides cover to juvenile fish, aiding in escapement from predation while providing foraging areas (due to increased invertebrate abundance associated with habitat) for young bass or sunfish *Lepomis* sp. prey. However, excessive habitat complexity can negatively impact Largemouth Bass fisheries (Bettoli et al. 1992). When aquatic vegetation becomes too dense, juvenile Largemouth Bass do not transition to piscivory, resulting in depressed growth (Bettoli et al. 1992). Also, competition with abundant sunfish for forage may increase in heavily vegetated systems, further reducing growth rates of juvenile Largemouth Bass (Miranda and Pugh 1997). Stymied growth rates caused by overabundant vegetation can ultimately affect Largemouth Bass recruitment to adulthood.

Adult Largemouth Bass are also impacted by dense aquatic vegetation. In systems with dense beds of aquatic vegetation, Largemouth Bass can become stunted (Brown and Maceina 2002). Although prey abundances are usually high in heavily vegetated systems, foraging efficiency of adult Largemouth Bass is affected because forage fish have ample refuge resulting in decreased foraging rates (Savino and Stein 1982, Savino and Stein 1989). Further, stockpiling of adult fish occurs because predator-induced mortality of small fish is low (Savino and Stein 1982). Increased population density results in competition for forage resources that are mostly inaccessible due to the high vegetation densities.

Because high densities of aquatic vegetation negatively impact fish populations, our primary objective is to evaluate population characteristics of Largemouth Bass in Elmer Thomas Reservoir, Oklahoma, a small impoundment with a high abundance of Eurasian Milfoil *Myriophyllum spicatum*. Further, because dense vegetation can negatively affect angling (reduced catch rates), we inspected all adult Largemouth Bass for presence of hooking scars to attain a basic understanding of catchability of Largemouth Bass in this system. Finally, although it only represents a brief temporal span, diet of a subset of Largemouth Bass will be presented as foraging can be affected in heavily vegetated environments.

Methods

Study area

Elmer Thomas Reservoir (Figure 1), is a 135-ha reservoir located in southwest Oklahoma on the Wichita Mountains National Wildlife Refuge near Lawton, Oklahoma. The surrounding land area is composed of a cobblestone/granite substrate with little runoff influence from intermittent, ephemeral drainages resulting in minimal amounts of organic inflow to the watershed, hence the mesotrophic status of this system (OWRB 2016). The littoral portion of the lake is covered with dense beds of Eurasian Milfoil and intermittent pond weed *Potamogeton* spp. in water depths ranging from 0.3-4.6 m. Eurasian Milfoil became established in Elmer Thomas Reservoir prior to 1965 (Couch and Mace 1978). The coverage area of Elmer Thomas Reservoir by Eurasian Milfoil is 44.4% (vegetated area [60 ha]/total reservoir surface area [135 ha] x 100).

Study approach

Largemouth Bass were collected from Elmer Thomas Reservoir in April and August 2018 using boat electrofishing (pulsed DC, high voltage, Smith Root 7.5 GPP). The majority (~80%) of the littoral portion of the reservoir was surveyed between both seasonal sampling efforts to ensure that all size and age classes were represented in the sample. Our goal was to collect ten fish per 25 mm length bins to

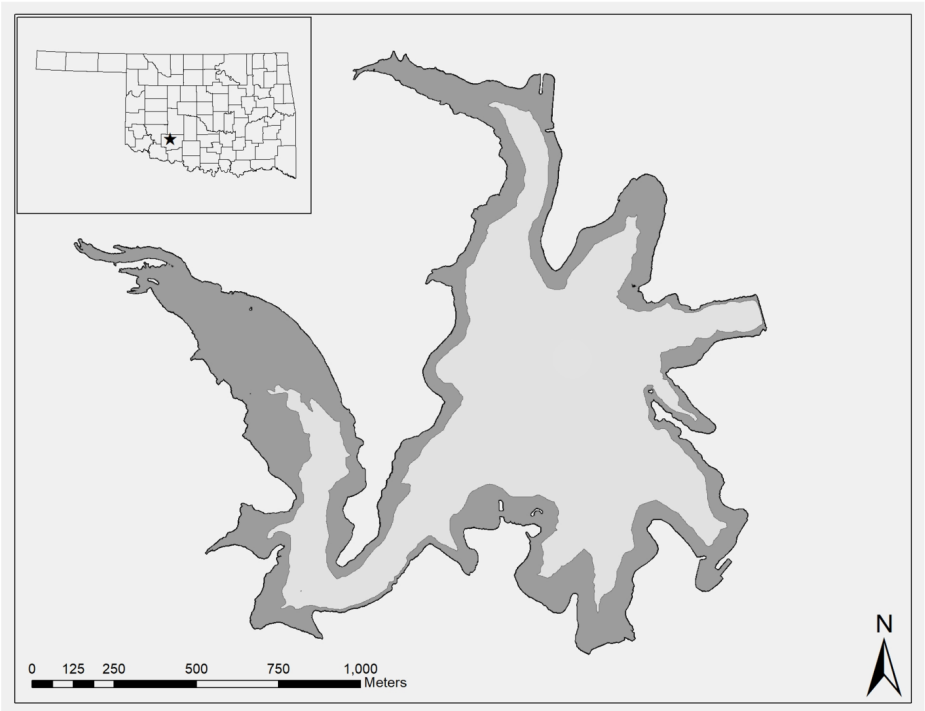


Figure 1. Map of Elmer Thomas Reservoir in Comanche County, Oklahoma. The dark gray area represents the extent of Eurasian Milfoil coverage.

ensure all ages were represented in the sample. Fish were measured for total length (TL; mm) and placed on ice to be processed at the J.A. Manning State Fish Hatchery (April) or the Oklahoma Fishery Research Laboratory in Norman, Oklahoma (August). In the laboratory, each Largemouth Bass was measured for TL, weighed (g) and sagittal otoliths removed for aging purposes.

Once otoliths were removed from fish collected during August, fish were then dissected to remove stomach contents from each fish. Diet items present in each stomach were identified to the finest taxonomic level possible, and enumerated. Largemouth Bass diets were described using percent occurrence, percent composition, and percent weight of prey items (Bowen 1996). Following dissection, fish were also inspected for presence of hooking scars or injuries, and any angling related damage was recorded, which was used to calculate angling hooking rate. Because the detection rate of known hook and line caught Largemouth Bass is imperfect (84%), the hooking rate of Largemouth Bass collected from Elmer Thomas Reservoir was corrected using a 1.19 multiplicative correction factor (Fernholz et al. 2018).

Otoliths were broken in the transverse plane by breaking it through the nucleus and polished with 2,000 grit wet/dry sandpaper. The broken otoliths were stood polished-side up in a black, clay filled dish, submersed in water, and viewed with a fiber optic light source under a dissecting microscope. Otoliths were viewed in random order by two independent readers and an age was estimated for each fish. When both readers disagreed on an estimated age, the otolith was reexamined by both readers to determine a final consensus age.

Size structure for Largemouth Bass was described using a length-frequency histogram and proportional size distribution (PSD, stock ≥ 200 mm, quality ≥ 300 mm, preferred ≥ 380 mm, memorable ≥ 510 mm; Gabelhouse 1984). Condition of Largemouth Bass was evaluated by calculating relative weight (W_r) using standard weight equations (W_s = -5.316

+ 3.191 × log10 TL) presented by Wege and Anderson (1978). Largemouth Bass growth was described with a von Bertalanffy growth model. Catch curves (Ricker 1975) were used to estimate instantaneous total mortality (*Z*) of Largemouth Bass. Total annual mortality (A) was then calculated as $1\text{-}e^{-z}$. Largemouth Bass < 2 years old were omitted from the catch-curve analysis as they are not fully recruited to the sampling gear. Further, any age groups having < 5 individuals were not included in the analyses (Miranda and Bettoli 2007).

Results and Discussion

A total of 191 Largemouth Bass were collected during the April (N=89) and August (N=102) sampling events to describe population characteristics at Elmer Thomas Reservoir. Largemouth Bass size ranged from 63 to 573 mm TL (Figure 2) and fish ages 0-13 years old were present in the sample. Because age-0 Largemouth Bass were not well represented in these samples, a separate collection of age-0 Largemouth Bass was conducted in late August to describe size structure of young-of-the-year fish. A total of 217 age-0 Largemouth Bass were collected in late August that ranged from 44 to 140 mm TL (mean TL = 63.4 mm; Figure 3).

Vegetation density appears to be affecting age-0 fish growth in Elmer Thomas Reservoir. Mean TL of age-0 Largemouth Bass captured during August at Elmer Thomas Reservoir was 63 mm, compared to a mean TL of 85 mm observed for other Oklahoma populations in August (Carlander 1977). Similarly, dense vegetation impacted feeding efficiency and first year growth of Largemouth Bass in Lake Conroe, Texas (Betolli et al. 1992), which can negatively affect first year survival (Ludsin and DeVries 1997). Although dense vegetation appears to be limiting growth of age-0 Largemouth Bass in Elmer Thomas Reservoir, this complex habitat can also promote age-0 fish survival through decreased predation and increased invertebrate forage, which may result in increased recruitment to age-1 (Savino and Stein 1982, Miranda and Pugh 1997).

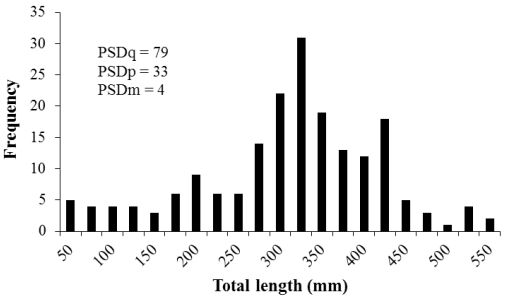


Figure 2. Length frequency distribution of Largemouth Bass collected from Elmer Thomas Reservoir during April and August 2018.

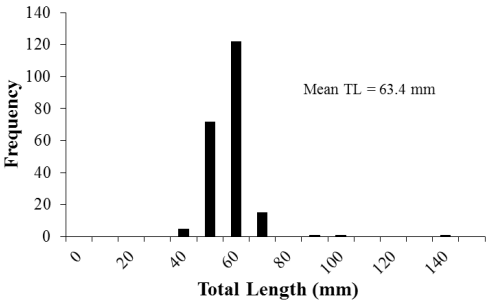


Figure 3. Length frequency distribution of age-0 Largemouth Bass collected from Elmer Thomas Reservoir during August 2018.

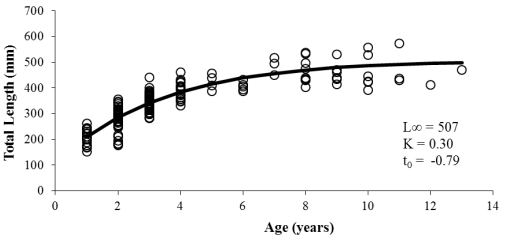


Figure 4. Von Bertalanffy growth curve for Largemouth Bass collected from Elmer Thomas Reservoir during April and August 2018.

Largemouth Bass that reach adulthood in Elmer Thomas Reservoir appear to stockpile between quality (≥ 300 mm) and preferred (≥ 380 mm) size classes, based on the high PSD (PSDq = 79). The length distribution for Largemouth Bass from Elmer Thomas Reservoir mirror the size structure of Largemouth Bass from the Spring Creek arm of Lake Seminole, Georgia

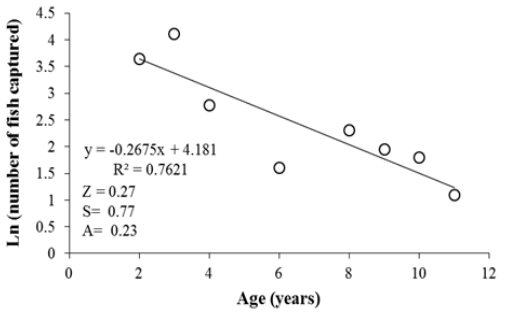


Figure 5. Catch-curve regression and total annual mortality (*A*) calculated from otolith age estimates for Largemouth Bass collected from Elmer Thomas Reservoir during April and August 2018.

where dense vegetation coverage has resulted in a length distribution skewed towards smaller fish (Brown and Maceina 2002). Largemouth Bass in the quality and preferred size classes range from ages 2-4, and the minimum length limit (355 mm TL) is reached in 3.43 years on average based on growth rates of this population (Figure 4). Condition of adult Largemouth Bass was well below average for fish collected from Elmer Thomas Reservoir (mean W_r = 88 in April and August). Similarly, Colle and Shireman (1980) documented lower Largemouth Bass condition in Florida lakes when vegetation coverage exceeded 40%. Decreased foraging success caused by high density of Eurasian Milfoil likely resulted in decreased growth and condition of the Elmer Thomas Reservoir Largemouth Bass.

Diet of adult Largemouth Bass from Elmer Thomas Reservoir may provide some insight to the overall poor condition of this population. Largemouth Bass diets were dominated by fish prey (Table 1), with age-0 Largemouth Bass dominating the diets by number (27.8%) and occurrence (19.6%). Summers (1980) also documented a high occurrence of age-0 Largemouth Bass in the August diets of adult Largemouth Bass in a densely vegetated small impoundment in Oklahoma. In that study, both adult and juvenile Largemouth Bass were observed on the outside edge of the vegetation, making the juvenile fish easy prey for the adults (Summers 1980). This observation may have resulted from a switch to an ambush feeding behavior to cope with high vegetation densities, which results in decreased foraging success (Savino and Stein 1982).

Catch-curve analysis indicated high survival (77%) of adult Largemouth Bass in Elmer Thomas Reservoir (Figure 5). The survival estimate of 77% at Elmer Thomas Reservoir was intermediate to survival estimates of 84% in the

Table 1. Diet of Largemouth Bass (N = 102) collected from Elmer Thomas Reservoir during August 2018 described using percent composition by number (%N_i), percent weight (%W_i), and percent occurrence (%O_i).

Diet Item	%N_i	%W_i	%O_i
Fish			
Bluegill *Lepomis macrochirus*	16.7	35.4	11.8
Channel Catfish *Ictalurus punctatus*	1.4	2.0	1.0
Green Sunfish *Lepomis cyanellus*	1.4	3.1	1.0
Largemouth Bass *Micropterus salmoides*	27.8	22.1	19.6
Sunfish *Lepomis sp.*	5.6	3.6	3.9
Unidentified Fish	23.6	8.3	16.7
Invertebrate			
Crayfish	22.2	18.8	15.7
Other			
Fishing Lure	1.4	6.7	1.0
Empty			50.0

Spring Creek arm (76% vegetation coverage) and 72% in the Flint-Chattahoochee arms (26-32% vegetation coverage) of Lake Seminole, Georgia (Brown and Maceina 2002). Higher Largemouth Bass survival rates are attributed to poor angler accessibility caused by dense vegetation coverage (Maceina and Reeves 1996, Brown and Maceina 2002). Higher survival may also result from a reduction in angler effort towards Largemouth Bass in response to increases in vegetation coverage (Slipke et al. 1998). Although vegetation coverage at Elmer Thomas Reservoir was high (44%), catchability of Largemouth Bass appears high (38%; 37 of 98 fish) based on the number of hooking scars present on fish ≥ 200 mm. When corrected for imperfect detection, 45% of Largemouth Bass inspected had hooking wounds. Furthermore, fishing lures were also found in diets of Largemouth Bass, suggesting that anglers are encountering Largemouth Bass at Elmer Thomas Reservoir.

These results suggest that the population characteristics of Largemouth Bass are negatively affected by vegetation abundance at Elmer Thomas Reservoir. A reduction in vegetation abundance could benefit Largemouth Bass by improving foraging efficiency that may improve condition and growth rates of this population. Further, a reduction in aquatic vegetation abundance would also improve shoreline fishing opportunities at Elmer Thomas Reservoir. Vegetation management using biological control (Grass Carp *Ctenopharyngodon idella*), herbicide application, or mechanical harvest should target an intermediate (~30%) vegetation coverage as a management goal. Largemouth Bass collections to evaluate changes in population characteristics should coincide with any efforts to manage vegetation densities at Elmer Thomas Reservoir.

Acknowledgments

The authors thank John Perry, Ryan Ryswyk, Matthew Lyons, Micah Waters, and Shelby Jeter for assistance with field collections and laboratory work. We thank Jeff Tibbits for map creation and calculation of vegetation coverage. We thank Kurt Kuklinski for reviewing an earlier draft of this manuscript. Financial support for this publication was provided by the Sport Fish Restoration Program grant [F-66-D-8] to the Oklahoma Department of Wildlife Conservation.

References

Betolli, P.W, M.J. Maceina, R.L. Noble, and R.K. Betsill. 1992. Piscivory in largemouth bass as a function of aquatic vegetation abundance. North American Journal of Fisheries Management 12:509-516.

Bowen, S.H. 1996. Quantitative description of the diet. Pages 513-529 *in* B.R. Murphy and D.W. Willis, editors. Fisheries Techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Brown, S.J., and M.J. Maceina. 2002. The influence of disparate levels of submersed aquatic vegetation on largemouth bass population characteristics in a Georgia reservoir. Journal of Aquatic Plant Management 40:28-35.

Carlander, K.D. 1977. Handbook of freshwater fishery biology; Volume 2. Iowa State University Press, Ames, Iowa. 431 p.

Colle, D.E. and J.V. Shireman. 1980. Coefficients of condition for largemouth bass, bluegill, and redear sunfish in hydrilla-infested lakes. Transactions of the American Fisheries Society 109:521-531.

Couch, R. and P. Mace. 1978. Eurasian water milfoil in Oklahoma. Proceedings of the Oklahoma Academy of Science 58:4-5.

Fernholz, S., A. Sylvia, and M.J. Weber. 2018. Hook wound longevity and use as an indicator of largemouth bass catch-and-release angling pressure. North American Journal of Fisheries Management 38:759-768.

Gabelhouse, D.W. Jr. 1984. A length-categorization system to assess fish stocks. North American Journal of Fisheries Management 4:273-285.

Goodgame, L.S. and L.E. Miranda. 1993. Early growth and survival of age-0 largemouth bass in relation to parental size and swim-up time. Transactions of the American Fisheries Society 122:131-138.

Ludsin, S. A., and D. R. DeVries. 1997. First-year recruitment of largemouth bass: the interdependency of early life stages. Ecological Applications 7:1024–1038.

Maceina, M.J. and W.C. Reeves. 1996. Relations between submersed macrophyte abundance and largemouth bass tournament success on two Tennessee River impoundments. Journal of Aquatic Plant Management 34:33-38.

Mesing, C. L., R. L. Cailteux, P. A. Strickland, E. A. Long, and M. W. Rogers. 2008. Stocking of advanced fingerling largemouth bass to supplement year-classes in Lake Talquin, Florida. North American Journal of Fisheries Management 28:1762–1774.

Miranda, L.E., and L.L. Pugh. 1997. Relationship between vegetation coverage and abundance, size, and diet of juvenile largemouth bass during winter. North American Journal of Fisheries Management 17:601-610.

Miranda, L.E. and P.W. Bettoli. 2007. Mortality. Pages 229-277 *in* C.S. Guy and M. L. Brown, editors. Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda, MD.

Oklahoma Water Resources Board Beneficial Use Monitoring Program Report. 2016. http://www.owrb.ok.gov/quality/monitoring/bump/pdf_bump/Lakes/ElmerThomas.pdf . Accessed September 2018.

Ricker, W.E. 1975. Computation and interpretation of biological statistics in fish populations. Fisheries Research Board of Canada, Bulletin 191.

Santucci, V. J. and D. H. Wahl. 1993. Factors influencing survival and growth of stocked walleye (*Stizostedion vitreum*) in a centrarchid-dominated impoundment. Canadian Journal of Fisheries and Aquatic Sciences 50:1548-1558.

Savino, J.F. and R.A. Stein. 1982. Predator-prey interaction between largemouth bass and bluegills as influenced by simulated, submersed vegetation. Transactions of the American Fisheries Society 111:255-266.

Savino, J.F. and R.A. Stein. 1989. Behavioural interactions between fish predators and their prey: effects of plant density. Animal Behavior 37:211-321.

Slipke, J.W., M.J. Maceina, and J.M. Grizzle. 1998. Analysis of the recreational fishery and angler attitudes toward hydrilla in Lake Seminole, a southeastern reservoir. Journal of Aquatic Plant Management 36:101-107.

Summers, G.L. 1980. Food of adult largemouth bass in a small impoundment with dense aquatic vegetation. Proceeding of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies 34:130-136.

U.S. Department of the Interior, U.S. Fish and Wildlife Service, and U.S. Department of Commerce, U.S. Census Bureau. 2016 National Survey of Fishing, Hunting, and Wildlife-Associated Recreation.

Wege, G.J., and R.O. Anderson. 1978. Relative weight (W_r): a new index of condition for largemouth bass. Pages 79-91 *in* G.D. Novinger and J.G. Dillard, editors. New approaches to the management of small impoundments. American Fisheries Society, North Central Division, Special Publication 5, Bethesda, Maryland.

Submitted October 9, 2018 Accepted November 20, 2018

Three New True Bug (Hemiptera: Miridae) Records for Oklahoma

Stephen W. Chordas, III
Center for Life Sciences Education, The Ohio State University, Columbus, OH 43210

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Over the past few years, several new geographic records for hemipterans have been documented for Oklahoma (Henry et al. 2010; Chordas and McAllister 2012, 2016; Chordas et al. 2017; McAllister and Robison 2017) as well as description of new taxa (Henry and Sweet 2015). Here, we provide new distributional records for three true bugs of the family Miridae in the state.

Between May and October 2018, various hemipterans were collected below a night light at a residence in Hochatown, McCurtain County. Specimens were collected with an insect aspirator and transferred to individual vials containing 70% (v/v) ethanol. They were subsequently shipped to the senior author (SWC) for identification. Voucher specimens were deposited in the C. A. Triplehorn Collection at The Ohio State University, Columbus, Ohio. The following specimens were identified.

Three male *Phytocoris breviusculus* Reuter, 1876 were taken with the following collection data: ***Oklahoma***: McCurtain County, off Halibut Bay Road in Hochatown (34° 10' 17.0286"N, 94° 45' 5.7414"W); 11 V 2018; C. T. McAllister (CTM), collector (unique museum specimen code: OSUC 620944). Photographs of this bug are available on BugGuide (https://bugguide.net/node/view/309222). Habitat of the area consisted of various hardwoods (*Quercus* spp.) and pines (*Pinus* spp.) in Ouachita uplands. In addition, a single male *Tropidosteptes cardinalis* Uhler, 1878 (Fig. 1) was collected by CTM from the same site (on 7 V 2018, OSUC 620945) and a *Pilophorus crassipes* Heidemann, 1892 was collected by CTM there on 1 IX 2018 (OSUC 620946). Photographs of *P. crassipes* are available on BugGuide (https://bugguide.net/node/view/290667).

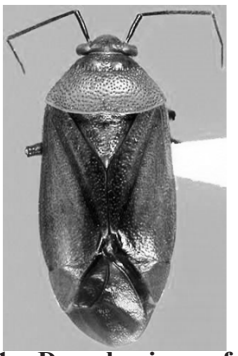


Figure 1. Dorsal view of *Tropidosteptes cardinalis*.

Phytocoris breviusculus is a relatively small (3.9–4.6 mm) mirid of the juniperanus group that possesses a wing membrane sprinkled with dark or minute pale spots. This bug is attracted to lights and can be also found on a wide variety of plants, but most commonly occurring on cedars (*Juniperus* spp.) (Stonedahl 1988) which are present at the study site. It has also been reported that *P. breviusculus* is at least partially predaceous on mites and scale insects (Henry et al. 2005). It has been recorded from the eastern US from Pennsylvania to Kansas and further south to Alabama, Mississippi, and Texas (Henry and Wheeler 1988); however, this mirid has not been previously documented from Oklahoma (Fig. 2A) so we report *P. breviusculus* here as a

Figures 2A–C. Three new bugs (Miridae) for Oklahoma. A. Distribution of *Phytocoris breviusculus* in North America north of México. Light shade = prior literature records (Henry and Wheeler 1988; Henry et al. 2005); dark shade = new state record. B. Distribution of *Tropidosteptes cardinalis* in North America north of México. Light shade = prior literature records (Henry and Wheeler 1988; Maw et al. 2000; Henry et al. 2005; Chordas et al. 2011); dark shade = new state record. C. Distribution of *Pilophorus crassipes* in North America north of México. Light shade = prior literature records (Henry and Wheeler 1988; Maw et al. 2000; Henry et al. 2005); dark shade = new state record.

new geographic record.

Tropidosteptes cardinalis is a bright reddish-colored mirid (see also color fig. 28 of this species in Chordas et al. [2011]). It is known from adjacent Arkansas (Chordas et al. 2011), Missouri, and Texas as well as other US states and eastern Canada (Fig. 2B) but this plant bug had not previously been documented in the refereed literature for Oklahoma.

Pilophorus crassipes is small (3 to 5 mm) brown and black colored plant bug with striking silvery bands on the hemelytra; a characteristic present in species of this genus. The hind tibia of this species is distinctly curved (Knight 1941). Records of this plant bug are concentrated around the Great Lakes region (Fig. 2C) and extend as far south as Louisiana but this species had not previously been reported in the refereed literature for Oklahoma.

Eleven other hemipterans within eight families were collected during this six-month period and all have been reported previously from Oklahoma including the following taxa: **Alydidae:** *Alydus pilosulus* Herrich-Schaeffer, 1847; *Megalostomus quinquespinosus* (Say, 1825); **Berytidae:** *Jalysus wickhami* (Van Duzee, 1906); **Coreidae:** *Acanthocephala declivis* (Say, 1823); **Miridae:** *Pseudatomoscelis seriatus* (Reuter, 1876); **Pentatomidae:** *Banasa euchlor*a Stål, 1872; *Thyanta custator* (Fabricius, 1803); **Reduviidae:** *Microtomus purcis* (Drury, 1782); **Rhyparochromidae:** *Ozophora picturata* Uhler, 1871; *Pseudopachybrachius basali*s (Dallas, 1852); **Scutelleridae:** *Stethaulax marmorata* (Say, 1831). Of these, only *A. pilosulus* is considered uncommon in the state.

Additional collections using various sampling techniques for hemipterans in Oklahoma 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 the state.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit to CTM. We thank Dr. Thomas J. Henry (USNM Smithsonian Institution, Washington, D.C.) for confirming the identity of these bugs.

References

Chordas III SW, McAllister CT. 2012. The southern pine seed bug, *Leptoglossus corculus* (Hemiptera: Coreidae): New for Oklahoma. Proc Okla Acad Sci 92:73–74.

Chordas III SW, McAllister CT. 2016. First report of the plant bug, *Collaria oculata* (Reuter, 1871) (Hemiptera: Miridae) from Oklahoma. Proc Okla Acad Sci 96:99–100.

Chordas III SW, Tumlison R. 2016. Four uncommon assassin bugs (Hemiptera: Reduviidae: Emesinae) new for Arkansas, U.S.A. Entomol News 126:77–82.

Chordas III SW, Tumlison R, McAllister CT. 2018. First report of the true bug *Pseudopachybranchius vinctus* (Hemiptera: Rhyparochromidae) from Arkansas and Oklahoma, U.S.A. Entomol News 127:269–272.

Chordas III SW, Tumlison R, Robison HW, Kremers J. 2011. Twenty-three true bug records for Arkansas, with two for Ohio, U.S.A. J Ark Acad Sci 65:153–159.

Henry TJ, Covell Jr CV, Wheeler Jr AG. 2005. An annotated list of the plant bugs, or Miridae (Hemiptera: Heteroptera), of Kentucky. J NY Entomol Soc 113:24–76.

Henry TJ, Hevel GF, Chordas S. 2010. Additional records of the little-known *Corixidea major* (Heteroptera: Schizopteridae) from Arkansas and Oklahoma. Proc Entomol Soc Wash 112:475–477.

Henry TJ, Sweet MH. 2015. *Wheelerodemus muhlenbergiae*, a new genus and new species of Blissidae (Hemiptera: Heteroptera: Lygaeoidea) from Oklahoma and Texas. Proc Entomol Soc Wash 117:151–161.

Henry TJ, Wheeler AG. 1988. Family Miridae, Hahn 1833, The Plant Bugs. In: Henry TJ and Froeschner RC, editors. Catalog of the Heteroptera, or true bugs, of Canada and the continental United States. New York (NY): E. J. Brill. p 251–507.

Knight HH. 1941. The plant bugs, or Miridae, of Illinois. Ill Nat Hist Surv Bull 22: 1–234.

Maw HEL, Foottit RG, Hamilton KGA, Scudder GGE. 2000. Checklist of the Hemiptera of Canada and Alaska. Ottawa (Ontario, Canada): NRC Research Press. 220 p.

McAllister CT, Robison HW. 2017. Two new state records for Hemiptera (Coreidae, Reduviidae) from Oklahoma. Proc Okla Acad Sci 97:47–49.

Stonedahl GM. 1988. Revision of the mirine genus *Phytocoris* Fallén (Heteroptera, Miridae) for western North America. Bull Amer Mus Nat Hist 188:1–264

Submitted October 10, 2018 Accepted December 8, 2018

Parasites (Cestoda, Nematoda, Mollusca) of Western Starhead Topminnow, *Fundulus blairae* (Cypriniformes: Fundulidae), from the Red River Drainage, Southwestern Arkansas

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Donald G. Cloutman
P. O. Box 197, Burdett, KS 67523

Stanley E. Trauth
Department of Biological Sciences, Arkansas State University (Emeritus), State University, AR 72467

Charles R. Bursey
Department of Biology, Pennsylvania State University, Shenango Valley Campus, Sharon, PA 16146

Henry W. Robison
9717 Wild Mountain Drive, Sherwood, AR 72120

Abstract: Between July 2016 and October 2017, and again during May and September 2018, 64 Western Starhead Topminnows, *Fundulus blairae*, were collected from a single site in the Red River drainage of southwestern Arkansas and examined for gill/fin ectoparasites and coelomic endoparasites. Twenty-nine (45%) were infected, including 8 (13%) with *Proteocephalus* sp. plerocercoids, 1 (2%) with a metacestode, *Valipora minuta*, 23 (36%) with encapsulated larval nematodes, *Spiroxys* sp., 1 (2%) with larval *Eustrongylides* sp., and 1 (2%) with an unknown glochidium. Four (7%) possessed a multiple infection of various parasites. No monogeneans or myxozoans were found on the gills of any fish nor were myxozoans found in the gallbladder. We document five new parasite host records in this contribution which represents only the second published report of any kind of parasite from this host.

Introduction

The Western Starhead Topminnow, *Fundulus blairae* Wiley and Hall, 1975 is a small fundulid that reaches a maximum total length of 80 mm and inhabits heavily-vegetated barrow ditches, oxbow lakes, sloughs, streams, and swampy backwaters (Wiley 1980; Ross 2001). The species is a surface-feeding insectivore (Miller and Robison 2004). Its range includes the Gulf Slope drainages from the Escambia River, Alabama and Florida, west to the Brazos River, Texas, and north into the Red River drainage of southwestern Arkansas and southeastern Oklahoma (Page and Burr 2011). In Arkansas, *F. blairae* has a very restricted distribution, is considered rare in the state (Robison 1977; Robison and Buchanan 1988), and listed as S2 (imperiled) by NatureServe (2018).

Little is known about the parasites of *F. blairae*, and Hoffman (1999) does not provide any taxa from this topminnow. A trematode, *Plagiocirrus loboides* Curran, Overstreet, and Tkach, 2007, has been described from *F. blairae* (as *F. dispar blairae*) from the Pascagoula River, George County, Mississippi (Curran et al. 2007). Here, after surveying 64 *F. blairae* from Arkansas, we document five new parasite host records.

Methods

Between July 2016 and October 2017, and again during May and September 2018, 64 juvenile and adult *F. blairae* (mean ± 1SD total length [TL] = 40.5 ± 7.8, range 32–66 mm) were collected from a small lake (backwater of the Rolling Fork River) in Sevier County and examined for parasites. Because these fish are elusive and difficult to capture, we used a 244 cm long-pole dipnet with a 41 long × 42 cm wide basket to collect specimens. The study site was typical of habitat for *F. blairae* as it was heavily vegetated with duckweed (*Lemna minor*), watermilfoil (*Myriophyllum*), and pondweed (*Potamogeton*). Topminnows were placed in containers with aerated water from their collection site and necropsied within 24 hr. We followed accepted guidelines for processing specimens (Use of Fishes in Research Committee 2014); they were overdosed with a concentrated tricaine methanesulfonate solution, and a mid–ventral incision was made to expose the gastrointestinal tract and internal viscera, which were placed in Petri dishes containing normal saline (0.9%). The stomach, intestinal tract, heart, liver, mesenteries, and urinary bladder were examined under a stereoscopic microscope at 20–30×. The gall bladder was scanned for myxozoans by placing whole tissue under coverslip pressure on a microscopic slide and examining by light microscopy. All 64 fish were preserved in 10% formalin, and their gills sets (left and right gills, 1–4) were examined for monogeneans and myxozoans under a stereomicroscope at 20–30×. Tissues suspected of being infected with helminths were fixed in 10% neutral buffered formalin and processed following standard histological methods (Presnell and Schreibman 1997) with sectioning at 8–10 µm followed by staining with hematoxylin and eosin. Voucher specimens (photovouchers or slides) of parasites were deposited in the Harold W. Manter Laboratory of Parasitology, University of Nebraska, Lincoln, Nebraska. Host voucher specimens were deposited in the Henderson State University Museum (HSU), Arkadelphia, Arkansas as HSU 3644.

Results and Discussion

Twenty-nine (45%) *F. blairae* were infected, 8 (13%) with *Proteocephalus* sp. plerocercoids, 1 (2%) with a *Valipora minuta* metacestode, 23 (36%) with encapsulated larval nematodes, *Spiroxys* sp., 1 (2%) with larval *Eustrongylides* sp., and 1 (2%) with an unknown glochidium; 4 (7%) possessed a multiple infection of various parasites. No myxozoans or monogeneans were found on the gills and no myxozoans were found in the gallbladder. Specific information on each parasite follows.

Cestoda: Proteocephalidea: Proteocephalidae *Proteocephalus* sp. (Figs. 1A–C)

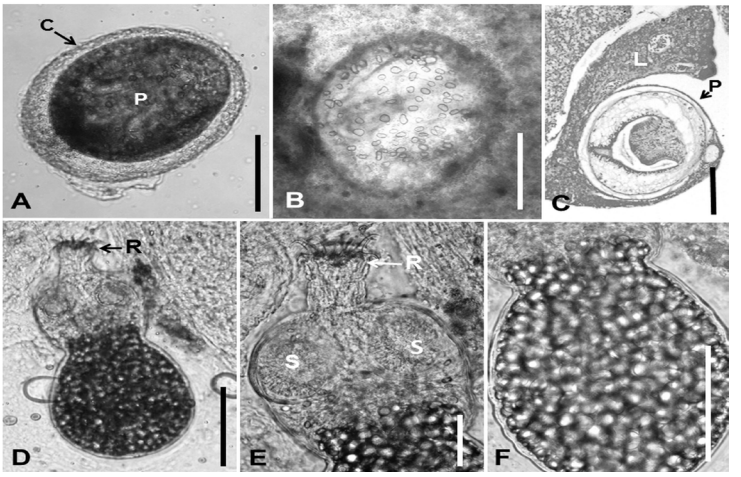
***Hosts and locality*:** 8 *F. blairae* (56.0 ± 3.1, 51–59 mm TL); 5 collected on 22 May 2017, 1 collected on 15 May 2018, and 2 collected on 30 September 2018 from just east of West Otis off St. Hwy 24, Sevier County, Arkansas (33° 57’ 14.6088”N, 94° 25’ 37.452”W).

Prevalence: 8/64 (13%).

***Intensity*:** Numerous plerocercoids (not counted) in each host.

Site of infection: Mesenteries and liver tissue.

***Other reported fundulid hosts*:** Northern Studfish, *Fundulus catenatus*; Golden Topminnow, *F. chrysotus*; Banded Killifish, *F. diaphanus*; Gulf Killifish, *F. grandis*; Mummichog, *F. heteroclitus*; Blackstripe Topminnow, *F. notatus*; Starhead Topminnow, *F. dispar* (Bangham 1941; Amin 1990; Hoffman 1999; McAllister et al. 2016a, b).



Figures 1A–F. Cestodes of *Fundulus blairae*. A. *Proteocephalus* sp. plerocercoid (P) within capsule (C); scale bar = 250 µm. B. *Proteocephalus* sp. plerocercoid showing numerous calcareous corpuscles; scale bar = 250 µm. C. *Proteocephalus* sp. plerocercoid (P) encapsulated in liver (L) tissue; scale bar = 250 µm. D. *Valipora minuta* larvae from gallbladder showing rostellum (R) with hooks; scale bar = 200 µm. E. Higher magnification of anterior view of same *V. minuta* showing rostellum (R) with hooks and suckers (S); scale bar = 100 µm. F. Posterior view of same *V. minuta*; scale bar = 250 µm.

Geographic range of genus in North America: The genus has been reported in nearly every U.S. state (see Hoffman 1999; McAllister et al. 2016a, b).

Additional Arkansas records in fishes: see McAllister et al. (2016a).

Specimens deposited**:** HWML 139402 (slide).

Remarks**:** Because these were plerocercoids, specific identification was not possible. This is the initial report of *Proteocephalus* sp. in *F. blairae*.

Cyclophyllidea: Gryporhynchidae
***Valipora minuta* (Coil, 1950) Baer and Bona, 1960 (Figs. 1D–F)**

Host and locality**:** 1 *F. blairae* (54 mm TL) collected on 30 September 2018 from same locale noted herein.

Prevalence**:** 1/64 (2%).

Intensity: A single metacestode.

Site of infection: Gall bladder epithelium.

Other reported fundulid hosts**:** None.

Other reported fish hosts: The larval form (metacestode) of the family infects more than 100 freshwater fish species and the adults, as well, are intestinal tapeworm parasites of fish-eating birds, mainly those in the Palearctic realm (Scholz et al. 2004). *Valipora minuta* occurs in a wide-variety of fishes of the families Centrarchidae, Heptateridae, and Poeciliidae, thus exhibiting a euryxenous type of host specificity (Scholz et al. 2004).

Geographic range of genus in fishes of North America**: USA:** Arkansas (Hoffman 1999; Scholz et al. 2004); Texas (Davis and Huffman 1975). **México:** Guerrero, Quintana Roo, Yucatán (Scholz et al. 2004). Coil (1950) described *V. minuta* from a green heron (*Butorides virescens*) from Indiana.

Additional Arkansas records in fishes: Spotted Bass, *Micropterus punctulatus*, Largemouth Bass, *M. salmoides* (Hoffman 1999).

Specimens deposited: HWML 139884 (photovoucher).

***Remarks*:** This specimen is tentatively identified as *V. minuta* based on its delicate hooks and shape of the smaller hooks (see fig. 2E, length of larger hooks ~35–40 µm). Identification of gryporhynchid larvae is almost always based on the number, shape, and size of rostellar hooks, which are situated in one layer. However, our specimen was not processed properly and future specimens should be prepared according to the GAP method of Malmberg (1957). We nevertheless document a new host record for this cestode.

Nematoda: Spirurida: Gnathostomidae
***Spiroxys* sp. (larvae) (Figs. 2A–E)**

***Hosts and locality*:** 23 *F. blairae* (48.0 ± 5.7, 39–54 mm TL); 2 collected on 9 July 2016, 1 collected on 21 August 2016, 1 collected on 22 May 2017; 7 collected on 8 October 2017; 4 collected on 15 May 2018; and 8 collected on 30 September 2018 from same locale noted herein.

Prevalence: 23/64 (36%).

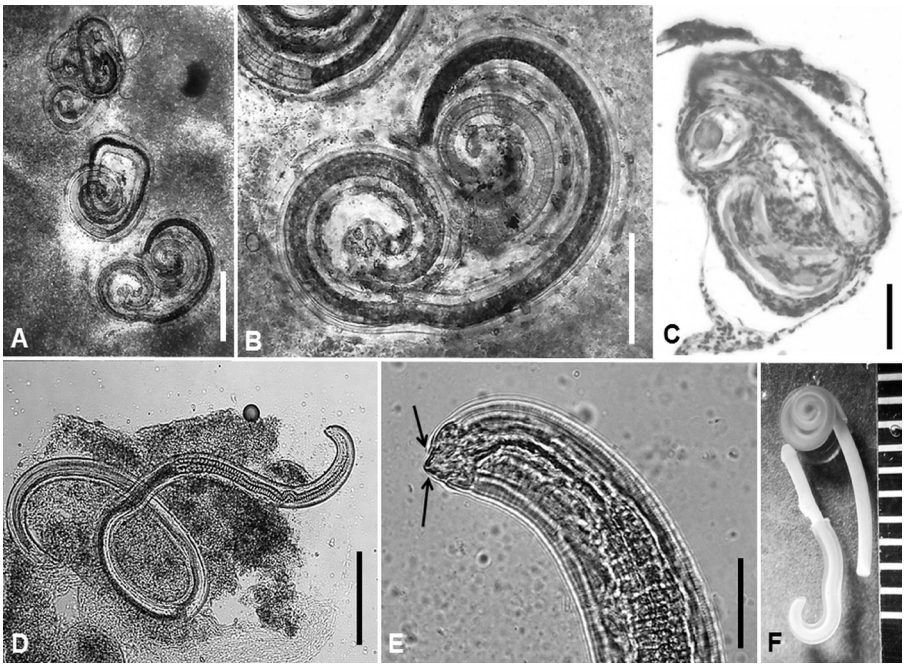
Intensity: Numerous larval worms (not counted) in each host.

Site of infection: Encapsulated in mesenteries and liver tissue (Figs. 2A–D).

Other reported fundulid hosts: *F. diaphanus* (Amin 1984); *F. notatus* (McAllister et al. 2016a); Bayou Topminnow, *F. notti* (Hoffman 1999).

***Geographic range of genus in fishes of North America*: USA:** Alabama, Arkansas, California, Georgia, New York, North Dakota, Ohio, Oklahoma, Pennsylvania, Texas, West Virginia, Wisconsin. **Canada:** British Columbia, Ontario. **México:** Campeche, Durango, Guanajuato, Hidalgo, Jalisco, Michoacán, Oaxaca, Querétaro, Quintana Roo, San Luis Potosi, State of México, Tabasco, Veracruz, Yucatán (see Salgado-Maldonado 2006; McAllister et al. 2016a).

Additional Arkansas records in fundulids:



Figures 2A–E. Nematodes of *Fundulus blairae*. A. Three encapsulated *Spiroxys* sp. in liver tissue; scale bar = 250 µm. B. Higher magnification of single encapsulated *Spiroxys* sp. in liver tissue; scale bar = 250 µm. C. Cross section of *Spiroxys* sp. encapsulated in mesenteries; scale bar = 25 µm. D. Single *Spiroxys* sp. teased from encapsulation. Scale bar = 125 µm. E. Anterior end of *Spiroxys* sp. showing characteristic lips (arrows); scale bar = 25 µm. F. *Eustrongylides* sp. nematode removed from cyst in coelomic cavity; scale = each bar on ruler is 1.0 mm.

F. notatus (McAllister et al. 2016a).

Specimens deposited: HWML 139373 (photovoucher).

***Remarks*:** Because these were larval specimens, we were unable to assign them to species. Adult worms are found in the intestinal tract of amphibians and the stomach of turtles; the first intermediate host is the copepod *Cyclops* (see Hedrick 1935).

Dioctophymatoidea: Dioctophymatidae
***Eustrongylides* sp. (larvae) (Fig. 2F)**

Host and locality: 1 *F. blairae* (54 mm TL) collected on 30 September 2018 from same site noted herein.

Prevalence: 1/64 (2%).

Intensity: 1 worm.

Site of infection: Encapsulated in coelomic cavity.

Other reported fundulid hosts: Summarized by McAllister et al. (2016a).

Geographic range of genus in North America: **USA:** Arkansas, Florida, Maine, Maryland, Massachusetts, Montana, Nevada, New York, Ohio, Oklahoma, Oregon, Tennessee, Texas, Utah, Wyoming. **Canada:** British Columbia, Ontario. **México:** Campeche, Guanajuato, Hidalgo, Jalisco, Michoacán, Morelos, Oaxaca, San Luis Potosi, Veracruz, Yucatán (see McAllister et al. 2016a).

Additional Arkansas records in fish: Pirate Perch, *Apheroderus sayanus*, Ozark Bass, *Ambloplites constellatus*, Grass Carp, *Ctenopharyngodon idella*, Northern Studfish, *F. catenatus*, Golden Topminnow, *F. chrysotus*, and Blackspotted Topminnow, *F. olivaceus* (McAllister et al. 2015, 2016a, b).

Specimens deposited: HWML 139885 (photovoucher).

Remarks: As adults, nematodes of the genus *Eustrongylides* are found in the proventriculus of piscivorous wading birds, with larvae encysted in the body cavity and musculature of fishes (Hoffman 1999). In the life cycle, early larval development occurs in the blood vessels of first intermediate host (freshwater oligochaetes) after they ingest infective eggs (Measures, 1988a), fishes are second intermediate hosts (Measures 1988b) and piscivorous birds are generally considered to be the definitive host (Spaulding and Forrester 1993; Franson and Custer 1994). However, predatory fish that ingest infected fish serve as paratenic, or transport, hosts (Xiong et al. 2013). Specific identification of *Eustrongylides* requires rearing larvae in an avian host, and our study did not include this experimental transmission. However, we report, for the first time, *Eustrongylides* sp., from *F. blairae*.

Mollusca: Bivalvia
Glochidia (Figs. 3A–B)

***Host and locality*:** 1 *F. blairae* (60 mm TL) collected on 15 May 2018 from same locale noted herein.

Prevalence: 1/64 (2%).

Intensity: Three glochidia.

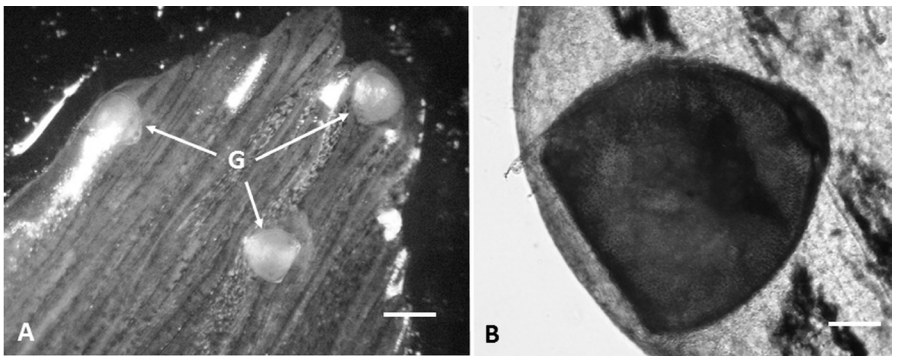
Site of infection: Encapsulated in caudal fin (Figs. 3A–B).

***Other reported fundulid hosts*:** *F. diaphanus*, Canada (Margolis and Arthur 1979).

Additional Arkansas records in fundulids: None.

Specimens deposited: HWML 139852 (photovoucher).

***Remarks*:** Larvae of most freshwater clams go through an obligate parasitic stage on the gills or fins of various fishes (Coker et al. 1921). We made no attempt to collect mussels at the study locale and, although it is not possible to identify the parent species of this glochidium, we document a new host record.



Figures 3A–B. Glochidia from *Fundulus blairae*. A. Three glochidia (G, arrows) in caudal fin; scale bar = 1.0 mm. B. Higher magnification of single glochidium in caudal fin; scale bar = 200 µm.

In summary, we examined a large sample of *F. blairae* from a single location in southwestern Arkansas and its parasite fauna is depauperate. This parasite fauna included five taxa, and four were represented by larval specimens, indicating these small topminnows are forage species that, as part of the life cycle, pass their parasites onto larger fish, reptilian, or bird definitive hosts. Additional populations from a variety of aquatic sites should be surveyed in an attempt to find additional parasites in this rarely studied fish.

Acknowledgments

We thank Drs. Scott L. Gardner and Gabor Racz (HWML) and Renn Tumlison (HSU) for expert curatorial assistance. We also thank the Arkansas Game and Fish Commission for Scientific Collecting Permits issued to CTM and HWR, and James T. McAllister, III (University of Arkansas-Fayetteville, AR) and Nikolas H. McAllister (Lukfata Elementary School, Broken Bow, OK) for assistance with collecting. Dr. Tomáš Scholz (Institute of Parasitology, České Budějovice) provided valuable information on gryporhynchid cestodes.

References

Amin OM. 1984. Camallanid and other nematode parasites of lake fishes in southeastern Wisconsin. Proc Helminthol Soc Wash 51:78–84.

Amin OM. 1990. Cestoda from lake fishes in Wisconsin: Occurrence of *Proteocephalus* in *Esox* and other fish species. J Helminthol Soc Wash 57:132–139.

Bangham RV. 1941. Parasites from fish of Buckeye Lake, Ohio. Ohio J Sci 41:441–448.

Coil WH. 1950. The genus *Ophiovalipora* Hsü, 1935 (Cestoda: Dilepididae) with the description of *Ophiovalipora minuta* sp. nov. from the green heron (*Butorides virescens* L.). J Parasitol 36:55–61.

Coker REA, Shira SF, Clark HW, Howard AD. 1921. Natural history and propagation of freshwater mussels. Bull US Bur Fish 37:75–182.

Curran SS, Overstreet RM, Tkach VV. 2007. Phylogenetic affinities of *Plagiocirrus* Van Cleave and Mueller, 1932 with the description of a new species from the Pascagoula River, Mississippi. J Parasitol 93:1452–1458.

Davis J, Huffman DG. 1975. The ecology of the helminth parasites of *Gambusia affinis* and *Gambusia geiseri* (Osteichthyes: Poeciliidae) from the area of San Marcos, Texas. Aquat Stn Southwest Texas State Univ, San Marcos, Res. Rep. No. 75–1, 161 p.

Franson JC, Custer TW. 1994. Prevalence of eustrongylidosis in wading birds from colonies in California, Texas, and Rhode Island, USA. Col Waterbirds 17:168–172.

Hedrick LR. 1935. The life history and morphology of *Spiroxys contortus* (Rudolphi); Nematoda: Spiruridae. Trans Amer Microsc Soc 54:307–335.

Hoffman GL. 1999. Parasites of North American freshwater fishes. Second Ed. Ithaca (NY): Comstock Publishing Associates. 539 p.

Malmberg G. 1957. Om förekomsten av *Gyrodactylus* på svenska fiskar. Särtryck ur Skrifter utgivna av Södra Sveriges Fiskeriförening, Årsskrift 1956:19–76.

Margolis L, Arthur JR. 1979. Synopsis of the parasites of fishes of Canada. Bulletin 199, Dept. Fish Oceans, Sci. Info. Publ. Branch, Ottawa, Bull. No. 199. 269 p.

McAllister CT, Bursey CR, Fayton TJ, Cloutman DG, Robison HW, Connior MB, Trauth SE. 2016a. Helminth parasites of the Blackstripe Topminnow, *Fundulus notatus* (Cyprinidontiformes: Fundulidae), from Arkansas and Oklahoma, U.S.A. Comp Parasitol 83:227–236.

McAllister CT, Bursey CR, Fayton TJ, Robison HW, Trauth SE. 2015. New host and geographic distributional records for *Eustrongylides* sp. (Nematoda: Dioctophymatoidea: Dioctophymatidae) from eight vertebrates from Arkansas, Oklahoma and Texas. Proc Okla Acad Sci 95:26–32.

McAllister CT, Bursey CR, Font WF, Robison HW, Trauth SE, Cloutman DG, Fayton TJ.2016b. Helminth parasites of the Northern Studfish, *Fundulus catenatus* (Cypriniformes: Fundulidae) from the Ouachitas and Ozarks of Arkansas, U.S.A. Comp Parasitol 83:78–87.

Measures LN. 1988a. The development of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in oligochaetes. J Parasitol 74:294–304.

Measures LN. 1988b. The development and pathogenesis of *Eustrongylides tubifex* (Nematoda: Dioctophymatoidea) in piscivorous birds. Can J Zool 66:2223–2232.

Miller RJ, Robison HW. 2004. Fishes of Oklahoma. Norman (OK): University of Oklahoma Press. 450 p.

NatureServe. 2018. NatureServe Explorer: An online encyclopedia of life [online]. Version 7.1. NatureServe, Arlington, Virginia. Available from: http://explorer.natureserve.org. (Accessed: September 30, 2018).

Page LM, Burr BM. 2011. Peterson field guide to freshwater fishes of North America north of Mexico. Second Ed. Boston (MA): Houghton Mifflin Harcourt. 663 p.

Presnell JK, Schreibman MP. 1997. Humason's animal tissue techniques. Fifth edition. Baltimore (MD): Johns Hopkins University Press. 600 p.

Robison HW. 1977. *Fundulus blairae* Wiley and Hall (Cyprinodontidae) in Arkansas. Southwest Nat 22:544.

Robison HW, Buchanan TM. 1988. Fishes of Arkansas. Fayetteville (AR): University of Arkansas Press. 536 p.

Ross ST. 2001. The inland fishes of Mississippi. Jackson (MS): University Press of Mississippi. 624 p.

Salgado-Maldonado G. 2006. Checklist of helminth parasites of freshwater fishes of Mexico. Zootaxa 1324:1–357.

Scholz T, Bray RA, Kuchta R, Řepová R. 2004. Larvae of gryporhynchid cestodes (Cyclophyllidea) from fish: a review. Fol Parasitol 51:131-152.

Spaulding MG, Forrester DJ. 1993. Pathogenesis of *Eustrongylides ignotus* (Nematoda: Dioctophymatoidea) in Ciconiiformes. J Wildl Dis 29:250–260.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. Bethesda (MD): American Fisheries Society. Available from: https://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf (Accessed September 25, 2018).

Wiley EO. 1980. *Fundulus blairae* (Wiley and Hall), Blair's Starhead Topminnow. In Lee DS et al., editors. Atlas of North American Freshwater Fishes. Raleigh (NC): North Carolina State Museum of Natural History. p 508.

Xiong FW, Li X, Wu SG, Zou H, Wang GT. 2013. Molecular phylogeny and host specificity of the larval *Eustrongylides* (Nematoda: Dioctophmidae) from freshwater fish in China. J Parasitol 99:137–144.

Submitted October 10, 2018 Accepted November 18, 2018

Parasites (Cnidaria, Trematoda, Cestoda, Nematoda, Crustacea) of Select Fishes (Lepisosteidae, Catostomidae, Hiodontidae, Cyprinidae, Ictaluridae) of Lake Texoma, Oklahoma

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Anindo Choudhury
Division of Natural Sciences, St. Norbert College, DePere, WI 54115

Donald G. Cloutman
P. O. Box 197, Burdett, KS 67523

Stanley E. Trauth
Department of Biological Sciences, Arkansas State University (Emeritus), State University, AR 72467

Henry W. Robison
9717 Wild Mountain Drive, Sherwood, AR 72120

Abstract: Twenty-seven fishes, including a Longnose Gar (*Lepisosteus osseus*), a Shortnose Gar (*L. platostomus*), three Goldeye (*Hiodon alosoides*), eight Highfin Carpsucker (*Carpiodes velifer*), a Bigmouth Buffalo (*Ictiobus cyprinellus*), four Smallmouth Buffalo (*I. bubalus*), a Common Carp (*Cyprinus carpio*), five Blue Catfish (*Ictalurus furcatus*), two Channel Catfish (*Ictalurus punctatus*), and a Flathead Catfish (*Pylodictus olivaris*) were collected in February 2017 and February 2018 from several sites on Lake Texoma, Oklahoma, and examined for parasites. Thirteen taxa of parasites were found, including two myxozoans (*Henneguya* spp.), seven cestodes (*Proteocephalus ambloplitis, Essexiella fimbratum, Megathylacoides* sp., *Pseudoglaridacris confusa*, *Promonobothrium currani, Khawia* sp., and *Bothriocephalus* sp.), two digeneans (*Crepidostomum illinoiense* and *Macroderoides* sp.), a larval nematode (*Contracaecum* sp.), and a copepod (*Ergasilus cerastes*) were harbored by these hosts. We document several new host and distributional records for these parasites, including new state records for Oklahoma.

Introduction

Lake Texoma, Oklahoma and Texas, is one of the largest reservoirs in the United States, with a total water volume of 3.115242 km^3 (2,525,568 acre-ft). The U.S. Army Corps of Engineers built Lake Texoma in the mid-1940s, and it is stocked with various game fishes. Denison Dam, located between Oklahoma and Texas on the Red River, impounded the lake with the primary outflow being the Red River and primary inflows include the Red and Washita rivers.

Over much of the last half-century or more,

the lake has been a mecca for several studies on parasites of various fishes (Sneed 1950; Self 1954; Self and Timmons 1955; Self and Campbell 1956; Roberts 1957; McDaniel 1963; Hopkins 1966; Mackiewicz 1964, 1968, 1969, 1970). We are not aware of studies conducted in recent years on the parasitic fauna of fishes of Lake Texoma. Here, we include additional information on some parasites of select fishes, including new host and distributional records and the first report of a *Henneguya* from the lake.

Methods

On 22 February 2017 and 28 February 2018, 27 fishes were collected with gill nets placed by members of the Oklahoma Department of Wildlife Conservation in Bryan and Marshall counties at the Red River near Cardinal Cove, the Washita Arm near Bridgeview Marina, the mouth of Catfish Bay (west of the Willow Springs boat ramp), and 0.8 km south of the railroad bridge. Fishes collected included: one Longnose Gar (*Lepisosteus osseus*), one Shortnose Gar (*L. platostomus*), three Goldeye (*Hiodon alosoides*), eight Highfin Carpsucker (*Carpiodes velifer*), four Smallmouth Buffalo (*I. bubalus*), one Bigmouth Buffalo (*Ictiobus cyprinellus*), one Common Carp (*Cyprinus carpio*), five Blue Catfish (*Ictalurus furcatus*), two Channel Catfish (*Ictalurus punctatus*), and one Flathead Catfish (*Pylodictus olivaris*). Specimens were donated to CTM, stored briefly on ice, and examined for internal parasites within 24 hr. Fishes were measured for total length (TL) and those still alive were killed by immersion in a concentrated tricaine methanesulfonate solution following accepted guidelines (Use of Fishes in Research Committee 2014), and preserved in 10% formalin after initial examination. Gills were removed from the hosts previously preserved in 10% formalin and examined for parasites under a stereomicroscope at 20–30×. Parasites were picked directly from the gills with small forceps or needles. Myxozoan (*Henneguya*) plasmodia and myxospores were placed in a drop of water on microscope slides and observed as wet mounts. Measurements (to the nearest 0.5 µm) and attempts to identify *Henneguya* were according to criteria in Kudo (1929), Griffin et al. (2009), Wagner (2016), and Leis et al. (2017). Crustaceans (*Ergasilus*) were cleared briefly in a drop of lactic acid on slides and then mounted in Grey and Wess medium, and coverslip ringed with fingernail polish. Specimens of *Ergasilus* were identified using Roberts (1970). A mid-ventral incision was made from the mouth to the anus of host fishes to expose the viscera, and the entire gastrointestinal tract and organs placed in Petri dishes containing 0.9% saline were examined for helminths under a stereomicroscope at 20–30×. Trematodes and cestodes were fixed in near boiling tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate, and mounted in Canada balsam. Nematodes were fixed in a similar manner and preserved in 70% (v/v) ethanol. They were later cleared by placing them in a mixture of 5% or 10% glycerin in 70% ethanol in an uncovered dish and allowing the ethanol (and water) to evaporate. Cleared nematodes were studied as temporary mounts in glycerol.

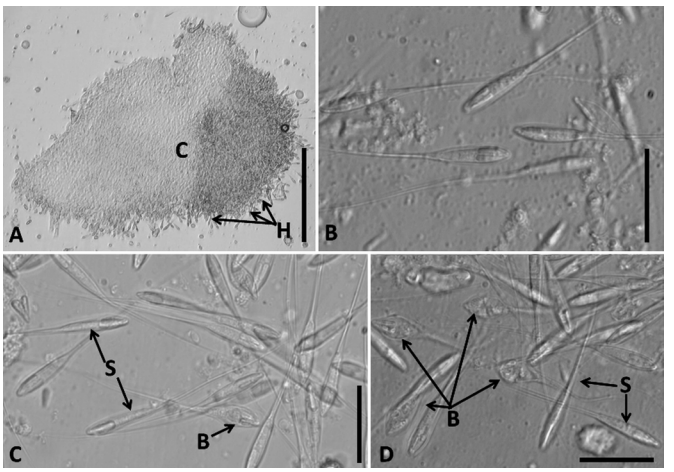
Prevalence, intensity, and range of infection were calculated according to Bush et al. (1997). Voucher specimens of parasites were deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska. Photovouchers of the hosts or those preserved in 10% formalin were deposited in the Henderson State University Museum (HSU), Arkadelphia, Arkansas.

Results and Discussion

Thirteen taxa of parasites were found, including two myxozoans, seven cestodes, two digeneans, a nematode, and a copepod. An annotated list of the parasites found and the host data follows.

Cnidaria: Myxosporea: Myxidiidae

***Henneguya exilis* Kudo, 1929 –** An 850 mm TL *I. furcatus* possessed a myxozoan, *H. exilis* (HWML 139857, Figs. 1A–D) on its gills. A description of this myxozoan follows: plasmodia (cyst) spherical, 280 diameter (*n* = 1). Myxospores with long caudal process,



Figures 1A–D. ***Henneguya exilis*** **from the gills of** ***Ictalurus furcatus*****. A. Ruptured plasmodia showing hundreds of** ***H. exilis*** **(H) along the edge of cyst; scale bar = 200 µm. B. Mostly frontal view of slender myxospores; scale bar = 20 µm. C. Mostly lateral views of slender spores (S) and frontal view of a broad morph (B); scale bar = 20 µm. D. Slender (S) and broad (B) morphs of spores; scale bar = 20 µm.**

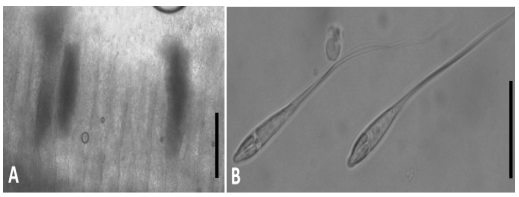
variable, with two morphs present in the same cyst, one with slender spore body and long caudal process, the other with broad spore body and shorter, usually separated caudal process. Slender morph (n = 20): total length of spore 59.5 (54.5–71.5). Spore body fusiform, widest in region near tips of polar capsules, length 17.1 (16.0–18.5), width 3.2 (3.0–3.5). Caudal process separated or not, length 42.4 (24.5–55.5). Two polar capsules, one usually slightly longer than the other; length of longest polar capsule 6.7 (6.0–7.5), width 1.2 (1.0–1.5); length of shorter polar capsule 6.3 (6.0–7.0), width 1.2 (1.0–1.5). Broad morph (n = 9): total length of spore 40.9 (33.5–45.0). Spore body ovoid to rhomboid, widest in region near tips of polar capsules, length 12.4 (10.0–15.0), width 5.2 (4.0–6.0). Caudal process usually separated, length 28.5 (23.0–32.0). Two polar capsules, one usually slightly longer than the other; length of longest polar capsule 5.5 (4.5–6.5), width 1.9 (1.5–2.5); length of shorter polar capsule 5.2 (4.0–6.0), width 1.8 (1.5–2.5).

Henneguya exilis was originally described from *I. punctatus* from the Rock River, Illinois (Kudo 1929). This myxozoan has also been reported from cultured *I. punctatus* in México (Rábago-Castro et al. 2013). It has been reported in other ictalurids, including Black Bullhead (*Ameiurus melas*) from Iowa (Kudo 1920) and Brown Bullhead (*A. nebulosus*) in North Carolina (Iwanowicz et al. 2008). Other localities for *H. exilis* include Arkansas, Mississippi, Nebraska, sites in lakes Erie and Michigan, and Ontario, Canada (see Hoffman 1999). Interestingly, only two *Henneguya* spp. have been previously reported from Oklahoma, *H. gambusi* Parker, Spall, and Warner, 1971 from Western Mosquitofish, *Gambusia affinis* (Parker et al. 1971) (Spall et al. 1971) and an unknown *Henneguya* sp. from *A. melas* (McAllister and Trauth 2015).

Due to generally high host and organ specificity (with some exceptions, e.g., *H. exilis*), species of *Henneguya* from ictalurid catfishes (see Wagner 2016) have been separated into "working forms," i.e., interlamellar, intralamellar, visceral, cutaneous, adipose, and gall bladder (Minchew 1977). Three species of *Henneguya* have been reported from *I. furcatus*: *M. limatula* Meglitsch, 1937, in the gall bladder (Meglitsch 1937; Minchew 1977); *H. exilis* in the gills (Rice and Jahn 1943); and *H. pellis* Minchew, 1977, in the dermis (Minchew 1977). The slender morph found in this study conforms closely with *H. exilis*. Our identification as *H. exilis* is tentative, as it is confounded by the presence of a broad morph. The broad

morph does not conform to any descriptions of *Henneguya* reported from ictalurids (Minchew 1977; Griffin et al. 2009; Wagner 2016; Leis et al. 2017). Spores of *Henneguya* are sometimes indistinguishable from others based on morphology (McAllister and Trauth 2015). 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 confirm our identification.

***Henneguya* sp. –** A 610 mm TL *P. olivaris* had cysts and spores of an unknown *Henneguya* sp. (HWML 139858 photovoucher, Figs. 2A–B) on its gills. A description of this myxozoan follows: cyst (n = 30) intralamellar, cylindrical, length 266 (96–582), width 94 (56–194). Myxospores (n = 30) with long caudal process. Total length of spore 78.2 (65.0–92.0). Spore body fusiform, widest in region near tips of polar capsules, length 17.8 (14.0–22.5), width 4.1 (3.5–4.5). Caudal process often not separated, but occasionally separated, especially near tip, length 60.4 (57.0–77.0). Two polar capsules, one usually slightly longer than the other, length of longest polar capsule 5.8 (5.0–6.5), width 1.1 (1.0–1.5); length of shorter polar capsule 5.5 (4.5–6.0), width 1.1 (1.0–1.5).



Figures 2A–B. *Henneguya* sp. from the gills of *Pylodictis olivaris*. A. Plasmodia (dark bodies), *in situ* within gills; scale bar = 200 µm. B. Frontal views of myxospores; scale bar = 20 µm.

Leis et al. (2017) described *Henneguya laseeae* from the gills of *P. olivaris* from the upper Mississippi River, Wisconsin and Iowa. Until now this was the only report of a myxozoan from *P. olivaris*. Our specimens from Lake Texoma differ from those of *H. laseeae* by the shape of the cyst (cylindrical versus spherical to ovoid) and length of the spore body (17.8 (14.0-22.5) versus 16.2 (15.1-17.0), respectively. The spore body of *Henneguya* sp. is proportionately longer in relation to width than that of *H. laseeae* (Figs. 2A–B, present paper; Leis et al. (2017). Based on the total length of spores, our specimens also resemble *H. longicauda* Minchew, 1977, previously reported from the gills of *I. punctatus*, and *H. pellis* Minchew, 1977, previously reported on the dermis of *I. furcatus* (Minchew 1977). Although there is slight overlap, the spore body of *Henneguya* sp. reported here is generally shorter (78.2 [65.0–92.0]) than that of *H. longicauda* (108.3 [91–127]) (Minchew 1977; Griffin et al. 2009; Wagner 2016). The spore body of *Henneguya* sp. is longer in relation to width (Fig. 2B) than that of *H. pellis* (Minchew 1977). This is the second report of a species of *Henneguya* from *P. olivaris* and it is likely a new species, but we withhold a description pending SSU rDNA studies.

Cestoda: Caryophyllidea: Caryophyllaeidae

Pseudoglaridacris confusa (Hunter, 1929) **Oros, Uhrovič and Scholz, 2018**. **–** Adult worms (HWML 139881) were found in the intestines of a 560 mm TL *I. cyprinellus*, and the tapeworm has been previously reported from Lake Texoma in *I. bubalus* and Black Buffalo, *I. niger* (Self and Campbell 1956). The finding in our study extends the host range of this tapeworm to *I. cyprinellus*. Type hosts of this tapeworm include *Ictiobus* spp. from Mississippi, and the species appears to be widely distributed in North America, including Arizona, Connecticut, Idaho, Iowa, New York, North Dakota, Wisconsin, and Canada (Hoffman, 1999). *Pseudoglaridacris confusa* was previously known in the literature as *Glaridacris confusus* Hunter, 1927, but a review by Oros et al. (2018) showed that species in North America do not belong in the genus *Glaridacris*.

Promonobothrium currani **Oros, Brabec, Kuchta, Choudhury, and Scholz, 2016. –**These caryophyllidean tapeworms (HWML 139880) were collected from the intestines of a 560 mm TL *I. cyprinellus* and a 370 mm TL *I. bubalus*. This tapeworm was first described by Oros et

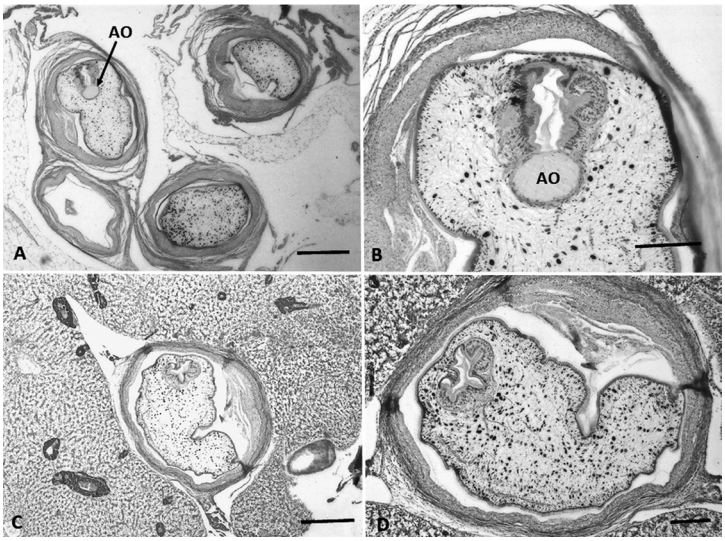
al. (2016) from *I. bubalus* and *I. niger* from Chotard Lake, Mississippi. Records from this study represent the second report of this parasite, and extend its geographic and host range. We document the first report of this parasite from Oklahoma and *I. cyprinellus* is a new host record. One other species of *Promonobothrium*, *P. ingens* (Hunter, 1927) Scholz, Oros, Choudhury, Brabec and Waeschenbach, 2015, was reported from *I. bubalus* in Lake Texoma (Self and Campbell 1956) as '*Monobothrium ingens*', the former name of the species (Scholz et al. 2015). We collected a single, somewhat contracted specimen from *I. bubalus* that also likely belongs to this species.

***Khawia* sp. (Yamaguti, 1934) Hsü, 1935**. – One adult worm (HWML 110617) was found in the intestine of a 650 mm TL *C. carpio*. Hoffman (1999) mentions two species in North America, *K. iowensis* Calentine and Ulmer, 1961, and *K. sinensis* Hsü, 1935 (Calentine and Ulmer 1961; Williams and Sutherland 1981). Scholz et al. (2011b) determined that *K. iowensis* is actually *K. japonensis* (Yamaguti, 1934), and therefore an introduced species, as is its host, *C. carpio* in North America. The tapeworm in our collection resembles *K. japonensis*. To our knowledge, this is only the second record of a *Khawia* species in Oklahoma from *C. carpio* (Spall 1969) but the first from a Common Carp in Lake Texoma.

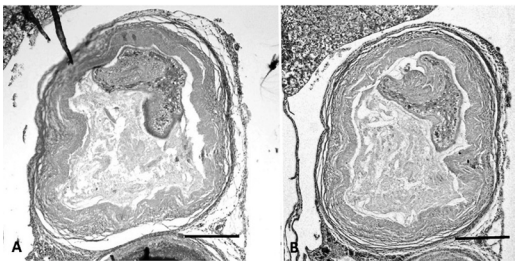
Onchoproteocephalidea: Proteocephalidae

***Proteocephalus ambloplitis* (Leidy, 1887) Benedict, 1900. –** Plerocercoids of *P. ambloplitis* (HWML 139890–139892, Figs. 3A–D, 4A–B) were observed in liver tissue of a 310 mm TL *I. punctatus*, a 500 mm TL *I. furcatus*, and a 610 mm TL *P. olivaris*. The "bass" tapeworm is known to cause pathologies to various second intermediate hosts (Hoffman 1999) and some fibrosis is observed in our histological samples (Figs. 3C–D). McDaniel and Bailey (1974) reported *P. ambloplitis* in centrarchids from the Little River (Cleveland County) and from Lake Texoma. Although the cestode has been previously reported in *I. punctatus* and *P. olivaris* (Hoffman 1999), to our knowledge, it has not been documented in *I. furcatus*. Here, we provide a new host record for *P. ambloplitis*.

***Essexiella fimbriatum* (Essex, 1927) Scholz, de Chambrier, Mariaux and Kuchta, 2011**. – This tapeworm (HWML 110615) was found



Figures 3A-D. *Proteocephalus ambloplitis* plerocercoids in liver tissue of *Ictalurus punctatus*. A. Four plerocercoids in liver tissue with one showing large glandular apical organ (AO); scale bar = 500 µm. B. Close-up of same plerocercoid in A showing AO; scale bar = 500 µm. C. Another view of different plerocercoid showing encapsulation and extensive fibrosis; scale bar = 500 µm. D. Higher magnification of plerocercoid in C; scale bar = 500 µm.



Figures 4A-B. *Proteocephalus ambloplitis* plerocercoids from liver tissue of catfishes. A. Plerocercoid from *Ictalurus furcatus*. B. Plerocercoid from *Pylodictis olivaris*; scale bars = 500 µm.

in the intestine of a 375 mm TL *I. punctatus*. It is widely distributed in this fish host (Hoffman, 1999). The genus is closely related to two other proteocephalidean genera in ictalurid catfishes, namely *Megathylacoides* Jones, Kerley and Sneed, 1956, and *Corallotaenia* (Freze, 1965) (Rosas Valdez et al. 2004). *Essexiella fimbriatum* is well known in the fish parasitology literature by its former name, *Corallobothrium fimbriatum* Essex, 1927, but a phylogenetic analysis that included the type species from Africa showed that North American *Corallobothrium* spp. belong in a separate genus, and *Essexiella* was established (Scholz et al. 2011a); this required a name change to *E. fimbriatum*. This species was previously reported from this host in Lake Texoma (Sneed, 1950) and in Lake Carl Blackwell (Spall 1969; Spall and Summerfelt 1969). In addition, McAllister et al. (2016) reported *E. fimbriatum* in a Yellow Bullhead (*Ameiurus natalis*) and Black Bullhead (*Ameiurus melas*) from the Red River drainage of the state.

Megathylacoides sp. – A strobilate, but immature specimen of this tapeworm (HWML 110616), was found in the intestine of a 310 mm TL *I. punctatus*. Species of *Megathylacoides* Jones, Kerley and Sneed, 1956, have been widely reported in ictalurid catfishes (Hoffman 1999; Pérez-Ponce de León and Choudhury 2002; Scholz et al. 2003). *Megathylacoides giganteum* (Essex, 1928) Freze, 1965, appears to be the most widely reported species (Hoffman 1999) in a variety of ictalurid catfishes, but the Channel Catfish seems to be its most common host (Hoffman 1999). Two other species, *M. procerum* Sneed, 1950 and *M. thompsoni* Sneed, 1950 were described from *I. furcatus* and *I. punctatus* respectively, from Lake Texoma (Sneed 1950) but the descriptions are incomplete, there are no illustrations, and no type specimens were deposited (Sneed 1950). A fourth species, *Megathylacoides tva* Jones, Kerley and Sneed, 1956 was described from *P. olivaris* in Tennessee (Jones et al. 1956); this fish host is also present in Lake Texoma but the scolex is distinctly different from the worm we collected in this study and, thus, can be ruled out. A fifth species, *Megathylacoides lamothei* (García-Prieto, 1990) Scholz, Rosas, Pérez-Ponce de León, Choudhury and de Chambrier, 2003, was described from *I. furcatus*, in México (García-Prieto, 1990) and this ictalurid is common in the U.S., including Lake Texoma. The scolex of the immature form collected in this study appears to resemble that of *M. lamothei*. Further studies, based on freshly collected mature specimens and molecular data, are necessary to clarify the taxonomy of this group of tapeworms.

Bothriocephalidea: Bothriocephalidae

Bothriocephalus **sp. –** Gravid specimens of a species of *Bothriocephalus* Rudolphi, 1808 were found in the intestines of two (143, 150 mm TL) *H. alosoides*. Self (1954) described *B. texomensis* from Goldeye in Lake Texoma. Scholz (1997) synonymized *B. texomensis* with *B. cuspidatus* Cooper, 1917. The morphology of the species collected in this study is consistent with that of *B. texomensis* but because the specimens were collected from previously frozen fish, we remain circumspect about a more definitive species diagnosis. The status of *B. texomensis* vis a vis its relationship to *B. cuspidatus* is currently being studied using morphological and molecular data (Choudhury and Scholz *pers. comm.*). The material has been retained by one us (AC) for a more detailed taxonomic study on North American *Bothriocephalus* species.

Trematoda: Digenea: Allocreadiidae

Crepidostomum illinoiense **Faust, 1918**. –These trematodes (HWML 110614) were collected from the intestines of a 143 mm TL *H. alosoides*. *Crepidostomum illinoiense* is

a common and widely distributed parasite of Goldeye (Hoffman 1999; Choudhury and Nelson 2000). The parasite has been reported from this host in Lake Texoma (Self 1954). Although the species was described based on young mature and immature specimens from White Crappie (*Pomoxis annularis*), hiodontid fishes (Goldeye and Mooneye, *Hiodon tergisus*) appear to be its principal hosts.

Macroderoididae

Macroderoides sp. – Two specimens (HWML 139883) were collected from the intestine of a 1,050 mm TL *L. osseus*. One specimen was stained and the other stored for molecular study. The single stained worm is morphologically most similar to *M. luki* Kusy and Barger, 2017, recently described from the Spotted Gar, *L. oculatus*, from Texas (Kusy and Barger 2017). To our knowledge, our report from a Longnose Gar appears to be the first record of a species of *Macroderoides* from any host in Oklahoma (Hoffman 1999).

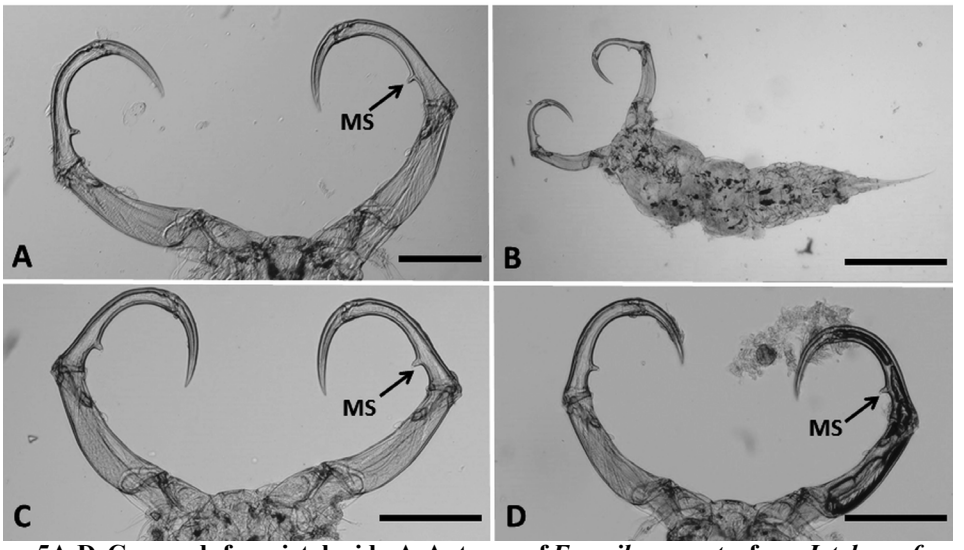
Nematoda: Secernentea: Ascaridida: Anisakidae

Contracaecum sp. – Third-stage larvae (L_3) of *Contracaecum* Railliet and Henry, 1915 (HWML 110613) were found encapsulated on the stomach and mesenteries in a 310 mm TL *I. punctatus*, each worm ensheathed in a thick connective tissue sleeve. Our specimens seemed intact and showed no signs of necrosis, suggesting that they were viable in their paratenic fish host and infective to their definitive bird hosts, particularly those of the family Phalacrocoracidae (cormorants) (Moravec 2009). *Contracaecum* L_3s are common in fish throughout North America (Hoffman 1999), but species determination at this larval stage is not possible. Furthermore, the precise number of *Contracaecum* species in North America remains unclear (D'Amelio et al. 2007).

Crustacea: Copepoda: Poecilostomatoidea: Ergasilidae

***Ergasilus cerastes* Roberts, 1969 –** Forty copepods (HWML 139478, Fig. 5A) were found on the gills of a single 850 mm TL *I. furcatus*; 45 specimens (HWML 139865, Fig. 5B–C) on the gills of two (310, 375 mm TL) *I. punctatus*; and 70 individuals (HWML 139477, Fig. 5D) on the gills of one 610 mm TL *P. olivaris*. This crustacean was originally described from *Ictalurus* sp. from a fish market in Washington, D. C. (Roberts 1969, 1970; Johnson and Rogers 1973). Other reported hosts and localities include *Ictalurus* spp. from Florida (Mueller 1936), White Catfish, *Ameiurus catus*, *A. nebulosus*, *I. furcatus*, and *I. punctatus* from Alabama and Mississippi (Johnson and Rogers 1973), and Freshwater Eel, *Anguilla rostrata* from South Carolina (Eversole 1981). Our specimens from all three catfish hosts from Lake Texoma conform to the description of *E. cerastes* in Roberts (1969, 1970). With the exception of an undocumented report from *A. rostrata* in South Carolina (Eversole 1981), all previous records of *E. cerastes* have been from species of *Ameiurus* and *Ictalurus* (Roberts 1969, 1970; Johnson and Rogers 1973; Hoffman 1999). Four other species of *Ergasilus* (*E. arthrosis* Roberts, 1969; *E. versicolor* Wilson, 1911; *E. cyprinaceus* Rogers, 1969; and *E. megaceros* Wilson, 1916) have been reported on North American catfishes (Johnson and Rogers 1973; Hoffman 1999), but *E. cerastes* was the only species of *Ergasilus* found in our samples of ictalurids from Lake Texoma. *Ergasilus cerastes* differs from all of these species by possessing a large blunt medial sensillum (Figs. 5A–D) near the midpoint (Roberts 1970). Johnson and Rogers (1973) noted that their records of *E. cerastes* were from mouths of river in estuarine areas. Our specimens from Lake Texoma represent the first *E. cerastes* found far inland and west of the Mississippi River, including Oklahoma, and the first reported from *P. olivaris*.

In conclusion, we document some new host and geographic records for these parasites. Most parasites (seven species) found in our hosts from Lake Texoma were cestodes. Only one nematode was recovered and that is somewhat surprising given that they are common in parasite communities of North American freshwater fishes (see Choudhury and Nadler 2018). Several taxa reported herein require additional study (including molecular approaches) to determine their specific identity. With the diverse



Figures 5A-D. Copepods from ictalurids. A. Antenna of *Ergasilus cerastes* from *Ictalurus furcatus*, showing unique large, blunt medial sensillum (MS) near its midpoint; scale bar = 200 µm. B. Whole mount (dorsal view) of *E. cerastes* from *I. punctatus*; scale bar = 500 µm. C. Antenna of same *E. cerastes* showing unique large, blunt medial sensillum (MS) near its midpoint; scale bar = 200 µm. D. Antenna of *E. cerastes* from *Pylodictis olivaris*, showing unique large, blunt medial sensillum (MS) near its midpoint; scale bar = 200 µm.

fish fauna of 180 species in Oklahoma (Miller and Robison 2004), we expect additional new host and geographic distribution records to be reported with surveys of at least some of the 71 species of Lake Texoma fishes (Riggs and Bonn 1959; Miller and Robison 2004), including the possibility of discovering new taxa.

Acknowledgments

We thank members of the Oklahoma Department of Wildlife Conservation, including Matt Mauck, David Routledge, and Cliff Sager for providing the fish reported in this manuscript. We also thank Drs. Scott Gardner and Gabor Racz (HWML) and Renn Tumlison (HSU) for expert curatorial assistance. Dr. Tomáš Scholz (Institute of Parasitology Biology Centre, Czech Republic) provided helpful information on the identification of the histological sections of *P. ambloplitus*. AC acknowledges the support of a St. Norbert College research grant, and CTM thanks Kendra Gross (Southeastern Oklahoma State University, Idabel, OK) and Margaret Sorrell (EOSC-Wilburton, OK) for providing laboratory space.

References

Bush AO, Lafferty KD, Lotz JM, Shostak AW. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. J Parasitol 83:575–583.

Calentine RL, Ulmer MJ. 1961. *Khawia iowensis* n. sp. (Cestoda: Caryophyllaeidae) from *Cyprinus carpio* L. in Iowa. J Parasitol 47:795–805.

Choudhury A, Nadler SA. 2018. Phylogenetic relationships of spiruromorph nematodes (Spirurida: Spiruromorpha) in North American freshwater fishes. J Parasitol 104:496–504.

Choudhury A, Nelson, PA. 2000. Redescription of *Crepidostomum opeongoensis* Caira, 1985 (Trematoda: Allocreadiidae) from fish hosts *Hiodon alosoides* and *Hiodon tergisus* (Osteichthyes: Hiodontidae). J Parasitol 86:1305–1312.

D'Amelio S, Barros NB, Ingrosso S, Fauquier DA, Russo R, Paggi L. 2007. Genetic characterization of members of the genus *Contracaecum* (Nematoda: Anisakidae) from fish-eating birds from west-central Florida, USA, with evidence of new species. Parasitol 134:1041–1051.

Eversole AG. 1981. New North American record of *Gyrodactylus anguillae*. Aquacult Mag 7:40–41.

Garcia-Prieto L. 1990. Descripción de una especie nueva del género *Choanoscolex* La Rue, 1911 (Cestoda: Proteocephalidae), parasite de *Ictalurus meridionalis* de Temascal, Oaxaca, México. Ann Inst Biol Univ Aut Méx Ser Zool 61:337–342.

Griffin MJ, Khoo LH, Torrans L, Bosworth BG, Quiniou SM, Gaunt PS, Pote LM. 2009. New data on *Henneguya pellis* (Myxozoa: Myxobolidae), a parasite of Blue Catfish *Ictalurus furcatus*. J Parasitol 95:1455–1467.

Hoffman GL. 1999. Parasites of North American freshwater fishes. 2nd ed. Ithaca (NY):Comstock Publishing Associates. 539 p.

Hopkins SH. 1966. Comparison of two freshwater shads *Dorosoma* (*Dorosoma*) *cepedianum* and *Dorosoma* (*Signalosa*) *petenense*, in Lake Texoma. J Parasitol 52:843.

Iwanowicz LR, Iwanowicz DD, Pote LM, Blazer VS, Schill WB. 2008. Morphology and 18S rDNA of *Henneguya gurlei* (Myxosporea) from *Ameiurus nebulosus* (Siluriformes) in North Carolina. J Parasitol 94:46–57.

Johnson SK, Rogers WA. 1973. Distribution of the genus *Ergasilus* in several Gulf of Mexico drainage basins. Agric Exp Sta Auburn Univ Bull 445:1–74.

Jones AW, Kerley C, Sneed KE. 1956. New species and a new subgenus of *Corallobothrium* (Cestoda: Proteocephala) from catfishes of the Mississippi Basin. J Tenn Acad Sci 31: 179–185.

Kudo RR. 1920. Studies on Myxosporidia. A synopsis of genera and species of Myxosporidia. Illinois Biol Monogr 5:1–265.

Kudo R. 1929. Histozoic myxosporidia found in fresh-water fishes of Illinois, U.S.A. Archiv für Protistenk 65:364–378.

Kusy H, Barger M. 2017. A new species of *Macroderoides* (Trematoda: Macroderoididae) from Spotted Gar, *Lepisosteus oculatus* (Lepisosteidae), in the Big Thicket National Preserve and surrounding areas, Texas, U.S.A. Comp Parasitol 84:28–31.

Leis EM, Rosser TG, Baumgartner WA, Friffin MJ. 2017. *Henneguya laseeae* n. sp. from Flathead Catfish (*Pylodictis olivaris*) in the upper Mississippi River. Parasit Res 116:81–89.

Mackiewicz JS. 1964. Variations and host-parasite relationships of caryophyllaeids (Cestoidea) from fish of Lake Texoma, Marshall County, Oklahoma. J Parasitol 50:31.

Mackiewicz JS. 1968. Two new caryophyllaeid cestodes from the Spotted Sucker, *Minytrema melanops* (Raf.) (Catostomidae). J Parasitol 54:808–813.

Mackiewicz JS. 1969. *Penarchigetes oklensis* gen. et sp. n. and *Biacetabulum carpiodi* sp. n. (Cestoidea: Caryophyllaeidae) from catostomid fish in North America. Proc Helminthol Soc Wash 36:119–126.

Mackiewicz JS. 1970. *Edlintonia ptychocheila* gen. n. sp. n. (Cestoidea: Capengentidae) and other caryophyllid tapeworms from cyprinid fishes of North America. Proc Helminthol Soc Wash 37:110–118.

McAllister CT, Bursey CR, Robison HW, Connior MB, Trauth SE. 2016. New records of helminth parasites (Trematoda, Cestoda, Nematoda) from fishes in the Arkansas and Red River drainages, Oklahoma. Proc Okla Acad Sci 96:83–92.

McAllister CT, Trauth SE. 2015. *Henneguya* sp. (Myxosporea: Bivalvulida: Myxobolidae) from the Black Bullhead, *Ameiurus melas* (Siluriformes: Ictaluridae), from southeastern Oklahoma. Proc Okla Acad Sci 95:79–82.

McDaniel JS. 1963. Parasites from the genus *Lepomis* (Centrarchidae) in Lake Texoma, Oklahoma. Trans Amer Microsc Soc 82:423–425.

Meglitsch PA. 1937. On some new and known Myxosporidia of the fishes of Illinois. J Parasitol 23:467–477.

Miller RJ, Robison HW. 2004. The fishes of Oklahoma. 2nd edition. Norman (OK): University of Oklahoma Press. 450 p.

Minchew CD. 1977. Five new species of *Henneguya* (Protozoa: Myxosporida) from ictalurid fishes. J Protozool 24:213–220.

Moravec F. 2009. Experimental studies on the development of *Contracaecum rudolphi* (Nematoda: Anisakidae) in copepod and fish paratenic hosts. Folia Parasitol 56:185–193.

Mueller JF. 1936. Notes on some parasitic copepods and a mite, chiefly from Florida fresh water fishes. Amer Midl Nat 17:807–815.

Oros M, Brabec J, Kuchta R, Choudhury A, Scholz T. 2016. A synoptic review of *Promonobothrium* Mackiewicz, 1968 (Cestoda: Caryophyllidea), parasites of suckers (Catostomidae) in North America, with description of two new species. Folia Parasitol 63:008. DOI: 10.14411/fp.2016.008.

Oros M, Uhrovič D, Scholz T. 2018. A new classification of *Glaridacris* Cooper, 1920 (Cestoda:Caryophyllidea), parasites of suckers (Catostomidae) in North America, including erection of *Pseudoglaridacris* n. gen. J Parasitol 104:60–69.

Parker JD, Spall RD, Warner MC. 1971. Two new myxosporidia, *Henneguya gambusi* sp. n. and *Myxosoma pharyngeus* sp. n., in the Mosquitofish, *Gambusia affinis* (Baird and Girard). J Parasitol 57:1297–1301.

Pérez-Ponce de León G, Choudhury A. 2002. Adult endohelminth parasites of ictalurid fishes (Osteichthyes: Ictaluridae) in Mexico: Empirical evidences for biogeographical patterns. Comp Parasitol 69:10–19.

Rábago-Castro JL, Gomez-Flores R, Tamez-Guerra P, Sánchez-Martínez JG, Aguirre-Guzmán G, Loredo-Osti J, Ramírez-Pfeiffer C, de la Cruz-Hernández I. 2013. Spatial and seasonal variations on *Henneguya exilis* prevalence on cage intensive cultured channel catfish (*Ictalurus punctatus*), in Tamaulipas, Mexico. Lat Amer J Aquat Res 41:194–198.

Rice VJ, Jahn TL. 1943. Myxosporidian parasites of some fishes of the Okoboji region. Proc Iowa Acad Sci 5:315–321.

Riggs CD, Bonn EW. 1959. An annotated list of the fishes of Lake Texoma, Oklahoma and Texas. Southwest Nat 4:157–168.

Roberts LS. 1957. Parasites of the Carp, *Cyprinus carpio* L., in Lake Texoma, Oklahoma. J Parasitol 43:54.

Roberts LS. 1969. *Ergasilus cerastes* sp. n. (Copepoda: Cyclopoida) from North American catfishes. J Parasitol 55:1266–1270.

Roberts LS. 1970. *Ergasilus* (Copepoda: Cyclopoida): Revision and key to species in North America. Trans Amer Microsc Soc 89:134–161.

Rosas-Valdez R, Choudhury A, Pérez-Ponce de León G. 2004. Phylogenetic analysis on genera of Corallobothriinae (Cestoda: Proteocephalidea) from North American ictalurid fishes, using partial sequences of the 28S ribosomal gene. J Parasitol 90:1123–1127.

Scholz T. 1997. A revision of the species of *Bothriocephalus* Rudolphi, 1808 (Cestoda:Pseudophyllidea) parasitic in American freshwater fishes. Syst Parasitol 32:85–107.

Scholz T, Brabec J, Král'ová-Hromadová I, Oros M, Bazsalovicsová E, Ermolenko A, Hanzelová V. 2011b. Revision of *Khawia* spp. (Cestoda: Caryophyllidea), parasites of cyprinid fish, including a key to their identification and molecular phylogeny. Folia Parasitol 58:197–223.

Scholz, T, de Chambrier A, Mariaux J, Kuchta R. 2011a. Redescription of *Corallobothrium solidum* (Cestoda: Proteocephalidea) and erection of a new genus, *Essexiella*, for tapeworms from channel catfishes (Ictaluridae). J Parasitol 97:1142–1151.

Scholz T, Oros M, Choudhury A, Brabec J, Waeschenbach A. 2015. New circumscription of freshwater fish parasites *Monobothrium* Diesing, 1863 and *Promonobothrium* Mackiewicz, 1968 (Cestoda: Caryophyllidea) using morphological and molecular evidence. J Parasitol 101:29–36.

Scholz T, Rosas RV, Choudhury A, Pérez-Ponce de León G, de Chambrier A. 2003. Taxonomic status of *Choanoscolex lamothei* García-Prieto, 1990 (Cestoda: Proteocephalidea) using morphological and molecular evidence. J Parasitol 89:1212–1219.

Self JT. 1954. Parasites of the Goldeye, *Hiodon alosoides* (Raf.), in Lake Texoma. J Parasitol 40:386–389.

Self JT, Campbell JW. 1956. A study of the helminth parasites of the buffalo fishes of Lake Texoma with a description of *Lissorchis gullaris*, n. sp. (Trematoda: Lissorchiidae). Trans Amer Micros Soc 75:397–401.

Self JT, Timmons HF. 1955. The parasites of the River Carpsucker (*Carpiodes carpio* Raf.) in Lake Texoma. Trans Amer Microsc Soc 74:350–352.

Sneed KW. 1950. The genus *Corallobothrium* from catfishes in Lake Texoma, Oklahoma, with a description of two new species. J Parasitol 6:43.

Spall RD. 1969. The endoparasitic helminths of fishes from Lake Carl Blackwell, Oklahoma. Proc Okla Acad Sci 49:91–99.

Spall RD, Summerfelt, RC. 1969. Host-parasite relationships of certain endoparasitic helminths of the channel catfish and white crappie in an Oklahoma reservoir. Bull Wildl Dis Assoc 5:48–67.

Wagner EJ. 2016. A guide to the identification of tailed Myxobolidae of the world: *Dicauda, Hennegoides, Henneguya, Laterocapsulata, Neohenneguya, Phlogospora, Tetrauronema, Trigonosporus,* and *Unicauda*. Logan (UT): Fish Creek Records. 167 p.

Williams DD, Sutherland DR. 1981. *Khawia sinensis* (Caryophyllidea: Lytocestidae) from *Cyprinus carpio* in North America. Proc Helm Soc Wash 48:253–255.

Use of Fishes in Research Committee (joint committee of the American Fisheries Society, the American Institute of Fishery Research Biologists, and the American Society of Ichthyologists and Herpetologists). 2014. Guidelines for the use of fishes in research. Bethesda (MD): American Fisheries Society. Available from: https://fisheries.org/docs/wp/Guidelines-for-Use-of-Fishes.pdf (Accessed October 14, 2018).

Submitted October 14, 2018 Accepted December 22, 2018

Endoparasites (Apicomplexa, Monogenoidea, Trematoda, Cestoda, Nematoda, Acanthocephala) from Eleven Reptiles (Testudines: Lacertilia: Ophidia) of McCurtain County, Oklahoma, Including the First Report of the Endogenous Stages of *Eimeria robisoni* (Eimeriidae)

Chris T. McAllister
Science and Mathematics Division, Eastern Oklahoma State College, Idabel, OK 74745

Charles R. Bursey
Department of Biology, Pennsylvania State University-Shenango, Sharon, PA 16146

Stanley E. Trauth
Department of Biological Sciences, Arkansas State University (Emeritus), State University, AR 72467

Michael A. Barger
Department of Biology, Peru State College, Peru, NE 68421

Abstract: Between September 2015 and May 2018, 11 reptiles, including a razor-backed musk turtle (*Sternotherus carinatus*), pallid spiny softshell (*Apalone spinifera pallida*), green anole (*Anolis carolinensis*), broadhead skink (*Plestiodon laticeps*), eastern hognose (*Heterodon platirhinos*), two eastern garter snakes (*Thamnophis sirtalis sirtalis*), prairie kingsnake (*Lampropeltis calligaster calligaster*), southern copperhead (*Agkistrodon contortrix contortrix*), and timber rattlesnake (*Crotalus horridus*) from various sites in McCurtain County, Oklahoma, were examined for endoparasites. Two coccidians (*Eimeria robisoni* and *Choleoeimeria* sp.), an intraerythrocytic hematozoan (*Hepatozoon* sp.), a monogenean (*Polystomoidella oblongum*), two digeneans (*Dasymetra conferta* and *Renifer ellipticus*), two tapeworms (*Testudotaenia testudo* and *Mesocestoides* sp. tetrathyridia), six nematodes (*Capillaria* sp. [ova], *Falcaustra affinis*, *Oswaldocruzia pipiens*, *Serpinema trispinosum*, *Spiroxys amydae* [larvae] and *Kalicephalus inermis coronellae*) and a larval acanthocephalan (*Neoechinorhynchus* sp.) were harbored by these hosts. We document several new host and distributional records for these parasites, including three taxa reported from *L. c. calligaster* for the first time. We also document novel information on the endogenous stages of the coccidian, *E. robisoni*.

Introduction

In the last decade, our parasitological surveys have attempted to help fill a void in our knowledge of various parasites of Oklahoma's amphibians and reptiles (McAllister et al. 2015, 2016, and references therein). Here, we supplement some of that lack of information by

reporting new host and distributional records for endoparasites from 11 reptiles from the foothills of the southwestern Ouachita Mountains and South Central Plains ecoregions of McCurtain County. In addition, we report on the endogenous stages of the coccidian, *Eimeria robisoni*, for the first time.

Methods

Between September 2015 and May 2018, adult specimens of razor-backed musk turtle (*Sternotherus carinatus*), pallid spiny softshell (*Apalone spinifera pallida*), green anole (*Anolis carolinensis*), broadhead skink (*Plestiodon laticeps*), eastern hognose (*Heterodon platirhinos*), prairie kingsnake (*Lampropeltis calligaster calligaster*), two eastern garter snakes (*Thamnophis sirtalis sirtalis*), southern copperhead (*Agkistrodon contortrix contortrix*), and timber rattlesnake (*Crotalus horridus*) were collected by hand, snake tong, or from dead on the road (DOR) from several sites in McCurtain County (Fig. 1), and examined for endoparasites. Specimens were placed in collection bags in the refrigerator (4°C), and necropsied within 24 hr. They were measured for snout-vent length (SVL) or carapace length (CL), killed by an intraperitoneal injection of sodium pentobarbital (Nembutal®) following accepted guidelines (SIH 2004), and examined for apicomplexan and helminth parasites. A bone saw was used to remove the plastron from turtles to expose the heart and a mid-ventral incision from mouth to cloaca was made to expose the same in other reptiles. Blood was obtained from all reptiles by making a small incision in their heart and taking a sample using ammonium heparinized (75 mm long) capillary tubes. Thin films were smeared onto glass slides, air-dried, fixed for 1 min in absolute methanol, stained for 20–30 min with Wright-Giemsa stain, and rinsed in phosphate buffer (pH = 7.0). Slides were scanned at 100× or 400× and when infected cells were found, photographs were taken and length and width (L × W) measurements were made on gamonts of an intraerythrocytic parasite (n = 20) using a calibrated ocular micrometer under a 1,000× oil immersion lens and are reported in micrometers as means ±1SD followed by the ranges. Feces

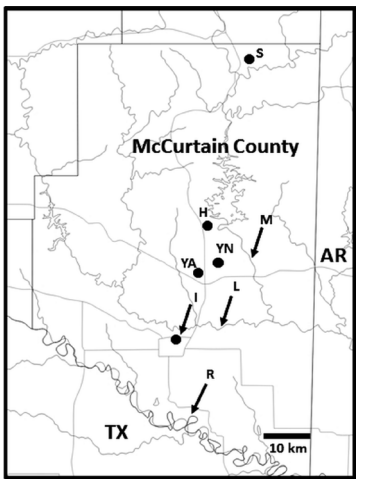


Figure 1. Map showing location of McCurtain County, Oklahoma, and sites (●) where reptilian hosts were collected. Abbreviations: H (Hochatown●); I (Idabel●); L (Little River); M (Mountain Fork River); R (Red River); S (Smithville●); YA (Yashau Creek●); YN (Yanubbee Creek●).

from the rectum was collected from all specimens and examined for coccidia and helminth eggs following the methods of McAllister et al. (2014). Intestinal tissues from *A. carolinensis* were placed in 10% neutral-buffered formalin and processed following Presnell and Schreibmann (1997) for examination of endogenous coccidial stages by light microscopy. Other visceral organs, particularly those of the GI tract from all specimens, were examined for helminths by removing and splitting organs lengthwise, placing separate organs in a Petri dish with 0.9% saline, and their contents scanned at 20–30× using a stereomicroscope. The liver and other suspected infected tissues from the turtle and mesenteries from two eastern garter snakes were also biopsied and specimens processed for examination by light microscopy following Presnell and Schreibman (1997). The conjunctival sac and blood of both turtles were examined for polystomatid monogenes and spirorchiid trematodes, respectively, per Snyder and Clopton (2005). Monogeneans, trematodes and cestodes were fixed in nearly boiling tap water without coverslip pressure, stained with acetocarmine, dehydrated in a graded ethanol series, cleared in methyl salicylate or xylene and mounted in Canada balsam. Nematodes were

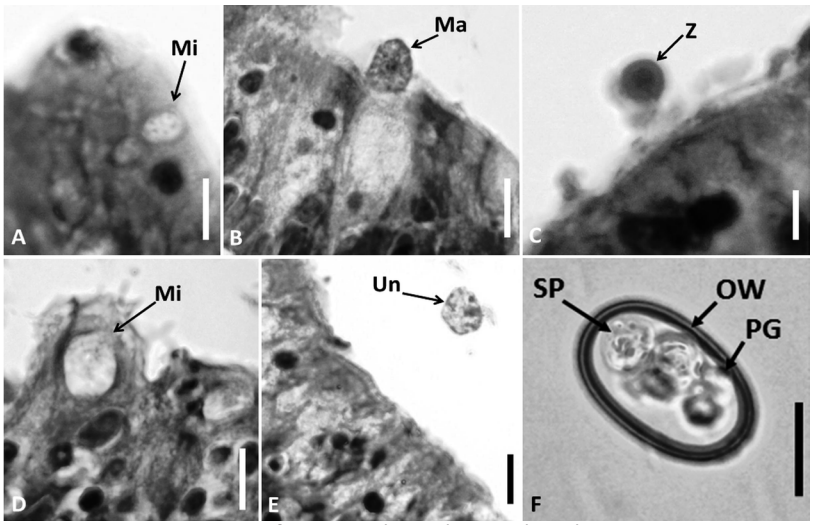
fixed in hot tap water and studied as temporary mounts on a microscopic slide in a drop of glycerol. Acanthocephalans were placed in a Petri dish with tap water overnight to allow for their proboscides to evert, after which they were transferred to 95% (v/v) DNA grade ethanol. Host vouchers are deposited in the Arkansas State University Museum of Zoology (ASUMZ) Herpetological Collection, State University, Arkansas, or the Henderson State University Herpetological Collection (HSU), Arkadelphia, Arkansas. Actual vouchers or photovouchers of parasites are deposited in the Harold W. Manter Laboratory of Parasitology (HWML), University of Nebraska, Lincoln, Nebraska.

Results and Discussion

Fifteen taxa of endoparasites, including three apicomplexans, one monogenean, two digeneans, two tapeworms, six nematodes, and an acanthocephalan were harbored by 11+ hosts. An annotated list of the parasites found and the host data follows.

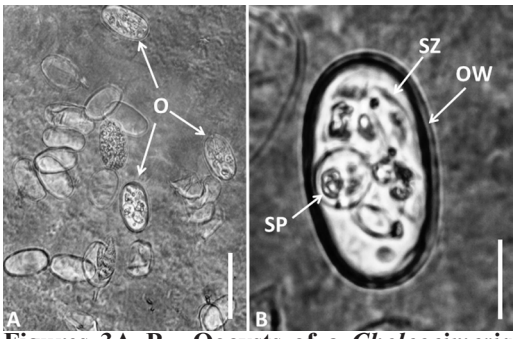
Apicomplexa: Coccidiasina: Eucoccidiorida: Eimeriidae

***Eimeria robisoni* – McAllister, Seville, and Connior, 2014.** – Sporulated oocysts (Fig. 2F) of *E. robisoni* (HWML 139886) were found to be passing in the feces of a single adult (53 mm SVL) *A. carolinensis* collected on 18 September 2016 from Hochatown (34° 09' 55.152"N, 94° 45'35.8776"W). Four other *A. carolinensis* from the same site collected between March 2013 and April 2014 were not passing oocysts. These oocysts matched the description of *E. robisoni* from Arkansas (see McAllister et al. 2014) quite well. The endogenous stages of *E. robisoni* from the intestine are reported here for the first time (Figs. 2A–E). However, these stages appear to be developing in the area of the brush border of epithelial cells (Figs. 2B–D) pushing out into the lumen. As such, they appear to fit nicely into the genus *Acroeimeria* (rather than *Eimeria*) where localization of endogenous development is reported to occur in the microvillus zone of intestinal epithelial cells of reptiles (see Paperna and Landsberg 1989). Further work will be necessary to resolve this placement including molecular analyses. Nevertheless, this eimerian has now been reported in *A. carolinensis* from Oklahoma and, as such, we suggest that additional populations of this anole in other states may as well be infected with the coccidian.



Figures 2A–F. Endogenous stages from the intestinal epithelium and a sporulated oocyst of *Eimeria robisoni* from feces of *Anolis carolinensis*. A. Mi (Microgamont); scale bar = 10 µm. B. Ma (Macrogamont); scale bar = 10 µm. C. Z (zygote); scale bar = 10 µm. D. Mi (microgamont); scale bar = 10 µm. E. Un (Unsporulated oocyst); scale bar = 10 µm. F. Sporulated oocyst, OW (oocyst wall); PG (polar granule); SP (sporocyst); scale bar = 10 µm.

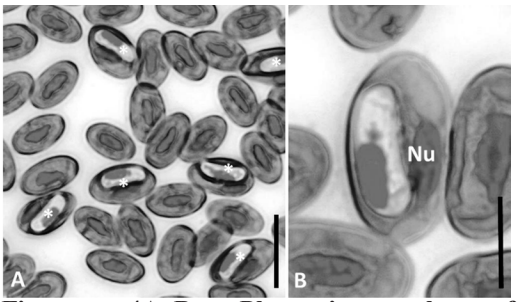
***Choleoeimeria* sp. –** Unsporulated and sporulated oocysts (Figs. 3A–B) of an unknown choleoeimerian (HWML 139887) were found in the feces of a 550 mm SVL *A. c. contortrix* collected on 21 September 2015 from near Yanubbee Creek, just north of Broken Bow off US 259 (34° 03' 45.9216"N, 94° 44' 17.955"W). Coccidians have not been previously reported from any taxon of copperhead (Duszynski and Upton 2009). Since we do not have endogenous stages from gall bladder tissues of this DOR snake, we are reporting this coccidian here with some hesitance. It could represent a pseudoparasite from a prey item of this viperid (perhaps a skink) that was just passing through the digestive tract of the snake. Additional research will be necessary to determine whether or not this is a true coccidian of *A. c. contortrix*.



Figures 3A–B. Oocysts of a *Choleoeimeria* sp. from feces of *Agkistrodon contortrix contortrix*. A. Sporulated oocysts (O, arrows); scale bar = 30 µm. B. Higher magnification of sporulated oocyst showing OW (oocyst wall), SP (sporocyst), and SZ (sporozoite); scale bar = 10 µm.

Adeleorina: Hepatozoidae

***Hepatozoon* sp. Miller, 1908** – About 40% of the red blood cells (rbc's) of a *L. c. calligaster* (adult male, 550 mm SVL), collected on 5 May 2018 from Smithville (34° 28' 0.4794"N, 94° 38' 37.6794"W) contained an intraerythrocytic hematozoan (HWML 139888) thought to belong to the genus *Hepatozoon* (Figs. 4A–B). Measurements (L × W) of bean-shaped gamonts were 16.5 × 6.1 (14–19 × 5–7) µm. In nearly every microscopic field scanned at 400×, infected rbc's could be seen (Fig. 4A). Hematozoans have been previously reported from two other snakes from



Figures 4A–B. Photomicrographs of hematozoans from *Lampropeltis calligaster calligaster*. A. Six gamonts (*) in a single microscopic field at 400× showing parasite intensity; scale bar = 20 µm. B. Single gamont showing nucleus (Nu) of host red blood cell; scale bar = 10 µm.

Oklahoma, the western rat snake, *Pantherophis obsoletus* and *C. horridus* (McAllister 2015). All hemogregarines of snakes are considered to be members of the genus *Hepatozoon* even when life-cycle data is not available, as in our case (Smith 1996; Smith and Desser 1997). We therefore document this hematozoan from *L. c. calligaster* to represent a *Hepatozoon* sp. This is the first time any haemogregarine has been reported from a prairie kingsnake.

Monogenoidea: Polystomatidea: Polystomatidae

***Polystomoidella oblongum* (Wright, 1879) Price, 1939. –** Five polystomes (specimens retained for further work) (Fig. 5) matching the description of *P. oblongum* (Price 1939) were found in the urinary bladder of a 115 mm CL *S. carinatus* collected on 9 October 2016 from Yanubbee Creek off Currence Road at Broken Bow (34° 02' 45.75"N, 94° 43' 19.66"W). Two *Polystomoidella* are known from North America, *P. oblongum* and *P. whartoni* (Wright, 1879) Price, 1939 (Du Preez and Morrison 2012). Both species have been reported from various kinosternid turtles (Ernst and Ernst 1977), and *P. oblongum* was reported previously from *S. carinatus* from Texas (Price 1939). It has also been reported from Oklahoma in common snapping turtle, *Chelydra serpentina* (Williams 1953) but has not, to date, been documented from *S. carinatus* in the state. Here, we report *P. oblongum* in a razor-backed musk turtle from Oklahoma for the first time.

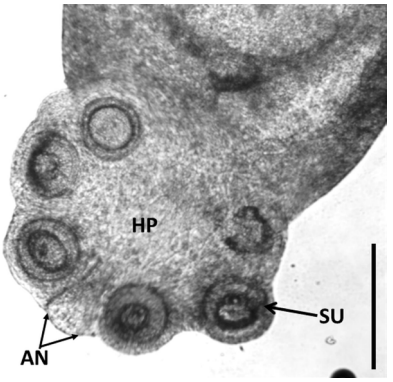


Figure 5. *Polystomoidella oblongum* from urinary bladder of *Sternotherus carinatus*; scale bar = 500 µm. Abbreviations: AN (paired anchors); HP (haptor), SU (sucker).

Trematoda: Digenea: Ochetosomatidae

***Dasymetra conferta* Nicoll, 1911 –** Thirty-five *D. conferta* (specimens retained) were found in the esophagus of an adult (650 mm SVL) *H. platirhinos* collected on 15 October 2017 from the same Hochatown site herein. McAllister and Bursey (2012) and McAllister et al. (2016) previously reported *D. conferta* from diamondback watersnake (*N. rhombifer*) from Yashau Creek (McCurtain County) and from blotched watersnake (*Nerodia erythrogaster transversa*) from Hochatown, respectively. The eastern hognose is a new host record for *D. conferta*.

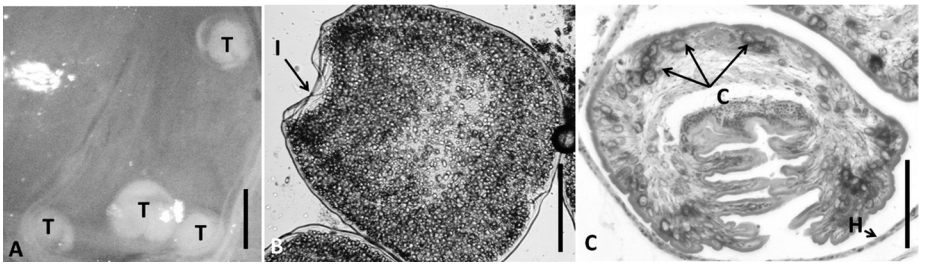
***Renifer ellipticus* Pratt, 1903 –** Three *R. ellipticus* (HWML 110494) were taken from the oral cavity and esophagus of an adult (505 mm SVL) *T. s. sirtalis* collected on 11 May 2018 from the same Hochatown site above. In addition, the same *L. c. calligaster* noted herein harbored two *R. ellipticus* (specimens retained) in its esophagus. This digenean was recently documented from a black racer (*Coluber constrictor priapus*) from the identical Hochatown site (McAllister et al. 2016). This digenean shows little host specificity as *R. ellipticus* has also been reported previously from *H. platirhinos* (locality not given), blue racer, *Coluber constrictor foxi* from Illinois (Dyer and Ballard 1989) as well as other colubrid snakes in North America, including *Coniophanes sp.,* indigo snake, *Drymarchion corais,* northern speckled racer, *Drymobius margaritiferus,* common kingsnake, *Lampropeltis getula,* northern cat-eyed snake, *Leptodeira septentrionalis, Micrurus sp., N. rhombifer,* blackneck garter snake, *Thamnophis cyrtopsis,* and eastern ribbonsnake, *Thamnophis sauritis* from Arizona, New Mexico, South Dakota, and Tabasco, México (see Ernst and Ernst 2006). We document two new host records for *R. ellipticus*.

Cestoda: Eucestoda: Proteocephalidea: Proteocephalidae

***Testudotaenia testudo* (Magath, 1924) de Chambrier, Coquille, Mariaux, and Tkach, 2009 –** Several gravid *T. testudo* were taken from the small intestine of an adult (360 mm CL) *A. s. pallida* collected on 23 September 2017 from Yashau Creek at Broken Bow (34° 02’27.0018”N, 94° 45’ 21.8046”W). Although this tapeworm has been reported previously from Oklahoma in red-eared sliders (*Trachemys scripta elegans*) and map turtles (*Graptemys geographica*), it was in an unpublished dissertation by McKnight (1959). This cestode was originally described by Magath (1924) from eastern spiny softshell (*A. spinifera spinifera*) from Minnesota. It has also been reported from *A. spinifera* (most likely Gulf Coast spiny softshell, *A. s. aspera*) from Louisiana (Acholonu 1970) and redescribed from eastern spiny softshells and bowfins (*Amia calva*) from Tennessee (de Chambrier et al. 2009). In addition, Brooks (1978) reported *Proteocephalus* (=*T.*) *testudo* from *T. s. elegans* from Nebraska but de Chambrier et al. (2009) noted his material “may represent another species” and we concur. We document the first published report of *T. testudo* from Oklahoma as well as a new host record for the tapeworm in the subspecies, *A. s. pallida*. Specimens are being retained for molecular studies (T Scholz, *pers. comm*.).

Cyclophyllidea: Mesocestoididae

***Mesocestoides* sp. –** Tetrathyridia of *Mesocestoides* sp. (Figs. 6A–C) were found in two *T. s. sirtalis* (505, 550 mm SVL) collected on 8 October 2016 and 12 May 2018, both from the same Hochatown site herein. This enigmatic cestode, for whom no complete life cycle is known, has been previously reported from *T. s. sirtalis* from Arkansas (McAllister et



Figures 6A–C. Tetrathyridia of *Mesocestoides* sp. from *Thamnophis sirtalis sirtalis*. A. Low power macroscopic view showing encapsulated tetrathyridia (T) in mesenteries; scale bar = 1.0 mm. B. Unstained whole mount of free tetrathyridium with invaginated scolex (I); scale bar = 250 µm. C. Brightfield microscopic view of stained histological section of tetrathyridium showing calcareous corpuscles (C) in a host-derived fibrotic capsule (H); scale bar = 250 µm.

al. 2014b); it has also been reported previously from Oklahoma in Sequoyah slimy salamander (*Plethodon sequoyah*), Hurter's spadefoot (*Scaphiopus hurterii*), plains spadefoot, (*Spea bombifrons*), American bullfrog (*Rana catesbeiana*), and ground skink (*Scincella lateralis*) (see McAllister et al. 2017c, 2018). It is a cosmopolitan genus that has been documented from a variety of amphibians and reptiles from the Asian, Australo-Papuan, Ethiopian, Nearctic, Neotropical, and Palearctic regions (Bursey et al. 2012; McAllister et al. 2014b). We report *Mesocestoides* sp. in Oklahoma specimens of *T. s. sirtalis* for the first time. These specimens are being processed further for molecular analysis of the genus *Mesocestoides* in reptiles (VV Tkach, *pers. comm.*).

Nematoda: Enoplida: Trichuroidea: Capillariidae

***Capillaria* sp.** – Ova of an unknown *Capillaria* sp. (HWML 139889, Fig. 7) was found to be passing in feces of a 1,000 mm SVL *C. horridus* collected on 10 May 2017 from the campus of Eastern Oklahoma State College, Idabel (33° 55' 16.0572'' N, 94° 46' 35.1084''W). *Capillaria* spp. has been reported previously from *C. horridus* from Virginia (Soloman 1974). *Capillaria* is the only known trichurid genera affecting reptiles and they have a direct life cycle; diagnosis is based on the presence of thick-shelled eggs with polar plugs at both ends (Fig. 7). We document this nematode from an Oklahoma *C. horridus* for the first time.

Ascaridida: Kathlaniidae

***Falcaustra affinis* (Leidy, 1856) Harwood, 1932 –** Twenty-three *F. affinis* (HWML 110495) were found in the rectum of the same *A. s. pallida* noted herein. Mackin (1936) reported *F. affinis* from the eastern river cooter, *Pseudemys concinna concinna* from Oklahoma. A similar species, *F. chelydrae* (Harwood, 1932) has been reported to occur in *A. spinifera* in Oklahoma by McKnight (1959) but that was in his unpublished dissertation. It is obvious that *F. affinis* is a common and widely distributed parasite of turtles (and anurans) of North America, including several species in the families Chelydridae and Emydidae from Arkansas, Florida, Georgia, Illinois, Indiana, Maryland, Ohio, Oklahoma, Oregon, Texas, and Wisconsin, Ontario, Canada, and México (Baker 1986, 1987). We report *F. affinis* from *A. s. pallida* for the first time.

Figure 7. *Capillaria* ova from feces of *Crotalus horridus* showing the characteristic bipolar plugs; scale bar = 30 µm.

Spirurida: Camallanata: Camallanidae

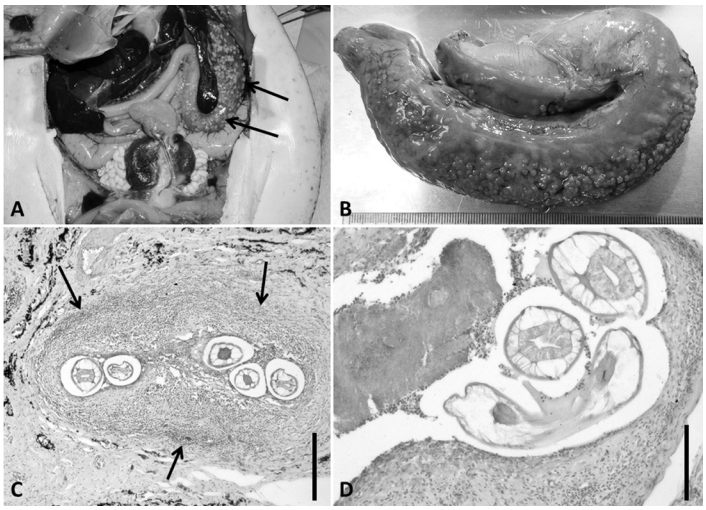
***Serpinema trispinosum* (Leidy, 1852) Yeh, 1960 –**Thirteen *S. trispinosum* (HWML 110496) were found in the small intestine of the same *A. s. pallida* noted herein. This nematode has previously been reported from *P. c. concinna* and *C. serpentina* (McAllister et al. 2015), both from McCurtain County. It has also been reported from at least 18 species of Nearctic turtles ranging from the Canadian border to Texas (Baker 1987; Wiles and Bolek 2015), including an older report from Oklahoma (Harwood 1931). In the life cycle, copepods serve as intermediate hosts, and paratenic hosts include lymnaeid snails, damselflies, anurans, and fish (Moravec and Vargas-Vázquez 1998). Prey items of *A. spinifera* recorded from other parts of its range are larval and adult aquatic insects, crayfish, and fish (Lagler 1943; Ernst and Barbour 1972; Cochran and McConville 1983). To our knowledge, this nematode has not been previously reported from *A. s. pallida*, so we document a new host record for *S. trispinosum* here.

Spirurata: Gnathostomatidae

***Spiroxys amydae* Cobb, 1929 (Figs. 8A–D)** – Nematodes of *S. amydae* (HWML 139893) were found encysted in the stomach, small intestine, and other visceral organs of the same *A. s. pallida* noted above. This nematode has been reported previously in encysted tissues of *A. s. aspera* from Mississippi (Cobb 1929; Hedrick 1935) and México (Peña-Rivera et al. 1994), and from *A. s. pallida* from Texas (Harwood 1932). Similar eroding lesions were reported from Florida softshell (*Apalone ferox*) from Florida (Foster et al. 1998). We report *S. amydae* from Oklahoma for the first time.

Strongylida: Diaphanocephaloidea: Diaphanocephalidae

***Kalicephalus inermis coronellae* (Ortlepp, 1923) Lichtenfels, 1980** – Two (male, female) *K. i. coronellae* (HWML 110417) were taken from the intestine of the same *L. c. calligaster* noted herein. This nematode has been reported from various colubrid and viperid snakes from the United States (Colorado, Florida, Georgia, Louisiana, Massachusetts, North Carolina, and Texas), Québec, Canada, and Guerrero, Michoacán, and Vera Cruz, México (Schad 1962; Baker 1987). This is the first time *K. i. coronellae* has been documented from a prairie kingsnake and from Oklahoma, and more importantly, only the third helminth reported



Figures 8A–D. *Spiroxys amydae* infection in *Apalone spinifera pallida*. A. Macroscopic view of infection in stomach and intestinal tract (arrows). B. Closer view of infection showing white lesions in sectioned stomach. C. Cross sectional microscopic view of infection in stomach showing five *S. amydae* producing a host granulomatous reaction (arrows); scale bar = 2.0 mm. D. Higher power microscopic view of cross section showing three *S. amydae*; scale bar = 1.0 mm.

from this host (McAllister et al. 2008; this study).

Trichostrongyloidea: Molineidae

***Oswaldocruzia pipiens* Walton, 1929** – An adult *P. laticeps* (102 mm SVL) collected on 20 August 2017 from Hochatown harbored eleven (9 male, 2 female) *O. pipiens* (HWML 110502) in its small intestine. This nematode shows little host specificity as it has been reported from various amphibians and reptiles, including salamanders, frogs and toads, skinks (*Scincella lateralis* and *Plestiodon fasciatus*) and other reptilian hosts from Arizona, Arkansas, California, Illinois, Indiana, Louisiana, Maryland, Ohio, Oklahoma, Texas, Washington, and México (see McAllister et al. 2014 for summary). We report a new host record for *O. pipiens* in *P. laticeps*.

Acanthocephala: Eoacanthocephala: Neoechinorhynchida: Neoechinorhyncidae

***Neoechinorhynchus* sp. –** Five juvenile (2 male, 3 female) *Neoechinorhynchus sp.* (HWML 139853–139854) were found in the intestinal tract of the same *A. s. pallida* noted herein. Since these worms were juveniles it was not possible to determine their specific identity. However, *N. chrysemydis* Cable and Hopp, *N. emyditoides* Fisher, and *Neoechinorhynchus* sp. have been reported from *A. spinifera* (subspecies not specified but most likely *A. s. aspera*, see Powell et al. [2016]) from southeastern Louisiana (Acholonu 1966, 1969). We therefore document acanthocephalans from the pallid spiny softshell for the first time.

In summary, we report some new host and geographic records for these parasites, and, more importantly, several for a colubrid snake that has rarely been reported to be harboring any parasite. Indeed, although much is known about the ecology of *L. c. calligaster* (Blaney 1979), information on its parasites is mostly lacking. Coccidian parasites (Apicomplexa) have been reported from this snake in Arkansas (McAllister et al. 1995, 2017b), Illinois (Anderson et al. 1968), and Texas (McAllister et al. 2017a). A single nematode, *Physaloptera abjecta* was documented from *L. c. calligaster* from Arkansas (McAllister et al. 2008). However, the current study is the initial report of an intraerythrocytic hematozoan from *L. c. calligaster* as well as the second and third helminth ever reported from this host. With the diverse reptilian fauna in Oklahoma (Sievert and Sievert 2011), we expect additional new host and geographic distribution records to be reported with extensive surveys, including the possibility of discovering new taxa.

Acknowledgments

The Oklahoma Department of Wildlife Conservation issued a Scientific Collecting Permit to CTM. Drs. Scott L. Gardner and Gabor Racz (HWML) and Renn Tumlison (HSU) provided expert curatorial assistance, Mr. Nikolas H. McAllister (Lukfata Elementary School, Broken Bow, OK) assisted with collections, Ms. Kylie Brecheisen (EOSC-Idabel, OK) provided the prairie kingsnake, and Dr. Donald W. Duszynski (University of New Mexico-Emeritus) gave expert advice on the endogenous coccidial stages. CTM also thanks Ms. Margaret Sorrell (EOSC-Wilburton, OK) and Ms. Kendra Gross (Southeastern Oklahoma State University, Idabel, OK) for providing lab space.

References

Acholonu AD. 1966. Studies on the acanthocephalan parasites of Louisiana turtles. Bull Wildl Dis Assoc 3:40.

Acholonu AD. 1969. Acanthocephala of Louisiana turtles with a redescription of *Neoechinorhynchus stunkardi* Cable and Fischer, 1961. Proc Helminthol Soc Wash 36:177–183.

Acholonu AD. 1970. On *Proteocephalus testudo* (Magath, 1924) (Cestoda: Proteocephalidae) from *Trionyx spinifer* (Chelonia) in Louisiana. J Wildl Dis 6:171–172.

Anderson DR, Duszynski DW, Marquardt WC. 1968. Three new coccidia (Protozoa: Telosporea) from kingsnakes, *Lampropeltis* spp., in Illinois, with a redescription of *Eimeria zamenis* Phisalix, 1921. J Parasitol 54:577–581.

Baker MR. 1986. *Falcaustra* species (Nematoda: Kathlaniidae) parasitic in turtles and frogs in Ontario. Can J Zool 64:228–237.

Baker MR. 1987. Synopsis of the Nematoda parasitic in amphibians and reptiles. Mem Univ Newfoundl Occ Pap Biol 11:1–325.

Blaney RM. 1979. *Lampropeltis calligaster* (Harlan) prairie kingsnake. Cat Amer Amph Rept 229.1–229.2.

Brooks DR. 1978. Systematic status of proteocephalid cestodes from reptiles and amphibians in North America with descriptions of three new species. Proc Helminthol Soc Wash 45: 1–28.

Bursey CR, Goldberg SR, Telford SR Jr, Vitt LJ. 2012. Metazoan endoparasites of 13 species of Central American anoles (Sauria: Polycrotidae: *Anolis*) with a review of the helminth communities of Caribbean, Mexican, North American, and South American anoles. Comp Parasitol 79:75–132.

Cochran PA, McConville DR. 1983. Feeding by *Trionyx spiniferus* in backwaters of the upper Mississippi River. J Herpetol 17:82–86.

Cobb NA. 1929. *Spiroxys amydae*. J Parasitol 15:217–218.

de Chambrier A, Coquille SC, Mariaux J, Tkach V. 2009. Redescription of *Testudotaenia testudo* (Magath, 1924) (Eucestoda: Proteocephalidea), a parasite of *Apalone spinifera* (Le Sueur) (Reptilia: Trionychidae) and *Amia calva* L. (Pisces: Amiidae) in North America and erection of the Testudotaeniinae n. subfam. Syst Parasitol 73:49–64.

Du Preez LH, Morrison C. 2012. Two new polystomes (Monogenea: Polystomatidae) from the eyes of North America freshwater turtles. Zootaxa 3392:47–59.

Duszynski DW, Upton SJ. 2009. The biology of coccidia (Apicomplexa) of snakes of the world: a scholarly handbook for identification and treatment. http//www.CreateSpace.com. 422 p.

Dyer WG, Ballard SR. 1989. Ochetosomatid digeneans from Illinois snakes. Trans Ill Acad Sci 82:159–162.

Ernst CH, Barbour RW. 1972. Turtles of the United States. Lexington (KY): University of Kentucky Press. 347 p.

Ernst CH, Ernst EM. 2006. Synopsis of helminths endoparasitic in snakes of the United States and Canada. SSAR Herpetol Circ No 34:1–86.

Ernst EM, Ernst CH. 1977. Synopsis of helminths endoparasitic in native turtles of the United States. Bull Maryland Herp Soc 13:1–75.

Foster GW, Kinsella JM, Moler PE, Johnson LM, Forrester DJ. 1998. Parasites of Florida softshell turtles (*Apalone ferox*) from southeastern Florida. J. Helminthol Soc Wash 65:62–64.

Harwood PD. 1931. Some parasites of Oklahoma turtles. J Parasitol 25:98–101.

Harwood PD. 1932. The helminths parasitic in the Amphibia and Reptilia of Houston, Texas, and vicinity. Proc US Natl Mus 81:1–71.

Hedrick LR. 1935. Taxonomy of the nematode genus *Spiroxys* (family Spiruridae). J. Parasitol 21:397–409.

Lagler KF. 1943. Food habits and economic relations of the turtles of Michigan with special reference to fish management. Amer Midl Nat 29:257–312.

Mackin JG. 1936. Studies on the morphology and life history of nematodes in the genus *Spironoura*. Ill Biol Monogr 14:1–64.

Magath TB. 1924. *Ophiotaenia testudo*, a new species from *Amyda spinifera*. J Parasitol 11: 44–49.

McAllister CT. 2015. Hematozoa (Apicomplexa: Haemogregarinidae, Hepatozoidae) from two turtles (Testudines: Chelydridae, Emydidae) and two snakes (Ophidia: Colubridae, Viperidae), in southeastern Oklahoma. Proc Okla Acad Sci 95:119–124.

McAllister CT, Bursey CR. 2012. New host and distributional records for helminth parasites (Trematoda, Cestoidea, Nematoda) of herpetofauna from southeastern Oklahoma. Proc Okla Acad Sci 92:29–35.

McAllister CT, Bursey CR, Connior MB. 2015. Helminth parasites (Trematoda, Cestoda, Nematoda, Acanthocephala) of herpetofauna from southeastern Oklahoma: New host and geographic records. Proc Okla Acad Sci 95:125–134.

McAllister CT, Bursey CR, Connior MB. 2016. New host and distributional records for helminth parasites (Trematoda, Cestoda, Nematoda) from amphibians (Caudata, Anura) and reptiles (Testudines: Ophidia) of Oklahoma. Proc Okla Acad Sci 96:76–82.

McAllister CT, Bursey CR, Connior MB. 2017a. New host and distributional records for parasites (Apicomplexa, Trematoda, Nematoda, Acanthocephala, Acarina) of Texas herpetofauna. Comp Parasitol 84:42–50.

McAllister CT, Bursey CR, Connior MB, Durden LA, Robison HW. 2014a. Helminth and arthropod parasites of the ground skink, *Scincella lateralis* (Sauria: Scincidae), from Arkansas and Oklahoma. Comp Parasitol 81:210–219.

McAllister CT, Bursey CR, Trauth SE. 2008. New host and geographic distribution records for some endoparasites (Myxosporea, Trematoda, Cestoidea, Nematoda) of amphibians and reptiles from Arkansas and Texas, U.S.A. Comp Parasitol 75:241–254.

McAllister CT, Connior MB, Bursey CR, Trauth SE, Robison HW, Conn DB. 2014b. Six new host records for *Mesocestoides* sp. (Cestoidea: Cyclophyllidea) from amphibians and reptiles of Arkansas, U.S.A. Comp Parasitol 81:278–283.

McAllister CT, Motriuk-Smith D, Seville RS, Connior MB, Trauth SE, Robison HW. 2017b. Coccidian parasites (Apicomplexa: Eimeriidae) of Arkansas herpetofauna: a summary with two new state records. J Ark Acad Sci 71:143–152.

McAllister CT, Seville RS, Connior MB. 2014. A new caryosporan and eimerian (Apicomplexa: Eimeriidae) from green anoles, *Anolis carolinensis* (Sauria: Dactyloidae), from Arkansas and Louisiana, with a summary of the coccidia of the Dactyloidae. J Parasitol 100:480–484.

McAllister CT, Tkach VV, Conn DB. 2018. Morphological and molecular characterization of post-larval pre-tetrathyridia of *Mesocestoides* sp. (Cestoda: Cyclophyllidea) from ground skink, *Scincella lateralis* (Sauria: Scincidae), from southeastern Oklahoma, USA. J Parasitol 104:246–253.

McAllister CT, Trauth SE, Conn DB. 2017c. First report of *Mesocestoides* sp. tetrathyridia (Cestoda: Cyclophyllidea) from the American bullfrog, *Rana catesbeiana* (Anura: Ranidae). Proc Okla Acad Sci 97:15–20.

McAllister CT, Upton SJ, Trauth SE, Dixon JR. 1995. Coccidian parasites (Apicomplexa) from snakes in the southcentral and southwestern United States: New host and geographic records. J Parasitol 81:63–68.

McKnight TJ. 1959. A taxonomic study of the helminth parasites of the turtles of Lake Texoma [PhD thesis]. Norman (OK): University of Oklahoma. 55 p. Available from: University Microfilms, Ann Arbor, MI: 59–2966.

Moravec F, Vargas-Vázquez, J. 1998. Some endohelminths from the freshwater turtle, *Trachemys scripta* from Yucatan, Mexico. J Nat Hist 32:455–456.

Paperna A, and GH Landsberg. 1989. Description and taxonomic discussion of eimerian coccidian from African and Levantine geckoes. South African J Zool 24:345–355.

Peña-Rivera A, Garza-Treviño E, Fuentes-Pensamiento R. 1994. Pathological changes caused by *Spiroxys amydae* in the stomach of *Apalone spinifera emoryi* (Chelonia: Trionychidae), from Nuevo León, México. Publ Biol, Fac Cien Biol, Univ Autónoma de Nuevo León 7:45–48.

Powell R, Conant R, Collins JT. 2016. Peterson field guide to reptiles and amphibians of eastern and central North America. 4th Edition. Boston (MA): Houghton Mifflin Harcourt. 494 p.

Presnell JK, Schreibman MP. 1997. Humason's animal tissue techniques. Fifth edition. Baltimore (MD): Johns Hopkins University Press. 600 p.

Price EW. 1939. North American monogenetic trematodes. IV. The family Polystomatidae (Polystomatoidea). Proc Helminthol Soc Wash 6:80–92.

Schad GA. 1962. Studies on the genus *Kalicephalus* (Nematoda: Diaphanocephaloidea). II. A taxonomic revision of the genus *Kalicephalus* Molin, 1861. Can J Zool 40:1035–1165.

Sievert G, Sievert L. 2011. A field guide to Oklahoma's amphibians and reptiles. Oklahoma City (OK): Oklahoma Department of Wildlife Conservation. 211 p.

Smith TG. 1996. The genus *Hepatozoon* (Apicomplexa: Adeleina). J Parasitol 82:565–585.

Smith TG, Desser SS. 1997. Phylogenetic analysis of the genus *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina). Syst Parasitol 36:213–221.

Snyder SD, Clopton RE. 2005. New methods for the collection and preservation of spirorchiid trematodes and polystomatid monogeneans from turtles. Comp Parasitol 72:102–107.

Society of Ichthyologists and Herpetologists (SIH). 2004. Guidelines for use of live amphibians and reptiles in field and laboratory research. Second Edition, Revised by the Herpetological Animal Care and Use Committee (HACC) of the American (Committee Chair: Beaupre SJ, Jacobson ER, Lillywhite HB, Zamudio K.). Available from: https://www.aaalac.org/accreditation/RefResources/SS_Amphib.pdf (Accessed September 30, 2018).

Solomon GB. 1974. Probable role of the timber rattlesnake, *Crotalus horridus*, in the release of *Capillaria hepatica* (Nematoda) eggs from small mammals. Virginia J Sci 25:182–184.

Wiles CM, Bolek MG. 2015. Damselflies (Zygoptera) as paratenic hosts for *Serpinema trispinosum* and its report form turtle hosts from Oklahoma, USA. Folia Parasitol 62:019. DOI: 10.14411/fp.2015.019.

Williams RW. 1953. Helminths of the snapping turtle, *Chelydra serpentina*, from Oklahoma, including the first report and description of the male of *Capillaria serpentina* Harwood, 1932. Trans Amer Microsc Soc 72:175–178.

Submitted October 14, 2018 Accepted November 18, 2018

Isolation of Four Mycobacteriophages from Oklahoma Soil and Testing Their Infectivity Against *Mycobacterium abscessus*

Ahmed K. Ali
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

Hari Kotturi
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Abstract: Mycobacteriophages are phages that infect genus mycobacteria. Mycobacteriophages have potential application in phage-based diagnostic and phage therapy for identification and treatment of diseases caused by pathogenic mycobacteria. Four mycobacteriophages: Irak (IR), Fulbright (F), Ibrahim (IB), and Ahmed (A) were isolated from soil samples collected from different locations from the University of Central Oklahoma campus using *Mycobacterium smegmatis* mc^2155 as a host. The genomic DNA of the purified phages was subjected to restriction digest using BamH1, Cla1, EcoR1, HaeIII, and HindIII enzymes. The restriction digestion patterns of the four phage genomes were distinct. Fulbright and Ibrahim phage genomes have been sequenced with genome lengths of 42396 (bp) and 42596 (bp) respectively. The ability of the four viruses to infect *Mycobacterium abscessus* was determined using the spot test. Here, we also report the isolation of a mycobacteriophage Fulbright from the Oklahoma soil which can infect *Mycobacterium abscessus*. Transmission electron microscopy showed that all phages have siphoviridae morphology with isometric heads and flexible, non-contractile tails.

Introduction

Mycobacteriophages are viruses that infect the members of genus mycobacteria. *Mycobacterium smegmatis*, a soil-borne, non-pathogenic bacterium was used as a host to isolate the first mycobacteriophage (Gardner and Weiser 1947). Later, mycobacteriophages gained considerable attention due to their application in diagnosis, typing, and control of pathogenic mycobacterial species, such as *Mycobacterium tuberculosis* and *Mycobacterium leprae* (Broxmeyer et al., 2002, McNerney and Traore 2005, Hatfull 2014).

As of August 2018, 9829 mycobacteriophages were isolated using different mycobacterial species as hosts. Approximately, 1623 have been sequenced (The Actinobacteriophage Database 2018). Mycobacteriophages display a large genetic diversity (Hatfull et al., 2006). Depending upon their nucleotide sequence and gene content comparison, mycobacteriophages have been assigned to clusters (A-Z). Some clusters are divided into subclusters based on nucleotide sequence relatedness and phages without any close relatives are referred to as singletons (Hatfull 2012).

The host range of bacteriophages is generally known to be narrow. For example, phages isolated using *Vibrio parahaemolyticus* were able to infect a few strains of this species, but not other *Vibrio* species (Koga et al., 1982). The constraints that restrict the host range include host restriction-modification system, receptor-attachment compatibility, and abortive infection. However, some bacteriophages like P1 (Yarmolinsky and Sternberg 1988), Mu (Harshey 1988), SN and BHR (Jensen et al., 1998) have a broad host range and can infect hosts of other genera. The ability of mycobacteriophages to infect other mycobacterial hosts has been tested (Jacobs-Sera et al., 2012). A wide range

of mycobacteriophages has been proven to traverse the species-barrier and infect other mycobacterial hosts of clinical importance such as *M. tuberculosis*, *M. avium* (Broxmeyer et al., 2002), *M. bovis*, and *M. ulcerans* (Rybniker et al., 2006).

M. abscessus is known to be a causative agent for a wide range of skin and pulmonary infections (Moore and Frerichs 1953, Sermet-Gaudelus et al., 2003, Koh et al., 2010, Lee et al., 2015). The increased resistance of *M. abscessus* to antibiotics (Nessar et al. 2012) has prompted interest in Mycobacteriophage-based phage therapy to control infections (Broxmeyer 2004, McNerney and Traore 2005). To date, prophage Araucaria is the only mycobacteriophage isolated from *M. abscessus* subsp. *bolletii*. This prophage was detected in the cultures of specimens taken from respiratory tract infections of patients with lung disorders. The morphological and genomic characteristics of Araucaria were extensively analyzed following the retrieval of the prophage genome from *M. abscessus* subsp. *bolletii* CIP108541^T (Sassi et al. 2013). We report the isolation, purification and the characterization of four new mycobacteriophages isolated from Oklahoma soil using *M. smegmatis* mc^{2}155 and testing their infectivity against *M. abscessus.*

Methods

Phage isolation and purification

Four soil samples were collected from the University of Central Oklahoma campus. The global positioning system (GPS) coordinates of the collected soil samples that yielded four phages are as follows: IR (35.655^0 N, 97.473^0 W), F (35.658^0 N, 97.474^0 W), IB (35.656^0 N, 97.474^0 W) and A (35.655^0 N, 97.473^0 W). Soil samples were enriched as previously described (Patton and Kotturi 2018). Briefly, three grams of a soil sample were enriched in 10 ml of 7H9 broth (Dubos and Middlebrook 1947) containing 10% Albumin Dextrose Complex (ADC), 1mM $CaCl_2$ and 1 ml of overnight *M. smegmatis* mc^{2}155 culture. The enriched soil samples were incubated at 37^{0}C for 24 hours. After incubation, samples were filter sterilized using a 0.22 μ filter. To screen the filtered sample for the presence of phages, five microliters of the filtrate was plated on *M. smegmatis* mc^{2}155 lawn using an agar overlay method. The plaque forming samples were serially diluted using 10-fold dilution series in phage buffer (10 mM Tris pH 7.5, 10 mM MgSO4, 1 mM $CaCl_2$, and 68.5 mM NaCl). A ten-microliter aliquot of the samples was plated again using the agar overlay method. For phage purification, a single plaque was picked, diluted in phage buffer and plated. Three plaque purifications were performed to obtain a pure clone of each virus. The purified phages were amplified by seeding eight plates with high titer phage lysate and followed by salt precipitation with 1 M NaCl, polyethylene glycol 8,000 (10% V/V). Phage stock solution was prepared by resuspending phage in phage buffer. The phage stock solution was used for all the experiments.

Sample preparation for electron microscopy

100 ul aliquot of high titer phage lysate (5x10^9 pfu/ml) was concentrated at 4^{0}C top speed in a microcentrifuge for 20 min. The supernatant was carefully removed, and then the pellet was resuspended by 50 ul of phage buffer. The phage suspension was placed on a carbon electron microscope grid and stained with 1% uranyl acetate. Images were taken using a ZEISS 10A conventional transmission electron microscope.

Phage DNA extraction, restriction enzyme digestion, and genome sequencing

High titer phage lysate (5x10^9 pfu/ml) was treated with DNaseI and RNaseA for 30 min at 37^{0}C. The genomic DNA (gDNA) was extracted using a sodium dodecyl sulfate (SDS)/ phenol:chloroform: isoamyl alcohol (PCI) (25:24:1 V/V) extraction method as previously described (Green and Sambrook 2012). The extracted gDNA was treated with five different restriction enzymes (BamHI, ClaI, EcoRI, HaeIII, and HindIII,) as per the manufacturer's recommendation (New England Biolabs). The restriction digests were visualized by ethidium bromide-stained agarose gel using Gel Doc. An undigested DNA sample was used as a control. Genomes of two viruses Fulbright and Ibrahim were sequenced using Illumina® Next-

Generation Sequencing Technology on a MiSeq platform.

Phage infectivity against *Mycobacterium abscessus*

The isolated phages were tested against *M. abscessus* using the protocol described before (Jacob Sera et al. 2012). Briefly, 3 ul phage lysates were spotted onto fresh lawns of *M. abscessus* grown on 7H10 supplemented with 10% ADC, 1 mM $CaCl_2$, at 37^0C. The plates were inspected for plaques formation after five days.

Results and Discussion

Four new mycobacteriophages Irak (IR), Fulbright (F), Ibrahim (IB) and Ahmed (A) were isolated from four soil samples in the summer of 2017. As described above, the phages were isolated by enrichment method, in which the soil was incubated with the host, *M. smegmatis* mc^2155, culture. Phage filtrates were serially diluted and plated with the bacterial host. Upon the inspection of plaques, phages (IR, F, and A) formed relatively large plaques (2.5-4 mm); however, the plaque size of phage (IB) was less than 1 mm diameter. IR, F and A formed clear and round plaques. However, (IB) formed plaques with a clear center and fuzzy halo (Figure 1. A, D, G, and J).

The genomic DNA of four isolated phages was digested with five different restriction enzymes (BamHI, ClaI, EcoRI, HaeIII, and HindIII). These restriction enzymes recognize and cut specific nucleotide sequences in the double-stranded DNA. Each phage DNA sample produced a unique restriction pattern (Figure 1. B, E, H, and K, lanes 3, 4, 5, 6, and 7). Untreated phage DNA samples (Figure 1. Lane 2) of each virus was used as a control for comparing restriction digests. The results indicated the presence of multiple restriction sites specific to HaeIII (Figure 1. B, E, H, and K lanes 6) among all phages even though their restriction patterns were distinct. Restriction enzyme digestion is a commonly used technique for determining the genomic fingerprints of the isolated phages and often facilitates grouping isolated phages into specific clusters. Gissendanner et al., (2014) proposed a tool relying on analyzing restriction endonuclease patterns, rather than genome sequencing, to facilitate assigning discovered mycobacteriophages into possible clusters. Restriction analysis also gives an insight into the diversity even though the phages were isolated using a common host (Hatfull 2011). Two of the four phage genomes have been sequenced. The genome length of phage Fulbright is 42396 (bp) with GC content of 66.3% and belongs to cluster N of mycobacteriophages. Phage Ibrahim belongs to cluster T with a GC content of 66.1% and genome length of 42596 (bp).

Morphological characteristics of all four mycobacteriophages were examined using transmission electron microscopy (Figure 1. C, F, I, and L). The four viruses isolated in this study exhibit Siphoviridae morphotype with isometric heads and long, flexible non-contractile tails. The average tail lengths of all four phages are 225 nm. This morphology is one of the most common morphologies in mycobacteriophages with tail lengths ranging from 110 to 300 nm (Hatfull et al., 2010, Pope et al., 2011).

Testing the host range of the isolated mycobacteriophages would contribute to the development of techniques for detecting and controlling pathogenic mycobacteria such as *M. tuberculosis* and *M. avium* in clinical samples (Alcaide et al. 2003, Perkins 2000). In this study, we examined the ability of the four isolated phages (IR, F, IB, and A) to infect and lyse *M. abscessus*. Purified lysates of the phages isolated using *M. smegmatis* mc^2155 were spotted on active cultures of *M. abscessus*. Our results indicate that mycobacteriophage F can effectively infect and lyse *M. abscessus*, but the other three phages (IR, IB, and A) failed to form any plaques (Figure 2. A, C, and D). Previous studies have shown that some mycobacteriophages were able to infect different species of mycobacteria through mutations in tail fiber genes (Jacobs-Sera et al., 2012).

The specificity of the infection process is often restricted by different mechanisms like restriction-modification system of the host, receptor-attachment site interaction (Hyman and

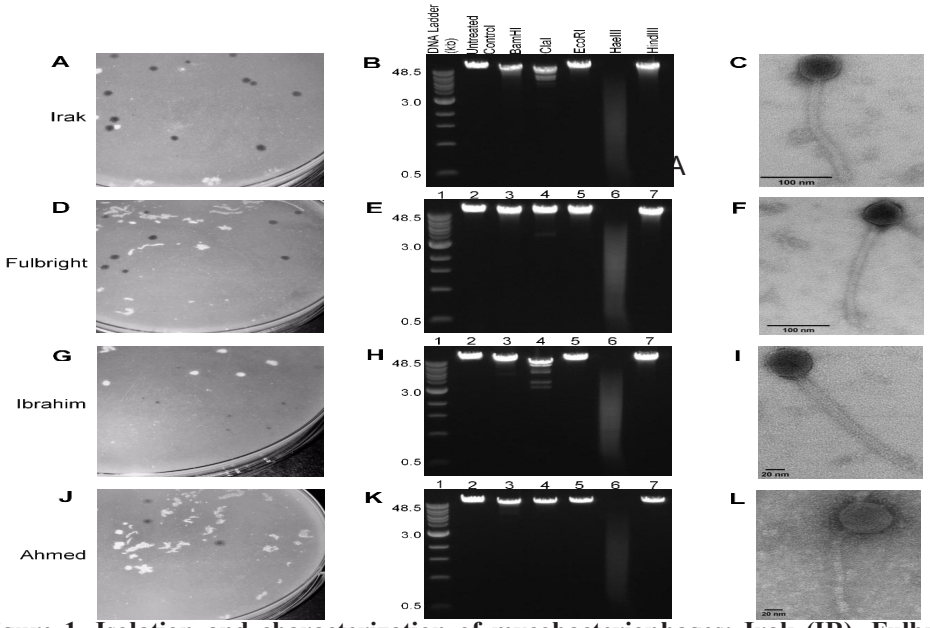


Figure 1. Isolation and characterization of mycobacteriophages: Irak (IR), Fulbright (F), Ibrahim (IB), and Ahmed (A). Panel A, E, D and J represent plaque morphology on *M. smegmatis* mc²155 lawns. Panel B, E, H, and K represents restriction digest patterns of the four phages genomic DNA with five restriction enzymes (BamHI (lane3), ClaI (lane4), EcoRI (lane5), HaeIII (lane6), and HindIII (lane7); Lane 1 is a 1-kb size marker; Lane 2 contains undigested phage genomic DNA. Panel C, F, I and L represent transmission electron microscope images of the four phages with a Siphoviridae morphotype.

Abedon, 2010), and abortive infection (Fineran et al. 2009). Although bacteriophage infection process is specific and its underlying mechanism is poorly understood, testing the host range of newly-isolated phages is a worthy task.

In this work, we report the isolation of characterization of four mycobacteriophages from Oklahoma soil. The genome of two of the four viruses has been sequenced. One of the isolated phages has a broad host range infecting *M. smegmatis* and *M. abscessus*. In our future work, we will further investigate the host range of Fulbright and would explore the possibility of using this phage in phage therapy.

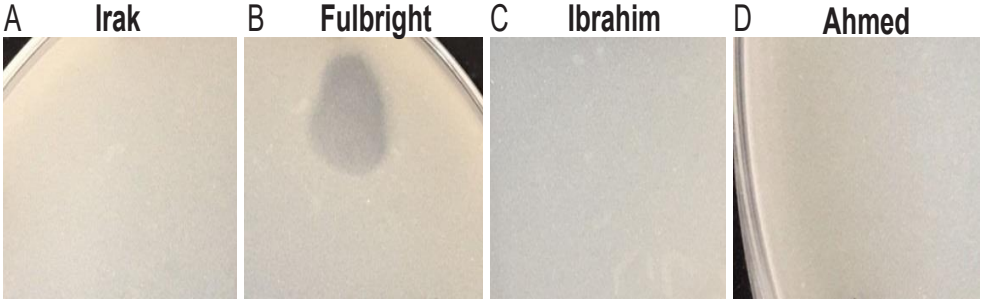


Figure 2. Host range of the four mycobacteriophages against *M. abscessus*. (A, B, C, and D) Irak (IR), Fulbright (F), Ibrahim (IB), and Ahmed (A) phage lysates spotted on *M. abscessus* lawns. (B) Mycobacteriophage Fulbright infects *M. abscessus*.

Acknowledgment

We would like to thank Dr. Graham Hatfull at the University of Pittsburg for sharing *M. smegmatis* mc^{2}155. *M. abscessus*, strain MC1518 was obtained from BEI Resources, NIAID, NIH. We would like 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.

References

Alcaide F, Gali N, Dominguez J, Berlanga P, Blanco S, Orus P and Martin R. 2003. Usefulness of a new mycobacteriophage-based technique for rapid diagnosis of pulmonary tuberculosis. J. Clin. Microbiol. 41: 2867-2871.

Broxmeyer L, Sosnowska EM, Chaco´n O, Wagner D, McGarvey J, Barletta RG, Bermudez LE. 2002. Killing of *Mycobacterium avium* and *Mycobacterium tuberculosis* by a mycobacteriophage delivered by a nonvirulent Mycobacterium: A model for phage therapy of intracellular bacterial pathogens. J. Infect. Dis. 186: 1155-1160.

Broxmeyer L. 2004. Bacteriophages: antibacterials with a future?. Med. Hypotheses. 62: 889-893.

Dubos RJ, and Middlebrook G. 1947. Media for tubercle bacilli. Am. Rev. Tuberc. 56:334-345.

Fineran PC, Blower TR, Foulds IJ, Humphreys DP, Lilley KS, Salmond GP. 2009. The phage abortive infection system, ToxIN, functions as a protein-RNA toxin-antitoxin pair. Proc Natl Acad Sci U S A. 106:894–899.

Gardner GM, and Weiser RS. 1947. A bacteriophage for *Mycobacterium smegmatis*. Proc. Soc. Exp. Biol. Med. 66**:**205-206.

Gissendanner CR, Wiedemeier AMD, Wiedemeier PD, Minton RL, Bhuiyan S, Harmson JS, and Findley AM. 2014. A web-based restriction endonuclease tool for mycobacteriophage cluster prediction. J. Basic Microbiol. 54: 1-6.

Green MR, and Sambrook J. 2012. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, New York, NY: Cold Spring Harbor Laboratory Press.

Harshey RM. 1988. Phage Mu. In: Callendar R, ed. The bacteriophages. New York, NY: Plenum Press. 193-234.

Hatfull, GF, Hendrix, RW. 2011. Bacteriophages and their genomes. Curr. Opin. Virol. 1: 298-303.

Hatfull GF, Jacobs-Sera D, Lawrence JG, Pope WH, Russell DA, Ko CC, Weber RJ, Patel MC, Germane KL, Edgar RH, Hoyte NN, Bowman CA, Tantoco AT, Paladin EC, Myers MS, Smith AL, Grace MS, Pham TT, O'Brien MB, Vogelsberger AM, Hryckowian AJ, Wynalek JL, Donis-Keller H, Bogel MW, Peebles CL, Cresawn SG, Hendrix RW. 2010. Comparative genomic analysis of sixty mycobacteriophage genomes: Genome clustering, gene acquisition and gene size. J. Mol. Biol. 397(1): 119–143.

Hatfull GF, Pedulla ML, Jacobs-Sera D, Cichon PM, Foley A, Ford ME, Gonda RM, Houtz JM, Hryckowian AJ, Kelchner VA, Namburi S, Pajcini KV, et al. 2006. Exploring the mycobacteriophage metaproteome: Phage genomics as an educational platform. PLoS Genet. 2(6):e92.

Hatfull GF. 2012. The Secret Lives of Mycobacteriophages. In: Lobocka M, and Szybalski WT, eds. Advances in virus research. Vol. 82, Burlington: Academic Press. 179-288.

Hatfull GF. 2014. Mycobacteriophages: Windows into tuberculosis. PLoS Pathog. 10(3): e1003953.

Hyman P, Abedon ST. 2010. Bacteriophage host range and bacterial resistance. Adv. Appl. Microbiol. 70:217–248.

Jacobs-Sera D, Marinelli L, Bowman C, Broussard G, Guerrero C, Boyle M, Petrova Z, Dedrick R, Pope W, Science Education Alliance Phage Hunters Advancing Genomics and Evolutionary Science (SEAPHAGES) program, Modlin RL, Hendrix RW, Hatfull1 GF. 2012. On the nature of mycobacteriophage diversity and host preference. Virol. 434 (2): 187-201.

Jensen EC, Schrader HS, Rieland B, Thompson TL, Lee KW, Nickerson KW, Kokjohn TA. 1998. Prevalence of broad host range lytic bacteriophages of *Sphaerotilus natans*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 64 (2): 575-580.

Koga T, Toyoshima S, Kawata T. 1982. Morphological varieties and host ranges of *Vibrio parahaemolyticus* bacteriophages isolated from seawater. Appl. Environ. Microbiol. 44 (2): 466-470.

Lee MR, Sheng WH, Hung CC, Yu CJ, Lee LN, Hsueh PR. 2015. *Mycobacterium abscessus* complex infections in humans. Emerg. Infect. Dis. 21 (9): 1638-1646.

McNerney R, and Traore´ H. 2005. Mycobacteriophage and their application to disease control. J. Appl. Microbiol. 99: 223-233.

Moore M, Frerichs JB. 1953. An unusual acid-fast infection of the knee with subcutaneous, abscess-like lesions of the gluteal region. J. Investig. Dermatol. 20 (2): 133-169.

Nessar R, Cambau E, Reyrat JM, Murray A, Gicquel B. 2012. *Mycobacterium abscessus*: a new antibiotic nightmare. J. Antimicrob. Chemother. 67: 810–818.

Patton CJ, Kotturi H. 2018. Genomic sequence Mycobacteriophage OKCentral 2016. Genome Announc. 6(8): e01208-17.

Perkins, MD. 2000. New diagnostic tools for tuberculosis. Int. J. Tuberc. Lung Dis. 4:S182–S188.

Pope WH, Jacobs-Sera D, Russell DA, Peebles CL, Al-Atrache Z, Alcoser TA, Alexander LM, Alfano MB, Alford ST, et al. 2011. Expanding the diversity of mycobacteriophages: Insights into genome architecture and evolution. PLoS One 6 (1): e16329.

Rybniker J, Kramme S, Small PL. 2006. Host range of 14 mycobacteriophages in *Mycobacterium ulcerans* and seven other mycobacteria including *Mycobacterium tuberculosis* – application for identification and susceptibility testing. J. Med. Microbiol. 55: 37-42.

Sassi M, Bebeacua C, Drancourt M, Cambillaua C. 2013. The First Structure of a Mycobacteriophage, the *Mycobacterium abscessus* subsp. *bolletii* Phage Araucaria. J. Virol. 87 (14): 8099-8109.

Sermet-Gaudelus I, Bourgeois ML, Pierre-Audigier C, Offredo C, Guillemot D, Halley S, Akoua-Koffi C, Vincent V, Sivadon-Tardy V, Ferroni A, Berche P, Scheinmann P, Lenoir G, Gaillard JL. 2003. *Mycobacterium abscessus* and Children with Cystic Fibrosis. Emerg. Infect. Dis. 9 (12): 1587-1591.

The Actinobacteriophage Database. 2018. Host Genus: *Mycobacterium* [online]. Available from: http://phagesdb.org. (Accessed August 7, 2018).

Yarmolinsky RM, and Sternberg N. 1988. Bacteriophage P1. In: Callendar R, ed. The bacteriophages. New York, NY: Plenum Press. 291-438.

Submitted August 15, 2018 Accepted December 3, 2018

Nasal Carriage of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* (MRSA) in Students at the University of Central Oklahoma

Rachael Baalman
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Yuhang Shang
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Jonathon Ryan Johnston
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Mohamed K. Fakhr
Department of Biology, University of Tulsa, Tulsa, OK 74104

Robert Brennan
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Abstract: Nasal carriage of *Staphylococcus aureus* has been identified as a significant risk factor for subsequent infections and is a target for decolonization approaches. The efficacy of the decolonization methods may be dependent on the load and type of *S. aureus* present in the nose of individuals in the community. The objectives of this study were to determine the rates of carriage for *S. aureus* and MRSA, quantify the level of *S. aureus* and MRSA carriage, and to determine the relatedness of the *S. aureus* and MRSA isolates recovered from a healthy student population. Nasal swab specimens were collected from 247 healthy University of Central Oklahoma students, serially diluted, and cultured onto blood agar plates containing 4% NaCl for qualitative and quantitative analysis. Methicillin resistance was determined with cefoxitin disk diffusion and PCR for *mecA*. Relatedness was determined by *spa* sequence typing. *S. aureus* prevalence was 21.5% (MRSA 2.4%), with a geometric mean of 1,820 CFU/swab (MRSA 412 CFU/swab). Twenty-two different spa types were identified among the 42 *spa* positive *S. aureus*/MRSA positive samples. *S. aureus*/MRSA carriage rates were similar to other studies. *spa* typing revealed a high degree of carriage diversity.

Introduction

In the past few decades, the rate of infections caused by *S. aureus* has increased. *S. aureus* has an incredible ability to become resistant to antibiotics (DeLeo et al. 2010). Two years after methicillin was introduced in 1961, resistant species were found (DeLeo et al. 2010). Methicillin-resistant *S. aureus* (MRSA) has become a major cause of nosocomial infections worldwide (Hallin et al. 2007) and the National Nosocomial Infection Surveillance (NNIS) System showed an increase in infections caused by MRSA in ICU patients over time (Boucher and Corey 2008). MRSA kills approximately 19,000 hospitalized patients every year (Boucher and Corey 2008). The rise in MRSA infections has increased health care costs due to its multidrug resistance (Buehlmann et al.

2008); these isolates are resistant to all β-lactam drugs (cephalosporins and penicillins) (Gorwitz et al. 2008). Some of the more serious diseases commonly caused by MRSA are bacteremia, endocarditis, and pneumonia (Hallin et al. 2008; Boucher and Corey 2008; Gorwitz et al. 2008).

S. aureus is considered to be part of the normal flora because it is present in one in three people without causing an associated disease (DeLeo et al. 2010). The anterior nares are the most frequently colonized site (Wertheim et al. 2005). Colonization is a high risk factor for subsequent infections despite most colonized individuals not developing a disease (Gorwitz et al. 2008). An association was first found between *S. aureus* nasal carriage and staphylococcal disease in the early 1930's (Wertheim et al. 2005).

In the past 20 years, concern has shifted to healthy individuals with MRSA infections who have had no contact with a health care facility (Klevens et al. 2006). The strains causing disease in the community are distinctly different from those found in a hospital setting and tend to be less resistant to non-β-lactam drugs (Klevens et al. 2006). The conclusion drawn from healthy individuals getting community-associated MRSA (CA-MRSA) is that these strains have greater virulence than hospital-associated MRSA (HA-MRSA) (DeLeo et al. 2010). In the USA, MRSA has become one of the leading causes of death by a single infectious agent (DeLeo et al. 2010; Hallin et al. 2008); it has also become a problem in almost all industrialized countries, although not all are at the same level (DeLeo et al 2010). MRSA strains acquire resistance through the acquisition of a mobile exogenous element called staphylococcal chromosomal cassette *mec* (SCC*mec*) which contains the *mecA* gene that encodes for resistance to β-lactam antibiotics (DeLeo et al. 2010; Hallin et al. 2008). While there are no risk factors for CA-MRSA it seems to be linked to person-to-person contact with a colonized individual and occurs more frequently among prison inmates, athletes, individuals using intravenous drugs, and children in day cares (DeLeo et al. 2010; Boucher and Corey 2008; Buehlmann et al. 2008).

In order to reduce the numbers of staphylococcal infections in hospitalized patients, many hospitals administer MRSA pre-admission screening in order to control high risk patients. Guidelines have recently been put in place for anti-staphylococcal drugs to be used for temporary decolonization (Wertheim et al. 2005; Rohr et al. 2003). These treatments include mupirocin, which is a topical intranasal cream, and bathing in an antiseptic-detergent (Rohr et al. 2003). It has been shown that these methods significantly decrease nasal and whole-body, though not to the same extent, MRSA colonization (Rohr et al. 2003). The decrease in colonization led to fewer incidences of subsequent infections in MSSA and MRSA patients (Buehlmann et al. 2008).

Within the *Staphylococcus aureus* species there are numerous strains, most of which can be characterized by their *spa* gene. One study found that MRSA isolates with *spa* type t041 were more difficult to decolonize than other *spa* types, although the difference was not statistically significant (Beuhlmann et al. 2008).

The goal of this study was to quantify and characterize *S. aureus* isolates found colonizing the anterior nares in a healthy population of college students. In order to develop safe and effective decolonization strategies to reduce the potential for post-surgical *S. aureus* and MRSA infections, we first need to understand the epidemiology of those found in healthy individuals to allow development of the most effective strategies. The efficacy of these methods may be dependent on the load and type of *S. aureus* present. Not all strains of *S. aureus* or MRSA react to antibiotics or decolonization efforts in the same manner. Our general hypothesis is that carriage levels and *spa* types of MSSA and MRSA are highly variable in a healthy student population. To test this hypothesis, we determined the rates of carriage, quantified the level of carriage, and determined the *spa* types of the *S. aureus* and MRSA isolates obtained.

Materials and Methods

Specimen collection, isolation, identification and characterization: Nasal swab samples were collected from students at the University of Central Oklahoma from July 2010 to July 2012. Students were only allowed to participate after signing the informed consent as approved by the Institutional Review Board (UCO IRB# 10104). Samples were collected from the external nares using the Copan Eswab. The swabs were rotated 3 times in each nostril and then placed into the Copan liquid and transport tube for elution of the sample from the swab. Samples were serially diluted 10-fold and plated onto 5% sheep blood agar plates with 4% NaCl and incubated at 35°C for 24-48 hours. Colonies morphologically consistent with *S. aureus* were counted as colony forming units (CFU). Gram staining, catalase, and tube coagulase tests were performed to confirm the colonies were *S. aureus*. Methicillin resistance was determined with cefoxitin disk diffusion, PCR for *mec*A, and PBP2a agglutination.

Molecular analysis: A previously described multiplex PCR was used for the detection of the Panton-Valentine leukocidin (*pvl*) gene, the *spa* gene and the *mec*A gene (Larsen et al. 2008)**.**

***spa* typing:** Samples confirmed to be positive for *S. aureus* or MRSA were subjected to *spa* typing. Amplification of the *spa* repeat region for typing was done according to Ridom GmbH protocol for DNA sequencing of the *spa* gene. DNA for amplification was obtained by washing a loopful of bacterial cells with distilled H_2O and incubated with 200 µl of 6% InstaGene matrix solution (BIO-RAD, Hercules, CA.) for 20 minutes at 56 °C. The suspension was vortexed and heated for 8 minutes at 100 °C and centrifuged at 8,000 x g for 3 minutes. Twenty microliters of the supernatants was used for PCR amplification. PCR analysis was performed using spa-1113f (5'-TAAAGACGATCCTTCGGTGAG-3'), and spa-1514r (5'-CAGCAGTAGTGCTGCCGTTGCTT-3') primers and Qiagen Taq PCR master mix. Amplification of the *spa* repeat region was initiated at 80°C for 5 minutes, followed by 35 cycles of 94°C for 45 sec, 60°C for 45 sec, 72°C for 90 sec, and a final extension at 72° for 10 minutes. To ascertain the presence of the *spa* gene, the amplicons were run through a 2% agarose gel (UltraPure™ LMP Agarose). PCR products were sequenced by Eton Bioscience. The *spa* sequence types were assigned using the BioNumerics Software version 6.0 (Applied Math, Austin, TX, USA) through the Ridom Spa Server.

Statistical analysis: Significant differences between male and female carriage rates were determined using a two-tailed student's t-test.

Results and Discussion

Nasal swab samples were collected from 247 subjects (100 male, 147 female). Of these, 21.5% (53/247; 29 male, 24 female) were positive for *S. aureus* nasal colonization with a mean (Log_{10}) CFU per nares culture of 2.98±1.17 (2.96±1.04 for males, 3.00±1.33 for females). The geometric mean per nares culture for all subjects was 1,820 (1,505 male, 2,282 female) (Table 1).

11% of the *S. aureus* isolates were methicillin resistant. The carriage rate among males was higher than females although not significantly different ($p = 0.27$). Carriage levels among males were lower than females although not significantly different. ($p = 0.12$).

Figure 1 shows multiplex PCR results for representative MRSA and MSSA isolates.

Only two out of 53 *S. aureus* isolates were *pvl* positive, both of which were also *mec*A positive. One of the *pvl* positive isolates (N063A) is shown in figure 1. MRSA isolates in lanes 5 and 7 were PCR negative for *spa*, but were confirmed to be *S. aureus* through positive catalase and coagulase tests. ATCC BAA1707 was included as a *pvl* positive MRSA control. The *spa* sequencing resulted in 22 different *spa* types (figure 2A).

As it is shown in the clustering dendrogram of figure 2A, four of the isolates were *spa* type t3297, four isolates were *spa* type t012, and

Table 1: *Staphylococcus aureus* and MRSA nasal carriage among healthy university students.

Subject Population	% (+) for nasal colonization	Mean Log_{10}±SD, CFU per nares culture	Geometric mean per nares culture
S. aureus			
All subjects	21.5.0% (53/247)	2.98 ± 1.17	1,820
Male subjects	29.0% (29/100)	2.96 ± 1.04	1,505
Female subjects	16.3% (24/147)	3.00 ± 1.33	2,282
MRSA			
All subjects	2.4% (6/247)	2.04 ± 1.65	412
Male subjects	4.0% (4/100)	2.15 ± 1.33	359
Female subjects	1.4% (2/147)	1.82 ± 2.88	545

three were *spa* type t216. The other 19 *spa* types identified were found in only one or two isolates. A minimal spanning tree was constructed using the BioNumerics software version 6.0 (Applied Math, Austin, TX, USA) grouped the 22 *spa* types into 19 distinct clusters (figure 2B). The distribution of these clusters highlights the variability of the *spa* types detected in this study. Eleven of our 53 *S. aureus*/MRSA isolates were *spa* negative, but were confirmed to be *S. aureus* through positive catalase and coagulase tests.

In this study, the nasal carriage rates of *S. aureus* (21.5%) and MRSA (2.4%) were within the ranges of previous reports (Kluytmans et al. 1997; Chatterjee et al. 2009; Askarian et al. 2009; Chen et al. 2012; Sharma et al. 2014). A small number of similar studies of *S. aureus* and MRSA nasal carriage among the general population of healthy college students have been conducted. A study published in 2009 from Texas State University reported nasal carriage rates of 29.6% for *S. aureus* and 7.4% for MRSA (Rohde et al. 2009). In a study published in 2013 from a historically black college in Virginia, the nasal carriage rate of MRSA was only 0.65% (Shen et al. 2013). Among collegiate student athletes, MRSA carriage rates have been reported to be

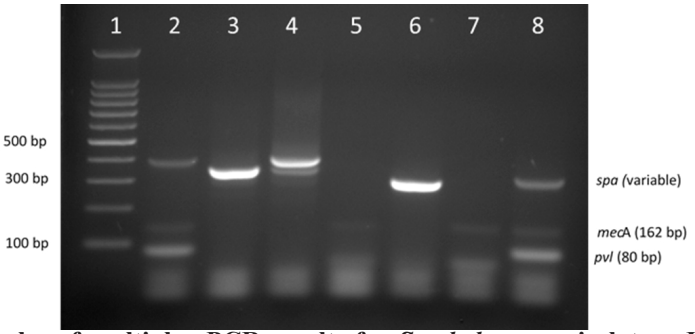
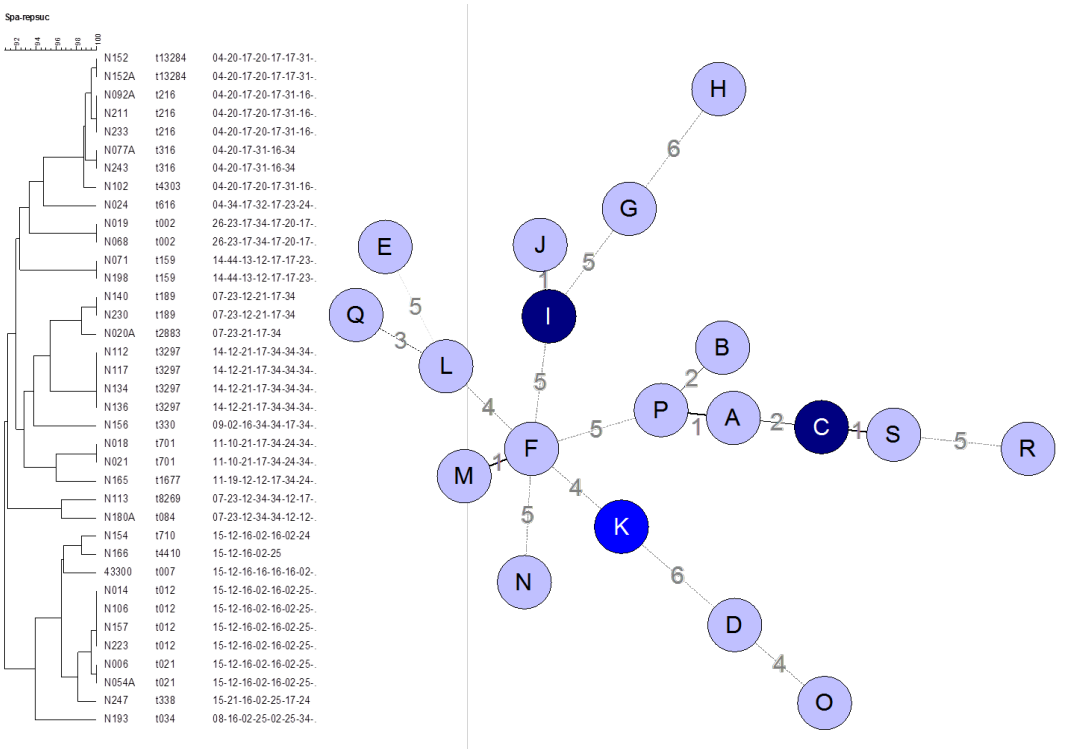


Figure 1. Examples of multiplex PCR results for *Staphylococcus* isolates. Lanes: 1, 100-bp DNA ladder; 2, isolate N063A; 3, isolate N064; 4, isolate N068; 5, isolate N071; 6, isolate N077A; 7, N091A; 8, ATCC BAA1707.



(A) (B)

Figure 2. A) A representative dendrogram showing spa typing of the recovered *Staphylococcus aureus* strains including MRSA. B) Minimum spanning tree showing the clustering of the recovered Staphylococcus aureus strains including MRSA. Each circle represents one of the 19 spa clusters of the 22 different spa types detected in this study. The letter inside each circle represents a cluster/group. The darker the color of the circle is proportional to the number of strains represented in each cluster. Thick lines denote closer association between the types and thin lines denote less while the dashed lines denote the least association.

anywhere from 0.65% to 34.9% (Rackham et al. 2013; Champion et al. 2014). Carriage rates between studies are often difficult to compare due to differences in sample sizes, culturing techniques, and demographics. In this study, we were interested in comparing the carriage rates between males and females. Our results showed that the carriage rate was not significantly different (p=0.27), which is in agreement with studies conducted by Askarian et al. and Sharma et al. in both healthcare and community settings (Askarian et al. 2009; Sharma et al. 2014). However, Chen and colleagues found carriage rates of *S. aureus* to be significantly higher in males than in females in a study carried out among Taiwanese university medical students. Based on our findings and the findings of others, whether or not gender is a factor associated with *S. aureus* and MRSA nasal carriage remains unclear. There are a number of other factors such as age, healthcare exposure, occupation, and health status that alone or in combination likely play more of role in nasal carriage than just gender. In our study the average carriage levels were slightly higher in females than in males for both MSSA and MRSA. A study published by Mermel et al. in 2010 found that in hospitalized patients the average MRSA carriage levels were slightly higher in males than in females (Mermal et al. 2010). Interestingly the average

MRSA carriage levels in the Mermel study were approximately two times higher than what we found in our study. Additional studies would be needed to determine if carriage levels in MSSA or MRSA positive patients are consistently higher than MSSA and MRSA positive healthy individuals in the community.

Panton-valentine leukocidin (PVL) is a pore-forming cytotoxin and *pvl* positive *S. aureus* and methicillin resistant *S. aureus* strains have been associated with more severe skin and soft tissue infections, bone and joint infections, and necrotizing pneumonias (Lina et al. 1999; Badiou et al. 2010). Historically, *pvl* rates have been reported to be much higher in *S. aureus* isolates associated with primary necrotic infections than those not associated with these types of infections. (Lina et al. 1999; Couppié et al. 1994; Prévost et al. 1995). However, very little is known about the prevalence of *pvl* in *S. aureus* isolates not associated with infection at all. A study by Harbarth and colleagues reported a 4:1 ratio between colonization and infection with community associated MRSA possessing *pvl* and suggests that surveillance of *S. aureus* carriers is important to understanding the true prevalence of PVL-producing strains (Harbarth et al. 2005). In our study none of the methicillin sensitive *S. aureus* (MSSA) isolates were *pvl* positive but 33% (2/6) of the MRSA isolates were *pvl* positive, which is in contrast to several reports of *pvl* positive rates in community-associated MRSA being greater than 75% (Naimi et al. 2003; Shukla et al. 2004; Naas et al. 2005). It may indeed be the case that the overall prevalence of *pvl* in MRSA is well below 75% but additional studies including many more healthy subjects is needed determine this. Since PVL producing *S. aureus* infections can be cause for altering therapeutic approaches, some countries such as England and France are now testing for PVL production by clinical isolates of *S. aureus* (Gillet et al. 2007; Health Protection Agency 2008; Etienne and Dumitrescu 2009). With this being the case, it is entirely possible that decolonization efforts could also be impacted by the presence of *pvl* positive MSSA or MRSA in carriers. As *pvl* positive strains can be clinically challenging when associated with infections, it would be beneficial to understand the efficacy of decolonization strategies to minimize the potential for infection by these strains and knowing the true prevalence of these strains as Harbarth et al. suggested may be critical to successful decolonization efforts.

spa typing is a technique used to distinguish strains of *S. aureus* with a resolution that is comparable to MLST and PFGE (Badiou et al. 2010; Koreen et al. 2004). The *spa* typing of our MSSA and MRSA isolates revealed a wide variety of *S. aureus* strains present in this university setting. Interestingly, *spa* type t3297, which was one of our most common *spa* types (4/37) was also found to be the most common *spa* type among the MSSA isolates (31/38) 81.6% from burn centers in the southeast of China (Chen et al. 2012). *spa* type t012, which was equally represented in our study, was shown to be the most common *spa* type to be found among healthy nasal carriers in a study conducted in Norway (Sangvik et al. 2011). The authors of that study also showed that the prevalence of t012 decreased significantly with age and that males had a lower risk of t012 carriage than females. In our study, all of the subjects were college age students, therefore we are not able to perform age associated analysis. With the relatively small number of t012 isolates (4), we found them to be equally distributed between males and females.

In conclusion, our study found carriage rates in a healthy UCO college student population to be consistent with earlier reports and we found no difference between the carriage rates among men and women. The diversity of the isolates obtained in this study as indicated by the variety of *spa* types identified does suggest that any decolonization method developed should show efficacy against a broad range of isolates.

Acknowledgments

Funding for this project was provided by the University of Central Oklahoma Office of Research and Grants and Student Research, Creative, and Scholarly Activities (RCSA) Grant Program.

References

Askarian M., Zeinalzadeh A., Japoni A., Alborzi A., Memish Z.A. 2009. Prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* and its antibiotic susceptibility pattern in healthcare workers at Namazi hospital, Shiraz, Iran. J Infect Dis. 13:e241-e247.

Badiou C., Dumitrescu O., George N., Forbes A.R.N., Drougka E., Chan K. S., Ramdani-Bouguessa N., Meugnier H., Bes M, Tazir M., Siliopoulou I., Nimmo G. R., Hulten K. G., and Lina G. 2010. Rapid detection of *Staphylococcus aureus* Panton-Valentine Leukocidin in clinical specimens by Enzyme-Linked Immunosorbent Assay and Immunochromatographic tests. J Clin Microbiol. 48(4):1384-90.

Boucher H.W., and Corery G.R. 2008. Epidemiology of methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis. 46(Suppl 5):S344-9.

Buehlmann M., Frei R., Fenner L., Dangel M., Fluckiger U., and Widmer A.F. 2008. Highly effective regimen for decolonization of methicillin-resistant *Staphylococcus aureus* carriers. Infect Control Hosp Epidemiol. 29:510-16.

Champion A.E., Goodwin T.A., Brolinson P.G., Were S.R., Prater M.R., and Inzana T.J. 2014. Prevalence and characterization of methicillin-resistant *Staphylococcus aureus* isolates from healthy university student athletes. Annals of Clinical Microbiology and Antimicrobials 13:33. doi:10.1186/s12941-014-0033-5.

Chatterjee S.S., Ray P., Aggarwal A., Das A., and Sharma M. 2009. A community-based study on nasal carriage of *Staphylococcus aureus*. Ind J Med Res. Vol 130. No. 6:742-48.

Chen C.S., Chen C.Y., and Huang Y.C. 2012. Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students at a Taiwanese university. Int J Infect Dis. 16:e799-e803.

Couppié P.B., Cribier, Prévost, Grosshans E., Piémont Y. 1994. Leukocidin from *Staphylococcus aureus* and cutaneous infections: an epidemiological study. Arch Dematol. 130:1208-09.

DeLeo F.R., Otto M., Kreiswirth B.N., and Chambers H.F. 2010. Community-associated methicillin-resistant *Staphylococcus aureus*. Lancet. 375:1557-68.

Etienne J., and Dumitrescu O. 2009. Panton-Valentine leucocidin associated *Staphylococcus aureus* infections. BMJ. 339:b4083.

Gillet Y., Vanhems P., Lina G., Bes M., Vandernesch F., Floret D., and Etienne J. 2007. Factors associated with the high fatality rate of Panton-Valentine leucocidin associated *Staphylococcus aureus* necrotizing pneumonia. Clin Infect Dis. 45:315-321.

Gorwitz R.J., Kruszon-Moran D., McAllister S. K., McQuillan G., McDougal L.K., forsheim G.E., Jensen B.J., Killgore G., Tenover F.C., and Kuehnert M.J. 2008. Changes in the prevalence of nasal colonization with *Staphylococcus aureus* in the United States, 2001-2004. J Infect Dis. 197:1226-34.

Hallin M., Denis O., Deplano A., De Mendonca R., De Ryck R., Rottiers S., and Struelens M.J. 2007. Genetic relatedness between methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*: results of a national survey. J Antimicrob Chemother. 59:465-72.

Harbarth S., Francois P., Shrenzel J., Frankhauser-Rodriguez C., Hugonnet S., Koessler T., Huyghe A, and Pittet D. 2005. Community-associated methicillin-resistant *Staphylococcus aureus*. Emerg Infect Dis. 11:962-965.

Harmsen D., Claus H., Witte W., Rothgänger J., Claus H., Trunwald D., and Vogel U. 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol. 41(12):5442-48.

Health Protection Agency. 2008. Guidance on the diagnosis and management of PVL-associated *Staphylococcus aureus* infections (PVL-SA) in England. Health Protection Agency, London, United Kingdom. www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1226478464166.

Klevens R.M., Edwards J.R., Tenover F.C., McDonald L.C., Horan T., Gaynes R., and the National Nosocomial Infections Surveillance System. 2006. Changes in the epidemiology of methicillin-resistant *Staphylococcus aureus* in intensive care units in US hospitals, 1992-2003. Clin Infect Dis. 42:389-91.

Kluytmans J., van Belkum A., and Verbrugh H. 1997. Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev. 10:505-20.

Koreen L., Ramaswamy S.V., Graviss E.A., Naidich S., Musser J.M., and Kreiswirth B.N. 2004. *spa* typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. J Clin Microbiol. 42(2):792-799.

Larsen A.R., Stegger M., and Sørum M. 2008. *spa* typing directly from a *mec*A, *spa* and *pvl* multiplex PCR assay – a cost effective improvement for methicillin-resistant *Staphylococcus aureus* surveillance. Clin Microbiol Infect. 14:611-614.

Lina G., Piémont Y., Godail-Gamot F., Ges M., Peter M.O., Gaudocon V., Vandernesch F., and Etienne J. 1999. Involvement of Panton-Valentine Leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. Clin Infect Dis. 29:1128-32.

Mermal L.A., Eells S.J., Acharya M.K., Cartony J.M., Dacus D., Fadem S., Gay E.A., Gordon S., Lonks J.R., Perl T.M., McDougal L.K., McGowan J.E., Maxey G., Morse D., and Tenover F.C. 2010. Quantitative analysis and molecular fingerprinting of methicillin-resistant *Staphylococcus aureus* nasal colonization in different patient populations: a prospective, multicenter study. Infect Control Hosp Epidemiol. 31(6):592-597.

Nass T., Forineau N., Spicq C., Robert J., Jarlier V., and Nordmann P. 2005. Three year survey of community-acquired methicillin-resistant *Staphylococcus aureus* producing Panton-Valentine leucocidin in a French university hospital. J Hosp Infect. 61:321-329.

Naimi T.S., LeDell K.H., Como-Sabetti K., Borchardt S. M., Boxrud D.J., Etienne J., Johnson S.K., Vandenesch F., Fridkin S., O'Boyle C., Danila R.N., and Lynfield R. 2003. Comparison of community and health care-associated methicillin-resistant *Staphylococcus aureus* infection. JAMA. 290(22):2976-84.

Prévost G., Couppié P., PrévostP., et al. 1995. Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. J Med Microbiol. 42:237-245.

Rackham D., Ray S., Franks A., Bielak K., and Pinn T. 2010. Community-associated methicillin-resistant *Staphylococcus aureus* nasal carriage in a college student athlete population. Clin J Sport Med. 20(3):185-188.

Rohde R.E., Denham R., and Brannon A. 2009. Methicillin resistant *Staphylococcus aureus*: Carriage rates and characterization of students in a Texas university. Clin Lab Sci. 22(2)176-184.

Rohr U., Mueller C., Wilhelm M., Muhr G., and Gatermann S. 2003. Methicillin-resistant *Staphylococcus aureus* whole-body decolonization among hospitalized patients with variable site colonization by using mupirocin in combination with octenidine dihydrochloride. J Hosp Infect. 54:305-309.

Sangvik M., Olsen R.S., Olsen K., Simonsen G.S., Furberg A.S., and Ericson Sollid J.U. 2011. Age and gender-associated *Staphylococcus aureus spa* types found among nasal carriers in a general population: the Tromsø staph and skin study. J Clin Microbiol. 49(12):4213-18.

Sharma Y., Jain S., Signh H., and Govil V. 2014. *Staphylococcus aureus*: Screening for nasal carriers in a community setting with special reference to MRSA. Scientifica vol. 2014. doi. org/10.1155/2014/479048.

Shen H., Akoda E., and Zhang K. 2013. Methicillin-resistant *Staphylococcus aureus* carriage among students at a historically black university: A case study. Int J Microbiology vol 2013. doi.org/10.1155/2013/989834.

Shukla S.K., Stemper M.E., Ramaswamy S.V., Conradt J.M., Reich R., Graviss E.A., and Reed K.D. 2004. Molecular characteristics of nosocomial and Native American community-associated methicillin-resistant *Staphylococcus aureus* clones from rural Wisconsin. J Clin Microbiol. 42(8):3752-57.

Wertheim H.F.L., Melles D.C., Vos M.C., van Leeuwen W., van Belkum A., Verbrugh H.A., and Nouwn J.L. 2005. The role of nasal carriage in *Staphylococcus aureus* infections. Lancet Infect Dis. 5:751-62.

Submitted August 30, 2018 Accepted December 3, 2018

Modifying the Redlich-Kwong-Soave Equation of State

James H. McNeill
Department of Natural Sciences, Middle Georgia State University, Macon, GA 31206

James E. Bidlack
Department of Biology, University of Central Oklahoma, Edmond, OK 73034

Abstract: The Redlich-Kwong-Soave equation of state for real gases and liquids has been modified in order to improve the match of calculated liquid molar volumes with measured data by allowing the *b*-parameter to be a function of the temperature and molar volume of real gases and liquids. Molecular dynamics simulations of the noble gas krypton, using the hard-sphere potential for a large number of krypton atoms, were performed to evaluate how the *b*-parameter varies with gas molar volume. The results of these simulations were applied when modifying the original Redlich-Kwong-Soave equation of state. In the modified Redlich-Kwong-Soave equation of state, the *a*-parameter is kept constant. Also, in this modified version, the measured critical molar volume is employed as well as the measured critical temperature and critical pressure values in parameter determination at the critical point. From this determination of parameters for a number of real gases, the same parameters can be approximately calculated using the measured critical compressibility factor avoiding numerical procedures. This results in an equation of state that matches closely with the measured critical point for many gases as well as improving the match between the model and measured liquid molar volumes.

Introduction

The first equation of state developed to model real gases at relatively high temperatures and low pressures is the ideal gas equation:

$$PV = nRT \quad (1)$$

P is the pressure of the gas, V is the volume of the gas, n is the number of moles of the gas, T is the temperature of a gas in absolute temperature scale, and R is the ideal gas constant. If one divides through the ideal gas equation with the number of moles of gas, one has the general expression below in terms of gas molar volume v.

$$Pv = RT \quad (2)$$

The gas molar volume v is the unit volume occupied by one mole of a gas and is equal to the gas volume V divided by the number n of moles of gas.

$$v = V/n \quad (3)$$

However, at low temperatures and high pressures, real gases deviate from the ideal gas expression given in Equation 1. At sufficiently low temperatures and high pressures, any real gas will condense into the liquid phase and an equilibrium situation takes place between the liquid and gas phases of any pure substance. The first attempt to model real gases and liquids was accomplished by van der Waals using the following mathematical relation.

$$P = nRT/(V - nb_{vdW}) - a_{vdW}\, n^2/V^2 \quad (4)$$

In the van der Waals expression, Equation 4, b_{vdW} is the molar volume of space occupied by individual gas atoms, in the case of a noble gas, or gas molecules which is much less than the volume of the gas itself at relatively low pressures. It is this space occupied by gaseous

particles which prevent any real gas volume going to zero at extremely large pressures. The a_{vdW} term represents the weak attractive forces amongst gaseous atoms or molecules which is responsible for the liquid phase at temperatures below the critical temperature value T_c of any pure gas. Above the critical temperature value, it is impossible to liquefy any gas, because only below the critical temperature both the liquid and vapor phases coexist. In theory, above the critical temperature, the average kinetic energy of gas atoms or molecules is sufficient to overcome the weak attractive forces that occur when two gas atoms or molecules come into close contact (Barrow, 1979). Above the critical temperature value, it is impossible to liquefy any gas due to the average kinetic energy of gas atoms or molecules successfully overcoming the weak attractive forces that occur when two gas atoms or molecules come into close contact. If one substitutes Equation 3 into Equation 4, then the van der Waals equation of state can be more conveniently represented as a function of gas molar volume v instead.

$$P = RT/(v - b_{vdW}) - a_{vdW}/v^2 \quad (5)$$

Unlike the ideal gas equation, one must experimentally determine the numerical values of parameters a_{vdW} and b_{vdW} in the van der Waals expression. The exact values of these two parameters depend upon the chemical composition of any pure gas. To determine their values, one has to use differential calculus and the definition of the critical point of a real gas. The critical point takes place when a real gas is at its critical temperature value T_c and its pressure value P is equal to its critical pressure value P_c. The critical pressure value P_c is that value the equilibrium vapor pressure of a pure liquid approaches to in the limit of the critical temperature, and likewise in the limit of the temperature approaching the critical temperature value at the critical pressure value, the liquid molar volume v of the liquid approaches its critical molar volume v_c. Or, at the critical temperature and pressure, the molar volume of the liquid is equal to that of its gas molar volume instead of being less than the gas molar volume. At the critical point of any pure gas, the first and second derivative of the van der Waals expression, Equation 5, with respect to the molar volume v are both equal to zero.

$$(\partial P/\partial v) = 0 \quad (\text{At } T = T_c, P = P_c \text{ and } v = v_c) \quad (6)$$

$$(\partial^2 P/\partial v^2) = 0 \quad (\text{At } T = T_c, P = P_c \text{ and } v = v_c) \quad (7)$$

By using Equations 6 and 7 along with the following expression one can determine the numerical values of a_{vdW} and b_{vdW}:

$$P_c = RT_c/(v_c - b_{vdW}) - a_{vdW}/v_c^2 \quad (8)$$

When van der Waals first studied real gases in the early part of the 20th century, it was practically impossible to measure the critical molar volume v_c of a liquid with the technology available at that time. When solving for parameters a_{vdW} and b_{vdW}, they were determined to be the following two functions of the measured critical pressure and critical temperature values.

$$a_{vdW} = (27/64)\, R^2\, T_c^2 / P_c \quad (9)$$

$$b_{vdW} = RT_c/(8P_c) \quad (10)$$

And the estimated critical molar volume v_c is three times the numerical value of the van der Waals b-parameter.

$$v_c = 3b_{vdW} \quad (11)$$

The first error in the van der Waals expression is that it overly estimates the values of most measured critical molar volumes determined with today's technology. Also, it over-estimates the pressures of most real gases as compared to high pressure data measured at temperatures above the critical temperature value where condensation is unable to take place. To improve this model, Redlich and Kwong later on developed the following expression.

$$P = RT/(v - b) - a/\{T^{1/2}\,[v(v + b)]\} \quad (12)$$

Division by the square root of the absolute temperature T compensates for the increase of overcoming weak intermolecular forces of attraction with an increase of temperature. With

the inclusion of the b parameter in the Redlich-Kwong equation of state for real gases and liquids, Equation 12, in the subtraction term, Equation 12 can be rearranged to similar format of the original van der Waals equation into the following equation.

$$P = RT/(v - b) - \{a/[T^{1/2}(1 + b/v)]\} / v^2 \quad (13)$$

Thus, by the Redlich-Kwong equation of state, the van der Waals a_{vdW} parameter is the following function of temperature and molar volume.

$$a_{vdW} = a/[T^{1/2}(1 + b/v)] \quad (14)$$

Thus, in view of Equation 13, the decrease in molar volume allows the van der Waals a_{vdW} parameter approach zero in the limit of zero molar volume because at sufficiently low molar volumes, atoms and molecules then would begin to overlap their electron clouds. This equation will then model decreasing attractive forces of gaseous atoms and molecules in the limit of zero molar volume along with the positive term in the original van der Waals equation of state. Again using the differential calculus given in Equations 6 and 7, the a and b parameters and the critical molar volume are approximately equal to the following expressions given below to a limited number of significant figures.

$$a = 0.42748\ R^2\ T_c^{\,2}/P_c \quad (15)$$

$$b = 0.08664\ R\ T_c\ /P_c \quad (16)$$

$$v_c = 0.38473\ b \quad (17)$$

When using differential calculus for the Redlich-Kwong equation, numerical techniques must be utilized in a computer program in order to evaluate the fractions given in Equations 15 to 17. The Redlich-Kwong expression yielded a much improved model when comparing the measured high pressure data of a real gas at temperatures above the measured critical temperature value. Yet, still the calculated critical molar volume v_c by the Redlich-Kwong equation of state given in Equation 17 over predicts the measured values, but closer than that by the van der Waals equation. Another problem with the Redlich-Kwong function is that with the a parameter divided by the square-root of the absolute temperature scale over estimates the weak attractive forces amongst gas atoms below the critical temperature value, especially as the temperature drops below the freezing point of any substance.

To avoid this problem for low temperatures concerning the Redlich-Kwong equation, Soave modified the Redlich-Kwong equation in the following expression referred to as the Redlich-Kwong-Soave equation of state (Prausnitz et al., 1998).

$$P = R\ T / (v - b) - a(T,\omega) / [v\ (v + b)] \quad (18)$$

For this expression, $a(T,\omega)$ is the next function of the absolute temperature T, the measured critical pressure value P_c, the measured critical temperature value T_c, and the measured acentric factor ω of a real gas or liquid:

$$a(T,\omega) = [0.42748\ R^2\ T_c^{\,2} / P_c]\{1 + [0.480 +$$

$$1.574\omega - 0.176\omega^2][1 - (T/T_c)^{1/2}]\}^2 \quad (19)$$

However, the b parameter and the critical molar volume v_c in the Redlich-Kwong-Soave equation of state are still defined by Equations 16 and 17. Thus, the calculated critical molar volumes are larger than that observed experimentally. By definition (Prausnitz et al., 1998), the measured acentric factor ω is equal to the following equation involving the observed equilibrium liquid-vapor pressure value P_{equil}, at an absolute temperature value equal to 70% of the measured critical temperature value T_c of any pure substance, and the measured critical pressure value P_c:

$$\omega = -\log_{10}(P_{equil}/P_c) - 1$$

$$(\text{At } T = 0.7\ T_c) \quad (20)$$

For noble gases, the acentric factors are nearly equal to zero, but for other gases and liquids acentric factors are measured to be greater than zero. The measured acentric factor is useful

in observing non-ideal properties of gases and liquids in general, such that larger the value of the acentric factor less ideal gas behavior of real gases and liquids. When utilizing the Redlich-Kwong-Soave equation of state, Equation 18, the model still matches well with observed high pressure data, and the *a* value will not approach infinity in the limit as the temperature goes to absolute zero. However, the value of *a* will reach a zero value at sufficiently high temperatures and then approach infinity as the temperature goes to infinity. Another important disagreement between the previously discussed models is that they all also over predict the measured liquid molar volumes of liquids studied because the *b* parameter is held constant. This is a common error in all the three previous models of real gases and liquids discussed thus far. An additional modification is needed to take into account how the *b* parameter is a function of both the gas molar volume and absolute temperature.

The Modified Redlich-Kwong-Soave Equation of State for Real Gases and Liquids

In theory, the *b*-parameter includes the space occupied by gas atoms or molecules, and the space they cannot occupy due to hindrance during collisions in space. The numerical value of the *b* parameter should decrease with increasing molar density and approach a constant value greater than zero in the limit of infinite pressures if the atoms making up the gas are hard-spheres. However, atoms that make up matter are not hard spheres but atoms behave instead like spheres with a soft outer periphery and a nearly incompressible inner spherical core. Thus, for real gases, the *b* parameter is a function of the absolute temperature as well as the gas molar density. The next function presented in Equation 21 is the modified Redlich-Kwong-Soave equation which matches measured liquid molar volumes below the critical temperature as well as high pressure data above critical temperature value:

$$P = RT / \{v - b_0 - b_1(T)\, exp[-k(T)/v]\} - a / [v\,(v + b_0)] \quad (21)$$

In this modified version, the effective atomic or molecular molar volume *b* of the original Redlich-Kwong-Soave equation has been replaced by the following negative exponential function of temperature and molar volume *v*:

$$b(T) = b_0 + b_1(T)\, exp[-k(T)/v] \quad (22)$$

In Equation 22, $b_1(T)$ and $k(T)$ are evaluated functions of temperature derived from analyzing listed liquid-vapor equilibrium data below critical temperatures and high pressure data above the critical temperature values for a number of pure substances (Green and Southhard, 2018). The substitution of the constant *b*-parameter with a function of temperature and molar volume, Equation 22, is originally based upon the study of computer simulations for the noble gas krypton using the hard-sphere potential (See the next section titled "The Modification with Regards to the *b*-Parameter."). In this modified version of the Redlich-Kwong-Soave equation (Equation 21), the *a*-parameter is assumed to be constant and also the b_0-parameter. To best match up with measured liquid molar volume values, the b_0-parameter is set equal to 0.2632 times the measured critical molar volume v_c of any real gas or liquid:

$$b_0 = 0.2632\, v_c \quad (23)$$

Furthermore, at the critical temperature value T_c the parameters $b_1(T_c)$ and $k(T_c)$ were observed approximately to be the following functions of the critical compressibility factor Z_c and measured critical molar volume v_c:

$$b_1(T_c) \cong v_c\,[-2030.1Z_c^4 + 1983.8Z_c^3 - 738.11Z_c^2 + 121.16Z_c - 6.2489] \quad (24)$$

$$k(T_c) \cong v_c\,[-22.92\,Z_c^4 + 19.098Z_c^3 - 6.1684Z_c^2 + 0.7599Z_c + 1.0437] \quad (25)$$

This was accomplished by curve-fitting the

evaluated b_0-, $b_1(T_c)$-, $k(T_c)$-, and a-parameters from measured critical point data of real gases and liquids using expressions given in Equations 46 to 47 as well as Equation 23. The data utilized are listed in Perry's Chemical Engineer's Handbook, 6th Edition. Referring to Equations 24 and 25, the critical compressibility factor Z_c is the following function of the measured critical pressure P_c, the measured critical temperature T_c, and the measured critical molar volume v_c at the critical point including the ideal gas constant R:

$$Z_c = P_c v_c /(RT_c) \tag{26}$$

Concerning the a-parameter, from the analyses performed it is observed to be nearly modeled by the following function of the measured critical pressure and measured critical temperature including the experimental critical compressibility factor and ideal gas constant R (Figure 1):

$$a \cong [(R\,T_c)^2/P_c][-881.16Z_c^4 + 848.14Z_c^3 - 318.38Z_c^2 + 55.96Z_c - 3.1858] \tag{27}$$

At and above the measured critical temperature value, the evaluated high pressure data of the noble gases neon, argon, krypton, and xenon reveal that $b_1(T)$ and $k(T)$ approximately follow the two next functions of temperature (Figure 2):

$$b_1(T) \cong b_1(T_c)\{0.1691 + 0.8247exp[-0.6928(T/T_c)] + 0.4611exp[-0.09207\,(T/T_c)]\}/1.002135 \tag{28}$$

$$k(T) \cong k(T_c)\{0.4871 + 0.2138exp[-0.5003(T/T_c)] + 0.4138exp[-0.05789(T/T_c)]\}/1.007263 \tag{29}$$

For temperatures below the critical temperature value ($T < T_c$) in the liquid-vapor region, the $b_1(T)$ and $k(T)$ functions were observed to approximately match up with the following cubic and quadratic functions of temperature, respectively, for non-polar and polar substances both (Figure 3):

$$b_1(T) \cong b_1(T_c)\,[\beta_0 + \beta_1\,(T/T_c) + \beta_2\,(T/T_c)^2 + \beta_3\,(T/T_c)^3]/[\beta_0 + \beta_1 + \beta_2 + \beta_3] \tag{30}$$

$$k(T) \cong k(T_c)\,[\kappa_0 + \kappa_1\,(T/T_c) + \kappa_2\,(T/T_c)^2]\,/\,[\kappa_0 + \kappa_1 + \kappa_2] \tag{31}$$

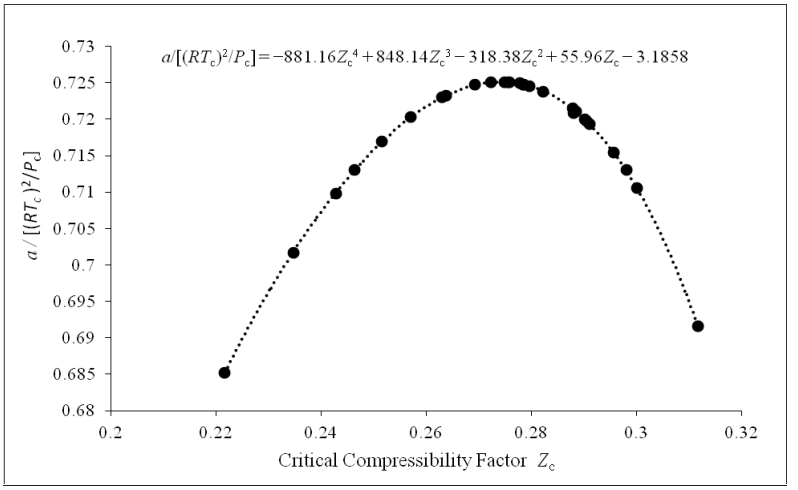


Figure 1. Evaluated $a/[(R\,T_c)^2/P_c]$ ratios for a number of non-polar and polar molecular compounds at the measured critical point using Perry's Chemical Engineers' Handbook (Green and Southard, 2018). The dotted line is the displayed fit to the data points.

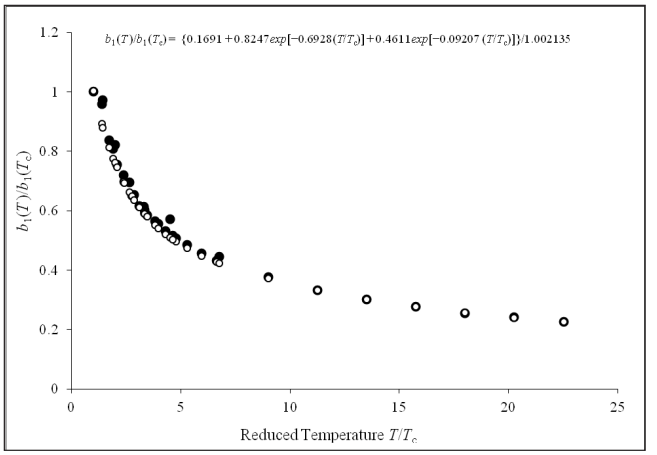


Figure 2. Evaluation of the $b_1(T)/b_1(T_c)$ ratios from the measured high-pressure data of the noble gases neon, argon, krypton, and xenon at temperatures above the critical temperature value using Perry's Chemical Engineers' Handbook (Green and Southard, 2018). The filled in circles (●) are from the data, and the open circles (o) are from the negative exponential fit of the data points.

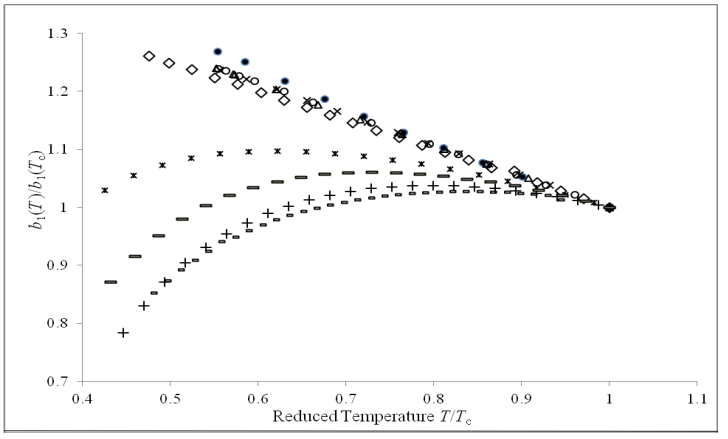


Figure 3. Evaluated $b_1(T)/b_1(T_c)$ ratios from measured liquid-vapor equilibrium data of noble gases neon, argon, krypton, xenon; hydrocarbons methane, ethane, propane, and butane; and water using Perry's Chemical Engineers' Handbook (Green and Southard, 2018). The filled-in circles (•) represent data for neon; circles (o) represent data for argon; triangles (Δ) represent data for krypton; crosses (×) represent data for xenon; diamonds (◊) represent data for methane; asterisks (*) represent data for ethane; long dashes (–) represent data for propane; pluses (+) represent data for butane; and short dashes (-) represent data for water.

The β_0-, β_1-, β_2-, and β_3-parameters in the cubic expression of Equation 30 for non-polar compounds were observed to approximately equal the following linear functions of the acentric factor ω (Figure 4):

$$\beta_0 \cong -11.393\,\omega + 1.4017 \quad (32)$$

$$\beta_1 \cong 32.772\,\omega - 0.0942 \quad (33)$$

$$\beta_2 \cong -32.655\,\omega - 0.5220 \quad (34)$$

$$\beta_3 \cong 11.282\,\omega + 0.2158 \quad (35)$$

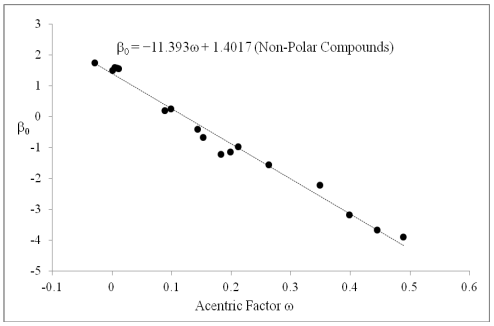


Figure 4. Evaluated β_0 values for a number of non-polar compounds using Perry's Chemical Engineers' Handbook (Green and Southard, 2018). The dotted line is the linear fit to the data, and the non-polar compounds analyzed were the hydrocarbons stated in the text, and all the noble gases except helium.

And for polar substances, the following approximate linear expressions of the acentric factor apply instead:

$$\beta_0 \cong -7.062\,\omega + 1.7997 \quad (36)$$

$$\beta_1 \cong 22.000\,\omega - 2.2642 \quad (37)$$

$$\beta_2 \cong -24.101\,\omega + 2.7785 \quad (38)$$

$$\beta_3 \cong 9.199\,\omega - 1.3223 \quad (39)$$

Because non-polar and polar substances experience different types of intermolecular interactions, it is not surprising that different numerical constants result in linear functions of the acentric factor. The κ_0-, κ_1-, and κ_2-parameters in the quadratic expression of Equation 31 for non-polar compounds are similarly approximately equal to the following linear functions of the acentric factor ω (Figure 5):

$$\kappa_0 \cong -2.089\,\omega + 1.1043 \quad (40)$$

$$\kappa_1 \cong 4.346\,\omega - 0.0688 \quad (41)$$

$$\kappa_2 \cong -2.276\,\omega - 0.0355 \quad (42)$$

And for polar substances, the following approximate similar linear expressions apply.

$$\kappa_0 \cong -1.355\,\omega + 1.1620 \quad (43)$$

$$\kappa_1 \cong 2.685\,\omega - 0.1886 \quad (44)$$

$$\kappa_2 \cong -1.337\,\omega + 0.0268 \quad (45)$$

Due to the slight curvature of the $k(T)$ parameter, a quadratic expression was utilized instead of a cubic function. For non-polar substances, data from all noble gases except helium were analyzed, and the hydrocarbons whose measured data were studied are linear saturated hydrocarbons methane to butane and heptane to decane, and also ethylene, propylene, isobutene, benzene and toluene. Concerning polar substances, data from ammonia, carbon monoxide, chloromethane, and water were studied.

Concerning the critical point at P_c, T_c, and v_c, the numerical value of the b_0-parameter was calculated using Equation 23, and then the numerical values of the a-parameter and the temperature dependent parameters $b_1(T_c)$ and $k(T_c)$ were initially evaluated using the following three expressions:

$$P_c = R\,T_c / \{v_c - b_0 - b_1(T_c)\,exp[-k(T_c)/v_c]\} - a / [v_c\,(v_c + b_0)] \quad (46)$$

$$(\partial P_c/\partial v_c)_T = 0 \quad (\text{at } T = T_c) \quad (47)$$

$$(\partial^2 P_c/\partial v_c^{\,2})_T = 0 \quad (\text{at } T = T_c) \quad (48)$$

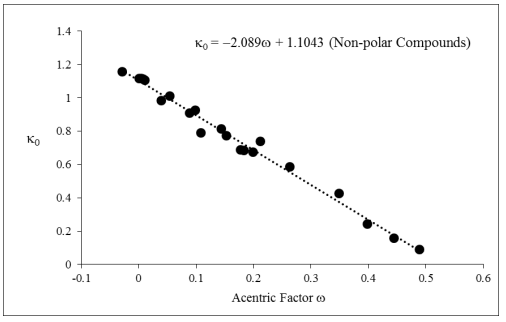


Figure 5. Evaluated κ_0 values for non-polar noble gases and hydrocarbons using Perry's Chemical Engineers' Handbook (Green and Southard, 2018). The dotted line is the linear fit to the data, and the non-polar compounds analyzed were the hydrocarbons stated in the text, and all the noble gases except helium.

In addition, for any particular real gas concerning the evaluated values of b_0-, $b_1(T_c)$-, and $k(T_c)$-parameters, there is the hypothetical minimum molar volume v_{min} at the limit of infinite pressures:

$$v_{min} - \{ b_0 + b_1(T_c)\, exp[-k(T_c)/v_{min}]\} = 0 \quad (\text{at } T = T_c) \tag{49}$$

For the expressions given in Equations 46 to 48, there is the complication that with three equations of state there are four parameters to be evaluated: a, b_0, $b_1(T_c)$, and $k(T_c)$. The fourth mathematical condition used was the expression in Equation 23 to calculate the constant b_0-parameter as a linear function of the measured critical liquid molar volumes. A number of gases in the liquid phase were evaluated by variation of the b_0-parameter to obtain the best calculated liquid molar volumes in comparison to experimental values. The constant value which yielded the best overall averages is the value of 0.2632 in Equation 23 for the constant b_0-parameter as a linear function of the measured critical molar volume v_c.

Of course, a computer program is necessary to evaluate the parameters a, $b_1(T_c)$, $k(T_c)$, and v_{min} at the critical point due to the complexity of the non-linear functions in Equations 46 to 48. Yet, from the evaluation of a number of real gases and liquids, curve fitting of these parameters obtained from numerical analyses of data in Equations 46 to 48 resulted in the expressions given in Equations 24, 25, and 27. Table 1 displays how closely the modified Redlich-Kwong-Soave equation of state matches measured critical point data when utilizing Equations 24, 25, and 27 for the measured critical point data to evaluate the a-parameter and parameters $b_1(T_c)$ and $k(T_c)$. Concerning the minimum volume v_{min} in the limit of infinite pressure, minimum molar volume varies slightly with temperature when using the modified Redlich-Kwong-Soave equation of state for temperatures below and above the critical temperature value. In theory, the value of v_{min} hypothetically should be constant or nearly constant.

Concerning the liquid phase, Figure 6 displays how well the modified Redlich-Kwong-Soave expression matches up with measured liquid molar volume data for the hydrocarbon butane at different temperatures as compared to the original Redlich-Kwong-Soave equation. With regards to high pressures above the critical temperature, Figure 7 displays the same comparison for high pressure data of butane at 500 Kelvin. The matches for both versions of the Redlich-Kwong-Soave equations of state are equally well at high pressures for temperatures above the critical temperature value.

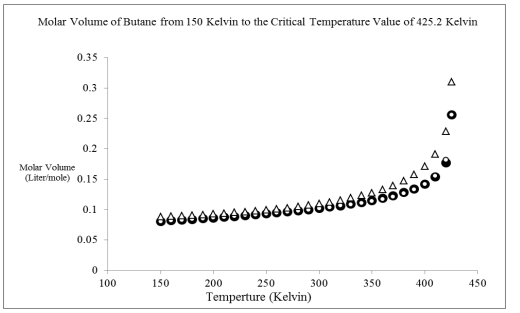


Figure 6. Liquid molar volume of butane from 150 Kelvin up to the critical temperature value of 425.2 Kelvin. The black circles are from experimental measurements, white triangles calculated values from the Redlich-Kwong-Soave equation of state, and the white circles from calculated liquid molar volumes using the modified Redlich-Kwong-Soave equation of state.

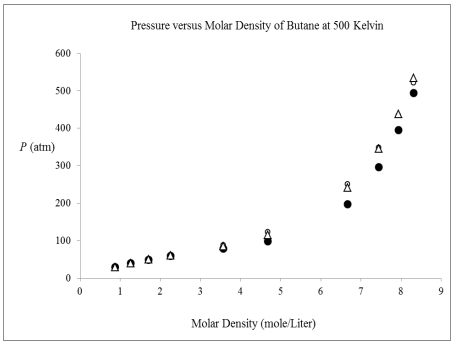


Figure 7. High pressure data of butane at 500 Kelvin. The black circles are from experimental measurements, white triangles from Redlich-Kwong-Soave equation of state, and the white circles from the modified Redlich-Kwong-Soave equation of state.

In addition to the evaluation of $b_1(T_c)$, $k(T_c)$ and a using measured critical point data, the other mathematical challenge was how to determine the general temperature variations of parameters $b_1(T)$ and $k(T)$ below and above the critical temperature value in order to derive general expressions in Equations 28 to 45. A series of iterations and algorithms in FORTRAN were developed to evaluate the b_1 and k values at different temperatures in both the liquid-vapor region and high pressure data above the critical temperature, for each real gas or liquid studied (Press et al., 1992). In all instances, not only was the b_0-parameter kept constant calculated using Equation 23, but also the minimum molar volume v_{min} was assumed to be constant when calculated by Equation 49 from the value of for a, $b_1(T_c)$, and $k(T_c)$ in Equations 46 to 48. Determining values of $b_1(T)$ and $k(T)$ for high pressure data above critical temperature value was accomplished simply by curve fitting experimental high pressure data observed at different measured molar gas volumes at different constant temperature values. At a given temperature, the $b_1(T)$ value determined was the number which yielded a minimum chi-square value while keeping v_{min} evaluated at the critical temperature constant, and the value of $k(T)$ was evaluated using the next expression from rearranging Equation 49.

$$k(T) = -v_{min} \log_e[(v_{min} - b_0) / b_1(T)] \qquad (50)$$

However, evaluation for the $b(T)$ value below the critical temperature incorporated the following integral set equal to zero.

$$\int_{v_{liq}}^{v_{gas}} (P_{equil} - P_{mRKS})\, dv = 0 \qquad (51)$$

P_{equil} is the measured equilibrium liquid-vapor pressure at a measured temperature value below the critical temperature, and P_{mRKS} is the modified Redlich-Kwong-Soave expression in Equation 21. Theoretically, this integral is equal to zero between the lower limit, the liquid molar volume v_{liq}, and the upper limit being the vapor molar volume v_{gas}, because the molar Gibbs free energy value of both liquid and vapor phases are equal at equilibrium conditions (Hirschfelder et al., 1954). The algorithm varied the value of $b_1(T)$, determining the value of $k(T)$ using the expression in Equation 50 by again assuming

Table 1. Comparison between measured critical point data along with that calculated using the modified Redlich-Kwong-Soave equation incorporating parameter derivation using measured critical compressibility factors in Equations 24, 25, and 27.

Gas	Measured T_c (Kelvin)	Calculated T_c (Kelvin)	Measured P_c (Atm)	Calculated P_c (Atm)	Measured v_c (Liter/mole)	Calculated v_c (Liter/mole)
Neon	44.4	44.4	26.18	26.23	0.04177	0.04177
Argon	150.9	151.0	48.34	48.42	0.07458	0.07457
Krypton	209.39	209.54	54.24	54.34	0.09201	0.09199
Xenon	289.7	289.9	57.45	57.50	0.1194	0.1194
Methane	190.6	190.7	45.39	45.47	0.1000	0.09998
Ethane	305.3	305.5	48.07	48.16	0.1471	0.1471
Butane	425.2	425.5	37.46	37.54	0.2560	0.2559
CO_2	304.2	304.4	72.86	73.02	0.09440	0.09438
O_2	154.77	154.82	50.20	50.23	0.07885	0.07887
N_2	126.25	126.34	33.52	33.58	0.09216	0.09215
Water	647.31	647.52	218.31	218.50	0.05711	0.05712
Ammonia	405.4	405.6	111.5	111.7	0.07246	0.07247
CO	132.91	133.01	34.50	34.56	0.09347	0.09346

the hypothetical minimum molar volume v_{min} is constant, until the integral expression in Equation 51 is equal to zero. Then for any particular real gas or liquid, the values of $b_1(T)$ and $k(T)$ at different temperatures were compared to the $b_1(T_c)$ and $k(T_c)$ values calculated using Equations 24 to 25 from measured critical point data. This resulted in the data shown in Figure 3, and a closer analysis of data below the critical temperature values resulted in an observation of the linear dependence of the β_i (i = 0 to 3) and κ_i (i = 0 to 2) values upon the acentric factor ω when curve fitting the data.

Concerning high pressure data for butane, the calculated pressures using the modified Redlich-Kwong-Soave equation was accomplished simply by applying Equations 21 to 25 and 27 to 29 for each measured gas molar volume and likewise Equations 18 and 19 for the original version. However, for the calculated liquid molar and vapor molar volumes in both the original Redlich-Kwong-Soave equation and the modified version, the expression in Equation 51 was used.

The Modification with Regards to the *b*-Parameter

In the Redlich-Kwong-Soave equation of state, the *b*-parameter represents the atomic or molecular molar volume of a real gas which is due to the gas atoms and molecules occupying space, or a small fraction of the gas volume at low pressure since atoms are not point masses. In theory [3], this *b*-parameter is not constant but dependent upon the gas molar density and absolute temperature as well. By assuming this parameter *b* is constant at all gas densities and temperature values, the Redlich-Kwong-Soave equation does not match up well with measured liquid molar volumes at temperatures below the critical temperature value. Therefore, it was necessary to determine how this parameter varies with gas molar density at least by using the hard-sphere model. This was accomplished by developing a FORTRAN computer program that models the collision dynamics of a monatomic gas contained within a cubic gas volume simulating gas atoms as hard spheres. A large number of simulations were performed for the noble gas krypton at different molar densities with a simulation temperature equal to 300 Kelvin. In addition, at each specific molar density simulated at 300 Kelvin, the number of krypton atoms was also varied. It was necessary to do simulations at constant temperature and constant molar density using a different large number of krypton atoms in order to obtain correct extrapolations to an extremely large number of gas atoms such as Avogadro's number.

For a single simulation, there is first the input value N for a large number of gas atoms, from several hundred to over ten-thousand, to be encased within a microscopically sized cubic volume that has edge lengths on the order from less than 100 to more than 1,000 Ångstroms (1Ångstrom = 10^{-10} meter) . Then, the atoms are initially positioned in the face-centered cubic unit cell arrangement, with the separation distances amongst the atoms established by the specific molar density input value. Afterwards, a Gaussian random number generator (Press et al., 1992) is utilized to establish a Maxwellian velocity distribution for the assigned gas temperature value. Then the simulation begins and continues until a very large number of inter atomic collisions take place. When the simulation is completed, the pressures at each of the six flat square walls are evaluated. The average simulation pressure value $< P >$ is determined by calculating the averages of the simulation pressures P_i at all six flat square walls:

$$< P > \; = \; \sum_{i=1}^{6} P_i / 6 \qquad (52)$$

And for the final determined simulation pressure value $< P >$, the standard deviation was calculated to get an estimated uncertainty in the atomic molar volume *b*. When figuring out the numerical value of the *b*-parameter at a given molar density and temperature value using the hard-sphere model, it is necessary to take edge effects into account since the number of gas atoms in any simulation is much less than Avogadro's number. When doing so, the

pressure observed at any of the six flat walls of the cube is computed as:

$$P_i = \left(\sum_{j=1}^{N_i} 2 m v_j / t \right) / (l - 2r)^2 \qquad (53)$$

In Equation 53, l is the edge length of the microscopic cubic gas volume of the simulation, r is the hard-sphere atomic radius, m is the mass of the hard-sphere, v_j is the velocity component of the colliding atom whose direction is perpendicular to the flat square wall, N_i is the total number of collisions at the wall, and t is the total simulation time.

For the hard sphere model, the equation of state of such a hypothetical gas is the following mathematical expression:

$$P = R T / (v - b) \qquad (54)$$

In Equation 54, the effective atomic molar volume b for one mole of hard-spheres is the following function of gas pressure P, temperature T, and molar volume v:

$$b = v - R T / P \qquad (55)$$

If one divides Equation 55 by Avogadro's number N_A, one obtains the effective atomic volume v_{eff} for a hard-sphere atom:

$$v_{eff} = v_{atom} - (R/N_A) T / P \qquad (56)$$

In Equation 56, the term v_{atom} represents the gas volume in units of Liters per atom. Since edge effects must be taken into account because the number of gas atoms in a single simulation is much less than Avogadro's number, Equation 56 becomes the following expression when using the average simulation pressure value $< P >$:

$$v_{eff} = (l - 2r)^3/N - (R/N_A) T / < P > \qquad (57)$$

The next challenge was then to determine how to extrapolate the simulation results to that for Avogadro's number of hard spheres where there are practically no edge effects. At zero moles per Liter or zero molar density, theoretically (Hirshfelder et al., 1954) the ratio of the effective atomic volume v_{eff} and atomic volume v_{atom} for hard-spheres is exactly equal to four:

$$v_{eff}/v_{atom} = 4 \qquad (58)$$

Theoretically this ratio decreases in value with increasing molar density until maximum molar density is attained at nearly infinite pressures, and for the face-centered cubic unit cell arrangement, this ratio at maximum molar density is about 1.35 at extremely large pressure values:

$$v_{eff}/v_{atom} = (3 \times 2^{1/2})/\pi \cong 1.35$$

(In the limit of infinite pressure) (59)

For the noble gas krypton using the hard-sphere model, the atomic volume v_{atom} is calculated by setting the atomic diameter equal to the sigma-parameter in the Lennard-Jones potential evaluated from viscosity data (Bird et al., 2006):

$$v_{atom} = (4\pi/3) (\sigma/2)^3 = (4/3) \pi r^3 \qquad (60)$$

It was observed that the ratio of the effective atomic volume and atomic volume (v_{eff}/v_{atom}) increases in value with the ratio $(l - 2r)^3/l^3$. The smallest number in one simulation was 172 atoms, and then additional simulations were performed at 365, 666, 1099, 1688, 2457, 3430, 4631, 6084, and 7813 atoms with molar density of 15 moles per Liter at 300 Kelvin. A plot of the ratio v_{eff}/v_{atom} versus the ratio $(l - 2r)^3/l^3$ can be fitted with a polynomial. Logically, if there is one mole of hard-spheres, Avogadro's number, the ratio $(l - 2r)^3/l^3$ will be nearly equal to one. Hence, extrapolation to one yields the simulation ratio of v_{eff}/v_{atom} for an astronomically large number of hard spheres. Once this value is determined, then the b-parameter at the simulation molar density can be evaluated for the expression in Equation 55. After a large number of simulations were performed and extrapolations done, the ratio of the effective atomic volume and atomic volume (v_{eff}/v_{atom}) versus molar density ($1/v$) at 300 Kelvin for

krypton using the hard-sphere potential was observe to follow a negative exponential function. The simulation data revealed that the effective atomic molar volume b in the hard-sphere model is approximately modeled by the following negative exponential cubic function of the gas molar density:

$$b = b_0 + b_1 \, exp[\,-(k_1/v - k_2/v^2 + k_3/v^3)\,] \quad (61)$$

And the numerical values of the constants in Equation 61 have to be determined from curve fitting the simulation data of a gas when using the hard-sphere model. Of course, the values of these constants depend upon the size of the hard-sphere and what type of initial arrangement was employed when beginning the simulations.

Because atoms are not hard-spheres but behave more like spheres with a soft, penetrable outer periphery and an infinitely hard inner spherical core, the negative exponential function in Equation 22 was employed to best match experimental data of real gases when modifying the Redlich-Kwong-Soave equation of state.

Conclusion: Why not modify the van der Waals Equation of State for Real Gases and Liquids?

The first equation of state developed for real gases and liquids is the van der Waals equation. Thus, one may ask the question why not apply the same variation of the b-parameter for this first equation of state.

$$P = RT / \{v - b_0 - b(T)\exp[-k(T)/v)]\} - a / v^2 \quad (62)$$

There is a serious problem when applying this modification to the tradition van der Waals equation of state for real gases and liquids, because for some polar liquids, such as methyl alcohol and others, instead of having one critical point, there are two instead. In reality, real gases and liquids only have one critical point.

However, the modified Redlich-Kwong-Soave gas equation can be rearranged mathematically to the following expression resembling a modified van der Waals expression.

$$P = RT / \{v - b_0 - b_1(T)\,exp[-k(T)/v]\} - [a/(1 + b_0/v)] / v^2 \quad (63)$$

In Equation 63, the numerator in the subtraction term represents the fact that the van der Waals a-parameter in the modified Redlich-Kwong-Soave equation has dependence upon the gas molar volume v such that in the limit of zero molar volume, the term in the numerator approaches zero for this subtraction term. In theory, this correlates with the fact that at such high pressures gaseous atoms or molecules will begin to overlap their electron clouds enhancing repulsive forces. Thus, the repulsive forces for real gaseous atoms and molecules are not completely represented by the positive first term only in both the van der Waals and Redlich-Kwong-Soave equations of state.

References

Barrow, Gordon M. 1979. Physical Chemistry. Fourth edition. New York (NY): The McGraw-Hill Companies. 832 p.

Bird, RB, Steward WE, Lightfoot EN. 2006. Transport Phenomena. Second edition. New York (NY): John Wiley & Sons, Inc. 905 p.

Green DW, Southhard MZ. 2018. Perry's Chemical Engineers' Handbook. Ninth edition. New York (NY): The McGraw-Hill Companies. 2272 p.

Hirschfelder JO, Curtiss CF, Bird RB. 1954. Molecular Theory of Gases and Liquids. New York (NY): John Wiley & Sons, Inc. 1219 p.

Prausnitz JM, Lichtenthaler,RN, de Azevedo EG. 1998. Molecular Thermodynamics of Fluid-Phase Equilibria. Third edition. International Series in the Physical and Chemical Engineering Sciences. Upper Saddle River (NJ): Prentice Hall, Inc. 896 p.

Press WH, Flannery BP, Teukolsky SA, Vetterling WT. 1992. Numerical Recipes in Fortran 77: The Art of Scientific Computing. Second edition. Cambridge (UK): Cambridge University Press. 933 p.

Submitted October 12, 2018 Accepted November 18, 2018

Abstracts of the

107th Oklahoma Academy of Science Technical Meeting

November 2, 2018

Southwestern Oklahoma State University – Weatherford

PSEUDOMONAS SYRINGAE MOTILITY APPENDAGES: ROLES IN SURFACE COLONIZATION AND SURVIVAL

Jennifer L. Absire and Regina S. McGrane, Southwestern Oklahoma State University

Outstanding Undergraduate Poster

Pseudomonas syringae is a bacterial phytopathogen that causes disease in economically important crops. *P. syringae* is found in many different environments, each with varying levels of moisture. Therefore, the appendage it relies on for motility varies. The removal of genes encoding for these motility factors significantly impairs pathogenicity. The objective of this project was to evaluate the impact of *P. syringae* motility related appendages on colonization of varying environments. To characterize the role of flagella and pili, movement of deletion mutants was observed in saturated, liquid, and natural surface environments. Flagella mutants had significantly reduced motility in both saturated and liquid conditions, whereas pili mutants had significantly reduced motility in saturated conditions only. This indicates that flagella are the most relied upon appendage for motility and that pili have minor impacts. Similarly, on simulated natural surfaces flagella were the primary movement appendage. To characterize the impact of flagella and pili on colonization of plants, attachment to leaves and seeds as well as growth on leaves were evaluated. Mutants lacking flagella or both flagella and pili were impaired in leaf colonization but showed seed attachment similar to the wild type. However, mutants lacking both the flagella and pili had greater levels of attachment to leaves. To characterize the impact of flagella and pili on antibiotic sensitivity, mutants and the wild type were evaluated using a Kirby-Bauer sensitivity assay. Mutants were more sensitive to ciprofloxacin than the wild type, which is opposite to observations made in a different *P. syringae* strain. These results indicate that removal of the genes encoding for motility factors such as pili and flagella, directly impacts pathogenicity. Limiting the motility of *P. syringae* could halt or decrease the symptoms associated with this disease-causing pathogen and increase crop yields of host plants.

IOT SEMI-TRUCK MONITORING & SAFETY SYSTEMS

Mohamed Afify, **Mohamed Keblwei, and Nesreen Alsbou,** University of Central Oklahoma

Outstanding Undergraduate Paper in Engineering Science Section

The Trucks accidents are deadly and cost the companies a lot of money each year. We are designing a system to prevent the Truck accidents by design a mechanical device to enhance the safety system of the trucks by implementing a blind spot system and LIDAR system to detect the obstacles and alert the driver. We also installing a system that can detect the sudden braking of the vehicle in front of the truck to except any distance a few seconds before. We designing a medical vest also to give readings of the driver health conditions to prevent accidents as heart attacks or the driver falling in sleep.

ASSESSMENT OF IRON OXIDE NANOPARTICLES AS CHEMICAL TRACERS IN OKLAHOMA GROUNDWATER

Kelsey H. Anderson, Brian Bigelow, Rabeka Lashbrook, and Randall D. Maples, East Central University

It is important to be able to assess the risks of contamination of groundwater associated with the aquifer recharge process. Nanoparticles such as magnetite (Fe3O4) may offer several advantages over traditional chemical tracers including stability in undesirable conditions, detection at low concentrations, and ability to functionalize to suit a variety of different uses. This study begins assessing the potential of iron oxide nanoparticles as chemical tracers in groundwater using columns packed with glass beads, limestone, sandstone and dolostone from the local area and used water from local sources including tap water, creek water and the Arbuckle-Simpson aquifer as small scale environmental simulations. We then measured the recovery of the nanoparticles after flowing through the columns.

USING GROUNDWATER FAUNA TO DETERMINE SUBTERRANEAN HYDRAULIC CONNECTIONS IN KARSTIC AQUIFERS: A CASE STUDY FROM THE ARBUCKLE MOUNTAINS

Kevin Blackwood, Justin Harris, Laramie Edens, Kay Woodring, and Stacy Gantt-Blackwood, East Central University

Geology plays a significant role in both the storage and transport of groundwater, as well as the distribution and diversification of subterranean and aquatic organisms. The effect that stratigraphic and structural features might have on hydrogeology is by acting as either conduits or barriers to groundwater flow. Determining whether hydrologic connections exist between two terranes can be difficult, especially when time and hydraulic gradients might not be sufficient for the injection of chemical tracers or pumping tests might not be practical. By using endemic groundwater fauna (such as cave amphipods) as biological tracers, hydrogeological parameters may be deduced by comparing genotypic and phenotypic relationships between populations on either side of potential groundwater barriers due to allopatric isolation. Cave amphipods and groundwater isopods both occur in relative abundance within the study area, but cave amphipods are the most reliable for determining groundwater connections due to their restriction to the phreatic environment. Groundwater isopods have been documented traversing the vadose environment over films of water and may be capable of navigating around potential groundwater barriers. Therefore, groundwater isopods may be unreliable as biological tracers. Looking at examples from the Arbuckle Mountains of southern Oklahoma, we examine genotypic and phenotypic relationships of endemic groundwater fauna, using DNA barcoding and taxonomic methods. By employing these techniques, we can assess the effectiveness of various types of stratigraphic and structural features as either pathways or as barriers in a region heavily altered by structural deformation and modified by karst processes.

EVALUATION OF DNA EXTRACTION PROTOCOLS FUNCTIONALITY FROM DRIED TESTUDINE BONE

Mariah Ewy, Alisha Howard, and Kenneth Andrews, East Central University

There are numerous papers written about DNA extraction out of bones and animal tissue. The variety of DNA extraction protocols and the lack of performance consistency across different organisms argues for a lack of robustness. A total of 3 different protocols have been evaluated attempting to extract DNA from dried testudine bone, or shell. The first evaluated protocol is the Armed Forces DNA Identification Laboratory (AFDIL) protocol. This protocol was written to obtain DNA from skeletal remains for identification. The second evaluated protocol was written by Cold Spring Harbor Laboratory and titled Using DNA Barcodes to Identify and Classify Living Things. This protocol was created to isolate DNA from plant, fungal, or animal samples. The third protocol evaluated was the Nature Protocol titled Ancient DNA Extraction from Bones and Teeth. The goal of this protocol is to maximize recovery of PCR-amplifiable DNA from bone and teeth, while limiting the amount of contaminants that can inhibit PCR. All protocols were altered slightly to fit the resources available in the lab where the experiments were conducted. Each extraction protocol was unique in its methodology. Polymerase Chain Reaction (PCR) was performed with all DNA extraction products to amplify specific single segments of DNA.

HEIRLOOM MICROBES: THE HISTORY AND LEGACY OF ANCIENT DAIRYING BACTERIA

Shannon Fulton and Paul Lawson, University of Oklahoma

Soninkhishig Tsolmon, Mongolian Dietetic Association

Christina Warinner, Jessica Hendy, Matthäus Rest, Sanjeet Kumar, and Bjorn Reichhardt, Max Planck Institute for the Science of Human History

Human communities have utilized microbes for thousands of years as exemplified by the development of dairy products such as yoghurts and cheeses. The adoption of dairy foods into the adult human diet, and its consequent effect on the human genome, is a clear example of gene-culture co-evolution. The mechanisms of this process are not well understood, although we know that milk must have been heavily processed in order to create digestible products, with microbes likely playing a key role. Modern dairy production methods use microbial strains that are highly regulated in order to maintain hygiene standards and reproducibility. Subsequently, we remain unaware of the vast microbial diversity involved in ancient food preparation, and the impact this microbial diversity may have had on flavors and textures. With food globalization and industrialization, traditional methods of dairying and their unique microbial cultures are now being rapidly lost. It is known that lactic acid bacteria (LAB) are primarily responsible for the natural fermentation of animal milk and its by-products. Therefore, this study focuses on the screening of LAB in traditional dairy products from Central Asia (Mongolia) using culture dependent isolation of representation organisms to explore their genetic identification and deposit in microbial culture collections. Various dairy products, including raw and boiled animal milk, were collected from nomadic herding communities in Mongolia. A series of enrichments were designed to enable characterization of the LAB communities of the cow and yak derived Mongolian dairy samples. Two growth mediums, MRS and M17, containing different carbohydrate sources and nutritional components were selected for incubation in a reduced oxygen environment at 27.9°C and 45°C in order to select for mesophilic and thermophilic LAB, particularly *Streptococcus thermophilus*. Species belonging to the genera *Lactobacillus*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Pediococcus*, *Leuconostoc*, *Weissella*, *Carnobacterium*, *Gluconobacter*, *Lelliottia*, *Bacillus*, *Anoxybacillus* and *Brevibacillus* were recovered.

142

EXTRACTION AND CHARACTERIZATION OF DIHYDROXYACETONE FROM SUGAR CANE

William Gathright and Amanda J. Nichols, Oklahoma Christian University

Best Undergraduate Paper of the Academy and Outstanding Undergraduate Paper in Physical Sciences Section

Dihydroxyacetone is a chemical interest because of its ability to react with the amino acids in the outer layer of skin, giving the skin a bronzed look. As a result of its behavior, dihydroxyacetone is the typical active ingredient in self-tanners. Industry usually synthetically makes the compound, but it is naturally found in sugar beet and sugar cane. No published extraction procedure for dihydroxyacetone from sugar cane or sugar beets could be found. Preliminary methods were developed in order to isolate and characterize dihydroxyacetone from sugar cane and commercial sugar beet deer feed. Reflux boiling in ethanol was used as the extraction technique. Characterization techniques included high-performance liquid chromatography (HPLC) analysis and Fourier-transform infrared spectroscopy (FTIR) analysis. A preliminary characterization method was developed that used gelatin to mimic the self-tanning process on skin. Color changes of the gelatin 'skin' was observed between the extracted dihydroxyacetone and commercial self-tanners. One research area of chemical education focuses on the development of experiments appropriate for an undergraduate laboratory. These experimental methods can be used in a personal care products-themed lab component of an introductory chemistry course.

UNDERSTANDING GENE EXPRESSION REGULATION THROUGH CHARACTERIZATION OF TRANSCRIPTION START SITES IN *DROSOPHILA ELEGANS*

Amy Giemza, Sidney Wilkins and Lindsey J. Long, Oklahoma Christian University

The Genomics Education Partnership (GEP), Washington University in St. Louis

Outstanding Undergraduate Paper in Biological Science-Zoology Section

Every living organism is composed of genes. The position of these genes on a chromosome is important for proper regulation of gene expression. Alteration of positioning or gene regulatory elements can lead to diseases such as cancer. Understanding the mechanisms of gene regulation can help explain why genes are misregulated in diseases and, furthermore, how to abrogate this misregulation. Because highly important DNA sequences are often conserved throughout evolution, our strategy was to identify gene regulatory elements in various species related to *Drosophila melanogaster* as the genome of this species is fully annotated. Transcription start sites (TSSs) are where transcription of DNA into RNA begins which ultimately leads to the production of proteins. Our specific research focused on identifying TSS positions for several genes and compiling the data to distinguish trends for the regulatory elements that surround the TSS of genes that are classically "on" or "off." To pinpoint the location of the putative TSS for the *D. melanogaster* unc-13 ortholog in *D. elegans*, we aligned the *D. melanogaster* unc-13 sequence to the *D. elegans* genome to search for homology. The identified search region was further scrutinized using lines of evidence derived from experimentation to identify the exact location of the TSS. Core promoter motifs in the region were also evaluated to investigate the possibility of alternative TSSs. Of particular interest, unc-13 had low sequence homology with the *D. melanogaster* ortholog and supplementary evidence was unsuccessful in difinitively identifying the TSS for all isoforms of the gene. Ultimately, the data supported that only a few of the isoforms of unc-13 were conserved in the evolution of this species.

MNIST DATASET ANALYSIS

Reid Kinder, East Central University

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

Teaching a computer to classify data accurately through multi-layer neural network processing is known as deep learning. The MNIST dataset was used to explore and compare machine learning processes to deep learning through packages such as SKLearn, and Tensorflow. Through SKLearn, different dimensional reduction techniques were used to manipulate the dataset, such as Principal Component Analysis (PCA) and T-Distributed Stochastic Neighbor Embedding (t-SNE). PCA and t-SNE were used to reduce the number of dimensions of the dataset, while conserving certain characteristics of the data. Finally, K-Nearest Neighbors (KNN) was used to classify the data after dimensional reduction. After this classification, a graphical representation of the data was presented. An accuracy greater than 85\% on the test set was achieved through this method. Tensorflow was also successfully applied to the data set. Through Tensorflow, we reached a result of greater than 95\%.

CLONING AND EXPRESSION OF *CHLAMYDIA TRACHOMATIS* INCLUSION MEMBRANE PROTEINS

Colleen (Denver) La Force, Kriti Shukla, Prakash Sah, Christina Bourne, and Erika Lutter, Oklahoma State University and University of Oklahoma

Outstanding Graduate Poster

Chlamydia trachomatis is an obligate intracellular human pathogen that resides inside host cells within a parasitophorous vacuole called an inclusion. In order to replicate and grow *Chlamydia* must usurp host cell proteins from within this vacuole. To do this, *Chlamydia* produce and secrete proteins, termed inclusion membrane proteins (Incs), that insert into the inclusion membrane with the N- and C- terminus facing the host cytosol. Currently, *C. trachomatis* is predicted to have 50 Incs, however very few of these have known functions. Very little knowledge about their function can be gained via bioinformatics analysis since they lack similarity to any proteins outside of *Chlamydia*. This has made characterizing Incs or identifying possible functions very difficult. The goal of this project is to clone and express the C-terminus of certain Incs which will be used to produce purified protein for future crystallography studies. This study focuses on the CT229-CT224 operon which is only found in human pathogens. Here, we present the cloning strategy of each Inc into the expression plasmid pET28a which will generate a C-terminus Inc fusion to a 6X His tag. To date CT226, CT227 and CT228 have been successfully cloned, verified by sequencing, and transformed into BL21 for expression studies. Protein production with these constructs has been induced with IPTG. Progress with the CT226 construct has proceeded to successful solubilization after expression, and re-folding is evident from the defined elution from size exclusion chromatography as an apparent trimer, in addition to strong amide bond signals in Fourier-transform infrared spectroscopy indicating approximately half of the protein adopts a helical conformation. By assessing their structures, insights may be gained as to possible functions based on similarity to other characterized proteins.

OKN RELIEVES OXIDATIVE STRESS INDUCED BY SEPSIS-ASSOCIATED ENCEPHALOPATHY

Tyler McKenzie, Redlands Community College,

Debra Saunders, Nataliya Smith, Shania Do, William Towler, Marvin Cruz, and Rheal Towner, Oklahoma Medical Research Foundation

Introduction: Sepsis-associated encephalopathy (SAE) is an oxidative stress-related disease of the brain caused by the introduction of bacteria, and it causes an increased permeability of the blood brain barrier (BBB). Neuroinflammation can lead to cognitive impairment which is linked with age. Methods: Lipopolysaccharide (LPS) induced rat models were used to compare OKN treated, untreated, and saline injected negative controls. OKN is a spin trapping compound for reactive oxygen and nitrogen species that prevents further damage to tissue from free radicals. Various Magnetic Resonance Imaging (MRI) techniques such as contrast enhancement, MRI perfusion, and MR spectroscopy were used to scan the brain for signs of a compromised BBB, vascular alterations, and metabolite changes, respectively. Results: MRI contrast enhancement showed that the OKN treatment was lowering MRI intensity within the cortex, hippocampus, thalamus, and peri-rhinal cortex. LPS-injected untreated rats had a greater MRI intensity post-contrast injection for week one. It was observed with MR spectroscopy that metabolites within the brain had also stabilized with the OKN treatment indicating the reduction of damaged tissue. Low perfusion rates were examined during post-LPS injection of both week one and three, which signaled constriction of the vessels within the brain. Conclusion: We can conclude the BBB and vascularity is negatively affected long-term by LPS. SAE promotes neuroinflammation which affects BBB permeability, vasoconstriction, and decreased brain metabolites. OKN proved to be an effective treatment with the rat models for minimizing the effects of SAE within the brain. Funding: OK-INBRE and NIH RO1NS092454.

STRUCTURE OF POXVIRUS A6 PROTEIN REVEALS A MECHANISM FOR STABILIZING OPEN-ENDED CRESCENT MEMBRANE

Prabhat Kumar Pathak, Shuxia Peng, and Junpeng Deng, Oklahoma State University

Best Graduate Paper of the Academy

Cellular membranes are maintained as closed compartments,broken up only transiently during membrane reorganization or lipid transportation. However, open-ended membranes, likely derived from scissions of the endoplasmic reticulum, persist in vaccinia virus-infected cells during the assembly of the viral envelope. A group of viral membrane assembly proteins (VMAPs) were identified as essential for this process. To understand the mechanism of VMAPs, we determined the 2.2-Å crystal structure of the largest member, named A6, which is a soluble protein with two distinct domains. The structure of A6 displays a novel protein fold composed mainly of alpha helices. The larger C-terminal domain forms a unique cage that encloses multiple glycerophospholipids with a lipid bilayer-like configuration. The smaller N-terminal domain does not bind lipid but negatively affects lipid binding by A6. Mutations of key hydrophobic residues lining the lipid-binding cage disrupt lipid binding and abolish viral replication. Our results reveal a protein modality for enclosing the lipid bilayer and provide molecular insight into a viral machinery involved in generating and/or stabilizing open-ended membranes.

TIMING OF AVIAN MIGRATION ONSET THROUGH THE OKLAHOMA CITY AREA 1995-2017 USING NOAA WEATHER DATA

Jennifer Prophet and Zach Jones, Southwestern Oklahoma State University

Outstanding Undergraduate Paper in Applied Ecology and Conservation Section

Timing and duration of avian migration patterns may be linked to large-scale climate patterns and reflect long-term shifts in average regional temperatures. The National Centers for Environmental Information (NCEI) provides a publicly-available archive of National Oceanic and Atmospheric Administration (NOAA) weather radar data scans occurring every ten minutes dating back to 1995. Our primary data of interest are 1) the measure of reflectivity: the amount of power returning to a radar after hitting water, and 2) radial velocity: the movement of water relative to radar position. Aerial density and flight direction will be collected annually and compared to global and regional temperatures to determine effects on seasonal migrations. For the current study, we used KTLX (Oklahoma City) weather radar data to determine time, duration, intensity and direction of fall avian migration. Our analysis currently includes data from 1995-2017, with the fall migrations beginning in September and the spring migrations beginning in March. The global average temperature change over this time range was 2.358℃. More data will be analyzed within this range in order to develop more accurate results regarding the effect of temperature change on fall and spring migratory onset. In addition to migratory information, we have observed fine lines of insects caught in outflow air masses as well as regular sunrise takeoffs of waterfowl overwintering near Lake Hefner. We also have encountered radar signals caused by wind turbines, but this has not noticeably affected avian flight behavior or our data collection on the scale and altitudes being sampled.

COMPUTATIONAL STUDY OF VOLATILE ALUMINUM HYDROXIDE

Uendi Pustina and Dwight L. Myers, East Central University

Reactivity and compatibility of oxides with other materials and with each other plays a significant role in choice of materials for developing Thermal Barrier Coatings (TBCs) or Environmental Barrier Coatings (EBCs) for use in combustion environments. We are performing a computational study of the gas phase molecule aluminum hydroxide. The ultimate goal of this study is to obtain a reliable value of the enthalpy of formation of aluminum(III) hydroxide. The software we are using is the GAMESS ab initio package. Presently we are to the stage of optimizing the geometry of the molecule. Results to date will be presented.

EFFECT OF STORAGE TEMPERATURE ON BIOACTIVITY OF A COMMERCIAL *SACCHAROMYCES BOULARDII* PROBIOTIC FORMULATION

Reid Reding and Jonathan Hunt, Oklahoma Christian University

Outstanding Undergraduate Paper in Microbiology Section

The commercial probiotic industry is rapidly expanding and gaining traction among the common population. Many people see advertisements for probiotics making claims that they will contribute to better overall health and potentially help restore health after dealing with gastrointestinal issues, etc. Probiotics have helped many infections such as *C. difficile* and ulcerative colitis by restoring the gut microbiota after antibiotics have killed many of the beneficial microorganisms. Probiotics do this by competing with the pathogen for resources necessary to sustain life and by interfering with important metabolic pathway steps. Furthermore, the probiotics promote a healthy gut flora in general, which helps to eliminate symptoms caused by pathogenic species. Many manufacturing companies state that the probiotics need to be stored in a refrigerated area to maintain their efficacy; however, often times consumers forget to keep them in a refrigerator. The experiment at hand measured the viability of a commercial preparation of *Saccharomyces boulardii* probiotic under varied storage conditions (4ºC, 23ºC, and 30ºC) once a week over a four-week period to assess the necessity of specific storage conditions.

RAD4 IS REQUIRED FOR MAINTENANCE OF DNA INTEGRITY

Brandon Reed, Whitney Bohannan, and Lindsey Long, Oklahoma Christian University

Outstanding Undergraduate Paper in Biomedical Science Section

UV radiation exposure can cause bulky adducts (such as cross-linked thymine bases) in DNA. The cell uses the Nucleotide Excision Repair (NER) pathway as the primary mechanism to repair these adducts. Normally, these adducts are repaired; however, when not repaired, these adducts lead to diseases such as xeroderma pigmentosum and an increased risk of skin cancer. In *S. cerevisiae*, RAD4 encodes for protein that is a component of the NER pathway. Rad4 forms a heterodimer complex with Rad23 to form Nuclear Excision Repair Factor 2 (NEF2), which is necessary for the recognition of the damaged bases. After DNA damage recognition, the remaining repair machinery is recruited to remove the bulky adduct and restore the DNA integrity. It has been shown that deletion of the RAD4 gene in *S. cerevisiae* leads to increased UV sensitivity when compared to the wild type (wt) strain, due to the inability of the machinery to recognize and repair lesions caused by UV radiation. We also showed that the Δrad4 strain showed significantly lower viability after UV radiation exposure when compared to the wt strain that was exposed to the same UV dosage. To investigate as to whether this reduction in viability was related to unrepaired DNA mutations, we used a reverse mutation canavanine assay to calculate DNA mutation frequency. Δrad4 cells showed a higher DNA mutation frequency when compared to the wild type strain. These results indicate that RAD4 is critical for DNA damage repair after UV exposure, and failure to repair DNA damage results in DNA mutation and cell death.

BACTERIOPHAGE--A POTENTIAL REPLACEMENT FOR ANTIBIOTICS

Abbey Renner and Madison Snow, Oklahoma City University

Bacteriophage ("phage") are viruses that infect and replicate in bacteria. Since phage infect bacteria very specifically, there is considerable interest in exploiting them as antibacterial agents. Phage are found in natural environments such as soil, where there are complex bacterial ecosystems. We extracted phage from nutrient-rich soil by mixing the soil with a buffer (SM buffer), and filtering out the large particles and living organisms using a 0.45 micron syringe filtration system. Phage were added to cultured bacteria (*Bacillus cereus* or *Serratia marcesens*), mixed with top agar, and plated on LB-media. After incubation, the plates were checked for zones of clearing ("plaques"), indicating the presence of specific bacteriophage.

CLONING, SEQUENCING, AND IDENTIFICATION OF PHAGE P13, AN UNKNOWN *SALMONELLA* OR EHEC (ENTEROHEMORRHAGIC *E. COLI*) BACTERIOPHAGE

Ryan Sloan, W.J. Reddig, and Earl L. Blewett, Oklahoma State University-Center for Health Sciences

Divya Jaroni, Oklahoma State University-College of Agriculture

Bacteriophage have been isolated from the environment that specifically infect bacteria responsible for food poisoning. These bacterial viruses kill *Salmonella* and Enterohemorrhagic *E. coli* and may be useful in the food industry to reduce bacterial food contamination. We cloned and sequenced fragments of genomic DNA from one of these bacteriophage, P13. Bioinformatic analyses showed this bacterial virus was most similar to phage from the *Yersinia* genus and not that similar to phage from the *Salmonella* group.

USE OF ENCLOSURE SPACE BY LONG-TAILED MACAQUES AT MINDY'S MEMORY PRIMATE SANCTUARY

Madison A. Snow, Tesa J. Martin, Kyle J. Copp, and Laurie Kauffman, Oklahoma City University

Huyen Tran and Tephillah Jeyaraj-Powell, University of Central Oklahoma

In terms of behavioral benefits, naturalistic environments have shown promise for primates in captivity. With the goal of providing data which supports the use of particular structures to stimulate and encourage natural behaviors, we investigated how 11 male long-tailed macaques (*Macaca fascicularis*), located at Mindy's Memory Primate Sanctuary in Newcastle, Oklahoma, use their enclosure space. Data was collected on usage of the ground, platform, firehose, stumps, warm house, and enclosure wall space. We also compared the patterns of enclosure space use of former pet macaques versus former lab macaques. Data collection included use of a video camera to record and narrate for 15 minute periods. Our initial hypothesis was that there would be no differences in use of enclosure space or structures. We found no significant difference between former pet and lab monkeys with use of a 2x7 mixed ANOVA. However, there was significant difference is structure use although not as we had hypothesized. We will communicate our results with the primary sanctuary in order to provide an environment which will enhance the macaque's overall health.

OKLAHOMA ACADEMY OF SCIENCE

2018 OFFICERS

President
Dr. Adam Ryburn, OCU

President-Elect
Dr. Robert Mather, UCO

Editor, *Proceedings*
Dr. Mostafa S. Elshahed, OSU

Immediate Past President
Dr. Terry Conley, CU

Executive Secretary-Treasurer
Dr. David Bass, UCO

Recording Secretary
Dr. Karen Williams, ECU

Section	Chair	Vice-Chair
A: Biological Sciences	Dr. Michael Husak, CU	Dr. Michael Dunn, CU
B: Geology	Mr. Kevin Blackwood, ECU	VACANT
C: Physical Sciences	Dr. Weldon Wilson, UCO	Dr. Karen Williams, ECU
D: Social Sciences	Dr. Jaclyn Maass, UCO	Dr. J. Adam Randell, CU
E: Science Communication & Education	Dr. Kathleen Coughlan, Redlands Community College	VACANT
F: Geography	Dr. Brad Watkins, UCO	VACANT
G: Applied Ecology & Conservation	Dr. Zach Jones, SOSU	Dr. Jerry Bowen, RSU
H: Microbiology	Dr. Krithi Sankaranarayanan, OU	Dr. Charlie Biles, ECU
I: Engineering Sciences	Dr. Gang Xu, UCO	Dr. Nesreen Alsbou, UCO
J: Biochemistry & Biophysics	Dr. Junpeng Deng, OSU	Dr. Ellie Nguyen , OSU
K: Microscopy	Dr. Matt Lundwall, Phillips 66	VACANT
L. Mathematics and Computer Sciences	Dr. Quan Tran, USAO	Dr. Nicholas Jacob, ECU
M: Environmental Sciences	Dr. Charles Crittell, ECU	Dr. Dan McInnes, ECU
N: Biomedical Sciences	Dr. Landon Moore, UCO	Dr. Bill Luttrell, OC

Collegiate Academy of Science	*Director*	Dr. Jerry Bowen, RSU
OAS Technical Meeting, 2018	*Local Coordinator*	Dr. Peter Grant, SOSU
OAS Web Site	*Webmaster*	Dr. Adam K. Ryburn, OCU

OKLAHOMA ACADEMY OF SCIENCE

STATEMENT OF REVENUES COLLECTED AND EXPENSES PAID FOR THE YEAR ENDED DECEMBER 31, 2017

REVENUES COLLECTED		
Membership Dues:	$3,402.30	$3,402.30
Investment Income:	$54.02	$54.02
Meetings:		
Registration - Spring Meeting	$2,570.73	
Registration - Fall Meeting	$6,005.53	
Registration - Technical Meeting	$4,869.91	$13,446.17
Donations:	$870.00	$870.00
Other Income:	$7,501.49	$6,631.49
Total Revenue Collected		**$24,403.98**
EXPENSES PAID		
Stipends and other Compensation:		
Stipends	$6,141.24	
Social Security	$824.60	
Medicare	$192.84	$7,158.68
Professional Fees:		
Audit	$500.00	
Tax Preparation	$1,145.00	$1,645.00
Meeting Expenses:		
Spring Meeting	$2,031.38	
Fall Meeting	$4,595.00	
Technical Meeting	$1,390.76	$8,017.14
Dues:	$750.00	$750.00
POAS:	$2,871.44	$2,871.44
Woody Plants:	$115.16	$115.16
Other Expenditures:	$2,798.39	$2,798.39
Total Expenses Paid		**$23.355.81**
Revenues Collected Over Expenses Paid		**$1,048.17**

OKLAHOMA ACADEMY OF SCIENCE

STATEMENT OF ASSETS, LIABILITIES AND FUND BALANCE ARISING FROM CASH TRANSACTIONS DECEMBER 31, 2017

ASSETS

Cash:		
Checking Account	$28,945.24	
Savings Account	$3,276.10	
Endowment Savings Account	$2,132.08	$34,353.42
Investments:		
Certificate of Deposit	$60,000.00	$60,000.00
Total Assets:		**$94,353.42**

LIABILITIES AND FUND BALANCE

Liabilities:	$0.00	
Fund balance:		
Beginning operation fund balance	$92,445.24	
Excess revenues collected over expenses	$1,908.18	
Total Funds:		**$94,353.42**

INDEPENDENT AUDITOR'S REPORT

Executive Committee
Oklahoma Academy of Science

I have audited the accompanying statements of assets, liabilities and fund balance arising from cash transactions of the Oklahoma Academy of Science as of December 31, 2017, and the related statements of revenue collected and expenses paid for the year than ended. These financial statements are the responsibility of the Company's management. My responsibility is to express an opinion on these financial statements based on the audit.

I have conducted an audit in accordance with generally accepted auditing standards. An audit to obtain reasonable assurance about whether the financial statements are free of material misstatement and examining, on a test basis evidence supporting the amounts and disclosures in the financial statements. These financial statements were prepared on the basis of cash receipts and disbursements and this report prepared only for the internal use of the Executive Committee of the Oklahoma Academy of Science.

I find the financial statements referred to above present fairly, in all material respects, the assets, liabilities and fund balance arising from cash transactions of The Oklahoma Academy of Science as of December 31, 2017 and its revenue collected and expenses paid during the year then ended.

E. Pace, Retired
Assistant County Auditor

OKLAHOMA ACADEMY OF SCIENCE

Name_____Affiliation _____
Last First Middle

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

_____
City State Zip
OR (not both)

Mailing Address (for home delivery) _____
Street, P.O. Box, Route, etc. City State Zip

Telephone_____E Mail _____

Please indicate whether this is a Renewal or New Membership. What year? _____

Note all annual memberships expire December 31 if you do not prepay for the following year.
Membership Type (check one):
_ Life $500 Professional $30 Family $35 Undergraduate/Graduate Student $20
$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.
_____ D Social Sciences_____H Microbiology_____L Mathematics/Computer Sci_____Z Junior Academy

Make checks payable to the **Oklahoma Academy of Science**.

Mail completed form and payment to: Dr. David Bass, Executive Director, Oklahoma Academy of Science, University of Central Oklahoma, Campus Box 90, Edmond, OK 73034.

Members, please photocopy this form and give that to a non member colleague or student. Help strengthen OAS by recruitment!

DONATION FORM

Make tax deductible donations in memory or honor of a family member, friend, or colleague. All donated fund are placed as principle into the OAS Endowment Account and the interest generated funds the Academy's programs.

Donor _____

_____
Mailing Address (Street or PO Box) City State Zip

Honoree _____
Amount

Editorial Policies and Practices

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

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

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

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

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

Reviewer's Responsibilities

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

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

Brief Instructions to Authors

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

A. Submission Process.

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

poas@okstate.edu

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

The Editors review the MS and carefully select other reviewers as described in "Editorial Policies and Practices" (see p. 153); 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.

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

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

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

Abstract. You may submit an abstract of your presentation at the OAS Technical Meeting. For specific instructions, contact the Editor. Even though abstracts are not peer-reviewed, they must align with the policies and scope of the

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

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

C. Manuscript Organization.

1. General organization.

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

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

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

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

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

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

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

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

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

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

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

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

Examples of References in CBE Style and Format

Journal Articles

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

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

Books

Book with Authors:

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

Book with Editors:

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

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

Chapter in Book with Editors:

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

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

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

D. Review Process.

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

E. Page Charges

The OAS will publish accepted MSs with the implicit understanding that the author(s) will pay a charge per published page. Page charges are billed at the cost per page for the given issue: current rates of $90 per page for nonmembers of the Academy and $35 for members. All authors are expected to honor these page charges. Billing for page charges and receipt of payment are handled by the

Business Manager, who is also the Executive Secretary and Treasurer for the Academy.

Under exceptional circumstances, when no source of grant funds or other support exists, the author(s) may apply, at the time of submission, for a waiver of page charges.

F. Copyright Transfer

Before publication, authors must transfer copyright to the Oklahoma Academy of Science. All authors must sign, or the signing author must hold permission to sign for any coauthors. Copyright for papers reporting research by U.S. Government employees as part of their official duties will be transferred to the extent permitted by law.